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ARTICLE TYPE

A new pathway of formation of radial nematic droplets within a lipid-laden aqueous-liquid crystal interface†

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A new pathway of formation of liquid crystal (LC) droplets with radial LC ordering in presence of surfactants and lipids are reported. Study of interactions between enzymes with the topological defects in the LC mediates the response of these droplets to be exploited in applications such as sensing.

Recently, liquid crystal (LC) droplets are widely appreciated as a new class of functional materials owing to their large surface areas, rich phases, well-defined director configurations and unique tunable optical properties.[1-10] In particular, they offer routes to the development in designing simple, economic and convenient passive sensing devices that provide high spatial resolution of micrometers with a very high sensitivity. Therefore, it is very important to explore new pathways of preparation of stable and uniform LC droplets that will ultimately provide a simplified and robust LC sensing platform. Past reports establish the feasibility of formation of LC droplets via sequential ultra-sonication and vortexing of LC in water with emulsifying agents.[1,10-15] However, droplets prepared using these methods have less stability and broad size distribution that limits their widespread use in real applications. To address these issues, much progress has been made to stabilize the LC droplets with uniform sizes. For example, uniform silica particles coated with polyelectrolyte's multilayers (PEM), microfluidic devices, etc. have been used as templates for stable and uniform LC droplets.[5,12,16,17] These polyelectrolyte's and other surface active agents (e.g., surfactants) can assemble at the interface of the LC droplet giving rise to a stable director configuration that is governed by surface anchoring and bulk elastic energies of the LC. But, most of these techniques are either tedious or not suitable for large scale production. For instance, in case of silica particles coated with PEM for the preparation of LC droplets, silica cores need to be first etched with hydrofluoric acid (HF) to achieve hollow polymeric capsules followed by filling the capsules with LCs.[5,12,16] Because of highly corrosive nature of HF, the preparations of LC droplets become tedious and time-consuming. Similarly, in case of microfluidic devices, stream of co-flowing liquids were used to squeeze and break off LCs into spherical droplets.[17] Therefore, it is difficult to customize the size of the LC droplets as they depend on the dimensions of the channel. Herein, we report a new pathway for easy formation of spontaneous uniform LC droplets. While the techniques reported for the preparation of LC droplets in past have a life span of few hours,[18] our approach rendered with their stability for days to months. We observed spontaneous formation of well-developed LC droplets with radial defects in presence of phosphatidylcholine (PC) within the confined boundary created by grid system, suggesting new principles for the design of LC-based chemical and biological sensors.

Although observations reported in the past established the feasibility of formation of LC droplets with diameters in the micrometer-to-sub-micrometer range and their size dependent ordering within the LC droplets,[16] direct observations of the spontaneous evolution of LC droplets with radial LC ordering in presence of surfactants and lipids have not yet been known. The study reported in this paper reveals the first breakthrough that showed characteristic micrometer-scale LC droplet patterns in presence of lipids and their control over LC droplet size. We have also demonstrated that interactions of enzyme with the topological defects in the LC mediate the response of these droplets and thus provide new designs for stimuli-responsive soft materials.

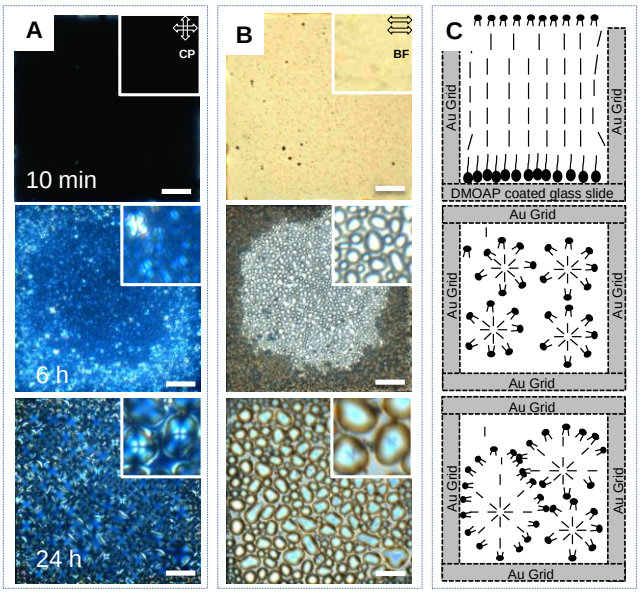

Fig. 1 A) Crossed polars (CP) and B) bright field (BF) images of aqueous-5CB interfaces within TEM grids supported on DMOAP coated glass slides upon exposure to 0.5 mg/mL phosphatidyl choline (PC) after 10 min, 6 h and 24 h, respectively. The insets (in A & B) show the corresponding high-magnification images that reveal the formation of stable and well-developed LC droplets exhibiting radial configuration. C)

Top to bottom: schematic illustration of the time-dependent formation of LC droplets with radial LC ordering. Scale bar = 40 µm.

We first performed an experiment to determine if the nematic ordering of 5CB (4'-pentyl-4-cyanobiphenyl) influences the organization and assembly of PC adsorbed to an aqueous-5CB interface. We hosted 5CB in the pores of 20 µm thick electron microscopy grids supported on N,N-dimethyl-n-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP) coated glass slides which induces homeotropic anchoring of the LC.[19] Subsequent immersion under an aqueous solution, the optical appearance of the LC became bright consistent with an orientation ordering transition induced by contact with water.[20] The results shown in Figure 1A were obtained by adsorbing PC (~0.5 mg/ml) to the aqueous-5CB interface that leads to change in the optical appearance of the LC from bright to dark within few minutes. On incubation for 3 h or more, we observed well developed droplets which were characterized by single point defect located at the centre of the droplets in the bright field optical micrograph (Figure 1B). We hypothesized that monolayer of PC rearranges itself around the LC resulting in the formation of LC droplets with defects (see below for details). The schematic representation and the process of the formation of droplets with time are shown in Figure 1C and Figure S1 (see Supporting Information), respectively.

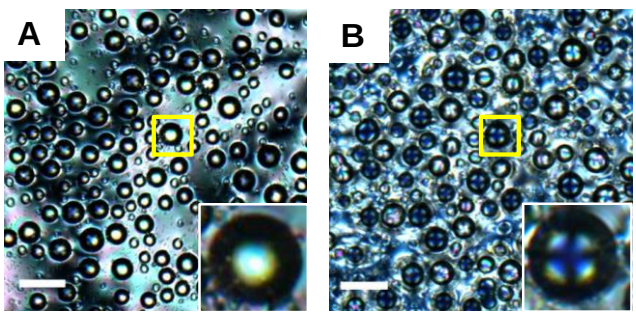

Fig. 2 (A) Polarized optical micrograph (crossed polars) of nematic 5CB hosted in gold grid supported on DMOAP-treated glass substrate in contact with water for 3 days. Water droplets formed spontaneously within the LC film. (B) Polarized optical micrograph of the aqueous-5CB system (as in Figure 2A) on exchanging the aqueous phase with the PC solution after 19 h. The well-developed water droplets reorganize to LC droplets with radial LC ordering. The inset in Figure 2A and Figure 2B shows the images of a water droplet and the corresponding LC droplet at high magnification, respectively. Scale bar = 40 µm.

To provide insight whether PC is responsible for the formation of LC droplets with radial LC ordering, we first incubated LC film (supported on DMOAP coated glass slides) in aqueous phase for three days followed by exchange of the aqueous phase with the PC solution. Past investigations reported that micrometer-thick film of LC supported on OTS (octadecyltrichlorosilane) treated glass substrates lead to the formation of water droplets when immersed under water.[21] On immersion of the LC film under water, we observed spontaneous evolution of well-developed water droplets formed at LC-DMOAP interface within 72 h (Figure 2A). Interestingly, exchanging the aqueous phase with the PC solution we observed topological defect formation within the droplet after 19 h. We note two observations from Figure 2B. First, regions of LCs around the droplets has transformed to homeotropic orientation, as evidenced by the black territory. Second, LC droplets with topological defects are formed in the same location where water droplets were previously present. We do not yet fully understand the reason of the formation of LC droplets from the water droplets in addition of PC. It may be hypothesized that in presence of PC, there could be a gradual replacement of water by LC which is stabilized through topological defects. Figure S2 shows the time lapse images of the organization of water droplets to well-defined LC droplets (see Supporting Information).

The results described above when combined, lead to following overall understanding of the LC droplet formation. In the formation of macro emulsion, the reduction of interfacial tension reduces the amount of mechanical work required to break the inner phase into dispersed particles. So, the use of surfactants decreases the surface tension as well as the rate of coalescence of the dispersed LC particles by forming mechanical, steric or electrical barriers around them. Theoretical approaches also reported in past for the formation of equilibrium state of a LC droplet which could be described as the minimum of the free energy functional (F), composed of both volume (F_v) and surface part (F_s).[22] The F_s can again be modeled as surface tension which has an isotropic part σ and an anisotropic part W_A (i.e., $F_s \sim 4\pi (\sigma r^2 + W_A r^2)$, r being the radius of the droplet). In case of cyanobiphenyl, being anchoring part W_A ($\sim 10^{-5}$ -10^{-6} J/m^2) much smaller than σ ($\sim 10^{-3} - 10^{-2}$ J/m^2), F_s ($\sim 4\pi\sigma r^2$) is dominated by the surface tension and thus proportional to r^2. It has been demonstrated that PC could decrease the σ to 10^{-3} -10^{-4} J/m^2 which in turn decreases the F_s subsequently.[23] In such cases, F reduced to a greater extent which result in the formation of LC droplets and stabilized by topological defects. Our understandings for the formation of LC droplets are also supported based on prior experiments. For example, the measured surface tension for the free interface (such as LC/argon and LC/glycerin interface) with no surfactant is of the order of 15-20 mJ/m^2.[22] In contrast, presence of small amount of lecithin in glycerin decreases the surface tension between the LC /glycerin to 10 mJ/m^2 for the cholesteric (Ch) and Smectic A (SmA) phase but drops to 3 mJ/m^2 in SmA*.[24] Kim et al. reported that the interface between 5CB/(water +cetyl trimethylammonium bromide (CTAB)) has a surface tension of the order of (1-6) mJ/m^2.[25] Similarly, plot of surface tension *vs.* temperature for 5CB/glycerin (no surfactant) showed that the values of surface tension are higher, again between 20 and 15 mJ/m^2.[22] Of course, glycerin and water are different matrices but adding a surfactant would generally decrease the interfacial tension between a LC and surrounding isotropic fluid. The value inevitably depends on the concrete materials as in the case of SmA*, it was decreased by a factor of 5-7, i.e., an order of magnitude.

In addition to reduction of interfacial tension, PC as reported imposes the anchoring boundary conditions for the nematic director.[26] The PC due to its hydrophobic part interacts with the alkyl chain of 5CB and results in the homeotropic ordering of LC. It then rearranges itself around the LC molecules and creates a radial arrangement around LC molecules where each droplet has a single point defect at the centre (called as *hedgehog*). Previous studies show the radial arrangement of LC droplets with lecithin.[26] Past reports by Terenjev demonstrated that the stability of nematic macro-emulsions is greatly enhanced with that of the

isotropic counterparts and thus the elastic constant of the LC and surface tension creates an energy barrier for coalescence.[27] This energy barrier of coalescence is ~ K^2/w. With K ~ 10^{-11} N and w~ 10^{-5} J/m^2, the energy barrier is very high (~ 10^{-17} J) as compared with the typical thermal energy at room temperature.[27] Hence, it is very likely that in presence of PC, there is an additional contribution of the elastic energy for the formation of topological defects in the confined nematic and the radial LC droplets are stable against coagulation compared with their isotropic counterparts.

In order to provide further insight into the microstructure of LC droplets in presence of PC, fluorescently tagged PC was used to examine the location of the surfactant (PC) in the co-assembly. Figure 3A shows the optical micrographs of 5CB hosted within gold grids supported on DMOAP coated glass slides in contact with PC mixed with fluorescent PC (NBD PC, see experimental section in Supporting Information for details) for 3 days. We observed that fluorescence was mainly present at the boundary (and also in between) of the droplets with no fluorescence at the centre of the droplets. This led us to conclude that the formation of PC monolayer stabilizes these droplets by rearranging itself around the droplets. A bright field optical image of the same is shown in Figure 3B. To understand that we have LC outside the droplet (not only inside) we performed a confocal fluorescent image with fluorescent N,N'-Bis(2,5-di-tert-butylphenyl)-3,4,9,10perylenedicarboximide (BTBP) compound which is known to align in the presence of LC.[28] As expected from the fluorescent (Figure 3C) and from the confocal image (Figure 3E), we observed more BTBP outside the droplet. This study suggests the background between the droplets is birefringence. A bright field micrograph for fluorescent and confocal was shown in Figure 3D and 3F, respectively.

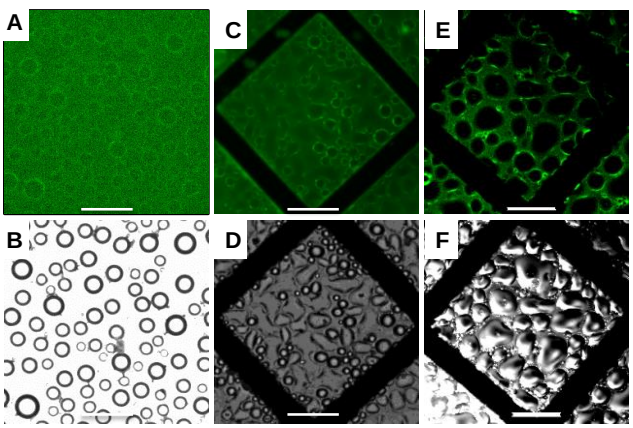

Fig. 3 (A) Fluorescent micrograph and B) the corresponding bright field image of nematic 5CB hosted in gold grid supported on DMOAP-treated glass substrate in contact with PC doped with 1 µM fluorescent PC (NBD PC) for 3 days. (C) and (E) represent fluorescent and confocal microscopy images of BTBP doped 5CB (hosted within TEM grids in contact with PC vesicles for 5 days, respectively. (D) and (F) correspond to the fluorescent and confocal bright field micrographs, respectively. Scale bar = 55 µm.

Next we sought to determine the stability of these droplets with time. It was found that the droplets produced in presence of PC are quite stable for a period of 20 days or more. As shown in Figure S3A (see Supporting Information), after incubation of PC at 5CB-aqueous interface for 3 days, the average droplets size reached to ~21.34 ± 4.67 µm and maintained for at least 5 days. A slight variation of the average size was observed within these days (i.e., from 21.34 ± 4.67 µm to 21.70 ± 4.70 µm). After 8 days of incubation (Figure S3B) the sizes increased to 32.63 ± 11.16 µm and changes continuously with time (Figure S3C, D). The time dependence growth of the droplet formation in presence of PC at 5CB-aqueous interface is shown in Figure S3E.

We investigated and compared the stability and size distribution of the LC droplets over the existing techniques. Past reports quantified the size distribution (with an average diameter of ~6 µm) of 5CB droplets formed by sequential sonication and vortex mixing.[18] In our case, we observed an increase of approximately three times(~21 µm) in the average size of the diameter of the LC droplets. Also, the techniques reported for the preparation of LC droplets in past have a life span of only few hours (Figure S4 and Figure S5 Supporting Information).[18] On the other hand, the droplets prepared by our method are quite stable for a period of several days. This is because in our case droplets formed in a confined boundary created by the grid system and thus stabilized (less mobile). These experiments also confirm the suitability of our approach towards a simple and robust platform using LC droplets for further applications.

Next we examined the stability of the obtained LC droplets (with defects) below and above the nematic (N) –isotropic (I) phase transition temperature (T_{N-I}) of 5CB (~35 °C). Below T_{N-I}, the LC droplets with radial LC ordering was observed and thus show a bright optical appearance under cross-polars (Figure S6, see Supporting Information). However, the optical response of the LC droplets exhibited a bright-dark optical appearance after it was heated above the T_{N-I} of 5CB. On cooling from isotropic temperature to N phase, we observed bright optical appearance of the LC droplets followed by the appearance of the topological defects within these droplets. These results indicate that the LC droplets (with radial configuration) were quite stable and reversible i.e., the optical responses remain unchanged by the temperature-induced phase transition of LCs.

In addition to PC, that induces the formation of stable droplets with radial LC configuration, we investigated the role of other surfactants and lipids and explored their behaviour on incubation at aqueous-5CB interfaces. We have chosen sodium dodecyl sulfate (SDS), CTAB, lysophosphatidic acid (LPA), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) and lipopolysaccharide (LPS) and studied their behaviour at aqueous-5CB interfaces. The goal of this experiment is mainly two fold. First, we sought to find out whether other lipids are also suitable for the spontaneous formation of LC droplets in addition to PC as described above. Second, we sought to demonstrate any additional parameter which can advance our understanding involved in the formation of droplets. All the surfactants we have chosen are ionic and therefore, can stabilize the droplets electrostatically. Figure S7 shows the optical images of nematic 5CB hosted within gold grids supported on DMOAP coated glass slides in contact with CTAB, DLPC, SDS, LPS and LPA with time. We monitored these systems carefully for 15 days and observed following two key points. First, within 3 days of incubation both CTAB and DLPC resulted in the formation of nice and well-developed droplets with radial LC ordering. Second, LPA does not lead to formation of stable droplets

whereas no droplet formation was observed in presence of LPS and SDS. These results, when combined indicate that (i) CTAB and DLPC are more surface active (in comparison to SDS & others) and thus resulted in more efficient reduction in surface tension.[5,23,29] (ii) Past reports also demonstrated that the LC droplets formed in water remained stable due to adsorption of hydroxide ions at the LC-water interface.[30] These hydroxide ions adsorption makes the LC droplet surface negatively charged. So, it can be concluded that in addition to efficient reduction of the surface tension, electrostatic attraction in presence of DLPC and CTAB (DLPC is zwitterionic and CTAB is positively charged) with LC interface (negatively charged) stabilized the LC droplets with respect to SDS, LPA and LPS (being negatively charged). Also negative surface charge density on DMOAP coated surfaces stabilizes the positively and zwitterionic droplets. These results above reveal for the first time that in addition to induce homeotropic alignment of the LC, ionic charge in the lipids can play an important role for the spontaneous formation of LC droplets.

The observations reported above are interesting to consider based on prior studies that have reported on the topological ordering of LCs within droplets to report interfacial enzymatic reactions.[6] Past reports established that PLA_2 triggers a transformation of the LC droplet from a radial configuration to bipolar configuration decorated with $_L$-DLPC.[6] Motivated by this we investigated the topological states encountered in the LC droplets during enzymatic degradation of PC using PLA_2. Figure 4 shows the corresponding polarized light micrographs of the LC droplets in response to the adsorption of PLA_2 (150 nM). Interestingly, upon introduction of PLA_2, an anchoring transition of the LC was observed from an initially homeotropic orientation (radial configuration in presence of PC) to a planar orientation with bipolar topological defect. These bipolar droplets were then further explored in detecting various bio-molecules such as bacterial phospholipids (LPS). Past reports demonstrated that LPS induced ordering transition in LC droplets from bipolar to radial configuration.[8] This ordering transition is not mediated by surface anchoring energy but rather consistent with the association of LPS with defects. We investigated this phenomena with the LC droplets formed through enzymatic degradation of PLA_2 (bipolar) and observed a structural transformation to radial configuration. The optical response of these LC droplets with bipolar configuration to radial configuration after adsorption of LPS is shown in Figure S8. This result, we believe, in principle, enable new pathways to exploit interfacial adsorbate-induced properties of LC droplets.

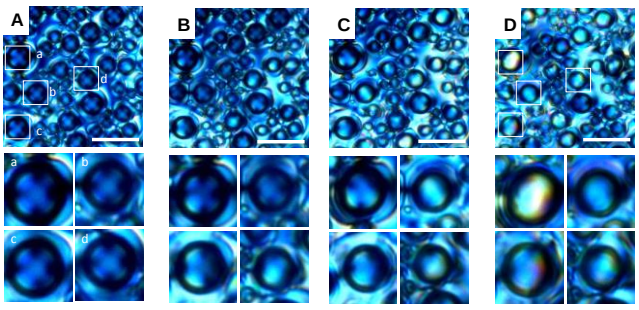

Fig. 4 Top row: (A-D) Optical images (crossed polars) of 5CB droplets covered with DLPC upon exposure of 150 nM PLA_2 into an aqueous solution of TBS (pH = 8.9) containing 10 mM $CaCl_2$. These droplets with radial defect were formed by contacting the 5CB interface laden with DLPC (0.1 mg/mL) for incubation period of 2 days. The second row depicts the change in anchoring transition of representative region of four selected droplets (a-d) from radial to bipolar upon adsorption of PLA_2. Scale bar = 40 µm.

In conclusion, the study establishes first to reveal direct observations of the spontaneous evolution of LC droplets with radial LC ordering in presence of surfactants and lipids. The formation of stable LC droplets are not only due to the reduction of interfacial tension between the LC and surrounding isotropic fluid but also have an additional stability mechanism (against coagulation) associated with the internal elasticity. Our observations also affirm that ionic charge can play an important role for the spontaneous formation of LC droplets with topological defects. Finally, we have shown that interactions of enzyme with the topological defects in the LC droplets can provide a means to the developments of new responsive soft materials.

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† Electronic Supplementary Information (ESI) available: Detailed procedure for the preparation of LC films, vesicles, LC droplets and their optical characterization and fluorescent imaging are described in the Supporting Information. See DOI: 10.1039/b000000x/

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