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Android integrated urea biosensor for public health awareness

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ABSTRACT

Integration of a biosensor with a wireless network on the Android 4.2.1 (Jelly Bean) platform has been demonstrated. The present study reports an android integrated user friendly Flow injection analysis-Enzyme thermistor (FIA-ET) urea biosensor system. This android-integrated biosensor system will facilitate enhanced consumer health and awareness alongside abridging the gap between the food testing laboratory and the concerned higher authorities. Data received from a flow injection mode urea biosensor has been exploited as an integration point among the analyst, the food consumer and the responsible higher authorities. Using the urea biosensor as an example, an alarm system has also been demonstrated both graphically and through text message on a mobile handset. The presented sensor integrated android system will also facilitate decision making support system in various fields of food quality monitoring and clinical analysis.

1. Introduction

Milk is consumed by people of all age groups. Thus, there is a need to monitor levels of urea adulteration in milk which are regarded as harmful for human health. Naturally occurring urea concentrations in milk are reported in the range 18–40 mg/dL, whereas the permissible level for urea in milk is 70 mg/dL as per the Food Safety Standards Authority of India [1]. Large amount of milk is processed daily in dairy industries. Milk received at the milk collection centers is sent for adulterant analysis. Mostly the sample analysis is done offline and also the source of adulteration is not known. There is a lack of continuous monitoring and in time feedback system which usually results in huge economic losses to industry as well as it poses health hazards to the society. In addition, recent incidents on milk adulteration reported in Indian states by FSSAI also emphasized the need for regular adulteration detection in milk. According to the survey on milk adulteration in 2011–13 around 65–70% of milk samples are adulterated with urea, detergents, water, and skimmed milk sample. In the tested samples about 17% milk samples were of packaged milk from dairy [2]. There is an urgent need for a decision support system wherein the sample quality can be immediately communicated back to the dairy plant for appropriate action. Making use of the android technology, we have developed an android integrated urea biosensor that will facilitate a decision support system in the milk supply chain. FIA-ET technology has been demonstrated for analysis of various analytes in food constituents such as, fructose [3], choline [4], glucose [5], and cholesterol [6]. Recently, FIA-ET has also been successfully demonstrated for urea detection in adulterated milk, environmental and clinical monitoring [7–10]. Thus this technology was selected for integration with android mobile technology. Traditional techniques for urea analysis are usually offline, wherein analyzed data is not shared directly with the user. With the recent advancement of information science and technology, it is possible to share the data directly with users by incorporating mobile computing tools. The availability of open source smartphones such as androids addresses such constraints [11]. In this work we have used an android mobile platform because of its availability as an open source. The open source code and permissive licensing allow the software to be freely modified and distributed by device manufacturers, wireless carriers and enthusiast developers [12]. In brief, the biosensor integration was achieved in two steps. At first, milk samples were tested for urea adulteration on FIA-ET biosensor and data generated was stored on a computer. Secondly, the analyzed data was received by means of a wireless network protocol as an application on the android handset used by the analyst. The android application is programmed in such a way that data seen by the application user (analyst) through graphical user interface (GUI) on the handset screen is in the chart format. The analyst has an active session with the sensor (ca. urea biosensor) and observes a stream of sample response signal in real-time. Another

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important requirement in food processing/milk supply chain/testing laboratories is integration of on-site sample analysis with the regulatory agencies and consumer. This requirement is addressed by incorporating an alarm system within the developed android application system to check variation of adulteration above threshold values. Hence any communication gap can be bridged between the analyst, user industry and consumer with the presented prototype. This prototype system will help creating enhanced consumer awareness and increases the level of surveillance by the responsible higher authorities as well as it helps process industry to identify the problem at the point of collection of milk.

2. Experimental

2.1. Chemicals and biochemicals

Enzyme urease (Jack beans lyophilized 5 IU/mg, EC 3.5.1.5), urea, sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$), disodium hydrogen phosphate monohydrate ($Na_2HPO_4.H_2O$), glutaraldehyde solution 25%, and tri-ethanol amine were obtained from Merck, Germany. Amino silanized control pore glass (CPG) spherical beads (Trisoperl, size 125–140 µm, pore size 50 nm) were purchased from VitraBio (Germany).

2.2. Instrumentation

The experimental setup consists of a peristaltic pump (Gilson Minipuls Evolution II, France), an injection valve (Rheodyne type-50, Cotati, USA), sample loop (0.1 mL), enzyme thermistor, Wheatstone bridge equipped with a chopper-stabilized amplifier and an android Smartphone. Other details of FIA-ET biosensor i.e. preparation of immobilized column, optimization of the FIA system are described elsewhere [8].

3. Result and discussions

3.1. Calibration of the FIA-ET biosensor for urea

For urea analysis, calibration standards (1–300 mM) were prepared by dilution of urea stock solution (300 mM) in 100 mM PB, pH 7.2. The immobilized urease column was mounted inside ET and allowed to equilibrate with the surrounding. The degassed carrier buffer was passed at an optimum flow rate of 0.5 mL/min at which a stable baseline was achieved. The spiked milk samples were injected and data were recorded in real time using a data acquisition system connected to the Wheatstone bridge. All measurements were carried out by injection of 0.1 mL sample at a flow rate of 0.5 mL/min. After each sample analysis, the sample loop was thoroughly rinsed with buffer (PB). The milk samples were spiked with known amounts of urea after matrix matching. Appropriate dilutions were injected into the FIA-ET system. Milk sample spiked with urea standard solutions (10–300 mM) were injected into the flow stream and response signal was recorded. Original response recorded from the FIA-ET biosensor is presented as Fig. 1. The calibration of the FIA-ET biosensor showed an excellent dynamic range for urea present in spiked milk (10–300 mM) with a good linearity. The minimum detection limit was found to be 10 mM. The Michaelis constant, K_M for urea was calculated using Line weaver–Burk plot and found to be 248.9 mM. The result is presented as Fig. 2 (R^2 = 0.994, % RSD = 0.007, n = 3).

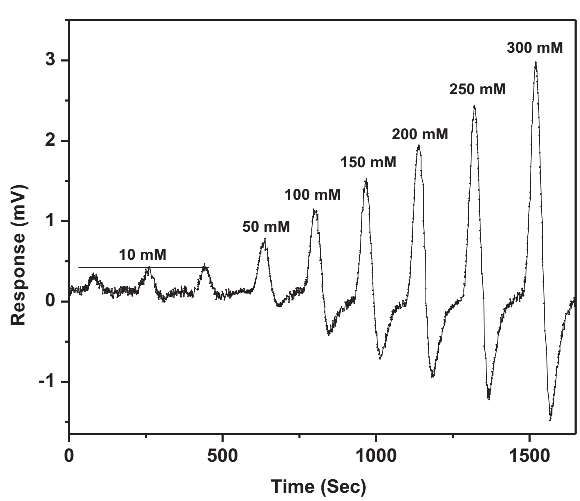

Fig. 1. Original response recorded from the FIA-ET biosensor for urea spiked (10–300 mM) milk samples in 100 mM PB, pH 7.2 for 0.1 mL of sample injection, flow rate 0.5 mL/min at 30 °C.

3.2. Reproducibility of the FIA-ET biosensor for milk urea analysis

Reproducibility of the FIA-ET biosensor was determined by measuring the known concentrations of urea at different time interval. Four concentrations of urea (10, 50, 100 and 200 mM) were selected from the calibration range and the response of urea was recorded 3 times in a day. The precision and reproducibility of the method was evaluated in terms of % RSD As observed from the Table 1, very good reproducibility was obtained for urea concentration with % RSD 0.50–1.42 (n = 3) for intraday analysis. The obtained results confirm the reproducibility of the sensor response.

3.3. Stability of the FIA-ET biosensor

Stability of the FIA-ET biosensor was monitored over the period of 200 days. About 15 measurements were made every week for 0.1 mL, 100 mM urea. The operational stability is over the studied

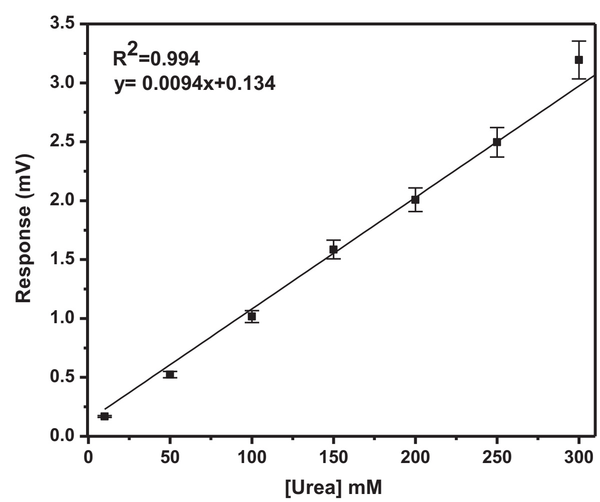

Fig. 2. Calibration plot obtained for urea spiked milk samples using FIA-ET biosensor in 100 mM PB, pH 7.2 for 0.1 mL of sample injection, flow rate 0.5 mL/min at 30 °C.

Table 1
Intraday reproducibility of the FIA-ET biosensor for urea analysis in milk.

Urea (mM)	Intra-day mean (mV) n = 3	Mean ± S.D.	% RSD
10	0.1407	0.14 ± 0.002	1.42
50	0.5963	0.59 ± 0.005	0.85
100	1.1267	1.12 ± 0.015	1.34
200	2.0100	2.01 ± 0.010	0.50

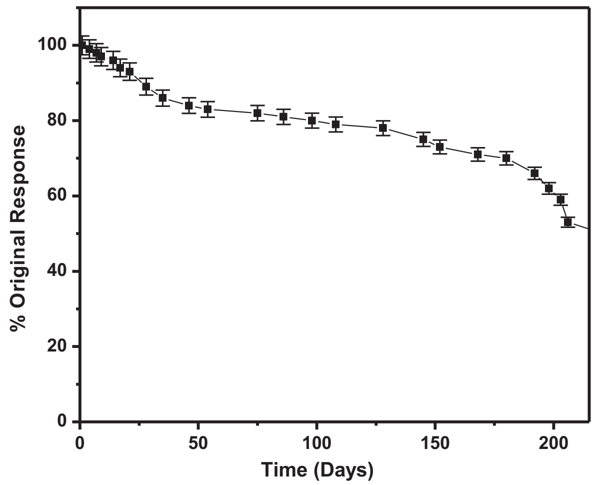

Fig. 3. Operational stability of FIA-ET milk urea biosensor, response to 0.1 mL, 100 mM urea, observed for 200 days at 30 °C. Samples were injected randomly during the period.

period is presented in Fig. 3. The immobilized urease inside the thermistor presented excellent stability, over the first 30 days the enzyme activity was found 90% of the original response and there was no appreciable loss of activity. However, after a period of 45 days operation, about 16% decay in response was observed. Similarly the response was monitored after 75, 150, 180, 198 and 200 days and signals recorded were respectively 82%, 73%, 70%, 62% and 53% of the original response.

4. Integration of the FIA-ET biosensor with android platform

4.1. Architecture of the proposed system

The sample response data from the sensor is stored on a laptop computer. This data is imported on the server wherein a database of each tested sample is maintained. The data is then sent to an android mobile handset. Our application is pre-installed on the mobile which displays data in chart format. The pictorial representation of the biosensor integrated android system is shown as Fig. 4.

The AChartEngine library for androids was used to develop the application. The AChartEngine is a charting library that can be used in android applications with open source software. The developed system can be deployed in various situations such as:

i. Milk collection centers
In milk collection centers milk from different vendors is collected. Even one bad sample of milk can contaminate other milk collected. Hence it is necessary that milk collected from each vendor is checked for contamination and only after checking it should be used for packaging.

ii. Agricultural fields for soil urea content
Farmers use fertilizers in their paddy fields. They are used for protection of fields from pest and for good growth, but the rate of fertilizer treatment usually tends to be neglected. Excessive use of fertilizers can increase the urea content in soil. Cattle feed on such paddy fields. Consumption of high concentrations urea affects the milk quality. This milk may eventually be consumed by human beings.

iii. Clinical analysis for urea in urine
If milk containing higher urea content than the permissible level is consumed by human beings it can be detected by clinical analysis of urea in urine samples. High concentration of urea in the human body can affect the kidneys. Hence it is important to address the problem of urea adulteration in milk.

4.2. The circumstance of system usage

In this section three potential applications of the presented system are discussed taking urea as an example. Fig. 5a represents the logical role of the alarm system in communicating data to milk collection center, consumer or higher authorities.

i. *Detection of urea adulterated milk at milk collection centers*
Milk collection centers receive milk from different suppliers. Hence such milk has to be checked for quality before processing in dairy plants. Urea being relatively cheap and rich in nitrogen is an economical choice to adulterate the milk. Presence of exceeding levels above cut-off limit for urea causes severe damage to the human health. If urea adulterated milk is mixed with other milk which may be of good quality it will in turn contaminate other milk and that can render entire milk unfit for consumption. Therefore a quick, economical and efficient system is needed to monitor milk urea levels before further processing of the milk. With the

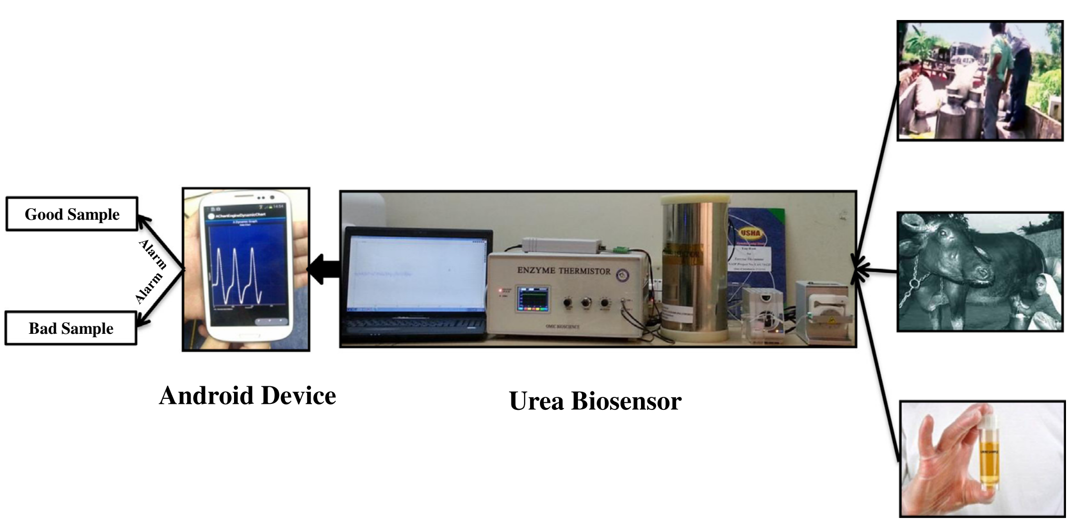

Fig. 4. Pictorial representation of android integrated urea biosensor system.

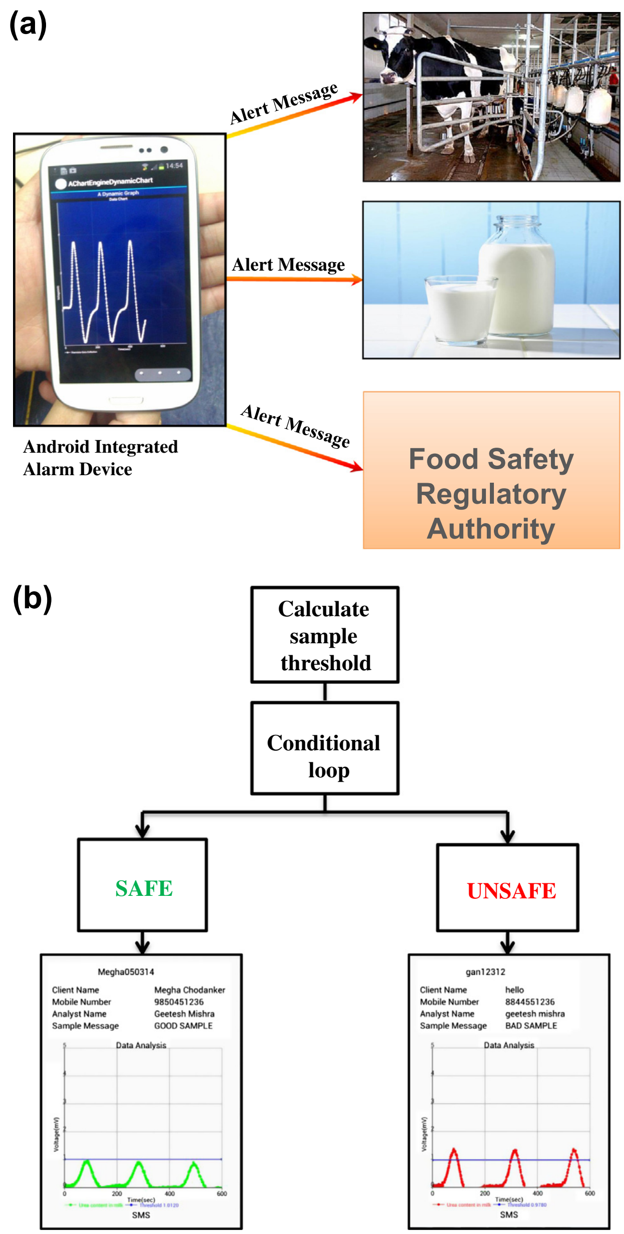

Fig. 5. (a) Logical representation of decision making alarm system with sensor data on graph. (b) Schematic representations of three ideal scenarios for urea analysis in milk.

presented prototype system the analyst can quickly monitor milk samples and thus bridge the gap between quality check and production.

ii. *Milk quality check for consumer at individual level*
Milk contains many essential nutrients, which makes it very important for energy provision, growth, and development of the human body. Since milk is consumed by people of all age groups on daily basis, it is important to keep check on its quality. If consumers (such as a small community or individuals) desire a quality assurance prior to consumption of the milk, this need is addressed by proposed system wherein the individual user can receive a text message report on his registered mobile number of the sample being good or bad.

iii. *Data analysis of the records collected for keeping track on adulteration rate*
Milk adulteration with synthetic chemicals like urea is a serious concern for human health and needs to be brought into the eyes of higher authorities. Continuous monitoring of milk over a period of time will show the rate at which adulteration is in practise. Hence by gathering data directly from the analyst the number of bad samples for which alarm is triggered can be obtained periodically or instantly. Since data collected over the years will keep increasing in quantity. An effective technique for data analysis is needed for efficient data analysis. In order to handle such huge data, data mining of records for further analysis can be done. The overall goal of the data mining process is to extract information from a data set and transform it into an understandable structure for further use. This highly processed data is then used for data analysis for better decision making. Hence by observing different data patterns milk adulteration can be kept under control by the authorities and minimized.

Even if the FIA-ET biosensor urea analysis in adulterated milk usually is done in offline mode, the results can be generated after the analysis is complete. Integration of the android-based decision making support system facilitates the real time data analysis through its alarm system. In the presented system, the android application is pre-built such that the permissible limits for urea by responsible higher authorities are marked on the graph. Data received on the handset from the server is passed through a conditional loop in the android application. Threshold value of the alarm system is set from the actual base line. If baseline is at zero and the concentration of urea is below 70 mg/dL (e.g. below 0.9580 mV as per the Time/Voltage graph used in our application), the sample is declared to be safe. If the urea concentration crosses threshold value, the built in alarm is activated in the form of a notification marked in red to the analyst/user indicating that the sample is bad. This threshold based alarm system is shown as Fig. 5b. If sample is within limit it is marked in green color. When the sample analysis is complete an SMS is sent on the registered mobile of that particular sample. This way a registered user need not to wait till reports are received. After receiving the SMS unsafe milk can immediately be discarded or used for other purposes than consumption. The analysis report can also be sent to the dairy industry or concerned authority.

4.3. Implementation results

In this section a system prototype has been implemented according to the design of the system presented in Fig. 4. An android handset with Android 4.2.1 (Jelly Bean) version was used to develop the android application. Data collected from biosensor is stored as excel file on a laptop computer with the help of Picolab's data logger (Picolab, U.K.). This excel file is then imported on server. The android application communicates with the server with the help of a network protocol HTTP transport library.

4.3.1. HTTP transport library

The function of HttpClient is to execute HTTP methods. Execution of an HTTP method involves one or several HTTP request/HTTP response exchanges, usually handled internally by HttpClient. The user is expected to provide a request object to execute and HttpClient is expected to transmit the request to the target server by returning a corresponding response object, or throw an exception if execution was unsuccessful. Data received from the server by HTTP request is displayed in the form of a graph on the android application.

4.3.2. AChartEngine library

We have used AChartEngine library to display the sensor values in line chart format. Threshold value for permissible urea content

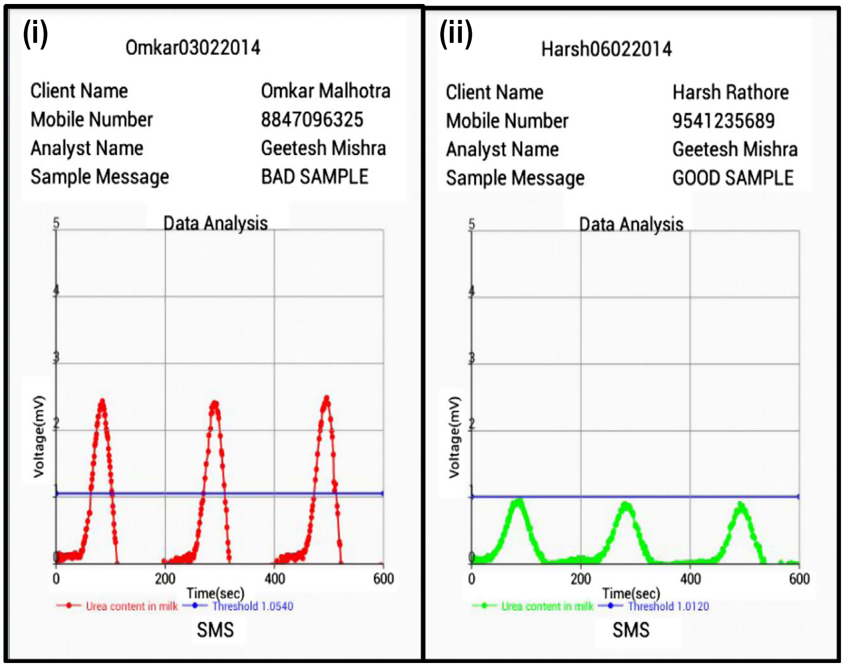

Fig. 6. Pictorial representation of (i) high concentration and (ii) low concentration of urea analyzed through android integrated FIA-ET urea biosensor.

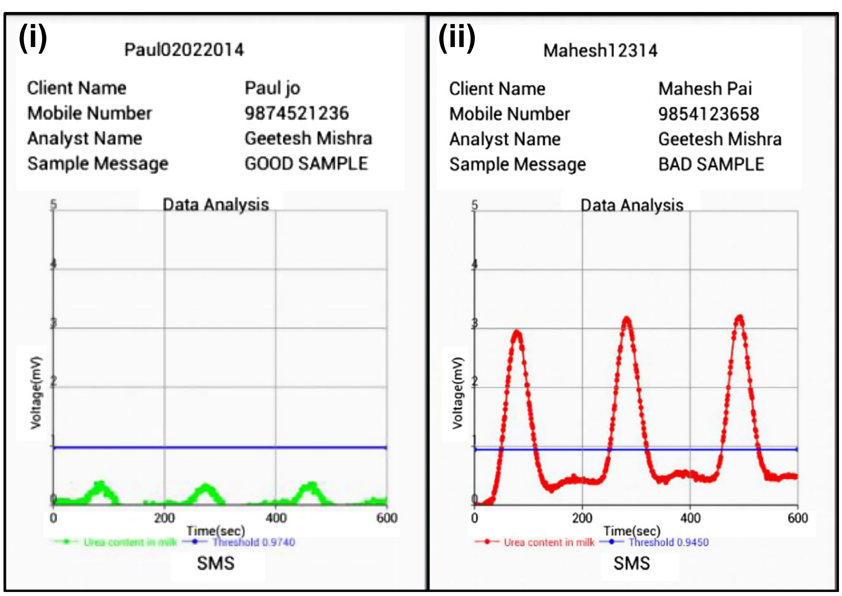

Fig. 7. Pictorial data of real sample analysis for (i) blank milk and (ii) spiked milk urea concentration.

in milk is marked on chart. If sample values cross threshold, chart signal will turn red. If the signal exceeds the threshold limit twice, it is termed as bad sample. This will enable the analyst sitting remotely or in the vicinity of the system to share or notify the sample result to either the milk supplier or consumer. The mode of communication to the analyst is through the chart and alarm whereas to the sample supplier it is through text message.

5. Facilitation of decision support system

Decision support system is provided for data analysis. Android application is pre-programmed for decision making. Where necessary, computations are made with the help of algorithms to facilitate decision making. The module is designed to give the best visualization effect to the analyst for better result analysis. The flow of the decision making is presented in Fig. 6 (i) that shows data results of an analyzed sample with high urea concentration. This is indicated by red signal and can be clearly figured out as a signal exceeding the threshold value and a message is sent to the person concerned stating that the sample is unsafe for consumption. The presented system has also been provided with a feature by which a higher authority can acquire direct access to the analysis database to identify the sources of bad samples for which alarm is triggered. Moreover, the database will also help processing plant authorities to identify channels supplying bad quality milk to the center. Fig. 6 (ii) represents the sample within the threshold limit hence message sent states that sample is safe and within permissible limit.

5.1. Real sample analysis

To check the practical applicability of the developed alarm system real milk samples were analyzed by the FIA-ET urea biosensor. Milk samples were spiked with known high concentration of urea. The milk samples were analyzed for presence of urea against a non-spiked milk sample, taken as blank. Milk samples with spiked urea concentration over the threshold limit activated the inbuilt alarm system and a text message was sent to the registered mobile number. Pictorial data for real spiked sample analysis and blank is shown as Fig. 7(i) and (ii) respectively.

5.2. Application end-users

The presented application is designed keeping into consideration two end users:

1) Application analyst
2) Consumer

The android application designed for the analyst consists of two functionalities:

a) Online sample
This involves the current sample analyzed. The details of the sample are noted in the information form by the analyst. The application is then redirected to the next view wherein details of sample feed along with the graphical representation of graph is displayed. Whether the sample analyzed is good or bad is displayed by the software in this view.
b) Offline sample
The database of all the samples analyzed is maintained in this block. The analyst can get the information about quality of earlier analyzed samples. Hence data history is maintained in this module. This data is also displayed in graph format for easy visualization and faster analysis.

5.3. Android application developed for consumer

The consumer will get result of the analyzed data in the form of text SMS on their registered mobile number thus getting instant and quick reply for the sample.

6. Conclusion

In this work, a novel low cost technology for FIA-ET biosensor integration with android mobile platform is presented. The remote access of visual interpretation has been demonstrated at the analyst level, which can be further extended to other user levels. In addition to this, we have also demonstrated an alarm system both graphically and through text message on an android mobile handset. The presented sensor integrated android system will facilitate enhanced quality assurance, decision making support system in various fields of food quality monitoring. Hence communication gap is bridged between the analyst, user industry and consumer with the presented prototype.

Conflict of interest

The authors declare that there is no conflict of interest.

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