

Vesicle-to-Micelle Oscillations and Spatial Patterns

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A pH oscillator is coupled to and controls rhythmic interconversion of nanoscopic vesicles and micelles made of fatty acids. When changes in pH are combined with diffusion, self-assembly produces spatially extended patterns of vesicle/micelle “stripes” or concentric “shells”.

Chemical oscillators supporting rhythmic concentration changes are among the most fascinating chemical systems with implications for biology,^{1,2} catalysis,³ electrochemistry,⁴ and fundamental research on nonlinear chemical phenomena.^{5–7} Despite tremendous progress in understanding the mechanisms underlying these phenomena,⁷ however, the use of chemical oscillators as control elements of other chemical processes (as is often the case with biological oscillations^{8,9}) remains relatively unexplored and limited to oscillating gels^{10–13} or systems in which pH oscillators shift complexation or precipitation equilibria.^{14,15} Here, we demonstrate that chemical oscillations can be coupled to and can control self-assembly of molecular components. Specifically, we describe a system in which a pH oscillator controls the assembly of and rhythmically interconverts nanoscopic oleic acid vesicles and micelles. In gel-supported systems where pH depends on both time and space, the assembling nanostructures can form spatially extended patterns including arrays of vesicle/micelle stripes in gel columns or concentric vesicle/micelle shells within gel particles. Such “stratified” vesicle/micelle media could be useful in controlled delivery applications.^{16,17}

Fatty acids are known to form bilayer membrane vesicles whose ability to encapsulate macromolecules and hydrophilic small molecules is useful in drug delivery applications.^{16,17} The vesicles form at pH values close to the acid's pK_a . At higher pHs, however, the vesicles transform into smaller, unilamellar micelles.

Our present work is based on oleic acid (OA) whose apparent pK_a in monolayers is ~ 8.3 ^{16,18} and lies within the range of pH oscillations (from ~ 7.0 to ~ 9.4) of the so-called methylene glycol–sulfite–gluconolactone (MGSG) system.^{19–21} The key features of the MGSG oscillator are (i) the dehydration of methylene glycol accompanied by an autocatalytic production of OH^- (positive feedback) and (ii) the catalyzed hydrolysis of gluconolactone by the autocatalytic species, OH^- (negative feedback).²²

In a typical arrangement (Figure 1a), pH oscillations were sustained in a 10 mL continuously stirred tank reactor (CSTR) at 22.0 ± 0.2 °C. The pH was monitored by a computer-interfaced pH electrode (Mettler Toledo), and the contents of the reactor were stirred by using a magnetic stirrer at 400 rpm. The CSTR was continuously fed by constant flows (1.52 mL/min in each channel) of two solutions: (i) sodium sulfite/sodium bisulfite buffer solution ($[\text{SO}_3^{2-}] = 0.01$ M, $[\text{HSO}_3^-] = 0.1$ M) containing 1% w/w oleic acid and (ii) 0.2 M formaldehyde and 0.0134 M gluconolactone. The volume of the reaction mixture in the CSTR was kept constant at 10 mL by removing reaction products at 3.04 mL/min.

The pH oscillations within the reactor had a period of ~ 2 min and were coupled to the self-assembly of OA (Figure 1b,c; see also the video in the Supporting Information). When the oscillator was in the “low” pH ~ 7.0 – 8.3 state, the solution contained significant fractions of both protonated and deprotonated forms of OA, specifically, $\chi_{\text{COOH}} = 1 - \chi_{\text{COO}^-} = (1 + 10^{\text{pH} - \text{p}K_a})^{-1}$, such that $\chi_{\text{COOH}} = 0.95$ for pH = 7 and $\chi_{\text{COOH}} = 0.5$ for pH = 8.3. Under such conditions, the OA molecules are known to form membrane (bilayer) vesicles, burying the hydrophobic chains and presenting inner and outer hydrophilic surfaces with interspersed protonated and deprotonated carboxylic groups.²³ Dynamic light scattering (DLS) measurements revealed that for pH ~ 7 these vesicles had

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(22) An important feature of the methylene glycol–sulfite reaction is the sulfite–bisulfite buffer, which compensates any changes in pH up to around pH 8 (at which point all sulfite has been consumed) followed by an autocatalytic evolution of OH^- . With high concentration of OH^- , the catalyzed hydrolysis of gluconolactone becomes significant, reducing the pH back to neutral.

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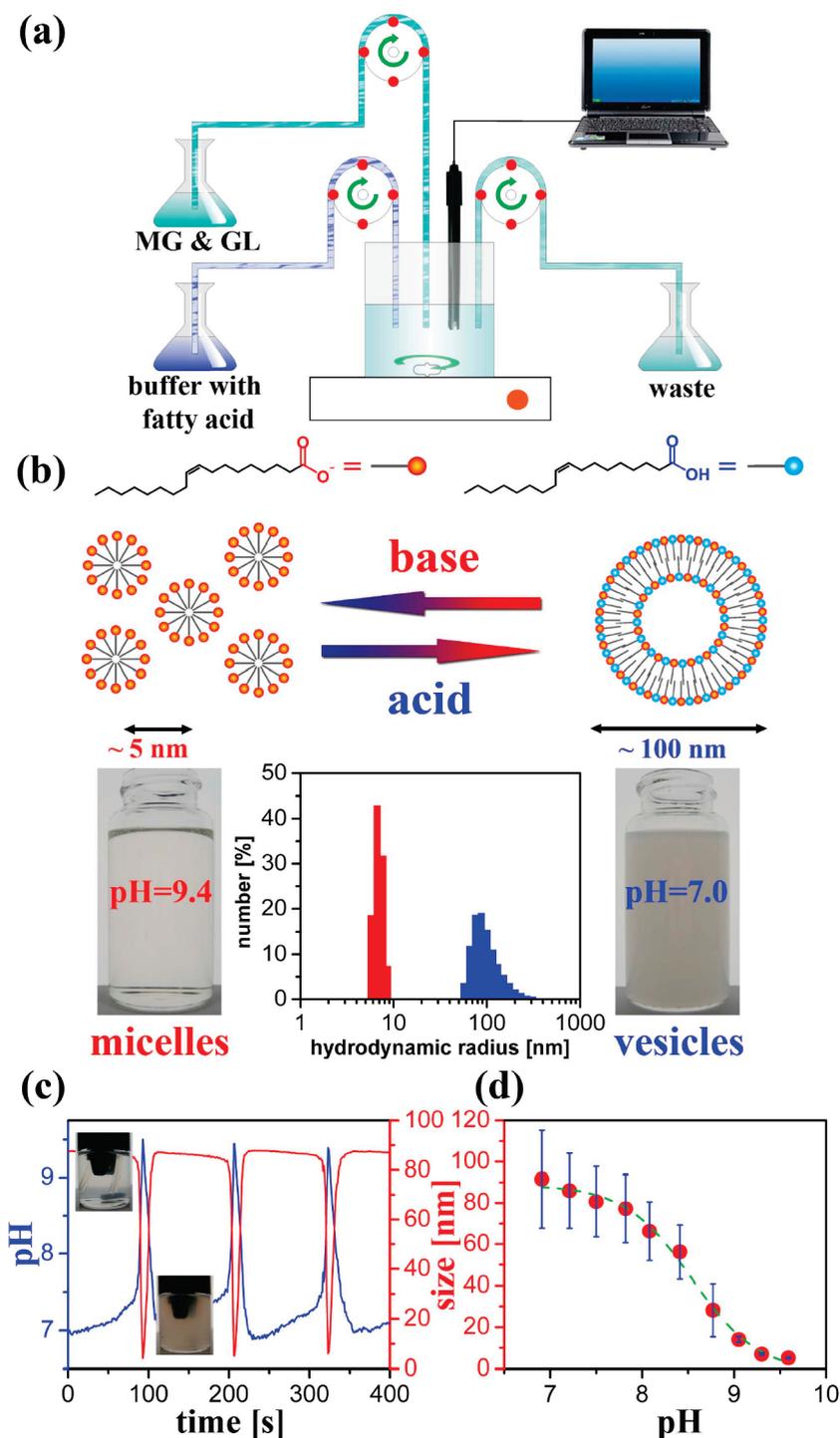


Figure 1. (a) Scheme of the experimental setup. (b) Self-assembly of oleic acid molecules into vesicles and micelles that interconvert as a result of pH oscillations between $\text{pH} = 7$ and 9.4 . The middle graph has the size distributions of vesicles (blue) and micelles (red) measured by DLS at, respectively, $\text{pH} = 7.0$ and $\text{pH} = 9.4$. Optical images illustrate that vesicle solutions have a milky appearance (due to scattering) while those of smaller micelles are transparent. (c) The curves show that pH changes are in phase with the size changes – as the pH spikes to above 9 , the size of the assemblies decreases. DLS data is measured in non-oscillating buffer solutions at appropriate pH and is summarized in (d).

hydrodynamic radii ca. 100 ± 25 nm with a zeta potential of ~ -66 mV and their solution appeared milky due to the Tyndall effect. When, however, the oscillator “spiked” to $\text{pH} \sim 9.4$, the solution turned transparent. This change was due to the fact that, at high pHs, the OAs became deprotonated and formed unilamellar micelles presenting COO^- head groups. These micelles were characterized by the zeta potential ~ -73 mV, and their size at the peak value of pH was 6.4 ± 0.4 nm. When the oscillator returned

to the low-pH state, the micelles reverted to vesicles and the cycle continued as long as the pH oscillations were sustained.

The vesicle-to-micelle transformation can translate into spatially extended patterns when the pH changes are coupled with transport phenomena such as diffusion. One example is illustrated in Figure 2a which shows a test tube filled partly with an agarose gel and originally containing a micellar solution ($\text{pH} = 11.0$) of OA. When the pH of the solution above the gel is changed,

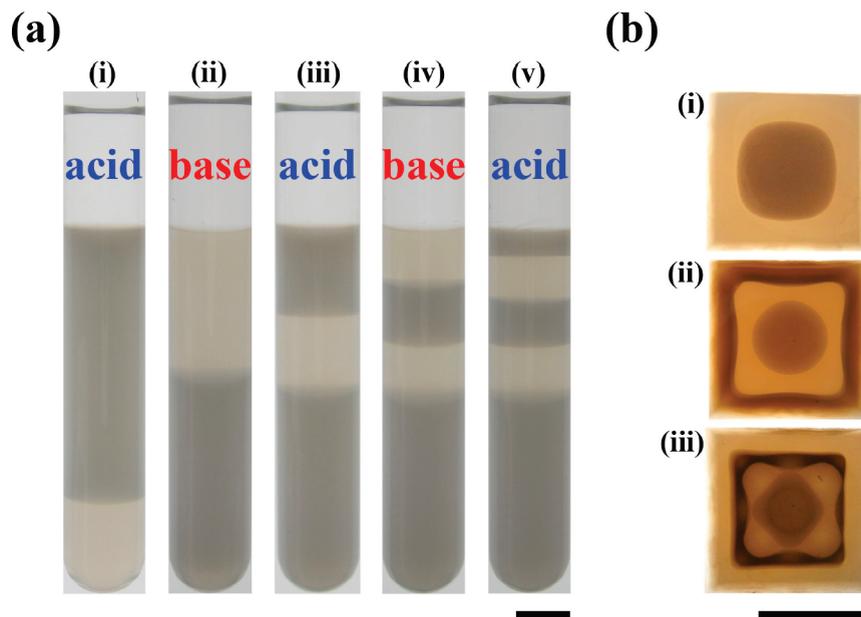


Figure 2. (a) Striped vesicle/micelle patterns formed in a test tube filled with a solution of OA in a 1% w/w agarose gel. Darker and transparent regions correspond to zones of vesicles and micelles, respectively. Solution on top of the gel was periodically changed from acid ($[\text{HCl}] = 0.1 \text{ M}$) to base ($[\text{NaOH}] = 0.1 \text{ M}$) at $t = 25 \text{ h}$ (i), $t = 45 \text{ h}$ (ii), $t = 50 \text{ h}$ (iii), $t = 52 \text{ h}$ (iv), and $t = 52.5 \text{ h}$ (v). (b) 3D vesicle/micelle structure in an agarose cube 1.5 cm on the side. The outer solution was changed from base ($[\text{NaOH}] = 0.1 \text{ M}$) to acid ($[\text{HCl}] = 0.1 \text{ M}$) at $t = 30 \text{ min}$ (i), $t = 37 \text{ min}$ (ii), and $t = 40 \text{ min}$ (iii). Scale bars correspond to 1 cm.

the pH front propagates down the test tube and causes formation of alternating opaque/transparent bands of, respectively, vesicles and micelles. In particular, each change of the solution's pH gives rise to one band. The fact that the boundaries between the consecutive bands appear sharp can be related to the sigmoidal dependence shown in Figure 1d, which features a relatively abrupt transition from vesicles for $\text{pH} \leq \text{p}K_a$ to micelles for $\text{pH} > \text{p}K_a$. Also, the process of band formation can be quantified by a reaction-diffusion model,²⁴ which accounts for the diffusion and reaction of hydrochloric acid (H^+) and sodium hydroxide (OH^-) whose migration through the gel determines local pH and controls the reversible vesicle-to-micelle transformation (see section 1 in the Supporting Information). This model predicts, among other things, that if the time interval ($\Delta\tau_n$) for exchanging of the solution's pH from $\text{pH} < \text{p}K_a$ to $\text{pH} > \text{p}K_a$ is exponentially decreasing with time (i.e., $\Delta\tau_n \sim e^{-cn}$, where c is a constant), the width of the bands formed in the gel is linearly proportional to the band position; this trend is reminiscent of the width law for the so-called Liesegang rings.^{25–27}

Lastly, the formation of the banded structures can be extended to three-dimensional objects. For example, a gel particle immersed sequentially into acidic and basic media develops a series of micelle/vesicle “shells” such as those shown in Figure 2b. We believe that such stratified gel particles can find applications in controlled delivery applications whereby different layers of vesicles or micelles would be released sequentially and/or disintegrate under different pH conditions. For such systems, it would be interesting to “reinforce” the micelles and vesicles via polymerization²⁸ and to couple the formation of these assemblies with encapsulation²⁹ of molecular and or macromolecular cargos.

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Supporting Information Available: Model description and movie illustrating the oscillations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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