



# Extraction, Characterization Of Saponin From Rice Husk And The Study Of Interaction(Mixed Micelle) With Anionic Surfactant SLS.

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**Abstract:** The Saponin was extracted from Rice Husk through various steps of procedure: Extraction with Ethanol, Re-extraction, Separation of Aqueous and Organic Phase, Evaporation, Drying and Weight Determination: The weight of obtained Saponin is 1730 mg and % yield of Saponin is 8.65 %. Characterization of Physical Property was done by Determination of Surface Tension and CMC by using various analytical techniques i.e. Stalagmometer, Conductivitymeter, Absorbance by Spectrophotometer. The CMC of Saponin is found 40 mM and surface tension is 59.29 mN/m. The CMC of SLS is found 8.0 mM and surface tension is 32.55 mN/m. The Study of Anionic-Nonionic mixed surfactant system: In present investigation, the interaction between Sodium Lauryl Sulphate (SLS) as an anionic surfactant and Saponin as a nonionic surfactant is carried out in aqueous medium. Saponin is capable to reduce CMC of ( Anionic Surfactant-SLS) and as a result CMC of mixed Surfactant systems were also reduced up to 0.6 mM. As the ratio of Saponin is increasing in the mixed Surfactant systems, the CMC of mixed micelles are found reducing: The CMC of mixed Surfactant 1:9 (Saponin + SLS) system is found 1.5 (mM) and Surface tension is found 62.40 mN/m. The CMC of mix Surfactant 3:7 (Saponin + SLS) system is found 0.9 (mM) and Surface tension is found 58.50 mN/m. The CMC of mix Surfactant 5:5 (Saponin + SLS) system is found 0.6 (mM) and Surface tension of water is also found reducing up to 50.59 mN/m.

**Index Terms:** % yield, CMC, Surface Tension, SLS, mixed surfactant system, mixed micelle.

## 1. INTRODUCTION

The term “surfactant” comes from “surface active agents”, which are molecules that adsorb on the water–surface interface and reduce water’s surface tension to enhance the cleaning of surfaces.[1,2] They are also known as amphiphiles because they have polar heads, also known as hydrophilic heads, that have an attraction for polar solvents, and nonpolar tails, also known as hydrophobic tails.[2,3] The molecular structures of these molecules help reduce the cohesive forces between water molecules, resulting in the lowering of surface tension .[1-5] They possess other qualities that allow them to be used in applications other than lowering surface tension [6,7] such as emulsifiers, [8-11] foaming agents, [12-16] corrosion inhibitors, [17-21] and antistatic agents. [22-26]

There are many negative environmental consequences of using synthetic surfactants, including their high levels of toxicity and poor biodegradability. These materials have a negative impact on wastewater treatment as well as aquatic microbial populations, fish and other aquatic life, and plant photochemical energy conversion efficiency.[27] With over 15 million tons of surfactants used worldwide each year and an estimated 60% of them ending up in the aquatic environment, it is urgently necessary to find substitutes that have fewer environmental impacts.[28-30] The origins and natural uses of biosurfactants are discussed, low toxicity and biodegradability. Surfactants are amphiphilic molecules, designed during the second world war as a substitute for the then existing cleansing agent like soap. Surfactants, now-a-days have become an essential household commodity especially for laundry purpose and are known as 'detergents'. The hydrophobic part of the surfactant is usually a hydrocarbon chain comprising of more than eight carbon atoms including both aliphatic or aromatic moieties, while the hydrophilic head usually consists of groups such as sulfate, sulfonate, polyoxyethylene, polyether, phosphate, carboxylate, etc.

In response to increasing natural surfactant demand and environmental concerns, natural plant-based surfactants have been replacing synthetic ones. Saponins belong to a class of plant metabolites with surfactant properties that are widely distributed in nature. They are eco-friendly because of their natural origin and biodegradable.

To date, many plant-based saponins have been investigated for their surface activity. An overview of Saponins with a particular focus on their surface-active properties have been studied. Works published in the past few decades, which report better surfactant relevant properties of Saponins than synthetic ones, were extensively studied.

The investigations on the potential surfactant application of Saponins are also documented. Moreover, some biological activities of Saponins such as antimicrobial activity, antidiabetic activity, adjuvant potentials, anticancer activity, and others are reported. Plants rich in Saponins are widely distributed in nature, offering great potential for the replacement of toxic synthetic surfactants in a variety of modern commercial products and these Saponins exhibit excellent surface and biological activities. New opportunities and challenges associated with the development of Saponin-based commercial formulations in the future are also discussed in detail. [31]

## II. MATERIAL AND METHODS

### 2.1 MATERIALS

SLS: Sodium Lauryl Sulphate, ethanol, diethyl ether, n-butanol, distilled water.

Sample: Asian Rice Peels

Sample Collection: Asian Rice Peels-20 gm was collected from Olpad, Surat.



Fig 2.1: Sample of Rice Peels

### 2.2 Determination of Total Saponin:

#### Step 1: Extraction with Ethanol

The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol were added. Samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C.

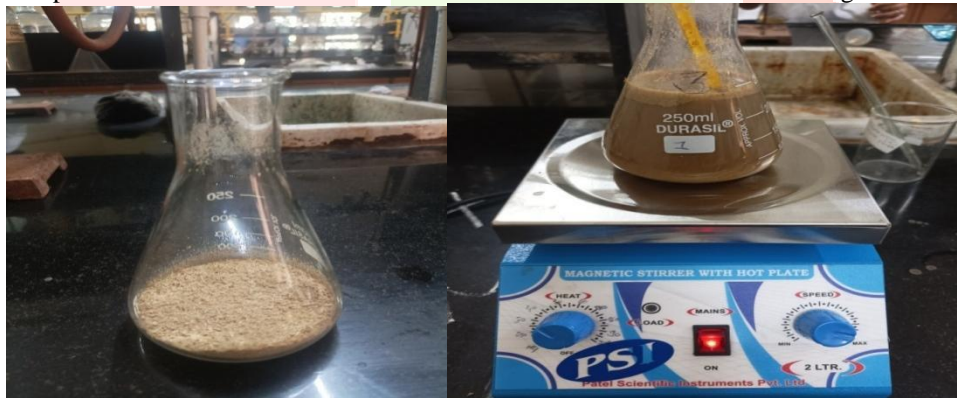


Fig 2.2.1 : Extraction with Ethanol

#### Step 2: Re-extraction

The mixture was filtered and the residue was re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 mL beaker.



Fig 2.2.2 : Re-extraction

#### Step 3: Separation of Aqueous and Organic Phase:

40 mL combined extract was taken into Separatory funnel and 20 mL of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride.



Fig 2.2.3 : Separation of Aqueous and Organic Phase

#### Step 4 : Evaporation, Drying and Weight Determination:

The above treated extract was heated in a water bath. After evaporation the same was dried in the oven to a constant weight; the Saponin content was calculated.



Fig 2.2.4 : Dry Saponin Yield

### 2.3 Characterization of Physical Property:[32]

#### 2.3.1 Determination of Surface Tension:

Surface tension was measured using a Stalagmometer at 30°C by counting number of drops falling from the upper mark to lower mark. Surface tension ( $\gamma$ ) was calculated by using the relationship.

$$\gamma = \frac{\text{No of drops of solvent (N}_0\text{)}}{\text{No of drops of sample (n)}}$$

The CMC was taken as the break point or intersection of straight line drawn through data representing the limiting surface tension above the CMC and the linear slope below the CMC. The reproducibility of the surface tension was up to  $\pm 1\%$ .

#### 2.3.2 Determination of Conductivity :

A digital conductivity meter (Equiptronic India) and a dipping type conductivity cell with platinized electrode was used for measuring the conductance of the surfactant solutions. All the data were obtained by direct concentration runs. i.e. solutions of the desired concentration were prepared by diluting the solution with water into clean dry cell and conductance was measured. Adequate time was allowed to attain the equilibrium for surfactant solutions. The temperature was maintained using a thermostatic bath at  $30 \pm 1^\circ\text{C}$ . The reproducibility of the conductance reading was  $\pm 0.1\%$ . The CMC was taken as the break point in Conductance.

#### 2.3.3 Determination of $\lambda$ max and Absorbance:

$\lambda$  max : 370 nm is Obtained for Saponin with help of Spectrophotometer (Equip-Tronics-EQ-824) UV-Visible Double beam Range – 190 nm to 1100 nm). The Absorbance was taken for all prepared aqueous solutions. The CMC was taken as the break point in Absorbance.

### III. RESULTS AND DISCUSSION:

- The Weight of Rice Husk Sample taken= 20,000 mg.
- The Weight of Saponin Obtained = 1730 mg.
- % Yield = (Obtained Weight/Sample Weight) x 100
- % Yield =  $(1730/20,000) \times 100$
- % Yield =  $0.0865 \times 100 = 8.65\%$
- % Yield of Saponin obtained is 8.65 %.

#### Foam Test :

Small quantity of the extract was shaken with 2 ml of water.

Persistence of foam produced for 10 minutes indicated the presence of Saponins.

#### Determination of CMC:

Determination of CMC by Measurement of Surface Tension and Conductance of Saponin, SLS and their mixtures in varying mole-fractions (0-1) are shown in Table 3.1 to 3.14 and Fig. 3.1 to 3.12. The general trend found for all the mixed system and individual surfactant is that the surface tension is decreasing with increasing concentration of surfactants and conductance is increasing with increasing concentration of surfactants.



### 3.1: Determination of CMC by Measurement of Surface Tension of Saponin

Table 3.1: Determination of CMC by Measurement of Surface Tension of Saponin

No.	Concentration (gm/l)	Surface Tension (mNm <sup>-1</sup> )
1	0.01	65.03
2	0.02	63.00
3	0.03	61.09
4	0.04	59.29
5	0.05	59.29
6	0.06	59.29
7	0.07	59.29
8	0.08	59.29

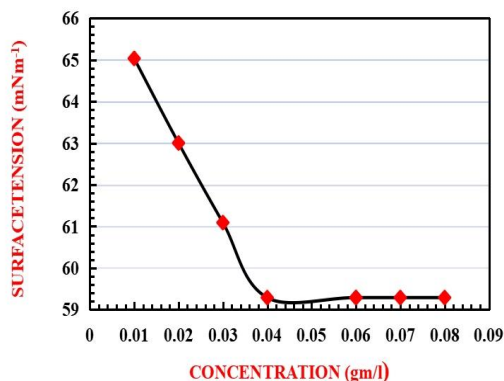


Fig 3.1: Determination of CMC by Measurement of Surface Tension of Saponin

- ❖ The CMC of Saponin is found 0.04 (gm/L) by Surface Tension Measurements.
- ❖ The Surface Tension of Saponin is found 59.29 (mNm<sup>-1</sup>) at CMC.

### 3.2: Determination of CMC by Measurement of Conductance of Saponin

Table 3.2: Determination of CMC by Measurement of Conductance of Saponin

No	Concentration (gm/l)	Conductance (ΩCm <sup>-1</sup> )
1	0.01	0.200
2	0.02	0.212
3	0.03	0.222
4	0.04	0.254
5	0.05	0.366
6	0.06	0.426
7	0.07	0.436
8	0.08	0.445

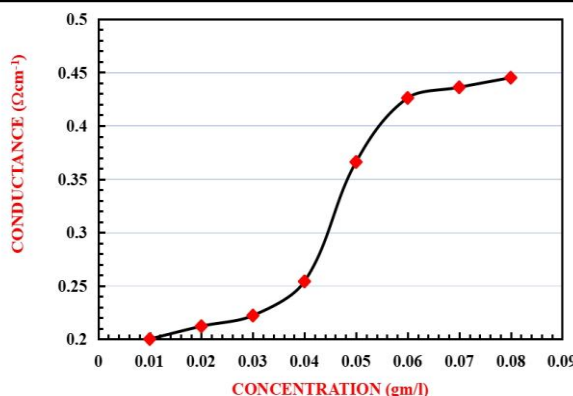


Fig 3.2: Determination of CMC by Measurement of Conductance of Saponin

- ❖ The CMC of Saponin is found 0.04 (gm/l) by Conductance Measurements.
- ❖ The Conductance of Saponin is 0.254 (ΩCm<sup>-1</sup>) at CMC.

### 3.3 Determination of Saponin λ max:

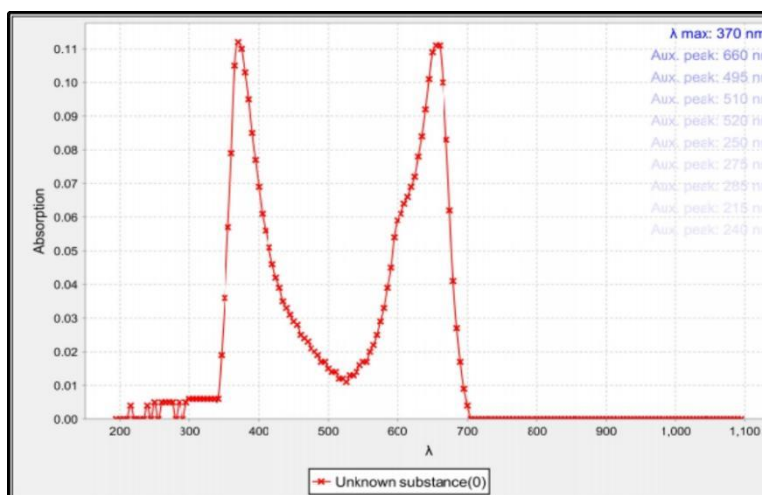


Fig 3.3: Saponin λ max Autoscanning with the help of Spectrophotometer

- ❖ λ max : 370 nm is obtained for Saponin with help of Spectrophotometer (Equip- Tronics-EQ-824) UV-Visible Double beam Range – 190 nm to 1100 nm)

### 3.4: Determination of CMC by Measurement of Absorbance of Saponin

Table: 3.4: Determination of CMC by Measurement of Absorbance of Saponin

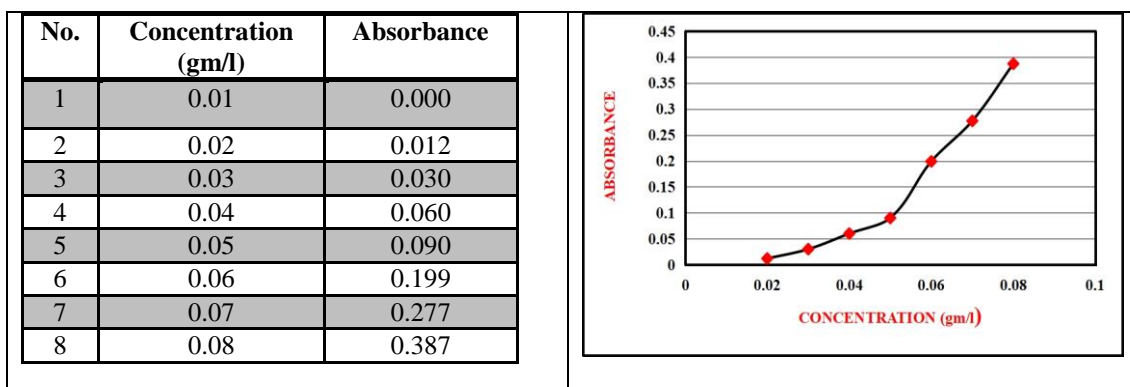


Fig 3.4: Determination of CMC by Measurement of Absorbance of Saponin

- ❖ The CMC of Saponin is found 0.04 (gm/l) by Absorbance Measurements.
- ❖ The CMC of Saponin is found 0.04 (gm/l) and confirmed by testing of three physical properties i.e. Surface Tension, Conductance and Absorbance Measurements.

### 3.5: Determination of CMC by Measurement of Surface Tension of SLS

Table 3.5: Determination of CMC by Measurement of Surface Tension of SLS

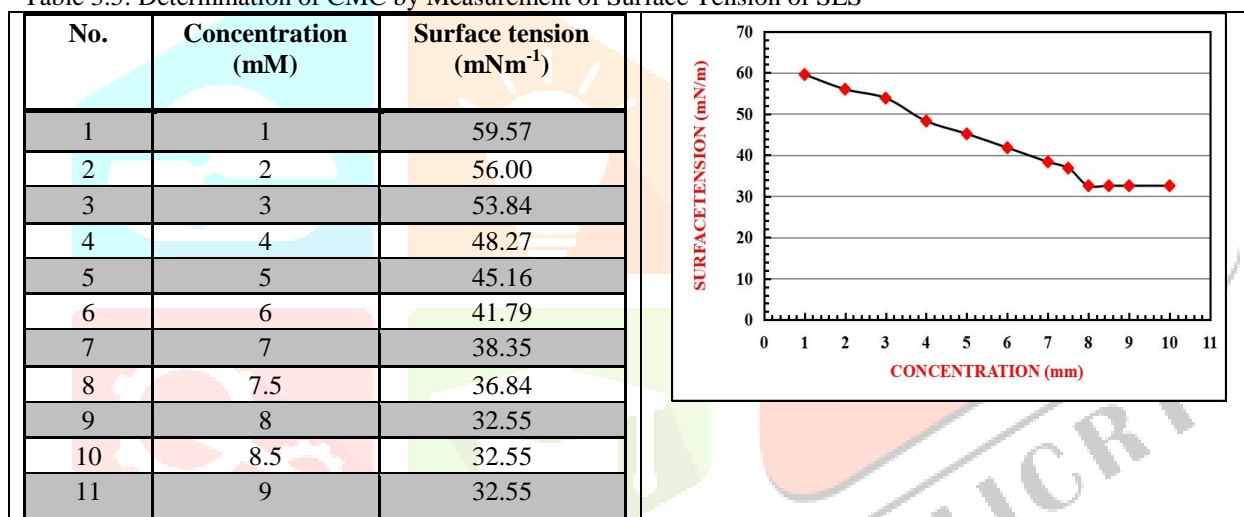


Fig 3.5: Determination of CMC by Measurement of Surface Tension of SLS

- ❖ The CMC of SLS is found 8.0 (mM) by Surface Tension Measurements.
- ❖ The Surface Tension of SLS is 32.55 (mNm<sup>-1</sup>) at CMC.

### 3.6: Determination of CMC by Measurement of Conductance of SLS

Table 3.6: Determination of CMC by Measurement of Conductance of SLS

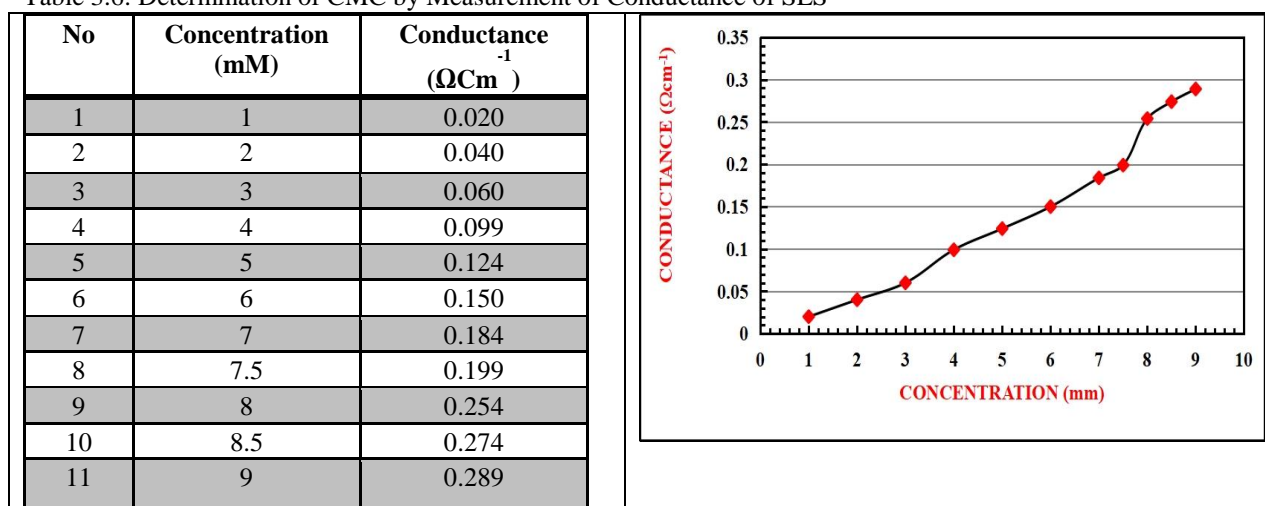


Fig 3.6: Determination of CMC by Measurement of Conductance of SLS

- ❖ The CMC of SLS is found 8.0 (mM) by Conductance Measurements.
- ❖ The Conductance of SLS is  $0.254 (\Omega\text{cm}^{-1})$  at CMC.

Determination of CMC by Measurement of Surface Tension and Conductance of (Saponin + SLS) in different mole fraction ratio in aqueous medium is carried out as following:

Table 3.7: Determination of CMC by Measurement of Surface Tension of 1:9 Solution (Saponin + SLS)

No	Concentration (mM)	Surface Tension ( $\text{mNm}^{-1}$ )
1	0.6	72.00
2	0.8	69.33
3	0.9	66.85
4	1.0	64.55
5	1.5	62.40
6	2.0	62.40

Fig 3.7: Determination of CMC by Measurement of Surface Tension of 1:9 solution (Saponin+ SLS )

- ❖ The CMC of 1:9 solution (Saponin + SLS) is found 1.5 (mM) by Surface Tension Measurements.
- ❖ The Surface Tension of 1:9 solution (Saponin+SLS) is found  $62.40 (\text{mNm}^{-1})$  at CMC.

Table 3.8 : Determination of CMC by Measurement of Conductance of 1:9 solution (Saponin + SLS)

No.	Concentration (mM)	Conductance ( $\Omega\text{cm}^{-1}$ )
1	0.06	0.130
2	0.08	0.136
3	0.1	0.140
4	0.5	0.156
5	0.6	0.160
6	0.8	0.170
7	0.9	0.180
8	1.0	0.189
9	1.5	0.209
10	2.0	0.209

Fig 3.8: Determination of CMC by Measurement of Conductance of 1:9 solution (Saponin + SLS)

The CMC of 1:9 Solution (Saponin + SLS) is found 1.5 (mM) and by Conductance Measurements.

The Conductance of 1:9 solution (Saponin + SLS) is found  $0.209 (\Omega\text{cm}^{-1})$  at CMC.

Table 3.9: Determination of CMC by Measurement of Surface Tension of 3:7 solution (Saponin + SLS)

No	Concentration (mM)	Surface Tension ( $\text{mNm}^{-1}$ )
1	0.5	72.00
2	0.6	69.33
3	0.8	64.55
4	0.9	58.50
5	1.0	58.50
6	1.5	58.50
7	2.0	58.50

Fig 3.9: Determination of CMC by Measurement of Surface Tension of 3:7 solution (Saponin + SLS)

- ❖ The CMC of 3:7 solution (Saponin + SLS) is found 0.9 (mM) by Surface Tension Measurements.
- ❖ The Surface Tension of 3:7 solution (Saponin + SLS ) is found  $58.509 (\text{mNm}^{-1})$  at CMC.

Table 3.10: Determination of CMC by Measurement of Conductance of 3:7 solution (Saponin + SLS)

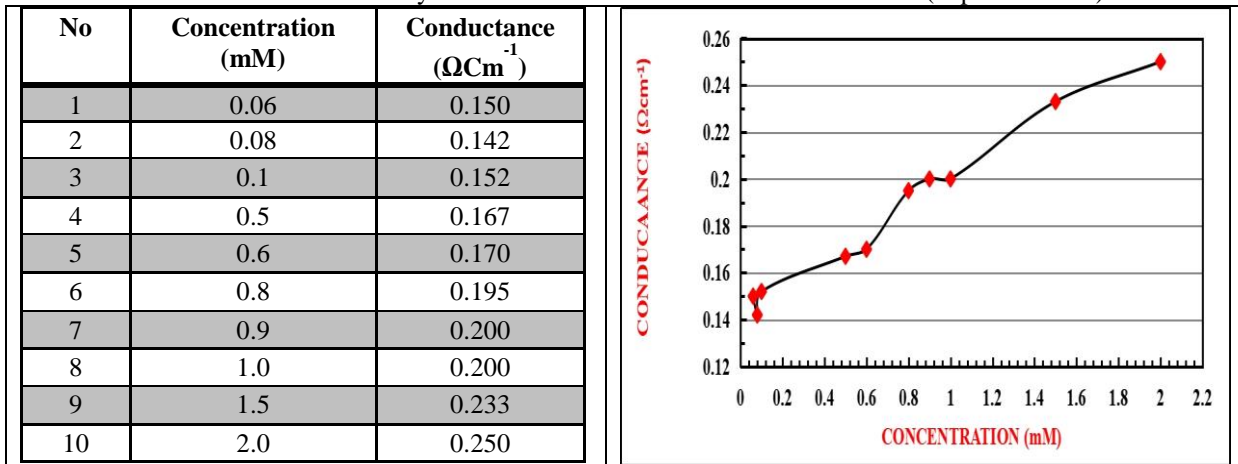


Fig 3.10: Determination of CMC by Measurement of Conductance of 3:7 solution (Saponin + SLS)

- ❖ The CMC of 3:7 solution (Saponin + SLS) is found 0.9 (mM) by Conductance Measurements.
- ❖ The Conductance of 3:7 Solution (Saponin +SLS) is 0.200 ( $\Omega\text{cm}^{-1}$ ) at CMC.

Table 3.11 : Determination of CMC by Measurement of Surface Tension of 5:5 solution (Saponin + SLS)

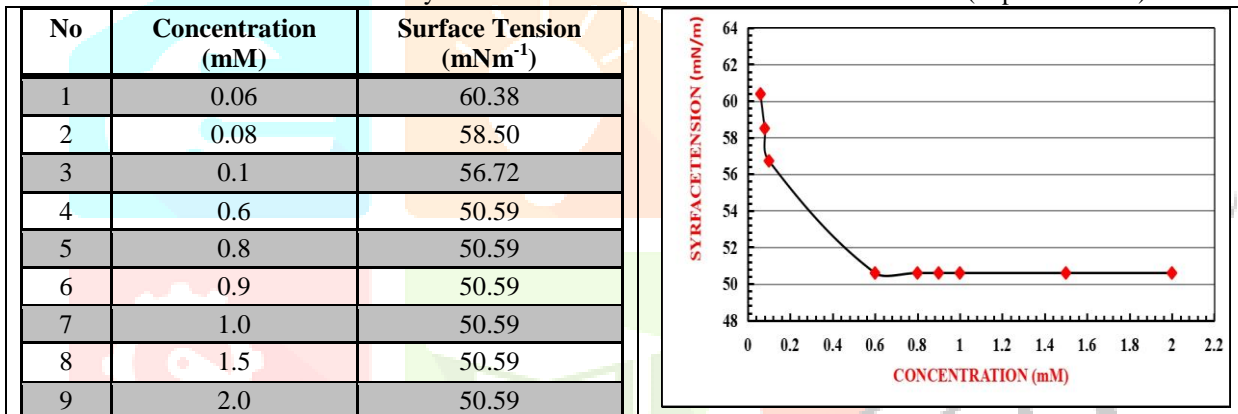


Fig 3.11: Determination of CMC by Measurement of Surface Tension of 5:5 solution (Saponin + SLS)

- ❖ The CMC of 5:5 solution (Saponin + SLS) is found 0.6 (mM) by Surface Tension Measurements.
- ❖ The Surface Tension of 5:5 solution (Saponin SLS) is found 50.59 ( $\text{mNm}^{-1}$ ) at CMC.

Table 3.12: Determination of CMC by Measurement of Conductance of 5:5 solution (Saponin + SLS)

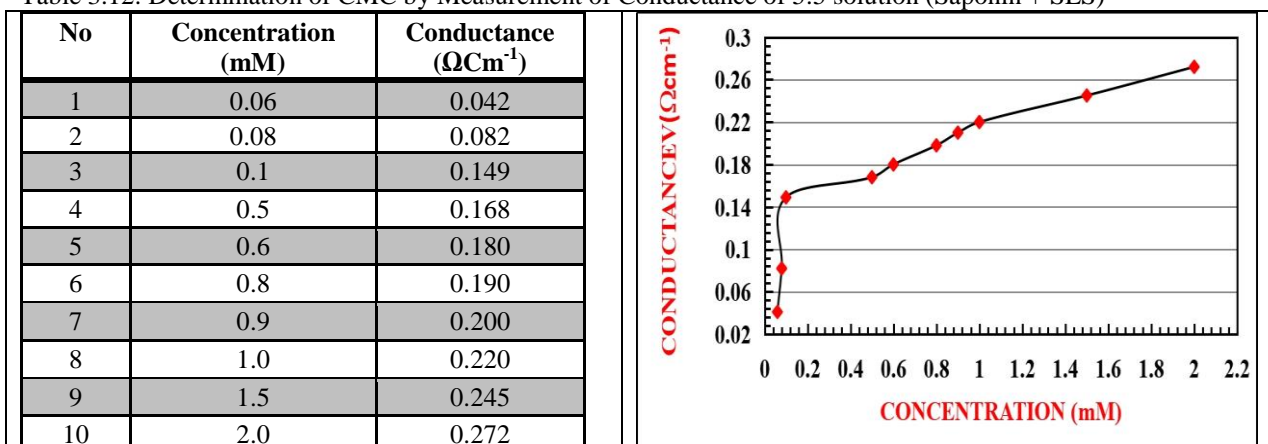


Fig 3.12: Determination of CMC by Measurement of Conductance of 5:5 solution (Saponin + SLS)

- ❖ The CMC of 5:5 solution (Saponin + SLS ) is found 0.6 (mM) by Conductance Measurements.
- ❖ The Conductance of 5:5 solution (Saponin + SLS ) is 0.180 ( $\Omega\text{cm}^{-1}$ ) at CMC.

Table 3.13: Interaction of Mixed Surfactant system:(Natural+Synthetic) - (Saponin+ SLS)

No.	Mixed Surfactant Systems	Determination of CMC By Physical Property		Physical Property Value of Surfactant System	
	Nonionic+Anionic	Surface Tension (mN/m)	Conductance ( $\Omega\text{Cm}^{-1}$ )	Surface Tension (mN/m)	Conductance ( $\Omega\text{Cm}^{-1}$ )
1	Distilled Water			72.00	0.500
		CMC (mM)	CMC (mM)		
2	Saponin+ SLS (0:1)	8.0	8.0	32.55	0.200
3	Saponin+ SLS (1:9)	1.5	1.5	62.40	0.209
4	Saponin+ SLS (3:7)	0.9	0.9	58.50	0.195
5	Saponin + SLS (5:5)	0.6	0.6	50.59	0.180
6	Saponin+ SLS (1:0)	40	40	59.29	0.250

Table 3.14: CMC of Anionic-Nonionic - Mixed Surfactant Systems

No	Saponin + SLS (mole-fraction) ratio	CMC (mM)By (Surface tension)	CMC (mM)By (Conductance)
1	0:1	8.0	8.0
2	1:9	1.5	1.5
3	3:7	0.9	0.9
4	5:5	0.6	0.6
5	1:0	40	40

#### Anionic-Nonionic Mixed Surfactant Systems: Synergism

- ❖ In present investigation, interaction between mixture of sodium Lauryl Sulphate (SLS) as an anionic surfactant and Saponin as a nonionic surfactant is studied.
- ❖ Saponin is capable to reduce CMC of (Anionic Surfactant-SLS) and as a result CMCs of mixed Surfactant systems were also reduced up to 0.6 mM.
- ❖ As the ratio of Saponin is increasing in the mixed Surfactant systems, the CMC of mixed micelles were found reducing.
- ❖ The CMC of mix Surfactant 1:9 Solution (Saponin + SLS) is found 1.5 (mM).
- ❖ The CMC of mix Surfactant 3:7 Solution (Saponin + SLS) is found 0.9 (mM).
- ❖ The CMC of mix Surfactant 5:5 Solution (Saponin + SLS) is found 0.6 (mM).

Critical micelle concentrations (CMC) were obtained from tensiometric studies on several binary surfactant mixtures (anionic-anionic, cationic-cationic, anionic-nonionic, and cationic-nonionic) in water at different mole fractions (0–1). The composition of mixed micelles and the interaction parameter  $\beta$ , evaluated from the CMC data for different systems using Rubingh's theory, are discussed. [32]

#### IV. CONCLUSION :

Above a particular concentration, surfactants form aggregates (micelles) in solution and these indirectly affect interfacial properties. The micelle formation determines monomer activities and hence surfactant adsorption at interfaces. The formation of mixed micelles, which contain more than one type of surfactant, is especially important since most commercially available surfactants are, in fact, mixtures. The superior properties of mixed surfactants as compared to a single surfactant and their relatively lower production cost have been brought out. The superiority in performance of mixtures of surfactants is attributed to the synergistic interaction among the surfactants. Interaction in mixtures of surface active molecules at the solution/air interface and in micelles and other aggregate have practical importance and have



received theoretical attention and experimental studies. Both critical micelle concentration (CMC) and distribution of surfactants between the micellar and aqueous phases play a very important role in describing the behaviour of these binary surfactant solutions.

The remarkable development experienced in non-ionic surfactants as important components in detergent formulations demands an understanding of their properties, not only as a separate entity but also as binary mixtures with other types of surfactants especially anionic surfactants. The formation of mixed micelles in the aqueous solution of mixtures of surfactants characterised by the values of CMC resulting from the surface-tension versus total concentration curves proves most interesting. The CMCs of mixtures of similarly structured ionic surfactants or nonionic surfactants, can be predicted by assuming that the ideal solution theory is obeyed in micellar phase. However, for mixtures of ionic and nonionic surfactant solutions, the CMC values obtained are much lower than those predicted by ideal solution theory. Rubingh successfully explained this non-ideal behaviour by using the phase separation model of micellization and regular solution approximation and derived equations, showing the interaction between the two surfactants. M. J. Rosen also derived expressions (relationships) for synergism in surface-tension reduction efficiency, mixed micelle formation and surface tension reduction effectiveness in aqueous solution of mixed surfactants based on non-ideal solution theory.

Anionic-Nonionic Mixed Surfactant Systems are useful in Synthesis of Detergent and Surfactant- consuming less amount of both the Anionic and Nonionic Surfactants – making it cost effective. From the present study, it is found that **The Optimum Ratio is 5:5 for Mixed Micelle of (Saponin + SLS) has lowest CMC value of 0.6 (mM)**. As Saponin-Nonionic Surfactant is extracted from Rice Husk - A Natural Resource/Agriculture solid waste – being useful here as a Raw Material, with minimum use of Chemical Reagents- Making it cost effective and Environment Friendly Sustainable Technology.

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