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Micellar Characterisation of Saponin from *Sapindus Mukorossi*

The micellar characteristics of a non-ionic, natural surfactant, saponin obtained from the soapnut tree, *Sapindus mukorossi*, were studied in aqueous solution. Critical micelle concentration of *Sapindus* saponin determined using conductivity measurements and UV absorption studies was 0.045 wt%. Increase in temperature and salt concentration led to decrease in the critical micelle concentration of *Sapindus* saponin. The critical micelle concentration was found to increase with increase in hardness of water and increase in pH. The micellar aggregation number was determined using cyclic voltammetry and was found to be between 13 and 21. The size of the *Sapindus* saponin micelles was determined using intrinsic viscosity measurements and was found to be independent of saponin concentration for concentrations above the CMC. Solubilisation of two types of crude oils and a vegetable oil was studied using micellar solubilisation technique. At lower concentrations of the surfactant, the micellar solubilisation of crude oils in saponin was better than synthetic surfactants like Triton X100[®] and SDS where as, the solubilisation of vegetable oil was better in synthetic surfactants.

Key words: *Sapindus* saponin, critical micelle concentration, solubilisation, natural surfactant

Mizellare Charakterisierung von Saponin aus *Sapindus Mukorossi*. Die mizellare Charakterisierung des nichtionischen natürlichen Tensids, Saponin, gewonnen aus dem Waschnussbaum, *Sapindus mukorossi*, wurde in wässriger Lösung untersucht. Die kritische Mizellbildungskonzentration von *Sapindus* Saponin wurde mittels Leitfähigkeitsmessung und UV-Absorptionsuntersuchungen zu 0,045 wt% bestimmt. Erhöhungen von Temperatur und Salzkonzentration führen zu einer Verringerung der kritischen Mizellbildungskonzentration von *Sapindus* Saponin. Durch Zunahme des Anteils an hartem Wasser und durch pH-Erhöhung steigt die kritische Mizellbildungskonzentration an. Die mizellare Aggregationszahl wurde mittels zyklischer Voltammetrie bestimmt und betrug zwischen 13 und 21. Die Größe der *Sapindus* Saponin Mizellen wurde durch intrinsische Viskositätsmessungen bestimmt und ist für Konzentrationen oberhalb der cmc unabhängig von der Saponin-Konzentration. Die Solubilisierung von zwei Rohölsorten und eines Pflanzenöles wurden unter Verwendung der mizellaren Solubilisierungstechnik untersucht. Bei niedrigeren Tensidkonzentrationen war die mizellare Solubilisierung der Rohöle in Saponin besser als die von synthetischen Tensiden, wie Triton X100[®] und SDS, wohingegen die Solubilisierung von Pflanzenöl in synthetischen Tensiden besser war.

Stichwörter: *Sapindus* saponin, kritische Mizellbildungskonzentration, Solubilisierung, natürliches Tensid

1 Introduction

Many naturally occurring surfactants have been attracting attention in the recent years from the viewpoint of ecologically adaptable and biologically safe materials with effective functional performance [1]. However, detailed studies of their physical and chemical properties are required for a better understanding of their behaviour and for their projected applications in pharmacology, detergents and in environ-

mental remediation. Examples of naturally occurring surfactants available are saponin, shellac, rosin, cholic acid, and liposaccharides [2]. Among these, the saponins are a class of large number of saponaceous substances produced by plants. Examples of plants, which have saponin, are soapnut, soybean [3], *Quillaja* bark [4] and *Fagonia indica* [5]. Soapnuts are obtained from the trees of *Sapindus mukorossi* and *Sapindus emarginatus*, found in India, Pakistan and other tropical and sub-tropical regions of the world. The outer pericarp of soapnuts contains 6–10 wt% of saponin depending on the weight of the fruit [6].

The outer pericarp of soapnuts has been traditionally used in India for fabric washing, bathing and in folk medicine due to the formation of lather or foam in water. However, there has not been much scientific study to understand its surfactant activities and micellar behaviour. There has been considerable amount of work carried out on the detergency and micellar activities of *Quillaja* saponin, which is also a naturally occurring saponin obtained from the bark of the tree, *Quillaja saponaria* found in Latin America. Bio-surfactants like *Quillaja* saponin are found to be effective in removing oil from contaminated soil [7]. It has been demonstrated to have a number of medicinal properties like, enhanced vaccine effectiveness, antitumour and antimicrobial activity [4], inhibition of hypercholesterolaemia in blood [8] etc. In the cholesterol extraction process, *Quillaja* saponin is shown to be an effective and non-toxic absorbent [9]. Recently, it has been shown that refined *Quillaja* extracts can suppress acid mist formation during copper electrowinning process [10]. *Quillaja* saponin was also found to be useful as a cost-effective process for the removal of heavy metals from incinerated municipal solid waste compared to the use of HCl and EDTA treatment [11]. Since the saponin obtained from soapnuts is not much different from that of *Quillaja* saponin in chemical structure, it may also exhibit similar properties. However, there has not been much study to understand the surfactant behaviour of *Sapindus* saponin. Preliminary studies by Komalapatti et al [6] using soapnut saponin (*Sapindus mukorossi*) indicated that the fruit extract could desorb and solubilise significant amount of hydrocarbons.

The main objective of the present study was to understand the micellar characteristics of *Sapindus* saponin and its ability to solubilise oils. The Critical Micelle Concentration (CMC), aggregation number and the size of micelles were determined to characterize the micellar behaviour. The effect of various parameters such as temperature, pH, salt concentration and hardness of water on the CMC of saponin was also investigated. Micellar solubilisation of two types of crude oils and a vegetable oil was carried out using saponin to examine its effectiveness in comparison to commercially available synthetic surfactants.

2 Materials and methods

2.1 Materials

The soapnut obtained from the species *Sapindus mukorossi*, found in Northern India, was used in the present study. Analytical grade ammonium sulphate (NH₄)₂SO₄ · 2H₂O, sodium

chloride (NaCl) and sodium dodecyl sulphate ($C_{12}H_{25}NaO_4S$) (SDS) were obtained from Merck Limited, Mumbai, India. Ferrocene ($C_{10}H_{10}Fe$), used in cyclic voltammetry studies was obtained from Merck, Germany. Potassium chloride (KCl), used as supporting electrolyte in cyclic voltammetry studies was obtained from SD Fine Chem Limited, Mumbai, India. Triton X100[®] was obtained from Sigma, India. Two grades of crude oils (Bombay High (BH) (specific gravity 0.8298, kinematic viscosity 2.77cs, pour point +12 °C, sulphur 0.2 wt%) and Forozan (specific gravity 0.8704, kinematic viscosity 9.3cs, pour point -18 °C, sulphur 2.3 wt%)) were obtained from Chennai Petroleum Corporation Limited, Chennai, India and the vegetable oil used in the study was refined sunflower oil (poly unsaturated linoleic acid, specific gravity 0.95, viscosity 33.3 cP) available commercially. All chemicals were used as received and all aqueous solutions were prepared using double distilled water.

2.2 Methods

Saponin was extracted from soapnut applying the method detailed by Rao et al [12]. The outer pericarp of the fruits were separated from the seeds and dried at 40 °C for 48 hours in an oven. It was ground to a fine powder and the water-soluble matter was extracted. This solution was centrifuged for 1 hour to separate water-insoluble matter. The extract was then treated with ammonium sulphate to precipitate the saponin. The saponin that separated on the top was skimmed off and dried at 40 °C. The ratio of the weight of dried saponin to the initial weight of pericarp taken gave the percentage of saponin extracted.

FT-IR, NMR and MALDI MS techniques were employed to analyse the basic structure and to get the molecular weight of *Sapindus* saponin. The FT-IR spectra were recorded at 25 °C using a Perkin Elmer1 FT-IR with a resolution of 1 cm^{-1} . NMR spectrum of *Sapindus* saponin solution was recorded at 23 °C using a JEOL GSX 400 NB FT-NMR spectrometer (400 MHz, 9.3 Tesla). Heavy water was used as solvent. MALDI TOF MS analysis was done on a Voyager-DE PRO Biospectrometry Workstation. Fluorescence spectral measurements were made on a Jobin Yvon Fluorolog-3-11 Spectrofluorimeter at 25 °C.

UV absorption spectra of *Sapindus* saponin solutions were recorded at 28 °C in a Cary 5E UV-spectrophotometer. Base line corrections were done before analysing the saponin solution. Double distilled water used for making the *sapindus* solutions, was used as the reference solution. Conductivity and pH measurements of aqueous solutions of saponin were made using a GENEI digital conductivity meter and Digital pH meter 7007 respectively. Conductivity measurements were made at 28 °C and pH measurements were done at 32 °C. Cells was calibrated using standard solutions before the conductivity and pH tests were conducted.

Surface tension was obtained from the contact angle between the surfactant drop and the glass substrate. Contact angle was measured using DigiDrop GBX instrument. A drop of saponin solution was carefully placed on glass plate and the equilibrium contact angle was measured. Contact angle measurements were carried out at 28 °C. Buffer tablets of different pH were used to prepare buffer solutions. These solutions were used to study the effect of varying pH on the CMC of aqueous saponin solution at 32 °C. The hardness of water is varied from 30 to 960 ppm. Water of known hardness (1200 ppm) was mixed with double distilled water (30 ppm) to get the required hardness.

All the electrochemical experiments were conducted using an ACM Gill Potentiostat in the three-electrode mode.

The working electrode was a platinum plate ($1\text{ cm} \times 1\text{ cm}$) and the auxiliary electrode was platinum, while the reference electrode was standard calomel electrode. The potential was scanned between -100 and 500 mV at the rate of 20, 60, and 120 mV sec^{-1} . The viscosities of aqueous *Sapindus* saponin solution were measured using an Ubbelohde viscometer. The effect of temperature on intrinsic viscosity was measured by immersing the viscometer in a temperature bath. Hardness of water was determined using the EDTA method.

Solubilisation experiments were carried out according to the method described by Urum et al. [7]. Saponin solution and crude oil were mixed for 24 hrs at constant speed and allowed to settle for 48 hrs. Saponin rich phase was collected and n-hexane was added for extraction. The contents were shaken for 30 mins and centrifuged for another 30 mins to separate the hexane and the surfactant solution. The centrifuged supernatant liquid was collected and its absorbance was measured using Cary 5E UV-spectrophotometer. Similar method was followed for the solubilisation of vegetable oil using saponin. The absorbance of vegetable and crude oils were measured at 300 and 400 nm respectively. From the absorbance values the concentration was calculated using a calibration curve, which is a plot of absorbance versus different concentrations of oil.

3 Results and discussion

3.1 Extraction

The percentage of water-soluble matter in the ground pericarp of soapnut was estimated as 33.2 wt% and the percentage of saponin extracted was 10.6 wt%. pH of the soapnut solution remained the same before and after extraction. However, the colour changed from reddish brown to pale yellow probably due to the removal of certain organic components

3.2 Spectroscopic analysis

The structure of the surfactants plays an important role in determining the micellar properties like size, shape, etc. [13]. Saponin, a non-ionic surfactant, is a glycoside. The hydrophilic part of saponin molecule consists of sugars such as D-glucose, D-xylose, L-arabinose, and L-rhamnose and glucuronic acid [14], while the hydrophobic portion of the saponin is comprised of sapindic acid and oleanolic acid [15]. Glucuronic acid is the only ionizable group of the molecule and the other acids are attached as ester bonds to the main structure [4].

The FT-IR analysis of *Sapindus* saponin showed signals for hydroxyl (polymeric) groups (3407 cm^{-1}), $-\text{CH}_2$ groups (2918 cm^{-1}), C=O stretching (1618 cm^{-1}), five member-ring ketone (1742 cm^{-1}) and $-\text{C}-\text{O}-\text{C}$ -groups (1044.91 cm^{-1}) [16].

^1H NMR spectrum of *Sapindus* saponin, showed signals for alkyl groups like methyl groups (δ 0.9, 1.1, and 1.2), anomeric protons and olifenic protons (δ 5.4), $-\text{OH}$ groups, and hydrogen directly attached to oxygen or nitrogen (δ 3.0 to 4.6) [16, 17]. The NMR spectra showed similarities to that of *Quillaja* saponin. From MALDI MS spectroscopy, the molecular weight of *Sapindus* saponin was found to be 1001.9. The reported molecular weight of saponin is between 719 and 1043 [5]. *Sapindus* saponin exhibits fluorescence with an excitation maximum at 345 nm and an emission maximum at 377 nm.

3.3 Critical Micelle Concentration (CMC)

There are various methods for the determination of CMC of a surfactant. In the present study conductivity measurements, UV absorbance and surface tension measurements were used

to determine the CMC of *Sapindus* saponin. Variation of conductivity and pH with varying concentration of saponin is shown in Fig. 1. Below the CMC, amphiphiles are present and the conductivity increases with increase in concentration. At the CMC, the rate of increase in conductivity reduces, indicating that amphiphiles start to aggregate and form micelles. From this method, the CMC of *Sapindus* saponin is found to be between 0.04–0.05 wt% where the slope of the conductivity versus concentration curve changes. It is reported that the increase in conductivity in the case of non-ionic *Quillaja* saponin is due to the hydrolysis of glucuronic groups present in *Quillaja* saponin [4]. The glucuronic group hydrolyses and gives carboxylic acid, which reduces the pH of the saponin solutions. The variation of pH as a function of concentration of *Sapindus* saponin is measured and is shown in Fig. 1. pH of the solution decreased with increasing saponin concentration, confirming the hydrolysis of glucuronic groups. In the case of non-ionic surfactants, like *Sapindus* saponin the hydrocarbon chain attraction is opposed by the requirements of hydrophilic groups for hydration and space. Therefore, the micellar structure is determined by an equilibrium between the repulsive forces among hydrophilic groups and the short-range attractive forces among the hydrophobic groups [13].

The non-ionic *Sapindus* saponin is found to absorb UV in the region of 290 to 240 nm with absorption maximum at 271 nm. This effect is seen only at very low concentrations, near the CMC. The absorbance (at 271 nm) of a series of samples with varying saponin concentrations is shown in Fig. 2. The slope of the curve changed abruptly at a concentration of 0.045 wt%. This was taken as the CMC of the *Sapindus* saponin. This is also in agreement with the earlier determination of CMC using conductivity measurements. The UV absorbance observed in *Sapindus* saponin is due to the presence of various natural chromophores [18]. The absorption maximum at 271 nm may be due to the presence of aromatic ring structure in *Sapindus* saponin [19].

Fig. 3 shows the variation of surface tension with saponin concentration at 28 °C. It is observed that the surface tension decreased up to 0.04 wt% saponin concentration and then remained constant. The concentration at which surface tension becomes a constant is considered as the critical micelle concentration. Surface tension measurements of saponin solution showed that the CMC is 0.04 wt%. The CMC values obtained using all the three methods are comparable and are in the experimental error range only. Hence, in the following discussions, the CMC of *Sapindus* saponin will be considered as 0.045 wt%. Tab. 1 compares the CMC of *Sapindus* saponin with other natural non-ionic surfactants such as *Quillaja* saponin [4], bile acid [20] and humic acid [21]. It is comparable to the CMC values of other natural surfactants. However, the CMC of *Sapindus* saponin and *Quillaja* saponin are well below those of synthetic surfactants like, Triton X100® [22], SDS [13] and CTAB [23]. This implies that micellar solubilisation would start at lower concentrations for these natural surfactants.

Generally, the CMC of saponin may vary depending upon its source. Natural products are known to have these variations due to the differences in the chemical and molecular structures depending on the source. This would change the hydrophobicity of the molecules and thus affect the CMC values. In general, the CMC values for non-ionic surfactants are one or two orders of magnitude less than ionic surfactants with the same hydrophobic group [13].

3.4 Effect of temperature and pH on the CMC

The effect of temperature on the CMC of saponin was studied between 25 and 60 °C. Temperature usually plays a

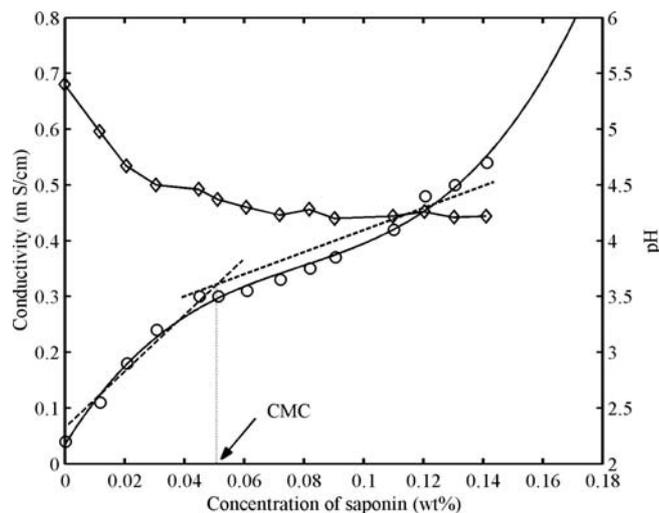


Figure 1 Variation of conductivity and pH with *Sapindus* saponin at various concentrations

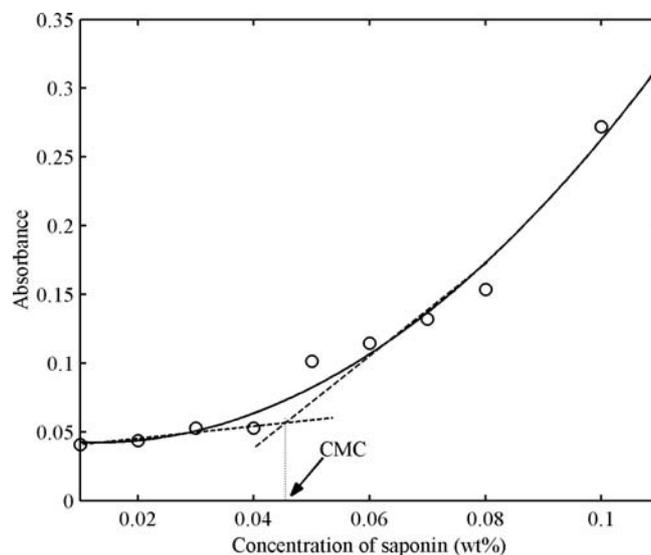


Figure 2 UV-Absorbance (at 271 nm) of *Sapindus* saponin at various concentrations

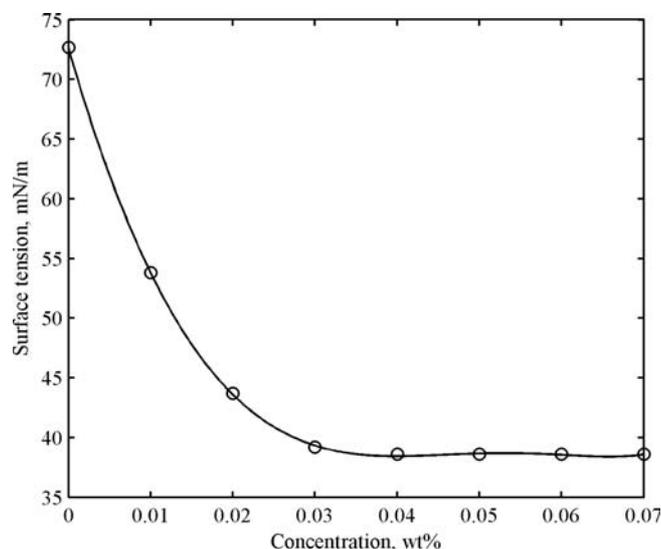


Figure 3 Variation of surface tension of *Sapindus* saponin at various concentrations

Surfactant	Critical micelle concentration (wt%)
<i>Sapindus</i> saponin	0.045
<i>Quillaja</i> saponin [4]	0.051
Bile salt (sodium cholate) [20]	0.068
Humic acid [21]	0.04
Triton X100® [22]	0.13
Sodium dodecyl sulphate [13]	0.224
Cetyl trimethyl ammonium bromide [23]	0.131

Table 1 Comparison of CMC of different surfactants

weak role in altering the CMC of a surfactant. However, the observed variation in CMC with temperature is complex. In many cases, the CMC of non-ionic surfactants are found to decrease with increasing temperature, while that of ionic surfactants are found to increase with increasing temperature [13]. The effect of temperature on the CMC of *Sapindus* saponin is shown in Fig. 4. The CMC values of *Sapindus* saponin marginally decrease with increase in temperature from 25 °C to 60 °C.

The effect of pH on the CMC of *Sapindus* saponin is shown in Fig. 5. It was observed that the CMC increases with increase in pH, with a significant rise in CMC above pH 7. Micelle formation in saponin is due to the glucuronic groups, and would depend on the aqueous condition. Hence, when the pH is increased, the net charge on the head group will vary. This variation in net charge on head group causes electrostatic repulsion between head groups, which tends to increase the CMC of non-ionic surfactants like saponin [4].

3.5 Effect of salt concentration and hardness of water on CMC

Addition of a salt such as sodium chloride significantly influences the electrostatic interaction of charged surfactants [4]. As a result, CMC values of ionic surfactants are appreciably reduced in the presence of a salt. In the case of non-ionic surfactants, it affects the CMC by partially screening the electrostatic repulsion between the head groups. In this case also, higher salt concentrations increase the hydrophobicity of the surfactant, resulting in a lower CMC. The effect of salt concentration on the CMC of *Sapindus* saponin has been studied and is shown in Fig. 6. As could be expected, the CMC of saponin decreases with increasing sodium chloride concentration.

Hardness of water is another important parameter that affects the surfactant behaviour. Hardness of water is mainly due to the salts of calcium and magnesium ions. The effect of increasing hardness of water on the CMC is in the order anionics > cationics > non-ionics, which indicates the degree of interaction of the hydrophilic group in the surfactant molecule with the ions present in hard water [8]. To study the effect of hardness of water on the CMC of saponin, the hardness of water was varied by adding increasing amounts of hard water (water with a total hardness of 1200 ppm by EDTA method) to double distilled water. Hardness of water was varied between 30 to 960 ppm by this method. The CMC of *Sapindus* saponin was found to increase with increase in hardness of water (Fig. 7). The binding constant of magnesium ions to polar groups is small. Hence, the effect of magnesium salts on the polar group is to decrease its surface potential, making the formation of the micelle difficult [24] which would result in an increase in the CMC.

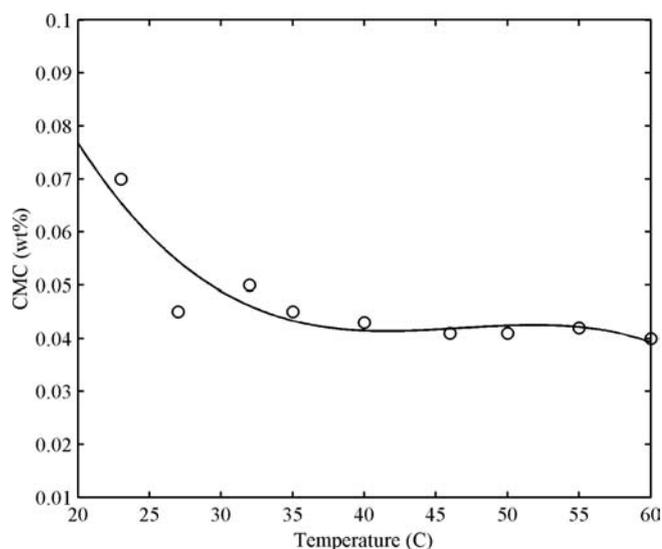


Figure 4 Effect of temperature on the critical micelle concentration of aqueous saponin solution

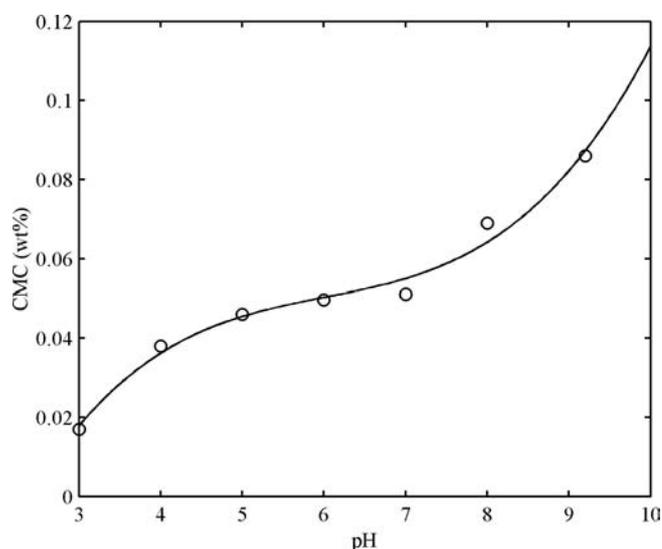


Figure 5 Effect of pH on the critical micelle concentration of aqueous saponin solution

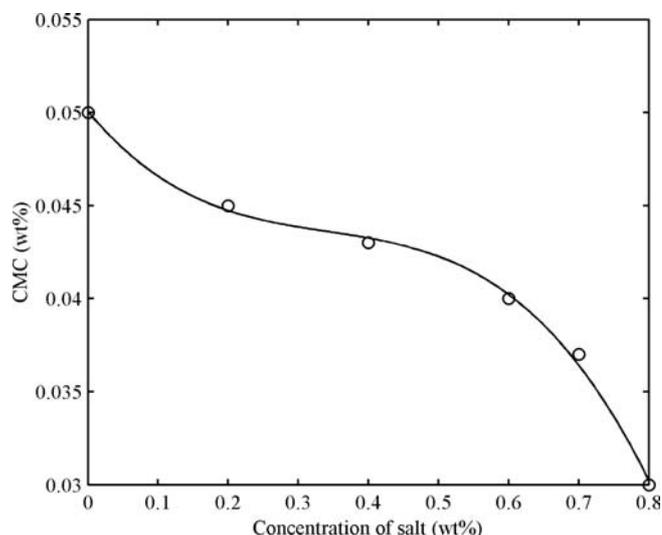


Figure 6 Effect of NaCl concentration on the critical micelle concentration of aqueous saponin solution

3.6 Size of saponin micelles and intrinsic viscosity

Micellar size and aggregation numbers are important parameters in studying the micellar solubilisation. The amount of hydrophobic groups that can be solubilised by a micelle depends on its size which is defined by its hydrodynamic radius. The effective hydrodynamic radii of the *Sapindus* saponin micelles were determined using cyclic voltammetry and by measuring the diffusion coefficient of the aggregates using the Stokes-Einstein equation [4] given by,

$$R = \frac{kT}{6\pi\mu D_0} \quad (1)$$

where, k is the Boltzmann's constant, T is the absolute temperature, μ is the viscosity of the solution at T , and D_0 is the diffusion coefficient.

The effective radii of the aggregates are determined using the assumption that the measured diffusion coefficient is that of isolated, spherical aggregates. It is generally assumed in voltammetric studies of micellar systems that inter-micellar interaction does not affect the current versus potential behavior significantly. It is well known that micellar structure and the extent of inter-micellar interactions are critically dependent on many variables including surfactant type and concentration, supporting electrolyte type and concentration, solution additives and temperature. Self-diffusion coefficients (D_s) for micellar systems are determined using a micelle immobilized electro active probe [25].

In the present study, Ferrocene was chosen as the electro active probe. Ferrocene undergoes an outer sphere one-electron redox process, which is known to be simple. In addition to this, it is stable and it has a reversible electrochemistry. More importantly, the very low solubility in water implies that molecules reside almost entirely inside the micellar structure and therefore the diffusion controlled current predominantly reflects the mobility of the micelles. For a reversible electrochemical reaction, the diffusion coefficient is obtained from Randles Sevcik equation [26],

$$i_p = 2.69 \times 10^5 n^{3/5} A D_0^{1/2} V^{1/2} C_0 \quad (2)$$

where, i_p is the anodic peak current in μA , n is the number of electrons transferred, A is the area of the electrode in cm^2 , D_0 is the diffusion coefficient in $cm^2 sec^{-1}$, V is the potential scan rate in $volt sec^{-1}$, and C_0 is the concentration of the electro active species. Saponin solutions were scanned at different scan rates. Fig. 8 illustrates the effect of scan rate on the voltammogram for saponin solution with CMC of 0.05 wt%. The behavior of ferrocene is reversible. The diffusion coefficient (D_0) is determined from the slope of the curve between anodic peak current and square root of the scan rate as shown in Fig. 9. As expected in Randles Sevcik equation; the peak current (i_p) versus $V^{1/2}$ is linear. The hydrodynamic radii were calculated from the diffusion coefficient using equation 2. The effect of concentration of saponin on the hydrodynamic radius of the micelle is shown in Fig. 10. It was found that the hydrodynamic radii of *Sapindus* saponin micelles decreased initially from 5.3 nm at 0.05 wt% saponin concentration to 4.9 nm at 0.12 wt% concentration and then remained constant at higher concentrations (upto 6 wt%) probably indicating there is no shape change upto 6 wt% saponin solution. In the case of *Quillaja* saponin, the hydrodynamic radii was reported to increase initially with increase in saponin concentration and then remained constant [4].

The self-assembling nature of *Sapindus* saponin molecules was further studied using intrinsic viscosity measurements [9]. Equation (1) coupled with the intrinsic viscosity can be

used to determine the aggregation number, N of micelles which is given by

$$N = \frac{10\pi N_{AV} R^3}{3[\eta] M_s} \quad (3)$$

where, R is the hydrodynamic radius measured by voltammetric techniques, $[\eta]$ is the measured intrinsic viscosity, N_{AV} is the Avogadro's number, and M_s is the molecular weight of the *Sapindus* saponin molecule. Intrinsic viscosity was measured using solutions of concentrations less than $0.5 gm dl^{-1}$. The aggregation number of *Sapindus* saponin micelles thus obtained was found to vary between 13 and 21. The aggregation number for *Quillaja* saponin is reported to be around 50. A micelle with a larger aggregation number generally has a better capacity to solubilise hydrophobic solutes [4] which means that *Quillaja* saponins are better than *Sapindus* saponin for solubilisation purposes. The effect of temperature on intrinsic viscosity of *Sapindus* saponin solutions is shown in Fig. 11. The intrinsic viscosity was found to decrease with increase in temperature.

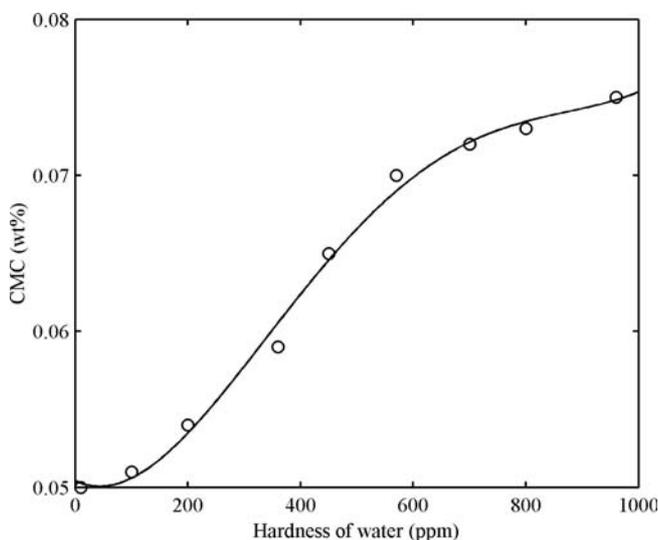


Figure 7 Effect of hardness of water on the critical micelle concentration of aqueous saponin solution

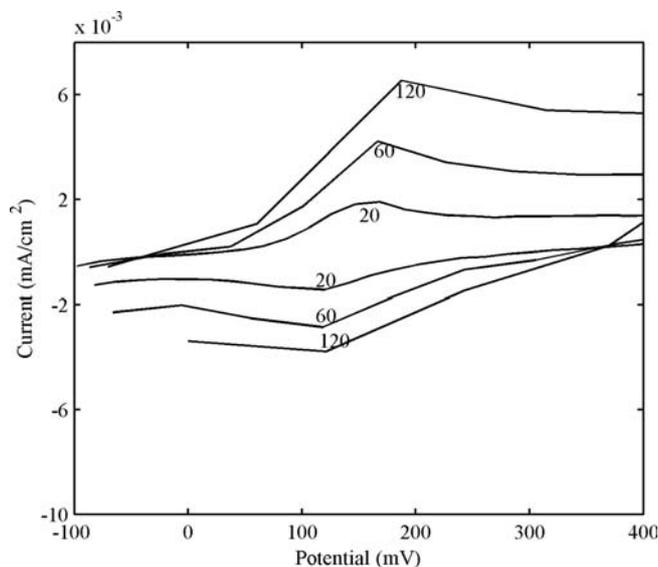


Figure 8 Cyclic voltammogram of solution containing 0.05-wt% of *Sapindus* saponin, 1mM ferrocene and 1M KCl at different scanning rates

3.7 Solubilisation of oils

The solubilisation of two different grades of crude oils and a vegetable oil in *Sapindus* saponin solution was studied. Fig. 12 and 13 show the plots of the ratios of concentration of surfactant solution (C) and CMC versus solubilisation of oil. The results are compared with two commercially used synthetic surfactants, Triton X100[®] (non-ionic surfactant) and SDS (ionic surfactant). C/CMC is used for plotting the graph since *Sapindus* saponin, Triton X100[®] and SDS have different CMC values. The CMC values of *Sapindus* saponin, Triton X100[®] and SDS are 0.045, 0.13 and 0.22 wt%, respectively. Using distilled water, solubilisation of crude oil was found to be 1.5×10^{-4} mg l⁻¹. Two different types of crude oils were used for the solubilisation studies. Below a C/CMC value of 1.5, *Sapindus* saponin shows higher solubility than Triton X100[®] and SDS. At higher concentrations of the surfactant, solubility in SDS is slightly higher than that

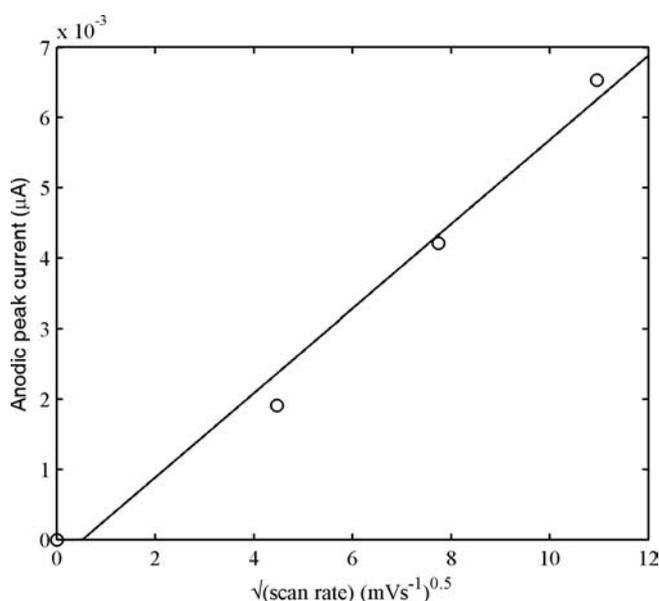


Figure 9 Anodic peak current versus \sqrt{v} (scan rate) for solution containing 0.05-wt% of *Sapindus* saponin, 1 mM ferrocene and 1M KCl

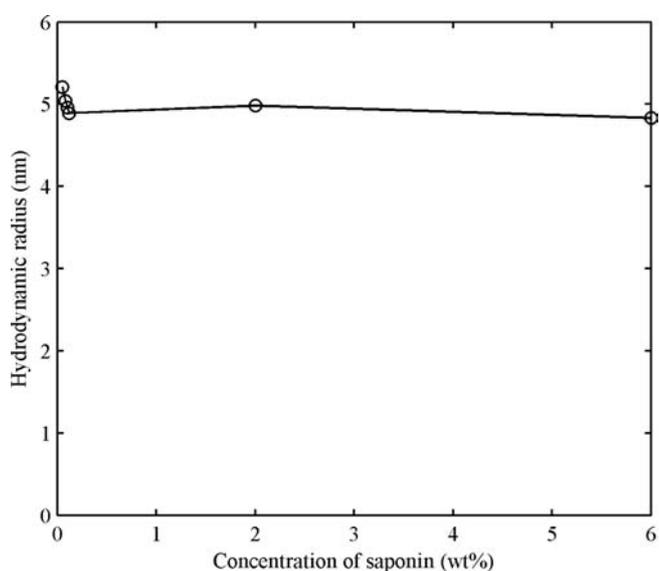


Figure 10 Effect of *Sapindus* saponin concentration on the hydrodynamic radius of saponin micelles

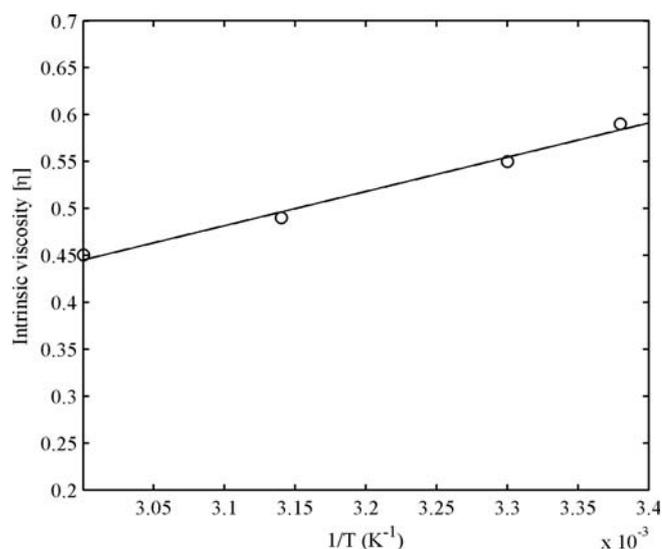


Figure 11 Effect of temperature on the intrinsic viscosity of *Sapindus* saponin micelles

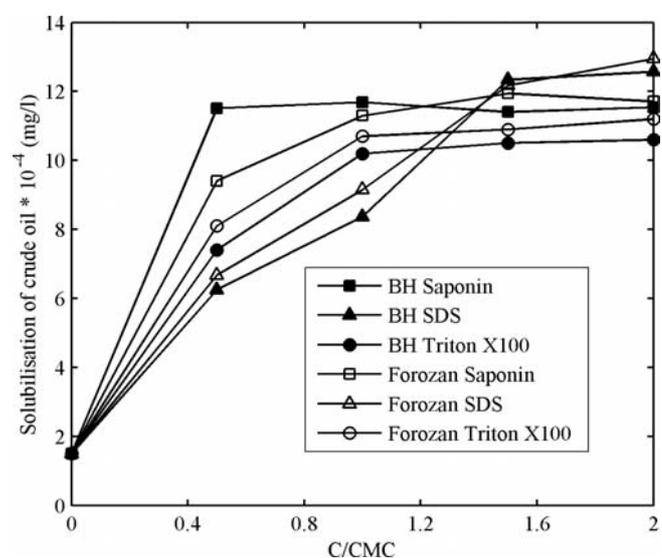


Figure 12 Solubilisation of crude oils (Bombay High (BH) and Forozan) in different surfactant solutions (*Sapindus* saponin, Triton X100[®], SDS)

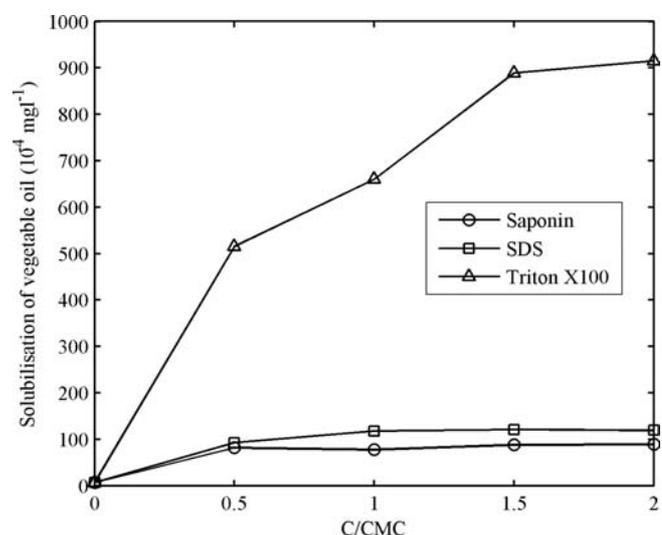


Figure 13 Solubilisation of vegetable oil in different surfactant solutions (*Sapindus* saponin, Triton X100[®] and SDS)

of *Sapindus* saponin and Triton X100[®] for both the crude oils. In general, the solubility of crude oil in *Sapindus* saponin is found to be higher than Triton X100[®]. When C/CMC = 1, *Sapindus* saponin requires only 0.045 wt% whereas SDS requires 0.22 wt% to solubilise the oil. Above C/CMC=1, solubility of crude oil remains constant for *Sapindus* saponin and Triton X100[®]. The solubility of vegetable oil is higher in both Triton X100[®] and SDS than in *Sapindus* saponin. Vegetable oil, SDS and Triton X100[®] are made up of aliphatic hydrocarbons, whereas saponin is a mixture of carbohydrates. Hence, solubilisation of vegetable oil in SDS and Triton X100[®] is higher than saponin.

4 Conclusions

Non-ionic, natural surfactant *Sapindus* saponin was studied for its surfactant activity by characterising its micellar aggregation behaviour and studying its solubilisation characteristics. UV absorbance, conductivity and surface tension measurements showed that the critical micelle concentration of *Sapindus* saponin is 0.045 wt% which is well below that of many synthetic surfactants. The CMC of *Sapindus* saponin was found to increase non-linearly with increase in hardness of water and pH. Similarly, the CMC of saponin was found to decrease non-linearly with increase in salt concentration and temperature. Size of the micelles was determined using cyclic voltammetry and was found to be between 5.5 to 4.83 nm. The micellar aggregation number of *Sapindus* saponin determined using viscosity measurements was between 13 and 21 which is lower than that of *Quillaja* saponin. At lower concentrations of the surfactant, the micellar solubilisation of crude oils in saponin was better than synthetic surfactants like Triton X100[®] and SDS where as, the solubilisation of vegetable oil was better in synthetic surfactants. Due to the low CMC value of *Sapindus* saponin, it could be further explored for potential applications as biodegradable surfactant in personal care products like shampoos. Solubilisation studies indicate that low concentrations of *Sapindus* saponin may have potential for bio-remediation of crude oil contaminated sites compared to synthetic surfactants which are required at higher concentrations.

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Nomenclature

- R – hydrodynamic radius in nm
 k – Boltzmann's constant in $1.38 \times 10^{-23} \text{ J K}^{-1}$
 T – temperature in K
 μ – viscosity of the solution at T
 D_0 – diffusion coefficient in $\text{m}^2 \text{ sec}^{-1}$
 i_p – anodic peak current in μA
 A – area of the electrode in m^2
 V – potential scan rate in volt sec^{-1}
 C_0 – concentration of the electroactive species mol m^{-3}
 N – micellar aggregation number
 N_{AV} – Avogadro's number 6.023×10^{23}
 M_s – molecular weight of the individual surfactant molecule.
 $[\eta]$ – intrinsic viscosity in dl gm^{-1}
 ΔG – Gibb's free energy in KJ mol^{-1}
 R – universal gas constant, $8.314 \text{ KJ Kmol}^{-1} \text{ K}^{-1}$

References

- Holmberg, K.: *Curr. Opin. Colloid Interf. Sci.* 6 (2001) 148.
- Ishigami, Y. and Suzuki, S.: *Prog. Org. Coat.* 31 (1997) 51.
- Berhow, M. A., Wagner, E. D., Vaughn, S. F. and Plewa, M. J.: *Mut. Res.* 448 (2000) 11.
- Mitra, S. and Dungan, S.: *J. Agric. Food Chem.* 45 (1997) 1587.
- Shaker, K. H., Bernhardt, M., Elgamil, M. H. A. and Seifert, K.: *Phytochemistry* 51 (1999) 1049.
- Kommalapatti, R. R., Valsaraj, K. T., Constant, W. D. and Roy, D.: *J. Haz. Mat.* 60 (1998) 73.
- Urum, K. and Pekdemir, T.: *Chemosphere* 57 (2004) 1139.
- Graf, C. S. and Mesa, C. L.: *Thermochim. Acta* 418 (2004) 79.
- Mitra, S. and Dungan, S. R.: *Colloids Surf. B: Biointerf.* 17 (2000) 117.
- Martin, R. M. S., Otero, A. F., Figueroa, M., Escobar, V. and Cruz, A.: *Hydrometallurgy* 77 (2005) 163.
- Hong, K. J., Tokunaga, S., Ishigami, Y. and Kajuchi, T.: *Chemosphere* 41 (2000) 345.
- Rao, A. S. V. S., Basa, S. C. and Srinivasulu, C.: *Research and Industry* 37 (1992) 209.
- Moroi, Y.: *Micelles – Theoretical and Applied Aspects*, Plenum Press, New York, 1992.
- Row, L. R. and Rukmini, C.: *Indian J. Chem.* 4 (1966) 36.
- Chatterjee, A. and Pakrashi, S. C.: *The Treatise on Indian Medicinal Plants*, National Institute of Science Communication, New Delhi, 1997.
- Nakanishi, K.: *Infrared Absorption Spectroscopy – Practical*, Nankodo Company, Tokyo, 1962.
- Nyberg, N. T., Baumann, H. and Kenne, L.: *Anal. Chem.* 75 (2003) 268.
- Paugam, M. F., Morin, G. T. and Smith, B. D.: *Tetrahed. Lett.* 34 (1993) 7841.
- Dyer, J. R.: *Applications of Absorption Spectroscopy of Organic Compounds*, Prentice Hall India, New Delhi, 1978.
- Reis, S., Mountainho, C. G., Matos, C., Castro, B. D., Gameiro, P. and Lima, J. L. F. C.: *Anal. Biochem.* 334 (2004) 117.
- Terashima, M., Fukushima, M. and Tanaka, S.: *Colloids Surf. A: Physicochem. Engg. Aspects* 247 (2004) 77.
- Gu, G., Yan, H., Chen, W. and Wang, W.: *J. Colloid Interf. Sci.* 178 (1996) 614.
- Okano, L. T., Quina, F. H. and Seoud, O. A. E.: *Langmuir* 16 (2000) 3119.
- Huang, Y. X., Tan, R. C., Li, Y. L., Yang, Y. Q., Yu, L. and He, Q. C.: *J. Colloid Interf. Sci.* 236 (2001) 28.
- Charlton, I. D. and Doherty, A. P.: *Anal. Chem.* 72 (2000) 687.
- Chokshi, K., Qutubuddin, S. and Hussam, A.: *J. Colloid Interf. Sci.* 129 (1989) 315.

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