

Effect of Degree, Type, and Position of Unsaturation on the pK_a of Long-Chain Fatty Acids

James R. Kanicky and Dinesh O. Shah¹

Center for Surface Science & Engineering, NSF-Engineering Research Center for Particle Science and Technology, Departments of Chemical Engineering and Anesthesiology, University of Florida, Gainesville, Florida 32611

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Titration of a series of C_{18} fatty acids yields pK_a values that decrease with an increasing degree of unsaturation in the fatty acid chain. The pK_a values of stearic, elaidic, oleic, linoleic, and linolenic acids were studied and compared to values of area per molecule in a spread monolayer of these acids. The decrease in pK_a was found to relate to melting point temperature and area per molecule in the spread fatty acid monolayer. The pK_a value was determined by first dissolving the fatty acid in a high pH solution ($pH > 10$) and subsequently titrating the solution with HCl to obtain the characteristic S-shaped curves used to calculate the pK_a values. The pK_a values of stearic, elaidic, oleic, linoleic, and linolenic acids were found to be 10.15, 9.95, 9.85, 9.24, and 8.28, respectively. These pK_a values were in the same order as area per molecule values of fatty acids in spread monolayers. This suggests that as area per molecule increases the intermolecular distance increases and pK_a decreases due to reduced cooperation between adjacent carboxyl groups. © 2002 Elsevier Science (USA)

Key Words: fatty acids; stearic; elaidic; oleic; linoleic; linolenic; pH; pK_a ; unsaturation; monolayer; molecular packing; area per molecule; intermolecular distance; limiting area; melting point temperature.

INTRODUCTION

The manner in which surfactant molecules align and pack at an interface is an important factor in systems involving soluble and insoluble interfacial films. Among the important properties of a surfactant that dictate the strength, elasticity, and stability of an adsorbed film are surface activity, chain length compatibility, and chain cohesion (1, 2), as well as the interaction between polar groups of the molecules in the monolayer (3–9). All of these factors contribute to the intermolecular distance and the overall stability of the monolayer.

In 1917, Irving Langmuir showed that monolayers of fatty acids of various chain lengths compress to the same limiting area, indicating that the different acids must all form films in which the molecules are orientated vertically with respect to the surface (10). It was further shown that fatty acids with a *cis* double bond in the chain showed limiting areas different than those of fatty

acids with a saturated chain. The addition of a double bond to an alkyl chain (1) limits flexibility of the chain in this region and (2) decreases adhesion between molecules (11, 12). It was found, for example, that a saturated fatty acid chain (e.g., stearic acid) has a limiting area of 21 \AA^2 , while an unsaturated chain (e.g., oleic acid) has a limiting area of 32 \AA^2 (2, 8).

Further evidence as to the effect of unsaturation on the packing of molecules at surfaces was shown by Shah and Schulman in the lecithin–cholesterol system (4, 5). They showed that the limiting areas of synthetic (dipalmitoyl), egg, and yeast lecithin monolayers depend on the degree of unsaturation of the fatty acid residues and that the addition of cholesterol to these systems serves to increase the area per molecule and fluidize the monolayer.

The compression data and limiting areas of the lecithin system obtained by surface pressure measurements were independently verified by an enzyme-catalyzed hydrolysis experiment (6). Shah and Schulman (6) measured the action of snake venom phospholipase A on lecithin monolayers. It was shown that a lecithin monolayer does not hydrolyze when it is compressed above a critical pressure. The critical surface pressure required to block the penetration of the enzyme into the monolayer increased with molecular area (i.e., dioleoyl lecithin > soybean lecithin > egg lecithin > dipalmitoyl lecithin), which corresponded to their area per molecule as well as to the increase in degree of unsaturation of the molecule. This and other experiments (7) corroborated previous limiting area results indicating that increasing the degree of unsaturation yields increased area per molecule in a spread monolayer. Furthermore, Rao and Shah have shown that the addition of a minimum of 20 mol% cholesterol to an arachidyl alcohol monolayer fluidizes the film and strikingly increases the evaporation of water through the film (13). Fang and Shah showed again using infrared imaging techniques that the molecular packing at the interface significantly affects heat transfer at the interface (14). These studies illustrate how monolayer packing and fluidity can influence important macroscopic phenomena.

The presence of a net charge on a surfactant polar group also affects its performance in an adsorbed monolayer. Fatty acids, for instance, can become ionized by an increase in the pH of the solution. If all the fatty acid molecules become ionized,

¹ To whom correspondence should be addressed. E-mail: shah@che.ufl.edu.

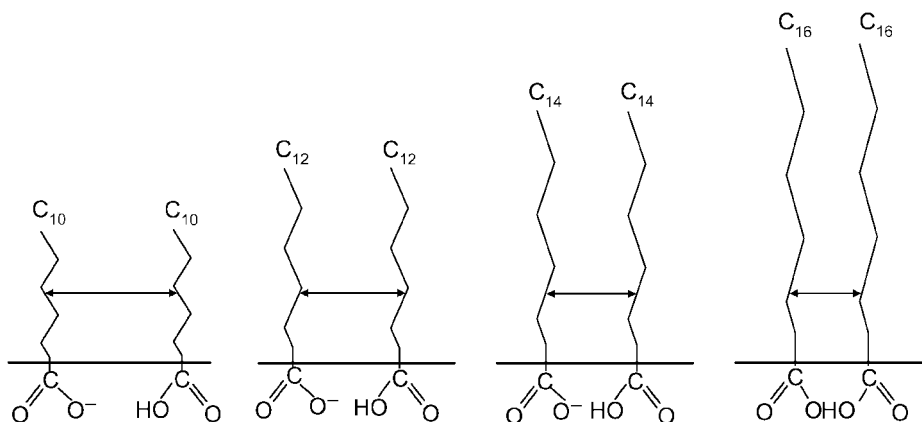


FIG. 1. Effect of chain length on the intermolecular distance between the fatty acid molecules in an adsorbed film.

repulsion between similarly charged molecules in the monolayer can result in an expansion of the monolayer at high pH (15, 16), which in turn can lead to a weak and unstable film. The change in monolayer characteristics of fatty acids as a function of pH of the bulk solution was first reported by Schulman and Hughes (17), who found that the surface potential (ΔV)–pH relation resembled an acid–base titration curve. For weakly ionized monolayers such as those created by fatty acids, it was proposed that the difference in pK_a between the surface and the bulk is small (18).

Most short-chain carboxylic acids have a pK_a value of *ca.* 4.8. For example, when acetic acid (CH_3COOH) and propionic acid ($\text{CH}_3\text{CH}_2\text{COOH}$) are dissolved in water, their pK_a values are found to be 4.74 and 4.87, respectively (19). The pK_a value represents the ionic environment of the solution where 50% of hydrogen atoms are removed from the carboxyl group by the existing OH^- ions in the solution. The pK_a can be decreased by attaching an electron-accepting substituent to stabilize the carboxylate anion (20). The converse effect—that of electron-releasing substituents lowering the acidity and raising the pK_a —is true, though less dramatic. The pK_a can also be raised by increasing the carbon chain length of the carboxylic acid. However, because electronic effects are not felt beyond two to three carbons, when the chain length is increased beyond about four carbons, the pK_a tends to level off. Increasing the chain length from pentanoic to hexanoic acid, for example, increases the pK_a from 4.82 to 4.83. Therefore, we know that *intramolecular* interactions (i.e., the effects on the carboxylate anion by the rest of the carbon chain) become negligible beyond four carbons in the alkyl chain.

However, in a previous work (21) it was shown that the pK_a values of long-chain fatty acids depend on the chain length of the fatty acid molecule and can increase to values much higher than those for the shorter chained analogs. These pK_a 's can reach values higher than 9 as the chain length increases to C_{16} and beyond. Heikkilä *et al.* (22) reported a pK_a for hexadecanoic acid (i.e., palmitic acid) of 9.7, and Peters (23) reported a pK_a of 8.5 for the same fatty acid. We find the pK_a of palmitic acid to be 8.6–

8.8 by titration (21). Christodoulou and Rosano (24) estimated pK_a values of 9.0 for octadecanoic (i.e., stearic, C_{18}) acid.

Figure 1 shows how the chain length of the fatty acid might affect its pK_a . It is known (25, 26) that, as the chain length increases, van der Waals interactions between the chains of adjacent molecules increase, bringing these molecules closer to each other. When this happens, the carboxylic acid groups of the fatty acids are also packed closer, shielding the hydrogen atom between the two oxygen atoms. The closer the molecules, the more strongly shielded the hydrogen atom and consequently the higher the pK_a (21).

This paper presents our studies on the observed pK_a values of C_{18} fatty acid solutions or aqueous dispersions and attempts to describe how the pK_a 's of these fatty acids can be influenced by the degree of unsaturation and subsequent packing behavior of the alkyl chains.

EXPERIMENTAL

Materials. Stearic acid (97+% purity) and oleic acid (97+% purity) were supplied by Fisher Scientific (Fair Lawn, NJ). Elaidic acid (99% purity) and linoleic acid (95% purity) were supplied by Sigma Chemical Company (St. Louis, MO). Linolenic acid (acid value, 203; saponification value, 200; iodine value, 192) was supplied by Pfaltz and Bauer (Waterbury, CT). Potassium hydroxide pellets and hydrochloric acid were supplied by Fisher Scientific (Fair Lawn, NJ). All solutions were prepared using water that was both deionized and distilled. All experiments were carried out at $20 \pm 1^\circ\text{C}$ unless otherwise stated.

Solution preparation. Each of the C_{18} fatty acids listed above were placed in a clean beaker containing 10 mol% excess solid potassium hydroxide pellets. Water was very slowly titrated to the mixture until all of the fatty acid and potassium hydroxide had dissolved, producing a clear solution for each fatty acid. Because C_{18} fatty acids are insoluble in water, it was assumed that this solution was composed of water-soluble potassium salts of these fatty acids. The concentrations of these fatty acid salt

solutions varied (stearic acid, 0.30 M; elaidic acid, 0.293 M; oleic acid, 0.35 M; linoleic acid, 0.66 M; linolenic acid, 0.61 M). The solutions were filtered with a 0.22 μm Cameo syringe filter and diluted with water to 100 mmol for determination of pK_a by titration.

Determination of pK_a . The pK_a values of the acids listed above were determined by titration at 20°C with 0.1 M hydrochloric acid using a METROHM 726 titroprocessor. The neutralization endpoint was first determined. This is the point of inflection on the S-shaped titration curve. The pK_a was subsequently calculated as the pH of the solution at half the neutralization volume (i.e., half the volume needed to reach the neutralization endpoint). Five titrations were performed on each solution and mean pK_a values for each fatty acid were calculated. Error bars were calculated using a 95% confidence interval.

RESULTS AND DISCUSSION

It was shown previously (21) that the molecules of a fatty acid monolayer are packed closest together when the pH of the solution is close to the pK_a of the fatty acid (Fig. 2). When the pH is very high, ionic repulsion between the carboxylate ions keeps the molecules apart. When the pH is close to the pK_a , approximately 50% of the acid groups in the adsorbed film are protonated. The added ion-dipole interaction between the ionized and unionized acid groups is absent when all of the acid groups are protonated (i.e., pH is very low).

Figure 3 shows the structures of the C_{18} fatty acids with the increasing degree of unsaturation studied. Stearic acid is completely saturated and consequently has a straight chain that can be packed tightly in a monolayer. Elaidic acid has a double bond at the number 9 carbon, but because the double bond is *trans* the molecule remains straight and can still pack well in a monolayer. Oleic, linoleic, and linolenic acids have increasing numbers of *cis* double bonds, resulting in chains that kink and bend at the position of the *cis* double bond (2). These molecules, therefore, become more and more difficult to pack easily in a monolayer.

Stearic acid has a limiting area of approximately 20 \AA^2 (27, 28) and packs into a solid state. Elaidic acid, even though it packs nicely, does not become fully condensed like stearic acid because of its double bond. Elaidic acid has a limiting area of approximately 31–33 \AA^2 (27). Oleic, linoleic, and linolenic acids do not pack as tightly and as a result form a liquid expanded state for which the limiting areas are approximately 41 \AA^2 for oleic acid (18, 28) and 48 \AA^2 for linoleic acid (28, 29). The limiting area for linolenic acid is a difficult value to obtain because of the inherent characteristics of the linolenic acid molecule. This molecule contains three unsaturated bonds and a carboxyl group. Together these polar groups seem to be sufficient to allow the molecule to leave the spread film when compressed to a sufficient pressure. Therefore, calculations of area per molecule may incorrectly assume a higher number of molecules in the monolayer and result in a low value of area per molecule (Fig. 2 of Ref. 29).

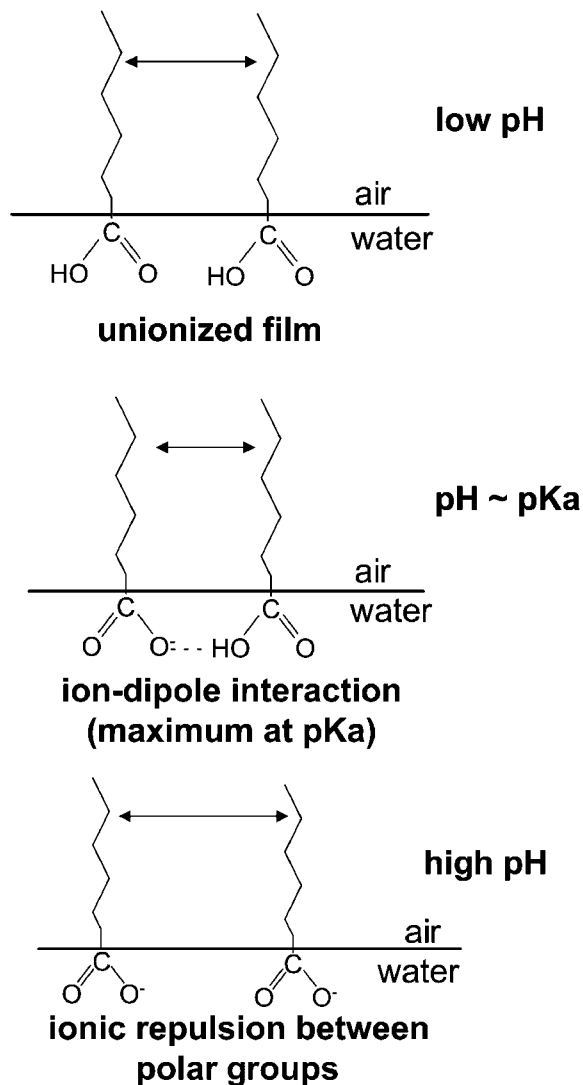


FIG. 2. A strong ion-dipole interaction among the carboxyl groups near the pK_a value decreases the intermolecular distance and area/molecule at the air/water interface.

Figure 4 is a schematic diagram of a typical pH titration curve obtained for fatty acids (30). When the pH of the solution is high, the solution is clear and contains only soluble potassium salts (Fig. 4a). As hydrochloric acid is added, the pH decreases until it is close to the pK_a of the fatty acid. At this point (Fig. 4b), crystals begin to appear in the solution. The pH then levels off and crystals continue forming (Fig. 4c) until total protonation of the fatty acids and precipitation of fatty acid crystals occur (Figs. 4d and 4e). The pK_a is calculated from the neutralization endpoint shown in Fig. 4.

Figure 5 shows the results of the titrations of the various C_{18} fatty acids with hydrochloric acid. As can be seen in this figure, stearic acid has the highest pK_a value ($pK_a = 10.15$), followed closely by elaidic acid ($pK_a = 9.95$), and then oleic acid ($pK_a = 9.85$), linoleic acid ($pK_a = 9.24$), and finally linolenic acid ($pK_a = 8.28$).

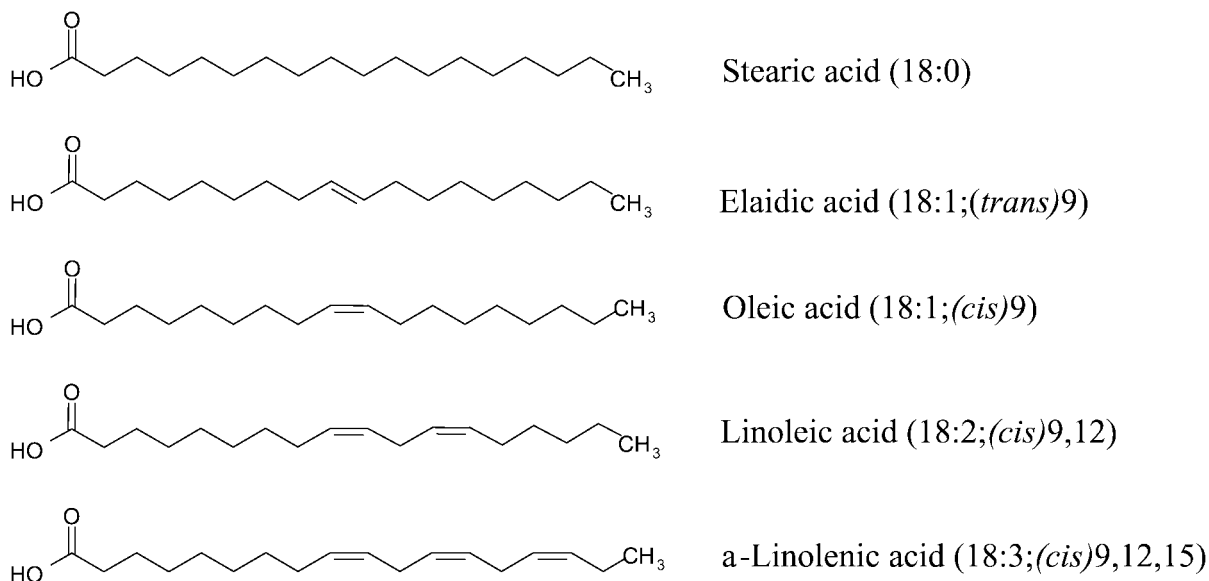


FIG. 3. Molecular structures of stearic, elaidic, oleic, linoleic, and α -linolenic acids.

The results are not surprising if they are interpreted in the manner described above. These C_{18} fatty acids have a tendency to be expelled from water in order to minimize the total energy of the system (i.e., to maximize entropy of the water molecules). Even when slightly soluble in the form of potassium salts, these long-chain fatty acids arrange themselves into oligomers or clusters in order to minimize the free energy of the system. Stearic

acid, with its straight alkyl chain, packs much tighter both in an adsorbed monolayer and in the small aggregates formed in solution. Therefore it is more difficult for hydroxide ions in solution to strip away the acid's proton. On the other end of the series, linolenic acid contains many kinks in its chain caused by three *cis* double bonds. These kinks prevent the molecules from packing closely, as is shown in Fig. 6. Elaidic acid, with

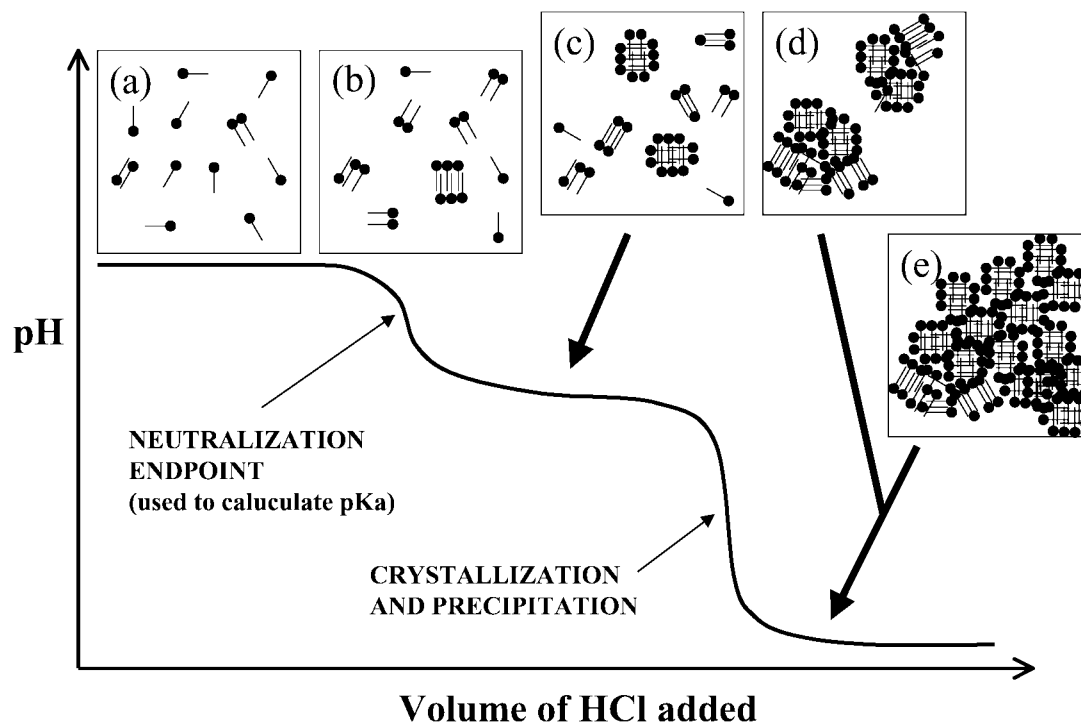


FIG. 4. Schematic diagram of a typical C_{18} fatty acid pH titration curve and the degree of aggregation vs pH expected in the aqueous solution. (a) At $pH > pK_a$ the solution is clear and contains soluble potassium salts. (b) At $pH \sim pK_a$ crystals begin to appear in solution. The crystals keep forming at lower pH values (c) until total conversion to insoluble fatty acid (d or e).

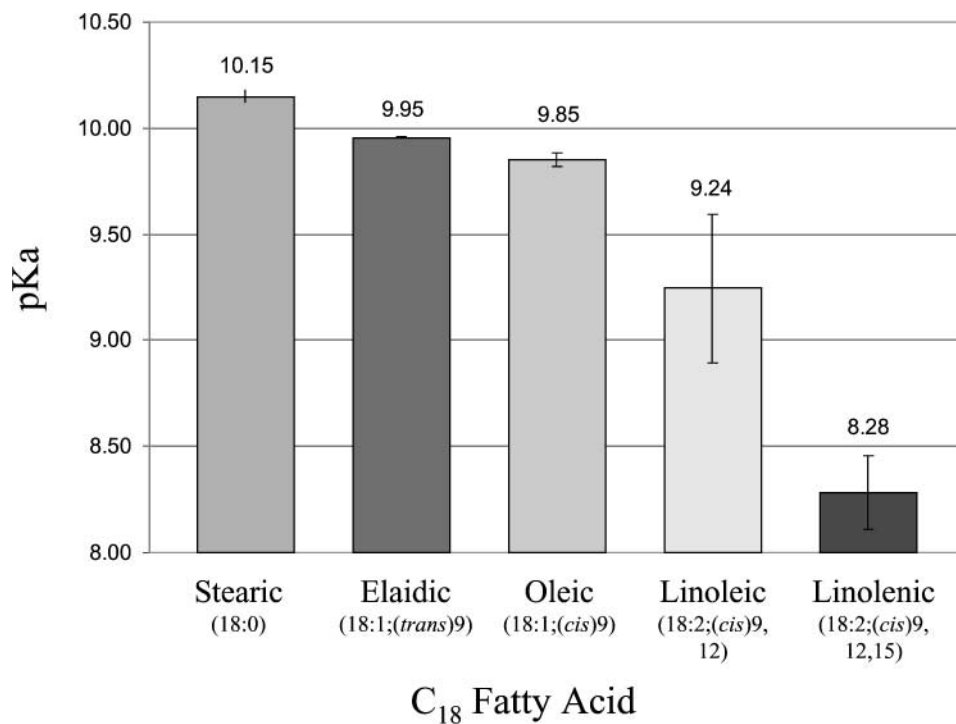


FIG. 5. pK_a values vs degree and nature of unsaturation of C_{18} fatty acids, obtained by acid–base titration at 20°C.

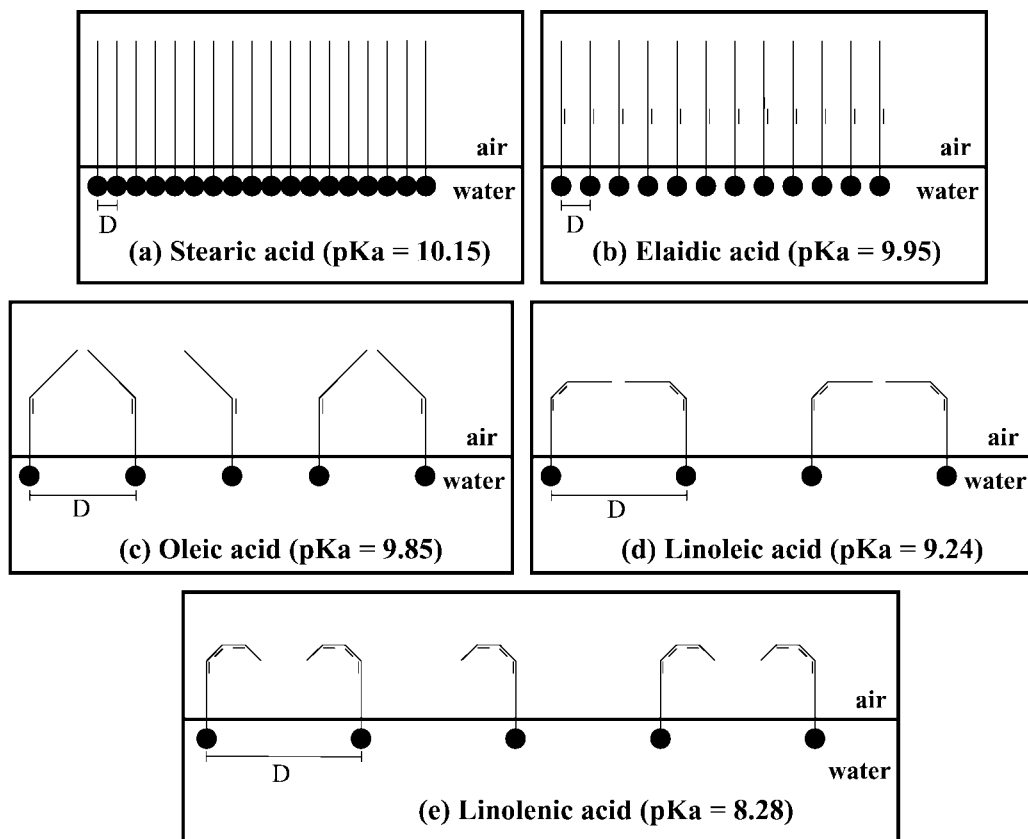


FIG. 6. Schematic representation of C_{18} fatty acid monolayers at the air/water interface. Note the effect of the degree of unsaturation on the area per molecule and the intermolecular distance, D , in the spread monolayers.

TABLE 1
Selected Physical Properties of C₁₈ Fatty Acids

C ₁₈ fatty acid	Structure and degree of saturation	Melting point temp. (°C) ^a	Limiting area of monolayer (Å ²)	Intermolecular distance (Å) in monolayer ^b	Observed pK _a
Stearic acid	18:0	69–71	20 ^c	4.47	10.15
Elaidic acid	18:1; (<i>trans</i>)9	44–45	31–33 ^d	5.57–5.74	9.95
Oleic acid	18:1; (<i>cis</i>)9	13–14	41 ^e	6.40	9.85
Linoleic acid	18:2; (<i>cis</i>)9,12	–5 – –1	48 ^f	6.93	9.24
α-Linolenic acid	18:3; (<i>cis</i>)9,12,15	–11 – –10	—	—	8.28

^a Sigma–Aldrich catalog.

^b Calculated from limiting area of monolayer data.

^c Refs. 27 and 28.

^d Ref. 27.

^e Refs. 18 and 28.

^f Refs. 28 and 29.

its *trans* double bond, packs much closer than its *cis* double bond counterpart oleic acid. As can be seen in Fig. 5, this results in a higher pK_a value as well. One must keep in mind that these molecules are always in a state of thermal kinetic motion. Even in a spread monolayer, fatty acid tails are spinning, rotating, and colliding with the adjacent molecules. These collisions result in increased intermolecular distance, *D*, as illustrated in Fig. 6.

Table 1 contains data on melting point temperatures (*T*_m), intermolecular distances between molecules in a spread monolayer, and the observed pK_a values of the fatty acids studied. It is interesting to note that the decrease in pK_a values corresponds to a decrease in melting point temperatures. This makes sense since the melting point temperature is an indication of the energy required to disrupt the molecular packing of crystals in the solid phase. For molecules that can pack very closely (e.g., stearic acid), *T*_m is relatively high. As degree of unsaturation increases, *T*_m decreases and so too does pK_a. However, melting point temperature and pK_a do not have such a strong correlation. For example, there is an approximate 30°C difference in *T*_m between elaidic acid and oleic acid, while only a 0.1 difference exists between their pK_a's.

Area/molecule and intermolecular distance calculations of fatty acid monolayers made from π-area curves leave uncertainties as to whether or not the fatty acids present at the surface are actually present in uniform spread molecules or if they have formed aggregates or islands at the surface. Fatty acid monolayer phase transitions can also be studied using methods such as X-ray diffraction (31–36), FT–Raman spectroscopy (35), and Brewster angle microscopy (BAM) (36, 37). A correlation between fatty acid monolayer phase transition and solution pH has been shown using the BAM technique (37). Specifically, spread monolayers of fatty acids show maximum resistance to phase transition under compression when the pH of the solution equals the pK_a, indicating that the L₂ phase, which is characteristic of fatty acid monolayers, is stabilized by the ion–dipole interactions between carboxylic acid and carboxylate headgroups (Fig. 10 of Ref. 37).

In summary, the results presented in this paper report for the first time the effect of the degree of unsaturation on the pK_a values of oleic, linoleic, and linolenic acids and further correlate the pK_a value of a long-chain fatty acid with the intermolecular distance between the fatty acid molecules. The greater the intermolecular distance, the lower the pK_a value of the acid.

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