

## Partitioning of Fatty Acids in Oil/Water Systems Analyzed by HPLC

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Received: 22 February 2007 / Accepted: 28 September 2007 / Published online: 12 December 2007  
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**Abstract** Surfactant/oil/water systems in which the surface-active substance is a mixture of an undissociated fatty acid (FA) and its sodium salt soap, exhibit the typical phase behavior and the general emulsion phenomenology produced by a formulation scan. The phase behavior transition is induced by changing the FA concentration in the system at a fixed alkaline (NaOH) content in water, which results in a variable lipophilic acid/hydrophilic salt ratio. The partitioning is reported for different FA types (octanoic to myristic) and alcohol co-surfactants between excess oil and water phases of Winsor three-phase optimum formulation system. The concentrations are determined by using a method reported in a previous work to quantify the total acid fraction in each phase.

**Keywords** Fatty acid · Formulation · Partition coefficient · Linear alcohols

### Introduction

Fatty acids (FA) are widely used in industry, and they are common compounds found in foods and pharmaceutical products [1–3]. FA can be ionized by a change in pH to produce a hydrophilic group, which results in an

amphiphilic compound with surfactant properties. As for most other surfactants, FA are able to produce microemulsions with oil and water, provided that a proper formulation is found. Such microemulsions are single-phase systems in which the normally immiscible oil and water phases are made compatible by a surfactant or surfactant/co-surfactant mixture.

In the 1970s, the enhanced oil recovery (EOR) potential of microemulsions drove an outstanding research effort on surfactant–oil–water systems (SOW) that resulted in a considerable improvement of the knowledge concerning the phase behavior of these systems [4–16]. Because of the current oil prices, the EOR methods are becoming attractive again, particularly those so-called alkaline-surfactant-polymer (ASP) flooding [4–6]. Since the partitioning coefficient is directly linked with the generalized formulation expression [17, 18], basic formulation studies are generally carried out with model species, such as FA, which must be analyzed in the different phases. To do so, several separation techniques have been used for the determination of FA in different samples [19–36].

Surfactant partitioning was first studied in the early 1960s, but very few experimental data were available, e.g., only for one surfactant–oil–water system containing polyethoxylated nonionic surfactants [37]. Since the partitioning study requires the measurement of the concentrations in both oil and water phases in the absence of micellar structure, it was carried out at extremely low concentration, below the critical micelle concentration (cmc) of such polyethoxylated surfactants in water. In the 1980s the three-phase so-called Winsor III phase behavior [7] systems were used to measure the partitioning by analyzing the concentration of the species in the excess oil and water phases, because all structures would collect in the microemulsion middle phase [9, 11].

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The behavior of polyethoxylated surfactant mixture partitioning was successfully interpreted by using a pseudophase model in which the middle-phase microemulsion is virtually stripped of its oil and water that are assumed to have the same composition as the excess water and oil phases. The composition of the remaining interfacial pseudophase, which commands the system properties, can be readily calculated assuming an ideal mixing rule. [38] The model has been successfully used to interpret the change in the adsorbed interfacial mixture with formulation as well as composition variables such as the total surfactant concentration, and the water-to-oil ratio [38, 39]. Since then, the partitioning has no longer been studied at low surfactant concentration, i.e., below the cmc, and Winsor's type III case, has been the preferred case.

FA are well known amphiphiles, which have been studied since the very beginning of surface science [40, 41]. The surface activity of FA strongly depends on the pH of the aqueous phase [42], and the interfacial tension of their solution against an oil phase often exhibits a minimum versus pH [43]. At the liquid–liquid interface, FA salts might be formed, whose surface activity and solubility in both phases strongly depend on the pH and the presence of ions.

In pH-dependent systems, it is useful to consider that the amphiphile is composed of a mixture of two surfactant species, a nonionic one, e.g., the undissociated acid most common at low pH, and its ionized counterpart, e.g., an acid salt which is generated at high pH. According to this assumption the overall surfactant can be viewed as a mixture of a lipophilic species and a hydrophilic one [4, 6, 44]. This approach has already been dealt with in general [45] and for acid–salt pairs, [4–6] although not with mixtures in which the partitioning [38, 45] of the different species is probably of overwhelming importance. Moreover, many crude oils contain a variety of naphthenic and other carboxylic acids. By contacting such crude oils with an alkaline aqueous solution, a surface-active mixture is formed at the oil–water interface by the neutralization reaction. It is composed of a mixture of carboxylic acid derivatives that is able to reduce the interfacial tension and eventually produce a spontaneous emulsification of the crude oil [46, 47].

The behavior of FA in oil/water system is affected by formulation variables such as the FA concentration, the pH and the co-surfactant type and concentration; the resulting interfacial amphiphilic mixture has been shown to depend on both the dissociation in water and the partitioning of the undissociated FA between water and the organic phase. This paper is dedicated to study the partition of different FA between oil and water. Moreover, the effect of different alcohol co-surfactants on the FA partitioning between the excess phases of a FA/*n*-heptane/water/microemulsion

system is also studied. The concentration of FA in each phase of the system is determined through an improved HPLC quantitative analysis.

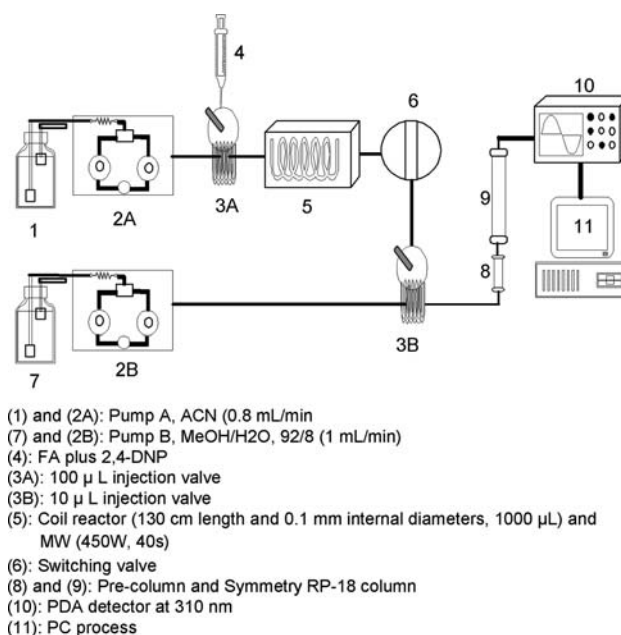
## Experimental Procedures

### Apparatus

HPLC separations were performed with a setup from Waters Corporation consisting of a model 510 pump and a U6K injector valve with a 10- $\mu$ L sample loop, a photodiode array detector (PDA) model 996 coupled to a PC loaded with Millennium software. The symmetry RP-18 column (3.0 mm  $\times$  20 mm i.d., 3.5  $\mu$ m) was purchased from Waters. The on-line derivatization system consisted of an isocratic high-pressure pump model 510 from Waters, a Rheodyne injection valve model 5020 and a switching valve model 5011 from Supelco. A domestic microwave oven (Kenmore) equipped with a 2,450 MHz magnetron with a nominal maximum power of 400 W was used as marketed. The coil reactor was introduced through the vent holes of the microwave oven in order to avoid drilling the walls. Figure 1 show a schematic diagram of the equipment setup [48].

### Materials

Methanol (MeOH), *n*-heptane and acetonitrile (ACN) used as solvents were HPLC grade products from Baker



**Fig. 1** Schematic diagram of an on-line derivatization-HPLC system

Chemicals. All solvents were ultrasonically degassed. The studied FA samples were as follows: caprylic acid (C8), capric acid (C10), lauric acid (C12), myristic acid (C14), palmitic acid (C16) and stearic acid (C18) all provided by Merck. They are referred to as *CN*, where *N* indicates the number of carbon atoms in the acid molecule. The effect of the alcohol in the partitioning coefficient of the Winsor-III system was studied using: ethanol, *n*-butanol, *n*-hexanol, *n*-octanol, *n*-decanol and *n*-dodecanol (4.0 vol.%) all provided by Merck. For the analysis of the FA in each phase (aqueous and oil phase), 2,4-dinitrophenylhydrazine (2,4-DNPH) (purity over 99%, Merck) was used to produce the derivatives 2,4-dinitrophenylhydrazone of FA [48].

### On-Line Derivatization-HPLC Procedure

Initially, a FA solution containing 0.050 mmol of each acid in ACN medium and 2,4-DNPH (1 mM) filled the loop of injection valve (3A, in Fig. 1). By switching the injection valve, the loop content was injected into the acetonitrile carrier stream and driven to the reactor coil located inside the microwave oven. In this system, the coil was wrapped around an assay tube. The microwave oven was switched on at 400 W for 40 s after sample injection, with the sample plug inside the reactor coil. Excessive heating was not generated because of the low power, so that bubble formation was avoided. After the derivatization process, the loop of the injection valve (3B, in Fig. 1) was automatically filled and switched so that the reaction products were sent to the HPLC analysis line. The procedure is described in more detail elsewhere [48].

### FA/*n*-Heptane/Water/Microemulsion System Preparation

FA-oil-water systems were studied according to the so-called one-dimensional scan technique [9, 10, 49], though not in the usual way in which the formulation is changed by varying the amount of alkaline substance at constant acid concentration in the system. Here, it is the alkaline amount which is fixed and the acid concentration which is varied. For each type of FA, a dozen test tubes were prepared, each containing: 10 mL of *n*-heptane (oil phase), 10 mL of distilled water (with NaOH and NaCl as indicated in Table 1) and the respective commercial acid (0.2–2.0 wt.%) which is introduced as an oil solution. The tubes were closed with a screw cap and placed to rest in a vertical position to equilibrate at 25 °C. They were gently stirred once a day for a period of 1 week to improve the contact between phases; then, they were left to rest for at least 2 weeks to fully equilibrate. After equilibration, the phase

**Table 1** Composition of phases

Acid	Acid (mol/L) <sup>a</sup>	mL NaOH (0.6 mol/L)	mL NaCl (0.6 mol/L)	Middle phase volume (mL)	Fi <sup>b</sup>
C8	0.125	0.1	9.9	0.3	0.030
C10	0.090	0.4	9.6	1.2	0.220
C12	0.070	0.8	9.2	2.1	0.400
C14	0.052	1.2	8.8	3.0	0.686
C16	0.039	1.6	8.4	3.9	0.768
C18	0.028	2.0	8.0	4.8	0.960

<sup>a</sup> Concentration of FA is expressed in terms of the total volume system

<sup>b</sup> Fi = (moles of added NaOH)/(initial moles of acid)

behavior is recorded and the three-phase system that exhibits an equal solubilization of oil and water into the middle-phase microemulsion is taken as optimum. The three phases are removed slowly with a long needle syringe taking special precautions to avoid contamination. In some cases, the phases are separated and left to equilibrate again to avoid cross-contamination before removing the sample. Then, oil and aqueous phase samples were evaporated to dryness in a convection oven at 60 °C or using a rotavapor under vacuum. The FA residue was then redissolved in anhydrous acetonitrile, and the total FA concentration in the different phases was determined by using the on-line derivatization-HPLC method.

### Alcohol Effect in the FA/*n*-Heptane/Water/Microemulsion System

Test tubes were prepared, each containing: 10 mL of *n*-heptane (oil phase), 10 mL of distilled water (with NaOH and NaCl as indicated in Table 1) and the respective commercial acid (1.0 wt.%) which is introduced as an oil solution in order to attain a three-phase system. Then, the respective alcohol (4.0 vol.%) was introduced as the external phase in the WIII system. After equilibration, the total FA concentration in the different phases was determined by using the on-line derivatization-HPLC method described previously.

## Results and Discussion

It is well known that most of the solvents used as a mobile phase in HPLC present a strong absorption in the far UV, which can interfere with FA peaks of absorption at 214 nm; however, when acids are derivatized with 2,4-dinitrophenylhydrazine, both sensitivity and selectivity are increased because of the shift of the strong absorption wavelength to 310 nm. The concentration of the each acid

in the system is determined after a calibration curve that relates the peak area with the mass of acid ( $r = 0.999$ ). A relative standard deviation below four was attained. The on-line derivatization-HPLC procedure is a handy experimental technique to measure FA concentrations and attain the partition coefficient in Winsor III system.

The following reports for the first time the analysis of FA in Winsor III, as a way of estimating their partition coefficients ( $K_r$ ), which allows a direct measurement of the influence of the formulation variables such as type and concentration of co-surfactant, type and concentration of surfactant and others. The partition coefficient ( $K_r$ ) of the undissociated acid between oil and water phases is the ratio of the FA concentration in the oil excess phase, essentially  $[HA_O]$ , to its concentration in the water excess phase  $[HA_W]$ , as indicated in Eq. 1.

$$K_r = \frac{[HA_O]}{[HA_W]} \quad (1)$$

According to previous studies [4–6] the dissociated (ionic) and undissociated (nonionic) species are likely to selectively partition or fractionate between water and oil.

#### Optimal Formulation of the *n*-Heptane/Water/Microemulsion Systems

The optimum formulation at the interface (and in the middle phase microemulsion) is a combination of the hydrophobic undissociated acid specie (AH) and the hydrophilic sodium soap specie (ANa or  $A^-$ ). Since the latter is produced by the reaction with NaOH, its amount is constant; hence the hydrophilic contribution is constant and it is in water. Consequently, it is the amount of FA added that produces the hydrophobic contribution. By changing the amount of FA, the hydrophobic contribution is scanned and at optimum formulation for three-phase behavior both contributions are exactly balanced at interface [7–10]. In order to observe the phase behavior of FA in an oil/water system, a scan formulation with different FA was performed. Figure 2 shows the concentration scan for C14 in an *n*-heptane/water system. The results showed that it is possible to obtain a wide range of WIII systems. The optimum system corresponds to the tube, in the scan, with the same water and oil phase volume. Table 1 show the optimum condition for the all FA studied.

It is worth remarking that in most scans the surfactant concentration is constant and the pH is the variable which changes when the amount of NaOH is changed. In such scans the pH exhibits a very slow change with the amount of NaOH at half neutralization, i.e., at optimum pH symbolized as  $pH^*$ , where the amount of acid and salt are the same in the water phase, because it is a buffer point.

In our case, the amount of NaOH is constant and the amount of acid is changed, and the neutralization curve is essentially the same, but upside down. At half neutralization there is a buffer point and close to optimum, the pH does not vary much as more acid is added. In this buffer zone that corresponds to three-phase behavior, there is an equilibrium between undissociated acid and salt. Since the pH does not vary much, the ratio between the two species is almost constant; hence the formulation is almost constant, which is why three-phase behavior is observed over a wide range. But since the amount of total acid and salt increases, the microemulsion volume increases. Then, when the pH becomes too acid, the equilibrium constant expression  $K_a$  in water indicates that an increase in  $H^+$  concentration implies a decrease in salt concentration. Thus, if less salt is present, hence less salt goes to interface, and even if the formulation has not changed significantly (and there is still three phase behavior) the total amount acid and salt decreases and the volume of middle phase decreases. This variation explains the change in volume of the middle phase microemulsion exhibited in Fig. 2.

The partitioning of FA between water and *n*-heptane can be expressed numerically by the variation in the logarithm of the partition coefficient as a linear function of the number of carbon atoms in the alkyl chain. In the partition coefficient study in three-phase behavior systems, the FA concentrations required to attain optimum formulation (three-phase system where the middle phase is in equilibrium with the same volume of water and oil excess phases) were found to be as follows (mol/L): 0.125 (for C8 acid), 0.090 (for C10), 0.070 (for C12), 0.052 (for C14), 0.039 (for C16) and 0.028 (for C18). As the FA becomes more hydrophobic, i.e., as its chain length increases, a lower

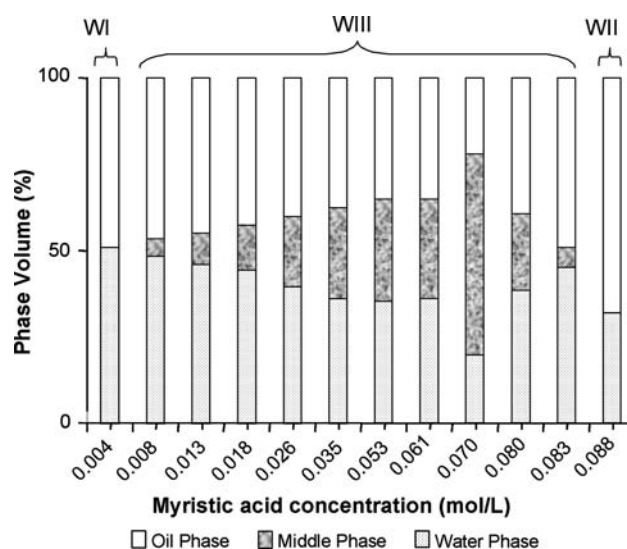
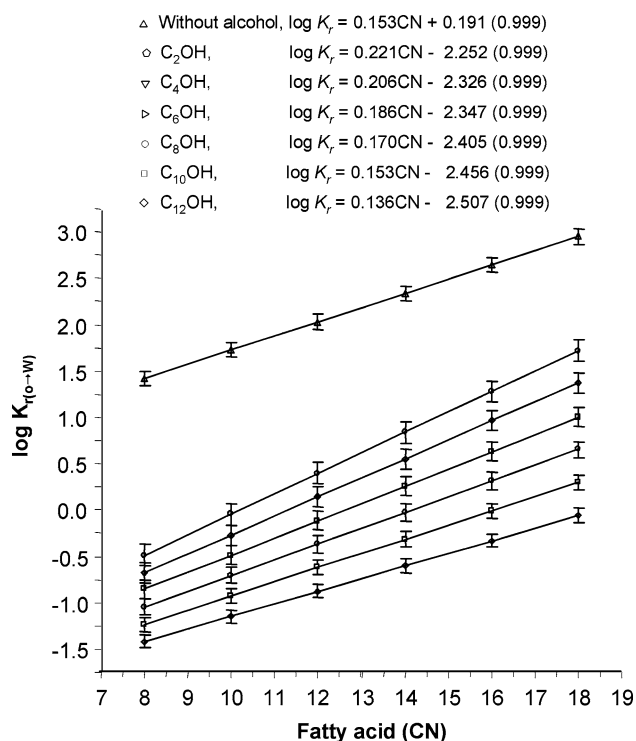


Fig. 2 Formulation scans for C14 acid in an *n*-heptane/water system

amount is required to attain optimum formulation for three-phase behavior (balanced formulation). This is of course because the acid is more hydrophobic. Moreover, the hydrophilicity of the soap slightly decreases as the FA chain increases [4, 5], hence requiring less hydrophobic acid to reach the balanced formulation.

#### Effect of Adding Alcohol on the Partition Coefficient in the *n*-Heptane/Water/Microemulsion Systems

The partitioning coefficient was calculated from FA concentrations between the excess oil and excess water phases of three-phase systems for different acids and different linear alcohols at 25 °C. Figure 3 shows a comparison of the variation of the partition coefficient versus the acid type (as *CN*) for FA (1.0 wt.%) /*n*-heptane/water systems in the following alcohol (4.0 vol.%): (filled triangles) no alcohol, (filled circles) ethanol, (filled diamonds) *n*-butanol, (filled squares) *n*-hexanol, (open circles) *n*-octanol, (open squares) *n*-decanol and (open diamonds) *n*-dodecanol. The data in Fig. 3 show that the variation in the logarithm of the partition coefficient follows the same linear rule as a function of the alkyl length *CN* with a slope in the 0.14–0.22 range according to the linear regression. Moreover, the partition coefficient of all FA decreases when the alkyl



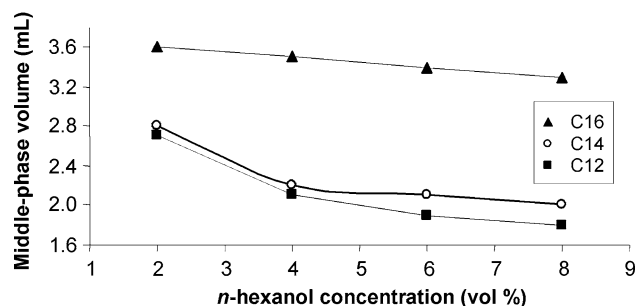
**Fig. 3** Partition coefficient of FA in *n*-heptane/water/microemulsion systems for linear alcohols (4.0 vol.%) studied. Bars indicate standard deviation of error 3%

chain length of alcohol increases. This trend corroborates the fact that the FA apparent lipophilicity at interface is increased by a lipophilic alcohol, although the effect is found to be stronger than for ethoxylated alkyl phenols [17, 18].

In absence of alcohol, the excess oil phase contains a much higher concentration of FA than the excess aqueous phase, because the acid concentration in the aqueous phase is limited by its solubility or its cmc. When alcohol is added the partition coefficient tends to decrease strongly, about two orders of magnitude for short alcohol and even more for lipophilic ones. This does not mean that the alcohol increases the hydrophilicity of the acid, but that its presence probably facilitates the dissolution of the undissociated acid in water which becomes slightly less polar. The presence of adsorbed lipophilic alcohol at interface is also likely to increase the hydrophobicity of the amphiphile mixture, thus requiring less undissociated acid to attain optimum formulation. As a consequence, the acid tends to appear as more hydrophilic, as corroborated by a decrease in the partition coefficient.

On the other hand, it is known that the addition of many alcohols of short to medium chain length, referred to above as co-surfactants, will increase the solubility in the aqueous phase of ionic surfactants. This effect works in favor of microemulsion formation as postulated above, especially from the standpoint of the solution properties of the surfactant. Moreover, the alcohols are less polar than water and are distributed between the bulk solution and the micelles. The more preference they have for the micelles, the more they stabilize them. A longer alkyl chain leads to a less favorable location in water and a more favorable location in the micelles.

On the other hand, when more alcohol is added to the three-phase system, the middle phase volume is reduced. Figure 4 shows, as more *n*-hexanol is added in the FA/*n*-heptane/water/microemulsion system, the middle-phase volume decreases, because the alcohol partitions more in excess phases. This change essentially follows the variation in the middle phase volume, so that the concentration of



**Fig. 4** Middle-phase volume variation by adding *n*-hexanol in the WIII system

FA in the middle phase remains roughly constant. The middle phase volume for C16 acid was higher due their more lipophilic character.

This study showed that the concentration for attaining the optimum microemulsion formulation decreases when the chain length FA increases, due to the lipophilic character of these surfactants. This is an additional argument for considering the partition coefficient to be a yardstick to render the physicochemical formulation concept. Furthermore, the FA with a long chain length are more appropriate for use in crude oil recovery due to their lipophilic character and capacity to form the oil/water emulsions. Consequently, the use of lipophilic alcohol leads to a more favorable microemulsion formation.

**Acknowledgments** This work was financially supported by various projects from the Venezuelan Ministry of Science and Technology (FONACIT G-2005000428 and G-2005000454) and from Zulia University of Research Council, (CONDES-LUZ CC-0322-06 and CC-0957-06).

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