Analysis of anhydrous glucose and human serum assisted by Raman spectroscopy.

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ABSTRACT

Raman spectroscopy has been considered like a potentially important clinical tool for real-time diagnosis of disease and evaluation of living tissue, whit the proposal to development noninvasive glucose measurements in a near future, with lower power than other reported studies, in this work are reported experimental tests made with a excitation source of semiconductor laser of 785 nm and 35 mW power.

Measurements were made to different glucose concentrations, with variation from 50 mg/dL to 6000 mg/dL. For this, three intervals with different ranges of concentration were analyzed, these tests were put into plastic sampling cells, making incise the beam vertically on sample. In the same way measurements to serum human are reported, for healthy volunteers had 12 hours fasting and non-fasting conditions, with it's corresponding values of glucose taken through a conventional glucometer.

Freeze-dried human serum was poured on object-holder, in the case of human serum reconstitute, it was used container in which were previously kept samples. Nine spectra per test were obtained and subsequently average was calculated, the spectra were studied in a range of 500 to 1700 cm^{-1}. This work explores the intensity variation of the bands of glucose in 1065 cm^{-1} and 1127 cm^{-1} as a function of glucose concentration. In the obtained results, there observes a behavior with positive slope in both substances, interrelation being observed between the measurements, being promissory for non-invasive measurement.

Keywords: Raman spectroscopy, anhydrous glucose, serum, non-invasive meausurements

1. INTRODUCTION AND ANTECEDENTS

Diabetes is a disease characterized for high and lows blood glucose levels as a result of defect in the insulin production, deficiency action of insulin, or both. Highlighting the importance to maintain a frequent monitoring of glucose concentration in diabetics patients for an effective treatment, this provides relevant information that can help to identify and prevent undesired periods of hypoglycemia and hyperglycemia.[1,2]

The number of diabetic people has increasing of 108 million in 1980 to 422 million in 2014, likewise the diabetes has rising faster into countries whit income middle and low. The diabetes can be treat, prevent or delay its consequences; that can be done, making a diet, physical activity, medicines and periodic tests for prevent complications.

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Among the conventional techniques for non-invasive blood glucose measurement are those which is necessary collect a blood sample through a finger prick, and putting it into an electrode, this glucose measurement is by means of electrochemical reactions.[3]

Non invasive analyte measurements in blood and tissue are an important goal for the laboratory medicine and a highlight point for diagnostic tests, specially glucose blood detections, as a way for better life quality of diabetic patients,[4,5] such as many of them should get a glucose test several times at day.

Among some of non-invasive techniques to measure glucose is Raman spectroscopy, in this technique an incident photon allows the transit of a molecule, of a vibrational or rotational state, to another.[6] Raman spetroscopy is dependent on rotational or vibrational energy states within a molecule, specific absorption bands are also seen with Raman spectroscopy, which can be used to identify and quantify molecules. The Raman signal is also susceptible to turbidity, haematocrit, skin thickness and melanin.[7]

Usually Raman spectrum is considered between 200-1800 cm^{-1}, because in this band, the Raman spectrum of glucose can be easily identify from others compounds.[6,8] On the other hand, others researches[9] had reported an analyzed spectral range from 700 to 1700 cm^{-1}, corresponding to fundamental modes associated with C-H, C-O, C-C, and C-N molecular bonds .

In addition has been used 785 nm at 300 mW, and acquisition time of 100 s.[10,11] In the same way has been reported glucose bands at 880, 1072, 1128 and 1370 cm $^{-1}$ corresponding to C-C stretching, C-O stretching, C-O-H bending and CH_2 wagging, respectively,[12,13] lactate bands centered at 855, 930, 1040, 1420 and 1455 cm^{-1} corresponding to C-COO stretching, CH_3 rocking, C-CH_3 stretching, COO stretching and CH_3 deformation, respectively.[9,14]

Others works showed characteristic peaks of glucose at 1125 cm^{-1}, this was using a diode laser operating at 785 nm for Raman spectra excitation with an average power of about 15 mW on the sample.[15]

In this paper we show experimental test made to different anhydrous glucose concentration (vary from 50 mg/dL to 6000 mg/dL), and to human serum. As a result of the art state, in this work we made an analysis of two Raman peaks at 1065 cm^{-1} and 1127 cm^{-1} as a function of intensity.

2. METHODOLOGY DESCRIPTION

For the measures by Raman spectroscopy it was used the next devices: an infrared semiconductor laser at 785 nm, with power from 1 to 450 mW; a retrospection Raman probe (180 ^{o}); an ocean optics spectrometer QE 65000; the samples were put into plastic cells. For interface and spectral analysis were used SpectraSuit and Origin Pro 8.

The optical arrangement is shows at figure 1. For this measurements the detector was put in a vertical way. It is worth mentioning that this measurements were made in a controlled environment (light outdoor), whit the purpose to reduce the noises effects from other wavelengths.

All spectrums shows in this work, were obtained whit 25 seconds of integration time, at 35 mW, and 9 spectrum were recollected for each sample, after the average was calculated an error minor to 10% was obtained. The electronic noise was measured with laser off and the resultant spectrum was subtracted of the samples measurements through the software.

Three intervals were made of anhydrous glucose concentration, the first range from 500 mg/dL to 6000 mg/dL, the second vary from 50 mg/dL to 500 mg/dL, and the last one from 200 mg/dL to 3000 mg/dL whit intervals of 200 mg/dL.

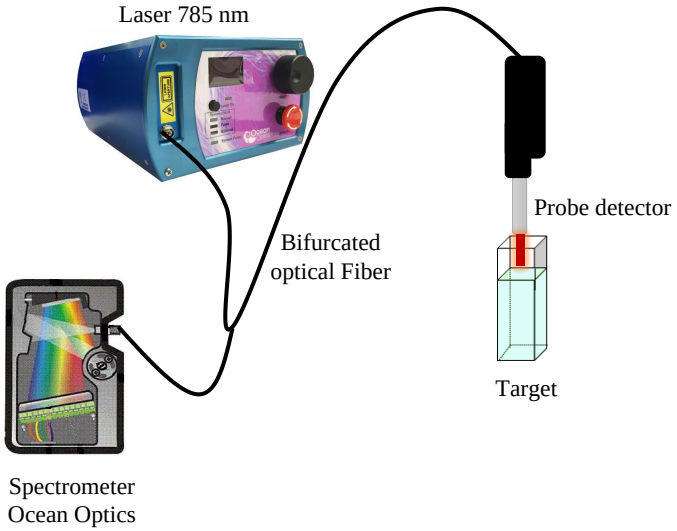


Figure 1. Raman spectroscopy diagram for glucose measurements.

In the same way we made human serum measurements under two conditions, freeze-dried serum and reconstituted serum. For the sampling, the volunteers were separate in two groups, one of them whit 8 hours to fasting and the other side non-fasting, after the blood extraction, the sample was centrifugate and the serum was freeze-dried whit the purpose of long its life. Freeze-dried process consist in dehydrate the sample and exposed it to fast freezing and eliminating the ice through light warming to the vacuum that it transforms in steam, this is the best way for drying of solutions organic without alter their qualitative and quantitative composition. For the reconstitute process we use previous freeze-dried serum, and then, it is necessary to add 1 ml of saline solution.

Is important highlighted that this research was realized under the volunteers assent based on the declaration of Helsinki which mentions the ethical beginning for the medical researches in human beings,[16] the glucose levels were measurement through a conventional glucometer One touch Ultra 2. the human serum glucose values are shows in the table 1.

Table 1. Human serum glucose concentration.

Volunteer 1	124 mg/dL
Volunteer 2	129 mg/dL
Volunteer 3	144 mg/dL
Volunteer 4	146 mg/dL
Volunteer 5	150 mg/dL
Volunteer 6	154 mg/dL
Volunteer 7	155 mg/dL

The spectral analysis was made through the software Origin Pro 8, using the *Positive Peak Algorithm*. This algorithm allows the analysis for importants bands from a spectrum, through manipulating of the spectrum baseline, this delete the fluorescence and shown clearly visualization of peaks. Results of the algorithm are shown in a table, peaks magnitudes and intensity values. The entire procedure is shows at figure 2. In figure 2 (a) it is observed 9 spectrum measurements from just one sample of anhydrous glucose at 500 mg/dL, after that we obtained the average spectrum, figure 2 (b); the positive peak algorithm was applied to average, figure 2 (c);

and the last step shows at figure 2 (d), the resulting spectrum. For this work were analyzed the peaks intensity as a function of glucose concentration.

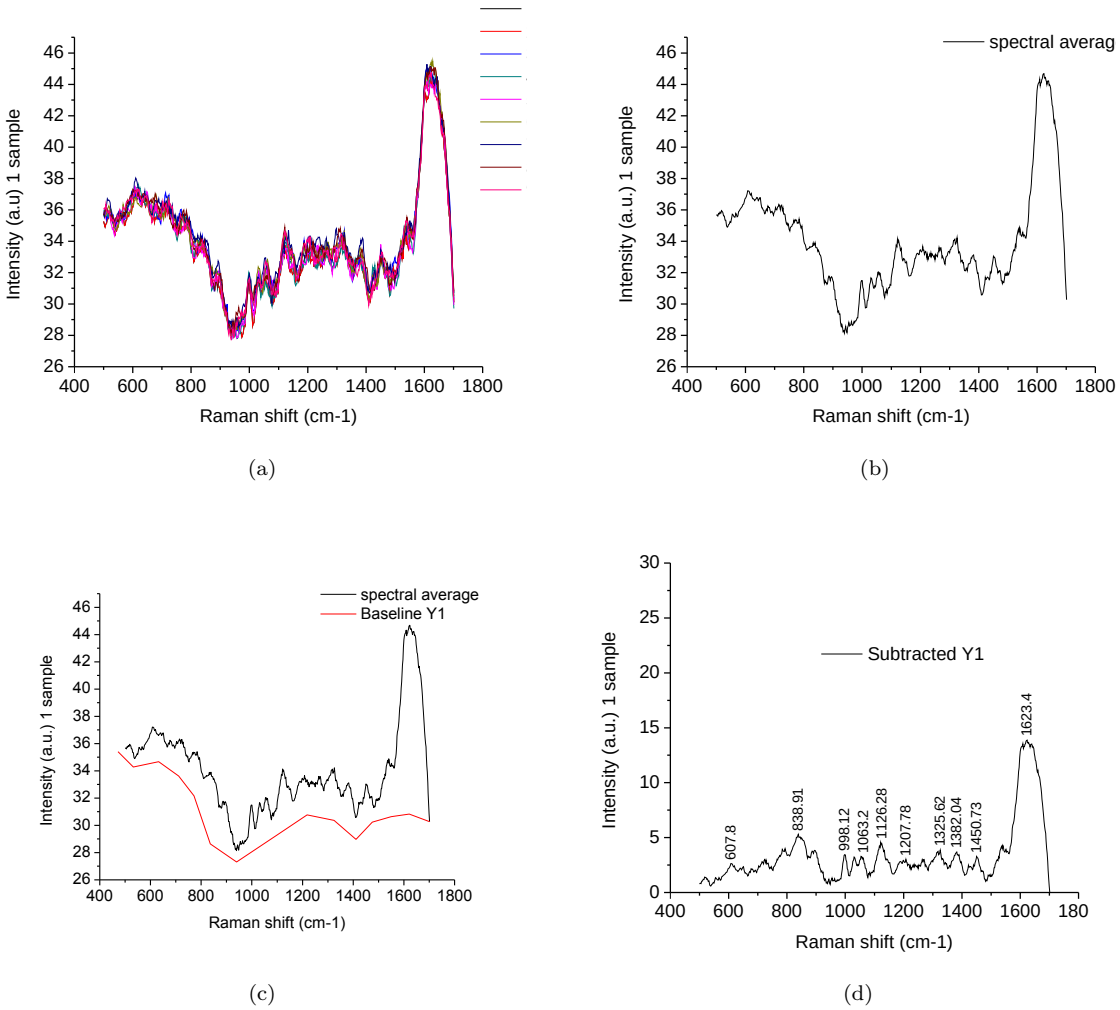


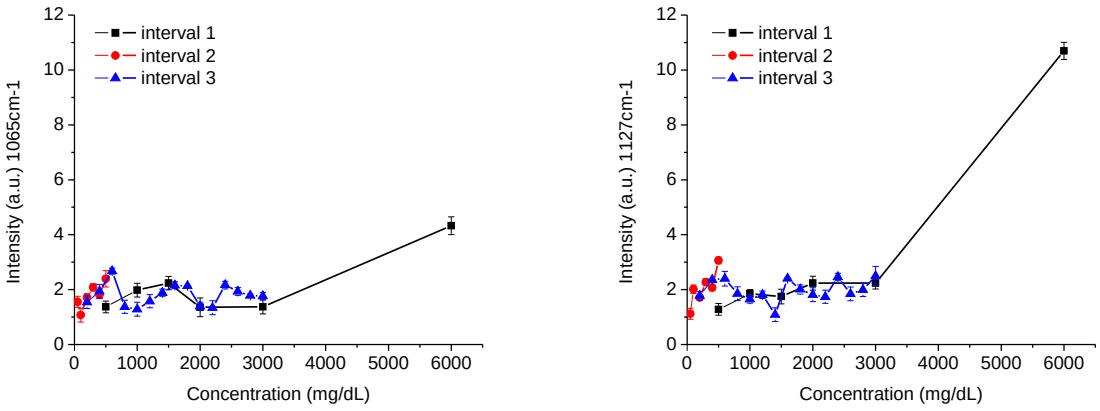
Figure 2. (a) Spectrum measured (b) Spectral average (c) Positive peak algorithm applied (d) Resulting spectrum

3. RESULTS AND DISCUSSION

We analyze the results in two parts, the first is going to be the anhydrous glucose results and the second part to the human serum results. Plots shows the results as a function of Raman peaks and glucose concentration from the analyzed substances.

3.1 Glucose anhydrous results

In the anhydrous glucose results, it is observed an intensity rising pattern as a function of glucose concentration, in the same way, overlapping the different intervals, those coincide each with others, see figure 3. As previously mentioned, we analyzed two Raman peaks, 1065 cm^{-1} and 1127 cm^{-1}. The figure 3 shows better sensibility at 1127 cm^{-1} than 1065 cm^{-1}, this may be due to the atomic links presented in the compound of glucose, as well as the peak of 1127 cm^{-1} is referenced mostly in the literature.



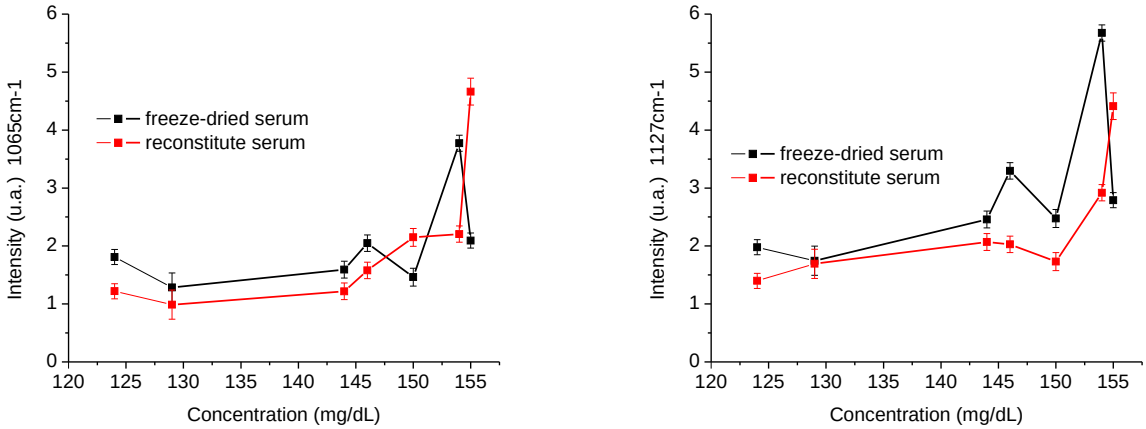
(a) (b)

Figure 3. (a) Comparative Raman peak 1065 cm^{-1} (b) Comparative Raman peak 1127 cm^{-1}

3.2 Human serum results

In the same way, we observe the human serum shows an intensity rising behavior as a function of glucose concentration, see figure 4. Plots shows raising behavior in both Raman peaks.

It is possible to be considered that the peak at 1127 cm^{-1} shows a major intensity change according to the glucose concentration (see figure 4 (b)). Likewise it's possible to observe that for re-constituted serum, the standard of behavior is more constant than the freeze-dried serum, showing a behavior lightly exponential.



(a) (b)

Figure 4. (a) Comparative Raman peak 1065 cm^{-1} (b) Comparative Raman peak 1127 cm^{-1}

4. CONCLUSIONS

In this paper, the Raman spectra of anhydrous glucose solutions and human serum were obtained, and two Raman peaks were using for the analysis. The most important part of this works is that the intensity rising in each analyzed Raman peak depends on the glucose concentration and the low light power used (35mW). We consider

this is a security advantage for an in vivo implementation. Likewise the measurements were made without any another compound, providing an easy method for a future application in a non invasive glucometer.

The results showed that this methodology can be used for noninvasive and quantitative analysis of blood glucose in vivo. The future work is going to applied this technique to diabetic patients, and to increase accuracy and precision of measurements, corroborating whit the Clarke error grid.

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