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# Chemical Waves and Pattern Formation in the 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine/Water Lamellar System

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The Belousov Zhabotinsky (BZ) reaction−diffusion system[1] is the most famous example in the wider class of oscillating chemical reactions.[2] Briefly, it consists of the catalytic oxidation of an organic substrate with active methylenic hydrogens (e.g., those of malonic acid) by $BrO_3^-$ in strong acidic solution. Among the most interesting features of the BZ reaction, its capability of generating waves and patterns[3−6] in various media has been documented. Examples include gels,[7,8] synthetic membranes,[9] mesoporous glasses,[10] ion-exchange resins,[11] Langmuir monolayers,[12] and arrays of metal electrodes.[13]

Recently, compartmentalization within microemulsion droplets[14] showed that peculiar patterns,[15−18] such as striped standing waves, inwardly rotating spirals, and Turing structures,[19,20] can be generated by the coherent coupling of randomly distributed domains, each of them representing an independent oscillator. Oscillating phenomena are also intrinsically related to the evolution and sustenance of self-organized biological systems.[21] Indeed, many living organisms exhibit chemical oscillations,[22,23] and the study of pattern formation in various environments, mimicking biological structures, is a subject of widespread interest.

Phosphatidylcholines are among the major components of biological membranes, and pure phospholipids bilayers are generally used as models for cell plasma membranes. In this work, we studied a BZ reaction, carried out in the anisotropic environment of the 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/water binary system, that presents layered aqueous domains separated by lipid bilayers.[24] The structural properties of this system were investigated by small-angle X-ray scattering to elucidate their actual features in the presence of the BZ reactants, that is, $H_2SO_4$ 0.5 M, $KBrO_3$ 0.5 M, malonic acid 0.5 M, ferroin 0.025 M, and KBr 0.16 M. In fact, lipid lamellar phases, as all surfactant/water equilibrium systems, tend to rearrange into structures that may differ significantly from those found in pure water, if the ionic strength and pH of the aqueous medium are deeply altered.[25] Modification of physical properties, such as volume and interfacial tension, may also occur as a consequence of chemical waves.[8] Therefore, to avoid possible complications coming from the reaction itself (e.g., layer spacing variations during the experiment), X-ray spectra were recorded on a "blank" system. This contained all the reactants in the same concentration as the true BZ mixture, but the catalyst ferroin was replaced by its inert analogue $Zn(o\text{-phen})_3$ in the same concentration. The typical smectic pattern of a lamellar stack (i.e., the 00L series of Bragg peaks with $q$-spacing in the ratio 1:2:3:...) was found for lipid content ranging from about 7 up to 40% w/w, as in the case of the DPPC/water system, and three orders of reflections were invariably detected. Small shifts in the maximum $q$ values were observed with respect to the pure water systems, but a single repeat distance indicated that only one phase was retained even in the presence of the BZ reactants. A typical example of this behavior is shown in Figure 1, which compares the SAXS spectra of DPPC 20% w/w in pure water and in water containing the BZ solution.

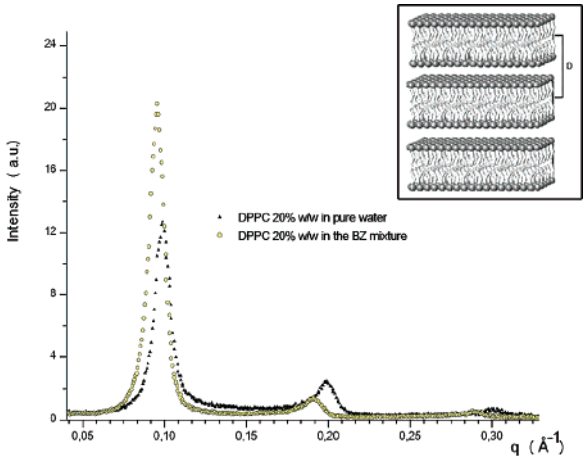


***Figure 1.*** Radially averaged SAXS intensity for the lamellar phase containing DPPC 20% w/w in pure water and in the BZ solution, with $Zn(o\text{-phen})_3$ replacing the catalyst ferroin. From the $q_{001}$ and $q_{002}$ Bragg reflections, repeat distances ($D = 2\pi/q_{max}$) of 65.7 and 63.6 Å were calculated for the BZ and reference system, respectively. Spectra were recorded on a pinhole camera with collimating optics and a rotating anode (Cu Kα) as the radiation source. Measuring samples were 1 mm thick, and accumulation time was 1 h. The inset reports a schematic drawing of the lipid and water arrangement in a lamellar phase.

For DDPC concentration higher than 40%, two distinct series of peaks were obtained, whereas for lipid contents below 5 to 6% macroscopic precipitation took place. The BZ reaction was thus carried out only in the monophasic systems.

Chemical decomposition of the lipid matrix might also occur in the reactive and acid environment of the BZ solution. This possibility was checked and ruled out by high-resolution NMR, an extremely sensible tool in this contest. In fact, narrow peaks, coming from isolated molecules, are able to show up from the broad lines of a lamellar phase even at concentrations of the order of 1% or less. $^1H$ spectra (600 MHz) were recorded on the blank system (to

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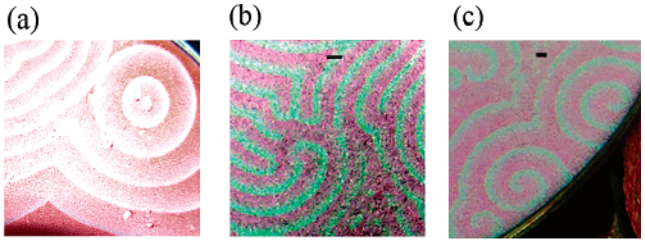


***Figure 2.*** (a) Pacemaker structures and traveling waves found when BZ/DPPC system was 10% w/w lipid content. (b) Stationary Turing-like labyrinthine structure formed in the middle of the Petri dish when BZ/DPPC system was 18% w/w lipid content. Such patterns, once they were formed, were able to persist for about 20 min without changing position. (c) Inwardly rotating spirals appeared at the edge of the reactor, and waves emerged at the boundary between the spirals when BZ/DPPC system was 18% w/w lipid content. Horizontal bar scales 1 mm.

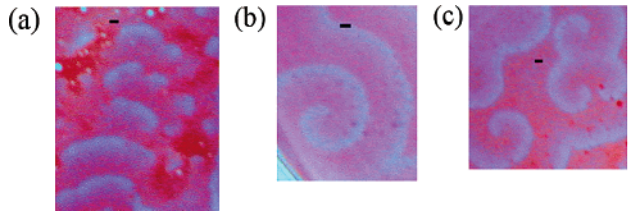


***Figure 3.*** BZ/DPPC system with 25% w/w lipid content, horizontal bar scales 1 mm. (a) Striped standing waves. (b) Inwardly rotating spirals. (c) Spiral-like structures, generated from collision between traveling waves and spirals.

avoid line broadening due to the paramagnetic Fe ions) at different times from the preparation, and no chemical alteration was found for at least 5 to 6 h, that is well after the periodical events had damped down in the corresponding ferroin-containing system.

Experiments on the BZ behavior were carried out at room temperature by mixing DPPC with all the reactants, except ferroin, in a beaker under stirring. When the solution became homogeneous, ferroin was added for a total volume of 2 mL. The BZ/DPPC system obtained was finally poured into a 6-cm diameter Petri dish and allowed to evolve. Photos were taken with a high-resolution digital camera. All chemicals were from SIGMA, except DPPC, which was purchase from Northern Lipids.

Until DPPC concentration was kept between 7 and 10% w/w, traveling waves and pacemaker structures were obtained. These patterns did not differ, to an appreciable extent, from those usually found when the BZ reaction is carried out in a bulk aqueous medium (Figure 2a). On the other hand, many interesting patterns were found when the DPPC concentration was increased. As an example, Figure 2b shows a stationary Turing-like labyrinthine structure that was observed in the BZ/DPPC system with 18% w/w lipid content. At the same concentration, we also found that inwardly rotating spirals coexisted with Turing structures in different regions of the sample (Figure 2c).

When the system reached 25% w/w of lipid content, inwardly rotating spirals and spiral-like structures (Figure 3b,c) coexisted with striped standing waves (Figure 3a).

In all the systems investigated, waves started at the edge of the reactor, except for a few cases in which pacemaker structures appeared in the middle of the dish, and slowly diffused toward the center. This phenomenon was probably favored by the zero-flux boundary conditions.

For lipid concentrations higher than 30% w/w, all systems exhibited only a few blue-filled circular "spots" and no waves or other interesting structures were observed. Again, patterns started from the edge of the dish and slowly formed the uniform reduced steady-state typical of the end of the process.

The above findings demonstrated that peculiar patterns, first discovered in AOT microemulsions,[15,16] were also formed in layered water domains. Thus, we showed that these structures are not exclusive of spherical droplets. In our case, the coupling mechanism between different aqueous compartments, which provide the observation of patterns at a macroscopic scale length, that is, centimeters, is likely due to membrane defects and permeability (i.e., interlayer crossing) of some intermediates. In this respect, the diffusion mechanism was different from the one found in microemulsions, were collisions among different droplets are thought to play a key role.[15] Moreover, we observed longer wavelengths with respect to the BZ carried out in the AOT/octane/water system,[15] and this could be because diffusion coefficients in the water layer of lamellar phases are usually higher than those in microemulsions.[25]

The system we investigated has some unique features, being the first one that really represents the model for a biological structure. Moreover, from a detailed knowledge of the system geometrical parameters, modelization for the anisotropic diffusion of the BZ reactants in layered water can be performed, and this will be the subject of a forthcoming work.

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