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Autopoietic Self-Reproducing Vesicles: A Simplified Kinetic Model

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A simplified kinetic model is presented in order to interpret the kinetics of the self-reproduction of vesicles. These experimental systems have been recently described (Walde P. et al., *J. Am. Chem. Soc.* **1994**, *116*, 11649) and consist of a biphasic system in which an insoluble surfactant precursor, e.g., an anhydride of a long-chain fatty acid, is overlaid on a basic aqueous solution and is eventually hydrolyzed into surfactant molecules that then assemble spontaneously into vesicles. Under these conditions, a catalytic effect can be observed due to the presence of the aggregates in the aqueous solution. To elucidate this effect within a theoretical framework, the hydrolysis has been described as an irreversible surface reaction that can take place both at the macroscopic interface and on the surface of vesicles. This reaction has been also assumed to be the limiting step so that all the others processes (e.g., formation of vesicles, absorption of molecules anhydride by vesicles, acid–base reactions, etc.) have been considered as equilibrium processes. On the basis of these assumptions, the catalytic effect has been interpreted as mainly due to the large growth of the reaction surface and it has been possible to kinetically describe two different self-reproducing systems of vesicles. The good agreement obtained between the experimental data and the theoretical forecasts supports the validity of this approach.

Introduction

In the last few years, a number of surfactant-based, self-reproducing¹ chemical systems were implemented in which the production of a surfactant is catalyzed by the presence in solution of the aggregates, which it spontaneously forms (micelles,² inverse micelles,^{3,4} and vesicles^{5,6}). Thus, during the same process, both the autocatalytic synthesis of amphiphilic molecules and the assembly of aggregates take place at the same time.

Interest in these systems is due to their being examples of self-bounded chemical structures able to reproduce themselves: *autopoietic self-reproducing systems*.⁷ The notion of the *autopoietic unit*,⁸ i.e., a structure capable of self-maintenance by means of processes all occurring within its boundary, has been regarded as a minimalistic definition of a living system. Moreover, it has been shown that self-reproduction is indeed one of the possible kinetic aspects of autopoiesis^{7,9} and therefore, autopoietic molecular aggregates can be considered as the most elementary chemical structures capable of simulating certain essential properties of the simplest living systems. It should also be recalled that some authors have underlined the importance of the role that supramolecular aggregates, such as vesicles and liposomes, may have played in the prebiological phases of the development of life on earth¹⁰ owing to their ability to realize microsystems having different chemical physical properties from those of the surrounding environment. Therefore, the experimental and theoretical study of the phenomenological behavior of these supramolecular systems can give new insights both into designing more complex chemical structures simulating living systems and into understanding the role that vesicles and liposomes could have in development of life on earth.

Until now, these self-reproducing vesicular systems^{5,6} have been described only experimentally, i.e., without a theoretical or kinetic background. The present work represents a first attempt to provide a kinetic description for this class of processes

and is partly based on previous theoretical studies regarding self-reproducing micelles.¹¹ It is organized in four different sections. In the first one, two different experimental implementations of autopoietic vesicular systems will be briefly described, as reported in literature (see ref 5). In the second, the kinetic model will be illustrated, discussing the main simplifying assumptions introduced, and different sets of differential equations will be obtained for the two different experimental systems. In the third section, the experimental data, available from ref 5, will be compared to the theoretical curves calculated by careful selection of the parameters, and finally, the results will be critically discussed.

1. Detailing the Two Autopoietic Systems of Vesicles

Two examples of autopoietic vesicle chemical systems have recently been described.⁵ Both consist of a biphasic system made up of a neat organic phase, overlaid on a basic aqueous solution. The organic phase is composed of the water-insoluble symmetrical anhydride of a long-chain carboxylic acid. These two systems differ from each other both for the different composition of the water solution and for the different anhydride used. In one case, the organic phase is composed of caprylic anhydride and the water solution consists of a sodium hydroxide solution, while in the other, a certain amount of oleic anhydride is overlaid on an aqueous buffered solution. In both cases, to ensure the homogeneity of the solution, the water phase is slowly but continuously stirred by means of a magnetic stirrer. Further on, we will refer to the first of these systems as the *caprylic hydroxide system*, while the second one will be indicated as the *oleic-buffered system*.

Under the conditions illustrated before, hydrolysis of the respective anhydride occurs in both systems and the total surfactant concentration in solution (i.e., the concentration of the acidic and basic forms of the surfactant present both as free monomers and as aggregates) increases over time. If vesicles are present in aqueous solution, the surfactant production is *very rapid* owing to the ability of these aggregates to catalyze the process. It should be clear that the two different experimental

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sets up are due to the different range of stability of the caprylic and oleic vesicle in the aqueous phase.¹² In particular, as reported in the literature, long-chain fatty acid vesicles can form spontaneously in aqueous solution provided that particular concentrations of surfactant (the so-called *critical aggregate concentration* cac) and correct pH values are reached. In the experiments performed by Walde et al.,⁵ the vesicles were present in solution from the beginning of the process in the oleic-buffered system, whereas in the caprylic hydroxide system they would appear only when the conditions for their stability were reached. In both cases, the anhydride was the limiting reagent and the reaction stopped when it was all consumed.

2. Theoretical Model

To kinetically describe both these experimental systems, we will use the same approach already utilized for the autopoietic self-replicating micellar systems.¹¹ The hydrolysis reaction will be treated as a chemically irreversible surface process,¹³ which can take place at the macroscopic interface, between the organic and water phases or on the microscopic surface of the aggregates. Moreover, a certain number of simplifying hypotheses will be introduced in order to make easier the mathematical treatment. The main assumption is to consider the surface hydrolysis as the slowest reaction compared with all the other processes occurring in the system, i.e., the vesicle formation, the process of molecule distribution between the organic and water phases, the solubilization of the hydrophobic compounds by the aggregates, and the acid–base reactions. Consequently, all these other processes can be assumed as equilibrium processes. Therefore, the macroscopic rate R ($\text{mol L}^{-1} \text{s}^{-1}$) of the overall process can be expressed, according to the common accepted approach for surface reactions at liquid interfaces,¹⁴ as follows:

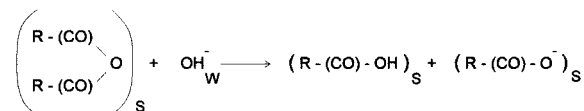
$$R = \sum_i^{\text{reaction surface}} \frac{k_i S_i}{v} [A]_{S_i} [\text{OH}^-] \quad (1)$$

where $[A]_{S_i}$, S_i , and k_i represent the anhydride surface concentration (mol dm^{-2}), the area (dm^2), and the surface hydrolysis constant ($\text{L mol}^{-1} \text{s}^{-1}$) relative to the i th reactive surface, respectively, while $[\text{OH}^-]$ and v are the aqueous bulk concentration of hydroxide (mol L^{-1}) and the volume (L) of the water solution.

On the basis of these hypotheses, the process can be divided into three separate stages, each with its own expression of the rate R : (a) *the initial slow stage*, which describes the reaction taking place only at the macroscopic interface, that is, when no aggregates are present in the system; (b) *the autocatalytic stage*, when vesicles appear in the aqueous solution and the reactive surface includes also the microscopic interface; (c) *the final stage*, when the organic phase has been completely consumed and the anhydride is present in the system only on the surface of the vesicles. Although three different expressions of the process rate will be used, their numerical values calculated at the time transition between two consecutive time stages will be the same.

In the forthcoming sections these three different expressions of R will be obtained for each stage of the process. At the beginning of each section some further assumptions used for both experimental systems will be discussed and also the common kinetic equation will be given. Then, the case of *caprylic hydroxide system* will be treated, obtaining the complete set of kinetic differential equations to be solved numerically.¹⁵ The equations for the *oleic-buffer system* will then be determined, assuming that the aqueous hydroxide concentration is constant.

SCHEME 1: Surface Hydrolysis Reaction^a



^a Index S represents a species absorbed on an interface surface, while index W represents a species in the water solution

2.a. Slow Stage: Hydrolysis in the Absence of Vesicles. Until the right conditions for vesicle formation in the aqueous solution are reached, hydrolysis will only take place on the macroscopic interface because both the caprylic and oleic anhydride are insoluble in water. So, the rate of the process will be given by the expression

$$R_I = \frac{k_0 S_0}{v} [A]_{S_0} [\text{OH}^-] \quad (2)$$

where the index I indicates that the rate is related to the first stage while index 0 indicates values with reference to the macroscopic surface.

The area of macroscopic interface S_0 can be expressed as a function of the total mole number of anhydride molecules A present in the organic phase as follows:

$$S_0 = \left(\frac{18\pi}{\delta_A^2} \right)^{1/3} A^{2/3} \quad (3)$$

δ_A being the density of neat anhydride (mol L^{-1}). Formula 3 was obtained by means of simple geometrical considerations assuming that the organic phase takes a hemispherical form. The surface concentration of anhydride $[A]_{S_0}$ is considered constant as long as anhydride is present in the organic phase.

The variations of the aqueous surfactant concentration and the anhydride moles in the organic phase are therefore linked to rate R_I as follows:

$$\frac{1}{2} \frac{d[\Sigma]}{dt} = -\frac{1}{v} \frac{dA}{dt} = R_I \quad (4)$$

where the factor 2 derives from the stoichiometry of anhydride hydrolysis (see Scheme 1). When the quantity of molecules present in the macroscopic interface is ignored, the anhydride and the total surfactant concentration are linked by the following conservation relationship:

$$\frac{A_0 - A}{v} = \frac{[\Sigma] - [\Sigma_0]}{2} \quad (5)$$

in which A_0 and $[\Sigma_0]$ represent the number of initial moles of anhydride and the initial surfactant concentration, respectively.

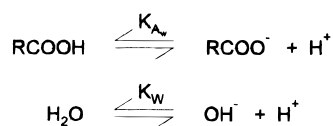
Caprylic Hydroxide System. Let us consider first the caprylic hydroxide system. To determine the complete set of differential equations, the variation of the hydroxide concentration with time must be found by considering that the hydroxide ions are also involved in the acid–base equilibrium processes according to Scheme 2. The concentration of hydroxide must therefore, at every moment, satisfy the conditions imposed by the equilibria

$$K_{A_w} = \frac{[\text{RCOO}_w^-][\text{H}^+]}{[\text{RCOOH}_w]} \quad K_w = [\text{OH}^-][\text{H}^+]$$

and the equations of conservation of mass

$$[\Sigma] = [\text{RCOOH}_w] + [\text{RCOO}_w^-]$$

SCHEME 2: Acid–Base Equilibrium in Aqueous Solution



and charge

$$C_S + [\text{H}^+] = [\text{OH}^-] + [\text{RCOO}_w^-]$$

C_S being the initial concentration of sodium hydroxide and index W labeling the surfactant molecules present in the aqueous solution. From the previous equations it is possible to obtain an expression that links the total surfactant concentration to the hydroxide concentration

$$[\Sigma] = \left(1 + \frac{K_W}{K_A[\text{OH}^-]}\right) \left(C_S + \frac{K_W}{[\text{OH}^-]} - [\text{OH}^-]\right) \quad (6)$$

By the derivation of both the members of this equation and taking into account eq 4, the rate of the variation of hydroxide concentration during the first stage of the process can be obtained:

$$\frac{d[\text{OH}^-]}{dt} = -2f_1([\text{OH}^-])R_I \quad (7)$$

where

$$f_1([\text{OH}^-]) = \frac{[\text{OH}^-]^3}{[\text{OH}^-]^3 + K_W \left(\frac{C_S}{K_{Aw}} + 1\right) [\text{OH}^-] + \frac{2K_W^2}{K_{Aw}}} \quad (8)$$

and $2f_1([\text{OH}^-])$ gives the exact mole number of OH^- consumed to hydrolyze 1 mol of anhydride. This number depends obviously on the pH and on the various thermodynamic constants of the acid–base equilibria so that $f_1([\text{OH}^-])$ can be seen as a “correction factor” due to the acid–base equilibria. To better understand the physical meaning of it, one should consider that in a highly basic environment for each mole of dissociated anhydride 2 mol of hydroxide will be needed, one for the dissociation and the other for the neutralization of the acid surfactant molecule produced. On the other hand, when the pH decreases, the number of hydroxide moles consumed for every mole of anhydride will be lower than 2, that is, not all the molecules of carboxylic acid formed will be instantly neutralized.

The set of differential equations for the first stage may therefore be written as follows.

$$\frac{1}{v} \frac{dA}{dt} = -k_I A^{2/3} [\text{OH}^-] \quad (9)$$

k_I being equal to

$$-\frac{d[\text{OH}^-]}{dt} = 2f_1([\text{OH}^-])k_I A^{2/3} [\text{OH}^-] \quad (10)$$

$$k_I = k_0 \sqrt[3]{\frac{18\pi}{\delta_A^2}} \frac{[A]_{S_0}}{v} \quad (11)$$

while the total surfactant concentration can be calculated through the conservation eq 5.

Oleic-Buffered System. For the oleic-buffered system the aqueous hydroxide concentration can be assumed to be constant throughout the process,¹⁶ so eq 9 can be easily solved to give the following

$$A(t) = \left(\sqrt[3]{A_0} - \frac{vk_I'}{3}t\right)^3 \quad (12)$$

where $k_I' = k_I[\text{OH}^-]$. Having now expressed the time course of the different species during the slow stage of the process for both experimental systems, we will consider, in the next paragraph, the more interesting autocatalytic stage.

2.b. Autocatalytic Stage: Hydrolysis in the Presence of Vesicles. In the presence of vesicles, the expression of the rate becomes the sum of two different contributions: the first due to the hydrolysis at the macroscopic surface, as seen before, and the second due to the hydrolysis taking place on the surface of vesicles. This last term has to take into account the polydispersity of vesicles both in regard to their size and the number of anhydride molecules absorbed. So the process rate takes the form

$$R_{II} = k_I A^{2/3} [\text{OH}^-] + \sum_i^{\text{vesicle size}} \sum_n^{\text{absorbed anhydride molecules}} \frac{k_I S_{i,n}}{v} [A]_{S_{i,n}} [\text{OH}^-] \quad (13)$$

where the first sum is extended to all sizes of vesicles and the second is extended to the number of anhydride molecules absorbed by vesicles of the same size. For the description of this stage of the process a few more simplifying assumptions will be made in order to make the discussion easier.

(i) *The formation of vesicles in solution is instantaneous and happens solely when the total surfactant concentration reaches the cac (critical aggregation concentration), according to the pseudophase model generally accepted in the literature to describe micellar systems at equilibrium.*¹⁷ In addition, we will assume that cac depends only on the type of surfactant.

(ii) *The vesicles will be taken to be spherical and polydisperse with a density probability function of the radius r_i of the following type:*

$$P(r_i) = \frac{2r_i}{r_0^2} \exp\left\{-\left(\frac{r_i}{r_0}\right)^2\right\} \quad (14)$$

where r_i is defined as the segment joining the center of the vesicle formed by i molecules of surfactant to the center of the bilayer (as shown in the upper insert of Figure 1) and r_0 is a constant linked to the vesicle average radius according to the formula

$$r_0 = \frac{2}{\sqrt{\pi}} r_{av}$$

Figure 1 shows the experimental data for vesicle size distribution, reported in ref 5 for the oleic-buffered system, and the probability density function 13 calculated for an r_0 value of 21.96 nm.

(iii) *The number of molecules i that forms a vesicle with radius r_i can be expressed as follows:*

$$i = \frac{8\pi}{a} \{r_i^2 + \rho^2\} \quad (15)$$

calculated by assuming that the average surface area occupied by a molecule of surfactant is a and that its average length is ρ . It should be underlined that in eq 15 the effect due to anhydride

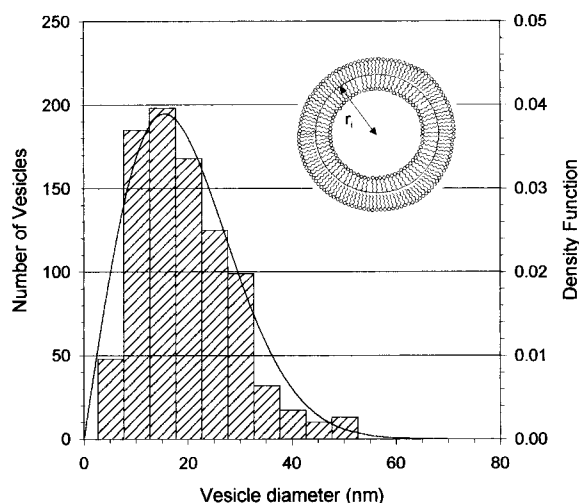
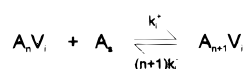


Figure 1. Distribution of vesicles among different classes of radius for the *oleic-buffered* system: experimental occurrences (from ref 5) on the left axis and distribution function on the right axis. The distribution function, see eq 13, was calculated for $r_0 = 21.96$ nm. In the upper insert on the right, the radius of a vesicle is shown, according to our definition.

SCHEME 3: Solubilization Process of Anhydride Molecules by Means of Vesicles



molecules in the vesicles has been ignored, based on the assumption that their number is very low compared to the number of surfactant molecules per vesicle.

(iv) *The process of solubilization of the anhydride molecules by the vesicles can be described by Scheme 3*, where the symbol $A_n V_i$ represents the vesicle formed by i molecules of surfactant that has absorbed n molecules of anhydride. Since we have supposed that this process is in equilibrium, the distribution function of the anhydride molecules among vesicles of a certain size results in a Poisson distribution:¹⁸

$$p_i(n) = \frac{[A_n V_i]}{\sum_n [A_n V_i]} = \frac{\bar{n}_i^n}{n!} \exp\{-\bar{n}_i\} \quad (16)$$

where $p_i(n)$ stands for the probability of finding among the aggregates formed by i surfactant molecules a vesicle containing n molecules of anhydride while \bar{n}_i stands for the average number of anhydride molecules absorbed by the vesicles of this size. This average value can be shown¹⁸ to be linked to the kinetic constants and to the surface concentration of anhydride according to the formula

$$\bar{n}_i = \frac{k_i^+}{k_i^-} [A]_{S_0}$$

(v) *The average number of anhydride molecules per vesicle is assumed to be proportional to the aggregate surface:*

$$\bar{n}_i = K r_i^2 [A]_{S_0} \quad (17)$$

(vi) *The kinetic constants k_i , corresponding to the hydrolysis on the surface of vesicles of different size, are assumed all to have a same value k_v . The hypothesis that underlies this assumption is that there is no relevant influence of the surfaces of different size vesicles.*

On the basis of the assumptions i–vi, it is possible to transform eq 13 into the following expression:

$$R_{II} = k_t A^{2/3} [\text{OH}^-] + k_{II} ([\Sigma] - \text{cac}) [\text{OH}^-] \quad (18)$$

where k_{II} is a pseudo-second-order kinetic constant defined as follows (see Appendix):

$$k_{II} = k_v K [A]_{S_0} \frac{r_0^2}{8\pi(\rho^2 + r_0^2)} \quad (19)$$

The second term on the right of eq 18 shows clearly the catalytic contribution of the vesicles to the kinetics of the process. The derivative of the total surfactant concentration as a function of time will be therefore given by

$$\frac{1}{2} \frac{d[\Sigma]}{dt} = \{k_t A^{2/3} + k_{II} ([\Sigma] - \text{cac})\} [\text{OH}^-] \quad (20)$$

while the equation of mass conservation becomes

$$-\frac{A - A_0}{v} = \frac{([\Sigma] - [\Sigma_0])}{2} + \sum_i^{\text{vesicle size}} \sum_n^{\text{absorbed anhydride molecules}} n [A_n V_i]$$

in which the double sum takes into account the molecules of anhydride dissolved by the vesicles. Under all these assumptions, it is easy to demonstrate (see Appendix) that

$$-\frac{A - A_0}{v} = \frac{([\Sigma] - [\Sigma_0])}{2} + \alpha ([\Sigma] - \text{cac}) \quad (21)$$

with

$$\alpha = K [A]_{S_0} \frac{r_0^2}{8\pi(\rho^2 + r_0^2)} \quad (22)$$

Therefore, the concentration of anhydride present in the aqueous solution solubilized by the vesicles $[A_v]$ can be expressed as follows:

$$[A_v] = \alpha ([\Sigma] - \text{cac}) \quad (23)$$

Deriving (21) and taking into account (20), it is possible to obtain

$$-\frac{1}{v} \frac{dA}{dt} = (1 + 2\alpha) \{k_t A^{2/3} + k_{II} ([\Sigma] - \text{cac})\} [\text{OH}^-] \quad (24)$$

which represents the differential equation for the disappearance of the anhydride in the organic phase.

Caprylic Hydroxide System. Analogous to the former stage (see eq 10), the decrease in hydroxide concentration in solution will be described by the following differential equation:

$$-\frac{d[\text{OH}^-]}{dt} = 2f_{II}([\text{OH}^-]) \{k_t A^{2/3} + k_{II} ([\Sigma] - \text{cac})\} [\text{OH}^-] \quad (25)$$

To calculate the “correction factor” $f_{II}([\text{OH}^-])$ for the second stage of the process, all the acid–base equilibria in which the hydroxide ion is involved must be taken into account. In addition to the reactions illustrated in Scheme 2, there is a further acid–base reaction (Scheme 4) that occurs on the surface of

SCHEME 4: Acid–Base Reaction on the Vesicle Surface

the aggregates with a different acid constant

$$K_{A_V} = \frac{[\text{RCOO}_V^-][\text{H}^+]}{[\text{RCOOH}_V]}$$

where index V indicates that the reaction occurs on the surface of the vesicles.

By means of the new expressions of the conservation laws of mass

$$[\Sigma] = [\text{RCOOH}_W] + [\text{RCOO}_W^-] + [\text{RCOOH}_V] + [\text{RCOO}_V^-]$$

and of charge

$$C_S + [\text{H}^+] = [\text{OH}^-] + [\text{RCOO}_W^-] + [\text{RCOO}_V^-]$$

it is possible to calculate, as seen before, the equation that links $[\Sigma]$ to the concentration of $[\text{OH}^-]$, and by the derivation of it and use of eq 20, the correction factor can be obtained:

$$f_{II}([\text{OH}^-]) = \frac{1}{1 + \frac{K_W}{[\text{OH}^-]^2} \left(\frac{C_S}{K_{A_V}} + 1 \right) + \frac{K_W^2}{[\text{OH}^-]^3} \frac{2}{K_{A_V}} + \frac{\text{cac } K_{A_W}(K_{A_V} - K_{A_W})}{K_{A_V} K_W \left(1 + \frac{K_{A_W}[\text{OH}^-]}{K_W} \right)^2}} \quad (26)$$

Oleic-Buffered System. Considering the hydroxide concentration as a constant, the differential equation set can be simplified to a single equation for the consumption of the anhydride in organic phase:

$$-\frac{1}{v} \frac{dA}{dt} = (1 + 2\alpha)k_I' A^{2/3} + k_{II}' \left(2 \frac{A_0 - A}{v} + [\Sigma_0] - \text{cac} \right) \quad (27)$$

where $k_I' = k_{II}[\text{OH}^-]$, and the concentration $[\Sigma]$ can be calculated by eq 21.

2.c. Final Stage: Hydrolysis after the Disappearance of the Organic Phase. Once all the anhydride in the organic has been consumed, both through hydrolysis on the macroscopic surface and through solubilization in the aqueous phase by the vesicles, the process continues only on the surface of the aggregates where the molecules of anhydride present are hydrolyzed. This stage is reached when the total surfactant concentration in solution attains the value

$$[\Sigma]_{II \rightarrow III} = \frac{1}{1 + 2\alpha} \left(\frac{2A_0}{v} + [\Sigma_0] + 2\alpha \text{cac} \right) \quad (28)$$

which is obtained by reducing to zero the number of moles of anhydride in the organic phase in the conservation eq 21. On the other hand, the concentration of anhydride in the aqueous phase becomes

$$[A_V]_{II \rightarrow III} = \frac{\alpha}{1 + 2\alpha} \left(\frac{2A_0}{v} + [\Sigma_0] + \text{cac} \right) \quad (29)$$

The kinetics of this final stage can be described by an expression

of the following type:

$$R_{III} = \frac{k_{III}}{v} [\bar{A}]_{S_V} \bar{S}_V [\text{OH}^-]$$

where k_{III} , $[\bar{A}]_{S_V}$, and \bar{S}_V stand for the average hydrolysis surface kinetic constant, the average surface concentration of anhydride molecules, and the average reaction surface relative to vesicles, respectively. The previous equation can be rewritten as follows:

$$R_{III} = k_{III}[A_V][\text{OH}^-] \quad (30)$$

while k_{III} can be obtained by equating eqs 18 and 30 and substituting the values $[\Sigma]_{II \rightarrow III}$ and $[A_V]_{II \rightarrow III}$:

$$k_{III} = k_{II}/\alpha \quad (31)$$

and by means of eqs 19 and 22, it is possible to obtain $k_{III} = k_V$, in agreement with the hypothesis vi. The total surfactant concentration $[\Sigma]$ can be determined according to the following equation of the mass conservation:

$$[\Sigma] = \frac{2A_0}{v} + [\Sigma_0] - 2[A_V] \quad (32)$$

Caprylic Hydroxide System. The set of differential equations to be solved for the present stage can be easily obtained, keeping in mind the previous considerations about acid–base equilibria:

$$\frac{d[\text{OH}^-]}{dt} = -2f_{II}([\text{OH}^-])k_{III}[A_V][\text{OH}^-] \quad (33)$$

Oleic-Buffered System. In the case of the buffered solution

$$\frac{d[A_V]}{dt} = -k_{III}[A_V][\text{OH}^-] \quad (34)$$

for the concentration of anhydride present in the water phase a simple exponential decay can be easily obtained from eq 34:

$$[A_V] = [A_V]_{II \rightarrow III} \exp(-k_{III}'t) \quad (35)$$

where $k_{III}' = k_{III}[\text{OH}^-]$.

3. Comparison between the Theoretical Results and the Experimental Data

In this section, the theoretical forecasts are set against the experimental data for the two chemical systems. In both experimental cases, for the differential equations that cannot be analytically solved, a numerical solution was obtained through a 4th-order Runge–Kutta method.¹⁹ The parameters used to fit the curves calculated to the experimental points were, in the case of *oleic-buffered system*, the three kinetic pseudoconstants k_I , k_{II} , and k_{III} , while for the *caprylic hydroxide system* only k_I and k_{II} were used. In fact, in this case, there are not enough experimental data to describe the last stage of the process, and therefore, we preferred to utilize the value of α obtained by the fit of the oleic-buffered system instead of using a new value of k_{III} . All the other parameters were assigned values to be found in the literature.

Oleic-Buffered System. Equation 12 provides the trend of anhydride moles in the organic phase during the first stage of the process if the initial surfactant concentration $[\Sigma_0]$ is lower than cac, while eq 5 allows the total surfactant aqueous concentration to be obtained. When $[\Sigma]$ in solution becomes

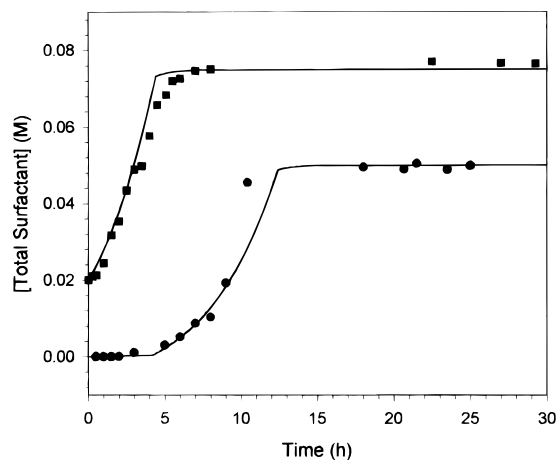


Figure 2. Basic hydrolysis of oleic anhydride in the presence of a buffered solution: experimental data (from ref 5) and theoretical plots. Total surfactant concentration is plotted against time for different initial amounts of reagents: $[\Sigma_0] = 20.0$ mM and $A_0 = 0.275$ mmol (■); $[\Sigma_0] = 0.0$ mM and $A_0 = 0.25$ mmol (●).

equal to the cac and the number of anhydride moles in the organic phase is equal to

$$A_{I-II} = A_0 + v \left(\frac{[\Sigma_0] - \text{cac}}{2} \right)$$

then the catalytic stage begins. Thus, the differential equation to be solved becomes eq 26. This equation was solved numerically, and the values obtained were used to calculate both the trend of the total surfactant concentration, through eq 21, and the concentration of anhydride in solution, through eq 23.

When the number of moles of anhydride in the organic phase has reached zero, the process enters the final stage. The lowering of the anhydride concentration in solution is described by eq 35, while eq 32 allows the course of the total surfactant to be calculated.

Figure 2 shows the experimental time evolution for the total surfactant concentration in solution (see ref 5) compared with the theoretical curves calculated assuming different initial surfactant concentrations: $[\Sigma_0] = 0.0$ and 20.0 mM and mole number of anhydride $A_0 = 0.250$ and 0.275 mol, respectively. The following values were used for the other required parameters:

pH	=	8.5	from ref 5
cac	=	0.4 mM	from ref 5
v	=	0.01 L	from ref 5
k_I	=	$1.08 \text{ mol}^{-2/3} \text{ s}^{-1}$	adjusted for the best fit
k_{II}	=	$11.11 \text{ L mol}^{-1} \text{ s}^{-1}$	adjusted for the best fit
k_{III}	=	$925.93 \text{ L mol}^{-1} \text{ s}^{-1}$	adjusted for the best fit

The value of α , calculated by the ratio k_{II}/k_{III} (see eq 31), is equal to 0.012. This value is in agreement with assumption iii, i.e., that the number of anhydride molecules absorbed by vesicles is so low so as not to disturb the process of aggregation.

In Figure 3 are reported the time courses of the mole number of anhydride in the organic phase and the concentration of anhydride in solution dissolved by the aggregates when $[\Sigma_0] = 0.0$ mM and $A_0 = 0.25$ mmol. In particular, the trend of the anhydride aqueous concentration shows a peak shape, as was experimentally observed.⁵

Caprylic Hydroxide System. In the slow stage, the set of differential eqs 9 and 10 was solved numerically, obtaining the time courses of the anhydride moles in the organic phase and the hydroxide concentration in aqueous solution, while the total surfactant concentration was calculated through the conservation eq 5.

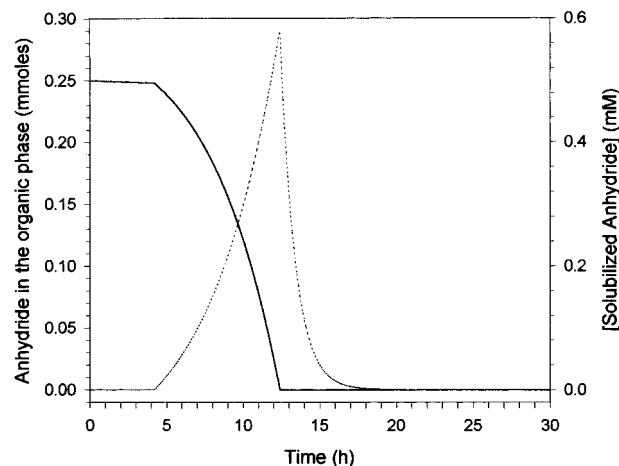


Figure 3. Theoretical plots of basic hydrolysis of oleic anhydride in the presence of a buffered solution. The amount of anhydride in the organic phase (on the left axis) and the concentration of anhydride solubilized in water solution (on the right axis) are reported against time for $[\Sigma_0] = 0.0$ mM and $A_0 = 0.25$ mmol.

In the catalytic stage, the differential equations to be solved are eqs 20, 24, and 25, with $f_{II}([\text{OH}^-])$ defined by eq 26, while the concentration value $[A_V]$ can be calculated by eq 23.

Finally, for the third stage, the set is constituted by the differential eqs 33 and 34 for the concentrations of hydroxide and of anhydride dissolved in the aqueous phase, respectively, while the total surfactant concentration is determined by eq 32 and the anhydride moles in the organic phase have reached zero.

Figure 4 shows the experimental data and the theoretical plot of the total surfactant concentration and pH obtained with the following parameter values:

$[\Sigma_0]$	=	0.0	from ref 5
A_0	=	2.50 mmol	from ref 5
$[\text{OH}^-]$	=	0.265 M	from ref 5
cac	=	0.220 M	from ref 5
v	=	0.010 L	from ref 5
$\text{p}K_{\text{AW}}$	=	4.8	from literature
$\text{p}K_{\text{AV}}$	=	8.0	from literature
k_I	=	$4.51 \times 10^{-5} \text{ mol}^{-2/3} \text{ s}^{-1}$	adjusted for the best fit
k_{II}	=	$2.78 \text{ L mol}^{-1} \text{ s}^{-1}$	adjusted for the best fit

In this case, the value of $\alpha = 0.012$, previously determined for the oleic-buffered system, has been utilized, so the kinetic constant of the third stage of the process could be obtained: $k_{III} = 231.67 \text{ L mol}^{-1} \text{ s}^{-1}$.

4. Conclusions

The agreement between the experimental data and the theoretical forecasts, shown in Figures 2 and 4, demonstrates how the same approach allows us to kinetically describe these two systems of autopoietic self-reproducing vesicles so different one from each other. It should be evident that this model is quite macroscopic and semiempirical in nature, but for this reason, it avoids a large-scale mathematical treatment, otherwise necessary, for the description of this class of processes. On the other hand, the main features of these systems have been sufficiently outlined and new insights about the mechanism of self-reproduction of long fatty acid vesicles have been achieved.

In particular, by our analysis, the slow stage has been interpreted as being due to the time needed to reach the stability conditions for the vesicles in aqueous solution. Therefore, the different length of this stage, observed for the two experimental systems, is directly related to the different values of cac: 0.220 M for the caprylic vesicles and 0.4 mM for the oleic ones, respectively, (in agreement with the values reported in the

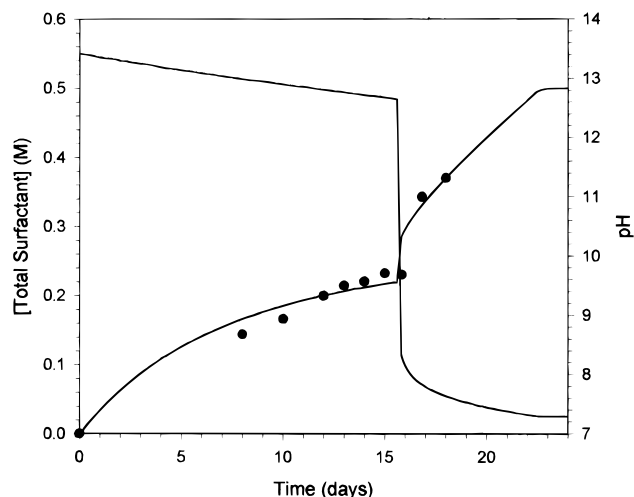


Figure 4. Basic hydrolysis of caprylic anhydride in the presence of a sodium hydroxide solution. The experimental data (from ref 5) and the theoretical plot of the total surfactant concentration are outlined against time on the left axis, while the calculated curve of pH is reported on the right axis.

literature⁵). However, the values of the pseudokinetic constant k_1 , used for fitting the experimental data, are extremely different from each other: $1.08 \text{ mol}^{-2/3} \text{ s}^{-1}$ for the oleic-buffered system and $0.45 \times 10^{-4} \text{ mol}^{-2/3} \text{ s}^{-1}$ for the other one, respectively. To account for this, it must be kept in mind that a certain amount of surfactant molecules will be absorbed at the macroscopic interface because they are able to decrease the surface energy. The ratio between surfactant molecules present in basic or acidic form will depend on the pH of the aqueous solution: at high pH values only molecules in basic form will be present. Therefore, the hydrolysis in the first stage occurs more slowly in the caprylic hydroxide system than in the oleic-buffered one, owing to the large surface concentration of negatively charged basic surfactant molecules present at the interface.

For the catalytic stage, we have shown that, if the assumptions i–iv are held, it can be generally described by a kinetic term as follows:

$$k[\text{surfactant in vesicles}][\text{OH}^-]$$

The pseudo kinetic constant k can be expressed as the product between the hydrolysis kinetic constant k_V for the vesicular surface multiplied for the anhydride molecules number α absorbed by vesicles for one molecule of surfactant, as shown by eqs 19 and 22. In this theoretical framework, the catalysis performed by the aggregates can be mainly ascribed to the extremely large increase of the reaction surface. Since the aqueous solubilization process of hydrophobic anhydride molecules by means of vesicles is so fast to be regarded as an equilibrium process, each aggregate can be considered continuously supplied by anhydride molecules so that, throughout the second stage, the average surface anhydride concentration on the vesicle boundary remains constantly equal to

$$[A]_{S_V} = \frac{\alpha}{N_A(a_S + \alpha a_A)}$$

a_A being the surface area of one anhydride molecule. This value is extremely low, 6.09×10^{-10} for $a_S = 32.0 \text{ \AA}^2$ and $a_A = 60.0 \text{ \AA}^2$, but the growth of the reaction surface is too large to account for the large speed up of the surfactant production. However, we can consider that while the macroscopic interface has an area of a few cm^2 , the microscopic interfacial area for a 0.1 M surfactant solution of vesicles is about 20.000 m^2 ,

calculated by using the former value of a_S . Therefore, the interfacial area increases by 6 orders of magnitude for a millimolar concentration of surfactant.

In conclusion, we wish to highlight that having considered the aggregation and solubilization processes as rapid processes leading to equilibrium allowed us to produce a sufficiently accurate description of highly complex systems such as auto-poietic self-reproduction vesicles and to ascribe the self-catalytic production of surfactant molecules to a cross-catalysis mechanism performed by the aggregates.

Appendix

To determine the expression of k_{II} , attention will be focused solely on the term that takes into account the contribution of the aggregates to anhydride hydrolysis in the second stage of the process:

$$\sum_i \sum_n \frac{\text{anhydride molecules}}{\text{vesicles}} \frac{k_{S_{i,n}}}{v} [A]_{S_{i,n}} [\text{OH}^-]$$

The anhydride surface concentration $[A]_{S_{i,n}}$ of vesicles made up of i surfactant molecules and n anhydride molecules and the total vesicle surface area $S_{i,n}$ can be expressed as follows:

$$[A]_{S_{i,n}} = \frac{n}{s_i N_A} \quad S_{i,n} = s_i v N_A [A_n V_i]$$

s_i being the surface of a vesicle with i molecules of surfactant and N_A Avogadro's number. With assumption vi, the former expression becomes

$$k_V \left\{ \sum_i \sum_n \frac{\text{absorbed anhydride molecules}}{\text{vesicle size}} n [A_n V_i] \right\} [\text{OH}^-] \quad (\text{A0})$$

From eq 16 one obtains

$$\sum_n \frac{\text{absorbed anhydride molecules}}{n} n [A_n V_i] = \bar{n}_i \sum_n \frac{\text{absorbed anhydride molecules}}{n} [A_n V_i] \quad (\text{A1})$$

which can be substituted in the previous equation together with eq 17 to give

$$k_V K [A]_{S_0} \left\{ \sum_i \frac{\text{vesicle size}}{r_i^2} \bar{n}_i \sum_n \frac{\text{absorbed anhydride molecules}}{n} [A_n V_i] \right\} [\text{OH}^-] \quad (\text{A2})$$

The probability of finding a vesicle formed by i molecules of surfactant is related to the macroscopic concentrations and to the density function of the vesicle radius as follows:

$$\frac{\sum_n \frac{\text{absorbed anhydride molecules}}{n} [A_n V_i]}{\sum_i \sum_n \frac{\text{absorbed anhydride molecules}}{n} [A_n V_i]} = \frac{2r_i}{r_0^2} \exp\{-(r_i/r_0)^2\} \Delta r_i \quad (\text{A3})$$

The denominator on the left side can be stated as

$$\sum_i^{\text{vesicle size}} \sum_n^{\text{absorbed anhydride molecules}} [A_n V_i] = \frac{[\Sigma] - \text{cac}}{\langle i \rangle}$$

where $\langle i \rangle$ denotes the average aggregation number of a vesicle. Therefore one has

$$\sum_n^{\text{absorbed anhydride molecules}} [A_n V_i] = \frac{[\Sigma] - \text{cac}}{\langle i \rangle} \frac{2r_i}{r_0^2} \exp\{-(r_i/r_0)^2\} \Delta r_i \quad (\text{A4})$$

By substitution of eqs A4 and 17 in eq A2, the following expression can be obtained:

$$k_V K[A]_{S_0} \sum_i^{\text{vesicle size}} \left\{ r_i^3 \exp[-(r_i/r_0)^2] \frac{2\Delta r_i}{r_0^2} \right\} \frac{[\Sigma] - \text{cac}}{\langle i \rangle} [\text{OH}^-] \quad (\text{A5})$$

and by replacement of the sum with an integral from 0 to $+\infty$, eq A5 becomes

$$k_V \left\{ K[A]_{S_0} \frac{r_0^2}{\langle i \rangle} ([\Sigma] - \text{cac}) \right\} [\text{OH}^-] \quad (\text{A6})$$

The average aggregation number $\langle i \rangle$ can be easily calculated by keeping in mind eq 15, which gives the aggregation number of a vesicle as a function of the aggregate radius:

$$\langle i \rangle = \int_0^\infty \frac{8\pi}{a} (r^2 + \rho^2) \frac{2r}{r_0^2} \exp\{-(r/r_0)^2\} dr = \frac{8\pi}{a} (r_0^2 + \rho^2) \quad (\text{A7})$$

Therefore, the expression of k_{II} can be found by comparing eqs A6 and A7 with eq 18.

Finally, α can be easily obtained keeping in mind that

$$\alpha([\Sigma] - \text{cac}) = \left\{ \sum_i^{\text{vesicle size}} \sum_n^{\text{absorbed anhydride molecules}} n[A_n V_i] \right\}$$

and comparing eqs A0 and A6.

References and Notes

(1) In this paper the term self-reproduction (as opposed to self-replication) is used according to a recent proposal.⁷

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- (15) We preferred to maintain explicitly the set of differential equations rather than reduce it to only one equation because in this way it becomes easier to switch from the *caprylic hydroxide* to the *oleic-buffered system* just by setting $d[\text{OH}^-]/dt \approx 0$.
- (16) The concentration $[\text{OH}^-]$ remains constant in the *oleic-buffered system* as a consequence of the presence of a buffer in the aqueous solution. The buffer is not involved in the kinetic processes but only in the acid–base equilibria. Therefore, $d[\text{OH}^-]/dt \approx 0$ cannot arise directly from the function $f_i([\text{OH}^-])$, as written in eq 8, because it has been obtained for the not-buffered *caprylic hydroxide system*.
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