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Quantification of Urea in Milk- A review of Existing Methods

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Abstract— The nutritional value of milk to human health needs no introduction. But it is alarming that many unscrupulous vendors are adulterating it with water, detergents, caustic soda, starch, formalin, urea, ammonium sulphate, sodium carbonate which have harmful effect on the human health. The greed for money has pushed them to the extent of producing synthetic milk which has no nutritional content. Many analytical techniques have been developed to qualitatively and quantitatively measure these adulterations. In this work we focus on the quantification of urea which is one amongst the many adulterants and provide an exhaustive literature survey of the available techniques for doing the same.
Milk being highly perishable product, it is desirable to test it at the earliest opportunity rather than taking it to laboratory for analysis. With this mindset we propose a new technique to quantify the amount of urea in milk.

Keywords—urea detection, biosensor, adulteration in milk, spectroscopic method, chemical method, piezoelectric crystal method, chromatographic method.

INTRODUCTION

Milk is an important food component in our regular diet. The milk that contains high solid non-fat is of good quality and valuable to the customers for its dietary value and flavor and also to the manufactures for the production of milk products, particularly relating to cheese yields [1].Other than fat, all solids present in milk are there in solids non-fats. Protein is the most essential component of milk because of its functional properties and nutritional value. The components like lactose, fat also add to the quality of milk and give a certain characteristic to milk products which are to a lesser degree. Table 1 gives a typical composition of the whole raw milk.

Table 1: average composition of whole raw milk

Components	**Quantity**
Protein	3.5%
Lactose	4.9%
Fat	3.5%
Minerals	0.7%
Water	87.4%
Total	**100%**

Milk provides calcium and is recommended to people of all age groups for regular intake. Hence adulteration becomes common due to the demand for milk. Urea in cow's milk varies from 20mg to 70mg/100mg of urea and urea content above this range is said to be adulterated or deliberately added. These unhygienic materials are mainly added to compensate the expenses of transportation, processing, storage and so on and hence to yield higher profit by cheap and low quality adulterants in order to increase the solid non-fat in milk. It is a common practice in many developing countries. The consumption of these adulterants causes vomiting, gastritis, nausea and even poisoning [2].

Traditional techniques based on chemical and quantitative methods were used to determine the presence of urea. Many urea biosensors have been developed based on several methods such as potentiometric, conductometric and amperometric transducers using electrochemical detection [3].

EXISTING DETECTION AND MEASUREMENTS METHODS

There are various quantitative and qualitative methods that have been developed for the detection and measurement of urea in milk. Some of them are:

1) Chemical method:

(a) Qualitative estimation- Urea is one of the natural constituent of milk and is present to an extent of 70 mg per 100 ml (700 ppm). Trichloroacetic acid (TCA) is added to precipitate the proteins in the milk. For the estimation of urea in milk, a test based on the use of Para-dimethylaminobenzaldehyde (DMAB) is performed [4].This method is based on the principle that urea forms a yellow complex with DMAB in a low acidic solution at room temperature. It is a preliminary test. There is no calibration required and hence it's the simplest of all. This method can be used for detection of urea but quantification is not achieved. Also, other drawbacks being wastage of chemicals and possibility of human contact with harmful reactants.

(b) Quantitative estimation- Pipette 5ml of aliquots of working standard solutions into 20x150ml (25mm) test tubes and add 5ml DMAB solution to each. Prepare a reagent blank of 5ml buffer and 5ml DMAB solution. Shake the tubes thoroughly and let it stand for 10 minutes. 10ml of milk sample is mixed with 10ml of TCA (Trichloroacetic acid) to precipitate the proteins and filtered using Whatman 42 filter paper. 5ml of this filtrate is then treated with 5ml of DMAB reagent to develop the color. Reagent blank is prepared by taking 5ml of diluting reagent and treating with 5ml of DMAB reagent. The optical density of the yellow color is measured at 420nm. From the standard curve the amount of urea in milk is calculated [4].This method is the most economical method adopted by many industries in India. However, exact amount of urea content cannot be quantified.

2) IR Spectrophotometer method:

The carbon dioxide evolved during the urea hydrolysis by urease can be quantized in milk by measuring the amount of C02 through optical method. This method works on the principal that carbon dioxide absorbs the infrared at a characteristic wavelength of 4260nm.The level of absorption is directly proportional to the quantity of urea present in milk. The contents of urea present in milk can be estimated using this method. Figure 1 shows the block diagram of a spectroscopic method.

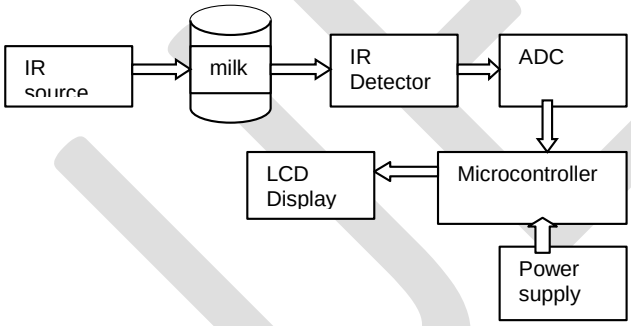

Figure 1: Block diagram of spectroscopy method

This method proves to be very promising and is used widely. It does not use any fragile material such as glass etc. The work proposed is very accurate but not cost effective. Also, Commercial range available is only up to 920 nm.

3) UV method:

This is a rapid, simple and enzymatic specific method that is used for the simultaneous determination of urea and ammonia (ammonium ions) in foodstuffs such as wine, fruit juice, bakery products, dairy products, egg products, meat and seafood, as well as in paper, fertilizers, pharmaceuticals, cosmetics, water and biological samples. This method is used to find adulterants as mentioned above by measuring the intensity of light in a part of the spectrum, especially transmitted or emitted by a particular spectrum. The working principle of this method is based on the following equation

$$\text{Urea} + H_20 \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

$$\text{2-Oxoglutarate} + \text{NADPH} + NH4^{+} \xrightarrow{\text{GIDH}} \text{L-Glutamate} + NADP^{+} + H_2O$$

The amount of NADP+ formed through the combined action of urease and glutamate dehydrogenase (GlDH), measured at 340 nm, is stoichiometric with the amount of urea and ammonia in sample volume.

This method involves determining the absorbance difference for both blank and sample (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{Ammonia}$. Determine the absorbance difference for both blank and sample (A2-A3). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining ΔA_{Urea} [5].

The concentration of ammonia (g/L) and urea (g/L), based on the ε of NADH at 340 nm (6300 L×mol-1×cm-1), are calculated as follows:

If the sample has been diluted or a different sample volume was used during the reaction, then the result must be multiplied by the corresponding dilution/concentration factor.

C (ammonia) = 0.07082x ΔAAmmonia [g/L]

C (urea) = 0.1258x ΔAUrea [g/L]

UV method is mainly used in industries for applications that require high accuracy. The reagents used in the simultaneous determination of urea and ammonia are not hazardous materials. However, the general safety measures that apply to all chemical substances should be followed. Also, The equipment is bulky, expensive, and excitation energy is required.

4) Teflon temperature sensing method:

RTD that is coated with Teflon is dipped into milk containing ammonia. The property of Teflon is such that it attracts ammonia towards it [6]. This increases the temperature and RTD is used to convert this change in temperature into an equivalent resistance change that is used in a bridge circuit. The unbalance in the bridge circuit is proportional to the level of ammonia present in the milk. The drawback in this method is that output signal needs to be amplified as it is very low. Figure 2 gives the general block diagram of Teflon temperature sensing method.

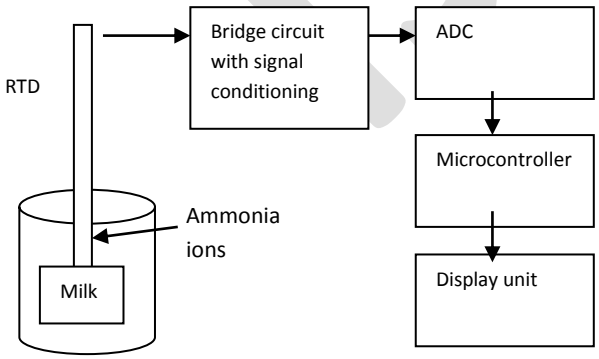

Figure 2: Block diagram for Teflon temperature sensing method

5) Piezoelectric Crystal Method:

Urea (CH_4N_2O), on hydrolysis in the presence of Urease gives ammonia and carbon dioxide. The two products of the reaction in turn exert a certain pressure on the piezoelectric sensor which converts the mechanical pressure into electrical signal. The signal is further amplified and programmed to give the quantity of ammonia present by using a calibration method [7].Figure 3, shows the block diagram of a piezoelectric crystal method.

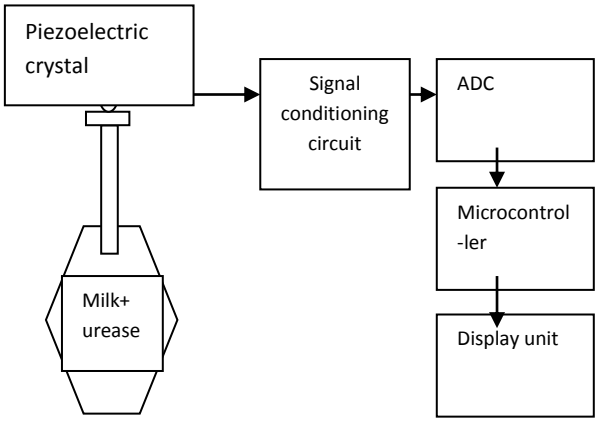

Figure 3: Block diagram of piezoelectric crystal method

The drawbacks of this method are that the output of the sensor is too low and that the crystal is prone to cracking.

6) Chromatographic method:

It is a method based on the liquid chromatography isotope dilution mass spectrometry (LC-IDMS) used to determine urea in milk. It is an indicator to check the diet status of lactating animals. The sequential addition of acetonitride and chloroform to milk precipitates proteins and separates them directly with the help of normal phase liquid chromatography where the derivatizing chemicals are not used. Milk should be treated twice the same way as mentioned above. To achieve accuracy, linearity and a very little uncertainty in the determination of milk urea, exact matching IDMS can be used[7].

PROPOSED METHOD

In recent years, various biosensors have been developed for food industries, environmental monitoring and clinical applications. The demand and interest in the development of portable devices for the detection, measurement and quantification of a specific analyte has led to the emergence of biosensors. A biosensor is a device which consists of a biological component or a sensing element for detection and a transducer for conversion of the biochemical signals to quantified measurable signal. Figure 4, shows schematic of a biosensor.

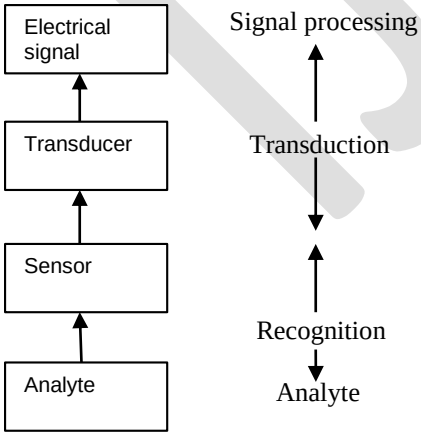

Figure 4: Schematic diagram of a biosensor

The objective of this work is to propose a new technique based on biosensor for the measurement of urea concentration in milk. This work requires hardware and software systems to quantify the urea concentration. The following reaction takes place when acetic acid is added to milk containing adulterated with urea-

$$\underset{\text{Urea}}{CH_4N_2O} + \underset{\text{water}}{H_2O} \xrightarrow{\text{urease}} \underset{\text{Carbamic acid}}{CH_3CONH_2} + \underset{\text{Ammonia gas}}{NH_3}$$

$$\underset{\text{Carbamic acid}}{CH_3CONH_2} \longrightarrow \underset{\text{ammonia gas}}{NH_3} + \underset{\text{carbon dioxide}}{C0_2}$$

Final products of hydrolysis of urea in presence of a catalyst, urease, are two ammonia molecules and carbon dioxide gas [4]. MQ641 ammonia gas sensor senses the amount of ammonia gas released and produces a potential equivalent to it . This signal is then sent to the microcontroller wherein the analog signal is converted to the digital form. The readings are noted for different urea quantity in milk and hence by two point calibration the lookup table is prepared, which is burnt onto the microcontroller .Finally, the amount of urea present in milk will be displayed on the LCD in terms of parts per million(ppm) .Figure 5 shows block diagram of the proposed method mentioned.

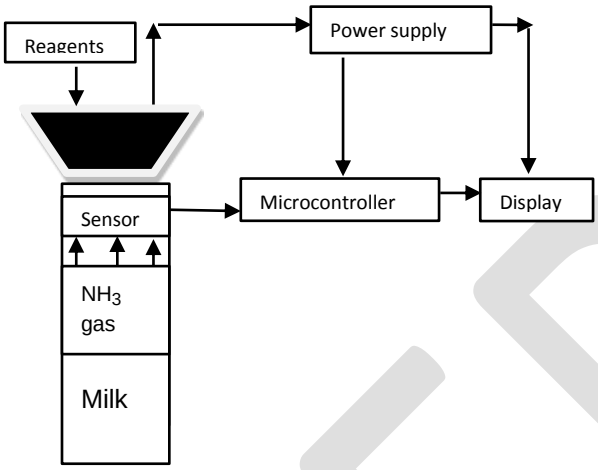

Figure 5: Block Diagram

We expect the output of the sensor to vary linearly with the amount of urea present in milk.

CONCLUSION

Milk is a very important food component of our regular diet. Milk adulterated with urea is a common practice because of increase in demands by the ever increasing population and to increase the SNF value. Since high quantity of urea is a threat to human life there is an utter necessity to develop and design a portable, accurate, sensitive and yet a cost effective method to detect as well as quantify the amount of urea in milk and hence deduce if the recorded level is harmful for human consumption. In this project, quantification of urea is carried out based on the principle that when ammonia gas is released the ammonia gas sensor produces a potential which is given as an input to the micro-controller. An LCD interface displays the percentage composition of urea in milk. This instrument will be tested for its accuracy, sensitivity and linearity by introducing known quantity of urea and calibrating the readings. An attempt is made to see that the instrument performs in-line of an ideal instrument giving an almost ideal response and to bring out the above-stated qualities which serve as a handy urea quantification instrument.

FUTURE SCOPE

The future effects will be oriented to optimize module performance, to increase accuracy and to improve user interface. Miniaturizing the system to a pocket size biosensor can be developed. Features such as alarm , data logging can also be implemented. The method proposed can be extended to determine various other adulterants in milk such as starch, glucose and hydrogen peroxide in a similar manner. Another important extension is in the field of blood analysis. It is said that blood containing 20mg-70 mg of urea is harmful to human health. Hence detection of urea content in blood can also be carried out on the similar lines in future.

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