ENZYME BASED SENSOR FOR DETECTION OF UREA IN MILK

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ABSTRACT
Enzyme based sensor for detection of urea in milk was constructed using a piezo-electric sensor, which measures the pressure of the gas, evolved in the sample. The sensor showed linear behavior for varying concentrations of urea in the samples. The time response of the sensor was evaluated and liquid to gas ratio of 1:2.5 was found to give satisfactory output by the sensor. The results indicate that this technique can be effectively used to detect urea levels in milk.

Introduction
The growing demand for rapid and easy measurement of monitoring food products has necessitated the use of sensors which have speed, sensitivity and stability over the conventional measurement technologies. Enzyme based sensors in this regard are new, substrate specific devices capable of fast responses and reproducible measurements. Milk is a common health drink consumed by people of all age groups. A large population in our country depends on milk from local suppliers. High water activity, moderate pH (6.4-6.8) and ample supply of nutrients make milk an excellent medium for microbial growth. This demands high standards of hygiene in its production and distribution. Due to the increasing demand, adulteration of milk by urea has been very common in our country. Urea a nitrogen containing molecule is found in milk. Milk urea nitrogen levels are known to vary with the amount of protein in the diet, amount of urine excreted, amount of water intake, dry matter intake, sampling methods, breed, parity, and days in milk, season and herd management. These levels increase with adulteration and are detrimental to human health. Therefore it is essential that household consumers test milk for the concentration of urea before consumption. In this work an attempt is made to study the detection of urea in milk using a piezo-electric sensor, which measures the pressure of the gas, evolved in the sample.

Urea determination has been carried out by many research groups and patented based on the classical spectrophotometric methods (7). The inconvenience of spectrophotometric determination is overcome in analyzers based on electrochemical methods. There are many methods to determine the presence of urea. Caras and Janata (1) constructed a probe by immobilizing penicillilase over a pH sensitive field effect transistor and found that differential mode of measurement was easily accomplished. Their probe had long lifetime rapid response time and was also economic. Pijanowska and Torbic (5) developed and characterized a urea biosensor...
based on pH ion selective field effect transistor with directly immobilized urease enzyme. Their biosensor had a response time of 80 seconds and lifetime of 35 days with stable analytical signals. Brzózka et al. (4) developed a durable NH\textsubscript{4}\textsuperscript{+} sensitive CHEMFET based sensor selecting proper matrices which showed responses and good selectivity even after 22 months of continuous exposure of the conditioning electrolyte. Chern. et al. (2) designed a sensor based on the use of urease and ionophore immobilized on to suitable membranes. This sensor could be adjusted to the physiological range of urea in human serum by choosing suitable buffer concentrations. The methods based on NH\textsubscript{4}\textsuperscript{+} based urea sensing suffers from the drawback that the presence of other cations influences the measurement. But all these methods exploit the presence of ammonium in the hydrolysis product. Therefore in the present investigation the gasometric technique for urea estimation was adopted and a simple setup was developed that could detect the concentration of urea in milk.

**Materials and Methods**

**Materials**
Sruthi brand milk produced by Sri Balamurugan Dairy Products, Chittoor, Andhra Pradesh, was used throughout the study. The composition of milk sample per ml was fat-6 mg; lactose-100 mg; Protein-7 mg; and calcium-2.5 mg. Thiosemicarbazide, 4-aminoantipyrine, ceric ammonium sulfate, phosphoric acid, sulfuric acid were of analytical reagent grade and, procured from Central Drug House Pvt. Ltd., Mumbai.

**Methods**
The reaction was carried out in a glass bottle of capacity 10 ml over which the sensor was mounted. The experimental set up is shown in Fig. 1.

3 ml of the milk was taken in the reaction cell and urease was added to it and the cell was kept undisturbed at a constant temperature for three minutes. 0.5 ml 2M citric acid was added to the contents in the reaction cell followed by vigorous shaking for

![Fig. 1. Experimental setup.](image-url)
20 seconds. The amount of gas released was an indication of the measure of urea in the reaction mixture. The charge from the piezoelectric crystal was connected to a charge amplifier (OPA111) as depicted in Fig. 2 so as to convert the weak charge output in terms of volts. A data acquisition card was used to convert the signals and monitoring was done using Lab View software.

Results and Discussion

Sensor calibration

Varying concentrations of urea were analyzed from 0 to 0.1 mg/ml for absorbance at 525 nm. (Fig. 3). After analyzing milk samples with and without solids content it was found that the solids in the milk affect the detection of urea (Fig. 4). Milk samples added with external urea at different dilutions were also analyzed. The sensor showed linear behavior for varying concentrations (Figures 5 and 6).

Sensor evaluation

The time response of the sensor was evaluated by checking the deflections for intermittent periods, so that satisfactory output was obtained after 180 seconds. Fig. 7 shows the response of the system at different time intervals. The ratio of the liquid to the gaseous volume in the reaction cell was tested for different values. A liquid to gas ratio of 1:2.5 was found to give satisfactory output by the sensor (Fig. 8).

The other parameters like dependence of lactose with the sensor output, temperature dependence, lifetime of the sensor, output change with urease activity was also determined and the results showed that this technique may be developed into a device for low cost, reliable urea measurement in milk.

Conclusions

The results obtained by testing milk for various concentrations of urea added in the constructed manometric biosensor were encouraging. The results indicate that this technique can be effectively used to detect urea levels in milk. Further research may be carried out for finding the effects of factors like temperature and residual pressure exerted by other gaseous components on the sensor.
Fig. 5. Sensor output (urea-0.5 mg/ml)

Fig. 6. Sensor output (urea-1.0 mg/ml)

Fig. 7. Sensor output vs time.

Fig. 8. Sensor output vs cell volume

REFERENCES