MEASUREMENT OF BLOOD UREA BASED IN OPTICAL SENSING. EMPLOYMENT IN THE HEMODIALYSIS TREATMENT.

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Abstract. - **The present work shows the development of an indirect, low-cost system to measure on-line blood urea concentration during dialysis procedures using non-invasive optical sensing in the visible range. Dissolved urea presents absorbance in the infrared, not showing any significant optical behavior in the visible range. Nevertheless, previous results obtained by the first author in Mexico displayed experimental evidence of a clear correlation between blood urea contents and optical absorbance in the red region obtained through the hemodialysis ducts. Our hypothesis considers that the cause of this dependence is the volume change of the red cells with the urea concentration due to the osmotic pressure and to the mechanisms of regulation of the cellular volume. The reduction of the volume of the cells would lead to a decrease of the optical absorbance. Cell volume changes should also be observed using electrical impedance spectroscopy; therefore this technique was used as a reference method. Measurements were first performed during experimental dialysis procedures, with yeast suspensions. Clinical measurements were carried out on a group of 30 patients during hemodialysis procedures in the Military Hospital in Mexico City. The most relevant interfering parameter was the water extraction (> 2% weight) along the dialysis treatment, given that changes the blood density, thus affecting the measurement principle.**

Introduction

The interest to develop new mechanisms for the on-line quantification of the blood urea by means of indirect techniques is increasing, due to their potential use to control the haemodialysis process. In particular, enzymatic biosensors are being incorporated to some haemodialysis systems and even to the disposable ultra filtration cartridges. Classical, off-line analytical techniques use colorimetric techniques at 550 to 590 nm after processing the blood samples with enzymatic reagents.

To reduce the cost of this measurement procedure, the first author of this work thought about the viability of using optical absorption acquisition in a non-invasive way across the tube through which the blood flows. In this sense, measures preliminary carried out at a single wavelength in the Clinic No. 25 of the I.M.S.S. in the city of Monterrey N.L. Mexico, already published [1] displayed a good correlation (r=0.87) with the concentration of urea for the group of patients not having other pathology than the renal disease.

However, measurements of urea dissolved in water and in physiologic serum don't show any significant absorbance in the visible range. Their absorbance peak is presented at 2.2 μm. Recent publications also propose NIR blood urea concentration measurement [2] and even flow-through measurements using FTIR in the 2.2 μm to 5.6 μm range [3]. Given that the observed optical absorption cannot be a consequence of the interaction of the light with the molecule of urea, it should be due to the interaction with blood components. The hypothesis we established proposed that the change in optical absorbance was due to the change of volume of cells suspended in blood, mainly erythrocytes. This would be due to the hiperosmolarity induced by the urea [4].

To validate the cell shrinkage hypothesis in the laboratory using a model simpler than blood, we employed suspensions of known concentration of yeast (*Saccharomyces Cerevisiae*) in saline solution. Urea concentration was varied in the suspension samples.

Measurements of optical absorbance were performed by means of two prototypes, the first operating at a single wavelength and the second including a fiber optic spectrophotometer. Electrical impedance spectroscopy was also acquired given its ability to validate the hypothesis of cell volume change. The results of the static measurements [5] were coherent with the established hypothesis, displaying a decreasing optical absorbance (increasing photodetector voltage) with the increasing urea concentration.

The dynamic behaviour of the measurement principle was also determined by provoking a sudden change in urea concentration (100g/l) in a 40 g/l (wet weight) yeast cell suspension (step response). An exponential behaviour with a time constant of 60 s was obtained. This time is short enough if compared with the typical dialysis session length.

After validation under static conditions, dynamic behaviour in an experimental dialyser was characterised both acquiring optical absorbance and electrical impedance spectroscopy. Clinical measurements were

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also performed only using optical absorbance measurement across the blood duct, given the intrinsic safety and sterility of this method. The results are presented in this communication.

Materials and Methods

Measurement prototypes

We arranged optical custom measurement systems based on photodiodes arrays connected to a PIC16F876 microcontroller, which implemented the acquisition and the physical interface between the optical sensors and the display and computer. The system is formed by an array of 128 photodiodes (Texas Instruments); the light source is a LED diode with a single wavelength of 620 nm. The physical construction of the system consists on a small dark chamber where the source of light (LED) and the semiconductor device used as sensor (photodiode array) are placed. They are located lengthwise regarding the probe of transparent plastic through which the liquid samples are passed. The linear disposition of photodiodes gives a better rejection of stray light conduced through the tube walls. The voltage resulting of integrating the current of each photodiode during a controlled time is used as the input signal of the 10 bit microcontroller A/D converter. The captured data are then transmitted via RS-232 to a personal computer. User's interface is developed with Visual Basic ver.6.0, performing the graphical representation of the data, and the determination of minimum, maximum, average and standard deviation values.

The impedance probe for static measurements was a prism shaped cell with 4 stainless steel rod electrodes (2 mm ∅, 4 cm long) at the bottom, while the flow-through probe used in the experimental dialysis measurements was a 3.5 cm ∅, 2 cm depth cylinder with a similar electrode set (2 cm long). Both probes were connected to a HP4192A impedance analyzer through a wideband front-end. Acquisition, calibration and model fitting were carried out with custom software developed under LabWindows to perform studies of microorganism suspensions [6].

For the experimental dialysis procedure, 1 litre of 40 g/l yeast in saline solution, with 2.5 g/l NaCl was prepared. The dialysis system (Figure 1) was composed by a Hemoflow F6HPS Fresenius dialysis cartridge (Polysulfone UF 8.5) and two peristaltic pumps. During 45 minutes dialysis time, the yeast suspension circulated repeatedly through the inner, closed circuit, while 5 litre of 2.5 g/l NaCl saline rinsing solution circulated through the outer, open circuit, both at 105 ml/min. No other components were added to avoid salt interchange between circuits that had provoked conductivity changes. The prepared urea concentrations were largely higher than those are usual in the blood, given the higher resistance of yeast to the osmotic stress.

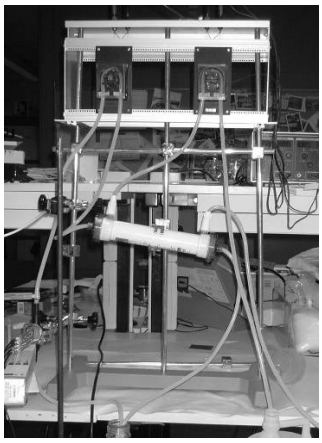

Figure 1: Experimental dialysis system with both the optical absorbance probe and the electrical impedance spectroscopy flow-through probe.

Clinical measurements protocol

To verify the performance of the proposed method in real conditions, clinical measurements were performed over 30 hemodialysis procedures in the Central Military Hospital in Mexico City, after the authorization of the medical protocol HCM160404. The optical chamber was placed along the blood duct (output line). The complete hemogram analysis was performed over blood samples for each patient (Dade Behring equipment). Blood Urea Nitrogen (BUN) and patient weight were obtained before and after the dialysis treatment.

The sensitivity between optical absorbance and urea content was calibrated by using the measurements of a young patient without any other pathology than the renal disease. Preliminary results [1] gave the best correlation for these cases.

Results

Experimental dialysis system

The direct results obtained with the prototype display the optical transmittance in the range of 600-620 nm, corresponding to a red LED (Figure 2).

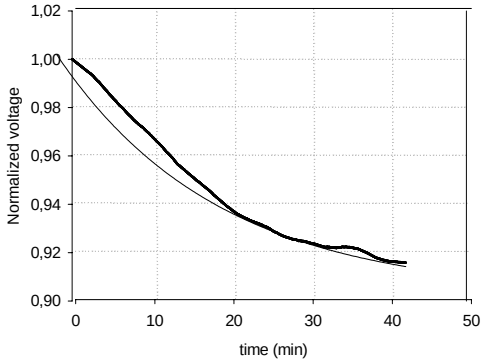

Figure 2: Normalized voltage time course obtained with the single wavelength system during the experimental dialysis procedure with yeast suspension.

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In the course of urea extraction, cells would increase in size due to the lowering of osmotic pressure thus increasing the bulk optical absorbance of the cell suspension and decreasing the optical transmittance (Figure 2). Results (not shown) were also confirmed using light scattering. The exponential fitting gives a time constant of 21 minutes, largely higher than the 1 minute time constant associated to the primary transduction mechanism (cell swelling) [5].

The results obtained with the impedance spectroscopy system in the range 10 kHz–10 MHz range were coherent with this behaviour. In the figure 3 we can observe that the ratio between low frequency and high frequency impedance magnitude increases monotonically, indicating a decrease of the extra cellular space if the medium conductivity stays constant, which is the case. A more extended analysis of the impedance results was presented at [7]. The biomass density estimator (not shown), also rises. Since there is not change in the number of cells, the increase of biomass density should be attributed to the augment of the average cell radius. This was confirmed with the decreasing time course of the central relaxation frequency of the impedance.

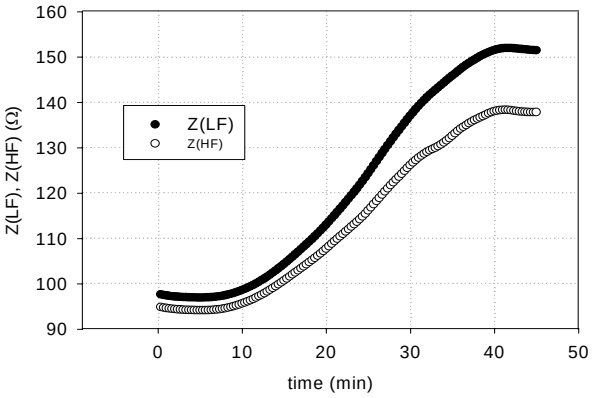

Figure 3: Evolution of the impedance value at low and high frequency with the urea concentration

Clinical measurements

During the haemodialysis treatment, some patients suffered hypotension episodes and were treated by injection of saline solution to compensate blood pressure. In these cases and given the evident change in blood density, the optical transmittance shows sudden peaks. For the normal cases, with a quiet and monotonous urea extraction, the optical transmittance presents an exponential decay, as corresponds to a diffusion process (Figure 4).

The exponential fitting of the transmittance time course allows the estimation and even the prediction of the dominant time constant in the cell swelling process. Typical values obtained range from 15 to 55 minutes.

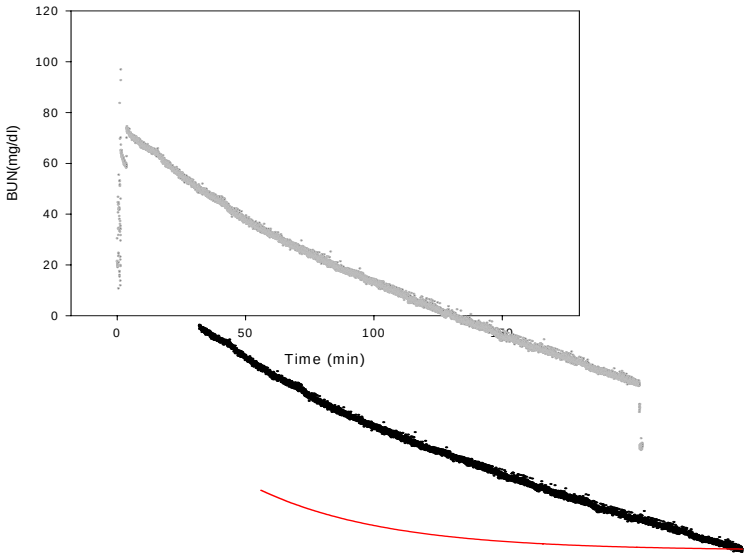

Figure 4: Evolution of the urea concentration estir
from the optical transmittance in a patient subje
haemodialysis in the Military Hospital in Mexico C

Figure 5 shows the correlation between the labor
BUN analysis and the prototype results (pre-dia
(R^2= 0.7).

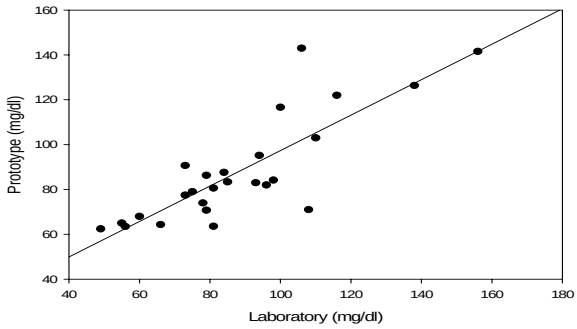

Figure 5: Experimental results showing the relatio
between urea estimation from optical transmittanc
blood urea concentration in hemodialysis patients.

Given the high dispersion of the results, we obt
the correlation between urea concentration and all
hemogram parameters. With the resolution of our a
measurements, we have not found any of the correla
was found relevant. Taking in consideration
information of dialysis process, we found that the
extraction was the main factor affecting
measurement. This is logical given that affects the
density and thus, the measurement principle.

We determined that the error in the measureme
the urea decrement estimation between pre and
analyses is kept below 10 % if the water extractio
been lower than 2 % of patient weight and grows
this value (Figure 6). If we limit the cases to tho
which the extracted water is below 1 litre, the corre
factor between the values of the urea concentr
(BUN) obtained with the prototype and the laborat
R^2= 0.9.

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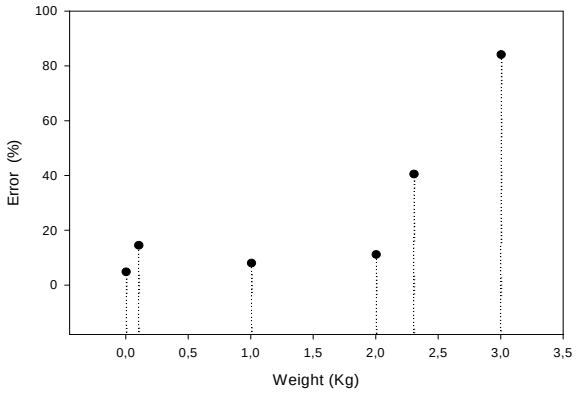

Figure 6: Relative Error in the estimation of extracted blood urea related with water extraction (weight loss) in haemodialysis patients.

Discussion and Conclusions

The results obtained with suspensions of Saccharomyces Cerevisiae confirm the preliminary results obtained in the hemodialysis blood circuit. Optical absorbance in the visible range decreases when urea concentration increases. Given that dissolved urea only presents absorbance peaks in the IR, we should infer that the absorbance change is due to urea induced changes in cell through the mechanisms of regulation of the cellular volume.

Measurements with the prototype show absorbance changes in a wide peak that corresponds to the bulk color of the yeast suspension. Single wavelength measurements at 620 nm allow obtaining a monotonic quasi-linear relationship between transmittance and urea concentration. In the case of the blood, the center of absorbance peak will change, but it will be obviously placed around the red color, given that the main population of blood cells subject to volume shrinkage will be erythrocytes.

Measurements confirm the proposed hypothesis. During the urea extraction both optical and electrical impedance measurements showed behaviour consistent with the cell volume increase.

The dynamic behaviour of the described mechanism shows a dominant time constant of the minute order. This means that the measurement system based on this phenomenon is potentially useful for monitoring and controlling the urea exchange in the dialysis process, that can take several hours and whose critical phase can last some tenths of minutes.

The statistical results between laboratory analysis and prototype show a direct dependence between both techniques. Considering all measures in patients, the dispersion is elevated in both cases. This dispersion lowers if we consider homogeneous patient groups. Independently of the accuracy of the urea estimation, this method provides a way for estimation and early prediction of urea extraction dynamics, through the exponential fitting of the estimator time course (τ). This parameter can help to adjust the settings of the haemodialysis machine and even be used as a control loop input.

Finally we conclude that there is scientific evidence of a correlation between the urea concentration and optical absorbance at 620nm. Due to its low cost, simplicity and the null interference with the dialysis system (intrinsical safety) it possible to consider its clinical use as a complementary method to monitor the dialysis performance and even its possible use in the process automatisation.

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