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Analysis of Near-infrared Raman Spectroscopy as a New Technique for a Transcutaneous Non-invasive Diagnosis of Blood Components

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Abstract. Near-infrared Raman spectroscopy can be a new technique for physical evaluations, allowing the measurement of lactic acid concentrations, in blood or muscles, during the physical activity in a transcutaneous non-invasive way. Lactic acid accumulation in the human body is one of the factors that leads to fatigue and therefore it should be continually monitored during physical training. Our proposal is to use Raman spectroscopy to monitor the lactic acid present in an athlete without interrupting his exercise for sample collection. The experimental set-up for Raman spectroscopy comprised a near infrared laser at 830 nm, a Kaiser f/1.8 spectrometer and a liquid nitrogen cooled CCD detector. The radiation from the exciting laser is blocked in the collecting system by Kaiser holographic filters. A personal computer controls the entire system, saving and processing the Raman spectra. Experiments were undertaken to verify the presence of lactic acid in the Raman spectra of solutions of lactic acid in human serum and in blood from a Wistar rat. After these two experiments, another was developed in vivo in a Wistar rat, injecting intraperitoneally 1 ml of a 0.12 mol/l lactic acid aqueous solution. An optical fibre catheter touching the skin of the rat groin, over the ileac vein collected the Raman signal. The presence of lactic acid was detected inside a live organism, in a transcutaneous non-invasive way. The minimum lactic acid concentration that the equipment can detect was also studied. An experiment was undertaken for that purpose, in which the laser illuminated directly a quartz cuvette containing solutions with decreasing lactic acid concentrations up to values near to the physiological level in the human body. The results indicated that the technique can be suitable for the physical evaluation of athletes.

Keywords: Blood components; Lactic acid; Near-infrared Raman spectroscopy; Transcutaneous diagnosis

INTRODUCTION

Over the last years, the use of lasers in the biomedical field have been widely disseminated. In particular, their use in the sportive area could become a powerful tool for predicting concentration rates of metabolites in an athlete. Knowledge of the metabolite concentrations in an athlete helps to take decisions about the training that is needed [1,2]. A well-oriented training allows the adjustment of the biological functions of the human body so as to reduce the spending of the energy reserves, and allowing physical activity to take place for a longer time.

The physical conditions of an athlete under physical activity can be determined by measuring the metabolite concentrations in blood and muscles. For instance, the lactic acid concentration is an excellent indicator of the energetic system of the athlete during physical exercise [2–7]. The level of lactic acid in the muscles of the body is strongly increased during intense activity [2,5], becoming a valuable source of chemical energy that is later consumed by the body [4,7,8]. Lactic acid is produced continuously in the human body, not only during intense exercise. Its level in blood when the body is at rest is low, around 10^{-3} mol/l of blood, reaching 22×10^{-3} mol/l during high intensity exercise [3,5]. The muscular lactic acid concentration at rest is a little higher than in blood, around 2.5×10^{-3} mol/kg of muscular mass. The coordination loss and chances of injury in an athlete increase when the muscular lactic acid level is high; it is important, therefore, to monitor lactic acid level during physical

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activity [2,9–13]. The accumulation of lactic acid is actually evaluated by measuring its concentration in an athlete's blood. After an exhausting activity, blood samples are collected from the athlete's ear and analysed by electro-enzymatic methods [1,5]. This procedure is painful, costly and can also cause blood contamination [2].

The development and characterisation of a method for determining the concentration of metabolites in blood in a non-invasive way is very important in the field of clinical analysis, as well as for evaluation of an athlete's conditioning. In this work, a new methodology is studied for evaluating the lactic acid concentration in blood and muscles, using the near-infrared Raman spectroscopy technique for transcutaneous diagnosis. In this technique, a low power laser beam is sent through the skin and the Raman radiation emitted by the metabolites in the blood is collected externally, without the need to extract samples for analysis. In this way, the determination of the lactic acid level in an athlete can be made in loco, without interrupting physical activity. The physical condition of an athlete can be evaluated at regular intervals when such a sensitive, non-invasive method is used. There is also less risk of contamination. The fact that there is no need to store and preserve material for analysis, will result in faster, cheaper and more reliable tests.

Near-infrared Raman Spectra of Lactic Acid

Spectroscopy, by emission or absorption, is a reliable and precise method for the chemical analysis of materials, since every chemical compound has its own characteristic spectrum [14–17]. The near-infrared Raman spectroscopy technique was chosen to monitor the lactic acid. The near-infrared choice was due to the deeper penetration in biological tissues and lower fluorescence emission. In the Raman effect, a specific optical frequency radiation (energy) is absorbed by the molecule under study and re-emitted at a lower frequency (energy). The energy difference between the absorbed and emitted photons corresponds to a vibrational transition of the molecule (Stokes radiation) [14,15,18]. The emission lines in the Raman effect are sharp and spectroscopically well resolved, being characteristics of the emitting material. Therefore, Raman spectroscopy is a powerful tool for identification of analytes [19]. Infrared absorption spectroscopy can also be used for such identification; meanwhile, the broad bands always present in the infrared spectra make it difficult to make a precise identification of analytes. The usefulness of infrared absorption spectroscopy is even less when is used to detect the presence of metabolites in blood because of the strong infrared light absorption of the water present in the blood [17,20]. The Raman spectrum of lactic acid has several sharp, well-resolved lines, within the spectral range of our system, that covers up to 1500 cm^{-1}. In all, there are eight spectral lines in the frequency range of interest: 730, 830, 866, 920, 1046, 1084, 1131 and 1457 cm^{-1}. Those Raman lines correspond to different vibrational modes of the lactic acid molecule; for instance, the strongest peak at 830 cm^{-1}, with a FWHM (Full Width at Half Maximum) linewidth of 25 cm^{-1}, is due to the strong stretching mode between a carbon atom and the carboxylic acid group (C-COOH) [18].

The highly distinct spectrum of lactic acid allows Raman spectroscopy to be used to detect its presence in a biological medium, even when other metabolites are also present. For collecting the Raman signal from a sample it is necessary to use sensitive, low-noise optical detectors, such as liquid nitrogen cooled CCD detectors. Band-pass filters should also be used in order to reduce back-scattering laser radiation, due to the reflection onto the sample surface or diffused inside the sample bulk. Typically, Raman signal is four orders of magnitude lower than the fluorescence of the sample, so spectra should be processed to eliminate the fluorescence contribution. For low Raman signals the spectrum should also be filtered using digital low pass frequency filters.

The use of near-infrared Raman spectroscopy to detect the presence of metabolites in blood in vitro and to measure their concentration has been intensively studied in recent years. Several metabolites are being investigated, such as glucose, urea, cholesterol, lactic acid and triglyceride, and many others [18–25]. So far, efforts are focused on the study of glucose content in blood, because of the huge number of diabetic patients for whom the monitoring of glucose is essential. Also, work has been done on the detection of cholesterol, but, to a lesser degree. On the other hand, only a few papers can be found in the literature concerning the detection and measurement of lactic acid concentrations [19,21,22,24].

MATERIALS AND METHODS

The lactic acid ($C_3H_6O_3$) used in our experiments was an aqueous solution at 86%, corresponding to a lactic acid concentration of 11 mol/l, from Sigma Chemical Co. (DL-Lactic acid, containing approximately the same concentration of D and L isomers.) Lactic acid solutions with different concentrations were prepared from this concentrated solution and used for in vitro experiments with Wistar rat blood and human serum, and for the transcutaneous in vivo experiment in rat. The human serum was obtained by centrifugation of human whole blood. The serum was stored under refrigeration to avoid degradation. The rat blood was extracted from a male Wistar rat, 300 g weight and about 3 months old, and heparin was added, to avoid blood coagulation before obtaining the Raman spectra. For the transcutaneous in vivo experiment the lactic acid solution was injected intraperitoneally into the Wistar rat.

Optical system

Two near infrared laser sources for the sample excitation were used, depending on the experiments that were carried out. For one set of experiments the sample was illuminated directly by the laser beam and the Raman radiation from the sample was collected and coupled into the spectrometer entrance slit by a set of lenses. The laser used in this case was a Ti:Saphire (Spectra Physics 3900S Model) pumped by a 6 W Argon laser (Spectra Physics 2017 Model), tuned at 830 nm. In the other set of experiments, an optical fibre bundle was used for exciting the sample and collecting the Raman signal. The laser source for this set-up was a diode laser operating at 830 nm (SDL 8630 Model).

The optical detection system was composed of a high coupling spectrometer (Kaiser Optical Systems, Inc. f/1.8i) and a liquid nitrogen cooled CCD, 'Deep Depletion' detector (Princeton Instruments EEv with 1024×256 pixels). The aperture of the entrance slit fixed at 200 μm gives a spectrometer resolution of the order of 10 cm^{-1}. The detection system is controlled by a personal computer that saves and processes the Raman spectra. The intensity of the excitation laser is strongly reduced in the collecting system by using a set of holographic notch filters from Kaiser Optical Systems, Inc.

The optical detection system, spectrometer and CCD detector, were calibrated using as a reference an organic compound, the indene C_9H_8, with a well-established Raman spectrum, presenting many sharp and well-resolved lines that cover the whole range of interest (700–1500 cm^{-1}) [26]. The contribution of the sample fluorescence to the Raman spectra was eliminated by subtracting a third-order polynomium fitting from the gross spectrum. For those spectra that present low intensity Raman peaks a filtering process was applied with a digital low pass frequency filter (scientific software Sigma-plot from Jandel Inc, USA).

For the experiments that employ fibre collecting optics an optical fibre cable developed by our Biomedical Instrumentation Laboratory (IP&D-UNIVAP) was used. This fibre bundle is composed by seven optical fibres with one central fibre for sample excitation, and the other six around the central one for signal collection. The seven fibres have a numerical aperture of 0.22 and a 200 μm core diameter. The fibres are placed inside Teflon tubing for flexibility and protection. The proximal end of the fibre bundle was configured in a linear array for coupling the radiation into the spectrometer slit entrance. A detailed description of that optical fibre catheter can be found elsewhere [27].

In order to compare spectra obtained under different experimental conditions, like different power of the excitation laser or signal acquisition times, all spectra were normalised by measuring peak intensities in counts/J; that means, the CCD count readings were divided by the laser energy. Typical values for excitation laser power and acquisition times were 100 mW and 20 s, respectively. The behaviour of the peak at 830 cm^{-1} was observed for all experiments, since this is the strongest peak of the lactic acid Raman spectrum.

RESULTS

Figure 1 shows the Raman spectrum of a lactic acid aqueous solution at 86%, in the spectral range 700–1500 cm^{-1}, the eight Raman peaks present in this region are well distinguished. This Raman spectrum was obtained using a quartz cuvette containing the liquid sample and as excitation source the TI:Saphire laser tuned to 830 nm.

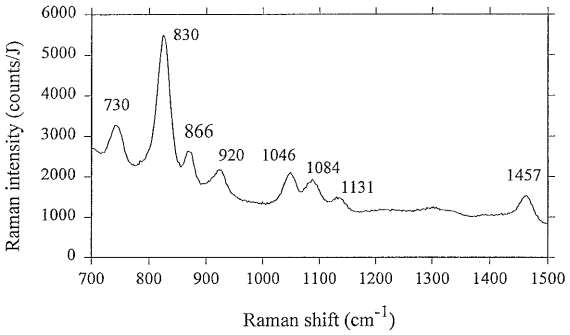

Fig. 1. Raman spectrum of lactic acid showing the eight Raman peaks present in the spectral range from 700 to 1500 cm^{-1}.

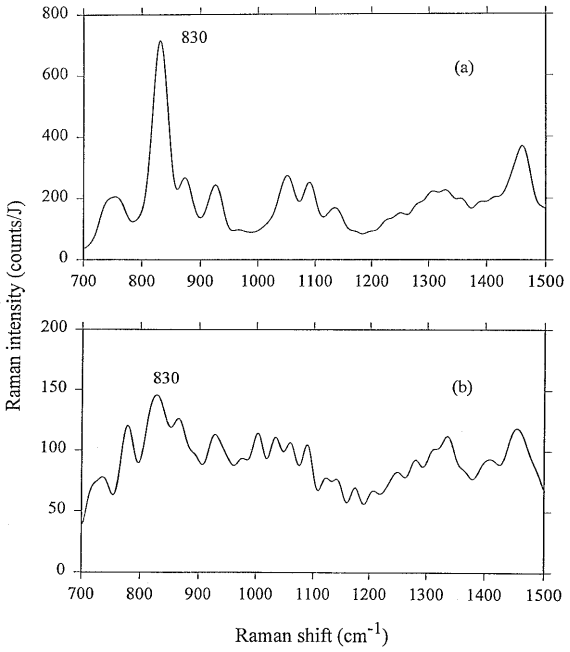

Fig. 2. Raman spectra of lactic acid in human serum, for the concentrations: (a) 1.92 mol/l, (b) 0.22 mol/l.

Raman spectra of lactic acid were obtained in human serum and in whole blood from a Wistar rat. The characteristic Raman peaks of this metabolite are clearly observed in those spectra, even when other metabolites are also present in the sample. The Raman emission from the sample was collected through a lens optical system, using the same geometry for both experiments. Spectra were taken with 100 mW laser power and 20 s acquisition time. To 1 ml of human serum in a quartz cuvette was added lactic acid to give solutions with concentrations from 0.22 to 1.92 mol/l. Raman spectra were taken for each concentration. Figure 2(a) and 2(b) show Raman spectra corresponding to the concentrations of 1.92 and 0.22 mol/l, respectively. It can be seen that for

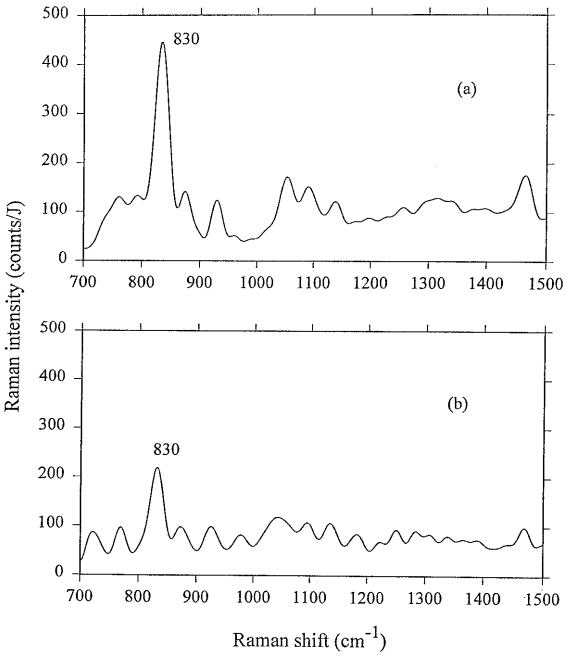

Fig. 3. Raman spectra of an aqueous solution of lactic acid at 1.05 mol/l, with rat blood added. Spectra correspond to blood amounts of (a) 20 μl, (b) 80 μl.

the highest concentration spectrum the lactic acid peak at 830 cm^{-1} strongly dominates over the Raman spectrum of the other metabolites present in human serum. Other peaks of the lactic acid can be also easily observed. The peak intensity at 830 cm^{-1} decreases when the lactic acid concentration is reduced. Peaks at 830 and 1457 cm^{-1} are still observable at the lowest concentration of 0.22 mol/l (Fig. 2(b)).

In the other experiment, 1 ml of a 1.05 mol/l aqueous solution of lactic acid was used. To this solution was added successively 20 μl of blood from a Wistar rat and the corresponding Raman spectra were obtained, the evolution of the intensity of the 830 cm^{-1} peak was observed. For blood quantities above 80 μl the blood in the solution coagulated and the intensity of the Raman peak was consequently strongly reduced. The spectra of the lactic acid solution with 20 and 80 μl of blood are shown in Fig. 3(a) and (b), respectively. The peak intensity is reduced when blood is added to the solution, due to the strong absorption and scattering in the blood. A reduction of the peak intensity by a factor of 2.3 (from 350 to 150 counts/J) was seen when the blood in the solution increased from 20 to 80 μl; but, it was still clearly observed at the highest blood concentration.

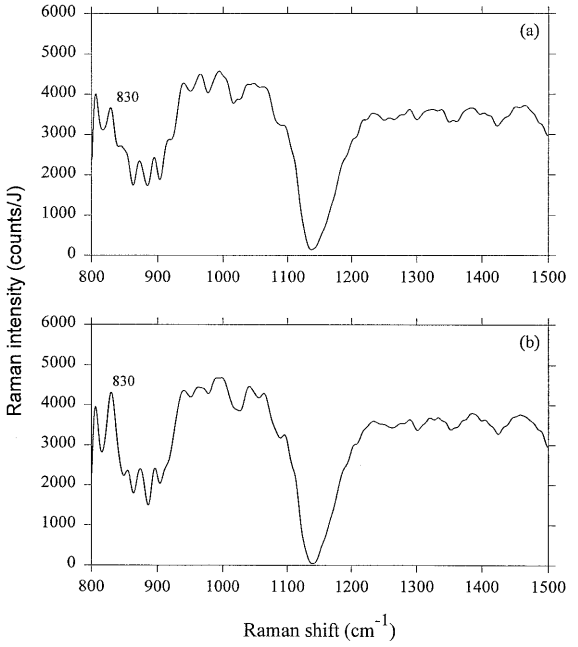

Fig. 4. Transcutaneous Raman spectra of lactic acid in a Wistar rat. Spectra correspond to times (a) before injecting the solution, (b) 30 s after the injection.

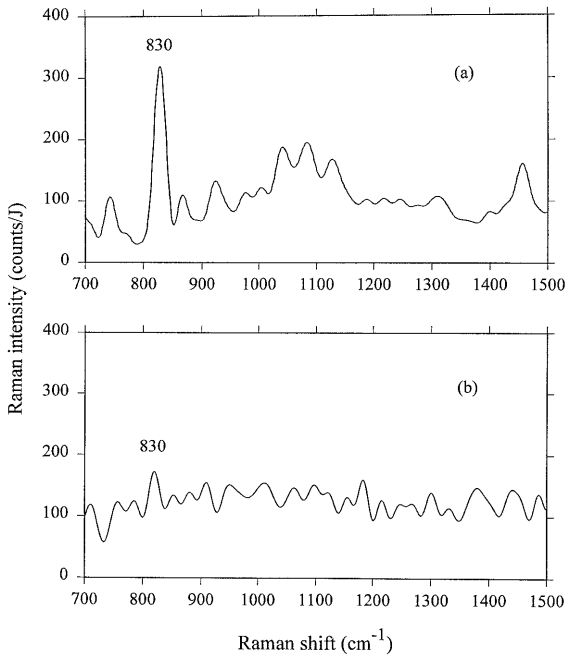

Fig. 5. Raman spectra for an aqueous lactic acid solution, using laser direct illumination of the sample cuvette; concentrations (a) 0.58 mol/l, (b) 0.0011 mol/l.

Following the two in vitro experiments in biological media (human serum and rat blood), an in vivo experiment, using a live Wistar rat was carried out. In this experiment 1 ml of a lactic acid aqueous solution at 0.12 mol/l, was injected intraperitoneally. The radiation of the exciting laser was introduced into the rat transcutaneously, using an optical fibre cable touching slightly the skin of the rat groin, above its ileac vein. The Raman signal generated inside the animal was also collected transcutaneously by the collecting fibres of the same optical fibre cable. The rat was anesthetised with Zoletil 50 (Virbac), to prevent animal movements that could alter the position of the optical cable. An optical fibre probe was chosen rather than an optical lenses system because of its flexibility for excitation and signal collection when such area of the animal is studied. Evolution of the acid lactic content in the animal's bloodstream was studied by taking Raman spectra at regular 30 s time intervals after injecting the solution.

The Raman spectra in Fig. 4(a) and (b) correspond to two different times: before injecting the lactic acid solution and 30 s later, respectively. The intensity of the Raman peak at 830 cm^{-1} increases with the elapsed time, reaches a maximum value after 30 s and decreases again later on, presenting slight intensity fluctuations in time. These fluctuations may be due to temporal variations of the lactic acid concentration in the rat bloodstream. This experiment indicated the possibility of detecting the presence of lactic acid in a live organism, in a transcutaneous non-invasive way and it also suggested that it could be used to monitor variations of the lactic acid content in the bloodstream.

In the next experiment the minimum concentration of lactic acid that the system can detect was determined. The system sensitivity determines the capability of the method to measure the lactic acid concentration in the blood of a live organism at rest, as well as during a physical exercise. Two different set-ups were used for studying the samples. In the first the laser beam stroke directly the cuvette containing the lactic acid solution and its concentration was reduced from 0.58 mol/l until the minimum at which the peak could still be observed was reached. Figure 5(a) and (b) shows the Raman spectra for 0.58 mol/l and 1.1×10^{-3} mol/l concentrations, respectively. The spectrum for distilled water in the quartz cell was taken as the reference spectrum and was subtracted from all the other spectra. It is still possible to observe the peak at 830 cm^{-1} for the lactic acid concentration of 1.1×10^{-3} mol/l, with a signal to noise ratio close to one. This concentration can be considered to be the minimum that can be detected

by our set-up. This limit is of the same order as the endogenous lactic acid blood content in the human body. However, during physical exercises the lactic acid concentration can increase by a factor of 20.

In the second set-up a piece of a biological tissue was placed between the laser beam and the sample cuvette and an optical fibre bundle was used to excite and collect the Raman signal from the sample. The biological tissue was a piece of skin from the groin area of a Wistar rat. The idea of inserting the tissue was to determine how the Raman signal is affected when it is collected through a biological tissue. This skin layer should introduce at least two important effects. First, the Raman radiation emitted by the sample is scattered and absorbed when passed though the tissue; therefore, the intensity of the signal collected by the optical cable is reduced. Secondly, the diffuse reflectivity of the skin increases the back scattering of the laser radiation. That back radiation is undesirable since it is also collected by the optical fibre bundle and can saturate the CCD detector. The fibre optical cable was positioned to form an angle with respect to the cuvette face in order to reduce the captured back radiation.

Measurements for the second set-up, i.e. using an optical fibre bundle and a piece of tissue, started with the lactic acid solution at 86% and then the concentration was reduced to search for the minimum for which the Raman peak can still be observed. Raman spectra were taken for each concentration. The spectra obtained for this configuration are shown in Fig. 6. Figure 6(a) shows the Raman spectrum corresponding to the distilled water, without lactic acid. In this spectrum there is an intense and broad peak around 800 cm^{-1}, due to the Raman radiation emitted by the silica of the optical fibres. This undesirable peak partially overlaps the peak of the lactic acid at 830 cm^{-1}. Figure 6(b) shows the Raman spectrum for the 86% lactic acid solution and in Fig. 6(c) the spectrum corresponding to 2.2×10^{-2} mol/l, that seems to be the detection limit in this configuration. The base spectrum shown in Fig. 6(a) was subtracted from the spectra in Fig. 6(b) and (c).

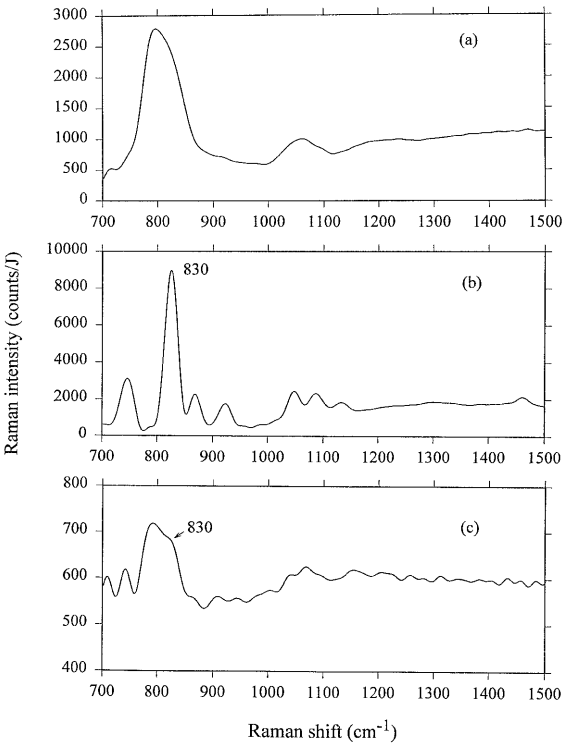

Fig. 6. Raman spectra of an aqueous lactic acid solution, using biological tissue and an optical fibre bundle for collecting the Raman signal. (a) Water, without lactic acid (reference spectrum), (b) lactic acid solution at 86%, (c) 0.022 mol/l concentration.

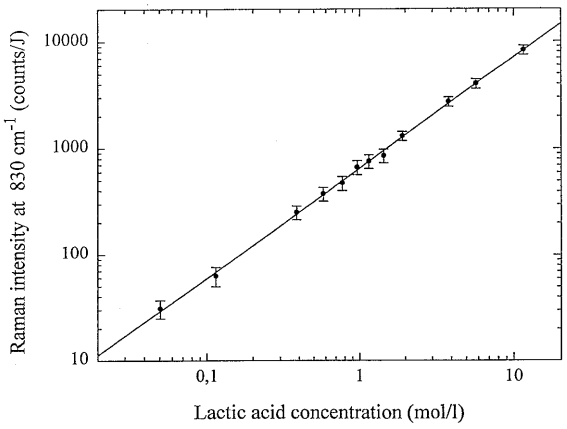

Fig. 7. Correlation between Raman peak intensities at 830 cm^{-1} and lactic acid concentrations.

Correlation Between Raman Intensity and Lactic Acid Concentration

Several lactic acid concentrations were studied for the above set-up, which includes cuvette containing the sample, biological tissue in front of the cuvette and optical fibre cable. For each concentration the intensity of the main Raman peak at 830 cm^{-1} was measured. The peak height was determined from a base line traced in the graph, eliminating the contribution of the silica broad line at 800 cm^{-1}. Figure 7 shows a plot of peak intensities versus lactic acid concentrations.

The plot starts at the concentration corresponding to the lactic acid aqueous solution at 86% spanning up to the minimum concentration for which is still possible to measure the peak height, approximately 5.5×10^{-2} mol/l. At the lower concentration of 2.2×10^{-2} mol/l it is possible to identify the peak, but no reliable measurement can be made. Figure 7 shows the correlation between the information given by the Raman spectroscopy and the lactic acid concentration. The experimental error for the peak intensity measurements can be estimated to be 10% for the highest concentrations, increasing up to 20% for the lowest ones. A plot with a two-axis logarithmic scale was chosen for an easier visualisation of the data, since lactic acid concentrations and Raman peak intensities varied by more than two orders of magnitude. It was found that the slope of the fitting curve is equal to one within the experimental errors, which means that the correlation should be linear.

CONCLUSIONS

The sequence of experiments we carried out shows the following.

The in vivo experiment shows that the near infrared Raman spectroscopy allows lactic acid to be identified in a live organism, through a transcutaneous non-invasive technique, that uses an optical fibre probe for Raman signal collection. Other experiments are being carried out in order to determine the minimum limit for detection in vivo of lactic acid.

It is possible through Raman spectroscopy to detect the presence of lactic acid in aqueous solutions at low concentrations. It is also possible when it is dissolved in human serum or in blood from a rat, even in the presence of many other metabolites that emit Raman radiation. In the experiment without the use of the optical fibre bundle it was possible to detect lactic acid at concentrations as low as 1.1×10^{-3} mol/l. This value is of the same order as the endogenous physiological level in the human body. During an intense physical exercise the lactic acid concentration can be much higher, reaching 2.2×10^{-2} mol/l in blood. So, the results obtained show that this technique has great potential for a transcutaneous and non-invasive diagnosis of lactic acid in athletes.

When biological tissue was placed in front of the cuvette containing the lactic acid solution at 86% we observed an intensity reduction of the collected Raman signal by a factor of two. This reduction is due to the absorption and strong light scattering in the biological tissue. Due to the diffuse reflectance in the rat skin some of the laser light reaching the sample is back scattered and captured by the collecting optical system. Therefore, this effect also increases the minimum limit that the system can detect. By comparing the obtained results we verified that the use of an optical fibre bundle, that also generates undesirable Raman radiation and a biological tissue that absorbs and scatters radiation, raises the detectable minimum from 1.1×10^{-3} to 2.2×10^{-2} mol/l. In summary, the tissue insertion increases the minimum by a factor of 2 and the introduction of the optical fibres by a factor of 100.

The use of an optical cable to excite and collect the Raman signal of the sample introduces an important contribution from the SiO_2 Raman emission of the optical fibres. One of the strongest Raman peaks in the optical fibre spectrum is localised in the 800 cm^{-1} region, covering the Raman signal from samples with very low concentrations of lactic acid. Therefore, in order to improve the sensitivity of the method it will be necessary to use optical fibre cables with dielectric filters on the end face of the fibres. These dielectric filters are basically a laser band pass for the excitation fibre and a rejecting laser frequency filter for the collecting fibres. The detection limit of lactic acid is expected to decrease drastically using those filters.

We can conclude from the analysis of our results that this technique is very promising for monitoring metabolites in the blood. The detecting limits can be optimised by improving the optical guide system and the laser excitation energy, increasing the signal to noise ratio. Procedures that certainly will reduce the detection limit are: improving in the signals processing; better noise filtering; and a statistical spectral treatment.

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REFERENCES

1. Matsudo V, Fisiologia da atividade esportiva. In: Tratado de fisiologia aplicada às ciências da saúde, Guanabara Koogan, Rio de Janeiro, Brazil, 1993: 1381–3.
2. Accusport: http://www.lactate.com
3. Fox EL, Bowers RW, Foss ML. The physiological basis of physical education and athletics. Madison Wis.: Brown & Benchmark Publishers, 1989.
4. McArdle WD, Katch FI, Katch VL. Exercise Physiology: Energy, Nutrition and Human Performance. Philadelphia: Lea & Febiger, 1991.
5. Billat LV. Use of blood lactate measurements for predictions of exercise performance and for control of training – recommendations for long-distance running. Sports Med 1996;22:157–75.
6. Bang O. The lactate content of the blood during and after muscular exercise in man. Skand Arch Physiol suppl 1936;10:51–82.
7. Tokmakidis SP, Léger LA, Pilianidis TC. Failure to obtain a unique threshold on the blood lactate concentration curve during exercise. Eur J Appl Physiol 1998;77:333–42.
8. Rodriguez FA, Banquells M, Pons V, Drobnic F, Galilea PA. A comparative-study of blood lactate analytic methods. Int J Sports Med 1992;13:462–6.
9. Gleeson M, Blannin AK, Walsh NP, Field CNE, Pritchard JC. Effect of exercise-induced muscle damage on the blood lactate response to incremental exercise in humans. Eur J Appl Physiol 1998;77:292–5.
10. Jacobs I. Lactate, muscle glicogen and exercise performance in man. Acta Physiol Scand [Suppl] 1981; 495:1–35.
11. Davis JA, Storer TW, Caiozzo VJ. Prediction of normal values for lactate threshold estimated by gas exchange in men and women. Eur J Appl Physiol 1997;76:157–64.
12. Farrell PA, Wilmore JH, Coyle EF, Billing JE, Costill DL. Plasma lactate accumulation and distance running performance. Med Sci Sports 1979;11:334–8.
13. Sjodin B, Jacobs I. Onset of blood lactate accumulation and marathon running performance. Int J Sports Med 1981;2:23–6.
14. Turrell G. In: Turrell G, Corset J. The Raman effect in Raman Microscopy, Developments and Applications. London: Academic Press, 1996:1–25.
15. Demtröder W. Laser Raman spectroscopy. In: Laser Spectroscopy – Basic Concepts and Instrumentation, 2nd edn. Berlin, Heidelberg and New York: Springer-Verlag, 1996:489–98.
16. McShane MJ, Coté GL, Spiegelman CH. Assessment of partial least-squares calibration and wavelength selection for complex near-infrared spectra. Appl Spectrosc 1998;52:878–84.
17. Chung H, Arnold MA, Rhiel M, Murhammer DW. Simultaneous measurements of glucose, glutamine, ammonia, lactate, and glutamate in aqueous solutions by near-infrared spectroscopy. Appl Spectrosc 1996; 50:270–6.
18. Wicksted JP, Erckens RJ, Motamedi M, March WF. Raman spectroscopic studies of metabolic concentrations in aqueous solutions and aqueous humor specimens. Appl Spectrosc 1995;49:987–93.
19. Berger AJ, Wang Y, Sammeth DM, Itzkan I, Kneipp K, Feld MS. Aqueous dissolved gas measurements using near-infrared Raman spectroscopy. Appl Spectrosc 1995;49:1164–9.
20. Vonach R, Buschmann J, Falkowski R, Schindler R, Lendl B, Kellner R. Application of mid-infrared transmission spectrometry to the direct determination of glucose in whole blood. Appl Spectrosc 1998;52:820–2.
21. Wang SY, Hasty CE, Watson P, Wicksted JP, Stith RD, March WF. Analysis of metabolites in aqueous solutions by using laser Raman spectroscopy. Appl Opt 1993;32:925–9.
22. Goetz MJ Jr, Coté GL, Erckens RJ, March WF, Motamedi M. Application of a multivariate technique to Raman spectra for quantification of body chemicals. IEEE Trans Biomed Eng 1995;42:728–31.
23. Berger AJ, Wang Y, Feld MS. Rapid, noninvasive concentration measurements of aqueous biological analytes by near-infrared Raman spectroscopy Appl Opt 1996;35:209–12.
24. Berger AJ, Koo TW, Itzakan I, Horowitz G, Feld MS. Multicomponent blood analysis by near-infrared Raman spectroscopy. Appl Opt 1999;38:2916–26.
25. Qu JY, Wilson BC, Suria D. Concentration measurements of multiple analytes in human sera by near-infrared laser Raman spectroscopy. Appl Opt 1999; 38:5491–8.
26. Wollman ST, Bohn PW. Evaluation of polynomial fitting functions for use with CCD arrays in Raman spectroscopy. Appl Spectrosc 1993;47:125–6.
27. Lima CJ de, Sathaiah S, Silveira L Jr, Zângaro RA, Pacheco MTT. Development of catheters with low fiber background signals for Raman spectroscopic diagnosis applications. Artif Organs 2000;24:231–4.

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