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Surface activity of a non-micelle forming compound containing a surface-active impurity

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Abstract

Purpose. The purpose of this study is to characterize the surface activity of a water-soluble compound and its ability to form aggregates/micelles. *Methods*. Aqueous solutions of the compound were prepared at various concentrations. Surface tension was determined using drop volume and Wilhelmy plate methods. Moreover, conductivity and osmolality measurements of aqueous solutions were also determined at various concentrations. *Results*. Even though the compound appeared to be surface active, no change in the slope was found of either molar conductivity (S cm^2/mol) versus square root of concentration ($R^2 = 0.994$) or osmolality (mOsm/kg) versus concentration ($R^2 = 0.999$). Moreover, no clear critical micelle concentration was observed when surface tension was plotted versus log concentration. These results indicated no micelle formation in these solutions. In order to investigate this behavior further, the main impurity in the compound was also tested. Surface tension measurements of solutions containing different concentrations of the impurity indicated that the impurity was more surface active than the compound. *Conclusions*. This study shows the importance of characterizing the behavior of surface-active compounds using multiple techniques. This work also emphasizes the importance of determining whether surface activity in aqueous solutions is due to the main compound, its impurities or both.

Keywords: Surface tension; Micelle; Bubble point; Impurity; Conductivity; Osmolality

1. Introduction

Molecules and ions that are adsorbed at interfaces are termed surface-active agents, or surfactants (amphiphile). The addition of a surfactant to a liquid system leads to a reduction in surface tension owing to these molecules or ions being adsorbed to the surface. Adsorption of surfactants in these binary systems is expressed quantitatively by Gibbs as follows:

$$\Gamma_2 = -\frac{1}{RT}\left(\frac{\partial\gamma}{\partial \ln c_2}\right) \quad (1)$$

in which Γ is the surface excess or surface concentration; γ the surface tension; c the bulk concentration of surfactant; R the gas constant; T is the absolute temperature. The above equation indicates that the concentration of amphiphile undergoing adsorption at the air–water interface increases and consequently γ decreases as the total concentration of amphiphile is raised. Eventually, a point is reached at which the interface becomes saturated with monomers. Consequently, γ levels off (Fig. 1). The concentration range, above which surface tension levels off, is referred to as the critical micelle concentration (CMC). However, not all surface-active molecules or ions form micelles (Mukerjee, 1974; King et al., 1989). Formation of micelles requires certain structural features, which are not necessarily present in all surface-active molecules or ions. One example is ethanol, which is surface active but does not form micelles. This behavior has also been seen with some drugs that are shown to be surface active, but do not from micelles (King et al., 1989). A number of examples in the literature indicate that different experimental approaches may give rise to apparent CMC values for systems exhibiting non-micellar behavior. In these cases, the application of the micellar hypothesis to such systems may be seriously misleading (Mukerjee, 1974).

Only a few reports in the pharmaceutical literature point out the importance of impurities in solutions of surface-active compounds. The effect of the purification of sodium lauryl sulfate (SLS) has been studied and shown that the shape of surface tension versus concentration curve changes with more pure grades

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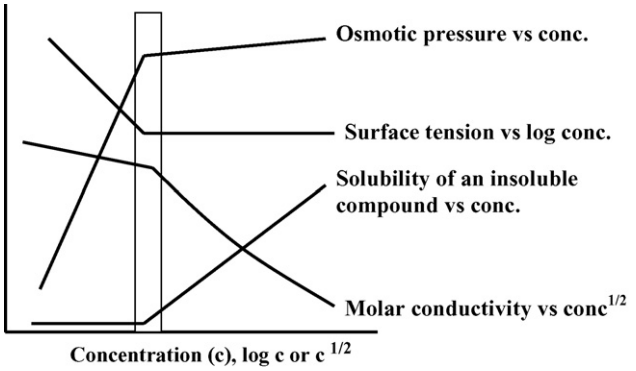
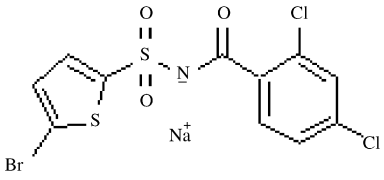


Fig. 1. Properties of surface-active agents showing sharp changes at the CMC.



2046742
API

Fig. 2. Structure of the API.

of SLS (i.e., the minima is no longer present with more pure grades of surfactant) (Arias et al., 1998). Moreover, the effect of SLS purity has been shown to affect the estimated CMC values as well as the in vitro dissolution when used in dissolution media (Crison et al., 1997). Other reports pointed out the effect of impurities in surface-active agents on stability of formulations (Nassar et al., 2004).

The current study was conducted for a highly water-soluble, sodium salt, active pharmaceutical ingredient (API) intended for parenteral delivery (compound number 2046742, Fig. 2). This compound is being evaluated in early clinical studies. During the development of a clinical formulation of the API, it was noted that the solution formulation depressed post-filtration bubble point; indicating that the API is surface active. The main objective of this work was to understand the surface-active properties of the API and its ability to form aggregates/micelles. This is important since surface activity will impact the acceptable minimum bubble point during parenteral drug product manufacture. In addition, such studies are helpful in understanding the behavior of the API in solutions and how future variations in formulation ingredients might affect it. Moreover, the surface activity of the main impurity in the API is characterized. This work illustrates the importance of characterizing the surface activity of such impurities for surface-active drugs to understand their contribution to the overall surface activity of the system.

2. Experimental

2.1. Surface tension measurements

A Lauda drop volume tensiometer (TVT2) was used to determine the surface tension of different concentrations of the API (34.3–343 mM) and impurity (1.02–6.11 mM). The experimental conditions were as follows:

- syringe volume: 2.5 mL;
- capillary radius: 1.346 mm;
- temperature: 25 °C;
- number of cycles: 2;
- drops/cycle: 3.

In addition, Wilhelmy plate method (KRUSS tensiometer) was also used to determine the surface tension of different concentration of the API (22.9–229 mM) in either water or normal saline (0.9% NaCl) at 25 °C.

2.2. Conductivity measurements

Conductivity of API solutions was measured at different concentrations (53.6–343 mM) using an Accumet AR50 Dual Channel pH/Ion/Conductivity meter and an Accumet conductivity cell (glass body conductivity cell, 0.1 cm). The conductivity cell was standardized using a conductivity standard (100 μS/cm ± 1.0% at 25 °C). The cell constant, K, was found to be 0.029 cm^{-1}.

A solution of the drug substance at 343 mM was prepared and the conductivity measured. This solution was subsequently diluted and conductivity measured at each dilution. Dilutions were made with purified water using a pipette. Conductivity values ranged between 3.06 and 0.792 mS/cm. Conductivity values of API solutions were not corrected for purified water conductivity (1.40 μS/cm). Molar conductivity of the API was then calculated from the conductivity measurements.

2.3. Osmolality measurements

A Fiske One-Ten Osmometer was used to investigate the effect of API concentration (94.1–229 mM) on the osmolality of its aqueous solutions. A sample of API solution (229 mM) was tested. This solution was subsequently diluted using a pipette and osmolality measured at each dilution.

3. Results and discussion

3.1. Surface tension

In the drop volume method for surface tension measurement, the weight of a droplet increases as its volume increases until the volume reaches a critical value at which the weight cannot be counterbalanced by the force exerted upwards by surface tension. Therefore, surface tension is proportional to the critical droplet weight as follows:

$$\gamma = \frac{mg}{2\pi r f} \quad (2)$$

where γ is the surface tension; m the critical drop mass; g the acceleration constant; r the radius of the capillary used; f is a correction factor; which depends on the nature of the liquid (Adamson and Gast, 1997).

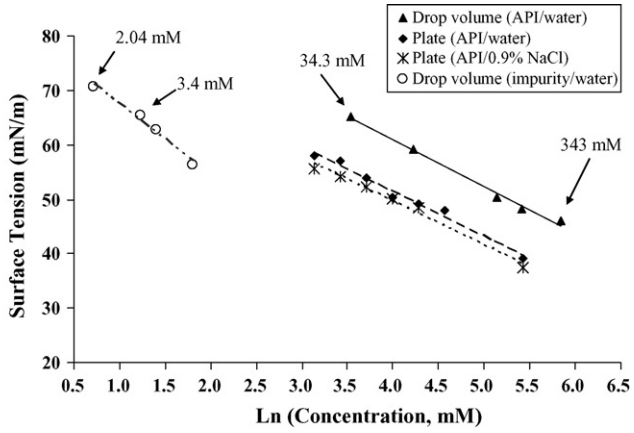


Fig. 3. Surface tension vs. ln(concentration) plots for API and impurity in either water or 0.9% NaCl using drop volume or Wilhelmy plate methods.

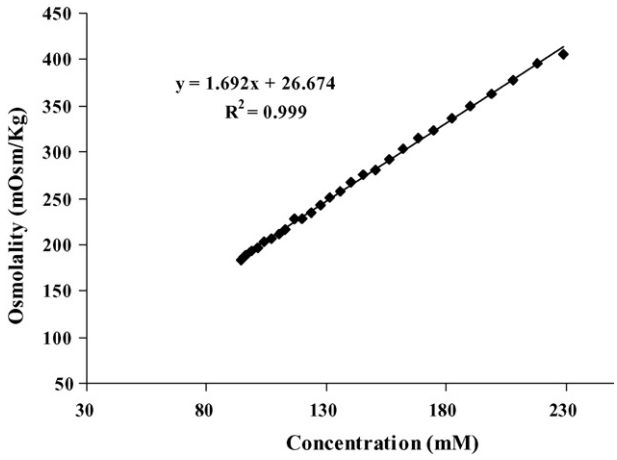


Fig. 4. Osmolality vs. API concentration.

Surface tension of API solutions as determined by drop volume method can be seen in Fig. 3. Standard deviations for surface tension measurements were ≤0.21 mN/m for all samples ($n = 3$ for drop volume method). Fig. 3 shows that as the concentration of API increases, surface tension decreases indicating that the API is surface active. The surface tension (drop volume method) versus ln API concentration curve does not show any distinct break (no leveling off of γ with ln concentration). Similar behavior is seen with surface tension data obtained by Wilhelmy plate method (Fig. 3). These results suggest that the API does not form micelles within the concentration range studied. The slope of γ versus ln concentration is proportional to surface excess (Γ) as apparent from Eq. (1). Table 1 summarizes the slopes of the plots in Fig. 3 and the corresponding estimated Γ values.

It is clear that the surface tension as determined by the drop volume method is different than that determined by the Wilhelmy plate method (Fig. 3—API solutions in water data). Such differences are not unusual and may be related to inaccuracies in the correction factors employed by the different methods. Estimated surface excess values (Γ), on the other hand, were found to be not significantly different ($p > 0.05$) irrespective of the method used for determining surface tension (Table 1).

The CMC of ionic surfactants is sensitive to ionic strength (Thibert et al., 1996; Yalkowsky, 1999). As ionic strength increases, polar head group repulsion decreases. Consequently, CMC decreases. A decrease in the CMC results in more surfactant molecules going to the bulk of the solution to form micelles rather than going to the surface. Therefore, Γ for ionic surfactants that form micelles is expected to decrease with ionic strength. The API is ionic (sodium salt) and is surface active. However, its surface excess in water is not significantly different than that in 0.9% NaCl ($p > 0.05$) as listed in Table 1. This further supports that the API does not form micelles within the concentration range studied.

3.2. Osmolality and conductivity

Many physical properties of surfactant solutions, in addition to surface tension, show consistent behavior with increasing concentration (Adamson and Gast, 1997). Sharp changes in properties occur in the region of the critical micelle concentration (Fig. 1). Such properties include osmotic pressure and molar conductivity (Florence and Attwood, 1988). In fact, such colligative properties have been used to estimate the CMC for some compounds (Streng et al., 1996).

A good linear correlation can be seen in Fig. 4 between osmolality and concentration up to 229 mM. The linear relationship indicates that the number of independent particles in solution is in excellent agreement with the total concentration of the API in solution and that no micellization/association takes place up to a concentration of 229 mM.

The change in molar conductivity with square root of concentration can be seen in Fig. 5. Conductivity is another solution property that is dependent on the number of independent species in solution. This has been utilized in previous investigations to characterize self-association (Coffman and Kildig, 1996; Streng et al., 1996). Similar to what is observed with the osmolality data, the relationship between molar conductivity with square root of concentration clearly indicates that no association or micelle formation takes place up to a concentration of 343 mM

Table 1
Summary of estimated surface excess (Γ) values and the slopes of surface tension vs. ln concentration plots

Sample	Method	$-d\gamma/d \ln$ concentration (mN/m)[a]	Estimated surface excess (μmol/m^2)
API/water	Drop volume	8.63	3.48
	Wilhelmy plate	8.21	3.31
API/0.9% NaCl	Wilhelmy plate	8.02	3.24
Impurity/water	Drop volume	13.00	5.25

[a] Standard error of slope estimation is ≤0.48 mN/m.

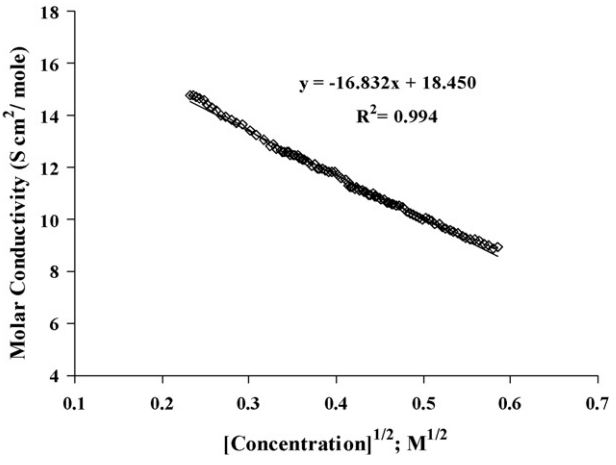


Fig. 5. Molar conductivity vs. square root of API concentration.

as can be seen in Fig. 5. Thus, both osmolality and conductivity data are in agreement with surface tension measurements indicating absence of micellization in solutions of this drug substance.

3.3. Surface activity of the main impurity

Due to structural similarities between the API and its major impurity (the impurity is a dimer), the impurity was studied to investigate if it was also surface active.

The major impurity in the API was found to be surface active (Fig. 3). In fact, the impurity is more surface active than the API. A 34.3 mM solution of the API is needed to lower the surface tension of water to 65.19 mN/m (Fig. 3). However, only a concentration of 3.4 mM of the impurity is needed to achieve similar reduction in surface tension. This is also apparent from the significantly larger surface excess value of the impurity ($p < 0.05$) when compared to that of the API (Table 1).

Based on the levels of the impurity in the API (approximately, 0.7 wt.%), a 343 mM solution of the drug substance would contain approximately 2.04 mM of the impurity. The surface tension of a 2.04 mM solution of the impurity is 70.61 mN/m (Fig. 3), which is much higher than the surface tension of a 343 mM solution of the drug substance (46.07 mN/m, Fig. 3; drop volume method). This indicates that even though the major impurity is more surface active than the API, it accounts only for a fraction of the observed surface activity in the API solutions. However, this clearly indicates that control over the level of the impurity in the drug substance is an important criterion when a product specific bubble point for the drug product needs to be determined.

4. Conclusions

Surface tension measurements of aqueous solutions of an investigational drug substance indicated that the drug substance is surface active. Several methods were used to investigate whether the API forms micelles. Results suggested that while the API is surface active, it does not form micelles within the concentration ranges studied. Due to structural similarities between the API and its main impurity, surface activity of the impurity has been characterized indicating that the impurity is more surface active than the API. This work highlights that not all surface-active molecules form micelles. This work also shows the importance of characterizing the effect of impurities on solution properties especially for parenteral formulations, where variations in the levels of such impurities might influence the filtration bubble point during product manufacture.

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