XXVI Congresso Brasileiro de Engenharia Biomédica

Armação de Búzios – RJ – Brasil

October 21st to 25th, 2018

Cystic Fibrosis Diagnostic Dedicated Device

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Background, Motivation and Objective. Cystic fibrosis (CF) is a genetic disease of recessive nature that causes dysfunction in the cystic fibrosis transmembrane conductance regulator (CFTR) channel, leading to alterations in the transport of Cl- through some membranes, such as those lining the sweat glands. Therefore, the [Cl-] in the sweat of a pathological individual is ≥60 mM, normal <30mM and between these values there is a need to confront different test results. The main method for diagnosis is based on a titration protocol described by Schales and Schales (1941), where the test setup is composed of the analyte, deionized water and the indicator (diphenylcarbazone). Thus, the reagent $(Hg(NO_3))_2$ is released in small known amounts in that solution, consuming the chloride. When all the chloride is consumed, the Hg_{2+} ions react with the indicator, leaving the translucent liquid solution color to form a complex in purple color and indicating the endpoint (EP) of the titration. The final calculation of the [Cl-] in mM is obtained by multiplying the reagent volume (mL) x 100 (eq. 1). The subjective perception of color tonality and the operator ability in this fully manual test often leads to an increased use of reagent, resulting in errors in the calculation of the [Cl-]. We proposed in this work a semi-instrumented prototype for CF diagnosis using optical detection of the EP in the Schales and Schales protocol, constituting an important part of a commercial version of our prototype under development.

Methods. Methods. The Beckman DU 800 spectrophotometer was used in order to determine the best wavelength to be monitored. At the exact moment of the occurrence of the EP (for the amount of reagent consuming the entire Cl-), 10 standard curves (4 different patterns, 10, 20, 50 and 100 mM) of spectral absorption were raised in a range from 450 nm to 750 nm, at 1 nm step. Thus, with the best wavelength found, a monochromatic LED was chosen as a light source. For the assembly of the prototype, the 3D Ultimaker 3 printer was used for the designing of a single-piece to fix the TCS 230 sensor, test tube fitting (1cm diameter), motor (5V DC) and support part coupled to the motor shaft with two magnets placed at 180◦; all with layer resolution of 20um (for 0.4mm nozzle) and 12.5 / 12.5 / 2.5um precision in the step for the X, Y and Z axes, respectively. These magnets create a variable magnetic field inside the tube by spinning a small magnetic bar and promoting liquid mixing. The TCS 230 sensor was adapted for this application. Tthe linght intensity is transduced into a 50% dutycicle electrical signal whose frequency value represents the light intensity measured by the Atmega328 microcontroller. The absorbance is calculated according to the expression, A= -log(F_N / F_0), where F_0 is the frequency related to the maximum luminous intensity measured with the white solution and F_N the frequency related to the light absorption by the analyte. Finally, in order to establish the criteria that would be implemented in software for the automatic detection of the EP, the absorbance values was monitored throughout the protocol, i.e., for each addition of reagent, one absorbance reading was made. In possession of these data, 2 criteria are established for the end-of-test: variation between measures (X_N/X_{N+1}) and biological limits. With these criteria, tests of repeatability (20 measures/day), reproducibility (3 different days) were performed for standard 30 mM [Cl-].

Results. Figure 1 presents 12 scans for the 10 mM of [Cl-]. Analyzing it together with data from the other 3 patterns (n = 40 curves) it was possible to calculate the average wavelength for the maximum absorbance, which was 545 ± 3,05nm. So this value was adopted as the best wavelength to be monitored. On figure 2, the absorption characteristics during the conduction of the entire protocol at this wavelength (not only in the EP as in figure 1) are plotted. However, commercial LEDs do not emit in a narrow range of λ's, so other lengths were analyzed and plotted together with figure 2. All these measurements were obtained by Beckman equipment and, as a result, was selected the green LED for this prototype. The experiment that gave rise to figure 2 in the Beckman equipment was repeated in the prototype, figure 3, since it is fundamental to define the end-of-test criteria. In order to make the definition of end-of-test criteria more evident, a horizontal bar graphic showing the percentage of variation between measurements was performed in Figure 4. Thus, it was determined that the end-of-test will occur when there is 50% of variance between measures and it is between 10 and 160 mM(biological limits). The sum of the random and systematic errors, as well as the coefficient of variation are summarized in Table 1.

Discussion and Conclusions. The amount of reagent that was expected for the EP of a 50 mM solution was 500uL (figure 4), as to be deduced from eq1., thus, the reading of the prototype was more sensitive than the equipment at that point. For the other standards, the results were similar. The design and the precise printing of the custom piece for the elements of the prototype gave a rectilinear beam and little diffraction, which maximizes the interaction of the photons and increases the sensitivity. The adaptation of the TCS for this application also proved satisfactory, which, together with the low-cost microcontroller (Atmega328p) capability of executing 16 millions of instructions per second was able to measure accurately up F_{out} to 50 kHz. Finally, to make semi-instrumented detection in this work, with coefficients of variation (Table 1) less than the total error and elimination of some operational errors and the subjectivity of the EP detection, make this prototype close to the medical decision levels and represent important step in the design of the first fully automatic low cost equipment specifically dedicated to the diagnosis of cystic fibrosis using optical principles.

Figure 1. Set of spectral absorption curves representing the characteristic absorbance of the molecular complex.

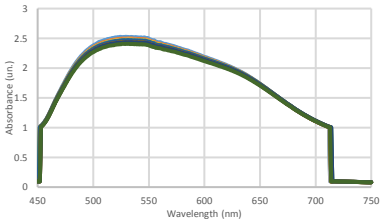

Figure 2. Absorption measurements for the entire protocol in a 50mM standard solution of [Cl-] in the Beckman equipment.

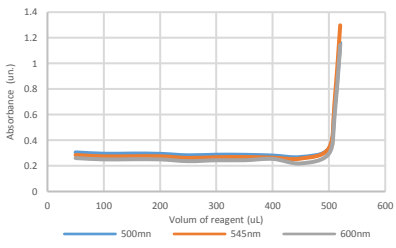

Figure 3. Absorption measurements in the prototype. For each addition of reagent one measure of absorption.

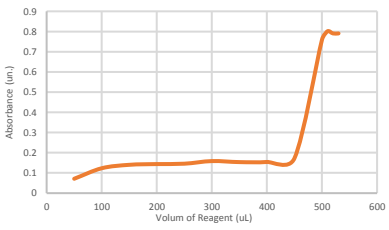

Figure 4 - Percentage of variance between measurements of the same experiments that gave rise to figures 2 and 3.

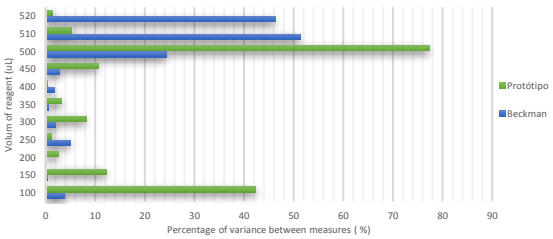

Table 1 – Measurements [Cl-] for 30 mM standard on 3 different days.

	Day 1	Day 2	Day 3
Average	29.85	29.76	30.24
STD.Deviation	0.71	0.62	0.44
Total Error	1.82	1.72	0.79
Coefficient of variation	0.02	0.02	0.014

Acknowledgment. To Eldorado Research Institute.

Keywords. Cystic Fibrosis Diagnostic; Dedicated Device; Optical Measurement; Titration.