LANGMUIR

pubs.acs.org/Langmuir Article

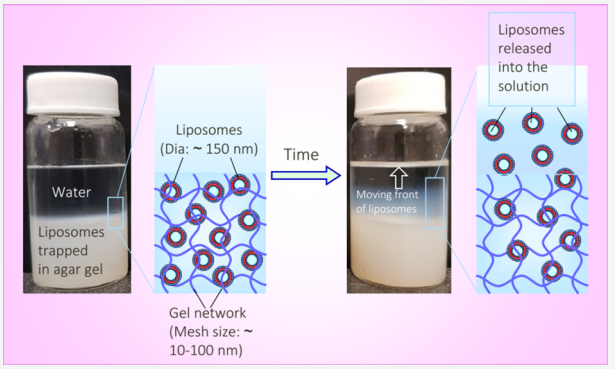
Liposomes Entrapped in Biopolymer Hydrogels Can Spontaneously Release into the External Solution

Benjamin R. Thompson, Brady C. Zarket, E. Hunter Lauten, Samiul Amin, Sivaramakrishnan Muthukrishnan, and Srinivasa R. Raghavan*

Cite This: *Langmuir* 2020, 36, 7268−7276 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Hydrogels of biopolymers such as agar and gelatin are widely used in many applications, and in many cases, the gels are loaded with nanoparticles. The polymer chains in these gels are cross-linked by physical bonds into three-dimensional networks, with the mesh size of these networks typically being 10−100 nm. One class of "soft" nanoparticles are liposomes, which have an aqueous core surrounded by a lipid bilayer. Solutes encapsulated in the liposomal core can be delivered externally over time. In this paper, we create liposomes with diameters ∼150 nm from an unsaturated phospholipid (lecithin) and embed them in agar gels (the aqueous phase also contains 0−50% of glycerol, which is an active ingredient in cosmetic products). Upon placing this gel in quiescent water, we find that the liposomes release out of the gel into the water over a period of 1−3 days, even though the gel remains intact. *This is a surprising result that runs contrary to our expectation that the liposomes would simply remain immobilized in the gel.* We show that the release rate of liposomes can be tuned by several variables: for example, the release rate increases as the agar concentration is lowered and the rate increases steadily with temperature. In addition to agar, release of liposomes also occurs out of other physical gels including those of agarose and gelatin. However, liposomes made from a saturated phospholipid do not release out of any gels. We discuss a possible mechanism for liposomal release, which involves intact liposomes deforming and squeezing through transient large pores that arise in physical networks such as agar. Our findings have relevance to transdermal delivery: they suggest the possibility of systematically delivering liposomes loaded with actives out of an intact matrix.

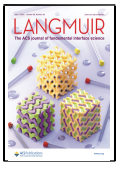


INTRODUCTION

Topical formulations (gels, creams, lotions, and pastes) are widely used in cosmetics and pharmaceutics.[1,2] These formulations typically contain an active ingredient (e.g., a hydrating agent or a drug) loaded into an aqueous matrix. When applied onto the skin, the active ingredient, which is typically a small molecule, is expected to diffuse through the outer layers of the skin and thereby get absorbed into the underlying tissue. A model material for studies on topical delivery is a hydrogel matrix loaded with the active.[1,2] Biocompatible hydrogels can be made from common biopolymers such as agar, agarose, gelatin, chitosan, alginate, pectin, and guar gum.[3,4] For example, agar is a polysaccharide extracted from algae that forms gels in water.[5,6] The agar gel structure is a three-dimensional (3-D) network of agar chains connected at "helical" junctions (i.e., two or more agar chains are intertwined into a helix at these junctions). The bonds holding the helices are physical bonds, which means that they can be broken easily. Thus, agar gels are thermoreversible: they can be "melted" to a sol (solution) by heating, and they revert to a gel upon cooling.[6] The network in an agar gel is expected to be rather dense: the "mesh size" of the network, which reflects the sizes of pores between network strands, has been estimated to be ∼100 nm.[7]

One route to enhancing the properties of topical formulations that has been widely explored is by incorporating nanoparticles into the formulation.[1,2] An example of soft nanoparticles are *liposomes*, i.e., vesicles formed from biocompatible lipids.[8−10] Liposomes have an aqueous core enclosed by a lipid bilayer, and the core can be loaded with actives. When such liposomes, with typical diameters ∼100 nm, are incorporated into hydrogels, several benefits are anticipated.[10−16] For example, an active in the liposomal core would face two transport barriers: one from the lipid bilayer and the other through the gel matrix. As a result, the delivery of

Received: March 3, 2020
Revised: May 22, 2020
Published: June 16, 2020



https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

the active from a liposome-bearing gel could be prolonged (as compared to the control case of a bare gel with the same active). Additionally, if the liposomes in the gel have responsive properties, the same would be conferred to the overall gel. For example, if the liposomes are pH-responsive but the gel matrix is not, the release of solute from a liposome-loaded gel would still show a sharp dependence on pH.[16] In all these studies, the liposomes are expected to be *immobilized* in the gel matrix, provided the bonds constituting the network remain intact (i.e., do not degrade over time).[10−16] The same assumption is also made in studies on liposome-loaded gels for purposes unrelated to solute delivery. One example is for sensing, where the molecules to be sensed are assumed to diffuse into the gel and trigger a response in the encapsulated liposomes (e.g., a color change), whereas the liposomes are not expected to diffuse out of the gel.[17,18]

In this paper, we focus on a system of liposomes encapsulated in hydrogels such as agar. Our original intent was to use this as a model system relevant for topical delivery and to study the release of solute from this liposome-loaded gel into the surrounding solution. In our typical experimental setup (Figure 1), the gel is placed in a vial, and an aqueous solution is introduced in the headspace above the gel. To our surprise, we observed that the *liposomes escape out of the gel into the solution* as time progresses. This can be seen visually from the upward-moving blue front in the solution (Figure 1; see below for details). This is a surprising result because we expected the liposomes to simply remain immobilized in the gel. To substantiate this unusual result, we have investigated a number of factors, including the concentrations of various components in the gel, as well as temperature. We have also studied different gel matrices (made from biopolymers other than agar) and also liposomes made with different phospholipids. Overall, we confirm the main result, i.e., that liposomes can indeed translocate across the gel−water interface in many (but not all) cases studied. Moreover, the rate of liposomal release can be systematically tuned by several of these variables. We conclude by discussing three possible mechanisms for liposomal translocation. The results from this study may inspire new designs for topical formulations in cosmetics and medicine.

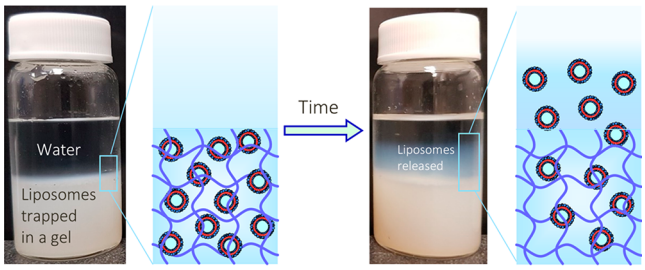


Figure 1. Schematic of the experimental setup in this study and the key finding. Initially, (left) liposomes are embedded in a hydrogel such as agar, and water is placed above the gel in a vial. Over time (1−3 days), some of the liposomes release out of the gel into the water above. The release of liposomes can be visually observed as an upward-moving blue front in the water.

RESULTS AND DISCUSSION

Liposomes in Agar Gels. Our typical system consists of liposomes from lecithin (soy-phosphatidylcholine or soy-PC) in agar gels. We formed liposomes using 2.5% lecithin and characterized them by dynamic light scattering (DLS). The hydrodynamic diameter (D_h) of the liposomes in deionized (DI) water from DLS was around 200 nm. We also prepared liposomes in aqueous solutions containing 10−50% of glycerol, which is a common hydrating agent in cosmetic formulations. Previous studies had shown that liposomes were stable in the presence of glycerol, and we confirm this finding.[19−21] The D_h of the liposomes decreased from 200 ± 10 nm without glycerol to 130 ± 20 nm with 50% glycerol, as shown by Figure S1 in the Supporting Information. The presence of liposomes in a 30% glycerol solution was also corroborated by cryotransmission electron microscopy (cryo-TEM). Figure S2 in the Supporting Information shows typical cryo-TEM images of this sample, which reveal spherical unilamellar liposomes. A size distribution was extracted from these images, and the average diameter of the liposomes is 70 ± 20 nm, which is smaller than the D_h measured by DLS. Such a discrepancy has also been noted in other studies.[22]

Agar is a natural biopolymer extracted from red seaweed.[5,6] It consists of agarose and agaropectin: the former is a nonionic constituent responsible for gelation, while the latter has sulfated groups that impart a net anionic character to the gel.[5,6] Agar is insoluble in cold water but dissolves at temperatures close to the boiling point of water, with the chains being random coils at this stage. Upon cooling below

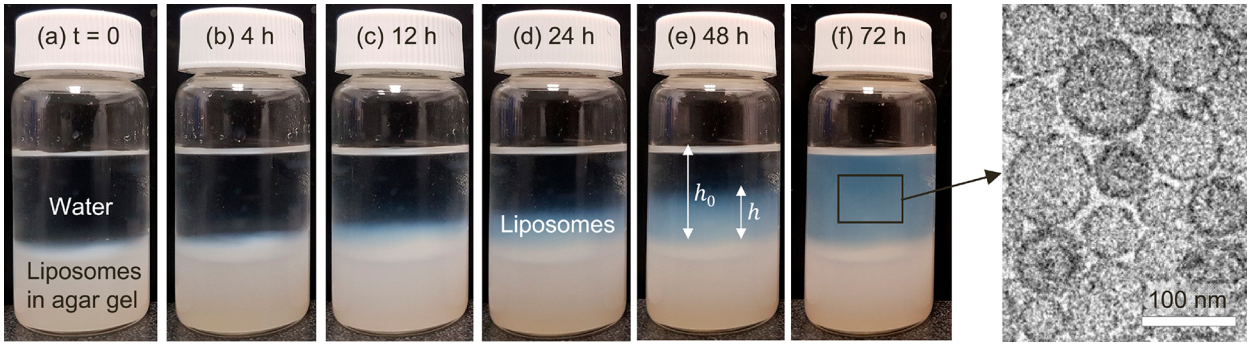


Figure 2. Release of liposomes over time from an agar gel to the solution above. (a) At t = 0, the agar gel containing liposomes is contacted with water in the headspace above it. Over a period of 3 days (72 h), the liposomes escape out of the gel into the water, which can be seen in the photos (b−f) as an upward-moving blue front. In the photo in (e), the height h of the blue front from the gel surface is marked, and this is normalized by h_0 to determine the extent of liposomal release by eq 1. After the blue front has filled the entire headspace, a sample of this liquid is withdrawn and studied by cryo-TEM. The image on the right confirms that the sample contains unilamellar liposomes.

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

~45 °C, the random coils transform into double helices, which aggregate at junction zones to form a 3-D network.[5,6] Once formed, this hydrogel will melt (i.e., become a sol) when heated to ~85 °C, and the sol will re-form upon cooling.[5] To embed liposomes in agar gels, we mixed an agar solution with a liposomal suspension in a 1:1 volume ratio at a temperature above 45 °C and then cooled the solution to room temperature to form the final gel.

Visual evidence confirms that the liposomes are present in the gel (Figure S3 in the Supporting Information). Liposomal suspensions have a bluish color because of the scattering of light by the liposomes (Figure S3A). A bare agar gel (without liposomes) is slightly bluish in color because the helical strands and/or their junctions (where the strands meet) weakly scatter light (Figure S3B). In comparison, the agar gel with liposomes is highly turbid (Figure S3C)—more so than the liposomes or the bare gel. The higher turbidity reflects the presence of liposomes embedded in the gel. The fact that liposomes can remain intact in an agar gel is further shown by data from small-angle neutron scattering (SANS). SANS spectra are provided in Figure S4 (Supporting Information) for a liposomal suspension in D_2O, for an agar gel (in D_2O) containing liposomes, and for liposomes in 30% and 50% v/v deuterated glycerol solutions. In all cases, the spectra show a slope of −2 at low values of the scattering vector q, which reflects the presence of liposomes.[23] The ability to encapsulate intact liposomes in gels is indeed to be expected, as it has been done previously with many other liposomes and in many gel matrices.[10−16]

Release of Liposomes from Agar Gels. In working with agar gels bearing liposomes, our initial aim was to encapsulate actives in the liposomes and study the release of these actives into the external solution. For this, we used the experimental setup shown in Figure 2. Here, a 1% agar gel with liposomes (2.5% lecithin) is at the bottom of a vial, and quiescent water is placed in the headspace above the gel at $t = 0$ (Figure 2a). As time progresses, we were interested in measuring the concentration of actives in the headspace. However, we found an unusual phenomenon, which can be seen from the photos at different times over a period of $t = 72$ h (Figure 2a−f). A blue front can be seen to arise from the gel−water interface and move upward over time. The height of the blue front from the gel surface is denoted by h (see Figure 2e), and we can experimentally measure h as a function of t. Eventually, the blue front extends all the way to the top of the headspace solution, i.e., up to a height h_0. We will show that the blue front represents *the release of liposomes out of the gel into the surrounding water*. Such a result is unexpected because we assumed that liposomes would be immobilized within the agar gel, consistent with previous studies on liposomes in gels.[10−16]

What exactly is the blue front? Does it really consist of liposomes? To ascertain this, we first conducted control experiments with a bare agar gel that did not contain liposomes. In this case, no blue front was observed in the headspace even over weeks. The gel−water interface remained intact, and there was no sign of any species leaking out of the gel. We also confirmed that the agar gel was unaffected by the water in the headspace over long times; that is, there was no degradation of the gel. This is in line with expectations because agar gels are known to remain stable and robust and furthermore do not swell at all in water.[24] Next, in the case of the agar gel with liposomes (Figure 2), we performed additional experiments. After the blue front had formed and extended to a significant height h, as shown in Figure 2e,f, we removed the liquid above the gel, mixed it until it became homogeneous, and studied it with a couple of techniques. DLS revealed structures with a diameter of 100 ± 20 nm in this liquid, which matched the sizes of liposomes embedded in the gel. Also, we conducted cryo-TEM on this liquid sample, and we again found unilamellar liposomes in it, as can be seen from the image in Figure 2. Thus, there is strong evidence that the structures released into the headspace from the liposomal gel are indeed liposomes.

Rate of Liposomal Release from Agar Gels. To gain insight into the unusual phenomenon documented by Figure 2, we studied the effects of various factors. From experiments like those shown in Figure 2, we can easily monitor the kinetics of liposomal release. Extraneous variables such as the size of the vial, the height (volume) of gel in the vial, and the height h_0 of water in the headspace above the gel were all held constant. The height of the liposomal front h (starting from the gel−water interface) was monitored over time, and this was normalized by h_0 to obtain an extent of liposomal release ϕ (in %)

$$\phi = \frac{h}{h_0} \tag{1}$$

First, we consider the effect of temperature on the release rate. The experiment shown in Figure 2 was done at room temperature (21 °C), and we repeated the experiment for the same sample (1% agar and 2.5% liposomes) at 4 and 35 °C. Figure 3 plots the extent of release ϕ at the three temperatures

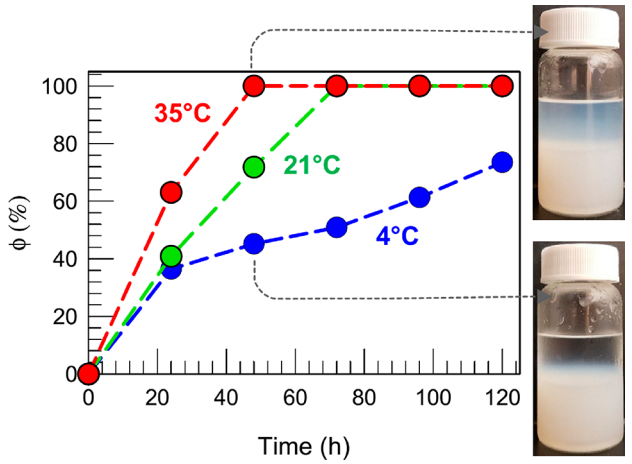


Figure 3. Effect of temperature on the release of liposomes from an agar gel. The gel is made of 1% agar and the concentration of liposomes in it is 2.5%. Release profiles are plotted at three temperatures. The y-axis represents the extent of liposomal release, as calculated from the height of the moving blue front by eq 1. The photos of the vials after 48 h illustrate the faster release at 35 °C compared to 4 °C.

(T). Liposome release occurs at all T and its *rate increases steadily with T*. That is, the plot of ϕ versus T is initially almost linear, and the slope of this line, which signifies the release rate, is greater at higher T. As examples, some vial photos at 48 h are included in Figure 3: note that the entire headspace is bluish at 35 °C (i.e., $h \approx h_0$), whereas at 4 °C, the blue front has only reached 40% of the headspace (i.e., $h \approx 0.4h_0$). It is worth emphasizing that the agar gel remains unaltered (in terms of its rheological properties) over the range of T studied.

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

The lecithin liposomes are also insensitive to T (in terms of their size). Thus, the results imply a faster transport of intact liposomes out of the intact gel at a higher T.

Next, we varied the concentration of liposomes embedded in the gel between 0.5 and 2.5% with the agar fixed at 1%. All experiments were done at 21 °C. Figure 4 shows that the

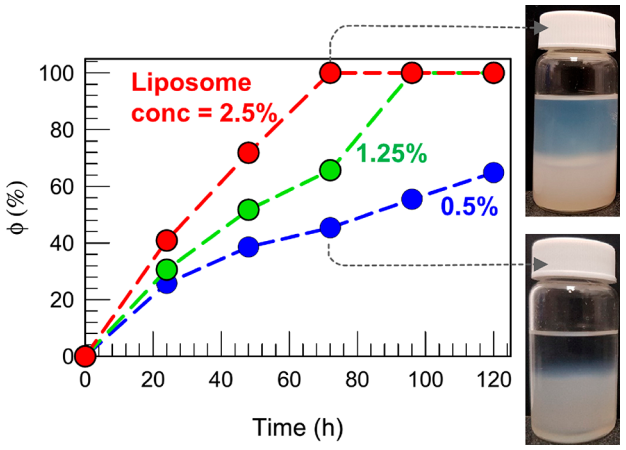


Figure 4. Effect of liposomal concentration on their release from an agar gel. The gel is made of 1% agar, and the studies were all done at 21 °C. Release profiles are plotted for three liposomal concentrations. The y-axis is the extent of liposomal release, as calculated from the height of the moving blue front by eq 1. The photos of the vials after 72 h illustrate the faster release at the highest liposomal concentration compared to the lowest.

release extent ϕ increases as the concentration of liposomes increases. As examples, the vial photos at 72 h in Figure 4 reveal that the entire headspace is bluish when there is 2.5% of liposomes, whereas in the case of 0.5% liposomes, the blue front has only moved up to 40% of the headspace. We then varied the concentration of agar from 0.5 to 2.5% while the liposome concentration was fixed at 2.5%. Increasing the agar increases the gel modulus (i.e., the stiffness of the gel) and decreases the mesh or pore size.[6,25] This is indicated by the rheological data in Figure S5 in the Supporting Information, and we will return to these aspects later in the paper. Figure 5 plots the release extent ϕ for various agar concentrations, and there is again a systematic trend: the release rate increases as the agar concentration decreases, that is, as the gel becomes softer and more porous. The vial photos at 72 h again clearly reveal the differences between the lowest (0.5%) and the highest (2.5%) agar content. We also see, once again, that the release curve is close to linear at the lowest agar concentration and nonlinear at higher concentrations. Note that a linear increase in ϕ (implying a constant rate of release) means that the release cannot be due to diffusion alone. Diffusive release would be reflected in a scaling of $\phi \sim t^{0.5}$ and in turn a decrease in release rate with time.[26]

In all the above samples, the agar gel contained 30% of glycerol. Is the glycerol somehow inducing the liposomes to be released? To test this aspect, we prepared an agar−liposome gel in DI water (no glycerol), with the other concentrations being identical to the sample shown in Figure 2 (1% agar and 2.5% liposomes). We monitored this sample over time using the same setup as above. Liposomal release into the headspace was also observed in this case, and the release rate was actually much faster than in Figure 2, as shown by Figure S6 in the

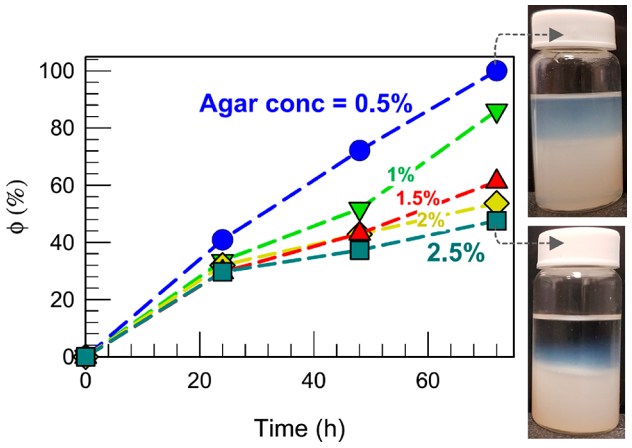


Figure 5. Effect of agar concentration on the release of liposomes from agar gels. All gels contain 2.5% liposomes, and the studies were done at 21 °C. Release profiles are plotted for five agar concentrations. The y-axis is the extent of liposomal release, as calculated from the height of the moving blue front by eq 1. The photos of the vials after 72 h illustrate the faster release at the lowest agar concentration compared to the highest.

Supporting Information. This indicates that the solvent composition in the gel is not the primary factor behind liposomal release. The glycerol has minor but important effects on several parameters including the viscosity of the continuous phase, the size of the liposomes, and the modulus of the agar gel. However, the phenomenon is quite general and occurs with aqueous samples as well. For the purpose of this paper, we will ignore this variable and continue to work with samples containing 30% glycerol.

Liposomal Release from Other Gels. Can liposomes be released out of gels other than agar? To study this aspect, liposomes (based on lecithin) were embedded into two other biopolymer gels (agarose and gelatin) and into two chemically cross-linked polymer gels. Agar and agarose gels are very similar;[3,27] as noted earlier, agarose is the component of agar that is responsible for gelation upon cooling a hot sol. Thus, agarose gels also contain helical junctions between the chains. One difference is that agarose chains are nonionic, whereas agar is anionic because of the sulfate groups in the agaropectin component. Since agaropectin is absent in agarose, its gels exhibit higher moduli than agar gels at a given concentration.[27] Here, we prepared 1% agarose gels with 2.5% liposomes. Release of liposomes from the gels into the external solution was indeed observed (Figure 6a), just as from agar. Next, we studied gels of gelatin, which is a denatured form of the protein collagen. Gelatin forms a gel by transitioning from random coils in the hot sol into triple helical junctions upon cooling.[3,28] The gels we studied contained 5% gelatin and 2.5% liposomes. As shown by Figure 6b, liposomes do get released from these gels into the external solution. The rate of liposomal release was comparable to that in 1% agar, with the entire headspace being filled with liposomes (i.e., ϕ reaching 100%) in about 72 h.

We have also attempted to study liposomes in gels formed by covalent cross-linking of monomers like acrylamide (AAm). To create these, we combined the liposomes with the monomer, cross-linker, initiator, and accelerator and conducted free-radical polymerization at room temperature (see Experimental Section for details).[29] The resulting gel showed no release of liposomes into the headspace water even over

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

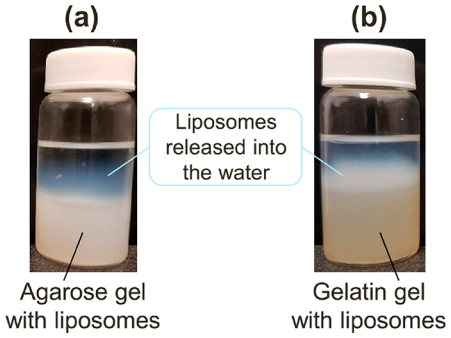


Figure 6. Liposomal release from other gels. All gels contained 2.5% liposomes, and the studies were done at 21 °C. The photos are of vials with the liposomal gel at the bottom and quiescent water on top for 48 h. Release of liposomes into the water occurs in the case of both 1% agarose (a) and 5% gelatin (b). This is evident from the moving blue front in the headspace water in both cases.

weeks. However, the results are difficult to interpret because it is not clear if cross-linking of the monomer occurs in the liposomal core as well as around the liposomes. In other words, the liposomes in this instance may get converted into nanogels or may become intertwined with the polymer network through covalent bonds—which would explain why the liposomes would not be free to diffuse. A revealing experiment in this regard is to contact the gel with a solution of the detergent Triton X-100, which is known to transform liposomes into micelles. If the detergent was added to the headspace water above a gel, then a downward-moving clear front would be seen in the gel, indicating the conversion of liposomes in the gel into micelles. Such a clear front is indeed seen in the case of an agar liposomal gel but not in the case of an AAm gel. For this reason, we have chosen to omit further discussion of covalent gels bearing liposomes.

Varying the Liposomal Composition. Does the release of liposomes depend on the nature of lipids that form the liposomal bilayer? Thus far, the liposomes we studied were formulated using lecithin, which has *cis*-unsaturations in both its alkyl tails (Figure 7a). Due to the unsaturations, bilayers of lecithin will be in a fluid state at room temperature and will freeze into a more rigid and ordered state only if cooled below their melting temperature T_m of −14 °C.[26,30] As a comparison, we studied liposomes of DPPC. This lipid has two saturated C_{16} tails (Figure 7) and thereby a much higher T_m of 41 °C.[30] Bilayers of DPPC will thus be in a frozen state at room temperature and will become more fluid only if heated above 45 °C. We prepared DPPC liposomes (D_h of 130 ± 20 nm) and embedded 2.5% of the liposomes in a 1% agar gel. Figure 7b shows this liposomal gel at room temperature (21 °C) with quiescent water above this sample. No release of liposomes into the water could be detected visually (or by DLS) over 48 h. We then repeated this experiment at temperatures of 50 and 70 °C, which are above the T_m of DPPC, and this scenario is also shown in Figure 7b. Again, no release of liposomes into the external solution was observed. Thus, the key result here is that liposomes release out of agar gels if they are made from lecithin (an unsaturated lipid) but not from DPPC (a saturated lipid).

DISCUSSION: MECHANISM FOR LIPOSOMAL RELEASE

We have provided compelling evidence for the time-dependent release of liposomes from gels such as agar into the external solution. To reiterate, this is a surprising and unexpected result because liposomes (with sizes >100 nm) are expected to remain immobilized in these gels based on previous studies[10−16] on similar systems. So why are the liposomes able to escape, i.e., what is the mechanism for this? There are three hypotheses to consider, and we will discuss each of them in order:

(1) The gel network has pores that are large enough for liposomes to pass through by simple diffusion.
(2) The pores in the gel are smaller than the liposomes, but the liposomes break up into unimers (individual lipids), which escape out and re-form liposomes in the external solution.
(3) The gels develop transient pores that are relatively large, and liposomes can squeeze through these pores.

Hypothesis 1. Our initial focus is on the pores in agar and other gels. The pore or mesh size ξ is the average size of pores in the network, i.e., of the spaces between adjacent cross-links.[25,31] This parameter is difficult to measure directly. There have been attempts to measure or indirectly estimate it using microscopic, scattering, and rheological techniques,[25] but each of these approaches is fraught with issues. Pore sizes for agar

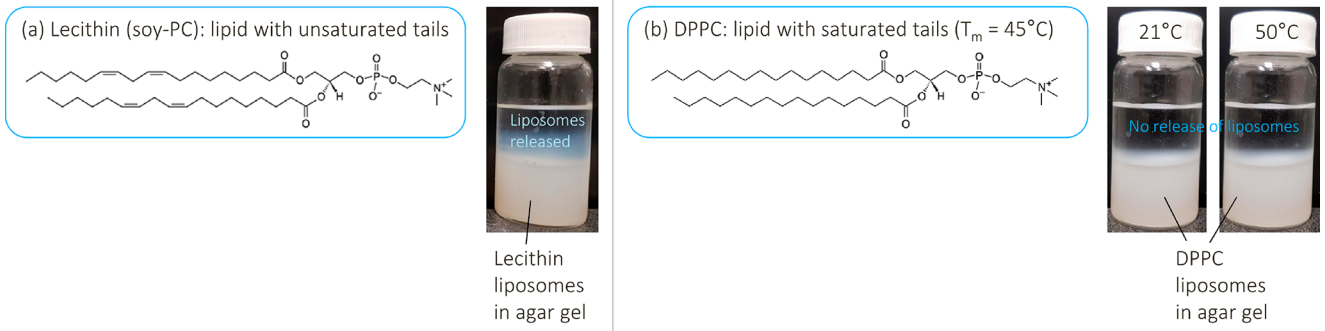


Figure 7. Effect of lipid composition on liposomal release from agar gels. (a) Studies thus far were performed with lecithin, a lipid that has two *cis*-unsaturations in each of its tails. As shown earlier, lecithin liposomes (2.5% concentration) get released from a 1% agar gel into the water above at room temperature (21 °C). (b) For comparison, liposomes of dipalmitoyl phosphatidyl-choline (DPPC), a lipid that has two saturated tails and a melting temperature T_m of 45 °C, were studied. The liposomes (2.5%) were again embedded in a 1% agar gel. In this case, no release of liposomes occurs; i.e., there is no moving blue front in the headspace water even after 48 h. The same results were obtained at both room temperature (21 °C) and 50 °C, which is above the T_m of the lipid.

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

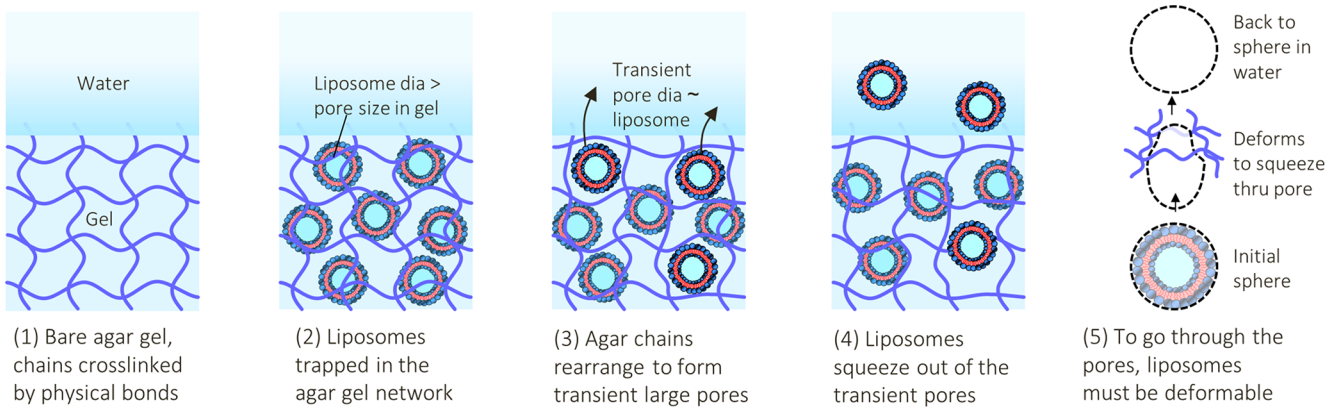


Figure 8. Schematics illustrating the hypothesized mechanism for liposomal release from physical gels such as agar. (1) The gel is formed by physical cross-linking of polymer chains into a 3-D network. The pore (mesh) size of the network is relatively small (≪ 100 nm). (2) Liposomes, with sizes >100 nm, are thus trapped in the gel. (3) It is hypothesized that the physical bonds between agar chains are sufficiently dynamic so that bonds can break and reform while preserving the connectivity of the network. As the chains rearrange, they temporarily form large pores. (4) The pores are closer in size (~100 nm) to the liposomes, and therefore, the liposomes can squeeze out of these pores into the water above. (5) In addition to a dynamic network, a further requirement for liposomal release is that the bilayer must be soft and deformable, so that the liposomes can squeeze through pores that are slightly smaller than their original size.

and agarose gels have been estimated to be ~100 nm for polymer concentrations similar to those used here (~1%).[7] Note that ξ will vary inversely with polymer concentration. In the case of gelatin gels, at concentrations around 5%, the pore sizes have been reported to be somewhat smaller at 5−50 nm.[32] Lastly, for chemically cross-linked gels such as AAm (at 10% monomer concentration), pore sizes of ~20 nm have been reported.[33]

One way to estimate pore sizes is from rheological data on the gels. ξ can be calculated from the following equation[25,31]

$$\xi = \left(\frac{k_B T}{G_0}\right)^{1/3} \tag{2}$$

where k_B is Boltzmann's constant, T is the absolute temperature, and G_0 is the plateau modulus. G_0 is the constant value of the elastic modulus at low frequencies, and it can be obtained from frequency sweeps on the gels. All the gels studied here showed the expected rheological profile, as illustrated for agar gels at two different concentrations in Figure S5 (Supporting Information). That is, for each gel, the elastic modulus G' is much higher than the viscous modulus G'' over the entire frequency range, and moreover, G' is nearly independent of frequency (indicating that the network is permanent and does not relax over time).[25,31] The constant value of G' for a given gel corresponds to G_0. The table in Figure S5 (Supporting Information) reports the values of G_0 for the different gels and also the corresponding values of ξ calculated using eq 2. ξ ranges from 5 nm at the low end (for a 2.5% agar gel) to 18 nm at the high end (for a 0.5% agar gel).

The above pore sizes—both our own estimates and those from the literature—appear to be smaller than the diameters of liposomes in our study, which are ~150 nm. Is it possible that the pore sizes of our gels are actually much larger and furthermore that they exceed the sizes of liposomes? If that were the case, as per Hypothesis 1, the liposomes would be readily able to escape out of the gels. We believe this is *highly unlikely*. For one thing, this hypothesis implies that there will be a distinct cutoff in liposomal release when the pore size exceeds the liposome diameter. However, our experiments with different agar concentrations did not show any cutoff—there was only a reduction in release rate at higher agar concentrations. Another experimental finding that conflicts with this hypothesis is with regard to DPPC liposomes. While lecithin liposomes escape out of 1% agar gels, DPPC liposomes do not. Since the two liposomes have similar sizes, this result cannot be explained based on pore size alone.

Hypothesis 2. Next, we consider the possibility that liposomal release has no connection to the pore size in the gel. This could be possible if it is *lipid molecules*, not liposomes, that are escaping out of the gel, with the lipids reconstituting into liposomes in the external solution over time. In this context, it is well known that small molecules such as dyes get rapidly released from both physical and chemical gels in a matter of hours (a fact that we have experimentally confirmed for all our gels).[2] From the standpoint of size, dye molecules as well as lipids will have a size of ~1 nm, which means that they will be smaller than the pore sizes of most gels. Also, liposomes are formed by the self-assembly of lipids, and therefore, there will be a dynamic equilibrium between liposomes and their constituent lipids.[26,30] The lipids in a given liposome could leave and incorporate into an adjacent liposome and vice versa. If individual lipids were to release from the liposomes, they could also, presumably, leave the gel.

However, we do not think Hypothesis 2 is likely either. For one thing, the dynamics of lipid exchange between liposomes is known to be very slow (timescale of hours to days).[26,30] The slow dynamics has to do with the molecular structure of lipids. If individual lipids leave a liposome, they would be surrounded by water. However, because of their two tails, lipids are insoluble in water, and it is unfavorable for lipids to remain in water (as opposed to being within a lipid bilayer, where the lipid tails will all be shielded from water).[26,30] Hypothesis 2 is also inconsistent with some of our experimental results. Specifically, we found faster release of lecithin liposomes as the agar concentration decreased. If it was lipid molecules that were being released, their small size should have allowed them to diffuse out of the larger gel pores regardless of the agar concentration (i.e., the kinetics should not have been sensitive to the agar concentration at all). Moreover, if lipids could diffuse out, then both DPPC and lecithin ought to do so, but experimentally, we find no release with DPPC liposomes.

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

Thus, many of our findings are unexplained by Hypothesis 2, which means a different mechanism is necessary.

Hypothesis 3. We now discuss the third possibility which revolves around the dynamics of polymer chains in physical gels such as agar. The chains in an agar gel are connected to other chains at junction zones. The precise nature of interchain bonds is not known but is expected to involve entanglements (topological constraints), as well as weak physical interactions such as van der Waals and hydrogen bonding interactions.[3,4] Are these bonds transient or permanent? The rheology (Figure S5) of agar gels indicates a permanent network; that is, the network itself remains intact over long time scales. However, because the bonds are weak, individual bonds may be able to break and reform while preserving the overall connectivity of the network. If so, the agar chains may be able to release temporarily from junction zones and thereby widen the pores. *This dynamic nature of bonds in agar gels could underpin the escape of liposomes.*

The central idea is schematically sketched in Figure 8. As the bonds between the chains break and reform, we hypothesize that "*transient pores*" with sizes around 100 nm are created temporarily (panel 3). These spaces may remain open only for a short time, but that time may be sufficient for liposomes to squeeze through. Liposomes may thus be able to translocate from one area in the gel to another, and liposomes close to the gel−water interface may be able to escape through these pores into the water (panel 4). The transient pores must be sufficiently large for liposomes to escape. As the agar concentration is increased, the pores will become smaller, and thus, it will become more difficult (i.e., there will be a lower probability) for liposomes to squeeze through, which explains their slower release. Conversely, such pores may be created more frequently at higher T due to more rapid segmental motions of the chains, which would account for the faster release of liposomes at higher T. All the three physical gels we studied (agar, agarose, and gelatin) appear to have sufficiently dynamic bonds to allow large pores to be formed and, in turn, for liposomes to be released through these pores. We should add that the constant release rate seen frequently in our data may be related to the dynamics of pore formation. As noted earlier, this result is inconsistent with the release being via diffusion of either liposomes or lipids, which also helps to rule out Hypotheses 1 and 2.

From the above discussion, one requirement for liposomal release is the emergence of large transient pores in the gel. This requirement is necessary but not sufficient, however. We postulate that a second condition must also be satisfied, which is that the liposomal bilayers must be *sufficiently flexible* to permit the liposomes to squeeze through the pores. The importance of this second condition is indicated by our studies with DPPC liposomes, which are unable to release out of agar gels. We hypothesize that DPPC liposomes are too rigid to pass through the pores. In contrast, liposomes of lecithin are likely to be soft and deformable; thereby, they can deform sufficiently to pass through pores that are slightly smaller than their diameters (panel 5). The deformability of lecithin liposomes seems to correlate with the low T_m (−14 °C) of the lipid. In the case of DPPC, no release was observed even at 70 °C, which is well above the T_m of the lipid (45 °C). Perhaps the DPPC bilayers are still too rigid to enable deformation and escape of the liposomes.

CONCLUSIONS

In this study, we encapsulated liposomes in a variety of gels. The liposomes were typically formed from an unsaturated phospholipid (lecithin) and had diameters ∼150 nm. The gels were formed by physical cross-linking of biopolymers such as agar, agarose, and gelatin. When the liposome-bearing gels were placed in contact with water, we found that the liposomes released out of the gel into the water over a period of 1−3 days. This result was observed with all the physical gels studied. To explain this surprising result, we have postulated a mechanism that stipulates two conditions for liposomal escape: **(a) the cross-links in the gel must be dynamic** (i.e., they break and reform) so that large, transient pores (∼100 nm) are created in the gel network, and **(b) the liposomes must be sufficiently flexible and deformable** so that they can squeeze through the above pores. This mechanism accounts for the results from our study, including the increase in release rate with increasing temperature, increasing liposome concentration, and decreasing concentration of agar. The fact that liposomes of a saturated phospholipid, DPPC, do not escape out of any gels suggests that these liposomes are too rigid to squeeze through pores. Based on this study, formulations of gels containing liposomes could be a useful class of materials in the cosmetic and medical industries. The ability to deliver intact liposomes loaded with actives at predictable rates could be valuable for many applications. Future work will involve more detailed modeling of the factors that affect liposomal release rates from such formulations.

EXPERIMENTAL SECTION

Materials. Lecithin (soy-PC, 95%) and DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids. The agar was a gift from TIC Gums (the product is termed agar−agar and is extracted from Gracilaria). Agarose, gelatin, chloroform, ethanol, and glycerol (>99.5%) were purchased from Sigma-Aldrich. Deuterium oxide (D_2O), deuterated glycerol (d_8), and deuterated ethanol (d_6) were obtained from Cambridge Isotopes Laboratories. The monomers acrylamide (AAm), *N*,*N*′-dimethyl-acrylamide, and *N*,*N*′-methylenebis(acrylamide) (BIS); the initiator ammonium persulfate (APS); and the accelerator *N*,*N*,*N*′,*N*′-tetra-methylethylenediamine (TEMED) were purchased from Sigma-Aldrich. Ultrapure DI water (resistivity = 18.2 MΩ·cm) was used in all experiments.

Preparation of Liposomes. Liposomes were typically prepared using the following method. First, lecithin was mixed with ethanol in a 1:0.79 mass ratio. Following this, water or aqueous glycerol solutions (10−50% v/v) were prepared and heated to 45 °C. Addition of the above solution to the lipid solution followed by subsequent tip sonication with an ultrasonic probe (20 W power, 1 min) led to the formation of liposomes. Liposomal suspensions were stored in a fridge for future use. For the SANS studies, the same method was used but with deuterated solvents. For DPPC liposomes, a thin-film method was used. In this case, the lipid was dissolved in chloroform and then a flow of nitrogen gas was used to remove some of the solvent. Thereafter, the remaining solvent was removed by vacuum. The lipid film was then rehydrated with water or glycerol solutions and tip-sonicated as mentioned above to obtain liposomes. Lecithin liposomes were also prepared using the thin-film method for comparison purposes, and it was confirmed that the resulting liposomes were near-identical.

Preparation of Liposome-Loaded Physical Gels. The majority of studies were done with agar gels, and the following procedure was used to encapsulate liposomes in these gels. First, an agar solution of a given concentration was prepared by adding an appropriate mass of agar powder to water (or to a water−glycerol solution) and then heating to 95 °C with stirring. The solution was then cooled to ∼60 °C and mixed with the liposomal suspension in a 1:1 volume ratio

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

until homogeneous. Subsequent cooling to room temperature resulted in agar gels with liposomes embedded within. For most experiments, the concentrations in the final sample were 2.5% w/v liposomes, 1.0% w/v agar, and 30% v/v glycerol. In the case of other physical gelators (agarose and gelatin), a similar procedure was used. The agarose concentration in the final gel was also 1% w/v (as for agar), while it was 5% w/v in the case of the gelatin gel.

Preparation of Liposome-Loaded Chemical Gels. The monomer acrylamide (AAm, 10% w/v) was dissolved in a degassed solution of liposomes (2.5% w/v). The cross-linker BIS (1 mol % of monomer) and the accelerant TEMED (1% v/v) were also added. Finally, the initiator APS solution (5% w/v, 100 μL) was added to 10 mL of the above solution. The mixture was left to polymerize at room temperature for an hour to form chemical gels containing liposomes.

Determination of Release Rate of Liposomes from Gels. To measure the release rate of liposomes, first a vial was taken with the gel (bearing liposomes) at its bottom, with the gel volume being 8 cm^3. Then, water (8 cm^3) was placed above the gel at $t = 0$, as shown in Figure 2, and the vial was left undisturbed. A bluish front was observed in the water, and this corresponded to liposomes due to their scattering of light (Tyndall effect). Images were taken at regular time intervals and analyzed using the ImageJ software. The height of the bluish front was thus recorded as a function of time, as shown in the figures. Most of the release studies were performed at room temperature (21 °C). For the studies as a function of temperature (Figure 3), one sample was kept in a fridge at 4 °C and another in an oven at 35 °C.

Dynamic Light Scattering (DLS). Sizes of nanostructures were measured at 25 °C using a Photocor-FC instrument equipped with a 5 mW laser source at 633 nm. The scattering angle was 90°. The autocorrelation function was measured using a logarithmic correlator and analyzed using the DynaLS software package to obtain the hydrodynamic radius. Each experiment was performed in triplicate, and the average sizes are reported in the paper.

Cryo-Transmission Electron Microscopy (Cryo-TEM). A small drop (~3 μL) of the sample of interest was pipetted onto a holey carbon grid, and the grid was plunged into liquid ethane at −173 °C using a Gatan Cryoplunge 3A. The vitrified specimen was then transferred to a sample holder (Gatan model 914) using a cryo-loading station, and this holder was then inserted into a microscope (JEOL JEM 2100 LaB6). An accelerating voltage of 100 kV was used during imaging.

Small-Angle Neutron Scattering (SANS). SANS experiments were performed at the National Institute of Standards and Technology (NIST), Gaithersburg, MD, on the NG-B (30 m) beamline. Neutrons with a wavelength λ of 6 Å were selected, and the range of scattering vector q accessed was from 0.004 to 0.4 $Å^{-1}$. The sample holders were either 2 or 5 mm quartz cells. The scattering spectra were corrected and placed on an absolute scale using calibration standards provided by NIST. The data are shown for the radially averaged, absolute intensity I as a function of $q = (4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle.

Rheology. Experiments on the gels were performed at 25 °C on an AR2000 stress-controlled rheometer (TA Instruments). The gels were cut into discs of diameter 20 mm and thickness 2 mm and were studied using 20 mm parallel plates. Dynamic stress-sweeps were first performed to identify the linear viscoelastic (LVE) region of the sample. Dynamic frequency sweeps were then conducted at a constant strain amplitude within the LVE region.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.0c00596.

Analysis of liposomes by DLS and cryo-TEM; visual appearance of liposomes and gels; SANS profiles of samples; rheology data on gels; comparison of liposomal release in water and 30% glycerol (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Srinivasa R. Raghavan − *Department of Chemical & Biomolecular Engineering, University of Maryland, College Park, Maryland 20742, United States;* orcid.org/0000-0003-0710-9845; Email: sraghava@umd.edu

Authors

Benjamin R. Thompson − *Department of Chemical & Biomolecular Engineering, University of Maryland, College Park, Maryland 20742, United States*

Brady C. Zarket − *L'Oréal Research and Innovation, Clark, New Jersey 07066, United States*

E. Hunter Lauten − *L'Oréal Research and Innovation, Clark, New Jersey 07066, United States*

Samiul Amin − *L'Oréal Research and Innovation, Clark, New Jersey 07066, United States;* orcid.org/0000-0002-3469-7358

Sivaramakrishnan Muthukrishnan − *L'Oréal Research and Innovation, Clark, New Jersey 07066, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.langmuir.0c00596

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge L'Oréal for financial support of the work. The Maryland Nanocenter and its AIM Lab are acknowledged for assistance with cryo-TEM imaging. We also thank the NCNR for facilitating the SANS experiments performed as part of this work. We acknowledge the following lab members for their assistance with the SANS experiments: Niti Agrawal, Sohyun Ahn, Leah Borden, Hema Choudhary, and Nikhil Subraveti.

■ REFERENCES

(1) *Cosmetic Formulation: Principles and Practice*; Benson, H. A. E., Roberts, M. S., Leite-Silva, V. R., Walters, K. A., Eds.; CRC Press: Boca Raton, 2019.

(2) *Principles of Polymer Science and Technology in Cosmetics and Personal Care*; Goddard, E. D., Gruber, J. V., Eds.; Marcel Dekker: New York, 1999.

(3) Djabourov, M.; Nishinari, K.; Ross-Murphy, S. B. *Physical Gels from Biological and Synthetic Polymers*; Cambridge University Press: Cambridge, 2013.

(4) Ross-Murphy, S. B.; Shatwell, K. P. Polysaccharide strong and weak gels. *Biorheology* **1993**, *30*, 217−227.

(5) Arnott, S.; Fulmer, A.; Scott, W. E.; Dea, I. C. M.; Moorhouse, R.; Rees, D. A. The agarose double helix and its function in agarose gel structure. *J. Mol. Biol.* **1974**, *90*, 269−284.

(6) Labropoulos, K. C.; Niesz, D. E.; Danforth, S. C.; Kevrekidis, P. G. Dynamic rheology of agar gels: theory and experiments. Part I. Development of a rheological model. *Carbohydr. Polym.* **2002**, *50*, 393−406.

(7) Holmes, D. L.; Stellwagen, N. C. The electric field dependence of DNA mobilities in agarose gels: A reinvestigation. *Electrophoresis* **1990**, *11*, 5−15.

(8) Allen, T. M.; Cullis, P. R. Liposomal drug delivery systems: From concept to clinical applications. *Adv. Drug Delivery Rev.* **2013**, *65*, 36−48.

(9) Pattni, B. S.; Chupin, V. V.; Torchilin, V. P. New developments in liposomal drug delivery. *Chem. Rev.* **2015**, *115*, 10938−10966.

(10) Mourtas, S.; Fotopoulou, S.; Duraj, S.; Sfika, V.; Tsakiroglou, C.; Antimisiaris, S. G. Liposomal drugs dispersed in hydrogels. *Colloids Surf., B* **2007**, *55*, 212−221.

(11) Weiner, A. L.; Carpenter-Green, S. S.; Soehngen, E. C.; Lenk, R. P.; Popescu, M. C. Liposome collagen gel matrix - a novel sustained drug delivery system. *J. Pharm. Sci.* **1985**, *74*, 922−925.
(12) Cohen, S.; Ban∼ó, M. C.; Chow, M.; Langer, R. Lipid alginate interactions render changes in phospholipid-bilayer permeability. *Biochim. Biophys. Acta* **1991**, *1063*, 95−102.
(13) Takagi, I.; Shimizu, H.; Yotsuyanagi, T. Application of alginate gel as a vehicle for liposomes .1. Factors affecting the loading of drug-containing liposomes and drug release. *Chem. Pharm. Bull.* **1996**, *44*, 1941−1947.
(14) Glavas-Dodov, M.; Goracinova, K.; Mladenovska, K.; Fredro-Kumbaradzi, E. Release profile of lidocaine HCl from topical liposomal gel formulation. *Int. J. Pharrm.* **2002**, *242*, 381−384.
(15) Ruel-Gariépy, E.; Leclair, G.; Hildgen, P.; Gupta, A.; Leroux, J.-C. Thermosensitive chitosan-based hydrogel containing liposomes for the delivery of hydrophilic molecules. *J. Controlled Release* **2002**, *82*, 373−383.
(16) Dowling, M. B.; Lee, J.-H.; Raghavan, S. R. pH-responsive Jello: Gelatin gels containing fatty acid vesicles. *Langmuir* **2009**, *25*, 8519−8525.
(17) Lee, H.-Y.; Tiwari, K. R.; Raghavan, S. R. Biopolymer capsules bearing polydiacetylenic vesicles as colorimetric sensors of pH and temperature. *Soft Matter* **2011**, *7*, 3273−3276.
(18) Silbert, L.; Ben Shlush, I.; Israel, E.; Porgador, A.; Kolusheva, S.; Jelinek, R. Rapid chromatic detection of bacteria by use of a new biomimetic polymer sensor. *Appl. Environ. Microbiol.* **2006**, *72*, 7339−7344.
(19) Manca, M. L.; Cencetti, C.; Matricardi, P.; Castangia, I.; Zaru, M.; Sales, O. D.; Nacher, A.; Valenti, D.; Maccioni, A. M.; Fadda, A. M.; Manconi, M. Glycerosomes: Use of hydrogenated soy phosphatidylcholine mixture and its effect on vesicle features and diclofenac skin penetration. *Int. J. Pharm.* **2016**, *511*, 198−204.
(20) Manca, M. L.; Manconi, M.; Zaru, M.; Valenti, D.; Peris, J. E.; Matricardi, P.; Maccioni, A. M.; Fadda, A. M. Glycerosomes: Investigation of role of 1,2-dimyristoyl-sn-glycero-3-phosphatidycholine (DMPC) on the assembling and skin delivery performances. *Int. J. Pharm.* **2017**, *532*, 401−407.
(21) Manca, M. L.; Zaru, M.; Manconi, M.; Lai, F.; Valenti, D.; Sinico, C.; Fadda, A. M. Glycerosomes: A new tool for effective dermal and transdermal drug delivery. *Int. J. Pharm.* **2013**, *455*, 66−74.
(22) Crawford, R.; Dogdas, B.; Keough, E.; Haas, R. M.; Wepukhulu, W.; Krotzer, S.; Burke, P. A.; Sepp-Lorenzino, L.; Bagchi, A.; Howell, B. J. Analysis of lipid nanoparticles by Cryo-EM for characterizing siRNA delivery vehicles. *Int. J. Pharm.* **2011**, *403*, 237−244.
(23) Lee, J.-H.; Gustin, J. P.; Chen, T.; Payne, G. F.; Raghavan, S. R. Vesicle-biopolymer gels: Networks of surfactant vesicles connected by associating biopolymers. *Langmuir* **2005**, *21*, 26−33.
(24) Adams, S.; Frith, W. J.; Stokes, J. R. Influence of particle modulus on the rheological properties of agar microgel suspensions. *J. Rheol.* **2004**, *48*, 1195−1213.
(25) Larson, R. G. *The Structure and Rheology of Complex Fluids*; Oxford University Press: New York, Oxford, 1999.
(26) Evans, D. F.; Wennerstrom, H. *The Colloidal Domain: Where Physics, Chemistry, Biology, and Technology Meet*; Wiley-VCH: New York, 2001.
(27) Clark, L. J.; Whalley, W. R.; Leigh, R. A.; Dexter, A. R.; Barraclough, P. B. Evaluation of agar and agarose gels for studying mechanical impedance in rice roots. *Plant Soil* **1999**, *207*, 37−43.
(28) Djabourov, M.; Leblond, J.; Papon, P. Gelation of aqueous gelatin solutions. I. Structural investigation. *J. Phys.* **1988**, *49*, 319−332.
(29) Gargava, A.; Arya, C.; Raghavan, S. R. Smart hydrogel-based valves inspired by the stomata in plants. *ACS Appl. Mater. Interfaces* **2016**, *8*, 18430−18438.
(30) Israelachvili, J. N. *Intermolecular and Surface Forces*, 3rd ed.; Academic Press: San Diego, 2011.
(31) Macosko, C. W. *Rheology: Principles, Measurements, and Applications*; Wiley-VCH: New York, 1994.
(32) Varghese, J. S.; Chellappa, N.; Fathima, N. N. "Gelatin−carrageenan hydrogels: Role of pore size distribution on drug delivery process. *Colloids Surf., B* **2014**, *113*, 346−351.
(33) Kabir, M. H.; Ahmed, K.; Furukawa, H. A low cost sensor based agriculture monitoring system using polymeric hydrogel. *J. Electrochem. Soc.* **2017**, *164*, B3107−B3112.

https://dx.doi.org/10.1021/acs.langmuir.0c00596
Langmuir 2020, 36, 7268−7276

Supporting Information for:

Liposomes Entrapped in Biopolymer Hydrogels Can Spontaneously Release into the External Solution

Benjamin R. Thompson,[1] Brady C. Zarket,[2] E. Hunter Lauten,[2] Samiul Amin,[2,3]
Sivaramakrishnan Muthukrishnan[2] and Srinivasa R. Raghavan[1]*

[1]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD 20742-2111
[2]L'Oreal Research and Innovation, Clark, New Jersey 07066, USA
[3]Current address: Chemical Engineering Department, Manhattan College, New York

*Corresponding author. Email: sraghava@umd.edu

Number of Figures: 6

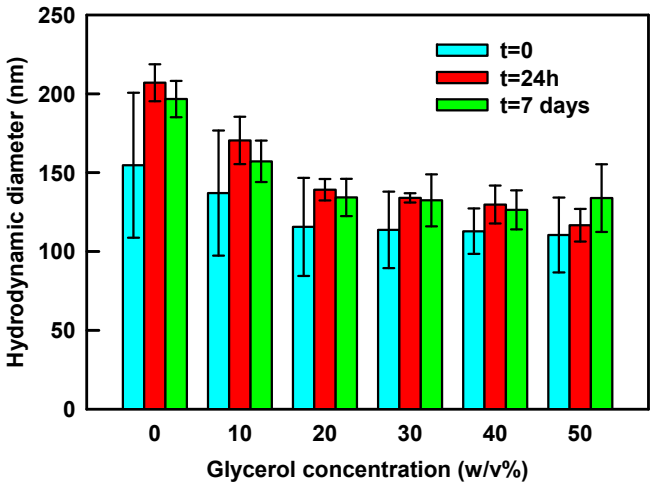


Figure S1. Sizes of lecithin (soy-PC) liposomes in water and aqueous glycerol solutions. The hydrodynamic diameters of the liposomes are measured over a period of one week. The lipid concentration was 0.2% w/v and the glycerol concentration was varied from 0 – 50% v/v. Each data point is the average of three different samples and the error bars represent the standard deviation.

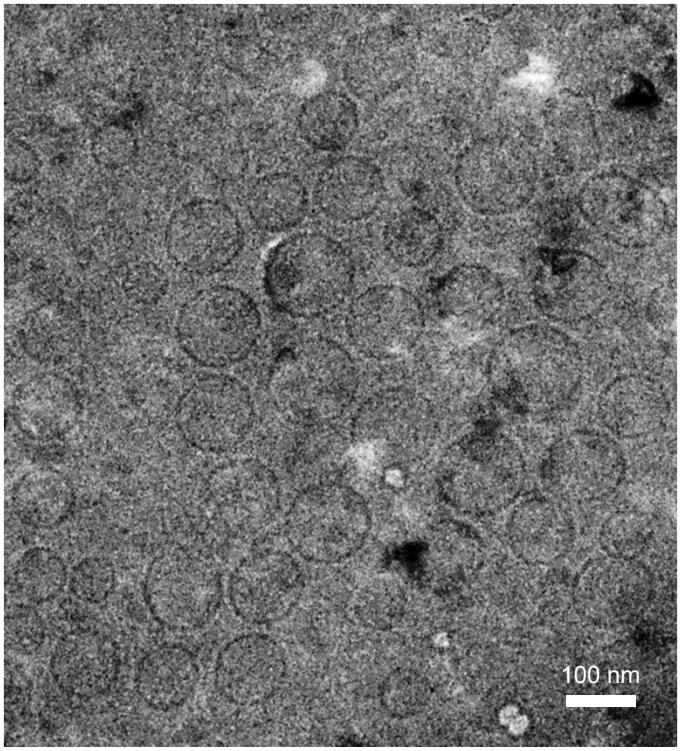


Figure S2. Cryo-TEM of lecithin liposomes in a 30% glycerol solution. Unilamellar liposomes are seen, and their average diameter, determind by image analysis from a population of 150 liposomes, was 70 ± 20 nm.

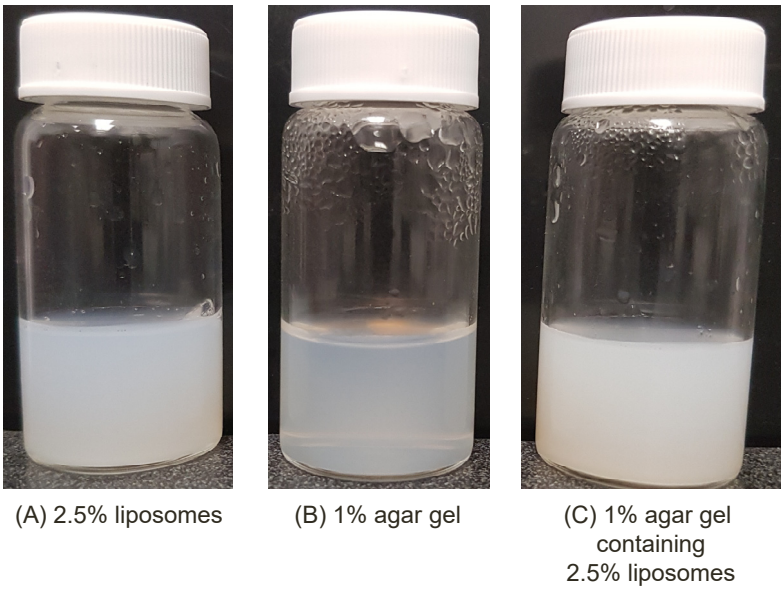


Figure S3. Visual appearance of various samples. (A) 2.5% lecithin liposomes. (B) 1% agar gel. (C) 1% agar gel containing 2.5% liposomes. The sample in (C) is considerably more turbid than that in (A) and (B).

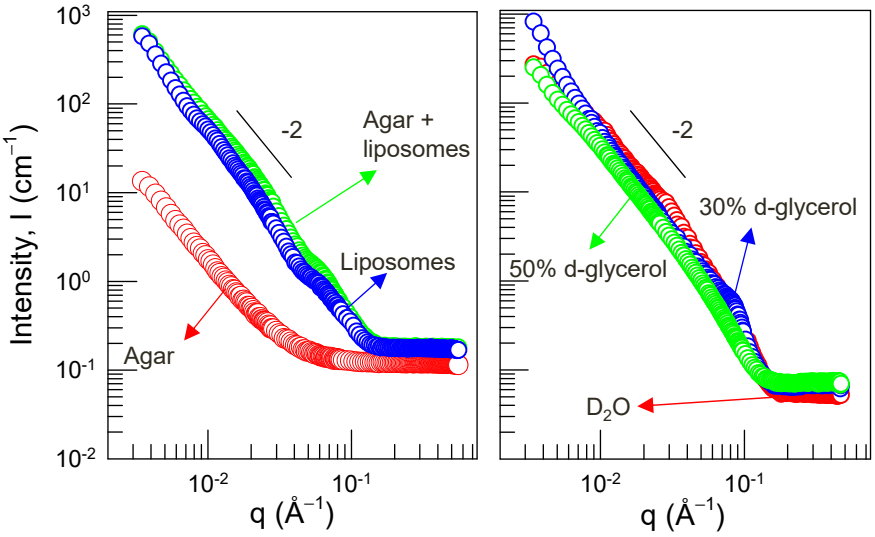
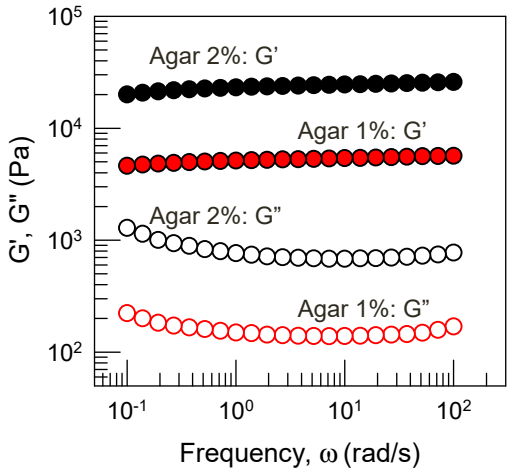


Figure S4. SANS scattering profiles for samples from this study. Each plot is for the scattered intensity *I* as a function of the scattering vector *q*. (Left) Data for a suspension of 1% liposomes, a 1% agar gel, and a 1% agar gel containing 1% liposomes embedded in it. All three samples are made using D_2O for scattering contrast. (Right) Data for 1% liposomal suspensions in D_2O or 30% or 50% d-glycerol. All samples containing liposomes show the –2 slope characteristic of bilayer scattering.



Polymer	G_0 / Pa	ξ / nm
Agar (0.5%)	698	18.0
Agar (1.0%)	4,610	9.6
Agar (1.5%)	10,800	7.2
Agar (2.0%)	19,990	5.9
Agar (2.5%)	30,080	5.2
Agarose (1.0%)	11,390	7.1
Gelatin (5.0%)	1,690	13.4
AAm (10%)	293	24.1

Figure S5. Rheology data on various hydrogels. (Left) Typical data from dynamic rheology for agar gels (1% and 2%). The data plotted are for the elastic modulus G' and the viscous modulus G'' as functions of the angular frequency ω. Both samples show the rheological response characteristic of a gel. Each sample can thus be characterized by its constant value of G', which is its gel modulus G_0. (Right) Values of G_0 for the various gels studied in this paper. From the G_0, the pore (mesh) size ξ of the gel is computed using eq 2, and this is shown in the right-hand column.

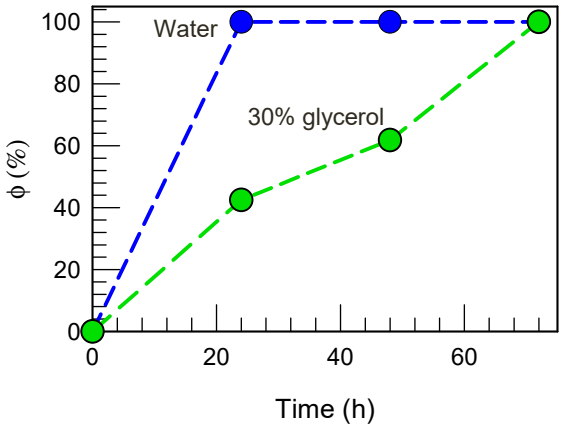


Figure S6. Comparison of liposomal release from agar gels made with water vs 30% glycerol. Both gels contain 2.5% liposomes and 1% agar, and the studies were done at 21°C. The y-axis is the extent of liposomal release, as calculated from the height of the moving blue front by eq 1.