International Journal of General Medicine and Pharmacy (IJGMP)
ISSN(P): 2319–3999; ISSN(E): 2319–4006
Vol. 10, Issue 1, Jan–Jun 2021; 1–6
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International Academy of Science, Engineering and Technology
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QUANTITATIVE ESTIMATION OF ADDED UREA CONTENT IN MILK BY UV-VISIBLE SPECTROSCOPY

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ABSTRACT

Urea is a natural constituent of milk and it forms a major part of the non-protein nitrogen in the milk. Urea concentration in milk is variable within herd. Urea content in natural milk varies from 20mg/100ml to 70mg/100ml.However, urea content above 70mg/100ml in milk indicates milk containing 'added urea'. The added urea in milk can be detected qualitatively by using UV-Visible spectroscopy

KEYWORDS: *Added Urea, DMAB, UV- Method*

Article History

Received: *22 Apr 2021* | ***Revised:*** *24 Apr 2021* | ***Accepted:*** *04 May 2021*

INTRODUCTION

Milk is a wholesome nutritious dairy product and is consumed by a majority of the population worldwide for drinking as such, as well as via dairy products. It is highly nutritious containing essential nutrients like energy providing lactose and fat, the bone forming calcium and other minerals, the body building proteins and health promoting vitamins required for the development for the all age groups. Unfortunately milk is being very easily adulterated throughout the world and significantly worse in developing and under developed countries due to the absence of advocate monitoring and lack of proper law enforcement. The nature of adulterants generally encountered in milk are water, removal of fat, addition of skimmed milk powder, reconstituted milk, thickening agents such as starch flour, glucose, urea, etc.., Preservatives such as neutralizers which usually consist of sodium bicarbonate, sodium carbonate, sodium hydroxide and calcium hydroxide. Some rarities include animal fats, aflatoxins and vegetable oil. Urea content more than 70mg/100ml is considered as "added urea" and the milk is said to be adulterated milk. The added urea compromises the quality of milk and also produces severe side effects like diarrhoea, acidity, malfunctioning of kidneys, damage of intestinal tract and digestive system. The study provides the knowledge of quality of milk available in local market.

Introduction to UV Spectroscopy

UV spectroscopy is an absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n, π or σ or electrons. These bonding (σ and π) and non-bonding (n) electrons absorb the radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the

nature of the electron present the molecular structure can be elucidated easily.

UV Spectroscopy Obeys the Beer-Lambert law,

Beer Law

This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

$$I=I_0*e^{-k1*c} \quad (1)$$

Where,

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

K_1=constant

Lambert's Law

This law can be stated as follows "When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light".

$$I=I_0*e^{-k2*l} \quad (2)$$

Where,

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

L = length of sample cell (cm.)

K_2=constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$$A = \log (I_0/I) = ƐCL \quad (3)$$

Where,

A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

L = length of sample cell (cm.)

Ɛ = molar absorptivity

IMETHODOLOGY

Quantiative Analysis

The test based on the use of para-dimethyl amino benzaldehyde can be used for the estimation of urea in milk after precipitation of milk proteins using trichloroacetic acid.

Reagents/Apparatus

- *p-Dimethyl amino benzaldehyde (DMAB) solution*: Dissolve 1.6 g DMAB in 100 ml ethyl alcohol and add 10 ml concentrate HCl. The reagent is stable for 1 month. Prepare new standard curve with each new batch of reagent.
- *Phosphate Buffer pH 7.0:* Dissolve 3.403 g anhydrous potassium dihydrogen orthophosphate (KH2PO4) and 4.355 g anhydrous dipotassium monohydrate orthophosphate (K2HPO4) separately in 100 ml of distilled water. Combine solutions and dilute to 1 litre with water.
- *Trichloroacetic acid (TCA) 24%, w/v*: Freshly prepared. 24.0 g TCA is dissolved in distilled water and volume made up to 100 ml.
- *Diluting Reagent*: Equal volumes of 24% TCA and phosphate buffer (pH 7.0) are mixed to make the diluting reagent.
- *Urea Standard Solution:*
    - Stock solution: 5 mg / ml. Dissolve 5 ± 0.001 g reagent grade urea in water and dilute to 1 litre with water.
    - Working solution: Pipette 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ml stock solution into 250 ml volumetric flask and dilute to volume with phosphate buffer.
    - Reference solution -Use standard solution containing 1.0 mg urea / 5 ml as reference standard. Store at less than 24°C.

Apparatus

- Spectrophotometer – Instrument with maximum band width 2.4 nm at 420 nm, with 1 cm cells
- Whatman filter paper: Grade 42.
- Funnels.
- Test tubes.

PROCEDURE

Preparation of Standard Curve

Pipette 5 ml aliquots of working standard solutions into 20 x150 mm (25 ml) test tubes and add 5 ml DMAB solution to each. Prepare reagent blank of 5 ml buffer and 5 ml DMAB solution. Shake tubes thoroughly and let stand for 10 minutes. Read absorbance (A) in 1 cm cell at 420 nm with reagent blank at zero A. Plot absorbance Vs concentration, urea Plot should be straight line.

Estimation of Urea in Milk

10 ml of milk sample is mixed with 10 ml of TCA to precipitate the proteins and filtered using Whatmann 42 filter paper. 5 ml of filtrate is then treated with 5 ml of DMAB reagent to develop the colour. Blank is prepared by taking 5 ml of diluting reagent and treating with 5 ml of DMAB reagent. The optical density of the yellow colour is measured at 420 nm. From standard curve the amount of urea in milk is calculated.

RESULTS AND DISCUSSIONS

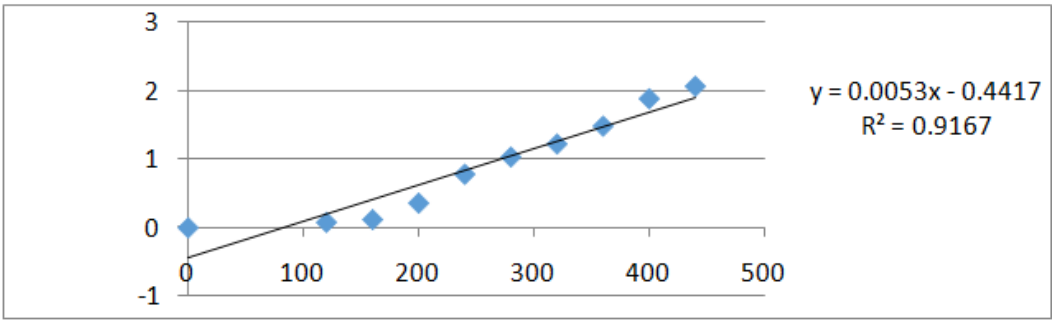

Figure 1: Standard Curve of Urea.

Table 1: Table Showing Absorbance Values of Various Locally Available Brands of Milk

MILK SAMPLES NAME	ABSORBANCE	UREA CONTENT (ppm per ml)
BRAND 1	0.5028	200
BRAND 2	0.5709	240
BRAND 3	0.5218	229
BRAND 4	0.5202	238
BRAND 5	0.5297	248

ACKNOWLEDGMENT

I wish to express my sincere gratitude to Mrs. Dr. M. Sumakanth, Principal, RBVRR Women's College of Pharmacy, for providing me an opportunity to carry out the analytical work. I sincerely thank Mrs. R. Swetha Sri, Assistant professor, department of pharmaceutical analysis, for her guidance and encouragement in carrying out this project work.

REFERENCES

1. *Khan Mohammad Khan, Hemant Krishna, Shovan Kumar Majumder & Pradeep Kumar Gupta Detection of Urea Adulteration in Milk Using Near-Infrared Raman Spectroscopy Food Analytical Methods.*
2. *Chauhan Mahipalsinh P, Arpita Agnihotri, AI Shaikh, SI Patel and KD Aparnathi*
3. *Evaluation of DMAB test for detection of urea mixed in milk and improvement in its efficacy. International Journal of Chemical Studies,2017,5(6)*
4. *Mohit Kamthania, Jyoti Saxena, Komal Saxena, D.K. Sharma Milk Adulteration: Methods of Detection &Remedial Measures National Conference on Synergetic Trends in engineering and Technology International Journal of Engineering and Technical Research*
5. *Tanzina Azad & Shoeb Ahmed Common milk adulteration and their detection techniques International Journal of Food contamination.*

6. *Dr. K. B. Ramesh, Khushboo K Gandhi, Pooja Valecha, Shradda Pai K, Sushma.*
7. *Quantification of Urea in Milk- A review of Existing Methods International Journal of Engineering Research and General Science Volume 3, Issue 2, March-April, 2015 ISSN 2091-2730 809*
8. *G. W. Watt and J. D. Chrisp Spectrophotometric method for determination of urea by Analytical Chemistry March 22,1954,26,3,452-453(Article)*
9. *Grant Ambernethy, Kerianne Higgs Rapid detection of economic adulterants in fresh milk by liquid chromatography Journal of Chromatography*
10. *Carina F Nascimento, Poliana Msantos, Edenir Rodrigues Pereria-Filho Recent advances on determination of milk adulterants Food chemistry 2017,221,1232-1244,*
11. *Manual of methods of analysis of food by fssai, Ministry of health and family welfare, Government ofindia, Newdelhi, 2016.*