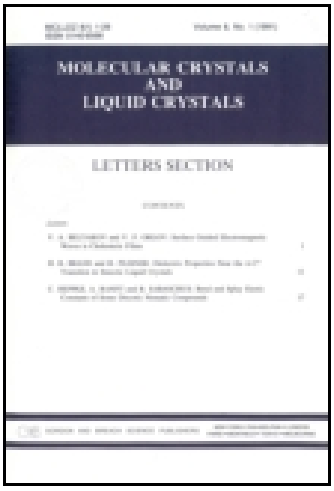

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Liquid Crystal-Based Sensors for Rapid Analysis of Fatty Acid Contamination in Biodiesel

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Liquid Crystal-Based Sensors for Rapid Analysis of Fatty Acid Contamination in Biodiesel

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Biodiesel is produced from various vegetable oils via a transesterification reaction. Emulsification of biodiesel during the transesterification reaction can occur, however, if free fatty acids are present in the feedstock oil. The emulsified product is not usable. Here we report a facile method using a liquid crystal (LC)-based sensing platform for detection of free fatty acids in oils at levels (1 wt%) relevant to biodiesel production. The approach involves the partitioning of fatty acid (doped into LC) to an aqueous-LC interface and an associated anchoring transition of the LC, leading to an optical read-out. Unlike other methods for detection of fatty acids (e.g, pH), this approach directly measures interfacial activity of fatty acids that underlies emulsion stability. We describe the influence of the molecular structures and concentrations of fatty acids, and the solution condition (pH), on fatty acid-triggered ordering transitions of LCs.

Keywords Liquid crystal-base sensors; fatty acid; biodiesel

Introduction

Biodiesel is an alternative diesel fuel derived from vegetable oils and animal fats. It has gained interest as a renewable source of liquid fuel [1, 2]. Vegetable oils used for preparation of biodiesel typically consist of triglyceride molecules formed by esterification of three long-chain fatty acids with a single glycerol molecule. Biodiesel refers to the alkyl esters of long-chain fatty acids that are formed by transesterification of triglycerides with alcohols via the reaction shown in Fig. 1a. In processes leading to the preparation of biodiesel, the presence of free fatty acids can lead to emulsification of the vegetable oil and aqueous glycerol products of the transesterification reaction, resulting in a failed batch of biodiesel. The acidity of the oil also reduces the conversion efficiency [3]. Different sources of oils used for biodiesel production contain different types and concentrations of fatty acids, as depicted in Table 1 [4]. Another low- cost feedstock for biodiesel is waste cooking oil from restaurants, which typically contain a level of free fatty acids that is greater than 2 wt% [5]. If it can be determined that more than 1 wt% free fatty acid is present in an oil to be used for biodiesel production, an acid-catalyzed esterification of the fatty acid can be performed, resulting in the elimination of the problems associated with emulsification [6]. This report

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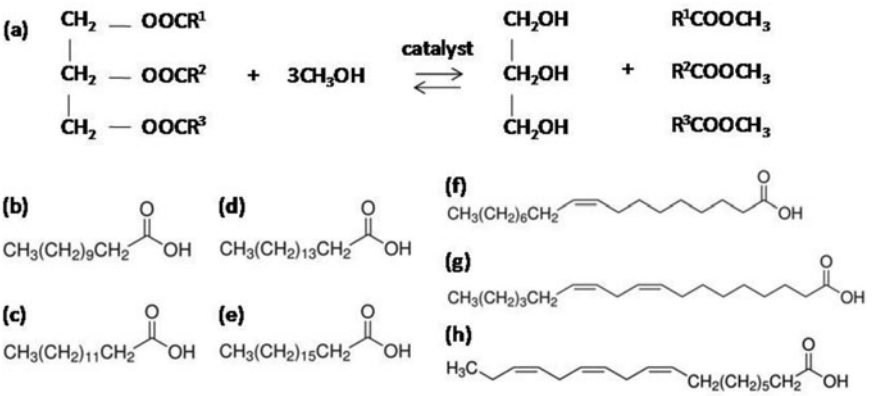

Figure 1. (a) Schematic illustration of transesterification of triglycerides with alcohols. The structures of the fatty acids used in experiments described below - (b) lauric acid, (c) myristic acid, (d) palmitic acid, (e) stearic acid, (f) oleic acid, (g) linoleic acid and (h) linolenic acid.

describes a facile method to detect the presence of fatty acid in an oil sample using a liquid crystal (LC, 4′-pentyl-4-cyanobiphenyl (5CB)).

The approach described in this paper is based on past studies of interfaces formed between aqueous phases and thermotropic liquid crystals (LCs) [7]. Specifically, Brake and co- workers demonstrated that adsorption and desorption of surfactant (sodium dodecyl sulfate (SDS)) at aqueous-LC interfaces could be observed via changes in the optical appearance of the LCs caused by surface anchoring transitions. Subsequent investigations revealed that the anchoring of the LCs was influenced by the molecular structure of surfactants and their interfacial organization [8–10]. Based on these reports and others [11], the so-called homeotropic orientation of LCs at these amphiphile-laden interfaces is generally understood to be a consequence of interactions (including interdigitation) of the hydrocarbon tails of the amphiphiles with the LCs. Because the LCs reorder on time-scales of tens of milliseconds, the time-dependent behavior of the LCs can be used to follow dynamic phenomena involving a range of amphiphilic species at these interfaces. Additionally, the surface sensitivity of LCs to biomolecules such as phospholipids, proteins and nucleic acids has been utilized as the basis of chemical and biological sensors [12, 13].

The study reported in this paper is focused on the assembly of fatty acids at aqueous-LC interfaces. Of specific relevance to our current studies, Hu and co-workers have demonstrated that an LC orientational transition from planar to homeotropic anchoring can be observed when an alkaline solution containing hydrogen peroxide is contacted with dodecanal-doped 5CB [14]. This result was attributed to the self-assembly of a dodecanoate monolayer formed at the aqueous-LC interface due to oxidation of aldehyde into carboxylic acid by hydrogen peroxide. A second study revolved around an investigation of 5CB doped with 4′-pentyl-biphenyl-4- carboxylic acid (PBA) [15]. Specifically, Bi and co-workers demonstrated that a planar to homeotropic anchoring transition of PBA-doped 5CB could be induced by a very small change in pH (from 6.9 to 7.0). The pH-driven optical response of the LC was attributed to the protonation and deprotonation of PBA at the aqueous-LC interface. The pH sensitivity of the PBA-doped LC was subsequently exploited for monitoring local pH changes resulting from enzymatic reactions.Here we report a study that builds from the above-mentioned studies to develop a platform for detection of free fatty acids contained in a sample of vegetable oil (vegetable oil is composed mainly

Table 1. Fatty acid compositions (wt%) of vegetable oils [4].

Fatty acid	Palm	Olive	Peanut	Rape	Soybean	Sunflower	Grape	Almond	Corn
Lauric (C12:0)	0.1	0	0	0	0	0	0	0	0
Myristic (C14:0)	0.7	0	0.1	0	0	0	0.1	0	0
Palmitic (C16:0)	36.7	11.6	8	4.9	11.3	6.2	6.9	10.4	6.5
Stearic (C18:0)	6.6	3.1	1.8	1.6	3.6	3.7	4	2.9	1.4
Oleic (C18:1)	46.1	75	53.3	33	24.9	25.2	19	77.1	65.6
Linoleic (C18:2)	8.6	7.8	28.4	20.4	53	63.1	69.1	7.6	25.2
Linolenic (C18:3)	0.3	0.6	0.3	7.9	6.1	0.2	0.3	0.8	0.1
Others	0.9	1.9	8.1	23	1.1	1.6	0.4	1.5	1

of triglycerides). Unlike triglycerides, fatty acids are readily ionized by an increase in the pH of the solution, leading to their increased interfacial activity. Since free fatty acids are dissolved in the vegetable oil phase, our initial experimental system reported below used fatty acid doped into the nematic 5CB. We hypothesized that the fatty acid in 5CB would partition to the aqueous-LC interface when contacted with an alkaline aqueous solution (pH 9) due to ionization of the carboxylic group of the fatty acid. This interfacial partitioning, we predicted, would result in an anchoring transition in the LC due to the interaction of the aliphatic tails of the interfacial fatty acid molecules with the LC, thereby indicating the presence of the fatty acid in the sample. Our results show that anchoring transitions are observed, and that they are dependent on the structure of the fatty acids (including length and degree of unsaturation of the hydrocarbon chains; see Fig. 1b-h for the structures of fatty acids used in our experiments). We also demonstrate that the fatty acid-triggered anchoring transitions can be used to detect 1 wt % fatty acid in vegetable oil.

Materials and Methods

Materials

Lauric acid, myristic acid, palmitic acid, stearic acid, *cis*-9-octadecenoic acid, *cis*-9,*cis*-12- octadecadienoic acid, *cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid, Na_2HPO_4 and octyltrichlorosilane (OTS) were obtained from Sigma-Aldrich (St. Louis, MO). Chloroform and Fisher's Finest Premium Grade glass slides were purchased from Fisher Scientific (Pittsburgh, PA). Gold- coated specimen grids (20 μm thickness, 283 μm grid spacing, and 50 μm bar width) were obtained from Electron Microscopy Sciences (Fort Washington, PA). The nematic LC 4′-pentyl-4-cyanobiphenyl was obtained from EMD Chemicals (Spring Valley, NY). Deionization of a distilled water source was performed with a Milli-Q system (Millipore, Bedford, MA) to produce water with a resistivity of 18.2 MΩ-cm.

Methods

Preparation of fatty acid-doped 5CB

Fatty acids were dissolved in chloroform at pre-determined concentrations and dispensed into glass vials in volumes that gave the desired concentration in 5CB upon mixing. The solution was dried using a stream of nitrogen and the vial was placed under vacuum for at least 1 h to remove chloroform.

Preparation of Interfaces of Nematic 5CB

A detailed description of the method used to prepare micrometer-thick films of LC hosted within gold specimen grids can be found in a previous publication [7]. Briefly, glass microscope slides were cleaned according to published procedures and coated with OTS. Gold specimen grids were placed onto the surface of the OTS- treated glass slides. The grids were filled with 5CB using a blunt-tipped glass syringe, and the excess LC was removed such that the grid was uniformly filled with LC. The LC-impregnated grid supported on an OTS-treated glass slide was immersed in a dish of de-ionized water. A buffer solution containing 0.1 M Na_2HPO_4 (pH 9, unless otherwise stated) was subsequently introduced into the dish.

Optical Characterization of LC Ordering

The orientation of the nematic phase of 5CB was determined by using plane-polarized light in transmission mode on an Olympus BX60 microscope with crossed polarizers. The gold grid hosting the film of 5CB was placed on a rotating stage located between polarizers. In-plane birefringence was indicated by a bright, colored appearance of the 5CB and the presence of brush textures when the sample was viewed between crossed polarizers. Homeotropic alignment of the LC was determined by first observing the absence of transmitted light during a 360° rotation of the sample between crossed polarizers. Insertion of a condenser below the stage and a Bertrand lens above the stage allowed conoscopic examination of the LC film. An interference pattern consisting of two crossed isogyres confirmed homeotropic alignment. All images were captured using a digital camera (Olympus C-4000 Zoom) mounted on the microscope. The camera was set to an f-stop of 2.8 and a shutter speed of 1/60 s.

Results

Anchoring of Fatty-Acid-Doped 5CB at Aqueous Interfaces

Previous studies have established that the anchoring of 5CB at an aqueous-LC interface can change from planar to homeotropic with increasing areal density of amphiphiles adsorbed at the interface [16, 17]. Therefore, we first sought to investigate the minimum concentration of palmitic acid (PA, C16) in 5CB required to induce an LC ordering transition. PA was selected based on its relevancy to fatty acid composition in vegetable oil (PA is a main component of palm oil; see Table 1). As mentioned in the Introduction, fatty acid is introduced into the LC because, as described below, the oil samples in which fatty acid are found are soluble in the LC. The fatty acid-containing LC was hosted in a TEM grid (supported on an OTS-treated glass microscope slide) and submerged into deionized water.

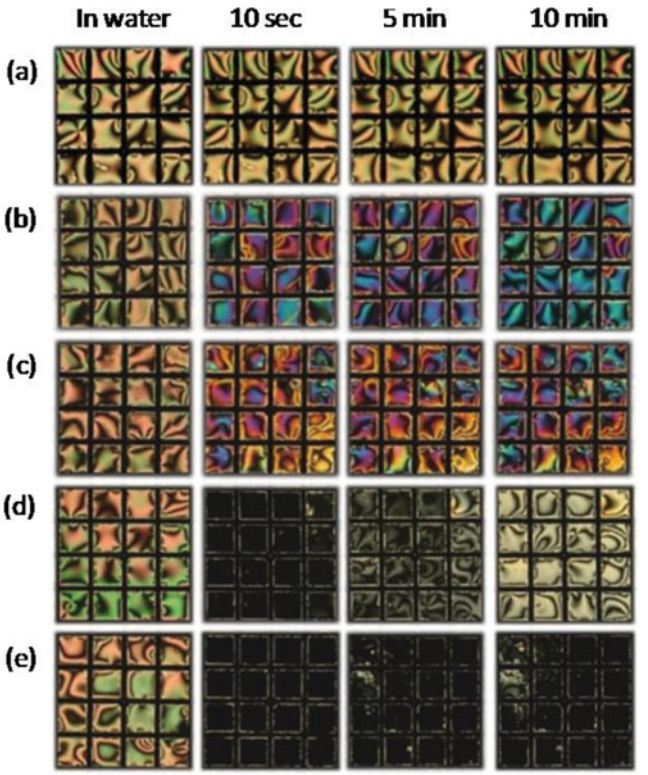

Figure 2. Optical images (crossed polarizers) of 5CB doped with palmitic acid before and after introduction of an alkaline buffer. The concentrations of PA in 5CB were (a) 0 mM, (b) 0.1 mM, (c) 0.15 mM, (d) 0.2 mM and (e) 0.3 mM,

An alkaline solution (0.1 M Na_2HPO_4 solution, pH 9) was subsequently introduced into the aqueous phase to change the pH.

The optical appearance of the LC (under crossed polars) doped with increasing concentrations of PA (0 mM to 0.3 mM) before and after addition of the alkaline solution are shown in Fig. 2. This range of concentrations of PA was selected because we calculated the concentration of PA in 5CB required for monolayer coverage at the aqueous-LC interface (assuming all the amphiphiles from the bulk partition to the aqueous-LC interface) to be 0.3 mM. From the results shown in Fig. 2, we make three observations. First, when submerged under deionized water (pH 5.5), the interference colors indicate that the LC films assumed a planar orientation at the LC interface, suggesting that most of the fatty acids remained in the bulk LC. Upon introduction of the alkaline solution and an associated increase in pH to 9, the LC film that did not contain fatty acid remained planar (Figure 2a). In contrast, a change in the orientation of the LC film containing PA was observed (Figs. 2b-e). We attribute this difference to the ionization of the carboxylic group of the fatty acid, causing it to adsorb or partition to the aqueous-LC interface (see below for the effect of pH and for additional discussion). Overall, this result supports our hypothesis that ordering transitions can be triggered by the accumulation of PA at the interface.

Second, inspection of Fig. 2 reveals that the minimum concentration of PA in 5CB required to induce a homeotropic orientation (dark appearance) immediately following the

increase in pH is 0.2 mM (Fig. 2d). This value is close to the threshold concentration of PA in 5CB required for monolayer coverage, and thus this result supports our hypothesis that the initial appearance of a homeotropic LC orientation (which is transient, see below) is driven by interfacial assembly of fatty acids. Below 0.2 mM, tilting of the LC was observed (resulting in bright interference colors; Fig. 2b-2c) following the shift in pH, suggesting that PA did partition to the interface, but that the interfacial density of fatty acid was too low to orient the LC homeotropically. This proposition is consistent with previous studies that have demonstrated that the orientations of LCs at aqueous-LC interfaces depend on the interfacial density of amphiphiles [18].

The third observation from Fig. 2 relates to the time-dependence of the LC orientational behavior (Figure 2d). Ten minutes after the change in pH, we observed the homeotropically-aligned LCs (0.2 mM) to start tilting away from the surface normal, as indicated by the re-appearance of grey and yellow interference colors. The tilt of the LC doped with the lower concentrations of PA (Fig. 2b-2c) also changed with time (as indicated by the shift to higher order interference colors). We interpret these observations to suggest that the fatty acids are desorbing from the LC interface to the aqueous phase. To further test this interpretation, we performed two additional experiments. In the first experiment, a small amount of PA was added to the aqueous phase (0.02 mM; we verified that this concentration of PA in the aqueous phase did not trigger an ordering transition of a film of pure 5CB). We hypothesized that the dynamics of the above-described anchoring transition (tilting away from the surface normal) would be slowed by the presence of PA in the aqueous phase since the net rate of desorption of PA would be lowered (due to a lower concentration driving force for desorption). Consistent with this hypothesis, our results show that the presence of 0.02 mM of PA in the aqueous phase caused the transient homeotropic state of the system to persist over longer intervals of time (Fig. S1a-b).

In the second experiment, we contacted a film of pure 5CB with an aqueous phase containing a concentration of PA (3 nM of PA in 6 mL aqueous buffer) equivalent to that which would be generated by dissolution of all PA added to the 5CB into the aqueous phase (0.2 mM in 0.1 μL 5CB). Our results show that this concentration of PA in the aqueous phase did not induce an anchoring transition in the 5CB (Fig. S1c). Overall, these two results support the proposal that PA is transiently present at the aqueous-LC interface and that its transient presence leads to the dynamic anchoring transition shown in Fig. 2.

Influence of Tail Length of Fatty Acids on the Anchoring Transitions of 5CB

Next, we investigated the influence of the tail lengths of the fatty acids (as described in Table 1) on the LC anchoring transitions. Past studies by Brake and co-workers revealed that the orientation of 5CB at aqueous interfaces was dependent on the length of the aliphatic chain of adsorbed amphiphiles. Specifically, surfactants with short alkyl chains ($n = 8$ for CnTAB and $n = 7$ and 12 for FCnAB) did not perturb the planar orientation of 5CB at the interface (up to concentrations at which the 5CB began to be solubilized by the surfactant). In the experiments described below, the length of the fatty acid was varied from C12 to C18 (Fig. 1b-1e), a range that is commonly found in vegetable oils (Table 1).

Inspection of Fig. 3a shows that a film of 5CB doped with 0.3 mM of lauric acid (C12) exhibited only tilted orientations (no homeotropic alignment). To determine if the absence of a homeotropic orientation is due to the equilibrium anchoring of 5CB by C12 or because of a high rate of desorption of the fatty acid, we performed two additional experiments. First, we increased the concentration of lauric acid to 3 mM in 5CB to increase the flux of

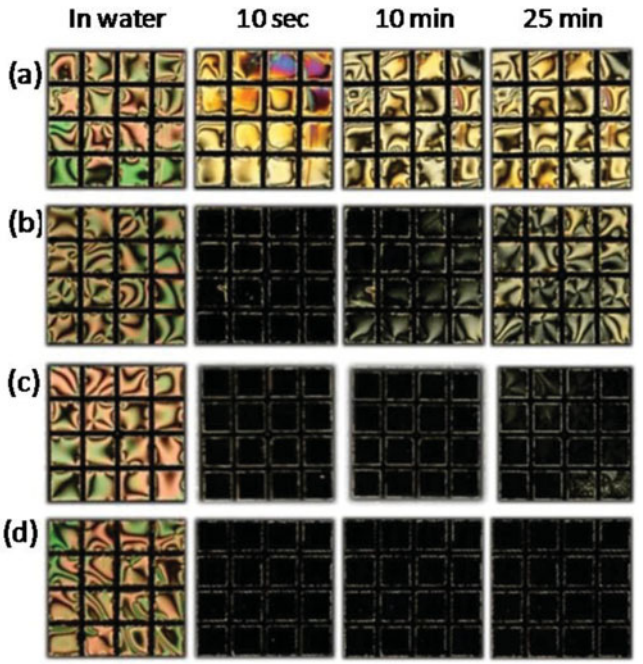

Figure 3. Optical images (crossed polarizers) of 5CB doped with fatty acids (0.3 mM in 5CB) with tail length of (a) C12, (b) C14, (c) C16 and (d) C18 before and after introduction of an alkaline buffer.

the fatty acid from the bulk of the LC to the aqueous-LC interface. Our results show that a short-lived homeotropic state was achieved upon introduction of an alkaline solution, but the LC quickly tilted away from the interface normal (See Fig. S2a). This suggests that a sufficiently high interfacial concentration C12 can induce a homeotropic orientation of LC. In the second experiment, 0.25 mM of lauric acid (in an alkaline buffer) was introduced into an *aqueous* phase that contacted a film of pure 5CB (no fatty acids doped into LC). A homeotropic orientation was exhibited by the LC in this experiment (Fig. S2b). These results, when combined, suggest that a homeotropic alignment can be induced by a high interfacial density of laurate at the LC interface, and that the absence of the homeotropic orientation in Fig. 3a was likely due to a high rate of desorption of laurate from the interface (leading to minimal transient accumulation of the amphiphile at the interface).

Figure 3b shows the response of a film of 5CB doped with myristic acid (C14) upon introduction of an alkaline solution. Although a homeotropic orientation was observed at short times, a gradual tilting of the nematic 5CB away from the surface normal was observed after 25 mins. For both palmitic and stearic acid (C16 and C18, respectively), a stable homeotropic orientation was observed to persist for at least 25 mins (Fig. 3c-3d). Overall, these results indicate that the tail length influences the tendency of the fatty acid to accumulate at the aqueous-LC interfaces. However, it is possible that the interaction of the fatty acid and LC (at fixed areal density) is influenced by the length of the fatty acid. Consistent with this proposition, Brake and co-workers attributed the effect of chain length to reflect a combination of lower surface excess concentrations (packing behavior) and lower penetration of 5CB into the surfactant monolayer as the aliphatic chain length of the surfactant decreased [9].

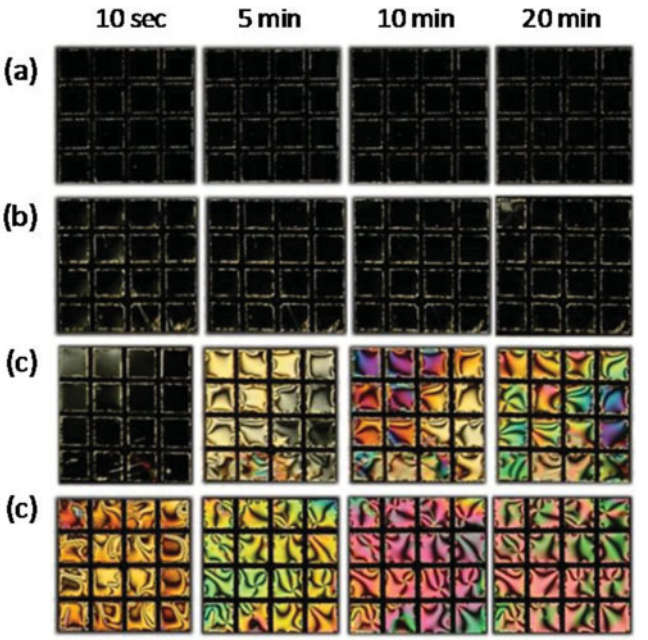

Figure 4. Optical images (crossed polarizers) of 5CB doped with fatty acid (0.4 mM in 5CB) comprised of tails with varying degree of unsaturation (or number of double bonds) - (a) C18:0, (b) C18:1, (c) C18:2 and (d) C18:3 - before and after introduction of an alkaline buffer.

Influence of Degree of Unsaturation of Fatty Acids on the Anchoring Transitions of 5CB.

Most vegetable oils are composed of fatty acids with varying degrees of unsaturation (see Table 1). The degree of unsaturation influences the conformations of the amphiphiles. We hypothesized that the degree of unsaturation would influence the interfacial interactions of fatty acids and 5CB. For example, Lockwood and co-workers have observed that branching of the aliphatic tails of surfactants can hinder homeotropic anchoring of LCs due to the impact of branching on the packing of surfactant at the interface [8]. Below we report an investigation of the degree of unsaturation of fatty acids on anchoring transitions of 5CB. We focused our investigation on fatty acids with a C18 chain, where the number of double bonds (unsaturated sites) in the chain varied from 0 to 3 (vegetable oils contain fatty acids with 0–3 double bonds; see Table 1 and Fig. 1f-h). Fig. 4 shows that homeotropic orientations were observed for C18 fatty acids that were saturated (denoted as C18:0) or had one or two *cis* double bonds (C18:1 or C18:2, see Fig. 4a-c). However, the homeotropic orientation observed for linoleic acid (C18:2)-doped 5CB was transient, resulting in tilted states after 5 mins (Fig. 4c).

Interestingly, linolenic acid (C18:3) did not cause a homeotropic orientation. Instead, it led only to transient tilted states of the 5CB (Fig. 4d).

We note that stearic acid (C18:0) has a limiting area of approximately 0.2 nm2 and packs into solid phase langmuir films. Oleic acid (C18:1) and linoleic acid (C18:2) do not pack as tightly and as a result form a liquid expanded state for which the limiting areas are approximately 0.41 nm2 and 0.48 nm2 respectively [19]. The limiting area for

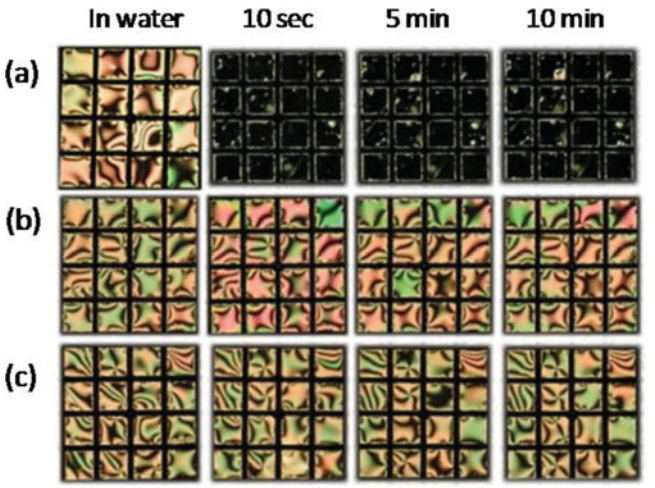

Figure 5. Optical images (crossed polarizers) of 5CB doped with 0.3 mM of palmitic acid and subsequently contacted with buffers of (a) pH 9.0, (b) pH 8.2 and (c) pH 7.8.

linolenic (C18:3) was not found in the literature, presumably because these molecules leave the spread film when compressed to a sufficient surface pressure (consistent with the propensity of C18:3 to desorb being higher than the other C18 fatty acids). To test if the interfacial concentration of the amphiphile (specifically, C18:3) at the aqueous-LC interface was impacting its interaction with the LC, we introduced linolenic acid into the aqueous phase in contact with a pure 5CB film (0.1 mM in alkaline buffer). Homeotropic orientation was observed in this case (see Fig. S3), suggesting that the degree of saturation impacts the surface concentration of the amphiphile, similar to the trend that was observed with varying tail lengths. In general, the solubility of molecules in water increases with degree of unsaturation (i.e., number of double bonds), and therefore we can expect that the driving force for desorption of fatty acids from the LC-aqueous inteface increases with degree of unsaturation of the fatty acids.

pH-Dependence of the Anchoring Transition of Fatty-Acid-Doped 5CB

We interpret the results above to reflect partitioning of fatty acids from bulk LC to an aqueous interface upon exposure to alkaline aqueous conditions. To confirm this interpretation, we varied the pH of the aqueous buffer (by adding 1 M HCl) that was contacted with 5CB doped with 0.3 mM of PA and characterized the effect of pH on the anchoring of the LC. Fig. 5 shows that a homeotropic orientation was observed only when the pH of the aqueous solution was above 8.2. The bulk p*Ka* of PA has been reported to be 8.6 – 8.8 [19], although we also note that the chain length and the environment (in this case, the LC interface) might affect its p*Ka*. Because the anchoring of the LC was not observed to change at values below the p*Ka* of PA, these results provide support for our interpretation of the homeotropic orientation of the LC as resulting from the ionization of the carboxylic acid group of the fatty acids during partitioning to the aqueous interface.

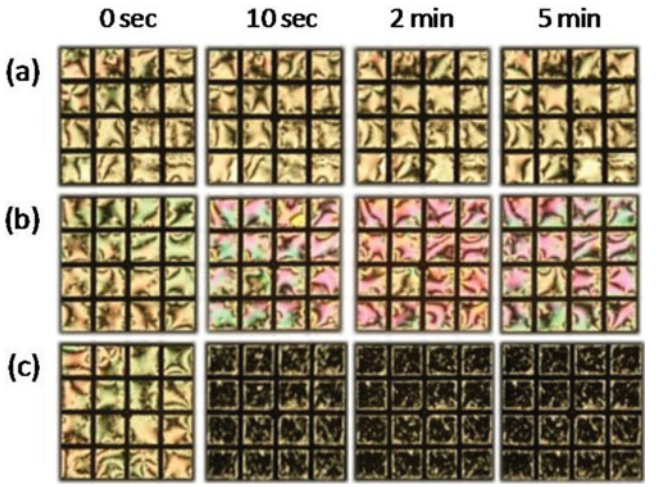

Figure 6. Optical images (crossed polarizers) of 5CB doped with 6.5 mM of glycerol tripalmitate and varying amounts of palmitic acid before and after introduction of an alkaline buffer. The concentrations of palmitic acid in 5CB were (a) 0 mM, (b) 0.1 mM and (c) 0.2 mM.

Anchoring Transitions of 5CB Induced by Fatty Acid-Contaminated Oil

As mentioned in the Introduction, triglyceride (TG) is the main component of vegetable oil (see also Fig. 1). Next, therefore, we sought to investigate the effect of TG on fatty acid-induced ordering transition of 5CB. In this experiment, a mixture of glycerol tripalmitate and PA was doped into 5CB to create a model oil with fatty acid contamination. The concentration of glycerol tripalmitate in 5CB was kept constant at 6.5 mM (or 5.25 g TG/L 5CB). Fig. 6 shows the response of the LC to varying concentrations of PA in 5CB in the presence of the TG. In the absence of PA, the alignment of TG-doped 5CB remained planar upon exposure to alkaline solutions, suggesting that unlike PA, TG does not induce an anchoring transition in the LC (Fig. 6a). At 0.1 mM of PA, a change in tilt of the LC was observed (Fig. 6b), and a further increase of the PA concentration to 0.2 mM led to an anchoring transition to a homeotropic orientation (Fig. 6c). Overall, these results demonstrate that the presence of TG does not appear to change the critical concentration of fatty acid required to induce an anchoring transition (0.2 mM; Fig. 2).

Significantly, concentration ratio of PA and TG used in the experiment (6.5 mM TG and 0.2 mM PA doped in 5CB) corresponds to 1 wt% PA in TG.

The final experiment that we report in this paper involved doping commercial cooking oils containing 1 wt% of PA into 5CB. Specifically, canola and soybean oil were used (we note that refined cooking oils have a free fatty acid content that is much less than 1 wt%). The mass of oil added into 5CB was the same as reported above (5.25 g/L 5CB), and 1 wt% of PA was added to both canola and soybean oil. The resulting mixture was introduced into 5CB using the methods described above. Fig. 7a and c shows that the presence of either pure canola or soybean oil (no added fatty acid) in 5CB did not trigger an anchoring transition in the LC, indicating that there are no components of these cooking oils that cause an LC response to the pH change. However, the LC films that contained canola or soybean oil doped with 1 wt% of PA exhibited a homeotropic orientation upon exposure to the alkaline solution. Overall, these results show that the LC-based system described in this paper is capable of detecting the presence of 1 wt% of fatty acid in either soybean or

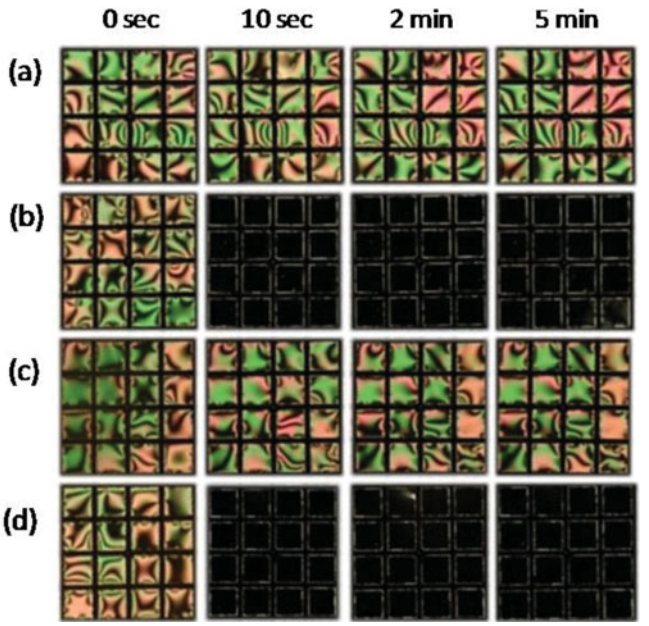

Figure 7. Optical images (crossed polarizers) of 5CB doped with canola oil, where the concentration of palmitic acid is (a) 0 wt% and (b) 1 wt% of the oil, following introduction of an alkaline buffer. Optical images (crossed polarizers) of 5CB doped with soybean oil, where the concentration of palmitic acid is (c) 0 wt% and (d) 1 wt% of the oil, upon introduction of an alkaline buffer.

canola oil. This result indicates that LC- based sensing can be used to analyze vegetable oils for the presence of free fatty acids.

Conclusion

The principal conclusion of the study reported in this manuscript is that thin films of nematic 5CB, when doped with vegetable oil samples contaminated with fatty acids, are capable of detecting fatty acids in the oil samples at concentrations relevant to biodiesel production. Specifically, we observed 5CB doped with fatty acid to change from a planar orientation to a tilted or homeotropic orientation upon exposure to an alkaline aqueous solution. The response of the LC is an easily detected optical signal, and is triggered by partitioning of fatty acids in 5CB to the aqueous-LC interface due to ionization of the carboxylic group of the fatty acids upon contact with an alkaline aqueous solution. For some fatty acids, we observed a transient homeotropic orientation of 5CB (i.e., gradual tilting away of the LC from the homeotropic orientation), which we attribute to desorption of the amphiphiles from the LC interface into the bulk aqueous phase. The dynamics of the LC response were observed to depend on both the tail lengths and degree of unsaturation of the hydrocarbon chain of the fatty acids. For instance, chains with shorter lengths and higher degree of unsaturation tended to cause transient tilting of the LC.

These findings provide guidance for the design of LC-based sensors for fatty acids. In comparison to existing fatty acid detection techniques such as gas chromatography/mass spectrometry (GC/MS), enzymatic (colorimetric/fluorometric) assays and pH-based titration, LC-based sensors measure the interfacial activity of the fatty acids (since the LC

response is induced by the amphiphilic nature of the species, and not solely on either the carboxylic group or the hydrocarbon chain). In contrast, the fatty acid detection technique most commonly employed by biodiesel homebrewers is based on use of a pH indicator. Any acids, including those that are not interfacially active, are measured in such an approach. In this context, the LC-approach is advantageous since it directly measures interfacial activity that is closely connected to emulsion stability. The LC-based detection techniques are passive (no power), are simpler and less expensive than techniques involving use of complex instrumentation such as GC/MS.

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