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Comparing the rates of absorption and weight loss during a desorption test using near infrared spectroscopy

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Abstract— The importance of determining skin hydration has over the years prompt the development of many instruments and methods, specifically designed to assess this parameter or water contents especially in the stratum corneum, and have greatly matured to suit different anatomical sites and measure multiple attributes. Of those, Near Infrared Spectroscopy (NIRS) has gained wide interest as a precise, safe, fast and noninvasive technique for determining skin hydration due to its high sensitivity to hydrogen bonding and ability to measure the amount of water in skin directly using the intensities of overtone and combination bands of OH and HOH water bonds occurring in the NIR region, that are good indicators of the state of skin hydration. This paper reports near infrared spectrophotometric measurements using a highly sophisticated spectrophotometer in the region of 1000-2500 nm to study the water uptake and dehydration properties of skin *in vitro* using samples of porcine skin. Initial results of pure liquid water and skin samples have clearly displayed the prominent bands associated with water content, and desorption tests have been able to verify changes in these bands associated with water content, although a clear correlation between the rates of weight loss and absorbance loss at various hydration periods has not yet been established. These preliminary results are expected to further explain the relationship between water and skin, and its role within, in hope to aid the future development of a portable instrument based on near infrared spectroscopy that would be capable of directly measuring skin hydration and/or water content in a fast and noninvasive manner.

I. INTRODUCTION

Without a doubt, the skin is the most complex organ of the human body, and one that requires constant care and attention [1]. In its widely appreciated context, the skin barrier function refers to the epidermal barrier to water permeability, and is also the largest organ of the body, an approximately two square meter, covering its entire surface [1,2]. In essence, it acts as a means for adjusting to variations in environmental temperatures through elegant controls that regulate the microcirculation, and as an interact impermeable barrier that precipitates the organism to intake water without flooding its internal organs, all whilst prohibiting the intrusion of various xenobiotics, and even includes touch, pain and pressure receptors. Large-scale damage to this part would make human life impossible, and its maintenance enhances our physical and mental health [1,2]. The outermost layer of the skin, known as the Stratum Corneum (SC) is a protein-enriched layer of corneocytes embedded in a lipid-enriched intercellular matrix made of 6-47 layers of keratinized cells, and is roughly 8-20 μm in thickness except on the palms and soles where it is much thicker [3,4]. Its structure follows that of the commonly named bricks and mortar model, where the corneocytes form the “bricks” as they are enclosed in a cornified cell envelope consisting of proteins such as loricrin, filaggrin, and involucrin, and covalently attached to the hydroxyceramide molecules of a lipid casing, all of which are embedded in lipid bilayers made up of various intercellular lipids including ceramides, cholesterol sulfate, free sterols and sterolesters, and free

fatty acids, which thus acts as the “mortar” [4]. This particular layer is primarily responsible for the regulation of water evaporation and the water-holding function of skin. It also acts as an essential permeable barrier and the presence of water within it or its water content has profound effects not only on its elastic and moisture properties, but also on its transport and optical properties, and may act to maintain the skin lipids in a preferred liquid crystalline arrangement.

However, although water plays an important role in the well-functioning of the skin, it is potentially a strong irritant and can cause skin damage [5,6]. Skin exposure to water can disrupt the barrier function of the SC by altering its physiological behaviour through extraction of water-soluble substances, or Natural Moisturizing Factors (NMFs) from the skin [5], which are amino acids, lactic acid, pyrrolidone carboxylic acid, and urea that were released following the breakdown of filaggrin in the mid-portion of the SC corneocytes, which then manifest an osmotic force and hence bind water [4]. Prolonged contact with water can also damage the SC barrier function by facilitating the penetration of foreign substances, and can even cause skin disease [5,6].

So far, various technologies have been implemented to assess the water content of the SC and other skin parameters noninvasively for moisture evaluation and/or for detecting abnormalities in the skin that may serve as indicators for underlying illnesses or skin conditions [7]. Previous measuring techniques have included those based on thermal, mechanical, imaging and spectroscopic methods, but with electrical probe-based measurements being the most commonly utilized today. Unfortunately though, the latter suffers several principal drawbacks due to the absence of a direct correlation between skin conductivity and water content, and that the flow of current can be influenced by variations in ion movements, and re-orientation of protein dipole moments, and so one must use such devices with utmost care to avoid inaccuracies that may rise due to the presence of salts in formulations or perspiration that can effect ion mobility, or from chemicals that can disturb the protein structure by altering the orientation of protein dipole moments [8]. Alternatively, Near Infrared Spectroscopy (NIRS) has the ability to measure the amount of water in skin directly using the intensities of overtone and combination bands of OH and HOH water bonds occurring in the NIR region, that are good indicators of the state of skin hydration [3]. Hence, this allows a more precise method of measurement, and with an added advantage in that its instrumentation can easily be equipped with fibre optic probes for *in vivo* measurements in various anatomical sites.

Looking at water alone, its absorption spectrum is one of extreme complexity since water molecules are capable of vibrating in many ways [9,10]. Such vibrations involve combinations of symmetric stretch (ν_1), asymmetric stretch (ν_3) and bending (ν_2) of its covalent bonds. As for liquid water, rotation of its molecules are entirely dominated by hydrogen bonding which restrict their movements, thus giving a type of vibration known liberation, where the water molecule is attracted to neighbouring molecules, so that it has a preferred orientation and cannot freely rotate [9]. Moreover, spectral lines of liquid water tend to be broader which causes overlapping of many of the absorption peaks, and in comparison to its gas state, the main stretching band in liquid water shifts to a higher wavelength, and the bending wavelength decreases by hydrogen bonding [9,10]. Within the skin, or biological systems in general, water interaction is more complicated. This is because water within such systems can either bind to biomolecules i.e. proteins or intermolecular to other water molecules through hydrogen bonding. As a result, two main types of water exists within the skin, namely bulk or free water and bound water, where the former represents water molecules non-hydrogen bonded to biomolecules but instead, are hydrogen bonded to other water molecules in a tetrahedral structure, and the latter describes those held tightly to other compounds by the colloidal micelles that forms an intimate part of the material rather than the tetrahedral structure [11]. This inconstant nature of water binding has been thought to exist from the unique ability of water molecules to constitute multiple hydrogen bonds with any combination of its two lone electron pairs and two protons.

Previous studies using NIRS technology were successful in obtaining information about these types of water present within the skin, and concluded that, water contents below 10% in the skin refer to water that

is tightly bound to protein, whilst higher contents, between 10-40%, represent less tightly bound water, and above 50%, water was similar to the form of bulk liquid. They also managed to obtain further details with regards to other skin constituents such as lipids and proteins [1,12,13]. Together, this has made the technique very attractable as a safe and noninvasive alternative, and whilst several earlier studies have applied it to study skin hydration and the efficacy of cosmetic products [6,8,12,13,14], many others continue to do so, and consecutive developments and advancements are being made in its instrumentation and chemometric analysis processes, thus extending its applications and utility in this field.

Although there has been interest in improving this technique for the purpose of skin hydration and water content measurements [15,16,17], a reliable handheld or portable measuring instrument of this type does not yet exist.

Overall, the absorption and scattering of liquid water belong to its physical chemistry, but are of great interest here since water is a major constituent of the skin and its overtones and combinations are clearly distinguishable in skin spectra. So, by presuming the intrinsic absorption properties of water will remain the same even when measured in the skin, the study will begin by determining the absorption spectrum of liquid water alone, using a highly sophisticated spectrophotometer. This assumption is almost always true as water dominates the NIR absorption spectra of various high water content intact soft tissues, such as the skin.

Therefore, the first part of this study will focus on obtaining a spectrum of liquid water alone in the NIR region, which by doing so, can then be used to detect its changes in skin and the change in skin water content under certain physiological conditions. Then, the objective is to compare spectroscopic measurements in the NIR region of pure liquid water against samples of porcine skin, and to conduct *in vitro* desorption experiments on porcine skin to investigate the mechanisms of water uptake and dehydration by the skin at various hydration periods, in order to aid the future development of a portable device based on NIR spectroscopy that would be capable of measuring skin water content in a safe, fast and noninvasive manner.

II. MATERIALS AND METHODS

A. Optical properties of liquid water

In order to obtain a spectrum of liquid water, the Lambda 1050 dual beam UV/Visible/NIR spectrophotometer (Perkin Elmer Corp, Massachusetts, USA) was used with increment steps of 1 nm, and a spectral region of 200-3300 nm, thus covering parts of the ultraviolet region, the entire visible and NIR, as well as parts of the mid-infrared region. The source was a deuterium lamp between 250-319.20 nm, and beyond that was a halogen tungsten lamp up to 3300 nm. The detector system was set to use a photomultiplier tube detector (PMT) for the shortest wavelengths from 250 nm until 860.80 nm, and an indium gallium arsenide detector (InGaAs) between 860.80-2500 nm. For the rest of the spectral region up to 3300 nm, a lead sulfide detector (PbS) was applied. Slits settings for the PMT detector were fixed at 2 nm, whilst InGaAs and PbS detectors were set on "servo mode", whereby the system monitors the reference beam energy and adjusts the slits accordingly to avoid over saturation of the detectors. Gain settings for the three detectors, which are applied for energy measurement in single beam mode, must be set to generate a useable spectrum. After a few trials, a gain of 3 was selected for the InGaAs and PbS detectors, whereas the PMT gain was set on auto to allow the instrument to determine the appropriate value. The response time for these detectors was set at 0.2 seconds for all. The lowest possible chopper cycle is 0.04 seconds, but since increasing the response time enhances the sensitivity of the instrument, the value chosen seemed to give a good balance between scan time and sensitivity. The front and rear attenuators enclosed in this instrument are used to select the attenuation in the sample and

reference beam, and in this case were set at 100% for the sample beam and reduced to 1 % for the reference beam. Prior to placing the sample of liquid water through the spectrometer, baseline corrections were performed on the spectrophotometer. This is to remove “background noise” or unnecessary peaks, resulting from carbon dioxide and oxygen impurities in the air, and is available in this instrument in three types, but only two of those, 100 %T/OA baseline, and 0 %T/blocked beam baseline were included. The former performs the correction whilst the sample beam and reference beam are set to 100 %, whilst the latter, sets the sample beam to 0 % and the reference beam to 100 %, and was applied due to the high absorbing nature of water in certain regions.

The sample of liquid water was contained in a rectangular quartz SUPRASIL cell, made of high purity synthetic fused silica material to ensure top performance, and with a light path length of 1 mm (P/N: B0631013, Perkin Elmer Corp, Massachusetts, USA). Liquid water was inserted into the cell through a syringe, which was then placed into the sample compartment whilst a blank duplicate cell was inserted into the reference compartment.

B. In vitro experiment on porcine skin

Firstly, ethics approval was sought by the University Senate Research Ethics Committee to allow testing on animal samples. The previous settings were maintained on the spectrophotometer and only the interval steps of measurements were changed to increments of 2 nm and the operating spectral region was reduced to 1000-2500 nm. Fresh porcine meat was acquired and kept frozen until ready for testing. Porcine skin samples were cut using a scalpel into a rectangular shape, of roughly 1.0 mm and 1.5 mm thickness. For the first part of the experiment, both samples were not hydrated or treated in any way, and were tested in their normal state to compare against the spectrum of liquid water. The 1 mm sample was then placed in a jar containing pure liquid water to measure its dehydration rate when it was placed in water for different periods of time. For the dehydration rate testing, it was chosen to leave the sample in water for 30 min, 60 min, 3 h, 12 h, 18 h and 24 h. Once the sample reached the required time in water, it was removed, dried, and weighed on the TR-214 model analytical balance (Denver Instruments GmbH, Gottingen, Germany), before placing it in the spectrophotometer. Measurements were then taken every 20 min from the initial reading, and lasted 3 hours. The porcine sample was weighed each time before the next measurement.

In order to directly place skin samples into the cell compartment of the spectrophotometer, a disposable polystyrene cell was used throughout the entire experiment which had a hole cut on both the front and rear of it in the area of the beam target, so that radiation from the spectrophotometer source was directly incident on the skin sample without striking the windows of the cell. The reference compartment was left blank. All spectra obtained during these experiments were available immediately at the end of each test, and were displayed on the UVWinlab software (Perkin Elmer Corp, Massachusetts, USA) that accompanies the Lambda 1050 spectrophotometer. The software was also used to perform quant scanning of data, but for further analysis, spectra were transferred to the UVWinlab Data Processor and Viewer program to carry out processes such as smoothing, calculating derivative spectra, and exporting data in excel format.

III. RESULTS AND DISCUSSION

The resulting absorption spectrum of liquid water is shown in Fig. 1, after undergoing smoothing to reduce the level of noise, as well as the effects of overlapping bands, and to suppress background effects. This process was carried out using the UV Winlab Data processor software which performs this function by detecting the sample data points from the spectrophotometer prior to storing it in an array in a data station, and then allows a pre-selected smoothing bandwidth to be entered into the data station

from the keyboard. Then, the software converts the bandwidth to a strength parameter, and thereafter each of the smoothed data points is stored before given as an output array of smoothed data points to form a modified spectral display, which identifies the components in the sample.

At first glance, water seems to have low absorption/high transmission throughout the near-UV (up to 400 nm) and the visible region between 400-760 nm, with no clear peaks being apparent. However, as it approaches the NIR region (760-2500 nm), its behaviour begins to shift slightly mainly from 1000 nm, and experiences dominant peaks near 1450 nm and between 1900-2000 nm. Beyond the NIR region and as the spectrum moves deeper into the infrared region, the behaviour of liquid water tends to change dramatically and becomes extremely highly absorbing.

The absorption spectrum of liquid water has been investigated by several studies in the past, and according to those, the spectrum of liquid water is normally dominated by overtone and combination bands at 1450 nm and around 1920-1940 nm, as well as weak broad combination bands near 1780 nm and 1200 nm. The band near 1920 nm in a water spectrum is known to represent the combination band from the fundamentals of OH and HOH bend, and the band at 1450 nm is the overtone of the OH stretching fundamental.

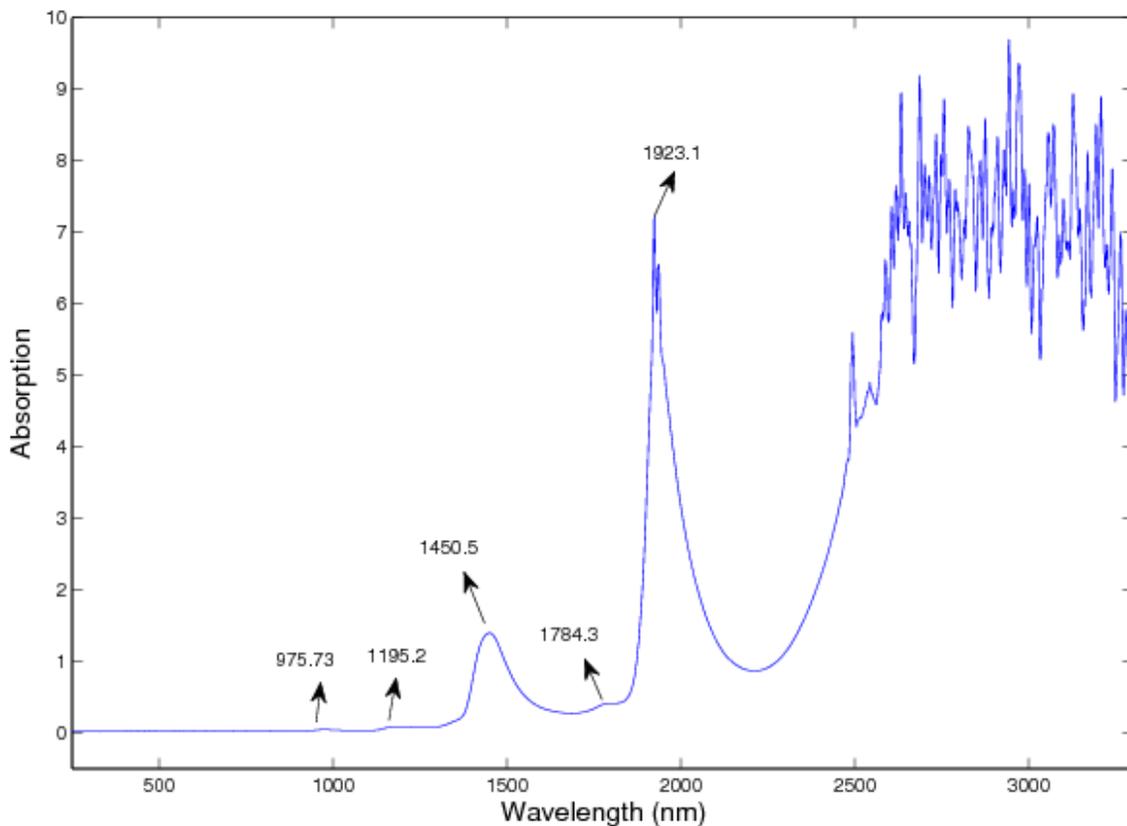


Fig. 1 Spectrum of liquid water smoothed to a factor of 20, and showing the dominant peaks at 1923.1 nm and 1450.5 nm, as well as the weaker bands at 1784.3 nm, 1195.2 nm and 975.73 nm.

Looking at the spectrum in Fig. 1, it can be seen that the values achieved here are slightly different from those reported previously reported, with the dominant overtone and combination bands being at 1450.5 nm and 1923.1, respectively, and the weak broad combination bands at 1784.3 nm, 1195.2 nm and 975.73 nm. Since the latter set of values may be quite difficult to identify, the spectrum was focused to clearly display these peaks in Figs. 2 and 3. More specifically, the strong peak around 1920 nm is thought to be a combination of the asymmetric stretch and bending of the water molecule, whereas those at about 1450 nm and 970 nm are accepted assignments that indicate combination bands involving the symmetric and

asymmetric stretching modes of the water molecule, although they are often wrongly referred to as the first and second overtones of the –OH stretch [18].

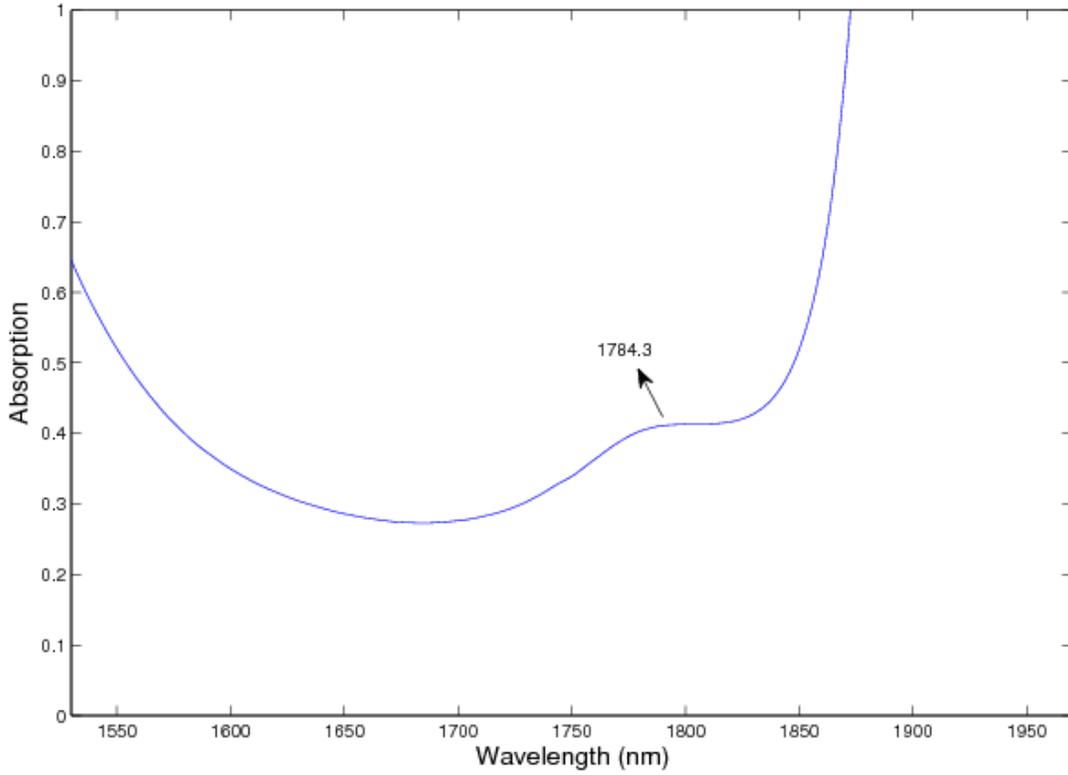


Fig. 2 spectrum of liquid water focused to show a weak combination band at 1784.3 nm.

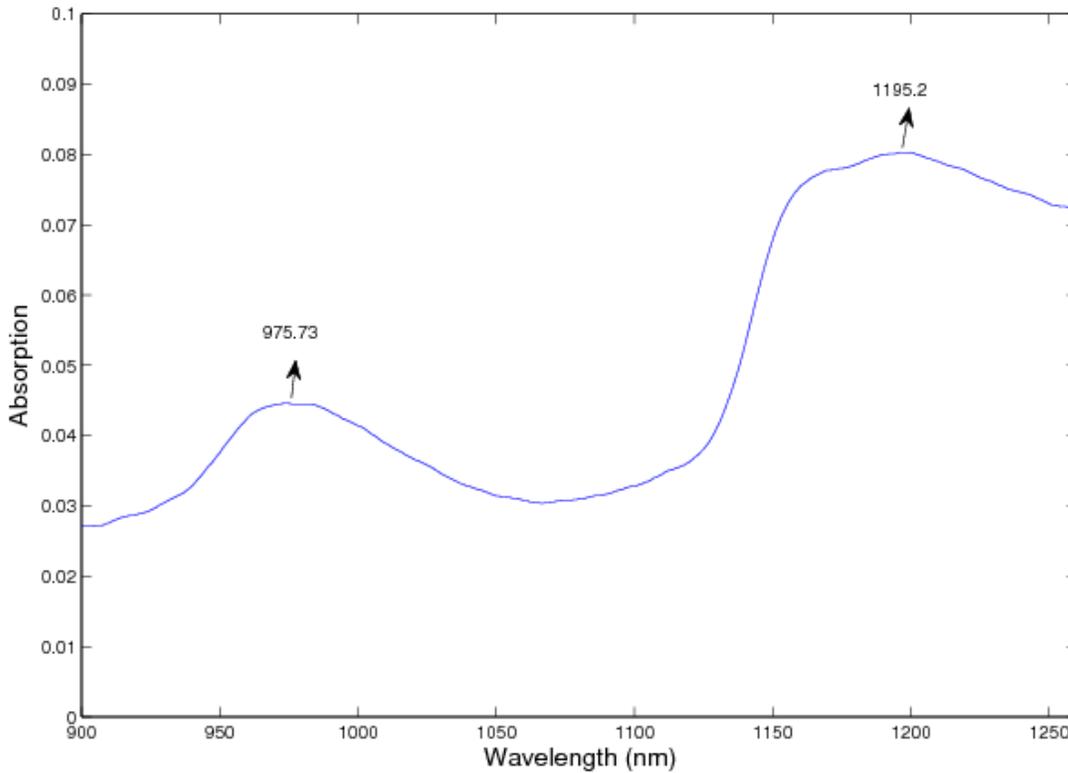


Fig. 3 spectrum of liquid water focused to highlight weak broad combination bands at 975.73 nm and 1195.2 nm.

Next, the spectrum of water obtained from the previous experiment, 1 mm of porcine skin, and 2 mm of porcine skin are shown in Fig. 4 for the region between 1300-2500 nm, after smoothing to a factor of 25. The large two peaks around 1920 nm and 1450 nm are clearly observable for both water and the skin samples but overall, both spectra of porcine skin have shifted upwards. This is because of the higher absorption coefficient of porcine skin or in other words, its ability to absorb more light in comparison to liquid water which is transparent.

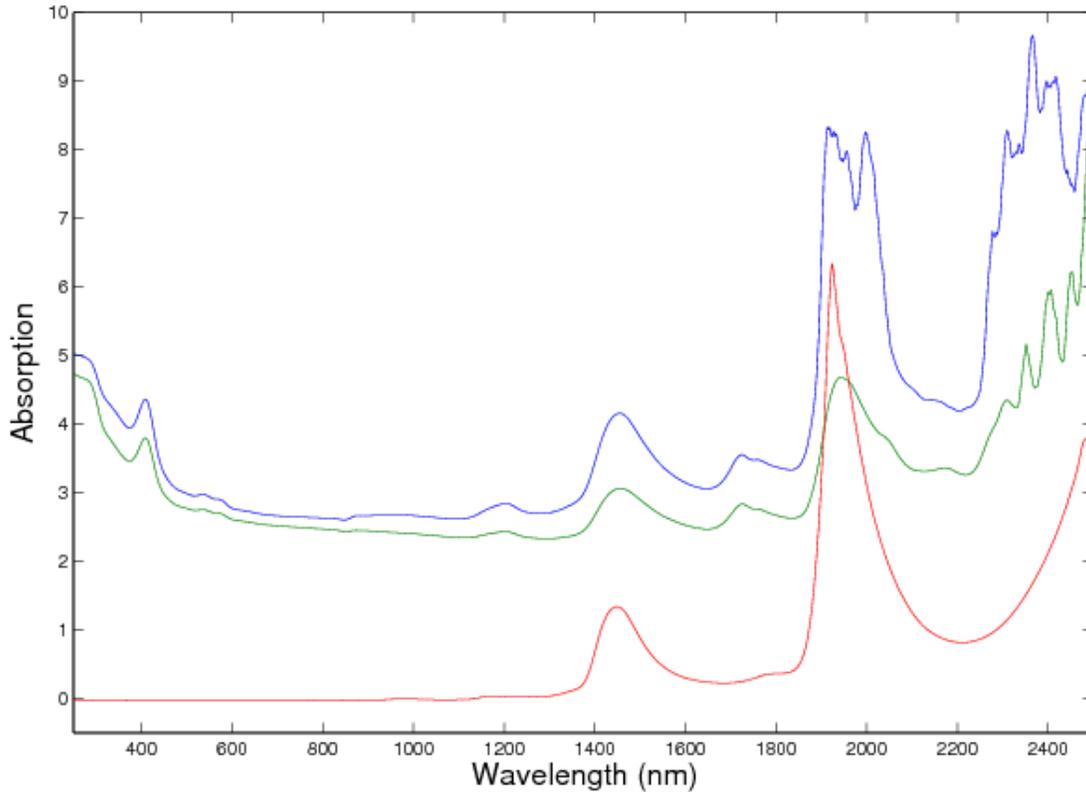


Fig. 4 Near infrared absorption spectra of porcine skin of 1 mm (Green) and 1.5 mm (Blue) thickness, and that of liquid water (Red).

As expected, the spectra are dominated by the overtone band of OH stretching fundamental and the combination band of OH and HOH bending at 1450 nm and around 1920 nm, respectively. According to the literature, a typical spectrum of porcine skin also contains combination and overtone bands of CH bonding at 2300 nm, 1760 nm and 1730 nm, NH bonding at 2050 nm and 1500 nm, and combination C=O bonding at 2180 nm from lipids and proteins within the skin, as well as a shoulder-like character at 2000 nm which indicates the presence of bound water [12,13]. The spectrum shown in Fig. 5 of 1 mm thickness porcine skin highlights these points and shows the exact values obtained here.

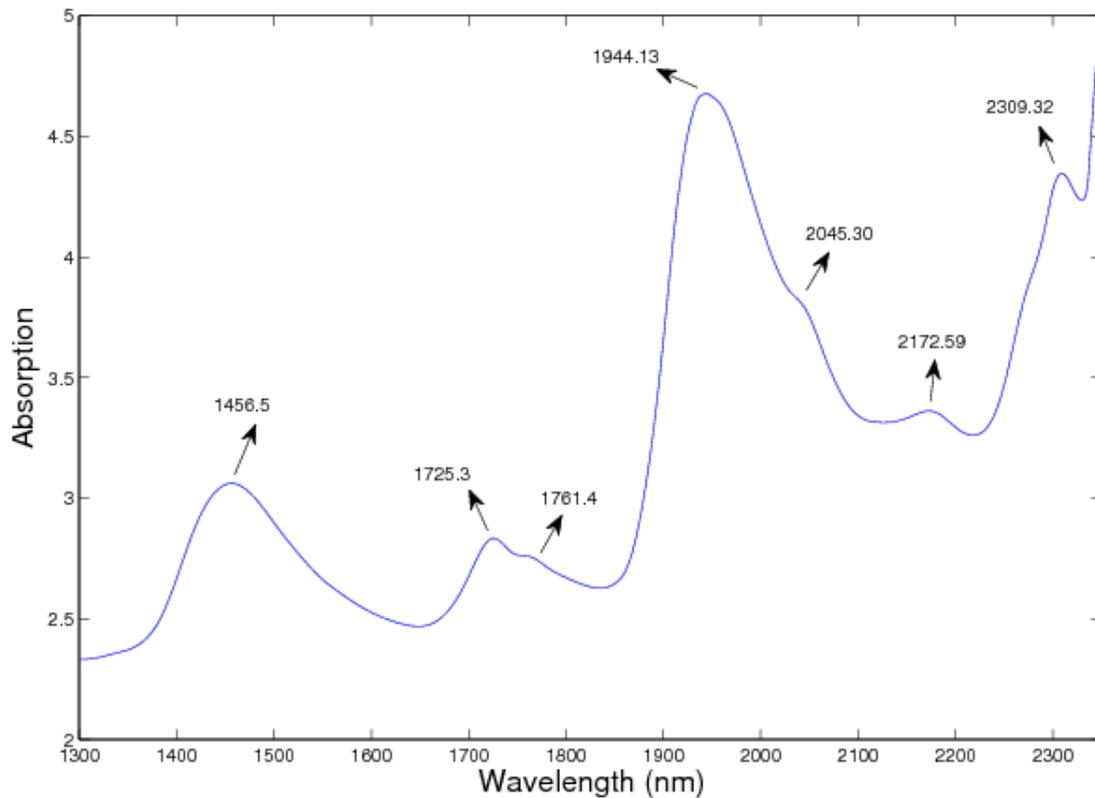


Fig. 5 Spectrum of the 1 mm thick sample of porcine skin with its distinguishing features highlighted. Values included for OH, NH, CH, and CO combination and overtone bands.

As for the evaporation and dehydration tests where the 1 mm sample of porcine skin was placed in water for different amounts of time (30 min, 60 min, 3 h, 12 h, 24 h and 46 h) then weighed and measured on the spectrophotometer over 3 hours, only the spectra of porcine skin left in water for 30 min are shown here in full in Fig. 6, and those from remaining tests have been focused on the 1450 nm band and are shown in Fig. 7.

For all the tests performed, both the weight and peak intensity of the sample decreased over time. However, this reduction in intensity bands is more distinguishable at 1450 nm as oppose to the 1920-1940 nm band, and so further calculations were based on this particular peak.

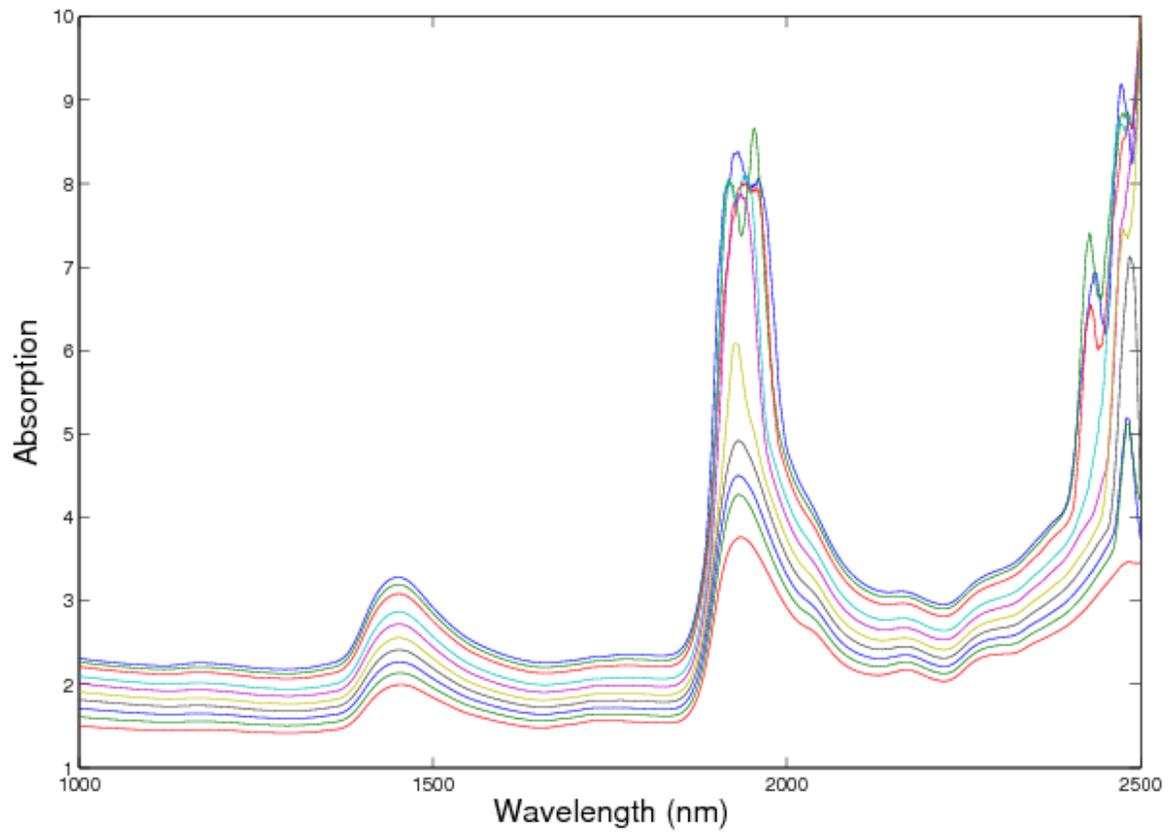


Fig. 6 Near infrared spectra of porcine skin left to hydrate for 30 min in water undergoing natural water desorption during a weight loss experiment.

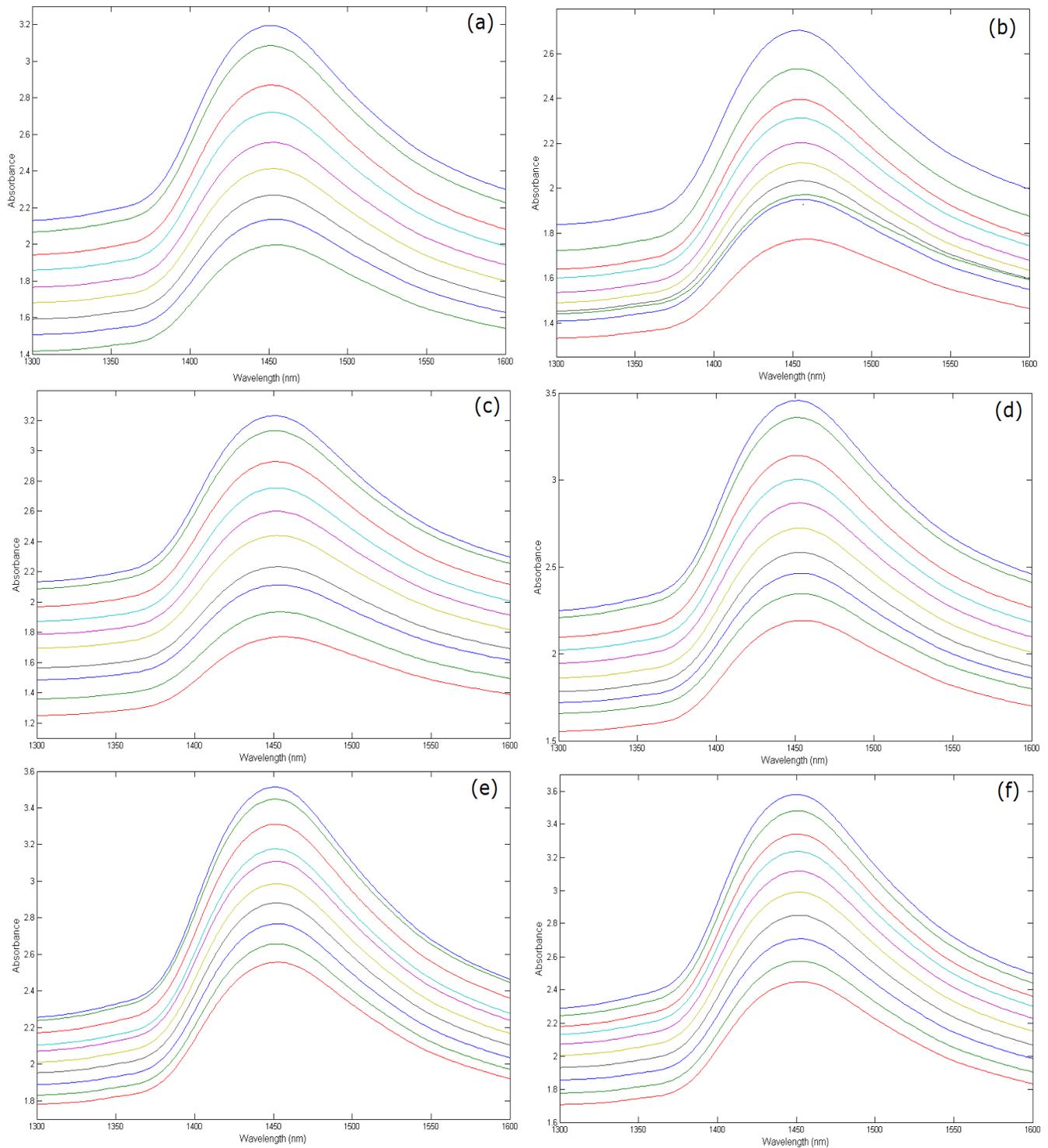


Fig. 7 NIR spectra focused at overtone peak of 1450 nm of porcine skin left to hydrate for; (a) 30 min, (b) 60 min, (c) 3 h, (d) 12 h, (e) 18 h, and (f) 24 h, undergoing natural water desorption during a weight loss experiment.

Moreover, the second derivative spectra were calculated for the spectra undergoing dehydration in Fig. 6, and are shown in Fig. 8. Here, a shift is seen from 1953.4 nm to 1963.8 nm of the water combination band as the content of water within the skin sample is reduced although another study [8] indicates that the shift occurs from 1915 nm to 1940 nm. The spectrum with the largest peaks is the second

derivative of the absorption spectrum obtained earlier where the sample had the highest water content or least dehydration period, whereas that with the smallest peaks belong to the absorption spectrum obtained when the sample was least hydrated. So, it follows that intensity decreased as the water content lessened but a wavelength shift is only apparent between 1949.8 and 1967.7 nm, and not on any of the other bands associated with NH, CH or CO bonds.

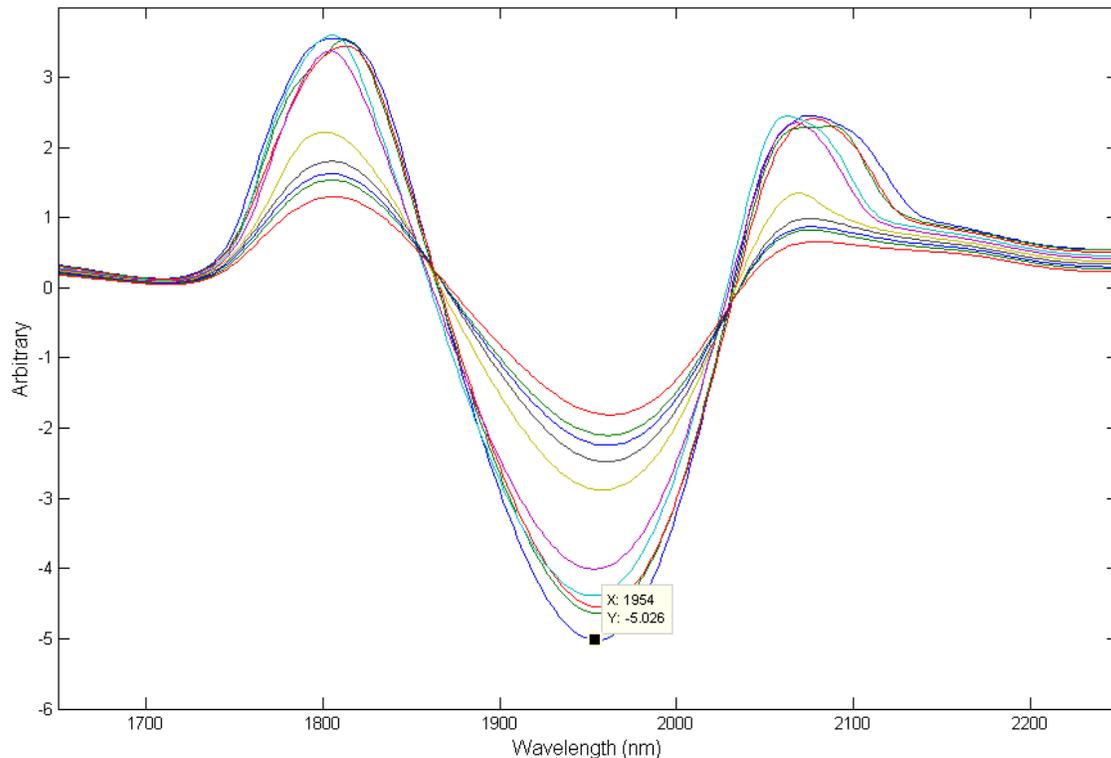


Fig. 8 Second derivative spectra of porcine skin undergoing desorption after 30 mins of hydration time.

As mentioned earlier, the weight of the sample decreased with increased desorption time. So for each test of different hydration period, the weight of the sample was greatest when measured immediately after removing it from the jar containing water and this value decreased over time, and was lowest at the end of the 3 hour testing period. This directly correlates with the resulting spectra since the magnitude or intensity of the 1450 nm band also decreased over time, but however, there does not seem to be a direct relationship between the duration of time spent in water by the sample and differences in weight, or intensity of the absorption peaks.

Furthermore, the rate of weight loss over the 3 hour testing period was calculated for each hydration time measurement, and is shown in Fig. 9, as well as the rate of absorption loss that occurred during the same interval which is shown in Fig. 10. Looking at both plots, there seem to be a conflicting relationship between the rate of weight loss and absorbance loss, as hydration periods whose rate of weight loss were higher compare to others had a reduced rate of absorption loss at the 1450 nm band and vice versa. However, it is not yet clear at this point whether a connection exists between the two rates and whether the duration of time spent in water had an effect on these results.

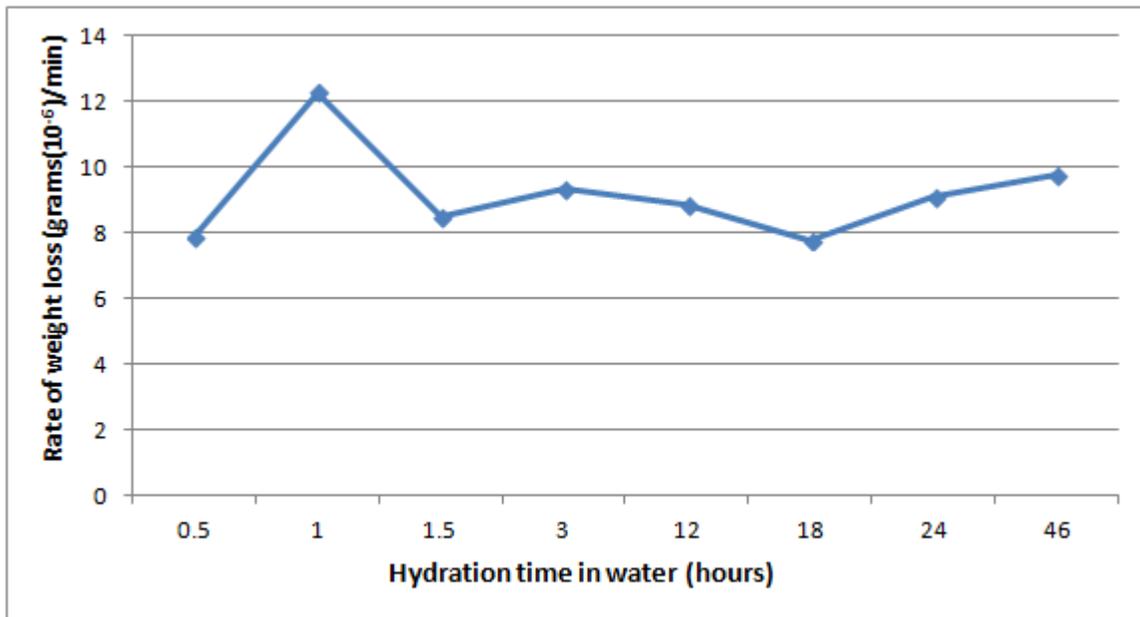


Fig. 9 A graph showing the rate of weight loss by the sample of porcine skin versus hydration periods.

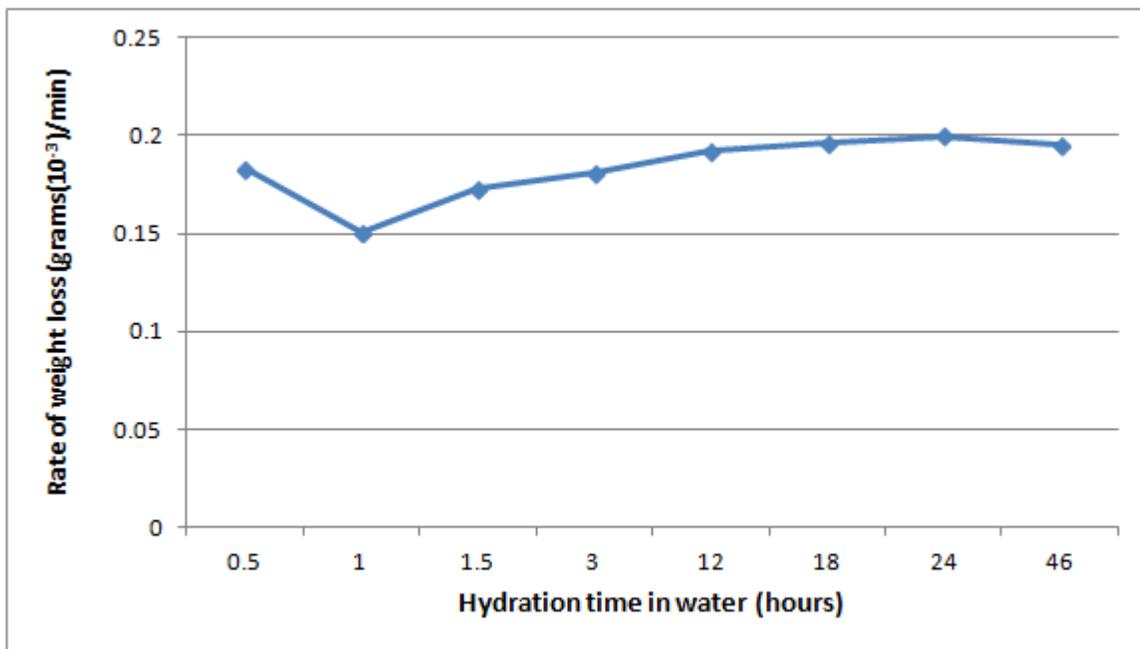


Fig. 10 A graph showing the rate of absorbance loss by the sample of porcine skin versus hydration periods.

IV. CONCLUSION AND FUTURE WORK

The importance of determining skin hydration has over the years has prompt the development of many instruments and methods, specifically designed to assess this parameter or water contents especially in the stratum corneum, and have greatly matured to suit different anatomical sites and measure multiple attributes. Of those, near infrared spectroscopy was chosen here to perform tests on liquid water and on porcine skin samples *in vitro*, mainly due to its high sensitivity to hydrogen bonding that causes near

infrared bands to become broader and to shift to lower frequencies, and its direct correlation to water content and concentration.

Using a highly advanced and accurate spectrophotometer, it has been possible to obtain direct absorption spectra of porcine skin samples *in vitro* that evidently showed the dominant combination and overtone bands around 1920 and at 1450 nm, and weaker bands belonging to CH, NH and CO bonds. The desorption tests were able to verify changes in these in accordance with water content but a clear relationship has not yet been established between hydration periods and the intensity of water absorption bands. Further work is required to examine the effects of different hydration periods on water contents of the skin, and their effect on the skins' ability to retain water. As for the other bands belonging to CH, NH and C=O bonding, these were apparent in the spectra of porcine skin, with the combination band of C=O bonding observed at 2171.3 nm, and combination and overtone bands at 2307.2, 1723.3 and 1760 nm which belonged to alkyl CH groups in skin lipids and protein. The NH band at 1500 nm became superimposed with the OH overtone at 1450 nm and resulted in a small shift in the latter to 1452.5 nm, whereas the second NH band at 2048 nm was nearly resolved due to the lower content of water within the sample. Nevertheless, there seem to be a conflicting or rather a slightly nonlinear correlation in relation to rates of weight loss experienced by the sample and rates of loss in intensities of water bands, which may relate to density changes occurring to the sample during dehydration that may affect its absorption and transmission properties, although this is not yet confirmed. Future work will focus on further analyzing these data to provide clearer understanding of this relationship and the contribution of barrier function to these results, as well as combining this method with fibre optic technology (sensors) to perform *in vivo* experiments on human participants. Finally, the preliminary results obtained here will be used to aid the development of a miniaturized optical hand held system which can be assembled either by building its individual components or by choosing ones available already in the market, that could reliably measure the hydration level of skin *in vivo*, or more specifically, the hydration in the SC in a fast and noninvasive manner, and that would be smaller in size to eliminate the use of current bench-top instruments, yet perform to the same standard, and could even be portable for wider use in various applications.

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