A Portable and Low Cost System to Blood Glucose, Cholesterol and Urea Identification

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Abstract: Over the last century there has been a considerable increase in human longevity and this made a large number of people to reach a critical age for development of several diseases. As a result of this increase in life expectancy health issues related, some examples are hypercholesterolemia, hyperglycemia and increased levels of blood urea. This paper presents a portable and low cost system using Artificial Neural Networks to blood metabolites identification. The system developed is based in amperometric biosensors and is able to perform the identification of glucose, cholesterol and urea concentrations in the blood. The main goals of this system is: the identification of three types of blood metabolites with their concentrations, the low cost of the entire system and the reuse capability of the biosensor.

Keywords: artificial neural networks, chemical and biological sensors, monitoring blood metabolites

1. INTRODUCTION

Hypercholesterolemia, which is the presence of high levels of cholesterol in the blood, is not a disease but a serious metabolic derangement due to the fact that cholesterol has an important role in the pathogenesis of atherosclerosis. Hyperglycemia, characterized by excess of sugar in the blood, is a disorder that can cause several complications such as amputation and blindness. Too much urea in the blood is a strong indicator that the individual may have liver and kidney diseases.

Having these problems in sight make it is easy to notice how important the control of glucose, cholesterol and blood urea levels is. Then comes the need to create a device to monitor the levels of these blood metabolites simultaneously and daily once they can rapidly change.

The clinical analysis methods that are currently being used in laboratories are expensive since they rely on duly qualified staff, large instruments, and appropriated place among other costs. Also, these tests bring some discomfort to the patient because of the need for a significant amount of biological fluid, which is usually collected with the use of needles and syringes, and thus can be considered invasive methods.

2. BIOSENSORS

The biosensors then emerge as an alternative to replace conventional methods from clinical laboratories. The analysis can be done by the patient himself at any time and place with the need of only a single drop of blood.

Other than being a non-invasive tool, the biosensors devices provide in the health system a considerable saving compared to conventional methods. Another advantage of using biosensors for monitoring blood metabolites is due to the fact that there are patients who require daily monitoring, for example, diabetic patients.

Biosensors are electronic devices capable of converting a biological reaction in an appropriate signal. This signal can be potentiometric, amperometric, conductimetric, optical, piezoelectric or enthalpymetric.

Figure 1 shows the basic components of a biosensor. In (a), we have the biocatalyst, where the biochemical reaction responsible for generating the signal occurs in (b) we have the transducer in (c) the amplifier and in (d) the results.
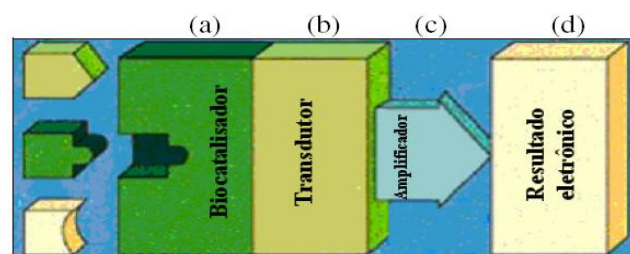

Figure 1. Representation of a biosensor: biocatalyst.

The first biosensor was developed by Clark and Lions in 1962, and this became known as enzymatic electrodes, it can be seen in the figure below.

The enzymatic electrodes or biosensors are used in a number of analytical determinations, in which the detection rate of glucose on the blood is highlighted.

From the invention of Clark and Lyons, several types of biosensors have been developed for different types of clinical analysis, obtaining the most prominent biomedical analysis such as the monitoring of hemometabolite in human blood (such as glucose, cholesterol and urea).
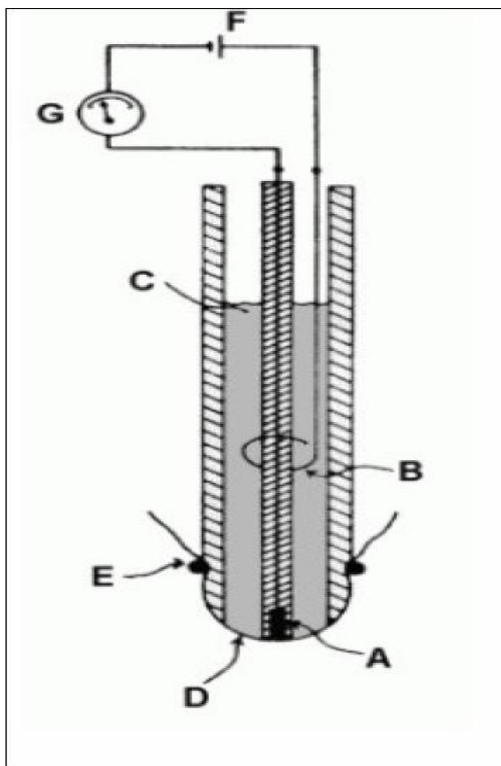

Figure 2 – Enzymatic biosensor by Clark e Lyons.

The biosensors are usually classified according to the signal that is generated in the transducer. The amperometric biosensors are the most used and researched, as it can be seen in Figure 3. But there are other types of biosensors based on other types of signals.
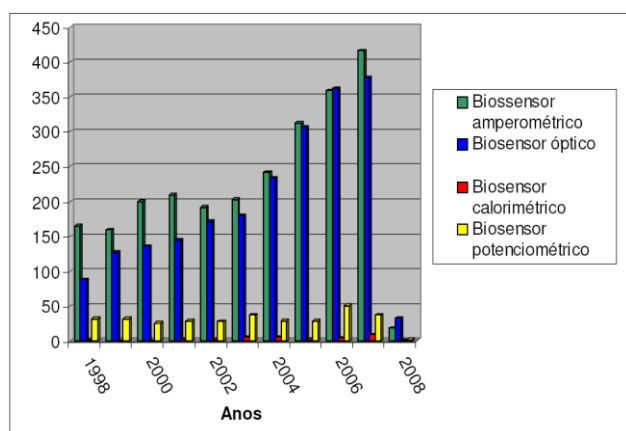

Figure 3 – Comparison chart for the most studied types of biosensors.

The amperometric biosensors provide the ability to capture an electrical signal that is proportional to the analyte concentration (hemometabolite analyzed). They are the most common biosensors found in the market and are shown in Figure 4.

Figure 4 – Commercial amperometric biosensors used in clinical analysis.

3. THE CHEMISTRY IN BIOSENSORS

The main components of a biosensor are the enzymes. The enzymes are biocatalysts that have high selectivity rate. They are proteins with the specific function of speed up chemical reactions that occur under unfavorable thermodynamically conditions. They considerably accelerate the speed of chemical reactions in biological systems when compared to corresponding non-catalyzed reactions. This is achieved by lowering the activation energy required for a chemical reaction, resulting in increased speed in the reaction and enabling the metabolism of living beings. The catalytic ability of enzymes makes them suitable for industrial applications such as in pharmaceuticals or food industry.

The enzyme acts on the substrate witch is transformed into a product. In the absence of enzyme little product is formed, otherwise, the reaction is processed at high speed. Enzymes are the most specific catalysts known for both the substrate and for the reaction performed on the substrate.

3.1 Immobilization

In a biosensor the enzyme is usually immobilized on the electrode surface along with another reagent that will react with one of the blood metabolites. In this study, the enzymes are immobilized using dendrimers.

Dendrimers are monodisperses macromolecules, highly branched, with well-defined structures and uniform molecular weight, as shown in Figure 5. This class of compounds has received great attention from researchers because they have molecular uniformity, multifunctional surface and the presence of internal cavities. These specific properties make dendrimers suitable for the immobilization of enzymes.

4. ARTIFICIAL NEURAL NETWORKS

Artificial Neural Networks are computational techniques presented in a mathematical model inspired by the neural structure of intelligent organisms and acquiring knowledge through experience, Figure 6 shows the structure of a neuron. An artificial neural network can have thousands of processing units. Furthermore the brain can have billions of neurons.
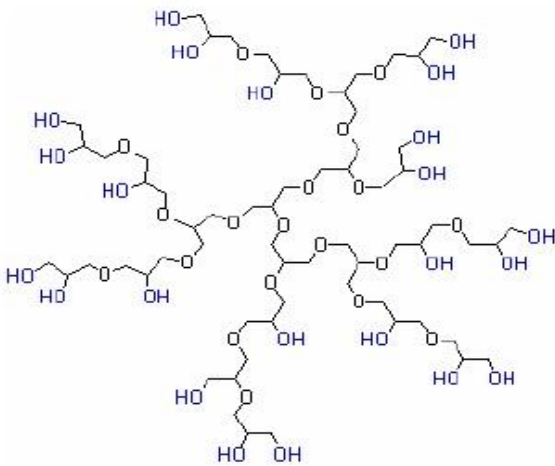
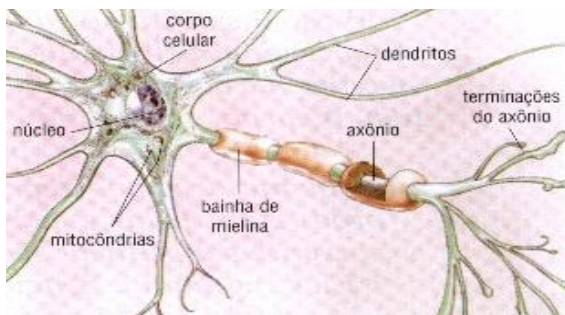*Figure 5 –* Illustration of a dendrimer structure.

Figure 6 – Delineation of the structure of the constituents of a biological neuron.

The nervous system consists of an extremely complex set of neurons. The neuron can be considered the basic unit of the structure of the brain and the nervous system. The communication is done through pulses, when a pulse is received the neuron processes it, and after a certain action threshold is reached, the neuron triggers a second pulse that produces a neurotransmitter substance which flows from the cell body to the axon. They have an essential role in determining the performance, behavior and ratiocination of human beings.

4.1 General features

The artificial neural models, try to bring the computer processing to the human brain, and they correspond to algorithms that mimic the biochemical process of the brain and are similar to this on two points:

a) Knowledge is gained through learning steps and
b) Synaptic weights are used to store knowledge.

Synapse is the name given to the connection between neurons, in it is assigned values that are called synaptic weights. The artificial neuron is a binary logical-mathematical device that tries to simulate the functions and behavior of a biological neuron. Thus, dendrites are represented by entries where connections with the artificial cell body are made through elements called weights that simulate synapses. Stimuli captured by the input are processed by the weighted

sum function of signals, and the threshold for triggering the biological neuron was replaced by the transfer function.

Combining several artificial neurons we can form the so-called Artificial Neural Network, a schematic example is shown in Figure 7. From an artificial neural network formed, a series of values can be applied to a neuron, and this is connected to others by the network. These values (or entries) are multiplied in the neuron by the weight value of their synapses. Then these values are added.

If this sum exceeds an established limit value, a signal is propagated through the output (axon) of this neuron. Then, this same step is performed by other neurons in the network. This means that neurons will face some kind of activation, depending on the inputs and the synaptic weights.

There are several ways to develop a neural network. It must be mounted according to the problems to be solved. In its architecture is determined the number of layers used (the layers are formed by neurons) and the number of neurons in each layer, the type of synapse used.
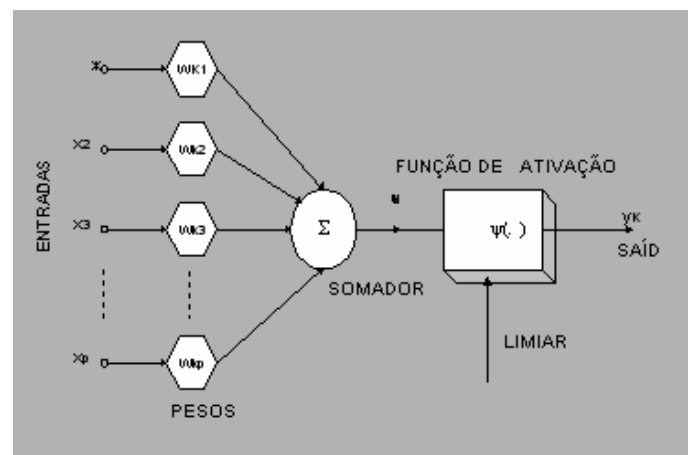

Figure 7 – Representation of the architecture of an artificial neural network (ANN).

4.2 Training

The most important property of neural networks is the ability to learn and thereby improve their performance. They learn by rote memorization, contact, examples, by analogy, by exploration and also by discovery. This is done through training, iterative process of adjustments applied to the weights. Learning occurs when the artificial neural network reaches a generalized solution to a class of problems. It is called learning algorithm a well-defined set of rules for solving a learning problem. There are many types of learning algorithms specific to certain models of ANNs (Artificial Neural Networks) they differ from each other by how the weights are modified.

The learning process of neural networks is performed when there are several significant changes in the synapses of neurons. These changes occur according to the activation of neurons. If some connections are used more frequently these are enhanced while others are weakened.

For this reason when an artificial neural network is deployed for a given application it takes time for it to be trained. There are basically three types of learning in artificial neural networks:

(i)   Supervised; in this type, the neural network receives a standardized set of inputs and corresponding output patterns, there are adjustments in synaptic weights until the error

between the output patterns generated by the network has a desired value;

(ii) Non-supervised; in this type, the neural network process the data to determine some properties of the data set. From these properties the learning is made;

(iii) Hybrid; this type is a merge of supervised and unsupervised. Thus, a layer can work as one type while the other layer works with the other type.

4.3 Multilayer perceptron

The artificial neural network is a system of neurons connected by synaptic connections and divided into input neurons that receive stimuli from the external environment, internal or hidden neurons and output neurons, which communicates with the exterior. The way to arrange the neurons into distinct layers is called a multilayer perceptron.

The multilayer perceptron, as the diagram in Figure 8, was designed to solve complex problems, which could not be resolved by the basic model of neural network, also known as single-layer perceptron, witch only has the input and output layer of neurons.
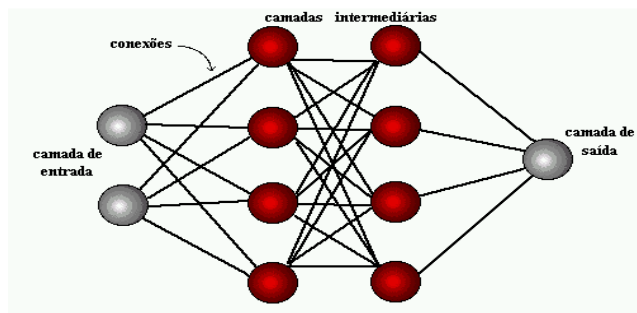

Figure 8 – Representation of a multilayer perceptron.

The internal neurons are of paramount importance for the neural network once it has been proven that without them it becomes impossible to solve problems not linearly separable. In other words we can say that a network is composed of several processing units, whose operation is quite simple. Usually the layers are classified into three groups:

- Input Layer: where patterns are presented to the network;

- Intermediate or Hidden Layers: where most of the processing is done through weighted connections, can be considered as features extractors;

- Layer Output: where the final result is completed and submitted.

4.4 The backpropagation algorithm

The backpropagation algorithm uses supervised learning this means he try to find iteratively the smallest difference between the desired outputs and the outputs obtained by the artificial neural network, with a minimum error. It works by adjusting the weights between the layers through the backpropagation by correcting the error detected, with other words, propagating the error from the output layer to the input layer in each iteration.

Networks using backpropagation work with the generalized delta rule suitable for multilayer networks. The default delta rule essentially program a descendent gradient in the squared sum of the error for linear activation functions, logical threshold function. Network without intermediate layers can solve problems where the error surface has the

shape of a paraboloid with only a minimum. In these cases we should use a network with intermediate layers. Still, the networks are subjected to problems with local minima.

The generalized delta rule, which will be discussed later, works when used on the network units with a semi-linear activation function, which is a continuous differentiable and non decreasing function. An activation function widely used in these cases is the sigmoid function.

The learning rate is a proportionality constant because this learning procedure requires only that the change in weight be proportional to η. However, the true gradient requires that infinitesimal steps be taken.

So the larger this constant the greater the change in weights, increasing the speed of learning, which can lead to a model oscillation on the error surface. The ideal would be to use the higher learning rate as possible that does not lead to an oscillation, resulting in a faster learning.

The training of MLP (Multilayer Perceptron) networks with backpropagation may require many steps in the training set, resulting in a considerably long time of training. If a local minimum is found the error for the training set stop its decrease and stay in highly acceptable value. One way to increase the learning rate without increasing the oscillation is to modify the generalized delta rule to include the term momentum, a constant that determines the effect of past changes in the weights of the current direction of movement in space of weights. This way the momentum term takes into account the effect of previous weight changes in the direction of the current movement in the space of weights. The momentum term becomes useful in error spaces containing long throat, with sharp curves and valleys with gentle descents.

Once the network is trained and the error is in a satisfactory level it can be used as a tool for classification of new data. For this, the network should be used only in feed-forward mode.

In other words, new entries are presented to the input layer then processed in the intermediate layers and the results are presented in the output layer, as in training, but without the backpropagation. In addition, you may need to preprocess the data, through standardization, escalations and format conversions to make them more appropriate for the network usage. In the interpretation of the network the output shown is the data model.

The ANNs using backpropagation as well as other types of ANNs can be seen as "black boxes" in which almost no one knows why the network reaches a certain result, where the models do not have explanations for the answers. In this regard many studies are performed to extract knowledge from ANNs and the creation of explanatory procedures, which in certain situations can justify the conduct of ANNs.

Another limitation is the training time of ANN (Artificial Neural Network) using backpropagation, which tends to be pretty slow. Sometimes it takes hundreds of cycles to reach an acceptable level of error, especially if it is being simulated in ordinary computers, because the CPU (Central Processing Unit) must calculate the functions for each unit and its connections separately, which can be problematic in very large networks or with large amounts of data.

It is very hard to define the optimal architecture of ANNs so that it is as large as necessary to obtain the necessary representations, while small enough to have a faster training. There are no clear rules to define how many artificial neurons

an intermediate layer must possess, how many layers there should exist, not even how each connection between units should be.

4.5 ANN development

4.5.1 Data Collection
The first step of development of an ANN is the collection of data of the problem and the separation into a training set and test set. This task requires a careful analysis of the problem in order to guarantee no ambiguities or data errors. Furthermore, the data must be meaningful and cover the entire problem domain and should not cover only the normal or routine operations but also the exceptions and the boundary conditions of the problem domain.

Typically all the data collected are separated into two categories: training data which are used for training the ANN and test data which will be used to verify the performance of ANN under real conditions of use. Besides this division, you can also use a subset of the training set, creating a validation set, used to verify the efficiency of ANN and also as a stopping criterion of the training. After determining the sets they are placed in random order for prevention tendencies associated with the order of presentation of data. In addition, you may need to pre-process the data through standardization, escalations and format conversions to make them more appropriate.

4.5.2 ANN Configuration
After collecting the data it is time to setup the configuration of the ANN, which can be divided into the following steps:
I.   Selection of an appropriate neural paradigm for the application.
II.   Topology determination of the ANN to be used (number of layers, number of artificial neurons in each layer, etc.).
III.   Determination of parameters of the learning algorithm and activation functions. This step has a huge impact on the system performance.
Usually these choices are made on an experimental basis. The setting of ANNs requires experience designers.

4.5.3 ANN Training
At this stage following the training algorithm chosen, as shown in Figure 9 the weights of the connections are adjusted. It is important to consider aspects such as the initialization of the ANN, the training mode and the time spent to train it. A good choice for weights initial values can significantly reduce the training time. Normally, the weights initial values of the network are random numbers and distributed uniformly in a defined range. The wrong choice of weights can lead to a premature saturation.

As to training, in practice the most widely used is the default mode due to the smaller data storage, in addition to being less susceptible to the problem of local minima. On the other hand, in batch mode if you have a better estimate of the gradient vector, which makes training more stable. The relative efficiency of the two training modes depends on the problem that is the case.
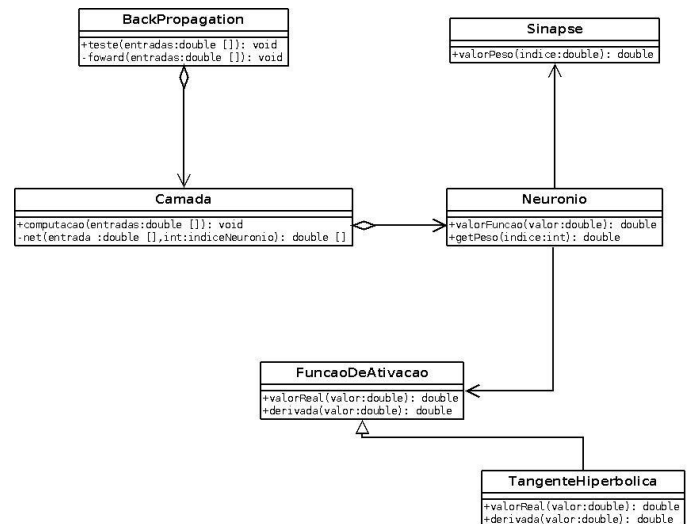

Figure 9 – Flowchart representation of the ANN used.

As for the training time several factors may influence the duration, but we always use some stopping criterion. In the backpropagation algorithm the stopping criterion is not well defined and it is usually used a maximum number of cycles. But it must be considered the average error rate per cycle and the generalizability of the ANN.

It may happen that at a given moment the generalization of the training process start to degenerate, in other words, causing a loss in the ability of generalization. So we must find a good stopping point with minimum error and maximum capacity of generalization. The flowchart of the backpropagation ANN for control and automation of the multienzyme biosensor device for monitoring the blood metabolites glucose, cholesterol and urea.

4.5.4 Tests
During this phase the test set is used to determine the performance of the ANN with data that were not previously used. The performance of the ANN at this stage is a good indication of its actual performance.

Other tests should be considered such as analyzing the behavior of the ANN using special entries and analysis of current weights of the ANN, because if there are very small values the associated connections can be considered insignificant and thus be eliminated. Conversely, values that are greater than others could indicate that there was a loss in the ability to generalize the ANN.

4.5.5 Integration
Finally with the ANN trained and evaluated, it can be integrated into an operating environment system of the application. For increased efficiency of the solution this system should include usability facilities as a convenient interface and an ease acquisition of data through spreadsheets, interfaces with signal processing units, or standard files.

We should periodically monitor the system performance also network maintenance should be done when needed or indicate the need of re-training to designers. Further improvements can be suggested when users are becoming more familiar with the system, these suggestions may be very useful in new versions or new products.

5. ANN DEVELOPMENT FOR BLOOD METABOLITES ANALYSIS

The biosensors are a promising tool to supplement the clinical analysis techniques due to its properties such as selectivity, low cost of construction and storage, potential for miniaturization, making it easy to build simple and portable equipment where the patient himself could do all the monitoring

The reading of recent literature shows that more and more problems in the area of analytical chemistry, such as the concentration analysis of components of a mixture, has been solved using ANNs, and it was observed that the multilayer perceptron using backpropagation algorithm has been the most widely used. This way, for teaching purposes an ANN was built, it uses the supervised learning and a feedback algorithm that is weights are modified during the computation so that the next weight will be given in terms of the current, the difference between the desired output and current output and the so-called learning rate. The development and testing process of the ANN will be described below.

The experimental procedure was basically divided into three stages. The first stage consisted by obtaining the reaction curves of the blood metabolites with the chemical components of the biosensor. In the second stage the data representing the curves were normalized and presented to the ANN. The third and final stage consisted of comparing the response data to validate the developed ANN and the method of analyzing curves proposed in this paper.

5.1 Obtaining Curves

The curves shown in this paper are obtained from the chemical reaction between the conductor and the blood metabolites present in the biosensor. As in any chemical reaction there are electron transferences, we used a Keitlhey voltage/current source to obtain each concentration curve of each of the three blood metabolites, a model 237 (K237) was used as shown in Figure 10, this voltage source is capable of responding which electric current was produced in a chemical reaction in a given time. This response is given as a time curve in the y-axis, measured in seconds, and electric current in the x-axis, measured in amperes.

The Keitlhey K237 is a source unit that measure with a high precision, essential to the realization of voltage measures between 10V to 1100 V, and current measures from 10 fA to 100 mA where these ranges are fundamental measures of low signals and any others that requires precision.

For automated control of any data acquisition, this instrument has IEEE-488 standard, which allows its programming via computer. With all these features this measuring unit source accurately characterize the environment in which a patient will measure the concentrations of their blood metabolites using a common amperometric meter as previously shown.

Figure 10 – Keitlhey measuring unit source model 237**.**

For each hemometabolite - cholesterol, urea and glucose - 181 samples were made starting at 0.2 mM (0.2 mmol / L) then 0.4 mM (0.4 mmol / L) and increasing the concentration with 0.2 mM for each sample up to a concentration of 36.2 mM (36.2 mmol / L). This way for all three blood metabolites we will be covering the concentrations for medical interest, because the normal concentration of these blood components are shown in Table 1. Is worth remembering that:

- Urea > 214 mg / dl (35.6 mmol / L) is indicative of acute renal failure.
- Glucose < 45 mg / dl (2.52 mmol / L) neurological symptoms of hypoglycemia, which can extend from a decrease in cognitive function even lead to unconsciousness. Glucose > 450 mg / dl (25.2 mmol / L) diabetic coma due to lack of insulin.

Cholesterol > 240 mg / dl (6.2 mmol / L) serious risk of heart disease.

Table 1 – Concentration of blood metabolites for medical interest.

Hemometabolite	Concentration of problems in humans (adults of both sexes)
Glucose	When the fasting glucose exceeds 11.2 mmol / L (200mg/dl of blood)
Cholesterol	<200 mg / dl (5.2 mmol / L) normal total blood cholesterol 200-239 mg / dl (5.2 to 6.2 mmol / L) limit of total cholesterol > 240 mg / dl (6.2 mmol / L) higher total cholesterol
Urea	When the urea concentration exceeds 8.3 mg / dl (1.4 mmol / L)

Figure 11 – Biosensor used for the reaction with hemometabolite.

Each of these concentrations was placed to react with the biosensor, shown in figure 11, producing curves as shown in Figure 12, generated through the measuring source.
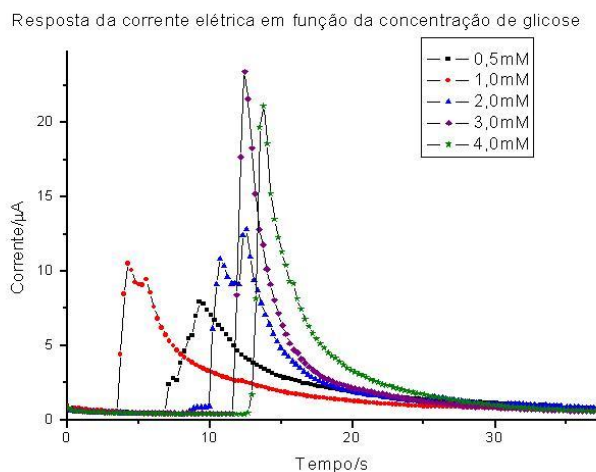

Figure 12 – Response of the electric current as a function of glucose concentration.

5. 2 Data preparation

With the curves of current vs. time, as shown in Figure 13, the data representing the curves were standardized to be presented to the ANN to be tested and validated. To standardize the data curves the following techniques were used:

1. Standardization by the range of variation, where the point to be standardized is subtracted by the lowest value point on the set and divided by the difference between the highest and the lowest points of the set.

$$y = (x - \min) \div (\max - min) \quad (4.1)$$

2. Standardization by standard deviation, where the point to be standardized is subtracted by the average of the set of points and divided by the standard deviation of the set of points.

$$y = (x - \mu) \div \sigma \quad (4.2)$$

These standardizations were chosen because they maintain the characteristics of the curves this can be seen in the figures below, because even after going through the transformations, the curves remain with the same format.
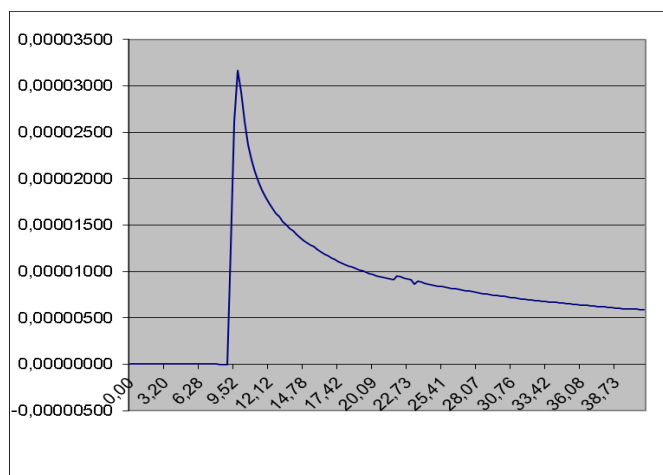

Figure 13 – Chart showing the current response time for the reaction of 20mM

Figure 13 shows the values for the reaction around 20 mM cholesterol with the reagent of the biosensor. In the y-axis is time in seconds of the chemical reaction and the x-axis is the electric current produced measured in uA.

The graphs presented in Figures 14 and 15 have the same data described in Figure 13, but their axes are respectively formatted using the standardization by the range of variation and the standardization by standard deviation.
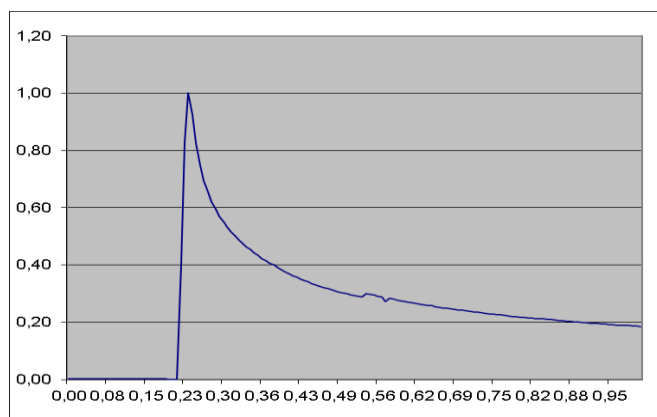

Figure 14 – Chart showing the current response time for the reaction of 20mM with axes standardized via standardization by the range of variation
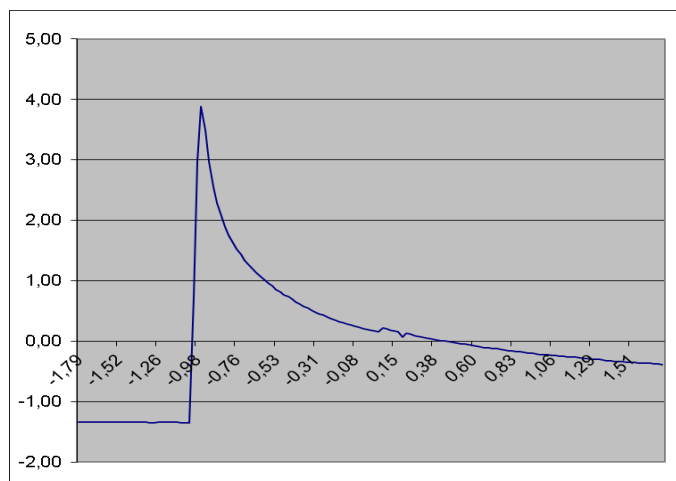

Figure 15 – Chart showing the current response time for the reaction of 20mM with axes standardized via standardization by the standard deviation.

The concentration values of the curves were also standardized using the same techniques. The blood metabolites were also identified numerically, but as in this case the identification job of the ANN is to classify the curve analyzed in one of the three classes of blood metabolites that are being studied in this article, the desired response for the classification is shown in Table 2.

Table 2 – Desired response for the classification of curves in three classes of blood metabolites.

	NEURON 1	NEURON 2	NEURON 3
CHOLESTEROL	1	0	0
UREA	0	1	0
GLUCOSE	0	0	1

5.3 Determination of the ANN structure

After the data is ready for presentation to the ANN, it was necessary to determine what structure of the ANN we were going use, how many nodes in the input layer, or how many input variables is needed for the ANN accurately determine what concentration of blood metabolites and which hemometabolite is being measured, and also in the hidden layer of the network how many nodes are needed. In addition to validate the ANN software that was developed during this work for its future use in other studies. For this purpose we used the Nets 3.0 and MATLAB R2008a, two highly renowned programs that works with neural networks.

5.3.1 Data separation for the formation of testing, validation and control groups

As a beginning of testing, due to the large number of curves that were available, 181 (one hundred and eighty-one) curves for each hemometabolite, the curves were divided into three groups. A group called test, with 327 (three hundred twenty-seven) curves with 109 (one hundred and nine) curves for each hemometabolite that was used for the learning of the ANN, 60% (sixty percent) of all curves. Another group called validation with 108 (one hundred and eight curves), 20% (twenty percent) of all curves, 36 (thirty six) curves for each hemometabolite used for testing (to verify that the values of free parameters of the ANN, synaptic weights, momentum and learning rate causes the ANN to determine which hemometabolite is being measured and what is its concentration) the ANN. Also another group called control, also with 108 (one hundred and eight) curves with 36 (thirty six) curves for each hemometabolite, 20% (twenty percent) of all curves, to ratify the free parameter values obtained, the table 3 summarizes the data curves.

Table 3 – Formation of validation, control and group tests.

	CHOLESTEROL	UREA	GLUCOSE	TOTAL
TEST	109	109	109	327
VALIDATION	36	36	36	108
CONTROL	36	36	36	108
TOTAL	181	181	181	543

5.3.2 Presentation of data to the ANN for tests

For testing purposes the data were presented as follows:

- The training mode is sequential, this means that after each presentation of a set of example the error backpropagation occurred for the correction of the synaptic weights.
- During the training phase of the ANN cholesterol, urea and glucose curves were alternately presented to the ANN, but respecting an order of increasing concentration value.
- For validation and control the curves were presented randomly to the ANN.
- Data using both standardization were presented, but in the first tests the standardization by the range of variation method was proved to be better be reaching better results, once this standardization produce results with values between 0 (zero) and 1 (um) which facilitates the ANN process of learning (SCHALKOFF, 97), furthermore, the data was standardized by the standard deviation were not used in the test.

5.3.3 First test

For all tests, the ANN used is the multilayer perceptron, feedforward, using the backpropagation algorithm to adjust the synaptic weights. For the first test we used the following structure of the ANN:

- Input layer with two neurons to receive the ordered pairs (electric current, time).
- A hidden layer with 10 (ten) computational neurons and with a nonlinear sigmoid activation function.
- The output layer with 4 (four) computational neurons and nonlinear sigmoid activation function.
- Learning rate equals to 0.6 and momentum equals to 0.3.

We used three different neural networks, one of them developed using MATLAB R2008a a second one using the Nets 3.0, and a third one using the ANN software developed during this work. The ANNs developed using MATLAB R2008a and Nets 3.0 are being used in this work also to check the performance of the ANN software developed in this work, this software is being called SJRNA in the text. The results obtained in the first trial are listed in Table 4.

Table 4 – Results of the first test

Simulator	Seasons	Hits of concentration	Hits of Hemometabolite
JRNA	3500	0%	100%
Nets 3.0	3500	0%	100%
MATLAB	3500	0%	100%

The 100% value of accuracy is considered as follows, for example:

1. Considering the concentration of 5.2 mmol and 5.4 mmol its standardized values are 0.138889 and 0.144444.

2. It is considered that there was an error by the ANN when the difference of the produced value by it is greater than 0.001, this error is enough so that the ANN can accurately determine with precision the concentration value, once the smallest difference between the two concentrations in sequence is 0.004, and to determine which class of hemometabolite the curve belongs this error is quite satisfactory, because there are only three classes to be checked and the worst output produced by the network had the following values: (0.9989 / 0.00108 / 0.001783) produced by the first test.

The three ANNs used in the first test were proven to be very effective concerning the identification of which hemometabolite belongs to the points of the analyzed curve, but were unable to identify the concentration of each curve, this was due to the existence of a large amount of ordered pairs (current, time) that belonged to more than one same curve. And another problem was the delay in making a training because each curve to be presented consists of 150 ordered pairs (current, time), this way the need to reduce the amount of data to be presented to the ANN was observed and also the need to introduce some other variables in order to the ANN have a reasonable performance in identifying the concentration.

5.3.4 Second Test

For the second test, due to the need to decrease the amount of ordered pairs representing each curve and the introduction of new variables to improve the performance of the ANN the

following changes in the training set and structure of ANN were made:

1. For each curve was calculated the area of the curve peak, as shown in Figure 16, and the breadth and these two data began to be used as input parameters of the ANN by increasing the number of entries from two to four.

2. To decrease the number of ordered pairs representing each curve only the ordered pairs from the peak were used, this way each curve is going to be represented by 40 (forty) ordered pairs (current, time).
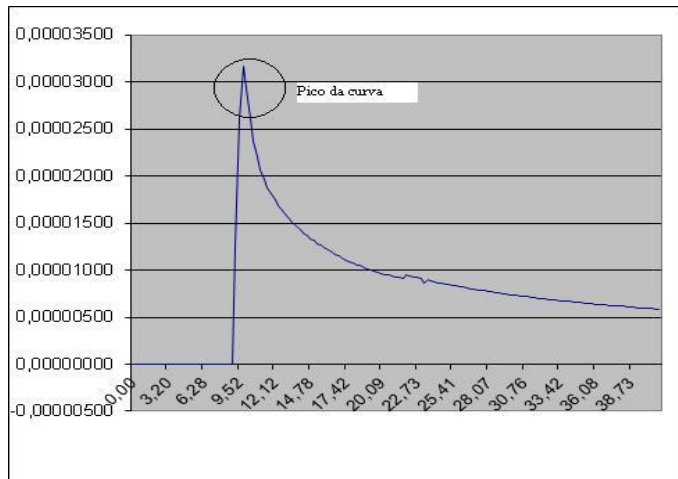

Figure 16 – The curve peak is shown in evidence.

With the changes the ANN configuration was as follows:
- Input layer with 4 (four) neurons to receive the parameters of the peak area, curve peak width and the ordered pairs current and time.
- Hidden layer with 10 (ten) computational neurons and sigmoid nonlinear activation function.
- The output layer with 4 (four) computational neurons and sigmoid nonlinear activation function.
- Learning rate equals to 0.6 and momentum equals to 0.3.

The results with the introduction of these changes can be seen in Table 5 below.

Table 5 – Results of the second test.

Simulator	Seasons	Hits of Concentration	Hits of blood metabolites
SJRNA	1300	97%	100%
Nets 3.0	1500	99%	100%
MATLAB	1100	99%	100%

The 100% value of accuracy is considered as follows, for example:
- ⚐ Considering the concentration of 5.2 mmol and 5.4 mmol its standardized values are 0.138889 and 0.144444.
- ⚐ It is considered that there was an error by the ANN when the difference of the produced value by it is greater than 0.001, this error is enough so that the ANN can accurately determine with precision the concentration value, once the smallest difference between the two concentrations in sequence is 0.004, and to determine which class of hemometabolite the

curve belongs this error is quite satisfactory, because there are only three classes to be checked and the worst output produced by the network had the following values: (0.9989 / 0.00108 / 0.001783) produced by the first test.

The new data show that the changes that were made are essential on determining the values of the curves, once the worst result the ANN had was not capable of distinguish only 16 curves that had concentration with the difference of only 0.2 mmol.

5.3.5 Third Test

In the third test all changes made in the second test were retained, but with the difference that we used two different ANNs, one to determine the concentration and another one to determine which class of hemometabolite belonged the analyzed curve. The ANN used to determine which class belonged the curve to be analyzed was given as follows:
- Input layer with two neurons to receive the ordered pairs (current, time).
- A hidden layer with 10 (ten) computational neurons and sigmoid nonlinear activation function.
- The output layer with 3 (three) computational neurons and sigmoid nonlinear activation function.
- Learning rate equals to 0.6 and momentum equals to 0.3.

The results are shown in the Table 6 below.

Table 6 – ANN test results to determine the class of the curve

Simulator	Seasons	Hits of Hemometabolite
SJRNA	1500	100%
Nets 3.0	1300	100%
MATLAB	1100	100%

The ANN used to determine the concentration of the analyzed curve was given as follows:
- Input layer with 4 (four) neurons to receive the parameters of the peak area, curve peak width and the ordered pairs current and time.
- Hidden layer with 10 (ten) computational neurons and sigmoid nonlinear activation function.
- The output layer with 1 (one) computational neuron and sigmoid nonlinear activation function.
- Learning rate equals to 0.6 and momentum equals to 0.3.

The results obtained with the introduction of these changes can be seen in the Table 7 below:

Table 7 – ANN test results to determine the concentration curve

Simulator	Seasons	Hits of Concentration
SJRNA	1800	100%
Nets 3.0	1500	100%
MATLAB	1300	100%

The separation into two ANNs was efficient because they began to accurately hit all the concentrations and blood metabolites classes.

6. CONCLUSION.

By analysis of the tests can be concluded that:

- The process described in this article to classify and determine the concentration of hemometabolites proved to be efficient, mainly when using specialized ANN to classification and determination of concentrations, as show the third test.

- Using the same methods for obtaining curves of chemical reactions discussed in this article. To determining concentration of hemometabolite, the ANN multilayer perceptron type that uses backpropagation algorithm to adjust the synaptic weights proved to be efficient and can used to determine concentrations of reagents of others chemical reactions.

REFERENCES

[1] BURTIS, C. A.; ASHWOOD, E. R. Tietz: Fundamentos de Química Clínica. 4 ed. Rio de Janeiro: Guanabara, 1998, 836 p.

[2] CLARK, L.; LYONS, C. Annals of the New York Academy of sciences, n. 29, p. 102-123, 1968.

[3] WILSON, G.S.; GIFFORD, R. Biosensor for real-time in vivo measurements. Biosensors and Bioeletronics, n. 20, p. 2388-2403, 2005.

[4] FERNANDES, E.G.R. Biossensores nanoestruturados para monitoração de glicose. 2005. 175 f. Dissertation (Materials Engineering Master Degree) – Federal University of Itajubá. Advisor: Prof. Dr. Álvaro A.A. de Queiroz.

[5] STRYER, L.; BERG, M.J.; TYMOCZKO, L.J. Bioquímica. Guanabara Koogan, 2002.

[6] Cinética enzimática. Disponible at <http://pt.wikipedia.org/wiki /Cin%C3%A9tica_enzim %C3%A1tica> January in 25. 2012.

[7] BOYER, R. Concepts in Biochemistry, 2 ed., New York, Chichester, Weinheim, Brisbane, Singapore, Toronto: John Wiley & Sons, 2002, p. 137–138.

[8] FERREIRA, J. P. M. Segredos da Catalise enzimática. Boletim de Biotecnologia.

[9] Universidade Católica Portuguesa, Porto. p. 22-29.

[10] DOMINGOS, J.B.; LONGHINOTTI, E.; MACHADO, V.G.; NOME, F. A química dos ésteres de fosfato. Química Nova, São Paulo, v. 26, n. 5, Set./Out. 2003.

[11] CARRIJO, R.M.C.; ROMERO, J.R. Oxidações catalíticas e eletrocatalíticas de substratos orgânicos. O cério como oxidante. Química Nova, v. 23, n. 3, 2000.

[12] SCHMIDT, H.L.; GUTBERLET, F.; SCHUHMANN, W.; Sens. Actuators B. p. 13-14, 366 p., 1993.

[13] OKAWA, Y.; NAGANO, M.; HIROTA, S.; KOBAYASHI, H.; OHNO, T.; WATANABE, M. Bios. Bioelect. v. 14, 229 p., 1999.

[14] SOTOMAYOR, M.D.P.T.; KUBOTA, L.T. Enzymeless biosensors: uma nova área para o desenvolvimento de sensores amperométricos. Química Nova, v. 25, n 1, p. 123-128, 2002.

[15] BERCHMANS, S.; VIJAYAVALLI, R. Surface Modification of Glassy Carbon by Riboflavin. Langmuir. n.11, p. 286-290, 1995.

[16] COIERA, E.W. Inteligência Artificial na Medicina Disponible at <http://dfm.ffclrp. usp.br/infobio/ceib /texto4.php> Accessed in January in 25. 2012

[17] GONGORA, A.D. O Que é Inteligência Artificial. Disponible at <http://www.pr.gov.br/batebyte/edicoes/ 2002/bb119/estagiario.htm> Accessed in January in 25. 2012.

[18] Uma introdução às Redes Neurais. Disponible at http://www.din.uem.br /ia/neurais/ Accessed in January in 25. 2012.

[19] Neural Networks. Disponible at <http://www.zsolutions.com /Neural.htm> Accessed in January in 25. 2012