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New Vesicle–Polymer Hybrids: The Parachute Architecture

M. Jung,[†] D. H. W. Hubert,[†] P. H. H. Bomans,[‡] P. M. Frederik,[‡] J. Meuldijk,^{†,§}
A. M. van Herk,[†] H. Fischer,^{||} and A. L. German^{*,†}

Laboratory of Polymer Chemistry, Eindhoven University of Technology, P.O. Box 513,
5600 MB Eindhoven, The Netherlands, Laboratory of Chemical Reactor Engineering and
Process Development, Eindhoven University of Technology, P.O. Box 513,
5600 MB Eindhoven, The Netherlands, TNO Eindhoven, P.O. Box 595,
5600 AN Eindhoven, The Netherlands, and Department of Pathology, Electron Microscopy,
University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands

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The influence of organized media on polymerization reactions results in many cases in interesting morphologies of the polymeric material. In the present study, vesicle bilayers were used as ordered medium for the free radical polymerization of styrene. Cryo-electron microscopy gives evidence that the polymerization induces phase-separation phenomena leading to parachute-like morphologies. On the basis of general knowledge about vesicles and polymerizations in heterogeneous media, explanations for the observed phenomena are given. Bearing in mind that vesicles are outstanding models for membrane mimetic chemistry, it becomes evident that these findings can be relevant to the investigation of, for example, membrane–protein interactions.

Introduction

The self-organized structure of vesicles is a fascinating phenomenon that attracted enormous interest not only in colloid chemistry but also in biochemistry, in a wide variety of chemical disciplines, and in material science.^{1–3}

Polymer chemists have been concerned with the polymerization of vesicles, mainly by investigating the polymerization of unsaturated amphiphilic building blocks of a vesicle.^{4,5} The concept was to immobilize the amphiphiles within the aggregate in order to improve vesicle stability and thus its lifetime. Particularly, drug delivery systems require an increased lifetime to allow delivery of their cargo to the desired place without premature vesicle breakup.⁶

A different approach to immobilize the colloidal structure of vesicles was suggested by Murtagh and Thomas.^{7,8} Their concept of polymerization *in* vesicles comprises the free radical polymerization of monomer molecules, inserted into the bilayer as host molecules. Although some publications^{9,10} have appeared following the pioneering study of Murtagh and Thomas,^{7,8} the question of the final

morphology could not be fully clarified and remained a matter of speculation. A strong influence of the confined medium on the polymerization reaction is expected, and the crucial question is how the growing macromolecules do arrange within the vesicle bilayer. Similar questions occur in biochemistry, where the interaction of proteins with lipid bilayers induces membrane rearrangements.¹¹

From a polymer chemist's point of view, it is advantageous to understand both routes, polymerization of vesicles and polymerization *in* vesicles, within the more general and accepted concepts of polymerization in organized media. In a first simplified approach it seems legitimate to focus on the lyotropic liquid crystalline character of vesicles, while neglecting all properties that result from the closed nature of the bilayer. Along these lines, polymerization *of* or *in* vesicles can be compared to polymerization *of* or *in* lyotropic liquid crystalline phases.

On one hand, polymerization of lamellar lyotropic liquid crystalline phases is a successful concept to stabilize ordered structures, both for planar mesophases^{12–16} and for vesicles. On the other hand, polymerization of monomers inserted as guest molecules in lamellar lyotropic crystalline phases often leads to a reorganization of the phases and eventually to an isotropic latex.^{16,17} Loss of entropy, through, for example, collapse of polymer chains, and the incompatibility between surfactant matrix and polymer molecules are the main driving forces for such phenomena. Consequently, a similar scenario could be expected for the polymerization *in* vesicles, although it has been suggested so far that hollow polymer particles were synthesized by polymerization *in* vesicles^{7–10} and

[†] Laboratory of Polymer Chemistry, Eindhoven University of Technology.

[‡] Department of Pathology, Electron Microscopy, University of Limburg.

[§] Laboratory of Chemical Reactor Engineering and Process Development, Eindhoven University of Technology.

^{||} TNO Eindhoven.

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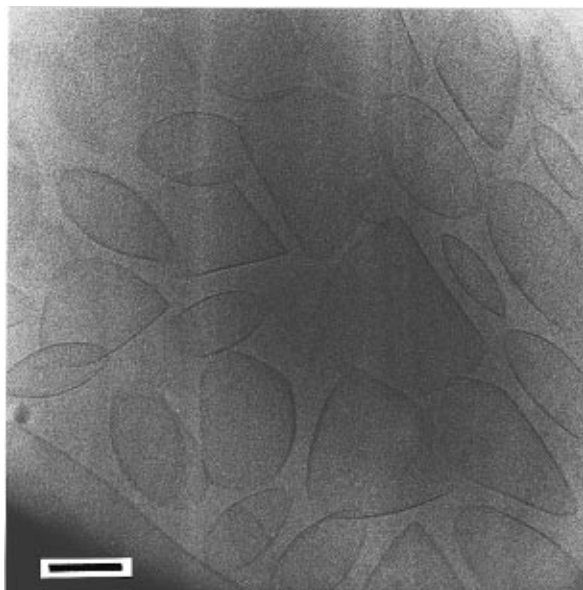


Figure 1. Cryo-TEM micrograph of a vesicle population obtained by extrusion of 10 mM DODAB. Ellipsoidal and lenslike geometries are dominating. The strong angularities are striking. The scale bar corresponds to 100 nm.

“no evidence of a separation of polymer from the vesicle system” was observed.⁷ Our present cryo-electron microscopy study clearly demonstrates that, at least under the present experimental conditions, interesting phase-separation phenomena occur within the vesicles during the course of the polymerization. Parachute-like structures are obtained, in which a single polymer particle is confined to the vesicle bilayer. To the best of our knowledge, these hybrid vesicle–polymer architectures have never been observed before.

Materials and Methods

Unilamellar vesicles with a number average diameter of 160 nm (dynamic light scattering, Malvern 4700) were prepared by extrusion of a dispersion of 10 mM dioctadecyldimethylammonium bromide (DODAB, Fluka) through three stacked 200 nm Millipore polycarbonate filters at 60 °C in three passes (pressure 5 bar). A highly turbid dispersion resulted from this procedure. Styrene (20 mM) and the photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DMPA, Ciba-Geigy) were added to the vesicle suspension. After stirring overnight at room temperature, monomer and initiator were readily absorbed. Photoinitiated polymerizations were performed at 25 °C using a pulsed excimer laser (XeF, 351 nm) at 2 Hz pulse frequency and 30 mJ energy per pulse. Dispersions of the polymeric products were colloidally stable for at least 6 months. Molecular weights of the polymer were determined by size exclusion chromatography to $M_w = 35\,000$ Da. Conversion was determined by HPLC analysis of the residual monomer.

Details concerning the use of cryo-electron microscopy have been described earlier.¹⁸

Results and Discussion

Figure 1 shows a typical cryo-electron micrograph of the vesicle population obtained by extrusion. A great variety of structures can be observed: ellipsoidal and lens-shaped geometries are dominating. Although such non-ideal vesicle morphologies have been reported earlier for DODAB¹⁹ and other synthetic surfactants (e.g. dihexadecyl phosphate²⁰), the origin of the angularities and strong

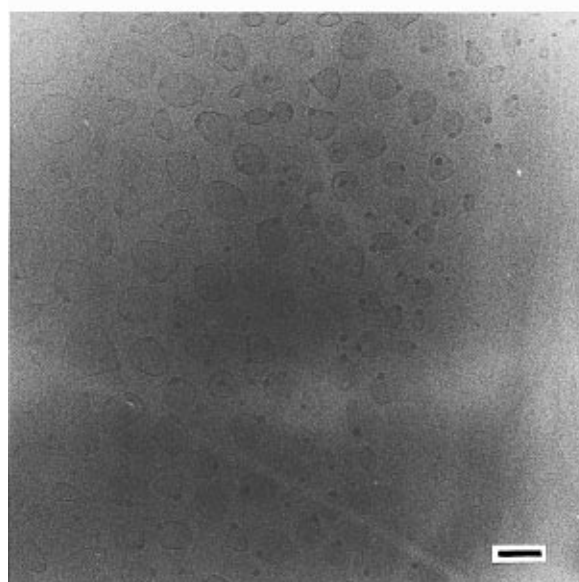
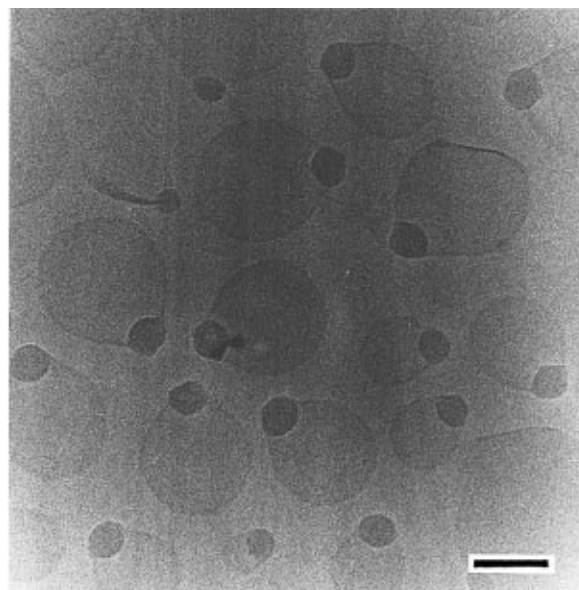


Figure 2. Cryo-TEM micrographs after polymerization of styrene in extruded DODAB vesicles. Phase separation occurs and gives rise to parachute-like structures. Small polymer spheres are linked to vesicle bilayers. A lower magnification (part b; bottom) shows all possible projections of the parachutes: from a side view a filled circle is linked to a cone; from a bird's-eye view a filled circle is enclosed by a larger circle or an ellipse, dependent on the orientation of the hybrid structures. The scale bar corresponds to 100 nm in part a (top) and to 200 nm in part b (bottom).

irregularities in the vesicle bilayer is still not well understood.¹⁹

After polymerization, however, exciting new structures dominate the micrographs (Figure 2a and b): small solid spheres are linked to bilayer structures, typically one sphere per vesicle, to form parachute-like geometries, where the bilayer resembles the unfolded parachute and the polymer sphere resembles its cargo. Especially, Figure 2b gives a view of all possible projections of three-dimensional perspectives of the parachutes.

When analyzing a representative series of micrographs at various magnifications, the following interesting main characteristics are found: (i) nearly all observed para-

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chutes contain only one polymer particle; (ii) the geometries of the bilayer structures seem less diverse than in the initial dispersion (parachutes are dominating, and only very few lenses or comet-like structures are present); (iii) smaller bilayer structures contain smaller polymer particles.

Clearly, a large number of polymer chains (approximately 1000 chains in a typical polymer particle of 50 nm diameter) are concentrated at one point in the vesicle bilayer. One could imagine at least two mechanisms that lead to such a polymer concentration:

First, in the beginning initiation may occur at one particular site in the vesicle bilayer. The growing polymer chain attracts more monomer, as the monomer is probably better soluble in its polymer than in the vesicle bilayer. As a consequence, a microenvironment develops where the propagation preferentially takes place due to an increased local monomer concentration.

The second mechanism assumes that polymerization starts all over in the vesicle bilayer and polymer chains are thus equally distributed over the bilayer. Subsequently, due to unfavorable polymer-surfactant interactions and restricted conformational freedom, the polymer chains migrate to one point where they coalesce into a spherical particle, reducing surface contact area and thus surface free energy.

The present results do not yet permit us to decide whether the polymer concentration is caused by the mechanism of monomer diffusion, by the mechanism of polymer diffusion, or by a combination of both. At first sight, it seems improbable that polymer diffusion leads to the observed phenomena as macromolecules¹¹ diffuse orders of magnitude (10^2 to 10^4 times) slower than small solutes²¹ ($D \approx 10^{-12}$ m²/s) within bilayers. Therefore, complete reorganization of the macromolecules within the bilayer is improbable during the time required for the growth of a single chain (≈ 1 s). Nevertheless, reorganization can occur on the time scale of polymerization (1 h) and certainly on the time scale of storage between synthesis and cryo-EM (i.e. several days).

Time-resolved cryo-EM measurements could help in discriminating between the monomer and polymer diffusion mechanisms.

A second interesting point of discussion is the nature of the colloidal stabilization of the growing polymer particle. The choice of the photoinitiator DMPA implies that the polymer particle cannot be stabilized in water by end groups stemming from initiator fragments. Consequently, another type of stabilization must be active, and one is inclined to ascribe a stabilizing effect to the bilayer-forming amphiphile. Due to the extreme low solubility of the amphiphile in water ($<10^{-15}$ M)²² and the low amphiphile exchange rate ($\approx 10^{-8}$ s⁻¹)¹ it becomes unlikely that amphiphiles are migrating from other vesicles toward the growing polymer surface in order to stabilize it. Hence, the total surface of one vesicle is fixed by the number of amphiphiles in the membrane and all stabilization of the newly created polymer occurs at the expense of its "own parental" vesicle. Again, two mechanisms could lead to polymer stabilization:

One could simply assume that the polymer particle develops within the midplane of the surfactant bilayer, thus splitting the two halves. The newly created polymer surface consumes the neighboring surfactant molecules for stabilization and induces a higher curvature of the

two bilayer halves that enclose the polymer particle, as can be seen from the micrographs (see Figure 2).

Another mechanism could be as follows: a polymer chain starts to grow within the midplane of the surfactant bilayer because the monomer is concentrated in the midplane.²³ After reaching a certain critical chain length, the chain becomes too long to be solubilized in the midplane and, as a consequence, the chain starts to orient parallel to the amphiphiles.²³ As the chain is still growing, the macromolecule is forced to leave the bilayer and is exposed to the surrounding water where the polymer is precipitated on the vesicle surface. Neighboring amphiphile molecules may then adsorb gradually on the polymer surface. Preferentially, the polymer chains will leave the bilayer via the inner half where the amphiphile density is lower,²⁴ so that the precipitation might occur in the inner compartment of the vesicle. The necessary polymer stabilization would thus consume more amphiphile molecules from the inner bilayer, half leading to an impoverishment of amphiphiles and finally to an increased asymmetry and curvature. The flip-flop mechanism of lipid bilayers will be far too slow to re-equilibrate a rising asymmetry between the bilayers, particularly below the phase transition temperature of the DODAB vesicles¹⁻³ at $T_m = 47$ °C.²⁵

Whatever the mechanism is, from the present results we are not yet able to conclude whether the polymer grows within a stabilized surrounding (i.e. inside the bilayer) or whether it creates its own stabilization after being exposed to the aqueous phase. Nevertheless, it seems highly probable that the mechanism of polymer stabilization and the peculiar parachute geometry are causally related.

A last observation that should be commented on is the size of the polystyrene spheres, which varies with the size of the parental vesicle. Apparently, only the monomer initially solubilized in the bilayer is polymerized and no monomer transport from other vesicles occurs. Such a behavior resembles the mechanism of miniemulsion polymerization²⁶ or the synthesis of microgels by microemulsion polymerization with complex amphiphiles,²⁷ where the monomer is expected to remain in its original droplet during polymerization, since monomer diffusion is hindered by a stiffer, less penetrable particle surface.

Conclusion

Summarizing, polymerization in DODAB vesicles leads to completely new vesicle-polymer hybrid structures. The findings not only contrast with earlier observations on this topic but also represent an interesting example of amphiphile-macromolecule interactions. While in natural systems the macromolecules (i.e. proteins) are always composed of hydrophobic parts that are solubilized by the membrane and hydrophilic parts that support the stabilization of the molecule,¹¹ in the present case we encounter a situation where an "unstabilized" polymer is integrated in the vesicle bilayer or "attached" to the vesicle. With respect to the initially discussed problem of polymerization in lamellar phases, these findings represent a unique case: although phase separation takes place, although the polymer leaves its template, and although a tendency to build a polymer latex can be observed—a

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final separation of polymer and vesicle does not occur. At this moment the precise mechanisms are still rather speculative, but the observation that *each* vesicle contains *one* polymer particle makes it highly likely that the compartmentalization of monomer together with a hindered intervesicular exchange of monomer and amphiphile can prevent a disproportional growth of polymer in only a small fraction of the vesicles at the expense of the others. The low solubility of DODAB and the high rigidity of DODAB bilayers—perhaps even the nonideal vesicle morphology observed after extrusion (Figure 1)—are considered to play central roles in this process. Currently,

investigations are underway to unravel the relevant mechanisms and to determine the scope of the present findings.

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