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Comparison of Different Methods Used For Detection of Urea In Milk By Quantification Of Ammonia

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ABSTRACT: Urea (CH_4N_2O), being a soluble agent is the commonly found adulterant in milk and other dairy products. The normal concentration of Urea in milk is expected to be around 10-14 mg/dl. Urea is generally added in milk to increase the Solid Not Fat (SNF) value. An investigation of different methods available for the detection and quantification of urea in milk is carried out in this paper. Also, a comparison is drawn between the various methods available and the optimal one is chosen in terms of factors like portability, durability, high sensitivity, accuracy and precision.

KEYWORDS: charge amplifier, liquid chromatography, manometric sensor, piezoelectric crystal , RTD, spectrophotometry, urea.

I.INTRODUCTION

Milk is commonly consumed by people of all age groups. Also, India is the largest producer and consumer of milk. According to a recent report, India is likely to produce 140.6 million tonnes of milk in 2014 and the demand is set to rise to 150 million tonnes of milk. To meet the growing demand, milk and its products have been adulterated to decrease the quality and increase the quantity for economic value. The common adulterants found in milk are urea; starch/blotting paper, glucose/sugar, caustic soda, refined vegetable oil (cheap cooking oil), white paint and common detergent or shampoo. These not only reduce the nutritious value of the beverage but also pose risk to health.
The supply of milk is predominantly from the local suppliers which many a times gets delivered to the consumers without pasteurisation. Hence, great care should be taken in the production and distribution process as water activity, moderate pH and ambient temperature is sufficient for the microbial activity in milk. In order to increase the SNF value which in turn increases the economic value of milk and to increase the productivity, urea, a nitrogen containing molecule is added as a common adulterant in milk [4].

The presence of urea in milk is detrimental to human health because they vary the amount of protein in the diet, amount of urine excreted, amount of water intake, dry matter intake.Therefore it is essential that the milk should be tested for purity before consumption. In this paper an attempt is made to study the different methods to estimate the presence of urea in milk. Section [II] discusses the various types of methods employed for detecting the presence of Urea in milk. Section [III] draws a comparison on the various methods available to detect ammonia that was discussed in the previous section. Section [IV] describes the best method that can be employed for the detection of urea in milk considering various factors like portability, durability, high sensitivity, accuracy and precision, less response time, etc. Finally, a conclusion is drawn in section [V] on the various methods existing to detect milk urea and the future enhancement that can be executed in the same.

The following table (Table 1) shows the constituents of milk and its percentage composition.

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COMPONENTS	PERCENTAGE COMPOSITION (%)
PROTEIN	3.2
WATER	87.8
FATTY ACIDS	3.6
CARBOHYDRATE	4.8
CHOLESTROL	0.014
CALCIUM	0.12
UREA	0.14

Table 1: Composition of milk

II.LITERATURE SURVEY

PAPER 1: Laurinavicius, V. ; Razumiene, J. ; Gureviciene, V. "Bioelectrochemical Conversion of Urea on Carbon Black Electrode and Application" Volume: 13 , Issue: 6 DOI: 10.1109/JSEN.2013.2250711 Publication Year: 2013 , Page(s): 2208 – 2213

A. Purpose: To develop a biosensor for the detection of urea in milk and hence calculate response time, coefficient of variation.

B. Outcome: The amperometric biosensor for urea determination is designed. The decomposition product of urea produced by urease is oxidized in an electrochemical way. Three types of the urea biosensor action are identified.

PAPER 2: Bamiedakis, N. ; Hutter, T. ; Penty, R.V. ; White, I.H. ;Elliott, S.R. " PCB-Integrated Optical Waveguide Sensors: An Ammonia Gas Sensor" Volume: 31 , Issue: 10 DOI: 10.1109/JLT.2013.2255582 , Publication Year: 2013 , Page(s): 1628 – 1635

A. Purpose: presents a novel platform for the formation of cost-effective PCB-integrated optical waveguide sensors.

B. Outcome:. The sensor operation relies on the change of the optical transmission characteristics of chemically functionalised optical waveguides in the presence of ammonia molecules and achieves a sensitivity of approximately 30 ppm and a linear response up to 600 ppm at room temperature.

PAPER 3: Inaba, A. ; Yoo, G. ; Takei, Y. ; Matsumoto, K. ; Shimoyama, I."A grapheneFET gas sensor gated by ionic liquid" Digital Object Identifier: 10.1109/MEMSYS.2013.6474408 Publication Year: 2013 , Page(s): 969 – 972

A. Purpose: report a gas sensor based on a field-effect transistor (FET) with a graphene channel and ionic liquid (IL) gate.

B. Outcome: The proposed sensor selectively detects low concentration gases at low gate voltage.It is demonstrated that this device was able to detect at least 30 ppm of ammonia (NH3) and 4000 ppm of carbon dioxide (CO2) at gate voltage below 1 V.

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III. METHODS AVAILABLE FOR UREA DETECTION

Urea is an important analyte for the diagnosis of diseases such as renal malfunction.There are many methods that are available to estimate the quantity of urea by measuring ammonia in milk. The different methods available for quantification of Urea are as following:

A. Spectroscopic method: Urea can be quantified in milk by measuring ammonia by an optical method. This method works on the principle that Infra Red absorbs ammonia at a characteristic wavelength of 1530 nm [2]. The level of absorption is directly proportional to the quantity of ammonia present in milk. This can be used to estimate the urea content in milk.

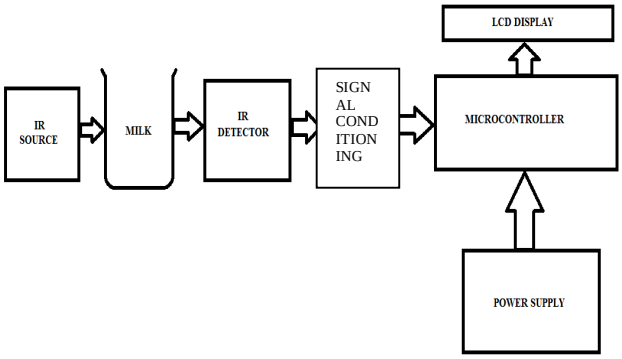

Fig 1: Spectroscopic method

B. Chromatographic method: A definitive method based on liquid chromatography isotope dilution mass spectrometry (LC-IDMS) is used to determine urea in milk, which is an indicator of nutrition status for the lactating animals. The sequential addition of acetonitrile and chloroform to milk, precipitates proteins which can be directly separated using normal phase liquid chromatography without chemical derivatization [3]. Milk should be treated twice the same way as mentioned above. To achieve high accuracy, high precision, good linearity and low uncertainty in the determination of milk urea, exact matching IDMS can be used.

C. Chemical method: Urea is a natural constituent of milk and is present to an extent of 70 mg per 100 ml (700 ppm). Trichloroacetic acid 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 [1]. 5ml DMAB solution is added to each of the 25ml test tube containing 5ml of working standard solution.5ml buffer is mixed with 5ml DMAB solution to prepare a reagent blank. This value of reagent blank is plotted against the urea concentration. This is expected to be linear. 10ml of Trichloroacetic acid(TCA) is added to 10ml of the milk sample to precipitate the proteins, which is then filtered through Whatman 42 filter paper. To develop the colour, 5ml of filtrate is added to 5ml of DMAB solution. The optical density of the colour so formed (yellow) is measured at 420 nm. Finally, the concentration of urea in milk is estimated from the standard curve.

D. Piezoelectric crystal method: Urea (CH_4N_2O), on hydrolysis in the presence of Urease gives ammonia and carbon dioxide [1]. The two products of the reaction in turn exert a certain pressure on the piezoelectric sensor which converts the mechanical pressure into electrical signal [7]. The signal is further amplified and programmed to give the quantity of ammonia present by using a calibration method.

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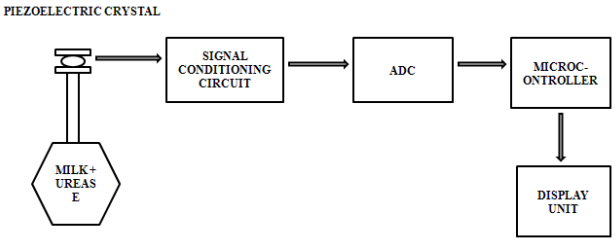

Fig 2: Piezoelectric crystal method

E. Teflon temperature sensing method: RTD that is covered with Teflon is dipped into milk containing ammonia. The property of Teflon is such that it attracts ammonia towards it [5]. 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.

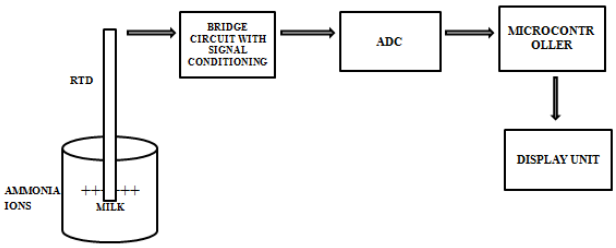

Fig 3: Teflon temperature sensing method

F. Potentiometric Biosensors Based on Silicon and Porous Silicon: A potentiometric biosensor, Electrolyte–Insulator-Semiconductor capacitor (EISCAP) shows a shift in the measured CV with changes in the pH of the electrolyte [6]. Ammonia and carbon dioxide liberated as a result of the enzymatic reaction dissolve in water thereby producing ammonium hydroxide and carbonic acid respectively.

Ammonium hydroxide is a strong base while carbonic acid is a weak acid. Hence pH of the electrolyte solution after the enzymatic reaction shifts towards the basic range. The change in pH is detected by CV measurements on the EISCAP and co-related to the concentration of urea. This gives an estimation of urea content present in milk.

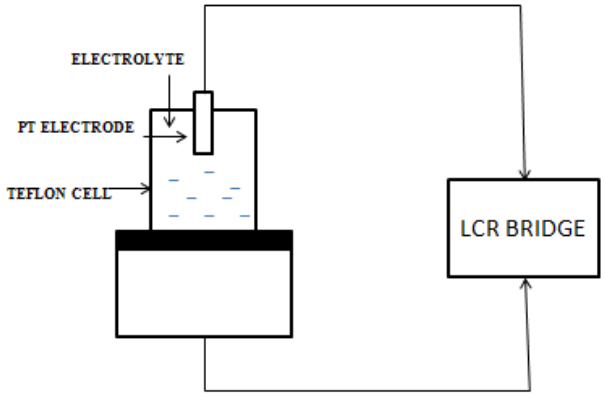

Fig 4: Potentiometric Biosensors Based on Silicon and Porous Silicon

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IV. COMPARISON OF AVAILABLE METHODS

As discussed in the previous section, there are various methods that are available for the detection of urea in milk. Each of their advantages and disadvantages are discussed and is as shown in Table1. An attempt to propose an optimal method that is used to attain portability, durability, high sensitivity, accuracy and precision is made.

METHODS	ADVANTAGES	DISADVANTAGES
IR Spectrophotometer method	Uses a physical rather than a chemical technique. Less sensitive to calibration errors.	Commercial range available only upto 920 nm.
Liquid Chromatography method	High accuracy, high precision, good linearity, Low uncertainty.	Not portable and expensive.
Chemical method	No calibration required.	Human contact with harmful reactants. Wastage of chemicals in testing
Piezoelectric crystal method	Low cost, high DC output voltage when compared to other methods.	Sensitive to stray gases present in the sample.
Teflon temperature sensing method	Selective absorption of Ammonia.	Output signal needs to be amplified as it is very low.
Electrolytic Capacitance method	High sensitivity and good reproducibility.	Calibration errors involved.

Table2: Comparison of various methods

V. OPTIMAL METHOD FOR DETECTION

There are various methods with which urea in milk can be quantified. Nevertheless, an optimal method is always desired. The comparison drawn between the various existing methods helps one to analyse the merits and demerits of them and to choose the best for the desired result. On study, it was clear that piezoelectric crystal method to detect the urea in milk stood out in optimality when compared to others due to its low cost, linear absorption of ammonia, portability, speed of response, etc.

Piezoelectric after excitation when inserted inside adulterated milk sample containing ammonia, ammonium ions are absorbed by the piezoelectric crystal which produces a vibration pulse that is given to a charge amplifier. This converts the charge stored in capacitor to a voltage signal. A signal conditioning circuit is used to calibrate the level of output voltage based on the quantity of urea present in milk. To obtain a digital value on the display unit, the signal conditioned output is given to ADC which can be interfaced with a microcontroller [6].

In the enzymatic method, urea on hydrolysis in the presence of urease gives ammonia and carbon dioxide. These two gases exert some amount of pressure on the piezoelectric crystal that causes vibration [7]. This pulse is given to a charge amplifier which converts the charge stored in the capacitor to a voltage level which is signal conditioned and

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given to an ADC for digital output. To display the quantity of ammonia, display unit is interfaced with microcontroller and based the voltage level obtained; it displays the corresponding concentration of urea in milk.

VI.CONCLUSION

A review of the various methods proposed to test milk for different concentrations of urea suggested that the manometric(piezoelectric method) method will give effective results. Further research in this area may be carried out to increase the sensitivity of the instrument by estimating the effects of external factors. Change in temperature, atmospheric pressure and also, residual pressure exerted by the stray gases present in the sample that affects the performance of the sensor can thus be minimized.

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