



Linking chemical degradation and physical instability of lipid vesicles

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Abstract

Lipid vesicles are applicable in a wide variety of uses including both academic and commercial avenues. In all cases, it is crucial to understand and connect the chemical degradation of constituent molecules – *i.e.*, lipids – with the physical instability of the vesicles since their function can be greatly altered if compromised. This chapter offers a concise overview of the two main pathways through which lipid molecules

chemically degrade – oxidation and hydrolysis. Next, it highlights factors that lead to physical instability of lipid vesicles. Within each section, relevant analytical techniques are included. We conclude by presenting preliminary data that highlights our recent efforts to relate lipid chemical degradation and vesicle instability.



1. Introduction

Lipid vesicles – or liposomes – are used in diverse applications from fundamental science to commercial products. In the lab, they are often used as simplified models to understand the physical properties of membranes that encapsulate and compartmentalize cells [1]. They are also highly tunable model systems used to reconstitute and study membrane proteins [2]. One of the first commercial applications of lipid vesicles was in drug delivery [3]. Since it was shown that encapsulating chemotherapeutic drugs in liposomes both improved the drug performance and reduced harmful side effects, liposome-based drugs are now used in fungal and viral infection treatments, macular degeneration inhibition, and local pain management. Next-generation liposome formulations are being explored for medical applications in stimuli-responsive drug delivery systems, gene delivery, and medical imaging and diagnostics. The advantages of encapsulating, protecting, and controlling the release of active molecules using liposomes also extend to a number of other applications including cosmetics [4], food products [5], agriculture [6], textile processing [7], and environmental remediation [8].

Whether on a lab bench or a pharmacy shelf, the performance of lipid vesicles in all applications critically depends on their chemical and physical stability. All lipids will chemically degrade given sufficient time. Chemical degradation of lipids has been shown to alter the physical properties of membranes and thereby affect protein function which could lead to confusing and inconsistent results in laboratory studies [9]. Lipid degradation products can cause immune responses and must be quantified over time to determine the shelf-life of liposome-based drug products [10]. Chemical degradation of lipids has also been shown to increase the permeability of vesicles or lead to structural rearrangement of the vesicles, both of which result in the release of the encapsulated cargoes and could have unintended consequences in applications where the physical properties and size of the vesicle carrier are important [11–13]. As such, realizing the full potential of lipid vesicles in both fundamental and applied applications, requires not just understanding their properties, but more importantly, quantifying how

these properties evolve over time. In this chapter, we provide an overview of lipid chemical degradation and vesicle instability and highlight the need for a stronger link between the two.



2. Chemical degradation

2.1 Defining stability and its importance

Stability is a fundamental aspect of any drug delivery system or commercial product, as it ensures the integrity and efficacy of the formulation over time. The stability of lipid-based carriers, particularly liposomes, depends on both their physical and chemical properties. Lipids, being the primary structural components of these systems, are prone to degradation through chemical reactions that alter their molecular structure. The two predominant degradation pathways of lipids are oxidation and hydrolysis, both of which can compromise lipid membrane integrity, leading to vesicle instability, leakage of encapsulated cargo, and ultimately, loss of functionality [14]. Understanding these degradation mechanisms is essential for optimizing lipid formulations and extending their shelf life in pharmaceutical applications.

2.2 Mechanism of oxidative degradation

Lipid oxidation is a free radical-driven process that disrupts the structural organization of vesicles, leading to progressive membrane destabilization. It typically begins when reactive oxygen species (ROS) or trace metal ions abstract a hydrogen atom from an unsaturated acyl chain, generating a lipid radical. This radical rapidly reacts with molecular oxygen, forming a peroxyl radical that further propagates oxidative damage. The oxidation of phospholipids follows a characteristic chain reaction, comprising initiation, propagation, and termination steps (Fig. 1). In the initiation phase, oxidation is triggered by exposure to light, elevated temperatures, or the presence of transition metal ions. Once initiated, lipid radicals interact with neighboring lipids, forming hydroperoxides that further destabilize the bilayer. The process terminates when radicals are either neutralized by antioxidants or recombine to form non-reactive species [14–16].

2.3 Mechanism of hydrolytic degradation

In addition to oxidation, hydrolysis represents another significant degradation pathway. Hydrolysis involves the cleavage of ester bonds in phospholipids through their reaction with water (H_2O), primarily at the sn-2 position,

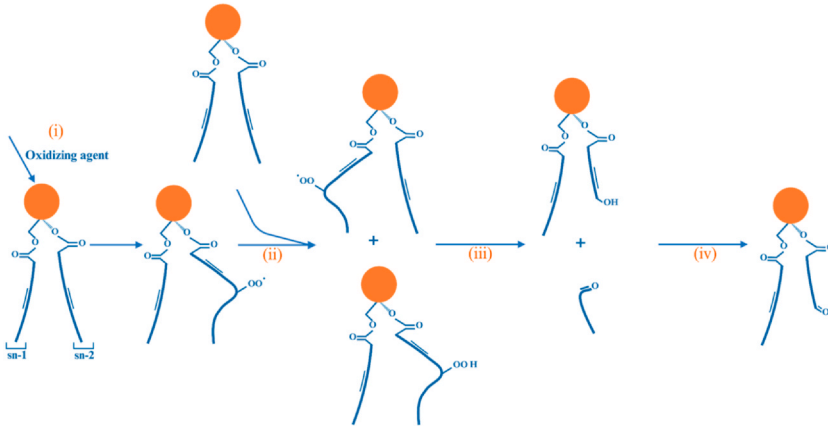


Fig. 1 The process of lipid oxidation. (i) Oxidation begins with the abstraction of an allylic hydrogen by singlet oxygen ($^1\text{O}_2$) or another reactive oxygen species, leading to the formation of a lipid radical, which then reacts with molecular oxygen to generate a peroxy radical. (ii) The peroxy radical propagates the oxidation by reacting with neighboring lipid molecules, resulting in the formation of a hydroperoxide ($-\text{OOH}$) group near the double bond. (iii) Further oxidative degradation causes lipid tail scission, producing a truncated acyl chain with a hydroxyl ($-\text{OH}$) group at one end and an aldehyde ($-\text{CHO}$) at the fragment's terminal. (iv) Continued oxidation converts the hydroxyl group into an aldehyde, further altering the lipid structure. <https://BioRender.com/k87b207>

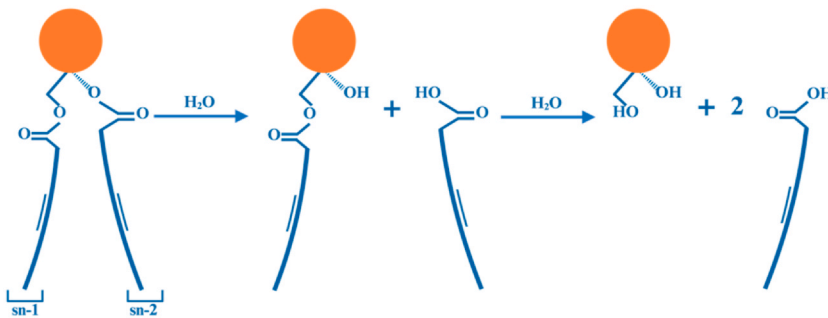


Fig. 2 The process of lipid hydrolysis. Water reacts with esters present on lipid molecules – primarily those on the sn-2 site – causing fracture of the lipid into a lysolipid and free fatty acid. The lysolipid can subsequently hydrolyze into an additional free fatty acid and isolated lipid headgroup. <https://BioRender.com/e68t457>

producing lysolipids and free fatty acids (Fig. 2) [17]. The lysolipids resulting from this initial reaction can be hydrolyzed again at the remaining ester bond. Collectively, lipid hydrolysis yields a mixture of lysolipids, free fatty acids, and cleaved lipid headgroups (such as, glycerol-3-phosphocholine).

2.4 Factors affecting degradation rates

Degradation rates are influenced by temperature, pH, and molecular composition, with degradation typically accelerated under extreme pH conditions, elevated temperatures, and enzymatic activity. In addition to storage conditions, oxidation rates in lipid structures are significantly influenced by the degree of fatty acid unsaturation. There is a direct correlation between the number of carbon-carbon double bonds on a lipid and the oxidation reaction rate. However, beyond a certain threshold, further increases in the number of double bonds may not substantially affect the initiation rate of oxidation, but does continue to elevate the total amount of oxidation products formed [18,19].

Hydrolysis can also be impacted by the lipid chemical structure, as shorter-chain lipids and negatively-charged lipids degrade faster due to increased molecular flexibility and promotion of acid-catalyzed hydrolysis from proton accumulation at the bilayer interface, respectively [20,21]. Moreover, hydrolysis follows pseudo-first order kinetics and is minimized at neutral pH, while acidic and basic conditions enhance ester bond cleavage, creating a V-shaped pH stability profile [22]. The temperature dependence of hydrolysis follows Arrhenius behavior with rates increasing at higher temperatures, though phase transitions alter activation energy making hydrolysis less pronounced in the gel phase where tighter packing reduces bond accessibility.

The aggregation state of lipids also plays a crucial role in susceptibility to degradation. Monomeric lipids – those present as isolated molecules in solution – and micellar lipids undergo hydrolysis at significantly higher rates than bilayer structures, where tight molecular packing provides steric hindrance against ion attack. Hydrolysis in unilamellar vesicles is estimated to be 7- to 11-fold slower than in micellar systems [20]. Meanwhile, the tight packing of lipids in bilayer structures promotes propagation rather than termination of oxidation reactions, and as a result, oxidation is 3- to 4-fold faster in unilamellar vesicles compared to dispersed micellar systems [23]. Both oxidation and hydrolysis in multilamellar vesicles is largely confined to the outermost layers due to limited diffusion of the reactive species deeper into structures [23].

2.5 Analytical techniques for monitoring degradation

Given the impact of oxidation and hydrolysis on lipid stability, analytical methods are essential for monitoring degradation and optimizing formulation conditions. A combination of spectroscopic and chromatographic

techniques is commonly employed to track lipid degradation. UV-Vis spectroscopy detects early-stage oxidation by monitoring the concentration of conjugated dienes, while fluorescence spectroscopy assays provides insights into secondary oxidation products, such as malondialdehyde (MDA). Nuclear magnetic resonance (NMR) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, and mass spectrometry can provide full structural detail enabling insights into chemical modifications associated with hydrolysis and oxidation. Chromatographic techniques, including thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), allow for the separation and quantification of intact and degraded lipid species, as well as byproducts like free fatty acids. Additionally, colorimetric assays, such as the thiobarbituric acid (TBA) test, measure lipid peroxidation levels by quantifying MDA, a key marker of oxidative stress [24]. Hydrolysis can also be assessed by tracking pH variations in aqueous dispersions, as the release of free fatty acids alters the acidity of the surrounding medium. The specific measurement(s) conducted should take into consideration sample quantity available, level of precision desired, and degradation product(s) of interest.



3. Physical instability

3.1 Linking chemical degradation to structural destabilization

Lipid vesicle stability is inherently linked to the chemical and physical properties of the bilayer, with degradation pathways playing a fundamental role in dictating membrane integrity. The relationship between chemical degradation and physical instability is influenced by several key factors, including lipid composition, phase behavior, external environmental conditions (temperature, pH, ionic strength), and interactions with surfactants or bile salts. As a result, structural transformations occur in response to degradation causing a transition in lipid assemblies from bilayers to micelles, bicelles, or other non-lamellar morphologies. Understanding these destabilization pathways is crucial for predicting vesicle performance in pharmaceutical and biomedical applications.

3.2 Molecular packing and self-assembly behavior

The relationship between molecular shape and self-assembly follows specific packing constraints, where small tails and large headgroups favor

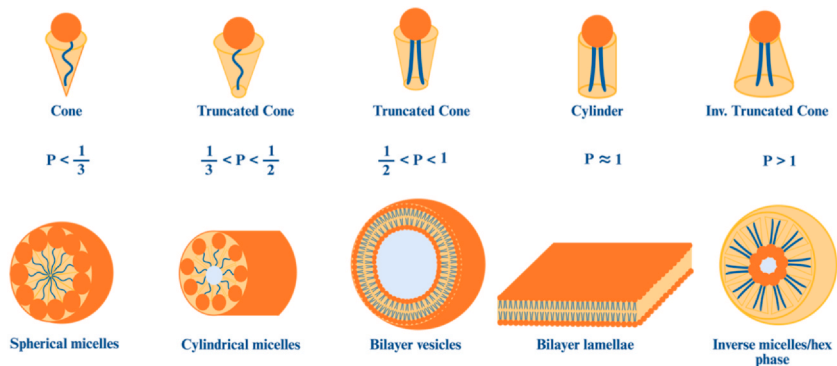


Fig. 3 Molecular shape of amphiphilic compounds based on hydrophilic headgroup size and hydrophobic tail size/number along with corresponding self-assembled structures. <https://BioRender.com/h17p909>

highly curved surfaces, while larger/longer tails and proportionally smaller headgroups prefer flat surfaces [25]. This structural preference, which dictates whether amphiphilic molecules self-organize into spherical or cylindrical micelles, bilayer vesicles, lamellae, or inverse micelles (Fig. 3), is quantified by the molecular packing parameter ($P = V/a_0l_0$), where the hydrophobic tail volume (V), optimal headgroup area (a_0), and chain length (l_0) collectively determine the preferred self-assembled structure [26].

Most lipids with two hydrocarbon chains and relatively small headgroups tend to form bilayers, as their packing parameter ($P \approx 1$) allows efficient lateral packing, with hydrophilic headgroups interacting with water while hydrophobic tails cluster inward, minimizing exposure to the aqueous environment. This reduces free energy, resulting in a stable, flexible structure, which explains why bilayers are the predominant form in biological membranes [26,27]. In contrast, single chain amphiphiles like lysolipids and free fatty acids, with $P < 1/3$ – $1/2$, adopt a conical shape, favoring micelle formation over bilayers and often remaining monomeric at low concentrations. When hydrophobic volume and headgroup size are more balanced ($1/3 < P < 1/2$), cylindrical micelles form, resulting in elongated structures instead of spherical micelles or bilayers [26,28]. In the other extreme when the headgroup size is smaller than the tail volume ($P > 1$), amphiphiles form inverted structures like the HII (inverted hexagonal) phase formed by many phosphatidylethanolamine (PE) lipids.

In addition to these classical morphologies, certain amphiphilic molecules can also spontaneously form lipid nanotubes (LNTs) in aqueous environments, driven purely by their intrinsic molecular geometry without

requiring co-lipids or external templates. This behavior is closely linked to asymmetry in the hydrophobic tails and the presence of structural features such as kinks, which promote partial interdigitation between opposing lipid layers. Such interdigitation facilitates chiral packing and introduces curvature stress that stabilizes the nanotubular morphology, expanding the range of self-assembled lipid structures beyond those typically predicted by packing parameter theory [29].

3.3 Effect of internal bilayer properties on stability

Molecular geometry and intermolecular forces play a crucial role in determining whether a bilayer remains stable or transitions into alternative structures. Molecules with a cylindrical shape and compact headgroup tend to form stable and well-defined vesicle structures, whereas those with a conical molecular shape and larger headgroup disrupt bilayer organization, promoting micelle-like assemblies. Mixing two molecules with different preferred packing parameters can, under certain conditions, drive transitions into other structures such as nanodiscs, illustrating the dynamic nature of self-assembly and molecular packing influences. Additionally, acyl chain length strongly influences bilayer stability, as shorter hydrocarbon chains accelerate bilayer disruption, making the system more susceptible to structural rearrangements [28,30].

Beyond influencing structural organization, molecular packing also plays a crucial role in chemical stability, as bilayer thickness, headgroup interactions, and molecular ordering directly affect degradation pathways. Tightly packed bilayers with strong headgroup interactions restrict water penetration reducing vulnerability to hydrolytic degradation, while loosely packed structures allow greater exposure to hydrolytic and oxidative agents making them more vulnerable to breakdown. The composition of headgroups also plays a role in degradation susceptibility, as variations in hydration and electrostatic interactions can alter lipid packing and molecular ordering. Furthermore, bilayer asymmetry can create localized instability, making certain lipid domains more prone to hydrolysis, ultimately influencing the overall stability of vesicles and their resistance to external stressors [31].

3.4 Effect of environmental factors on stability

Temperature and pH are critical external factors influencing the stability of self-assembled vesicular structures. At the lipid melting temperature – T_m , bilayers undergo a phase transition from the gel phase to the liquid-

crystalline phase significantly altering membrane properties, namely increasing bilayer fluidity, flexibility, and permeability. This transition can lead to inter-vesicle aggregation and fusion events that generate larger vesicular assemblies that sediment out of solution due to their size. However, the incorporation of stabilizing agents, such as cholesterol, has been shown to moderate these transitions by reducing abrupt shifts in membrane fluidity and maintaining structural cohesion [32].

Similarly, pH plays a crucial role in vesicle stability. Under acidic conditions, lipids within the bilayer orient their headgroups parallel to the vesicle surface whereas basic conditions promote a tilt of the headgroups relative to the bilayer surface. The most stable lipid orientation in bilayers is observed under physiological pH (7.4) where molecular interactions lead to the headgroups situated perpendicular to the vesicle surface. In some lipid systems, deviation from neutral pH can even cause a full structural transition from liposomes to an inverted hexagonal phase – this mechanism has been used for targeted drug delivery to acidic environments, such as tumors [33]. The presence of buffering agents and stabilizers like cholesterol further enhances structural resilience by maintaining membrane organization across different pH environments, mitigating the risk of premature structural breakdown [32].

3.5 Effect of lipid degradation on stability

Lipid oxidation profoundly impacts bilayer stability, permeability, and phase behavior by altering molecular packing and self-assembly properties. The accumulation of hydroperoxides expands the area per lipid headgroup and increases lateral pressure, which compromises mechanical stability and promotes vesicle fusion or aggregation. Simultaneously, oxidative fragmentation of acyl chains leads to membrane contraction and lipid loss potentially driving the formation of micellar structures and further destabilizing the vesicle architecture. As oxidation progresses, hydrophilic functional groups are introduced reducing acyl chain order and increasing lateral lipid diffusion, which weakens the hydrophobic barrier and facilitates water penetration even at low oxidation levels (2.5%–10%). Additionally, secondary oxidation products, such as aldehydes, ketones, and epoxides, further exacerbate instability by modifying the physicochemical properties of the bilayer, making it more permeable and prone to leakage. The degree of oxidation vulnerability is strongly influenced by lipid composition, with polyunsaturated species being particularly vulnerable

due to their multiple double bonds, which facilitate radical rearrangement and hydroperoxide formation [12,15,34].

While oxidation primarily disrupts bilayer integrity by introducing hydrophilic groups and disturbing molecular packing, hydrolysis leads to the breakdown of bilayer-forming molecules into smaller, more hydrophilic components, gradually shifting the system toward disordered or micellar phases. This degradation does not immediately affect structural dimensions, but as hydrolytic byproducts accumulate, bilayer destabilization reaches a critical threshold, triggering a transition from lamellar structures to micelles. This process is associated with increased membrane permeability, structural reorganization, and reductions in particle size, further emphasizing the role of molecular packing in determining stability under degradative conditions [13,35].

3.6 Effect of additives on stability

Beyond degradation pathways, interactions with surfactants and amphiphilic additives further influence bilayer stability and structural organization by modifying curvature, packing density, and phase transitions. While oxidation and hydrolysis progressively weaken bilayers, external amphiphiles actively drive structural rearrangements, shifting vesicular systems toward micellar phases or intermediate structures. These interactions introduce dynamic changes in membrane integrity demonstrating that physical instability arises not only from molecular degradation but also from alterations in self-assembly behavior.

A number of experimental phase diagrams have been published for mixtures of lipids and surfactants and provide a preview of the diversity of self-assembled structures that could be present in solution as lipids chemically degrade or other amphiphilic additives or cargos are added to the solution [36–38]. For example, incorporation of nonionic surfactants into bilayer systems follows a stepwise transformation, where initial bilayer structures gradually transition into micelles as surfactant concentration increases. At low concentrations, bilayers remain intact, but with more surfactant incorporation, disc-like aggregates form as transitional structures stabilizing interfacial tension. As interfacial forces weaken, these bicelles transition into cylindrical micelles, which ultimately breakdown into spherical micelles, completing the solubilization process. This progression demonstrates how surfactants influence self-assembly behavior, driving a shift from ordered bilayers to more disordered micellar phases [39].

Similarly, bile salts act as amphiphilic destabilizers driving bilayer solubilization in a concentration-dependent manner. At low concentrations, they adsorb onto bilayer surfaces, causing minor perturbations such as bilayer thinning. As concentration increases, destabilization progresses leading to a coexistence of vesicular and micellar phases where membrane integrity is partially compromised. At higher concentrations, complete bilayer fragmentation occurs forming mixed micelles. The efficiency of this solubilization process depends on amphiphile hydrophobicity, with more hydrophobic species interacting at lower concentrations, accelerating bilayer disruption. Additionally, temperature enhances this process, as increased molecular mobility facilitates bilayer insertion, promoting vesicle disintegration and micellar formation [40].

3.7 Macroscopic property changes due to structural instability

Structural modifications within self-assembled systems, particularly changes in vesicle size, lamellarity, and phase behavior, directly impact macroscopic solution properties such as turbidity and viscosity. These optical and rheological characteristics serve as indicators of aggregate stability, interactions, and dynamic behavior in suspension.

Turbidity, which reflects the extent of light scattering in a suspension, is primarily governed by vesicle size and structural organization. Larger vesicles scatter more light than smaller ones, leading to increased turbidity. Similarly, multilamellar vesicles exhibit higher turbidity than unilamellar vesicles due to their multiple bilayers' capacity to enhance light diffraction [41]. Beyond size and lamellarity, membrane composition plays a crucial role in determining turbidity by modulating vesicle aggregation, surface charge, and bilayer rigidity. Charged components influence electrostatic interactions, which can either increase or decrease turbidity, depending on ionic conditions. Membrane rigidity also affects light scattering, as rigid membranes resist aggregation, leading to lower turbidity shifts, whereas more fluid membranes deform easily, promoting clustering and amplifying scattering effects [42].

Similarly, viscosity is highly sensitive to vesicle size, lamellarity, and phase transitions, as these factors dictate how vesicles interact and resist flow. At comparable number density, larger and multilamellar vesicles (MLVs) significantly increase viscosity. Phase behavior further affects viscosity, with vesicles in the gel phase behaving as rigid, solid-like particles, increasing viscosity, whereas those in the fluid phase exhibit greater deformability [43].

3.8 Analytical techniques for assessing stability

Scattering and imaging techniques can be employed to monitor structural transformations and macroscopic property changes in lipid vesicles. Dynamic light scattering (DLS) is widely used to track size variations and changes in polydispersity providing insights into aggregation, fragmentation, and vesicle stability under different conditions. Complementary to DLS, static light scattering (SLS) assesses structural integrity by measuring absolute scattered light intensity before and after phase transitions, helping to identify disintegration or reorganization events [32]. Additionally, small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) are used to analyze bilayer thickness as well as the structure and phase behavior of the lipid assemblies, distinguishing between lamellar, cubic, hexagonal, and other arrangements. These scattering techniques provide high-resolution data on self-assembly transitions including kinetics thereof, particularly in the presence of external amphiphiles, bile salts, or surfactants, which can induce structural reorganization [39,44].

For direct visualization of solution-assemblies, cryogenic transmission electron microscopy (cryo-TEM) enables the structural characterization of vesicles, capturing morphological transitions such as vesicle-to-micelle conversion, bilayer fragmentation, and lamellar-to-disordered phase transitions. Optical microscopy, combined with fast Fourier transform (FFT) analysis, is useful for tracking mesoscopic changes, quantifying lattice periodicity, and identifying structural ordering effects induced by external stressors or chemical modifications. Finally, molecular dynamics (MD) simulations offer a theoretical framework for understanding curvature changes, lipid packing alterations, and hydration effects providing a detailed model of physical instability mechanisms [40,44].

Further insights into bilayer integrity and molecular rearrangement can be obtained using surface-sensitive techniques like neutron reflectometry (NR), which evaluates bilayer thickness and composition changes under different conditions, and quartz crystal microbalance (QCM) [45]. The latter is a powerful tool that can be used to measure membrane adsorption, mass deposition, and viscoelastic modifications during degradation or reorganization events. QCM captures real-time shifts in resonance frequency (Δf) and dissipation (ΔD), offering insight into lipid packing changes, bilayer integrity, and substrate interactions. This technique is particularly useful for studying the formation and destabilization of supported lipid bilayers, revealing how external factors such as oxidation, surfactants, or amphiphilic agents impact membrane properties [46,47].



4. Future outlook

With the susceptibility of lipids to chemical degradation and the sensitivity of vesicle structural stability on the composition of the component amphiphiles, a stronger link between chemical and physical stability is clearly needed. Most studies of chemical degradation focus on quantifying the breakdown of lipids and accumulation of degradation products over time, but do not perform equally in-depth characterization of the structures in solution over time. Yet, the self-assembled morphology has been shown to affect the chemical degradation kinetics [20,23]. Detailed studies of physical stability often degrade the dry lipids or pre-mix a lipid with a known amount of a single degradation product or surfactant to map how the assembled structures change with composition [9,38]. However, most lipid vesicle formulations are multicomponent mixtures that will contain numerous chemical degradation products, and the structural evolution over time as the lipids degrade will likely be more complicated than the phase and stability maps made for simplified two-component systems. Going forward, in-situ studies both quantifying chemical degradation and characterizing the structural evolution over time in the same systems are needed to better define stability.

While the two dominant chemical degradation pathways of lipids are well established, there are many areas where further research is needed. The majority of work on lipid hydrolysis has focused on glycerophospholipids; however, the limited data available suggest that other lipid classes, such as plasmalogens, are even more sensitive to hydrolysis [48]. Moreover, encapsulated drug molecules have been shown to catalyze the hydrolysis of phospholipid [49], and the ester carbonyl groups connecting the lipid headgroup to the acyl tails are also sensitive to cleavage by other nucleophiles in the membrane, such as the amine groups on peptides [50]. Studies of more complex vesicle formulations will require detailed chemical reaction kinetic modeling and thermodynamic analysis that give quantitative values to compare between formulations and assess their stability.

As an example of extracting quantitative information from more complex model systems, our first steps towards understanding the degradation kinetics of multicomponent lipid vesicles consist of monitoring the degradation of extruded vesicles composed of 60 mol% 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 40 mol% 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) using HPLC (Fig. 4). The plot shows decreasing concentrations of the intact lipid molecules over time, and these

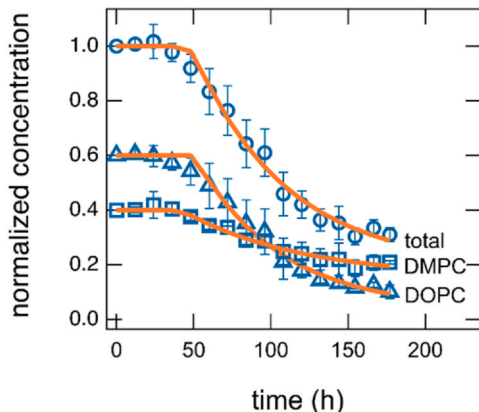


Fig. 4 HPLC data monitoring the loss of lipids in extruded vesicles composed of 60 % of DOPC and 40 % of DMPC that were subjected to accelerated aging conditions at 70 °C and pH 8.5. The loss of DMPC (squares) and DOPC (triangles) were monitored independently and fit (solid lines) with a first-order kinetic model. The degradation profiles of the individual lipids were normalized by the respective molar fractions and combined to capture the overall degradation (circles). Error bars on the data represent the standard deviation from three independent measurements.

changes were fit with a first-order kinetic model, allowing the extraction of key parameters such as the initial time delay before reaction initiation and the degradation rate constant. Notably, the results show that DOPC degrades faster than DMPC, and the total loss of lipid can be modeled as a sum of the components. Identifying the degradation products in addition to monitoring the loss of intact lipids will help identify the dominant chemical degradation pathways and provide a better understanding of the relationship between oxidation and hydrolysis in multi-component lipid vesicles [51,52].

Fully assessing the impact of chemical degradation of lipids on the physical stability of vesicles requires multimodal characterization methods. Our preliminary work provides a prime example of the need for complementary methods (Fig. 5). DLS measurements of extruded 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) vesicles indicate that the overall size has not changed after more than three months of storage at accelerated aging conditions. The similar sizes reported by the DLS results would fall within common acceptance criteria such that the sample would be deemed stable for >3.5 months [53]. However, the SANS data show that the two samples have completely different structures, where the data for the sample after 12 days is characteristic of unilamellar vesicles, as expected, while the

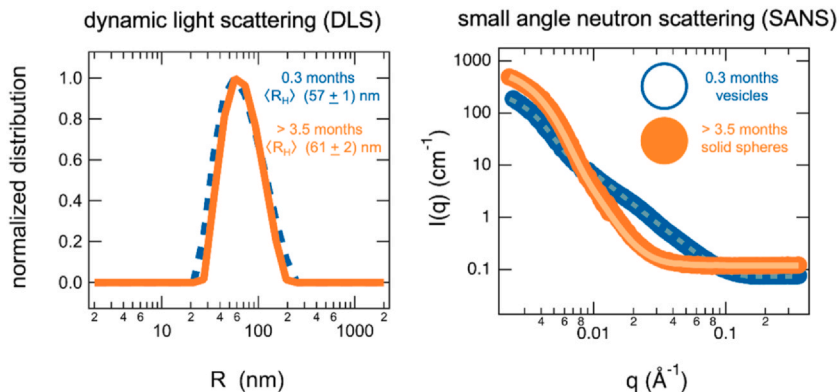


Fig. 5 Combined DLS and SANS data of extruded DOPC vesicles subjected to accelerated aging conditions at 55 °C for different periods of time. The DLS data suggests that both samples have similar sizes, while the SANS data shows that the two samples are completely different structures. Error bars on the hydrodynamic radii (R_H) in the figure represent one standard deviation of the values from Cumulants Analysis of three correlation functions measured for the same sample. SANS data were collected on the very small angle neutron (vSANS) instrument [54] at the National Institute of Standards and Technology (NIST) and fit with the respective models for vesicles and solid spheres in SasView (<https://www.sasview.org>).

data after 3.5 months is best fit as a solid sphere. Combining convenient, high-throughput techniques like light scattering with specialized nanoscale characterization techniques like cryo-TEM and SAXS/SANS will provide more complete and accurate pictures of vesicle physical stability.

Future studies combining detailed chemical analysis and chemical kinetic modeling with advanced structural characterization of solution assemblies will provide quantitative metrics to compare the stability of complex formulations. A strong link between chemical degradation and physical instability will help optimize the next generation of liposome technologies for all applications from the lab to the pharmacy.

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