UGC SPONSORED MINOR RESEARCH PROJECT

ON

DETECTION OF CHEMICAL ADDITIVES IN BRANDED MILK BY ULTRASONIC METHOD

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By

P.D. WANKAR
M.Sc., Ph. D.
Principle Investigator

R. L. CHANDANKHEDE
M.Sc., M. Phil.
Co- Investigator

Department of Physics
Jankidevi Bajaj College of Science
Wardha - 442001

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Report of the work done

Objectives of Project:

Experimentation involving Ultrasonic waves are extensively used to examine certain physical properties of the material in liquid as well as solid systems in basic science. Ultrasonic wave are used to extract information on the behavior of the microscopic particle of matter. In medical science the waves are used to determine structure and behaviour of bio-molecules.

The objective of the proposed work is to determine the various acoustic parameters like ultrasonic velocity, adiabatic compressibility, Rao's constant, acoustical impedance etc. in pure and adulterated milk. Comparison of thermo-acoustical properties of pure and adulterated milk can easily help us to detect the adulterated milk and comparing with standard adulterant properties, the substance added as adulterant can be detected.

Work done so far, results achieved, publication:

i) All the data required has been obtained
ii) Basic parameters like ultrasonic velocity, density and viscosity were measured experimentally.
iii) Different thermo acoustical parameters of milk and mixture were calculated with the help of mathematically calculation..
iv) The necessary graphs have been drawn
v) Results have been obtained, work is completed.
vi) One research papers based on above topics will be communicated for publication to " Kamla Nehru Journal of Science and Technology.", India.

Whether the Objectives were achieved: ---------- Yes the objectives of the project were achieved.

Achievements from the project:

i) A necessary experimental arrangement has been developed in the laboratory.
ii) By performing the experiment, different acoustical and thermo-acoustical parameters of milk and milk with additives are determined.

iii) The data for acoustical and thermo acoustical parameters were prepared and different graphs were drawn between concentration and parameters to analyzed the adulteration in the milk.

Summary of Project

Milk has traditionally been an important part of Indian diet, particularly for children, pregnant women, patients and senior citizens. For all of these categories, adulterated milk can be lethal, if not fatal. Our innocent population is at a big risk; the rising graph of cancers and kidney failures could well be sourced to inedible milk.

Adulteration of foodstuffs is a menace, which saps the vitality of common man. One of the commonly adulterated food is milk and milk products. Recent media reports reveal that many brands of milk commercially available in Kerala contain chemical additives such as sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), formalin (HCHO) etc. These are added to milk as neutralizers to preserve it for longer time and to prevent curdling. The continuous use of such milk may cause health hazards to the society. Since the frequency and quantity of milk consumed by infants and children are much more compared to adults, the health risk is more to them.

All ingredients are generally regarded as inedible by the human body, but our native adulterers are happily feeding India's cow-worshipping, milk-loving population this amazing diet.

Ours is a fast growing population. There is a huge, unending demand for food items and milk products. Criminals who want to make quick money are freely engaged in adulteration activities without fear of law and without conviction by courts of law.

A survey by the Indian Council for Medical Research (ICMR) warned that milk from 12 states had excessive pesticide residues, toxic metals and fungal particles which could lead to serious cancerous effects on the human body. According to Dr R.S. Khanna, managing director, Rajasthan Cooperative Dairy Federation, in a survey of more than 200 villages in a Rajasthan district, adulterated milk with vegetable fat, detergents, urea, salt and other elements were found in about 41 villages. Nirma, allegedly, was the preferred detergent adulterant. Apparently, the brand's smell and texture merged well with milk. Another survey is under way at the National Dairy Research Institute at Karnal in Haryana on the extent of adulteration.

According to Dr Anil Saxena, food scientist with the Delhi-based Food Research and Analysis Centre (FRAC), urea increases the nitrogen content in milk which ultimately determines the protein content. But urea makes milk acidic, so a dash of caustic soda is added to neutralise the acidity. Chemicals like formalin are used primarily for preservation. The use of urea and soap solution in milk, says Saxena, is harmful for the stomach and intestines while the use of caustic soda could lead to skin diseases and even be carcinogenic.

The Government is of the view that the situation is under control with self-regulation in cooperatives and random checking of samples apart from regular checks by food inspectors in districts. However, at most places there are not enough officials to take samples and test them.

But these tests can only determine the quantity of fat and SNF content in milk, while only very well-equipped and sophisticated laboratories will be able to detect the presence of urea, caustic soda or detergents.

Some state governments and larger milk agencies are taking steps to fight the menace. But unless synthetic milk is tackled on a war footing, notions about the sanctity of milk will have to be changed.

Experimental and Instrumentation

Instrumentation has played a key role in scientific investigations. The art of measurement is a wide discipline in both engineering and science, encompassing the area of detection, acquisition control and analysis of data. It involves the precise measurement and recording of physical, chemical or optical parameters and plays vital role in every branch of scientific research and industrial process interacting basically with control system, process instrumentation and data reduction.

Recent advances in electronic, physics, material science and other branches of science and technology have resulted in the development of many sophisticated and precision measuring devices and systems, catering to varied measurement problem in such discipline as science & technology, space, medicine and industry in general.

During the last four decades, the measuring techniques have improved considerably, meeting the exacting demands of scientists, engineers and technologists.

1. Ultrasonic cell:

The ultrasonic cell is a double walled stainless steel cell with silver plated surfaces. The capacity of ultrasonic cell is about 15 ml. The cell has an outer shell through which water is circulated. Piezoelectric crystal of resonant frequency 5 MHz is fixed at the bottom of the cell.

The experimental liquid is kept in the cell; the standing waves are formed between reflector and transducer.

2. Digital Ultrasonic Pulse Echo Velocity Meter (Model No : (VCT – 70 A) :

Automatic ultrasonic attenuation recorder (AUAR-102) is highly sensitive instrument used to measure ultrasonic velocity in solids and liquids. With the help of digital Ultrasonic Pulse Echo velocity Meter (Model No: VCT – 70 A) purchased from Vi Microsystems Pvt. Ltd. Chennai, the ultrasonic velocity of the liquid is measured automatically.

In this technique a burst of ultrasonic energy is introduced into the experimental sample under study.

The ultrasonic velocity is measured automatically and all the values of velocities are displayed by card reader on Laptop purchased under MRP scheme by interfacing with VCT-70 A. The accuracy of ultrasonic velocity measurements was ± 0.1m/s.

3. Thermo Bath:

A special thermostatic arrangement was done for determination of different parameters at different temperatures. The measurements of ultrasonic velocity, density and viscosity were measured at different temperatures and concentrations. The constant temperature water bath low temperature (LAB HOSP) purchased from Lab- Hosp Corporation Guregaon (E) MUMBAI under this scheme. This water bath is fully automatic with temperature sensors. The temperature is controlled automatically by thermostat at any point from room temperature 5^0 above the ambient to 99.9^0C having the accuracy of $\pm 1^0$C.

4. Density Measurement:

Density is measured by using specific gravity bottle and electronic balance.

The densities of the experimental liquids were measured by using the following relation.

$$\text{Density of liquid } (\rho) = \frac{\text{Mass of liquid}}{\text{Volume of liquid}}$$

The density of water is taken from the literature**.** The measurements were made in the temperature of 298K. The masses were measured by a digital balance Contech Make Mumbai with accuracy of ± 0.001 gm. The accuracy in density measurements was 0.001Kg/m^3.

5. Viscosity Measurement:

Ostwald's viscometer is the simplest, accurate and widely used viscometer. We have used Ostwald's viscometer to determine the viscosity in liquids owing to its versatility.

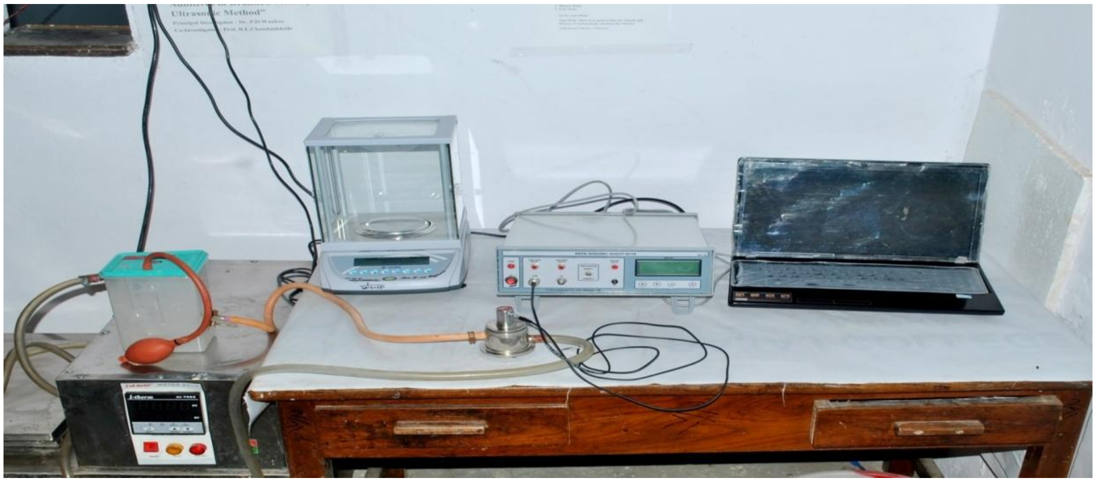
Prior to the measurement, the viscometer was calibrated with the help of standard and pure liquids viz. acetone, alcohol etc. The temperature of water bath was maintained constant for long time within an accuracy of $\pm 1^0$C. The time of falling of the liquid between viscometer marks is measured using an electronic digital timer. The viscosity was determined by using the following relation:

$$\eta_l = \eta_w \, x \, \left(\frac{t_l}{t_w} \; x \; \frac{\rho_l}{\rho_w}\right)$$

Where, η_l, ρ_l, t_l are respectively the viscosity, density and time of flow for experimental liquids. η_w, ρ_w and t_w are the corresponding quantities for double distilled water.

The data on viscosity of water was taken from literature**.** The measurements were made for the temperature298 K. The accuracy in viscosity measurements was ± 0.1 %.

Whole assembly of experimental setup is shown in **Figure.**



Complete experimental setup

6. Systems Under Investigation:

Different ultrasonic parameters like ultrasonic velocity, density, and viscosity were studied in the following systems:

1. Milk + Water
2. Milk + Water + Sodium Carbonate (Na_2CO_3)
3. Milk + Water + Sodium bicarbonate ($Na.H.CO_3$)
4. Milk + Water + Oxalic acid $(COOH)_2$
5. Milk + Water + Urea ($CO (NH_2)_2$)
6. Milk + Water + Melamine (($C_3H_6N_6$)
7. Milk + water + Formaline (HCHO)

7. Preparation of Experimental Mixtures:

All the pure additives were procured from the well known pharmaceutical company SRL Make, Mumbai with 99% purity. The pure milk for experimental purpose was purchased from Goras Bhandar, Wardha who provides pure milk to Wardha citizens. The milk solutions were prepared in doubled distilled water. Initially, pure milk with 50% dilution with water was taken as a stock solution. After that 1 wt % to 10 wt. % additives were added in to the mixture respectively and observations were carried out.

8. Mathematical Formulation

Thermodynamic parameters deduced from density measurement, ultrasonic velocity, and viscosity has been a matter of interest during recent years. These measurements have been used to interpret the results in terms of solute-solvent, solute-solute, solvent-solvent and ion-solvent interactions in binary and ternary systems containing electrolyte and non-electrolyte solutions. The properties which may be used for interpretation of molecular interaction and association in solutions of biomolecules are related to the density, viscosity, ultrasonic velocity, dielectric constant, refractive index, internal pressure, free volume etc. The present chapter deals with the detailed mathematical formulations used for illustrating ultrasonic and thermodynamic parameters obtained from ultrasonic and viscometric studies.

Formulation of Ultrasonic & Thermo-acoustical parameters:

Ultrasonic and Thermo-acoustical parameters can be calculated using following formulae.

1. **Adiabatic Compressibility (β_a):**

$$\beta_a = 1/ U^2 . \rho$$

Where, U - Ultrasonic velocity, ρ- Density of liquid

2. **Acoustic Impendance (Z):**

$$Z = U . \rho$$

3. **Free Length (L_f):**

$$L_f = K_j . \beta_a^{1/2}$$

Where, K_j –Temperature dependent constant (199.5 x 10^{-8}),
β_a - Adiabatic compressibility

4. **Surface tension (σ):**

$$\sigma = 6.63 \times 10^{-4} \rho . U^{1/2}$$

5. **Relaxation time (τ):**

$$\tau = 4/3 \beta_a . \eta$$

6. **Relative Association (R_A):**

$$R_A = (\rho / \rho_o) \times (U / U_o)^{1/3}$$

Microsoft Excel application software is used for evaluating these parameters and
Plotting the graphs.

Tables 1 to 7:

Ultrasonic and related thermo acoustical parameters, calculated at different concentration

of following mixtures are given in the respective tables from 1 to 7:
1. Milk + Water,
2. Milk + Water + Sodium Carbonate (Na_2CO_3)
3. Milk + Water + Sodium bicarbonate ($Na.H.CO_3$)
4. Milk + Water + Oxalic acid $(COOH)_2$
5. Milk + Water + Urea (CO $(NH_2)_2$)
6. Milk + Water + Melamine (($C_3H_6N_6$)
7. Milk + water + Formaline (H.CH.O)

The variation of ultrasonic and other related thermo-acoustical parameters with Wt % concentrations of the mixtures of Milk + Water, Milk + Water + Sodium Carbonate, Milk + Water + Sodium bicarbonate, Milk + Water + Oxalic acid ,Milk + Water + Urea, Milk + Water + Melamine, and Milk + water + Formaline are represented in the Tables 1 to 7.

Table No.1

Milk + Water

Wt %	Density ρ X 10^3 Kg/m^3	Viscosity η X 10^{-3} Pascal-Sec	Ultrasonic Velosity U m/s	Acoustic impedence Z x 10^3 Kg/m^2. s	Adiabatic Compr. β_a x 10^{-10} Kg^{-1}.m. s^2	Free length L_f x 10^{-16} m	Surface tension σ x 10^3 N/m	Relaxation time τ x 10^{-13} min.	Relative Association R_A
0	1.0709	1.328	1516.71	1624.2458	4.65525	4.6436	1176977	8.2429	0.362801259
2	1.06492	1.1947	1509.61	1598.166	4.67291	4.66123	1154043	7.44363	0.359086252
4	1.06084	1.1036	1506.51	1598.166	4.67418	4.6625	1142553	6.87791	0.356975932
6	1.05804	1.0653	1504.07	1591.367	4.67698	4.66529	1134012	6.64318	0.355457314
8	1.05568	1.0475	1502.13	1585.763	4.67865	4.66695	1127096	6.53451	0.35420558
10	1.05424	1.0348	1500.48	1581.865	4.68252	4.67081	1121863	6.46063	0.353334824

Table No. 2

Milk + Water + Sodium carbonate (Na_2Co_3)

Wt %	Density ρ X 10^3 Kg/m^3	Viscosity η X 10^{-3} Pascal-Sec	Ultrasonic Velocity U m/s	Acoustic impedence Z x 10^3 $Kg/m^2.s$	Adiabatic Compr. β_a x 10^{-10} $Kg^{-1}.m.s^2$	Free length L_f x 10^{-16} m	Surface tension σ x 10^3 N/m	Relaxation time τ x 10^{-13} min.	Relative Association R_A
0	1.0709	1.328	1516.71	1624.2458	4.65525	4.6436	1176977.02	1.2429	0.362801259
2	1.076	1.48044	1508.9	1623.52	4.72577	4.71396	1164371.35	1.02015	0.362639216
4	1.0875	1.75144	1539.15	1673.859	4.59065	4.57917	1249086	1.07203	0.373883065
6	1.0894	1.84712	1550.62	1689.202	4.53068	4.51935	1279394.94	1.11583	0.377310302
8	1.1258	2.09371	1579.87	1778.621	4.51042	4.49915	1398419.61	1.22114	0.397283179
10	1.1385	2.09989	1596.3	1817.412	4.46801	4.45684	1458785.69	1.25098	0.405948667

Table 3

Milk + Water + Sodium- bi-carbonate (Na H Co_3)

Wt %	Density ρ X 10^3 Kg/m^3	Viscosity η X 10^{-3} Pascal-Sec	Ultrasonic Velocity U m/s	Acoustic impedence Z x 10^3 $Kg/m^2.s$	Adiabatic Compres. β_a x 10^{-10} $Kg^{-1}.m.s^2$	Free length L_f x 10^{-16} m	Surface tension σ x 10^3 N/m	Relaxati-on time τ x 10^{-13} min.	Relative Association R_A
0	1.0709	1.3281	1516.711	1624.246	4.65525	4.6436	1176977	8.2429	0.362801259
2	1.0629	1.06298	1435.041	1536.24	5.19836	5.1854	996548.1	7.36767	0.343143775
4	1.0844	1.15041	1461.71	1585.078	5.07536	5.0627	1066801.7	7.78512	0.354052575
6	1.0963	1.20033	1451.839	1591.738	5.20135	5.1884	1056864	8.72445	0.355540166
8	1.1084	1.28391	1464.365	1623.102	5.16891	5.156	1096364.8	8.84853	0.362545808
10	1.1175	1.30225	1545.792	1727.515	4.67701	4.6653	1300273.1	8.12086	0.385868152

Table 4

Milk + Water + Oxalic Acid $(COOH)_2$

Wt %	**Density ρ X 10^3 Kg/m^3**	**Viscosity η X 10^{-3} Pascal-Sec**	**Ultrasonic Velosity U m/s**	**Acoustic impedence Z x 10^3 Kg/m^2.s**	**Adiabatic Compres. β_a x 10^{-10} Kg^{-1}.m. s^2**	**Free length L_f x 10^{-16} m**	**Surface tension σ x 10^3 N/m**	**Relaxation time τ x 10^{-13} min.**	**Relative Association R_A**
0	1.0709	1.328	1516.711	1624.2458	4.65525	4.6436	1176977	8.2429	0.362801
2	1.0608	1.02127	1484.59	1574.79	4.81287	4.8008	1093320	6.55365	0.351755
4	1.0674	1.09545	1501.251	1602.435	4.7361	4.7243	1137621.2	6.91754	0.35793
6	1.0737	1.20525	1507.198	1618.309	4.72662	4.7148	1158010.6	7.59567	0.361475
8	1.0784	1.46549	1502.576	1620.378	4.77647	4.7645	1152390.8	9.33316	0.361937
10	1.0842	2.62162	1493.529	1619.344	4.86069	4.8485	1137828.9	1.69905	0.361706

Table 5

Milk + Water + Urea ($CO(NH_2)_2$)

Wt %	Density ρ X 10^3 Kg/m^3	Viscosity η X 10^{-3} Pascal-Sec	Ultrasonic Velocity U m/s	Acoustic impedence Z x 10^3 $Kg/m^2.s$	Adiabatic Compres. β_a x 10^{-10} $Kg^{-1}.m.s^2$	Free length L_f x 10^{-16} m	Surface tension σ x 10^3 N/m	Relaxati-on time τ x 10^{-13} min.	Relative Association R_A
0	1.0709	1.32810	1516.711	1624.2458	4.65525	4.6436	1176977.016	8.2429	0.3628013
2	1.0624	1.05425	1503.277	1597.081	4.70121	4.68946	1136882.691	2.60834	0.3567337
4	1.0678	1.13821	1522.312	1625.584	4.60757	4.59635	1186659.279	3.99296	0.3631001
6	1.0762	1.27682	1552.115	1670.076	4.46647	4.4553	1267344.001	6.80384	0.3730381
8	1.0884	1.68099	1561.251	1699.266	4.46522	4.45406	1304719.867	7.85214	0.3795581
10	1.0961	2.3916	1571.11	1721.937	4.44015	4.42905	1338877.634	8.41587	0.384622

Table 6
Milk + Water + Melamine($C_3H_6N_6$)

Wt %	Density ρ X 103 Kg/m3	Viscosity η X 10-3 Pascal-Sec	Ultrasonic Velocity U m/s	Acoustic impedence Z x 103 Kg/m2.s	Adiabatic Compres. βa x 10-10 Kg-1.m.s2	Free length Lf x10-16 m	Surface tension σ x 103 N/m	Relaxation time τ x 10-13 min.	Relative Association RA
0	1.0709	1.328	1516.711	1624.2458	4.65525	4.6436	1176977	8.2429	0.36280126
2	1.0598	0.8524	1503.277	1593.173	4.68971	4.67798	1134100.4	5.33001	0.35586064
4	1.0612	0.9002	1506.382	1598.512	4.67639	4.66471	1142606.7	5.61291	0.35705327
6	1.0678	0.9254	1511.733	1614.289	4.67257	4.66089	1162096	5.76533	0.36057724
8	1.0691	0.96851	1515.285	1619.991	4.65618	4.64454	1171687.7	6.01274	0.36185092
10	1.0746	0.99132	1521.721	1635.181	4.64046	4.62885	1192741.5	6.13357	0.3652437

Table 7

Milk + Water + Formaline (HCHO)

Wt %	Density ρ X 10^3 Kg/m^3	Viscosity η X 10^{-3} Pascal-Sec	Ultrasonic Velocity U m/s	Acoustic impedence Z x 10^3 $Kg/m^2.s$	Adiabatic Compres. β_a x 10^{-10} $Kg^{-1}.m.s^2$	Free length L_f x 10^{-16} m	Surface tension σ x 10^3 N/m	Relaxation time τ x 10^{-13} min.	Relative Association R_A
0	1.0709	1.328	1516.71	1624.2458	4.65525	4.6436	1176977	8.2429	0.362801259
2	1.0573	0.92287	1501.18	1587.224	4.69183	4.68011	1126711	5.77327	0.35453195
S4	1.0588	0.93948	1506.79	1595.388	4.66347	4.65181	1140990	5.84167	0.356355449
6	1.0598	0.9412	1511.03	1601.445	4.64191	4.63031	1151770	5.82529	0.357708275
8	1.0636	0.94976	1514.57	1610.912	4.63658	4.62499	1164018	5.87152	0.359820224
10	1.0648	0.95697	1517.42	1615.753	4.62439	4.61283	1171924	5.90053	0.360904271

Results and Discussions

The pure milk contains proteins, fats, vitamins, minerals and carbohydrates etc. Casein is major protein present in the milk (3. 2%) **(Figure 1).** It is a dispersed in the form of large of tiny solid particles, which do not settle but remain in suspension.These particles are called as micelles and dispersion of micelles in the milk is referred as colloidal suspension. The building blocks of all proteins are the amino acids. It is rich source of nitrogen.

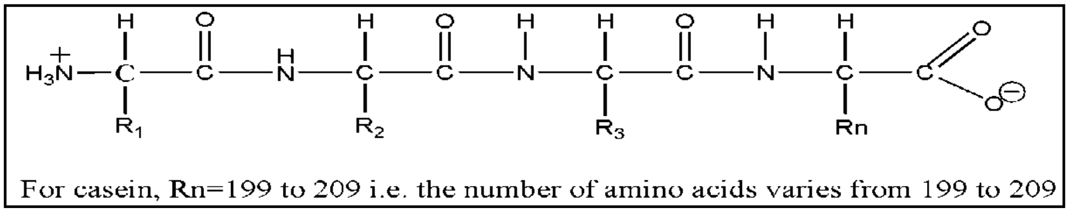


Figure 1- Structure of casein

The fat (3.4%) and fat soluble vitamins are also present in the milk in the form of emulsion. Triglyceride is a important part of fats **(Figure 2).** On hydrolysis, it gives glycerol and higher fatty acids.

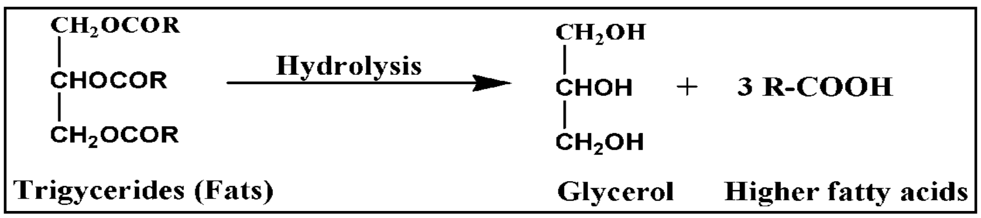


Figure 2- Structure of triglycerides

The principle carbohydrate (4.7%) in the milk is lactose which is known as milk sugar. On hydrolysis, it gives glucose and galactose (**Figure 3).**

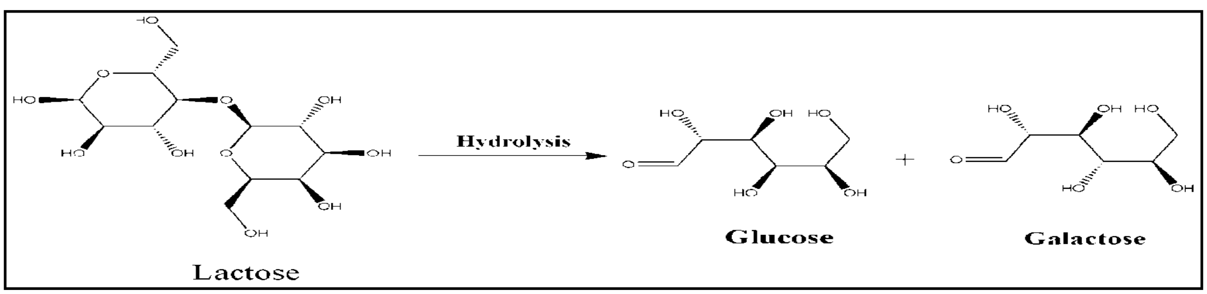


Figure 3- Structure of lactose

System 1 : (Milk + Water)

From the **Table data 1,** it is seen that, the density, viscosity and ultrasonic velocity decreases linearly with increase the % of water in the pure milk. The ultrasonic velocity, density and viscosity is a function of added water in the pure milk. It is seen that by adding water to the pure cow milk, there is a reduction in ultrasonic velocity, density and viscosity of solution with respective to pure milk because water has a lower viscosity than milk The decrease in the density and viscosity indicates that there is decrease in the intermolecular forces between proteins molecules present in the milk . The value of ultrasonic velocity decreases due to weak interaction between the milk component and water molecule.

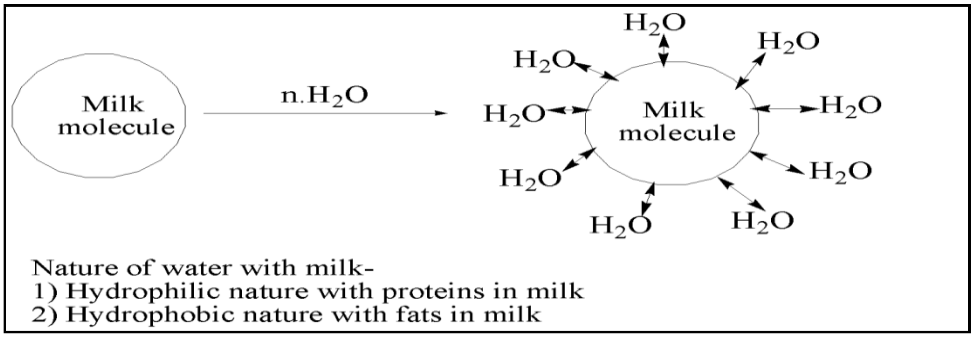


Figure 4 - Nature of water with milk

Similar trends are also found in the value of surface tension, relaxation time and relative association i.e. as concentration of water molecule increases from 2 wt % to 10 wt %, the value of respective parameters are decreasing linearly which are presented in **Table 1.**

Lower values of surface tension with increasing concentration is responsible for weak interaction in the mixture which is due to hydrophobic nature of protein and fat molecules present in milk while decreasing values of relaxation time occurs due to less time of molecule to relax in a free state that in the range of 10^{-13} second and also decreasing value of relative association shows that as concentration of water molecule increases suddenly, solvent structure breaks up and there is less interaction between protein molecules and water molecules.

The acoustical impedance decreases non-linearly with addition of water in the pure milk which is indicated in the data**.** This is due to the breaking of bonds present in the milk protein and represents weak interactions between solute-solute components present in the solution.

The values of adiabatic compressibility and intermolecular free length increases nonlinearly with increasing the concentration of water molecule due to weak interaction. As water is added in the pure milk, proteins get solvated and hence intermolecular forces of attraction becomes weak due to splitting of milk proteins.

System 2 : (Milk + Water + Sodium Carbonate- Na_2CO_3)

As the concentration of sodium carbonate (Na_2CO_3) increases from 2 wt % to 10 wt %, the values of density, viscosity, ultrasonic velocity, acoustical impedance, surface tension, relaxation time and relative association increases nonlinearly **shown in data of Table 2** while the values of adiabatic compressibility and intermolecular free length decreases nonlinearly. As Na_2CO_3 is salt, it ionizes in milk and water mixture and forms milk protein-Na_2CO_3 complex. Hence values of density, viscosity and ultrasonic velocity increases with respective to the pure milk which may be due to active interaction of ultrasound waves with the aqueous solution of milk protein-Na_2CO_3 complex and also decrease in adiabatic compressibility indicates the enhancement of the bond strength **.**

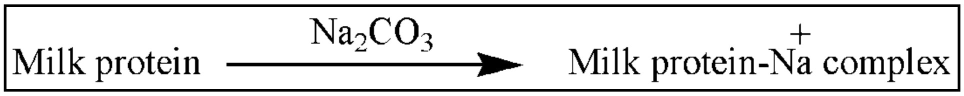


Figure 5- Structure of milk protein-Na_2CO_3 complex

The strong dipole-dipole interaction or hydrogen bond complex formation between the proteins molecules and other components present in the milk with Na_2CO_3 leads to the increase in ultrasonic velocity and decrease in adiabatic compressibility.

The higher value of acoustical impedance, relaxation time and relative association shows that milk protein-Na_2CO_3 complex interaction dominates over the solvent-solvent interactions while increasing values of surface tension shows the weak interaction which may be due to less interaction between hydrophobic fats and Na_2CO_3 present in milk-water mixture**.**

The value of adiabatic compressibility and intermolecular free length decrease nonlinearly which is showing the presence of strong solute-solvent interactions in the mixture and due to this structural rearrangement is also affected. The value of adiabatic compressibility and intermolecular free length increases up to addition of 4% of Na_2CO_3 and then decreases nonlinearly up to 10% addition of Na_2CO_3. This is due to weak interaction of milk protein-Na_2CO_3 complex and after addition of more and more $Na_2CO_{3,}$ strong complex is formed and more is the interaction. Hence stronger is the interaction which gives rise to stronger association**.**

System- 3 : (Milk + Water + Sodium- bi-carbonate - $NaHCO_3$)

From the **Table 3**, it is seen that as the concentration of $NaHCO_3$ increases from 2 wt % to 10 wt %, the values of density, viscosity, ultrasonic velocity, acoustical impedance, surface tension, relaxation time and relative association increases nonlinearly while the values of adiabatic compressibility and intermolecular free length shows irregular behaviour.

The density, viscosity and ultrasonic velocity of mixture increases nonlinearly with increasing concentration of $NaHCO_3$ which may be due to active interaction of ultrasonic waves with the aqueous solution of milk protein-$NaHCO_3$ complex **(Figure 6).** The increase in the values of density, viscosity and ultrasonic velocity also may be due to the cohesive forces and strong interaction and thus molecular association is responsible for the observed values**.**

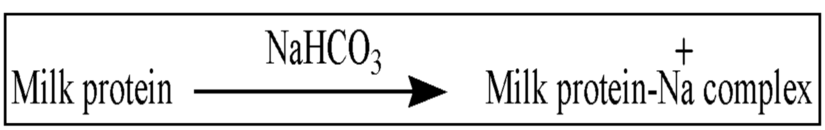


Figure 6- Structure of milk protein-$NaHCO_3$ complex

The value of acoustical impedance, relaxation time, relative association and surface tension increases nonlinearly which may be due to weak interaction initially and strong interaction after 2 wt % addition of $NaHCO_3$ in milk-water mixture. The graph of relative association verses concentration of $NaHCO_3$ concludes that more and more interaction among the components and chances of complexation is enhanced **.**

The nonlinear behaviour of adiabatic compressibility and intermolecular free length indicates weak interaction at lower concentration while after 6 wt %, the interaction becomes strong which may be due to colloidal formation of milk components in the milk-water mixture**.**

System- 4 : (Milk + Water + Oxalic acid $(COOH)_2$)

The trends of all the acoustical parameter related to the Milk + Water + Oxalic acid $(COOH)_2$ system are represented in the Table 4 .

It is found that the value of density and viscosity increases nonlinearly with increasing concentration of oxalic acid from 2 wt % to 10 wt %. This increase in density and viscosity may be due to addition of oxalic acid which causes the medium to be denser, while the value of ultrasonic velocity increases up to 6 wt % addition of oxalic acid but for higher concentration ultrasonic velocity decreases. The initially increase in ultrasonic velocity may be due to higher interaction of milk proteins with oxalic acid forming micelle i.e. coagulated proteins (**Figure 7)** in the suspension but later decrease in ultrasonic velocity may be weakening of interaction due to higher concentration of oxalic acid**.**

The value of acoustical impedance and relative association increase nonlinearly after 2% concentration of oxalic acid moiety. This is because of stronger interaction of milk proteins and oxalic acid molecules which suppressed the solute-solute and solvent-solvent interaction**.**

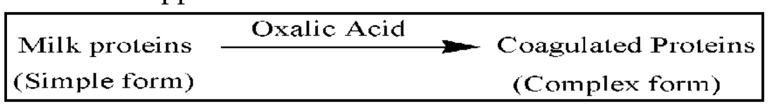


Figure 7- Reaction of Oxalic acid and Milk proteins

While the values of adiabatic compressibility, intermolecular free length, surface tension, relaxation time **Table 4** shows nonlinear behaviour. This nonlinear nature may be due to more concentration of oxalic acid which affects the nature of solute and solvent. From the data of intermolecular free length it is seen that after 6 wt % addition of oxalic acid, the interaction becomes weak as the value continuously increasing. The intermolecular free length increases on decrease of ultrasonic velocity and vice-versa.

System-5 : (Milk + Water + Urea $(CO(NH_2)_2)$)

It is seen that there is nonlinear increase in values of density, viscosity, ultrasonic velocity, acoustical impedance, relaxation time, surface tension and relative association and decrease in the values of adiabatic compressibility and intermolecular free length with increasing concentration of urea from 2 wt % to 10 wt % which are represented by the **Table 5**.

Addition of urea to the milk and water mixture which gives the foam to the milk (**Figure 8).** So due to the addition of urea to the milk, the medium becomes more and more dense and viscous. This may causes the increase in density, viscosity and ultrasonic velocity which shows the strong association and dipole-dipole interaction between the component molecules present in the Milk-Water-Urea mixture **.**

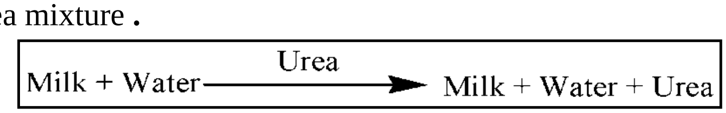


Figure 8 - Reaction of Urea and Milk

The value of acoustical impedance, relaxation time, relative association and surface tension show that all these values increases nonlinearly which may be due to weak interaction at initially and strong interaction after 2 wt % addition of urea in milk-water mixture. The graph of relative association verses concentration of urea suggests that more and more interaction among the components molecules observed **.**

The value of adiabatic compressibility and intermolecular free length decrease nonlinearly which shows the presence of strong and significant interactions in the mixture**.**

System- 6 : (Milk + Water + Melamine ($C_3H_6N_6$))

From the **Table 6** it is observed that as the concentration of melamine increases from 2 wt % to 10 wt %, the value of density, viscosity, ultrasonic velocity, acoustical impedance, surface tension, relative association and relaxation time increases while values of adiabatic compressibility and intermolecular free length decreases nonlinearly.

Due to addition of melamine to the milk and water mixture, the protein molecules present in the milk forms complex with melamine forming strong intermolecular hydrogen bonding (**Figure 9).** So medium becomes highly denser and viscous. This may causes the increase in density, viscosity and ultrasonic velocity which shows the significant association and strong dipole-dipole interaction between the milk components and melamine moiety present in the Milk-Water-melamine mixture**.**

$$\text{Milk + Water} \xrightarrow{\text{Melamine}} \text{Milk-Water-Melamine complex}$$

Figure 9- Reaction of Melamine and Milk

The higher value of acoustical impedance, relaxation time and relative association shows that milk protein-melamine complex interaction dominates over the solvent-solvent interactions while increasing values of surface tension shows the weak interaction due to hydrophobic end of fats present in the milk. The values of adiabatic compressibility and intermolecular free length decrease nonlinearly which shows the presence of strong solute-solvent interactions in the mixture**.** The relaxation time increase continuously as the concentration of melamine increase which indicates that there is small time lag i.e. in the range of 10^{-13} seconds between the collapse of old atmosphere and formation of new atmosphere and during this period, the ion is said to be relaxed.

System-7 : (Milk + Water + Formaline (HCHO))

It is seen that there is nonlinear increase in values of density, viscosity, ultrasonic velocity, acoustical impedance, relaxation time, surface tension and relative association and decrease in the values of adiabatic compressibility and intermolecular free length with increasing concentration of formaline from 2 wt % to 10 wt % which are represented by **Table 7.** Interaction of formaline with milk is given in **Figure 10.**

$$\text{Milk + Water} \xrightarrow{\text{Formaline}} \text{Milk + Water + Formaline}$$

Figure 10- Reaction of Formaline and Milk

As the concentration of formaline increases, the value of density and viscosity increases due to nature of medium to become more denser and property of formaline to form strong hydrogen bond with water present in the milk **.** The strong dipole-dipole interaction or hydrogen bond complex formation between the proteins molecules and other components present in the milk with Na_2CO_3 leads to the increase in ultrasonic velocity and decrease in adiabatic compressibility**.**

The data from **Table 7** shows the value of acoustical impedance, relaxation time, relative association and surface tension increases nonlinearly which may be due to weak interaction at initially and shows strong interaction after 2 wt % addition of formaline in milk-water mixture.

The relaxation time increase continuously as the concentration of formaline increases which shows that there is time where ions present in the mixture is said to be more relaxed. The value of adiabatic compressibility and intermolecular free length nonlinearly which shows the presence of strong solute-solvent interactions in the mixture**.**

Conclusion:

On adding additives such as water, sodium carbonate ($Na_2CO_{3)}$, sodium-bi-carbonate ($NaHCO_{3)}$, oxalic acid $(COOH)_2$, urea $(CO(NH_2)_2$, melamine ($C_3H_6N_6$) and formaline (HCHO) in the pure cow milk, it is observed that the values of density, viscosity and ultrasonic velocity of pure milk changes i.e. increases linearly with respective to that of the pure milk. The increase in the values of parameters on addition of various additives to pure milk may be due to increase in solute-solvent interactions.

The data produced in this work and the results obtained can be used as reference to detect the adulterant and indentify the various additives used as adulterant in locally available milk and different branded milk.