ANFIS Based Low Cost Blood Serum Analyzer

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Abstract— A predictive method based on Adaptive neuro fuzzy inference system (ANFIS) has been developed to study absorbance and pH effects on the equilibrium of blood serum. This technique has been used to analyze serum samples and to predict the calcium concentration in blood serum. The data acquisition system is designed and fabricated using LabVIEW. ANFIS model developed is based on the data collected from calcium test. *ANFIS is used to simulate different concentration of calcium (Ca2+) as a function of pH and absorbance, to correlate and predict calcium concentration. The sensor modules were designed and calibrated to measure pH and absorbance. ANFIS network was trained and validated using test data to predict the actual calcium concentration of blood serum.*

Index Terms— ANFIS, blood serum analysis, calcium concentration, pH, Fuzzy, RMSE, Correlation Coeeficient.

1 Introduction

Adaptive neuro fuzzy inference system (ANFIS) is a promising area of research for prediction and forecasting in the field of instrumentation and measurement. ANFIS are biologically inspired computational methods, which can be used to capture complex and non-linear relationships between data. The ANFIS basically consist of several non-linear processing units, called neurons or nodes and they are connected in a massive parallel architecture [3]. Calcium is the most prevalent cation, which plays important role in skeletal mineralization, blood coagulation and neuromuscular conduction. Estimation of calcium concentration in blood serum plays a vital role in identification of many deficiencies such as risk of hypertension, arteriosclerosis, Alzheimer's, colon cancer and premenstrual syndrome. Serum calcium level gives indication about thyroid diseases and serves as indicator for vitamins A and D deficiency. There are many methods for the determination of calcium ions, which involve atomic absorption spectrometry, potentiometry with ion selective electrodes, EDTA titration and spectrophotometry with variety of reagents. Among these methods, spectrophotometry is well-suited because of its simplicity and cost effective.

The basic principle of the spectrophotometry is the transmittance measurement which relies on Beer's law. For spectrophotometric determination of calcium, arsenazo III, a metallochromic indicator used as selective ligand and it forms a colored complex with calcium. Arsenazo III has high sensitivity and selectivity for calcium ions, forms stable complexes over a wide pH range. A study is made to find the effect of magnesium ion in absorbance, relative to Ca^{2+} ion concentration [10].

When, Mg^{2+} ion concentration is less than or equal to Ca^{2+} ion concentration, no change in absorbance is observed. When, Mg^{2+} ion concentration is 5-fold or above relative to Ca^{2+} ion concentration, increase in absorbance is observed. Hence, lower concentration of Mg^{2+} ion concentration does not interfere with calcium determination. The instrumentation system has been developed based on LPC1768 microcontroller, to measure the absorbance, calculate the concentration and to store the test result for future reference.

2 Hardware Design and Description

2.1 System Hardware

As shown in the Figure 1 the system hardware consists of light intensity measurement unit with phototransistor as light sensor and pH sensor for measuring the pH value of the solution. The LED(650nm) is used as a light source. The instrumental setup includes the ANFIS predictor, pH As shown in the Figure 1 the system hardware consists of light intensity measurement unit with phototransistor as light sensor and pH sensor for measuring the pH value of the solution. The LED (650nm) is used as a light source. The instrumental setup includes the ANFIS predictor, pH sensor and light sensor.The sensors were interfaced with the ANFIS predictor through LabVIEW. Standard values of pH and absorbance were used for validating the ANFIS based calcium concentration predictor.

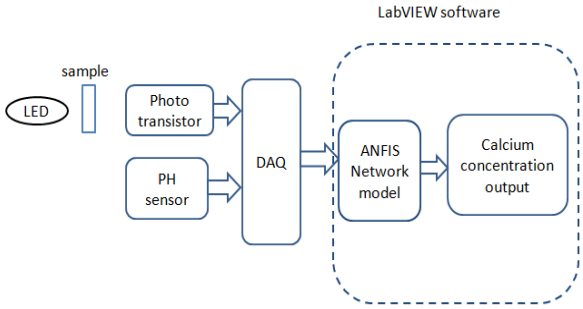

Fig 1: Block Diagram

2.2 LED (HLMP-Q106)

The subminiature solid state lamps utilize a highly optimized LED material technology, transparent substrate aluminum gallium arsenide (TS AlGaAs) is shown in Figure 2. This LED technology has a very high luminous efficiency, capable of producing high light output over a wide range of drive currents (500 µA to 50 mA). The color is deep red at a dominant wavelength of 644 nm deep red. TS AlGaAs is a flip-chip LED technology, die attached to the anode lead and wire bonded to the cathode lead. Available viewing angles are 75°, 35°, and 15°.

Fig 2: LED

2.3 Phototransistor

Phototransistors circuits may be adjusted for a selected sensitivity range and often do not require additional amplification. They can be applied in two modes, Active or Switch mode.

Fig 3: Phototransistor

Switch mode: means the phototransistor will either be "off" or "on" for a digital logic response to the object sensed.
Active mode: means the phototransistor generates an output response based on the light or irradiance level. IC (on) will be proportional to the coupled light intensity.

2.4 Light sensor

The circuit shown in Figure 4 is a common-collector amplifier with an output (V_{OUT}) increasing from low to high in response to light input. The phototransistor is used to detect the light intensity it is shown in the Figure 4. The output range varies from 0mv-1500mv.

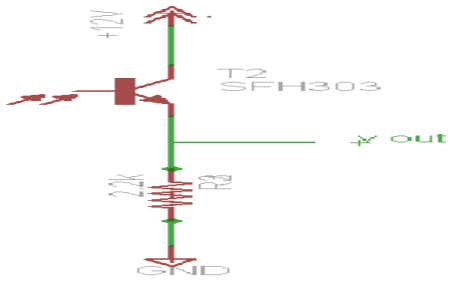

Fig 4: Circuit Diagram of Light Sensor

2.4.1 Transmittance, Absorbance, and the Beer-Lambert Law

As shown in the Figure 5 Beer-Lambert law defines transmittance as the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.

Fig 5: Absorbance of Sample

Absorbance is defined as the negative logarithm of the transmittance, and you will note that absorbance and transmittance bear an inverse relationship. Transmittance of light source is measured by Equation 1.
Transmittance,

$$= \frac{P}{P_0} = \frac{intensity\ of\ transmitted}{intensity\ of\ incident\ light} \qquad (1)$$

For the example of chlorophyll, if you have two colored solutions, you may deduce that the darker colored green solution appears darker because it absorbs more of the light falling on it [12]. Because the darker solution is also the more concentrated one, you can also say that the more concentrated one absorbs more of the light. That is, the absorbance increases as concentration increases.

$$Absorbance = -\log T = -\log P/Po \qquad (2)$$

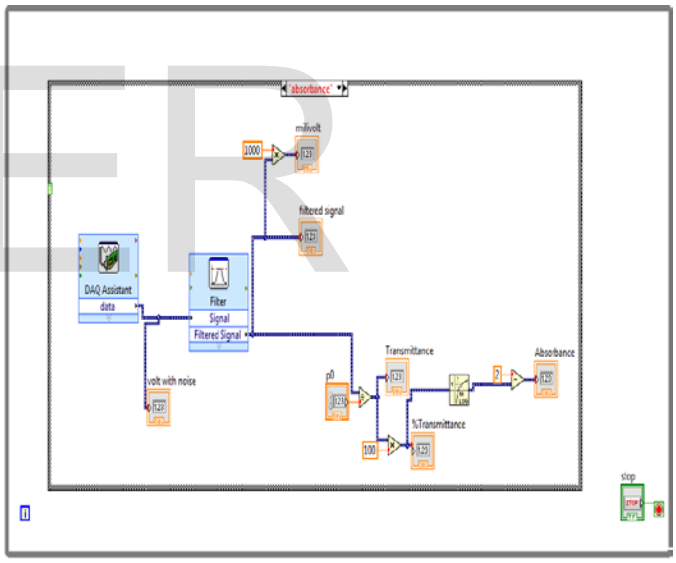

Fig 6: Block Diagram of Absorbance in LabVIEW

2.5 PH electrode

PH electrodes are electrochemical sensors used by many industries but are of particular importance to the water and wastewater industry. The sensor itself is similar to a battery. It generates a voltage output and has a useful service and shelf life.
OFFSET - Theoretically, when placed in 7.00 buffer at 25°C a pH electrode produces zero mill volts which the pH meter reads as 7.00 pH. The difference between these perfect readings and the electrode's actual reading is called the offset error.
SPAN - A perfect pH electrode, at 25°C produces 59.12mV per pH unit. The difference between this perfect reading and the electrode's actual reading is called the span error.

TYPICAL SPECIFICATION - Offset: 7.00 +/- 0.2 pH (+/- 12mV) SPAN: Better than 95% of theory; i.e. between 56.2 and 59.2 mV.

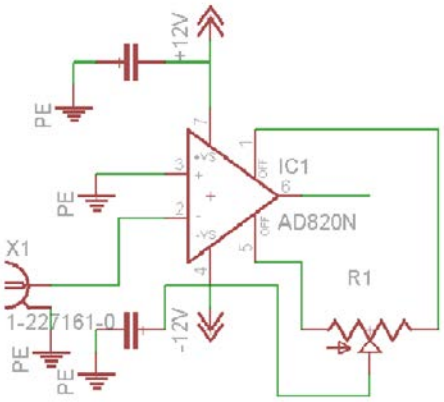

Fig 7: Circuit Diagram of Ph Sensor

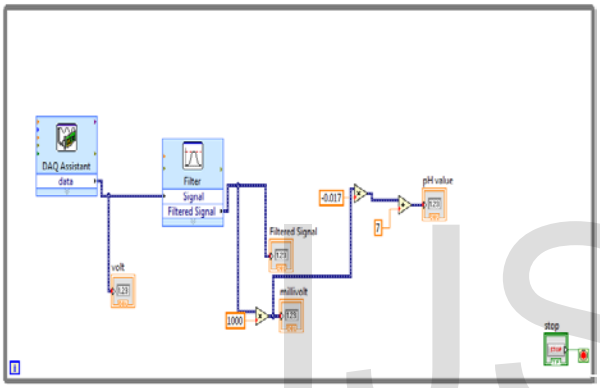

Fig 8: Block Diagram of pH measurement in LabVIEW

As shown in the Figure 7 attach a pH probe to a high-impedance input of an op amp and read the output with a digital voltmeter. Then, convert these readings to pH units using a calculator that can calculate the slope of a line. To calibrate the system, you can use pH standards. Generally, you would use three standards: 4-, 7-, and 10-pH units. These standards are inexpensive and available at any chemical-supply house. The calibration procedure is as follows:

1. Short the input leads together and adjusts the offset potentiometer such that the output reads 0 mV.
2. Place the pH probe in each standard and record the output (in millivolts) for each standard.
3. Enter the values in your calculator and determine the slope of the line. At approximately 24^{O}C, the equation for the slope of the line is Y=-0.017X+7. To obtain readings in tenths of volts, you multiply the equation by 10. The new equation is 10Y=-0.17X+70. The Figure 10 shows the graph representation for various pH standards of 4, 7, and 9.2 at different temperature. The output of pH sensor for standard buffer solution 4, 7, and 9.2pH.

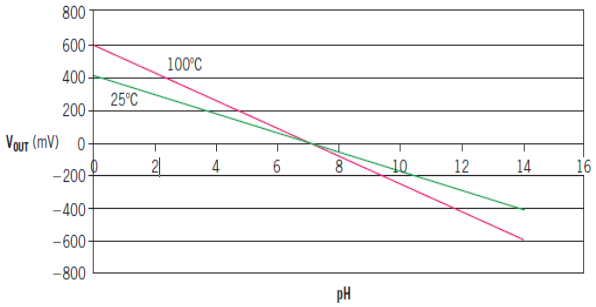

Fig 9: Graphical Representations for pH Value

3 Adaptive Neuro Fuzzy Inference System (ANFIS)

An adaptive network, as its name implies, is a network structure consisting of nodes and directional links through which the nodes are connected [1]. The ANFIS network is created for two inputs and one output which is shown in Figure 10.[9] Moreover, parts or all of the nodes are adaptive, which means each output of these nodes depends on the parameters pertaining to this node and the learning rule specifies how these parameters should be changed to minimize a prescribed error measure [7]. ANFIS is a multilayer feed-forward network where each node performs a particular function on incoming signals [3]. Both square and circle node symbols are used to represent different properties of adaptive learning. To perform desired input–output characteristics, adaptive learning parameters are updated based on gradient learning rules.
The acronym ANFIS derives its name from adaptive neuro-fuzzy inference system. Using a given input/output data set, the toolbox function ANFIS constructs a fuzzy inference system (FIS) whose membership function parameters are tuned (adjusted) using either a back-propagation algorithm alone or in combination with a least squares type of method. This adjustment allows your fuzzy systems to learn from the data they are modeling [6]. In the case of nonlinear mapping between the inputs and output, ANFIS shows better performance. ANFIS is used to map the inputs absorbance, pH and with the output calcium concentration [2]. It uses 14 samples for training and 10 samples for testing. Two membership functions are used for each input parameters and the membership function used was generalized bell-shaped (gbellmf) function. [4]The network was trained using 1000 epochs.

Layer 1 every node i in this layer is a square node with a node function Where x (or y) is the input to node i, and Ai (or Bi−2) is the linguistic label (small, large, etc.) associated with this node function, and where µAi (x) and µBi−2 (y) can adopt any fuzzy membership function.

Layer 2 every node *i* in this layer is a fixed node, marked by a circle node, labeled which multiplies the incoming signal sand outputs the product.

Layer 3 every node i in this layer is a fixed node, marked by a circle node, labeled N. The ith node calculates the ratio of the ith rule's firing strength to the sum of all rule's firing strengths.

Layer 4 every node i in this layer is a fixed node,

marked by a circle node, labeled N. The ith node calculates the ratio of the ith rule's firing strength to the sum of all rule's firing strengths: In this layer, the nodes are adaptive nodes. The output of each node in this layer is simply the product of the normalized firing strength and a first-order polynomial for a first-order Sugeno model.

Layer 5 the single node in this layer is a fixed node labeled, which computes the overall output as the summation of all incoming signals

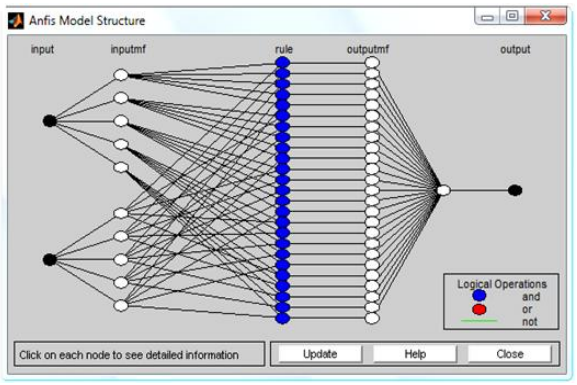

Fig 10: ANFIS Mode

Table 1: Data Samples Trained and Tested By ANFIS

S.No	pH	Absorbance	Concentration of calcium(mg/dl)
1	7.05	0.75	8.5
2	7.42	0.62	6.9001
3	7.10	0.75	7.3999
4	7.04	0.84	7.2
5	7.14	0.74	7.2701
6	7.18	0.70	7.61
7	7.2	0.75	8.5
8	7.42	0.60	6.9
9	7.48	0.64	7.0199
10	7.17	0.61	6.8
11	7.26	0.79	6.95
12	6.93	0.66	7.5
13	7.05	0.94	8.5
14	7.03	0.75	9.1597
15	7.10	0.75	7.3999
16	7.04	0.83	7.2923
17	7.14	0.75	7.458
18	7.09	0.70	7.1294
19	7.30	0.62	6.8541
20	7.42	0.62	6.9001
21	7.48	0.66	7.4364
22	6.64	0.69	8.5549
23	7.07	0.81	9.8886
24	7.13	0.66	6.9394

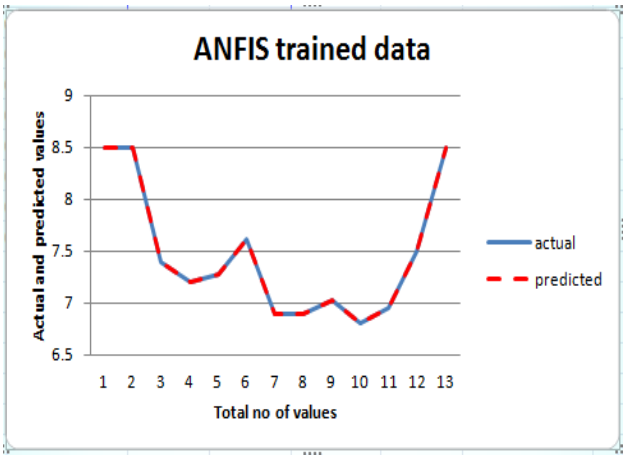

Fig 11: Graphical representation of ANFIS trained data

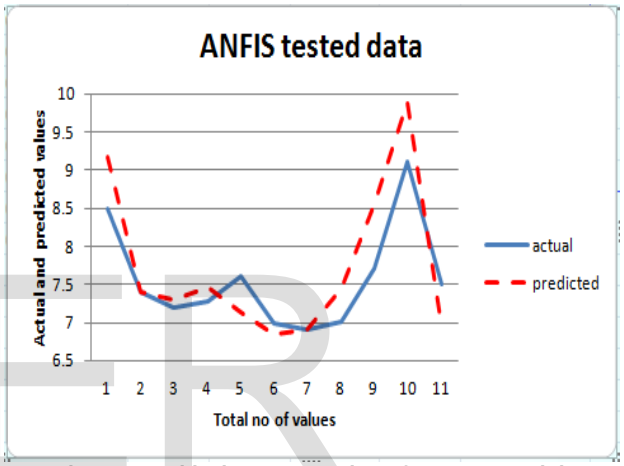

Fig 12: Graphical representation of ANFIS tested data

4 RESULT

This work has dealt with the analysis of effects of pH and absorbance on the complex formation of calcium in a human blood serum. The ANFIS is used as a method to simulate and predict the behavior of calcium as a function of pH and absorbance variations. A popular and well known MLP with back propagation algorithm is used to train ANFIS to model this complex system. The LabVIEW based data acquisition system is developed for calcium determination in serum samples. The proposed instrument with a LED source is very attractive, cost effective, simple and user friendly. The developed design is enough sensitive for use in the spectrophotometric determination of calcium using arsenazo III as a complexation reagent.

4.1 Performane Testing of Anfis Models

4.1.1 Root Mean Square Error (RMSE)

The Root Mean Square Error (RMSE) (also called the root mean square deviation, RMSD) is a frequently used measure of the difference between values predicted by a model and the values actually observed from the environment that is being modeled. These individual differences are also called residuals, and the RMSE serves to aggregate them into a single measure of predic-

tive power which is measured by Equation 3.

$$RMSE = \frac{1}{n}\sqrt{\sum_{i=1}^{n}\left(o_p - o_d\right)^2} \qquad (3)$$

Where n is the number of data, o_p is the predicted value and o_d is the real operating value.

4.1.2 Correlation Coefficient (R)

Correlation often measured as a correlation coefficient which is measured by formula in Equation 4. It indicates the strength and direction of a linear relationship between two variables (for example model output and observed values). A number of different coefficients are used for different situations. The best known is the Pearson product-moment correlation coefficient (also called Pearson correlation coefficient or the sample correlation coefficient), which is obtained by dividing the covariance of the two variables by the product of their standard deviations. If a series of n observations and n model values, then the Pearson product-moment correlation coefficient can be used to estimate the correlation between model and observations.

$$R = \frac{\sum_{i=1}^{n}(x_i - \bar{x}).(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2.\sum_{i=1}^{n}(y_i - \bar{y})^2}} \qquad (4)$$

A series of n measurements are written in terms of x_i and y_i where $\bar{x}$ and $\bar{y}$ are the sample means. The above mentioned performance metrics were tabulated in Table 2.

Table 2: ANFIS Performance

PERFORMANCE TESTING	ANFIS TESTING DATA
ROOT MEAN SQUARE ERROR (RMSE)	0.483011
CORRELATION COEFFICIENT (R)	0.918157

5 Conclusion

This work has dealt with the analysis of effects of pH and absorbance on the complex formation of calcium in a human blood serum. The ANFIS is used as a method to simulate and predict the behavior of calcium as a function of pH and absorbance variations. A popular and well known MLP with back propagation algorithm is used to train ANFIS to model this complex system. The LabVIEW based data acquisition system is developed for calcium determination in serum samples. The developed instrument with a LED source is very attractive, cost effective, simple and user friendly. The developed design is enough sensitive for use in the spectrophotometric determination of calcium using arsenazo III as a complexation reagent. With the availability of user-friendly software, optimum selection of ANFIS structure and parameters, it is possible to design a network depending upon the field requirement. For this study, two neurons in the input layer, one neuron in the output layer and five neurons in the hidden layer are chosen to reduce the RMS error. This network is scaled and properly trained to predict the unknown calcium concentration. In validation and testing stages, the correlation coefficients are 0.918157.

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