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Feature Article

Block copolymer vesicles—using concepts from polymer chemistry to mimic biomembranes

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Abstract

Owing to the increasing interest in self-assembled structures from block copolymer materials, we present here a review of recent literature concerning amphiphilic block copolymer vesicles. A vesicular morphology is applicable not only in such fields like delivery–release and biomineralization, but also has been utilized for preparation of nanoreactors and incorporation of biological macromolecules.

The organization of this paper is the following: we first provide the readers with the overview of the current literature concerning the vesicle preparation and most commonly used experimental methods applied for vesicle investigations. Next, we consider the vesicle formation in more detail and present the morphologies resulting from the interplay of factors influencing vesicular structures in solution. Further, membrane properties will be reviewed, and finally, we wish to focus on our group's achievements in studying nanocontainers from both ABA and ABC amphiphilic block copolymers.

Keywords: Amphiphilic block copolymers; Vesicles; Polymersomes

1. Introduction

Since the first definition of vesicular morphology in lipid systems [1], these colloidal assemblies have attracted attention in the fields of biology, chemistry and physics and found numerous practical applications in various branches of technology and industry. The most interesting examples could be delivery–release of various substances [2] (drugs and cosmetically active substances are especially interesting in this context), biomineralization [3], and reconstitution of biological molecules [4]. Presently, vesicles can be prepared from such compounds as phospholipids [5], surfactants [6] and block copolymers [2,7–13]. Their common feature is the presence of both a

Abbreviations: PS, polystyrene; PAA, poly(acrylic acid); THF, tetrahydrofurane; DMF, dimethylformamide; TEM, transmission electron microscopy; ITO, indium–tin–oxide; PDMS, poly(dimethylsiloxane); PEO, poly(ethylene oxide); PBD, polybutadiene; CWC, critical water concentration; P4VPMeI, poly(4-vinylpyridinum methyl iodide); SBT, polystyrene-*b*-polybutadiene-*b*-poly(*tert*-butyl methacrylate); st, polystyrene-*b*-poly(*tert*-butyl methacrylate); PI, polyisoprene; P4VPDecI, poly(4-vinylpyridinum decyl iodide); PMOXA, poly(methyloxazoline); PBO, poly (butylene oxide); DLS, dynamic light scattering; SLS, static light scattering SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering; SANS, small-angle neutron scattering PBA, poly(butyl acrylate); DIC, differential interference contrast; PEG, poly(ethylene glycol); TIRFM, total internal reflection fluorescence microscopy; LSCM, laser scanning confocal microscopy; PDLLA, poly(DL-lactide); PBS, phosphate buffer saline; SFM, scanning force microscopy; AFM, atomic force microscopy; STM, scanning tunelling microscopy; P2VPM, poly(2-vinylpyridinum methyl bromide); PEE, poly(ethylethylene); UV, ultraviolet; VIS, visible; FTIR, Fourier transform infra red; PCEMA, poly(2-cinnamoylethyl methacrylate); PFOA, perfluorooctanoic acid; DSC, differential scanning calorimetry; ADE, area-difference-elasticity (model); HX, hexanes; BSA, bovine serum albumin; SRA1, scavenger receptor A1; poly-G, poly(guanylic acid); BLM, black lipid membrane; OmpF, Matrix porin outer membrane protein F; LamB, maltoporin; DNA, deoxyrybonucleic acid; PGA, poly(L-glutamic acid); TMB, 3,3′-5,5′-tetramethyl benzidine.

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hydrophobic and a hydrophilic part in the molecular structure of the aggregate-forming compound. This structural specificity allows for aggregation of the hydrophobic fragments in a selective non-polar solvent, whereas the hydrophilic groups will have high affinity to the polar (aqueous) medium.

For a number of reasons, amphiphilic block copolymers have recently emerged from this group as a special class of material. First, there exists a plethora of possibilities of creating such polymers, in the context of both block composition and block length, thus offering the potential to engineer the most suitable polymers for certain applications. Secondly, as macromolecules, some of such polymers are very well suited to mimic biological macromolecular amphiphiles and therefore are subject to studies as a complementary component in various biocomposite materials [14].

In solution, structures from amphiphilic block copolymers undergo multiple transitions and may be present in various morphologies, such as micelles, rods, vesicles or larger aggregates. A number of factors, including both the structure of the amphiphile (chemical constitution and the relative lengths of the individual blocks) and the properties of the solution, such as concentration, pH, temperature and solvent can control the sizes and shapes of such aggregates.

Vesicles are hollow, lamellar spherical structures (Fig. 1), the dimensions of which range from nanometers to hundreds of micrometers and vary depending on the chemical constitution and size of a polymer, preparation method as well as environmental factors. Similarly, the wall thickness and the fluidity of the vesicular membranes strongly depend on the polymer used and experimental conditions.

Owing to their structural specificity, vesicles are fascinating systems for both theoretical and experimental studies. Many publications have recently been devoted to developing theoretical models of their formation and physicochemical properties. Such models are helpful in predicting the vesicles' behavior, however, they need to be verified by experimental results. From the experimental point of view, vesicles offer multiple application possibilities, and therefore much scientific activity focuses not only on understanding the behavior, but also on optimizing the systems to achieve particular properties, best suited for the foreseen applications.

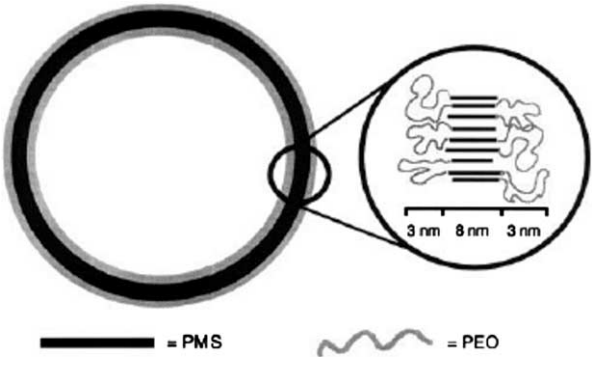

Fig. 1. A schematic representation of a vesicular morphology in a diblock copolymer system (from [9], with permission from The Royal Society of Chemistry).

Due to the hollow sphere morphology, vesicles can mainly be applied for encapsulation of various agents within the vesicle core and their further delivery in both synthetic and living systems. Additionally, vesicles have already been exploited as nanoreactors for controlled processes, which take place within their aqueous core [3]. Since the first observation of vesicular structure with lipids, there have been many studies to test the feasibility of such applications with lipid vesicles (liposomes). We wish to emphasize here that lipids are biocompatible, naturally occurring compounds and seem to be ideally suited for investigation in biological systems. However, lipid vesicles have a very poor stability and high membrane permeability, which are considerable limitations in applied science. In this context, it is important to note that block copolymer vesicles may have enhanced toughness and reduced water permeability [15].

The limitations of lipid vesicles were first addressed by introducing polymer 'scaffolding' for both liposomes and planar lipid membranes, which has a stabilizing effect on the membrane [16,17]. After this approach has proven successful, the next step was to create purely polymeric vesicles and verify their biocompatibility for further applications of the purely artificial (synthetic) systems in biological sciences.

A thorough understanding of vesicle formation and properties is essential for exploring new classes of materials to engineer nanocontainers of controlled structure and size for further applications. Therefore, this paper aims at providing a concise overview of the recent scientific literature on that topic.

2. Experimental preparation methods

All methods reported for liposome preparation are in general also valid for self-assembled vesicular structures of amphiphilic polymers (polymersomes). Preparation methods can be divided in two groups: solvent free techniques and techniques with the aid of organic solvents. In the first group, the amphiphile is brought in contact with the aqueous medium in its dry state and is subsequently hydrated to yield vesicles. This approach offers the advantage that no organic solvent is present any more in the system, which can be mandatory for certain applications.

In the second group of preparative methods, the block copolymer is first dissolved in an appropriate organic solvent and then mixed with water. The organic phase is subsequently excluded with an appropriate technique. This leads only to virtually solvent-free conditions, since it is not possible to completely remove all solvent. Solvent residues may interfere in biological and galenical applications and

they further fluidize membranes leading to decreased vesicle stabilities and promoted aggregation [18].

Depending on the system, each method can yield varying self-assembled superstructures (micelles, vesicles, tubes). Several factors such as the length and polydispersity of the individual blocks, additives (ions, homopolymers and surfactants), the nature and composition of the solvent, the water content as well as the temperature provide control over the types of self-assemblies produced [19], as discussed further.

Homogenization and the decrease of the size distribution of vesicle dispersion can be achieved through vortexing, freeze–thaw cycles, extrusion and sonication, or a combination of these methods. These steps usually also lead to the decrease of the mean vesicle diameter as well as lamellarity of vesicles [12,20,21].

2.1. Solvent free preparation methods

In the technique of film rehydration (film swelling), first described in 1969 [22], an amphiphile film is produced on a solid surface in the first step. This is achieved by dissolving the polymer in an appropriate solvent or solvent mixture, which is then evaporated by means of a rotary evaporator, high vacuum pump or nitrogen stream. The surfaces used commonly are glass [1,20,23,24] or roughened Teflon® [25,26]. The solvent should give a contact angle with the substrate as small as possible to yield extremely thin and fine layers [27].

In the second step, subsequent addition of aqueous buffer leads to the hydration of the film. The mechanism of the swelling procedure is proposed to be as follows: water permeates through defects in the polymer layers, this process being driven by hydration forces. Thus, the layers are successively inflated to form bulges, which yield vesicles upon separation from the surface [28]. The swelling process can be influenced by agitation: gentle methods as stirring and vortexing to vigorous techniques like turraxing or sonication are in use. The film rehydration method is reported to give rather small multilamellar vesicles with a broad size distribution.

The method of solid rehydration (bulk swelling) in a way resembles the film rehydration method; the only difference being that the amphiphile is not hydrated as a thin film on a surface but is directly hydrated as bulk powder. Therefore, longer or more vigorous agitation to completely hydrate the polymer is required [1,21,23,29,30].

Electroformation is the most suitable technique to achieve homogenous unilamellar giant polymersomes with diameters above one micrometer [10,21,26] (Fig. 2). This method is akin to the film rehydration method, however, instead of using a solid surface, the amphiphile film is spread on a pair of electrodes, made of either indium–tin–oxide (ITO) coated glass plates [31], platinum wires [32,33] or gold wires [3]. Instead of spreading a film, ITO coated glass plates can also be micro-patterned using poly(dimethylsiloxane) (PDMS) stamps [34]. After addition of a buffer, electric current (either AC or DC) is applied to facilitate hydration. The electric field affects the vesicle formation by decreasing membrane tension (and therefore leading to the increased number of defects in the layers), by inducing periodic motions (mechanical stress) through electroosmotic effects (only AC) and by increasing interlayer repulsion through electroviscous/electrostatic effects (mainly in the case of charged amphiphiles) [35].

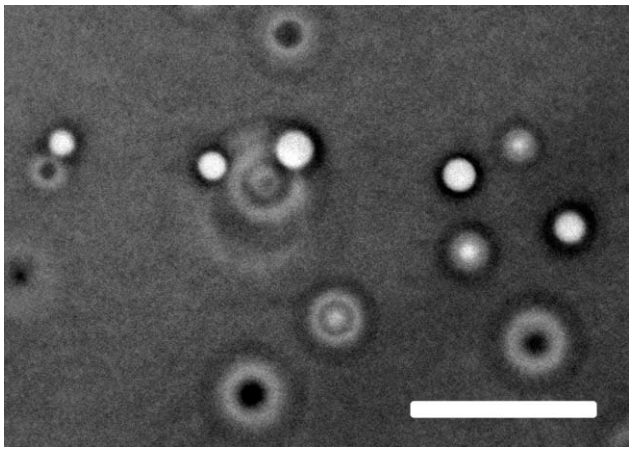

Fig. 2. Giant vesicles from PMOXA–PDMS–PMOXA prepared by electroformation with Au-plates, phase contrast microscopy image (scale bar 50 μm).

Diameters in the micrometer range and excellent monodispersity achieved by this method compete with low yields with respect to self-assembled particles as well as total volume attainable.

2.2. Solvent displacement techniques

Via the solvent injection method, the amphiphile is dissolved in an appropriate organic solvent or solvent mixture which is than added dropwise to an aqueous buffer under vigorous stirring [36]. This leads to the dispersion of vesicles of a rather broad size distribution. The polydispersity can be reduced by repeated extrusion [4]. This very fast and convenient method yields homogenous vesicles, however, the drawback is that the organic solvent is still contained in the vesicles and the surrounding liquid.

Alternatively, the amphiphile is dissolved in an organic solvent and mixed with an aqueous phase (this method is known as solvent evaporation (reverse-phase evaporation). The resulting two-phase system is agitated (e.g. by sonication) until the mixture becomes a homogenous dispersion. The organic solvent is then removed at room temperature under reduced pressure. To further exclude residual solvent, the vesicle suspension is dialyzed, centrifuged or ultrafiltered. This fast and easy technique can be used to prepare small multilamellar liposomes [37] as well as giant unilamellar lipid vesicles [38]. Application of this method to achieve polymersomes has not yet been

reported. In general, if solvent dialysis is applied, the amphiphile is dissolved in an organic solvent and added to an aqueous phase under vigorous agitation to yield a homogenous dispersion; the mixture is then dialysed against pure buffer. During dialysis, the concentration of organic solvent decreases so that vesicles form under conditions where their internal contents are continuously hyperosmotic [39,40]. The vesicle sizes and size distribution are reported to be smaller when a water-miscible solvent is used instead of a water-immiscible one [18]. Using this method, vesicles can be made in large numbers, however, the population is very heterogeneous [18]. In a modification of this method, ultrafiltration is applied instead of dialysis [9].

In another, similar method, known as detergent dialysis, a detergent is used to solubilize an amphiphile in a buffer. The detergent is subsequently removed by controlled dialysis [41].

3. Characterization techniques

In this section, we would like to present shortly several techniques, which are most commonly used in studies of vesicles. Owing to space limitations, we do not aim to characterize each technique in detail, and therefore we rather concentrate on physicochemical properties that can be learned from those measurements. The techniques reviewed here are compatible with those generally applied in colloid science and most importantly involve light scattering and various microscopies, however, other methods have also been applied and will be mentioned here.

Studies of mechanical properties of vesicles are described further (Section 6), as well as techniques applied for such investigations.

3.1. Scattering methods

The main tool for studying aggregation in solutions has long been turbidity measurements, practically realized in the form of static and dynamic light scattering experiments with the use of sophisticated instrumentation.

Laser light scattering is able to probe aggregates in the size range of 1–1000 nm [42]. After the beam of laser light passes through a polymer solution contained in an optically clear, cylindrical probe cell, most of the light will pass through the sample, but a small portion will be scattered. The intensity of the scattered light is next measured.

In dynamic light scattering (DLS), fluctuations of the intensity the scattered light in the microsecond timescale appear because of diffusive motions of particles in solution. From static light scattering (SLS), structural properties are available, such as weight averaged molecular weight $\langle M \rangle_w$, particle shape and size (via a particle scattering factor, P(q), formulas are available for many shapes [43]), and particle–particle as well as particle–solvent interactions via the second viral coefficient).

Laser light scattering has been, for example, employed in studies of ABA triblock copolymer vesicles from PMOXA–PDMS–PMOXA [4] to find the size polydispersity of vesicles, effect of intravesicular polymerization, critical aggregation concentration and vesicular morphology. In investigation of nanocapsule responsiveness [44], this method yielded information concerning the vesicular size with variations of pH, whereas in studies of block copolymers possessing a peptide sequence as a hydrophilic block [30] the hydrodynamic radius of particles was obtained, which depended strongly on the conformation of the poly(L-glutamate) segment.

High throughput scattering methods, such as combinatorial small-angle X-ray scattering (SAXS)/wide-angle X-ray scattering (WAXS), which provide information about structural features of colloidal size, have been used to study phase behavior over a concentration gradient of block copolymers in water [45]. The small-angle neutron scattering (SANS) technique is unique for studies of chain conformations and interaction parameters in the one-phase region. It allows investigations of the morphology and thermodynamics of polymer blends and copolymers. Additionally, structure and self-assembly of block copolymer, and control of drug encapsulation by multilamellar vesicles can be investigated [46]. Morphological and spatial segmental distribution in block copolymer–homopolymer mixture during vesicle formation was also studied with SAXS and SANS [47].

3.2. Microscopy

A very powerful method to investigate polymersomes is direct visualization by microscopy. Many important aspects like size, morphology or homogeneity can instantly be revealed. Most microscopy techniques are fast, easy and provide relatively straightforward specimen visualization. Some techniques, however, require a more complex data analysis.

Photons and electrons can serve as light source both featuring specific benefits and drawbacks. Light microscopy allows investigating vesicles under physiological conditions but is restricted due to limited resolution. Electron microscopy yields highly resolved images but specimens need to be dried, stained, sputtered or frozen. However, most microscopy techniques are highly suitable for polymersome analysis and frequently used as standard techniques.

3.2.1. Optical microscopy techniques

Optical microscopy offers the possibility to directly visualize polymeric vesicles under ‘physiological’ conditions. It is not necessary to dry or stain specimens; instead, they can be kept in aqueous buffer. The major drawback of light microscopy compared to electron microscopy is the limited resolution, due to which it is mandatory to have appropriate polymersome specimens of large size: giant

vesicles with diameters above one micron are best suited for such studies.

Transmission light microscopy. Although optical systems found in modern microscopes may be capable of producing high-resolution images at high magnifications, such a capability is worthless without sufficient contrast in the image. Contrast is not an inherent property of the specimen, but is dependent upon interaction of the specimen with light and the efficiency of the optical system used. Polymersomes neither absorb light nor seems staining with chemical dyes achievable. Therefore, contrast is so poor that the specimen remains essentially invisible and contrast has to be enhanced using other techniques.

Phase contrast microscopy. This contrast-enhancing optical technique was first described in 1934 by Zernike [48]. Phase contrast is ideal for thin, unstained objects, which barely exhibit any light absorption in the visible part of the spectrum. However very small differences exist between the refractive indices of the vesicles in the specimen and the surrounding aqueous solutions, of which the use is made to visualize differences in image contrast.

This technique provides an excellent method of improving contrast in unstained biological specimens without significant loss in resolution. For that reason, it is widely utilized to examine dynamic events in living cells. An example of phase contrast imaging in vesicular systems is provided in Fig. 3.

Direct visualization of polymeric aggregates, providing information on structural details [49] and the kinetics of transition between different aggregate morphologies [50] is possible in the micrometer regime. In addition, dynamics of morphological transformations can be continuously recorded [25].

Differential interference contrast. Through the mechanism quite different from phase contrast, differential interference contrast (DIC) converts gradients in specimen optical path length into amplitude differences, which can be visualized as improved contrast in the resulting image. Images produced in differential interference contrast microscopy have a distinctive shadow-cast relief-like appearance.

The optical components required for differential interference contrast microscopy do not obstruct the objective and condenser apertures as in phase contrast, thus enabling the instrument to be employed at full numerical aperture. The result is a dramatic improvement in resolution, elimination of halo artifacts and the ability to produce excellent images with relatively thick specimens [51].

The method is excellently suited for thick, non-stained specimens, as presented in Fig. 4. It is often employed in combination with fluorescence microscopy to reveal the cellular morphologies associated with fluorescent regions.

3.2.2. Fluorescence microscopy

Wide-field epifluorescence microscopy. In fluorescence microscopy, the excitation light irradiates a specimen and then the red-shifted emitted fluorescent light is separated from the brighter excitation light. Thus, unlike transmission light microscopy, only the emitted light from the specimen reaches the detector, allowing for sufficient contrast.

There are several important advantages of epifluorescence over transmission microscopy techniques:

(i) specific labeling with fluorochromes gives the ability to distinguish between non-fluorescing materials;

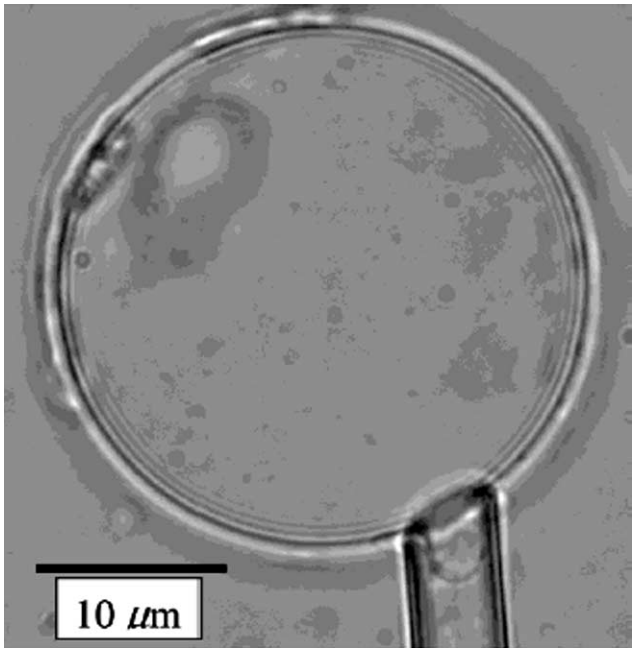

Fig. 3. Vesicles from PBA–PAA (70–30) in 1 wt% aqueous solution in THF; scale bar: 10 μm (from [23], with permission from ACS Publications Division).

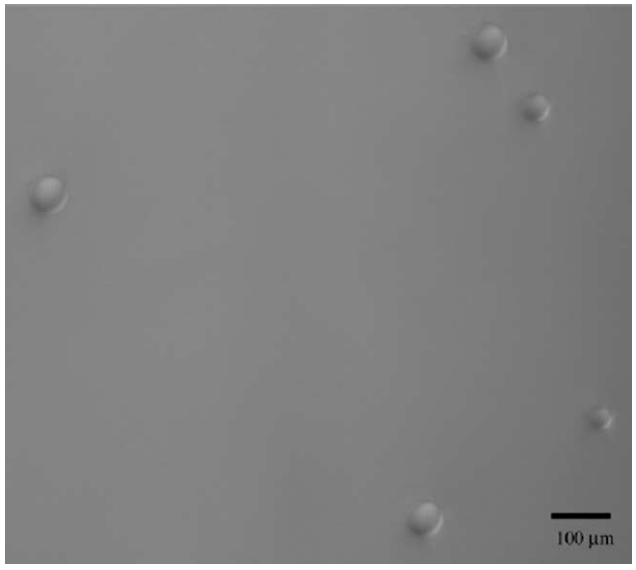

Fig. 4. A differential interference contrast micrograph of giant vesicles formed by PMOXA–PDMS–PMOXA triblock copolymer; scale bar: 100 μm (from [52], with permission from Elsevier B.V.).

(ii) multiple staining with different probes allows visualizing the presence of individual target molecules;
(iii) fluorescence microscopy reveals the presence of fluorescing material with exquisite sensitivity—as few as fifty fluorescent molecules per ml are sufficient to be detected.

Although a fluorescence microscope cannot provide resolution below the diffraction limit, the presence of fluorescing molecules below such limits is made visible [53].

Besides visualization of steady state structures (Fig. 5), fluorescence microscopy permits to study dynamic processes of macromolecules such as diffusion, binding constants, enzymatic reaction rates and a variety of reaction mechanisms in time-resolved measurements. In biological studies, fluorescent probes have been employed to monitor intracellular pH [54], local concentrations of important ions [55] and important cellular functions (endocytosis, exocytosis, signal transduction, and transmembrane potential generation) [56,57]. Such investigations provide an excellent basis for extending them further to synthetic systems, such as polymer vesicles.

Fluorescent labeling of polymeric vesicles can be achieved through different approaches. In most cases, the amphiphilic polymers used do not exhibit intrinsic fluorescence and therefore a dye needs to be encapsulated, or the vesicle membrane has to be stained. The first method features encapsulation of a water-soluble fluorescent dye during vesicle formation followed by a subsequent exclusion of the dye in the extracellular space (e.g. by size exclusion chromatography, dialysis, ultrafiltration, or centrifugation). To stain the membrane either a certain percentage of the membrane molecules are covalently linked to a fluorophore, or a lipophilic probe is aggregated in the hydrophobic part of the membrane [58].

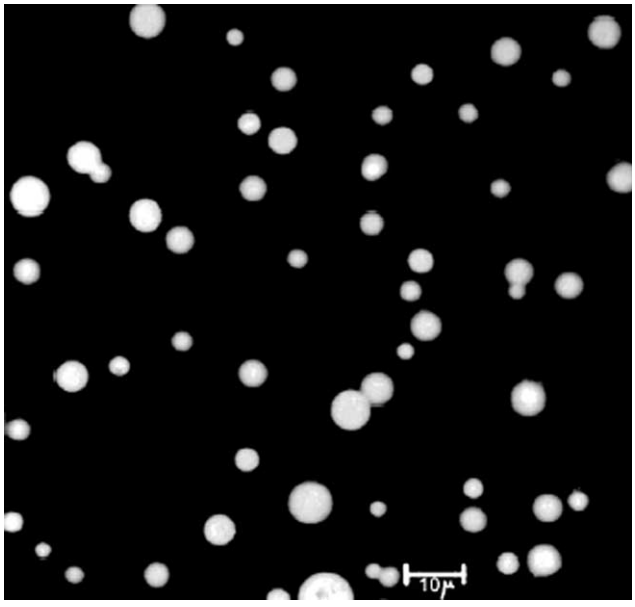

Fig. 5. Polymersomes from oligoanhydrides–PEG block copolymer under fluorescence microscope; scale bar: 10 μm (from [2], with permission from Elsevier B.V.).

Total internal reflection fluorescence microscopy. The analysis of images obtained with conventional wide-field fluorescence excitation is often obscured by background fluorescence emitted in out-of-focus planes. One solution to overcome this problem and to increase resolution in z plane is fluorescence excited by total internal reflection.

The principle of Total Internal Reflection Fluorescence Microscopy (TIRFM) is the refractive behavior of light upon the transition from an optically denser to an optically less dense medium. Such a transition produces a critical angle for total reflection, when a standing evanescent wave penetrates into the medium with lower refractive index. The intensity of this wave decays exponentially as distance from the interface increases. The depth of penetration depends on the angle, wavelength, and the ratio of refractive indices, which means that fluorophores further away from the interface are not excited. Background fluorescence is dramatically reduced, image contrast improved, and resolution significantly increased, to 200 nm or less [59].

TIRFM is also a useful tool for studying the reactions of individual molecules or objects adsorbed, adhered or bound to surfaces. Typical applications are membrane fusion of vesicles [60], conformational and orientation changes [61] and lateral mobility of molecules [62].

Confocal fluorescence microscopy. Two fundamentally different techniques are used in today's confocal microscopes. In Laser Scanning Confocal Microscopy (LSCM), optical slices of the specimen are imaged with high contrast and high resolution in x, y and z [63]. The maximum diffraction-limited resolution obtainable is enhanced by a factor of $\sqrt{2}$ compared to conventional light microscopy [64].

In contrast, spinning disk confocal microscopes offer the ability to readily capture images (up to 100 frames per second). Further arc-discharge lamps reduce specimen damage and enhance the detection of low fluorescence levels during real time image collection [65].

Besides high lateral resolution, contrast is dramatically improved over wide-field techniques due to the reduction in background fluorescence and improved signal-to-noise [66]. By moving the focus plane, optical slices can be put together to build up a three dimensional stack that can be digitally processed afterwards. LSCM offers the ability to adjust magnification electronically by varying the area scanned by the laser without having to change objectives. Its disadvantages are the limited number of excitation wavelengths available with common lasers (referred to as laser lines), which occur over very narrow bands and are expensive to produce in the ultraviolet region.

In confocal microscopy, the advantages of epifluorescence for vesicle investigation (e.g. exquisite sensitivity) are further improved. Optical slices with virtually no background fluorescence and enhanced resolution in x, y and z

are achievable. These slices can be further computed to a 3-D stack to give an even better impression of the vesicle morphology. Besides visualization of steady state structures, the study of dynamic processes is possible.

Confocal microscopy is therefore an extremely powerful technique for studying vesicles (example images are presented in Fig. 6), however, relatively high equipment costs limit its application as a standard tool.

3.2.3. Atomic force microscopy (AFM)

The most frequently applied technique for the determination of the topography of structures on solid substrates are scanning force microscopy (SFM) methods [67], practically realized as scanning tunneling microscopy (STM) on conducting substrates and atomic force microscopy (AFM) on both conducting and non-conducting surfaces. Both allow for obtaining precise (with a few Å resolution) images. The AFM principle stems from measuring the interaction force between the most exterior molecular layer of the sample and the tip, which is placed right above and moved with high precision along x and y axes at a given tip height above the surface. When the tip interacts with the surface, it undergoes vertical deflection, proportional to the interaction force with the surface molecules (in the range of 10^{-9}–10^{-10} N). This can be recorded as the swing of a very sensitive tip-integrated spring or the deflection of a laser beam, reflected from the upper part of the tip. In such a way, a surface inhomogeneity profile is obtained almost on the atom scale.

Presently, the AFM technique is the basic tool in laboratories investigating the properties of thin films on solid substrates, but it has also proven useful in studies of polymer vesicles [11,68]. The former paper concerns the polybutadiene-*b*-2-vinylpyridene system from which various structures have been observed on substrates such as graphite and mica (Fig. 7). Preferentially, the dry mica substrate favored the compressed vesicle formation upon the polymer adsorption, the deformation of vesicles being attributed to the drying process before sample imaging.

3.2.4. Transmission electron microscopy (TEM)

A transmission electron microscope was first developed in the 1930's after the effective role that wavelength has on the theoretical resolution became evident. The theoretical resolution of presently used electron microscopes is about hundred thousands times better than that of light microscopes. Additionally, the great advantage the electron microscope offers is about a 1000 fold increase in resolution and a 100 fold increase in depth of field.

There are several disadvantages of electron optics, though. Electrons are high-energy particles, which will easily be affected by any matter they encounter. When they do encounter matter, the interaction results in the emission of all the lower forms of energy, therefore, electrons cannot penetrate a specimen very deeply. In addition, for that reason a microscope has to be kept at a high vacuum. The TEM is ideal for studies in 'synthetic' systems, yet the disadvantage exists for biological samples, where the specimen is always dead. Owing to this fact, environmental scanning electron microscopes and cryo-TEMs are rather used in studying biological systems.

Presently, both TEM and cryo-TEM seem to be the most frequently used visualization methods for studying polymer aggregates in solution. Below, a few comments are presented concerning different TEM techniques.

TEM is the method of choice when studying surface and

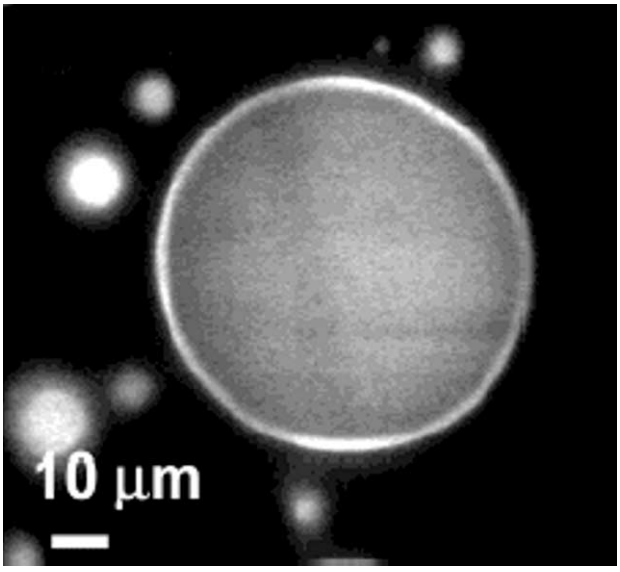

Fig. 6. LSCM images of polymersomes prepared from PEG–PDLLA in the presence of Nile red as a fluorescent probe, scale bar: 10 μm (from [18], with permission from ACS Publications Division).

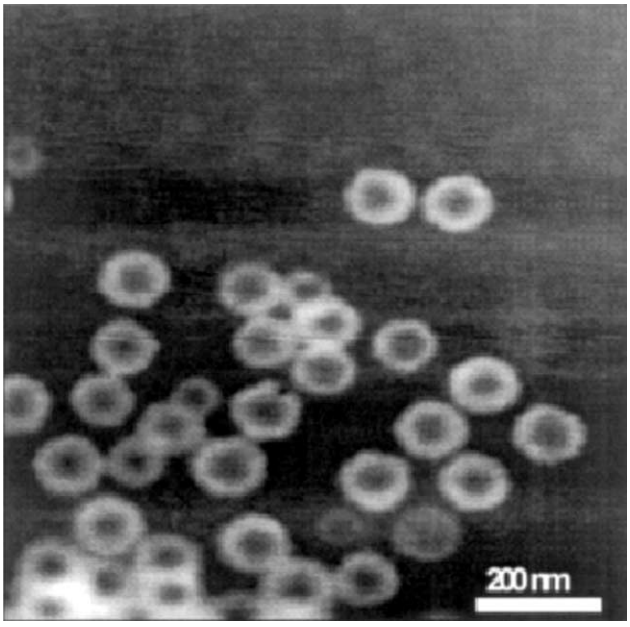

Fig. 7. Scanning force microscopy (SFM) image of $BPD_{210}-P2VPM_{99}$ vesicles on mica surface; scale bar: 200 nm (from [11], with permission from J. Wiley and Sons, Ltd).

sub-surface properties and is particularly advantageous for studying vesicles (Fig. 8). There are a huge variety of TEM techniques, which continue to evolve with the increased knowledge about the studied objects and advances in instrumentation.

Negative staining permits very high resolution imaging of surface details (Fig. 9). A mixture of stain and the suspended particles of interest is allowed to dry, and then the stain accumulates on the particles, which thus appear darker than their surroundings (hence 'negative' staining). However, surface heterogeneities in the particles also accumulate stain and so are revealed in fine detail.

Cryo-TEM offers another advantage: since specimens are frozen and viewed in vitreous ice, they are seen in a natural hydrated state, which is as close to their natural state as possible (Fig. 10). Indeed, when a sample is perfectly frozen, the osmotic effects due to chemical fixation are almost suppressed and dehydration is avoided. The above effects are responsible for aggregation and loss of biological material in classical preparation.

Cryo-TEM additionally allows investigating the phase behavior of macromolecules resulting from self-assembly in water: micellar polymorphism [71], spontaneous formation of vesicles and their transition to lamellar structures are observed [72]. Plane-polarized light microscopy and cryogenic scanning electron microscopy have been also used to characterize multilamellar vesicle structures [73].

Freeze-fracture reveals intra-vesicular details in three dimensions (Fig. 11). Samples are frozen rapidly in liquid nitrogen and fractured to reveal internal structure. The fracture surface is etched under vacuum and rotary shadowed with metal. The resulting replicas contain fine morphological details and have proven to be particularly useful for studies of lipid bilayers. Additionally, lyotropic behavior of amphiphilic ABA triblock copolymers in water has been investigated using polarized light optical microscopy and freeze-fracture TEM [74].

3.3. Other techniques

Depending on their research goals, many groups utilize a broader range of techniques than possible to present in more detail in this paper. Therefore, the interested readers are referred to original papers, whereas just a few examples will be described here.

UV and FTIR spectroscopies have been successfully applied in following the cross-linking of vesicles in the PI-*b*-PCEMA (2-cinnamoylethyl methacrylate) [75,76], based on the fact that absorption from CEMA disappears during the UV irradiation of a vesicle solution.

Fluorescence spectroscopy is normally applied in the studies of controlled encapsulation of materials within the nanocapsules and further release [75,77], as well as for finding the position of particular components in a membrane [69].

The degree of ionization of the corona blocks has been studied in various diblock copolymer systems with ζ potential measurements [78,79]. Such measurements allow for the precise evaluation of the inorganic acid/base influence on the formation and sizes of the resulting vesicles.

The self-assembly of PS-*b*-P4VP (4-vinyl pyridine) in the presence of perfluorooctanoic acid (PFOA) was studied with the use of differential scanning calorimetry in chloroform solutions [80]. From DSC results, conclusions can be drawn concerning the thermal changes in the system, namely, transition between various morphologies and the influence of various additives on the behavior of the system.

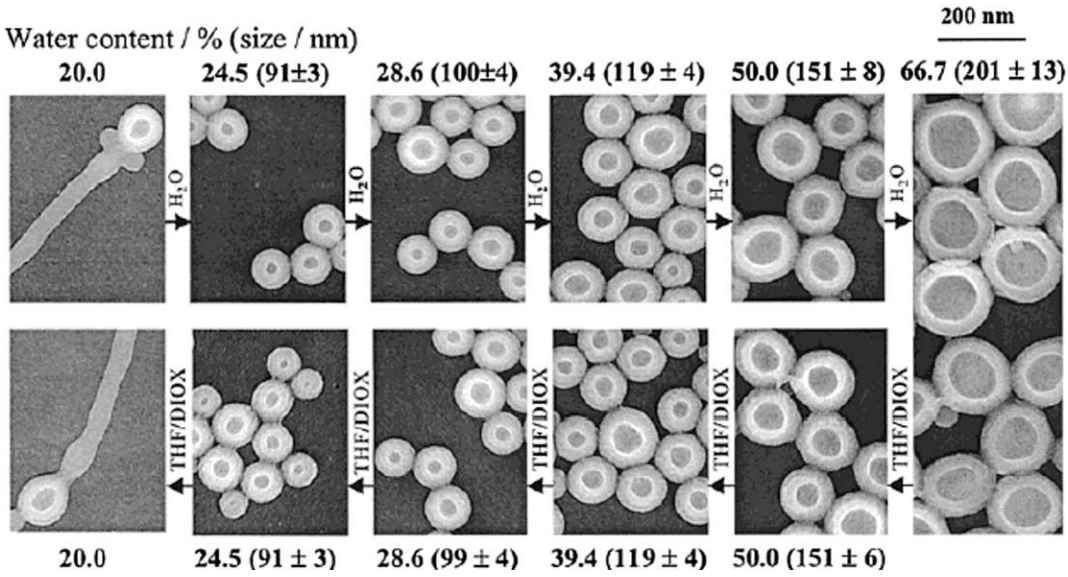

Fig. 8. Reversibility of vesicle sizes in response to changes of water contents for a PS_{300}-*b*-PAA_{44} system in THF/dioxane; scale bar: 200 nm (from [69], with permission from ACS Publications Division).

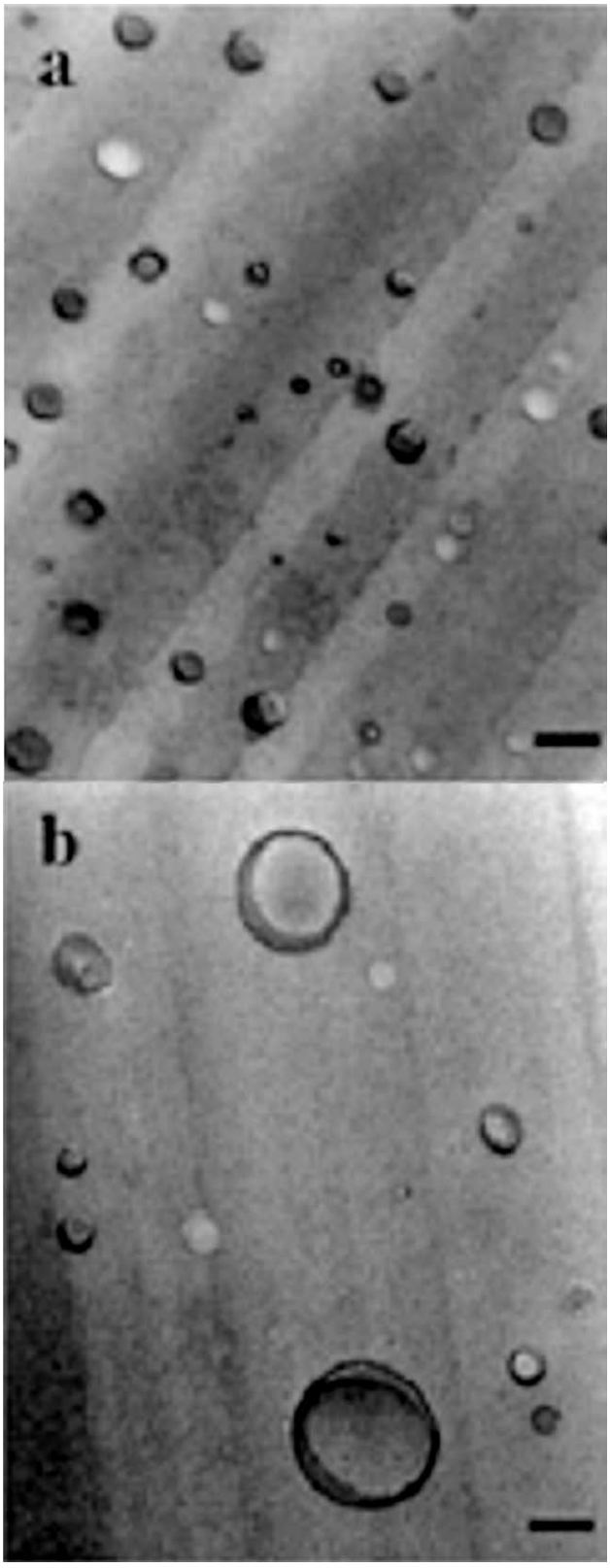

Fig. 9. Effect of the quiescent annealing time on the size and distribution of vesicles in the cured epoxy. The annealing time in solution increasing from (a) 2 to (b) 5 days; scale bar: 200 nm (from [70], with permission from Wiley Periodicals).

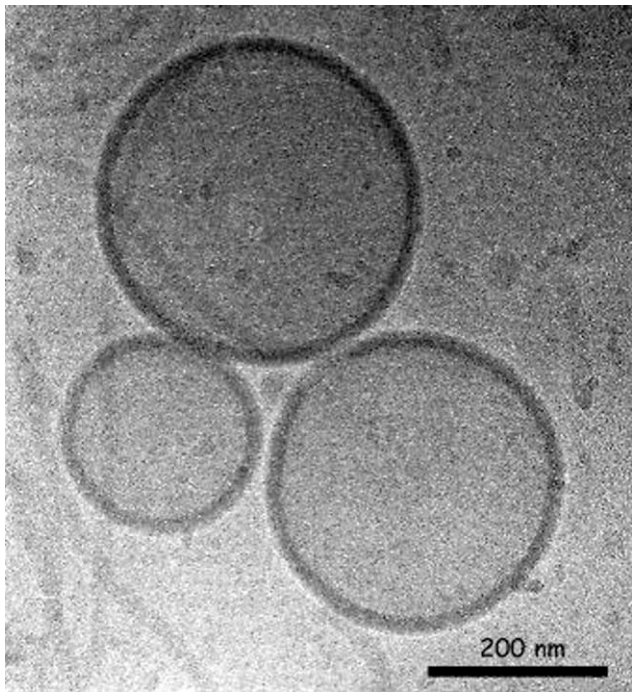

Fig. 10. A cryo-TEM image of PMOXA-*b*-PDMS-*b*-PMOXA vesicles prepared by film swelling in water; scale bar: 200 nm.

4. General aspects of vesicle formation

4.1. Thermodynamic and self-assembly considerations

The formation of nanostructures can essentially be achieved by two methods [81]: either by disintegration of a macroscopic phase of matter or by aggregation of free

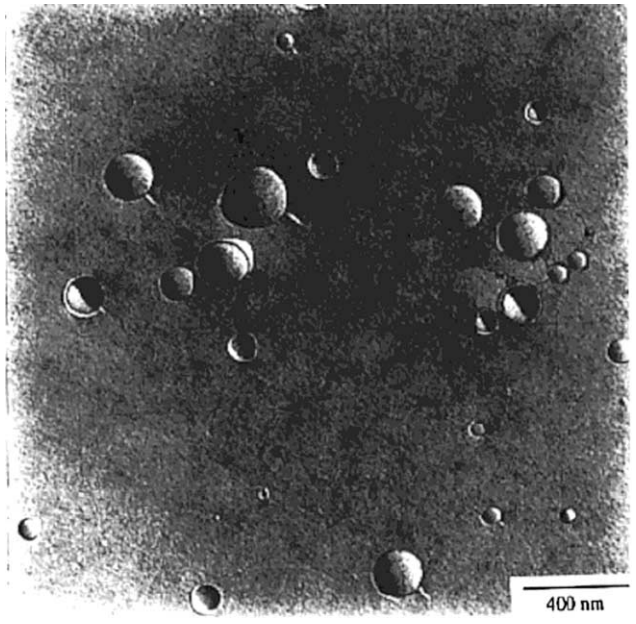

Fig. 11. A freeze-fracture electron micrograph of triblock copolymer vesicles; scale bar: 400 nm (from [4], with permission from ACS Publications Division).

molecules (or ions) into clusters. Both are easily realizable, yet certain prerequisites apply: the first method requires force for breaking interparticle bonds, whereas the second is actuated by a sufficiently high solution concentration, resulting, in turn, in equilibrium and stable structures which are sometimes unattainable by the first method (i.e. micelles, vesicles).

Similarly to lipids, amphiphilic block copolymers aggregate in solution to produce vesicular structures [82]. Even though the stability of lipid and polymer vesicles will inevitably vary due to their extremely different chemical composition, the principle of their formation remains essentially the same: both are held together solely by non-covalent interactions.

Block copolymers comprised of two or more chemically incompatible and dissimilar blocks can microphase separate into a variety of morphologies [83–85]. This self-assembly process [86] is driven by an unfavorable mixing enthalpy and a small mixing entropy, while covalent bonds between the blocks prevent macrophase separation. Depending on the polymers used and their volume fractions, various microphase separation morphologies are formed: spheres, lamellae, inverse spheres and several more complex shapes [87].

In polar media, such as water, the block copolymer macromolecules merge by their non-polar parts to form directly micelles or vesicles. Vesicles may also be found in non-polar media, as a result of mutual attraction of polar groups. Oriented amphiphilic molecules in vesicle membranes are able to move freely in the tangential direction (along the boundary between the polar and non-polar regions of a nanoparticle) and are only restricted in their movement along the normal. Therefore, vesicles can be viewed as liquids (two-dimensionally) and solid bodies (one-dimensionally). Owing to their two-dimensional fluidity multiple non-spherical shapes such as prolate, oblate, nanotubes etc. can be achieved.

In this section we review the publications devoted to understanding the vesiculation process in block copolymer systems based on the principles which have long been known for small molecules (lipids). We include here such issues as membrane curvature, thermodynamics and self-assembly. Additionally, the factors influencing vesicle formation, shape and size will be discussed.

Although a large number of papers have focused on lipid vesicles, not much theoretical work has been presented concerning vesicular assemblies from block copolymers. It has to be admitted here that polymers are far more complex for modeling than small-molecule amphiphiles, and this might be the limitation for computational analyses. On the other hand, with the constant progress in understanding such systems and with huge improvements in computer technologies each year, we believe that this gap will be filled and the computer modeling will become a standard tool for designing polymer aggregates of particular properties.

Döbereiner et al. [88] studied lipid vesicles both experimentally and using the area-difference-elasticity (ADE) model, which allowed to map the phase diagram of the system and find which shapes are stable. Thermal trajectories of vesicles were studied, and the results are considered the first direct confrontation between the vesicle-shape theory and experiment. The principles of the ADE theory will not be discussed here: the readers are referred to [89] and references concerning this model. It is just important to mention here, that good agreement of theory with experimental data was achieved for lipids, which indicates that the model could be appropriate for further extension to macromolecular systems, such as block copolymers (of course, after taking into account the polymer structural specificity). Extension of the above system to membrane interactions with dispersed nanoparticles has been presented in [90]. The membrane curvature changes upon the interactions, depending also on the particle size.

Statistical methods also found applications in membrane modeling, just to mention application of Monte Carlo simulations to pore formation in model (lipid) membranes in [91]. This way, a phase diagram is obtainable including pore shape fluctuations, instead of definite pore radii.

Two reports [92,93] give a detailed theoretical description of the phospholipid vesicle formation in water, including such parameters as membrane thickness, lipid chain packing and membrane asymmetry. For unilamellar vesicles, experimentally found values for energy balance and vesicle radii fit well with the theoretical predictions.

So far, studies of block copolymer vesicles have been limited to experimental papers, and despite of a large number of reports each year, some questions concerning the equilibrium nature of such morphologies have long remained unanswered. Since vesicles are experimentally found to be a part of the broader continuum of various morphologies, it is interesting to understand to which extent they would be stable under given conditions as well as in which direction the morphology would change upon a variation of external conditions.

The first approach to understand the thermodynamics of vesiculation in an A–B diblock copolymer system was given by Wang [94]. This study focuses on the stability of a bilayer membrane upon spherical or saddle-splay deformations, in particular whether and why a curved bilayer would be favored over a flat one. The calculations of free energy per diblock copolymer molecule for a general deformation lead to the conclusion that whenever the composition of the diblocks is sufficiently asymmetric with longer B (hydrophobic) blocks, the constituent monolayers will have a strong tendency to curve away from the aqueous phase. This results in flat bilayer instability with respect to spherical deformation; namely, spherical vesicles become favored over the flat bilayers. In this model, the transition from flat bilayers to spherical vesicles is ‘second order’, depending on the block length ratio. The lower free energy of vesicles as compared to flat bilayers is explained as follows: when each monolayer has a tendency to curve away

from the solvent, this new geometry decreases the free energy of the outer layer, whereas the free energy of the inner membrane increases. When—for the inner leaflet—the number of molecules per unit area decreases, the inner membrane free energy increase can be partly diminished. Moreover, since there are more molecules in the outer monolayer, the free energy decrease in the outer shell will more than compensate the free energy increase in the inner layer.

For example, stability of vesicles was experimentally verified for poly(styrene)-*b*-poly(acrylic acid) (PS-*b*-PAA) in dioxane-tetrahydrofurane (THF)–water or dimethylformamide (DMF)–THF–water [69,95]. It should be remembered, that in such systems as PS, for which the glass transition temperatures are high, vesicles are only obtainable after addition of an organic solvent, which fluidizes the membrane. After water is added to such a system, the aggregates become 'frozen', which, in turn, facilitates their further imaging and characterization.

Depending on external conditions, vesicles could be equilibrium morphology as their size and shape was governed by the water content in the system and could be changed reversibly while the vesicle wall thickness remained unchanged. The thermodynamic stabilization of vesicles resulted from the segregation of the hydrophilic chains of different lengths between the outside and inside of the vesicles.

Such segregation of polymer macromolecules into the inner and outer leaflet of the membrane is even enhanced due to the fact that polymers are generally polydisperse, which means that a given sample contains species of various masses and, consequently, block lengths.

The effect of polydispersity of the hydrophilic block in a PS-*b*-PAA system has been found to decrease the vesicle size with the increase of the polydispersity index of the PAA block [96,97]. (Polydispersity index (PI) is defined as the ratio of weight average molecular weight, M_w to the number average molecular weight, M_n, and is a measure of the width of the molecular weight distribution. Alternatively, polydispersity parameter is generally referred to as $p = (M_w/M_n) - 1$). As described above, segregation of the smaller hydrophilic chains to the inside of the vesicle bilayer takes place, whereas the longer chains would form the outer surface. Interestingly, TEM imaging indicates further that different polymers do not segregate into separate aggregates, namely, that mixing of various molecular mass polymers occurs before the vesiculation. In other words, under the same conditions, individual monodisperse component copolymers yield different vesicular morphologies than observed for their mixtures.

The morphology of block copolymer vesicles is determined by the bending elastic energy of their bilayer membrane. Although a single polymer vesicle is typically not in equilibrium with the bulk, forces acting on the membrane establish a local mechanical equilibrium [25]. Analogously to lipid vesicles, polymer vesicles will assume the shape which corresponds to the minimal elastic energy at a given vesicle volume and area [88]. The vesicle volume remains constant because of the osmotic balance across the membrane, which equilibrates the internal and external solute concentrations. On the other hand, the vesicle area is temperature-dependent due to thermal motions (such effects as change of solubility with temperature may also take place, but will not be considered here).

Apart from geometrical constraints, the most important parameter for determining the vesicle shape is the spontaneous curvature of its membrane [89]. Changes of this parameter, induced e.g. by temperature, control the vesicular morphology and may lead to transitions resulting in several shape classes. Various vesicle morphologies will be discussed in Section 5.

The self-assembly process in block copolymer systems leading to vesicle formation has been concisely reviewed by Antonietti and Förster [98], who consider vesiculation in terms of a bilayer formation, which will next bend (close) to form a vesicle. Classically, the shape of self-assembled structures is determined by the size of the hydrophobic blocks, which further influences the curvature of the hydrophilic–hydrophobic interface. The interface is described by two parameters [99], the mean curvature H and Gaussian curvature, K_G, defined by the two radii of curvature, R_1 and R_2:

$$H = \frac{1}{2}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{1}$$

$$K_G = \frac{1}{R_1 R_2} \tag{2}$$

Apart from forming vesicles, amphiphilic membranes are also capable to curve differently: saddle-shaped deformations arise from the 'frustration' experienced by the two monolayers because of their inability to curve with their natural curvature. Especially interesting in this context are minimal surfaces, whose mean curvature (H) is always zero, and the Gaussian curvature (K_G) is negative, as presented in Fig. 12, yet in this case, morphologies different from vesicles should be expected: saddle-splay deformations generally lead to bicontinuous phases.

According to [100], the interfacial curvature is related to the surfactant packing parameter as follows:

$$\frac{v}{al} = 1 - Hl + \frac{K_G l^2}{3} \tag{3}$$

where the symbols denote: v—hydrophobic volume of the amphiphile, a—the interfacial area of this volume, and l—the chain length normal to the interface. In general, the packing parameter is characteristic for the molecular shape and determines the geometry of the aggregates. For example, if it attains values $\leq 1/3$, spherical structures are formed [101]. As the value of the packing parameter increases, the morphology of the aggregates can change

from spherical to cylindrical, planar, vesicular, and, finally, to inverted spherical. The critical values of the packing parameter, for the transition from one morphology to another, can be easily obtained by using the radius of the aggregate core instead of l in the v/al.

In simple surfactant systems, the interfacial area (a) has been found to depend almost solely on the polar group, and since l can be estimated from modeling and calculations, the surfactant packing parameter has been proven useful for successfully predicting the morphologies of aggregates in such systems.

The surfactant packing parameter of a bilayer is unity, whereas both H and K are zero, and therefore, the v/al parameter value of 1 is realized by adjusting the balance between the size of the hydrophobic block and the interfacial area, a. In block copolymer systems, however, the above parameter will only give an estimate of the morphologies present in the system, yet the actual situation will depend on the intricate balance among a large number of forces [102].

At low concentration, lamellar (sheet-like) aggregates are formed in solution, and after they grow in size, the energy loss owing to surface tension will cause the aggregate closure into the vesicular form. Bending the bilayer disk to a closed shell requires the bending energy, E_{bend}. For a particular disk area, the disk radius will be twice as large as the vesicle radius, and therefore, the balance of line tension and bending energy defines the minimum aggregate number corresponding to the 'minimal vesicle size'. The resulting (minimal) vesicle radius will thus take the following form: $R_V = 2\kappa/\gamma$, where κ and γ are the bending modulus and surface tension, respectively. It is evident from the above formula that vesicles will preferentially appear in a system for which the bilayer bending elasticity is low and the surface tension is high.

In the context of morphological transitions,

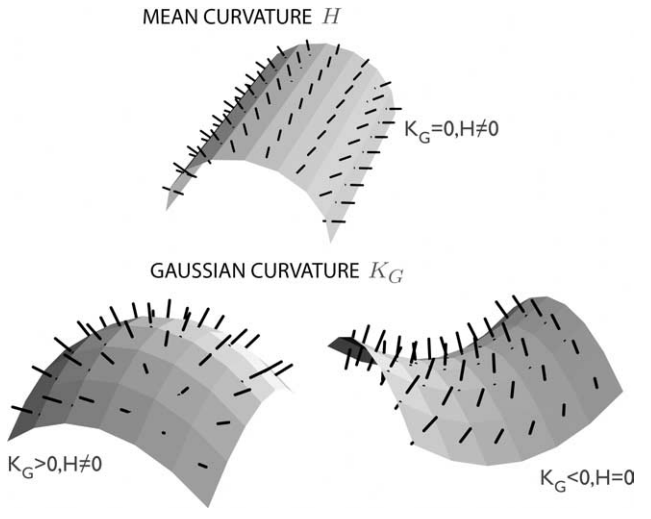

Fig. 12. A scheme of membrane curvatures, explanations in text (with permission from Dr Brian BiDonna, University of Minnesota, Minneapolis, USA).

thermodynamics of vesicle formation is considered in the PS-*b*-PAA system in dioxane–water [103]. After resolving the phase diagrams in this system, vesicular morphology is found as resulting from rods upon the addition of water to the system. Thus, the free energy of transferring one mol of single chains from rods to vesicles (ΔG_{RV}) and the equilibrium constant for the transition from rods to vesicles (K_{RV}) with an aggregation number of N_V can be roughly estimated as:

$$\Delta G_{RV} = -\frac{RT}{N_V}\ln K_{RV} \approx \frac{RT}{N_R}\ln\frac{C}{N_R} \tag{4}$$

$$K_{RV} = \left(\frac{C}{N_R}\right)^{-N_V/N_R} \tag{5}$$

where N_R is the aggregation number of rods and C is total polymer concentration. Based on the experimentally found boundaries of rod-to-vesicle transition, an aggregation number of 5000 has been calculated for vesicles. At 25 °C, the estimated free energy of transferring one mol of single chains from rods to vesicles ranges, for this particular system, ranges from −37 to −40 kJ/mol depending on water content in the system (above 15% wt. water). This result is much smaller than the corresponding value for the micelle-to-rod transition, indicating that at higher water content vesicles may become a thermodynamically favorable morphology.

In the PS-*b*-PEO family [104] as well as for PS-*b*-PAA copolymers [105], the vesicular morphology was revealed in solution as one of the possible aggregates. A morphology change may be observed after the degree of stretching of the hydrophobic chains increases causing the entropy decrease. Chain stretching is a parameter influenced by the external conditions, and therefore can hardly be considered independently, however, it is crucial for controlling membrane thickness. As the degree of stretching increases, the high stretching penalty for the hydrophobic chains in the core of a spherical micelle will change the morphology progressively from a sphere to a cylinder or bilayer in order to decrease the total free energy of micellization. The morphology and size of vesicles will be also influenced by the repulsion between the hydrophilic chains [105]: decrease of the repulsion between hydrophilic blocks leads to an increase of the aggregation number. Therefore, the dimensions of the core have to increase, which further stretches the polymer chain. As repulsion between PAA chains is related to (for example) environmental factors (solvent), the degree of stretching will be affected accordingly.

The ability to form vesicles in given physicochemical conditions has been proven to strongly depend on various factors, the major one being the properties of the polymer. The effect of such factors as the kind and ratio of hydrophilic/hydrophobic blocks, chain dynamics and polymer polydispersity on the vesicular sizes and shapes have

been investigated mainly by Eisenberg's group in systems like PS-*b*-PAA and PS-*b*-PEO (for the most recent overview of their work, see [19]), but contributions from other groups also appeared [23,72,73,106]. Various authors consider many factors, which indeed influence the system and combining them all in a uniform theory will be essential for the engineering of desired morphologies in various systems.

Detailed phase diagrams of the PS-*b*-PAA in dioxane—water were presented in [107], where the influence of the size of the polymers' building blocks was also discussed in detail. The PS block lengths studied were from 49 to 310 repeating units, whereas the PAA block lengths were from 7 to 26 units. At given water content in the system, the corona repulsion in the PAA region increases with increasing PAA block length, and therefore the morphologies of the aggregates present in the system should change in the direction of inverted structures to bilayers and to spheres. In addition, the longer the PAA block length, the larger the effective hydrodynamic radius of the vesicles. In the phase diagrams of the system, the increase in the PAA block length shifts the morphological boundaries to higher water contents due to the increase of the interactions of the PAA corona with the solvent. While decreasing the total block length the morphological boundaries shift to higher water content due to the increase of the interfacial tension between the PS blocks and the solvent.

In a PBO–PEO system, the two short-size repeating PEO units (at constant PBO block length) were found to produce a nearly perfect bilayer structure [73], where the thickness of the vesicle shells increases with the increasing ratio of PBO/PEO.

Similarly, self-assembly of novel dendritic building blocks (amphiphilic forms of the amide dendron, which form thermoreversible gels in organic solvents as well as vesicular structures in aqueous phase) have been studied. In such systems, a morphology shift has been observed from vesicles to rod-like and spherical micelles, depending on the increased volume fraction of a hydrophilic block [108]. Under certain conditions, even helical superstructures have been observed [109,110].

The influence of the block copolymer molecular mass (related strongly to the macromolecules' size and block lengths) on the thickness of the vesicular membranes was found for a series of PEO-*b*-PBD polymers [106]. As evidenced by TEM imaging, the wall thickness increased with the increasing block copolymer molecular mass in the range from 3600 to 20000 g/mol. The studied copolymers are in the strong segregation limit, where a balance of interfacial tension and chain entropy yields membrane thickness proportional to polymer molecular mass.

Apart from chain size, also the polymer architecture plays an important role in producing various morphologies of aggregates. An interesting study shows that the cyclization of a linear copolymer chain (of polystyrene–polyisoprene) induces a remarkable change in the micellar morphology. The micelles arising from linear diblock copolymers exhibit a monodisperse spherical shape (50 nm in diameter), whereas those formed from cyclic copolymers are long (>1 mm) cylindrical (wormlike) objects, resulting from the unidirectional self-assembly of 'sunflower-like' elementary micelles whose architecture strongly favors the core–core (PS–PS) attractions [111]. This way, via controlling the chemical structure of the polymer blocks, various nanostructures can be achieved.

In a number of publications, the solution properties have been related to the presence of vesicular morphology, as well as vesicle size. Zhang and Eisenberg [112] found that vesicles are preferentially formed at higher polymer concentrations as compared to concentrations at which micelles and rods are present. In the PS_{410}-*b*-PAA_{25} system studied in the above paper, the concentration (in DMF) required for vesicle formation was 4 wt%. The phase diagrams for polymer solutions of various PS–PAA polymers [103,107] revealed the same tendency. Again, by reference to thermodynamics of chain stretching, this effect can be immediately explained.

As mentioned earlier, the most common experimental method for vesicle preparation in block copolymer system is first dissolution of the material in a good solvent, common for all constituting blocks, followed by introduction of water, which acts as a precipitating solvent for the hydrophobic blocks. The formation of first colloidal particles (micelles) occurs at the critical water concentration (CWC), the value of which being an individual property of the studied system. With the increasing water content, changes in morphology of the aggregates can be observed, typically from micelles to rods and further to vesicles.

On the other hand, the common solvent in polymer solution controls the coiling of all blocks comprising the polymer chain; its nature will also affect the system in the sense that aggregates of various shapes and sizes may be observed, since the polarity of the solvent influences the repulsion between the hydrophilic blocks. Even more morphology control owing to the solvent dielectric constant can be expected in the case of ionic hydrophilic blocks. In general, the strength of the polymer–solvent interaction is described by the χ parameter, which is in turn related to solubility parameter (δ) and the dielectric constant (ε). Applying those parameters, the existence of various morphologies in different solvents could be explained [105].

The solvent influence on the aggregation in amphiphilic block copolymer solutions was investigated in such systems as PS-*b*-PAA, PS-*b*-P4VPMeI (poly(4-vinylpyridinum methyl iodide)) and PS-*b*-PEO [113,114]. Apart from the understanding of the solvent influence on multiple morphologies in solution, the major impact of these studies has been that the control of the interactions in the hydrophilic part of the vesicle membrane allows for very precise fine-tuning of the shapes and sizes of colloidal aggregates.

A few different types of forces have been found to facilitate vesicle formation, just to mention electrostatic interactions. Schrage et al. [115] consider a system consisting of oppositely charged block ionomers, in which the phase behavior is strongly affected by the additional energy contributions from electrostatic interactions. Since the block copolymers are studied in THF, the hydrophilic (ionic) blocks will form the middle layer of the vesicle membrane, whereas hydrophobic blocks will point to the vesicle inner and outer surface, respectively. Such a modular approach, just by mixing two different diblocks with the most simple primary structure, allows producing a library of complex superstructures with adjustable properties for special applications. In such systems, the formation of the superstructures is controlled by electrostatic interactions and/or other specific interactions like hydrogen bonding or donor–acceptor interactions [115].

The addition of small amounts of acids, bases or inorganic salts to the solution changes the interactions in the outer layer of the vesicles, especially in the cases where the hydrophilic block is ionic [78,116]. Again, the PS-*b*-PAA system has been exploited, and it has been proven that the addition of inorganics provides an excellent control of the aggregate morphologies, the more so because already minute (micro and millimolar) amounts of additives might induce drastic changes. Obviously, such additions are advantageous over other factors (just to mention the block length ratio or the solvent) via which various morphologies can be induced—this is due to ease in experimental preparations where no additional syntheses are necessary, needless to mention the low costs.

It should be mentioned here, that although being equilibrium morphology in multiple systems, the actual vesicle size is usually affected by the preparation procedures, via which so-called 'non-equilibrium' vesicles are obtained. This, in turn, allows for tailoring of vesicle size by experimental conditions and preparation methods, as will be discussed further.

4.2. Kinetic considerations

As mentioned above, the formation of block copolymer vesicles can be viewed as a result of transition from rod-like aggregates via flat, non-closed lamellar structures. The kinetics of such transitions has been explored in [50]. The transition steps are represented as follows:

$$\text{rod} \underset{k_{1b}}{\overset{k_{1f}}{\rightleftarrows}} \text{lamella} \underset{k_{2b}}{\overset{k_{2f}}{\rightleftarrows}} \text{vesicle}$$

where k_{1f} represents the rate constant of the formation of lamellae from rods and k_{1b} the reverse rate constant. Analogously, k_{2f} refers to the rate constant of vesicle formation from lamellae and k_{2b} the reverse rate constant. Therefore, the equations for the concentration of the three morphologies can be written as [117]:

$$\frac{dC_r}{dt} = k_{1f}C_r + k_{1b}C_l \tag{6}$$

$$\frac{dC_l}{dt} = k_{1f}C_r - \left(k_{1b} + k_{2f}\right)C_l + k_{2b}C_v \tag{7}$$

$$\frac{dC_v}{dt} = k_{2f}C_l - k_{2b}C_v \tag{8}$$

Above, C refers to the molar concentrations of each species, whereas the subscripts r, l, and v correspond to rods, lamellae and vesicles, respectively. Taking the above equations as the starting point, it is possible to calculate the concentrations of the individual species in solution in time and relate them to the experimental turbidity values.

The detailed mechanism of the morphological changes resulting in vesicles as well as the transition kinetics has been investigated for the PS_{310}-*b*-PAA_{52} [118]. Here, a jump in dioxane content has been employed to induce the vesicle to rod transition. The kinetics has been followed by turbidity measurements sensitive to changes in the size and shape of the aggregates. The turbidity curves for the vesicle to rod transitions upon water addition could be fitted by a single relaxation time (one time constant), owing to the fact that the closure of lamellae to form vesicles (being a slow step in the transition) contributes more to the turbidity than the fast lamellae formation. It is also noticed that the rod to vesicle transition occurs more slowly (the time constant is two orders of magnitude longer) than the reverse, even though it takes place at higher water content. The explanation for this fact is that the solvent ratio will also affect the mobility of the block chains. Since the organic solvent can fluidize the membrane, high water content will kinetically 'freeze' the morphology of the aggregates.

In [119], the kinetics of the increase of vesicle size after changing the solvent composition has been studied. The relaxation times of the process have been evaluated, and the factors influencing the vesicle size have been investigated in detail. The authors propose a fusion mechanism of vesicle formation as the content of water in the system increases. This mechanism is supported by TEM investigations. The differences between the values of relaxation times at different water content in the system were explained: the average relaxation times vary between 10 and 700 s, being strongly dependent on such parameters as water content, the magnitude of perturbation, the polymer concentration and the PAA block length. The slower rate of vesicle fusion at high water content is attributed to the decrease in both chain mobility and the vesicle collision frequency with the increasing water content.

5. Vesicle morphologies

5.1. Phase separation of polymers

In Section 4, we presented the major reasons for the

vesicular morphology appearing in solution, resulting mainly from polymer microphase separation owing to incompatibility of the building blocks. In general, multiple aggregate morphologies, such as micelles, rods, sheets (lamellae) and vesicles can be obtained from one polymer upon varying the polymer concentration and other solution properties.

In bulk, a variety of morphologies can be found, including lamellae, hexagonally ordered cylinders, modulated and perforated layers etc., depending on block length ratio. In context of membrane formation, lamellae are most interesting. At high concentrations, lyotropic liquid crystalline phases are observed, the stability of which varies with temperature [120].

In solutions, similarly to simple water-soluble amphiphiles, micelles were first observed, self-assembling spontaneously, because of incompatibility between the forming blocks. Additionally, vesicles of different morphologies were observed experimentally. In this section, we present the examples of various vesicular morphologies encountered in block copolymer systems.

5.2. Vesicles: small and giant vesicles

Block copolymer vesicles can be prepared in solution from a variety of different amphiphilic systems. Block copolymer systems can produce vesicles of a wide range of sizes; those in the range of 100–1000 nm have been explored extensively. Different factors, such as the absolute and relative block lengths [107], the polydispersity of the hydrophilic block, the presence of additives (ions [121], homopolymers [23], and surfactants [77,122]), the nature and composition of the solvent mixture (including water content [123]), and the temperature, provide control over the types of vesicles produced [19], as explained in detail in Section 4. Various types of vesicle morphologies are represented in Fig. 13.

Hollow nanoparticles with a polymeric shell can be prepared using vesicles formed by block copolymers in solution. Various systems are reported, not only in aqueous solution but also in organic solvents [125].

Nanocapsules (‘polymersomes’) are prepared using block copolymers, which possess enhanced toughness and reduced water permeability compared to liposomes. Those properties can be further enhanced when the shell is photo cross-linked [4,26,75]. The enhanced stability (for example in the bloodstream) of cross-linked nanoparticles and the ability to tune their size and to incorporate responsive or functional species are additional advantages offered by the use of polymers. A schematic drawing of an ABA triblock copolymer vesicle is given in Fig. 14.

Both unilamellar [12] and multilamellar vesicles have been observed. Particularly interesting morphologies were concentric vesicles of PS-*b*-PAA block copolymers with uniform spacing between the walls and multi-lamellar

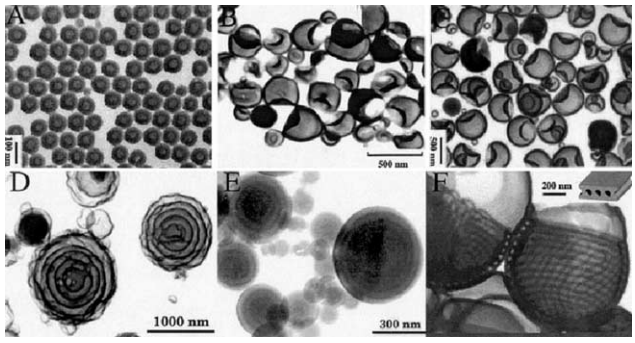

Fig. 13. Representative micrographs of various types of vesicles: (A) small uniform vesicles (PS_{410}-*b*-PAA_{13}), (B) large polydisperse vesicles (PS_{100}-*b*-PEO_{30}), (C) entrapped vesicles (PS_{200}-*b*-PAA_{20}), (D) hollow concentric vesicles (PS_{132}-*b*-PAA_{20}), (E) onions (PS_{260}-*b*-P4VPDecI$_{70}$), and (F) vesicles with tubes in the wall (PS_{100}-*b*-PEO_{30}) (from [124], with permission from J. Wiley and sons, Ltd).

onions, in which there was no spacing between the walls [126].

Diblock copolymers can also spontaneously form micrometer-sized, multilamellar vesicles (‘onions’) over a broad range of concentrations upon simple mixing with water [73], as evidenced by cryo-scanning electron microscopy, Fig. 15. The toughness of giant vesicles made from diblocks and their resistance to osmotic stress is of interest in order to test amphiphilic block copolymers as effective substitute for phospholipids. Such studies are motivated by the main disadvantage of liposomes, namely high membrane fluidity and poor stability. The enhanced stability of block copolymer systems makes them very well suited as models to investigate certain biological phenomena, especially as their shape transformations suggest analogies to biological cells [127]. What is evident from all structural characterization done on polymer vesicles is that the ’universal’ wall thickness of 3–4 nm, which is well known for natural lipid vesicles, presents no physical limitation on the amphiphilic assembly at the nano-scale [128].

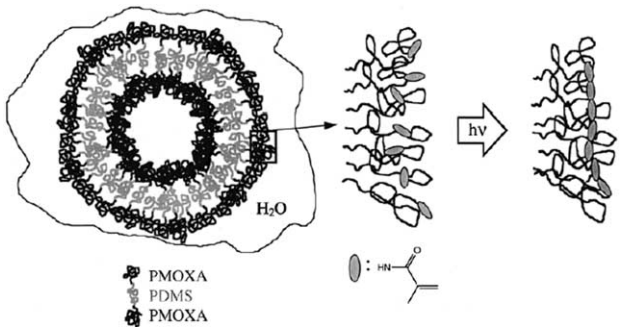

Fig. 14. A schematic representation of a PMOXA–PDMS–PMOXA vesicle in water. The intravesicular cross-linking of the individual polymer molecules to a nanocapsule is realized through UV irradiation of the polymerizable end groups of the triblock copolymer macromolecules (from [4], with permission from ACS Publications Division).

5.3. Hollow tubes

Tubes (rods) are one of multiple possible morphologies formed in solution as a result of self-assembly; the first example to yield stable block copolymer amphiphilic tubules has been reported in 1998 [114]. For example, fabrication of hollow tubes from self-associated star-shaped polymers in hydrophilic solvents was reported [129], when the polymer structure comprised of porphyrins as backbone and amphiphilic polymers as arms. Tubular vesicles (d= 2.4 μm) were also observed during preparation of giant vesicles from the diblock PBD-*b*-PEO, using an adapted standard swelling procedure. Morphological changes in those giant polymersomes were induced by temperature quenches [25]. Thermoreversible thread-like aggregates could also be formed in nematic solvent; rod-like vesicles have a uniform diameter of 2–3 μm and their long axis perfectly follows the interior field of the nematic matrix [130].

Soft, water-filled polymer tubes of nanometer-range diameters and several tens of μm in length have been prepared via self-assembly of amphiphilic ABA triblock macromonomers in aqueous media, the example image is presented in Fig. 16. The tubes are mechanically and chemically stable and can be loaded with water-soluble substances [131].

5.4. Other morphologies

Self-porating polymersomes have been prepared by blending a hydrolysable block copolymer with a vesicle-forming diblock copolymer; in such composites micro-environmental control of release is possible [132].

Vesicles with hollow rods running inside and parallel to the surfaces of the walls have been observed in solution of polystyrene-*b*-polyethylene diblocks [123,133], Fig. 13 F. This new, non-classical, morphology is an intermediate in the transition from vesicles to inverted hexagonally packed rods or hoops. Transmission electron microscopy revealed hollow rod substructures in vesicle walls, characterized by lower density of the copolymer chains. As compared to classical vesicles from the same polymer, the wall thickness is approximately two-fold higher, ca. 45–50 nm. In solution, the hollow rods are filled with water and water-soluble blocks (PEO). The interesting feature of this unusual morphology is various ‘patterns’ of such rods in vesicle walls, namely, rods were found to run in the same or in different directions, their shape could be straight or arranged in sets of helices. The mechanism for the formation of such morphology involves thickening of vesicle walls accompanied by the formation of the rods and thus decreasing the size of the hollow vesicular cores.

High-genus vesicles were studied by Förster’s group [88,98]. In this case, the vesicular wall is organized in a double bilayer connected by a lattice of passages or a tubular network with hexagonal symmetry. The detailed

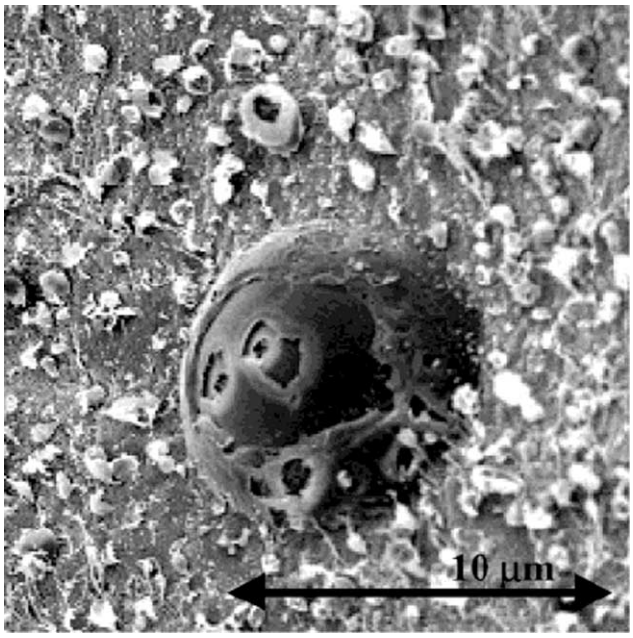

Fig. 15. A micrograph of a multilamellar vesicle found in a 1.0 wt% aqueous dispersion $PEO_{11}-PBO_{11}$; scale bar: 10 μm (from [73], with permission from ACS Publications Division).

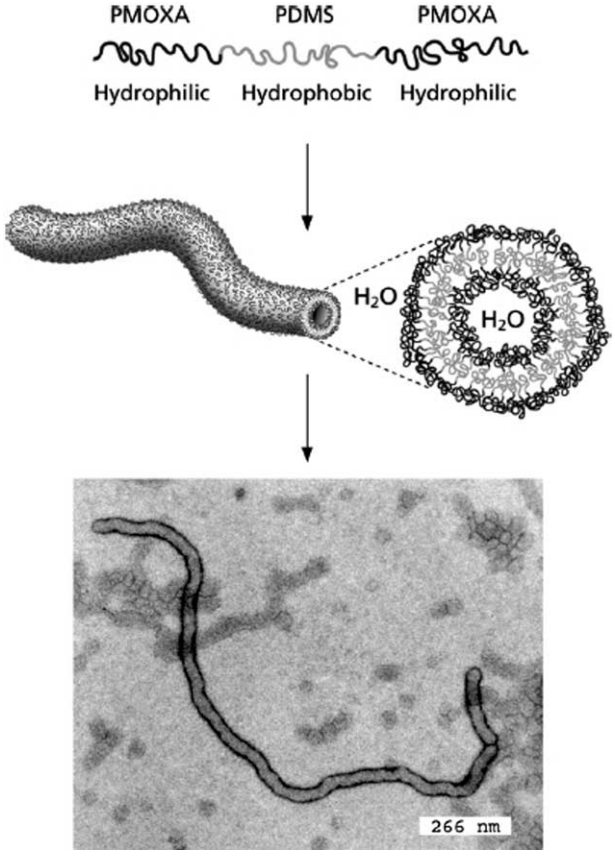

Fig. 16. Self-assembly of ABA triblock copolymers in aqueous solution; a TEM image of a polymer nanotube; scale bar: 266 nm (from [131], with permission from The Royal Society of Chemistry).

mechanism for the formation of this morphology remains under study; however, it is possible to control the shape and responsive properties of these vesicles through environmental stimuli, such as temperature and pH.

6. Mechanical properties of vesicles

Various experimental methods have been developed to determine the physical properties of biological membranes. Osmotic swelling and deformation in fluid shear [134] were first used to develop relationships between stress and strain on erythrocyte membranes. Later, micropipette aspiration was developed [135] to determine physical properties of membranes at high strains. The confined geometry of micropipette aspiration allows more accurate measurements of strain, and sensitive manometers provide exact measurements of membrane tension. More recently, techniques such as optical tweezers [136], tether pulling [137] and observation in high frequency fields [138] have been developed to provide more insight on the subtleties of membrane properties.

Micropipette aspiration is a technique applied widely to investigate the mechanical properties of living cells, but due to similar sizes, has also been applied to giant vesicles. It employs video microscopy to follow the vesicle shape as it is aspirated into a glass micropipette. Owing to the fact that the membrane deformation depends on the applied suction pressure, it is possible to evaluate the bulk vesicle viscosity by measuring its elongation into the pipette as a function of the suction pressure. With this technique, one can directly measure area compressibility modulus, bending moduli, lysis tension, lysis strain, and area expansion of membrane fluid phase. It has to be mentioned here that this technique is limited to giant vesicles (micrometer size) due to the necessity of optical microscopy visualization.

In [10], micropipette aspiration was applied to polymersomes from PEO–PEE in order to study the membrane elastic behavior. While the membrane elasticity was comparable to fluid-state lipid membranes, and the bending modules were in the same range as reported previously for pure and mixed lipid membranes, the permeability of polymersomes was highly decreased as compared to liposomes. In the same system, the sustainable critical strain of polymer vesicles was reported to highly exceed the value typical for lipid membranes (0.19 and 0.03–0.06, respectively), providing the evidence for polymersome enhanced toughness, which originates from membrane thickness [21,128,139].

Covalently cross-linked polymersomes have been studied in [140], revealing even higher toughness and durability properties. In contrast to non-cross-linked polymer membranes, which reveal fluid-like character, such cross-linked vesicles would rather respond as more solid-like upon deformation. In addition, enhanced stability towards environmental conditions was observed for such vesicles.

The membrane bending rigidity and its dependence on hydrophobic thickness, d (and thus indirectly the molecular mass) of PEO–PEE vesicles was studied in [141]. The bending rigidity scales as d^2, in agreement with existing theories concerning lipid membranes, thus providing a way to engineer vesicle properties by choosing various polymer blocks.

Electromechanical measurements using the same technique were performed to determine the vesicle breakdown potential at various membrane tensions [142]. The most remarkable result is that the robustness of vesicle membranes can be orders of magnitude larger than of liposomes [143].

Similarly, the membrane robustness has been tested for giant free standing ABA triblock copolymer films, applying the ‘black lipid membrane’ technique [144]. The energy barrier of the copolymer membrane (with a mean hydrophobic thickness of 10 nm) against rupture revealed its high stability, which could even be enhanced by polymerization of the end groups within the membrane.

7. Applications of block copolymer vesicles: biomembrane aspects

Polymeric vesicles have attracted considerable attention in recent years, since they could be models for biological membranes and have versatile structures with several practical applications. Their high mechanical stability, resistance to many external stimuli [21] and ability to encapsulate both hydrophilic and hydrophobic compounds make them excellent candidates for use in medical, pharmaceutical, and environmental fields. In biomedical sciences, for example, the recently studied behavior of some polymer vesicles in vivo showed utility in delaying vesicle clearance from the blood circulation [24]. The alternative routes to achieve control of the permeability of polymer nanocapsules with emphasis on the amphiphilic block copolymers have been reviewed in [145].

In this section, we provide the recent achievements in the field of application of block copolymer vesicles. In this context, we especially wish to focus on our group’s contributions to this field.

Polymer vesicles have a great potential for encapsulation of various species within their hollow cavities [146] and the further release due to membrane diffusion, vesicle breaking, or after the application of a stimulus to the system. Another advantage is that one can engineer and precisely control the properties of the vesicles by the polymer composition and the environmental conditions. Biodegradable micelles and vesicles, able to encapsulate and release hydrophilic drugs, are particularly useful for pharmaceutical use [2]. Active loading of doxorubicin (anticancer drug) into polymeric vesicles has also been reported [147]. Loading of purely

polymeric nanocontainers is possible in various systems, for example in PI-*b*-PCEMA [75] and in PEO-*b*-PEE as well as PEO-*b*-PBD systems [21].

In [75], after vesicle formation in THF/HX, the loading followed by equilibrating the nanospheres with Rhodamine B in methanolic solution. The authors were able to prove that the majority of the dye after incubation with vesicles was actually located within the vesicular hollow interiors. The delivery of Rhodamine B was investigated by fluorescence measurements and, additionally, the fine-tuning of the release was proven possible by changing the concentration of ethanol in the surrounding water/ethanol mixture.

Lee et al. [21] studied the encapsulation of proteins: globins (myoglobin and hemoglobin) and bovine serum albumin (BSA). They proved the possibility to encapsulate the above proteins, however, the loading efficiency was low, and since encapsulation in such systems is not yet fully understood, further studies are necessary. In addition, an issue remains of the protein functionality after encapsulation and subsequent release.

Biomedical applications of ABA triblock copolymers (PMOXA–PDMS–PMOXA) have been addressed in [148], focusing on specific targeting of cells using vesicles as drug carriers. Such approach allows avoiding multiple side effects resulting from the drug molecules within the body being involved not only in therapy, but also undergoing various undesirable physiological pathways. Highly stable and biocompatible synthetic ABA copolymer vesicles were used as a delivery system, whereas activated macrophages and their scavenger receptor A1 (SRA1) were a model target. It was possible to make vesicles target the cells after they have been functionalized with the oligonucleotide poly(guanylic acid) (poly-G), which is a specific ligand for the SRA1 receptor. Linking the ligand to vesicle membrane was accomplished via a biotin-streptavidin complex, Fig. 17. Further loading of the nanocontainers with fluorescent labels allowed the microscopic observation of the binding and uptake of the vesicles by the cells. The major result from this study was achieving high receptor specificity of vesicle uptake. In addition, the absence of unspecific binding showed that uncontrolled uptake of the carrier by cells can be overcome using specific nanocontainer building blocks, exhibiting very low polymer–protein interaction.

An interesting issue in the studies of polymer vesicles is their responsiveness to external stimuli, a useful feature for the delivery of encapsulated substances. Whenever the delivery of the encapsulated material takes place, destabilization of the polymer membrane should first occur. Design of the oxidation-responsive vesicles from block copolymers of ethylene glycol and propylene sulfide [20], was the first example of the use of oxidative conversions to destabilize nanocontainers [149]. Redox-active organometallic vesicles from poly(dimethylsiloxane-*b*-ferrocenylsilane) diblock copolymer have also been reported [150].

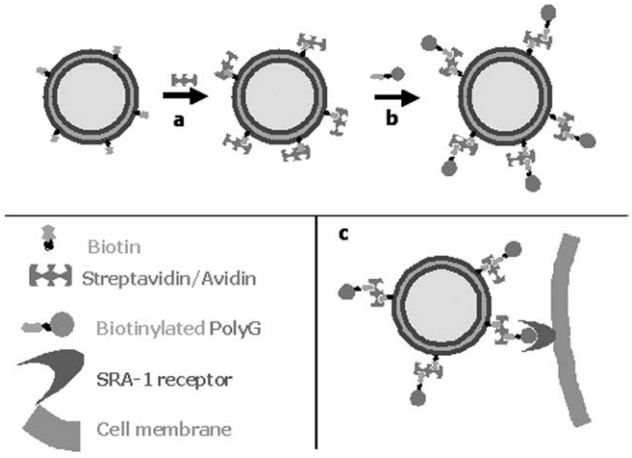

Fig. 17. Schematic representation of the coupling of polyG with polymeric nanocontainers via biotin-streptavidin affinity interaction, (a) coupling of biotinylated nanocontainers with streptavidin, (b) subsequent incubation with biotinylated polyguanilic acid to render ligand-labeled nanocontainers, (c) schematic representation of the mode of action by receptor-ligand targeting.

Polybutadiene-*b*-poly(L-glutamic acid) (PBD-*b*-PGA) diblock copolymers were found to form well-defined vesicles in aqueous solutions at basic pH. One can manipulate the size of these aggregates reversibly as a function of the pH and ionic strength applied [151].

In a number of applications, loading of vesicles has been approached using a concept from Nature, where cell membrane proteins allow for transport of various species to the inside of a cell, and for removal of compounds to the outside medium. Following this idea, incorporation of (cell) membrane channel proteins in the polymer vesicles' wall was performed.

Membrane proteins offer an excellent channel for transporting small molecules and ions, either specifically or non-specifically. The transport may be directed or the substances can move freely in both directions via the channel. Insertion of membrane proteins in polymer-stabilized lipid membranes has been successful [152], however, for long time pure polymer membranes have been thought an inappropriate system for such insertion, due to the thickness incompatibility. Namely, lipid membranes offer the 'universal' thickness of ca. 4 nm, which is also the height of protein channels. On the other hand, polymer membranes are at least two-fold thicker, and their size is very much dependent on the polymer used, preparative method and environmental conditions, as discussed before. Therefore, their dimensional mismatch was considered too large to create a chemically favorable environment for the inserted protein. Indeed, one could rather imagine adsorption of the protein to the membrane or the formation of protein domains within the membrane instead of random insertion.

Recently, it has been shown experimentally that functional incorporation of membrane proteins into block copolymer membranes is feasible, yet the question

concerning the mechanism of such an insertion remained. Indeed, the experimental approaches employed planar membranes from a triblock copolymer [13], which was also found to form vesicles in solution [4]. The BLM technique allows for the measurements of conductance across the membrane, which varies upon the insertion of pore-forming species into the film. Two well-characterized channel proteins: OmpF and LamB, naturally found in Gram-negative bacteria were subject to this study, which focused not only the insertion of proteins themselves, but also their functionality within the polymer membranes. A fully functional incorporation of porins into the artificial (non-physiological) environment of a polymer membrane is possible. Further applications are foreseen, such as creating protein–polymer hybrid materials for diagnostics, sensors and drug delivery.

The above problem has been theoretically considered by Pata and Dan [153], who proved via mean field calculations that such insertion can be possible. Conventional lipid bilayers are relatively (vertically) incompressible, due to the limited number of possible conformations of lipid molecules in the membrane. Therefore, they cannot support a perturbation in thickness and already a small dimensional mismatch will result in a huge energy penalty that prohibits protein insertion. In block copolymer membranes, however, the hydrophobic chains are in an unfavorable, stretched conformation inside the membrane core. Therefore, a local compression of the membrane around a protein (Fig. 18) increases local surface tension energy but decreases the stretching energy. This facilitates then the protein incorporation. Additionally, polydispersity of the synthetic block copolymers might further support this process, since shorter chains may segregate around the protein.

The successful incorporation of membrane proteins into planar polymer membranes, supported further by theoretical considerations, were a motivation to make one step further, aiming at creating protein-reconstituted polymer vesicular membranes [154,155]. Using the PDMS–PMOXA–PDMS amphiphilic triblock copolymer, vesicles were prepared, containing the OmpF protein inserted randomly into the vesicular membrane.

To find out whether one could employ such vesicles as nanoreactors, the goal was to encapsulate a substance, which would catalyze a reaction within the vesicles. The inserted channel protein, OmpF, controls the permeability of the membrane, because it serves as a channel to bring substrates to the inside of vesicles, and transport the resulting reaction products to the outside medium. A β-lactam antibiotic, ampicillin, was the model reaction system. After the drug diffusion to within a vesicle, hydrolysis takes place by the encapsulated enzyme, β-lactamase, and the resulting product, ampicillinoic acid, is released through the channel to the outside.

The possible utility of triblock copolymer nanocontainers in gene therapy has been presented in [156], which describes the successful delivery of phage DNA to the inside of the

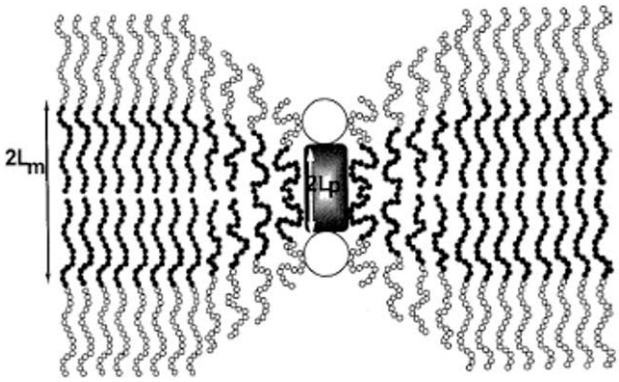

Fig. 18. Conformation of AB diblock copolymer chains near a protein inserted in a polymeric bilayer. Matching a protein whose height, which is half the thickness of the membrane, is easily obtained through polymer chains stretching (from [153], with permission from The Biophysical Society).

vesicles through another channel protein, LamB. Such DNA translocation across a non-physiological membrane was possible after reconstitution of the protein in the vesicular membrane. In natural systems, this protein is a receptor for λ phage and triggers the ejection of the phage's DNA into the bacterium cells. After LamB functional insertion into the polymer membrane, the phages attach to it specifically and inject their DNA through the channel to the inside of the vesicles (see Fig. 19). The amount of DNA is measurable via fluorescence experiments after the DNA labeling with a dye.

Block copolymers vesicles can also act as 'nanoreactors' for the synthesis of inorganic nanoparticles [15]. Pore-forming transmembrane protein incorporation within triblock copolymer membranes allows for the confinement of a biomineralization reaction in the interior of the vesicles [3], as presented in Fig. 20.

Phosphate anions encapsulation within the block copolymer vesicles takes place during the vesicle formation. To allow the transport of calcium cations from the surrounding medium to the vesicular cavities, three different species were reconstituted in the vesicle membrane: Lasalocid A and *N*,*N*-dicyclohexyl-*N*′,*N*″-dioctadecyl-3-oxapentane-1,5-diamide are selective cation carriers, whereas alamethicin, a pore forming peptide, would allow for non-specific transport. The authors were able to successfully control the local concentration of Ca^{2+} ions inside the vesicles, proving once again the functional incorporation of ionophores in the polymer membrane. In addition, they demonstrated controlled biomimetic mineralization, thus opening a broad field for studying crystallization in many systems with the application of vesicular membranes reconstituted with various channel proteins.

Although the first approaches of protein insertion into polymer membranes have been successful from the protein functionality point of view, one should remember that most membrane proteins are asymmetric, and in natural membranes, have a well-defined orientation. In symmetric AB and ABA block copolymer membranes, however, the

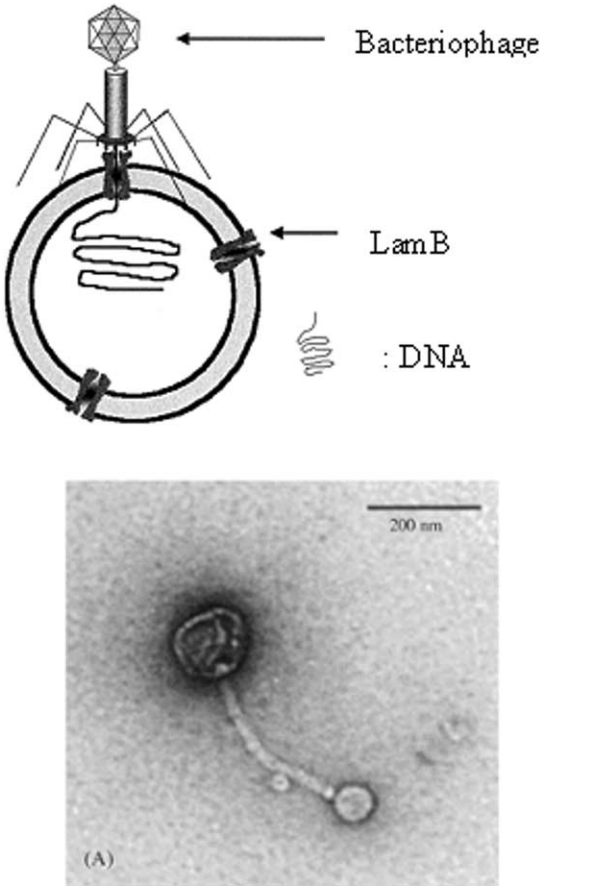

Fig. 19. A schematic representation of a DNA-loaded PMOXA–PDMS–PMOXA vesicle (above). The λ phage binds to a LamB protein incorporated in the polymer vesicle wall, and the DNA is transferred across the membrane. Below: A micrograph of a complex formed between the phage and a vesicle bearing LamB protein at 37 °C; scale bar: 200 nm (from [156], with permission from The National Academy of Sciences, USA).

protein macromolecules are usually inserted randomly. The issue of membrane protein orientation in polymer membranes was the motivation for the synthesis of amphiphilic ABC triblock copolymers with water-soluble blocks A and C and a hydrophobic middle block, B.

Such a synthesis and characterization of vesicles from PEO–PDMS–PMOXA was reported in [157]. ABC copolymers form asymmetric membranes due to segregation of the hydrophilic blocks as a result of their molecular incompatibility. It was shown that, for nanometer-sized vesicles, also the orientation of the membrane would be controlled. This seems to be governed by the relative size of the two hydrophilic blocks. Generally, the shorter amphiphilic block (A or C) points to the vesicle interior, whereas the longer one is directed to the outside medium. This finding is in agreement with thermodynamic considerations presented previously and supported by results from Eisenberg's group.

In a recent paper [158], directed protein insertion in ABC triblock copolymer vesicles was investigated, using

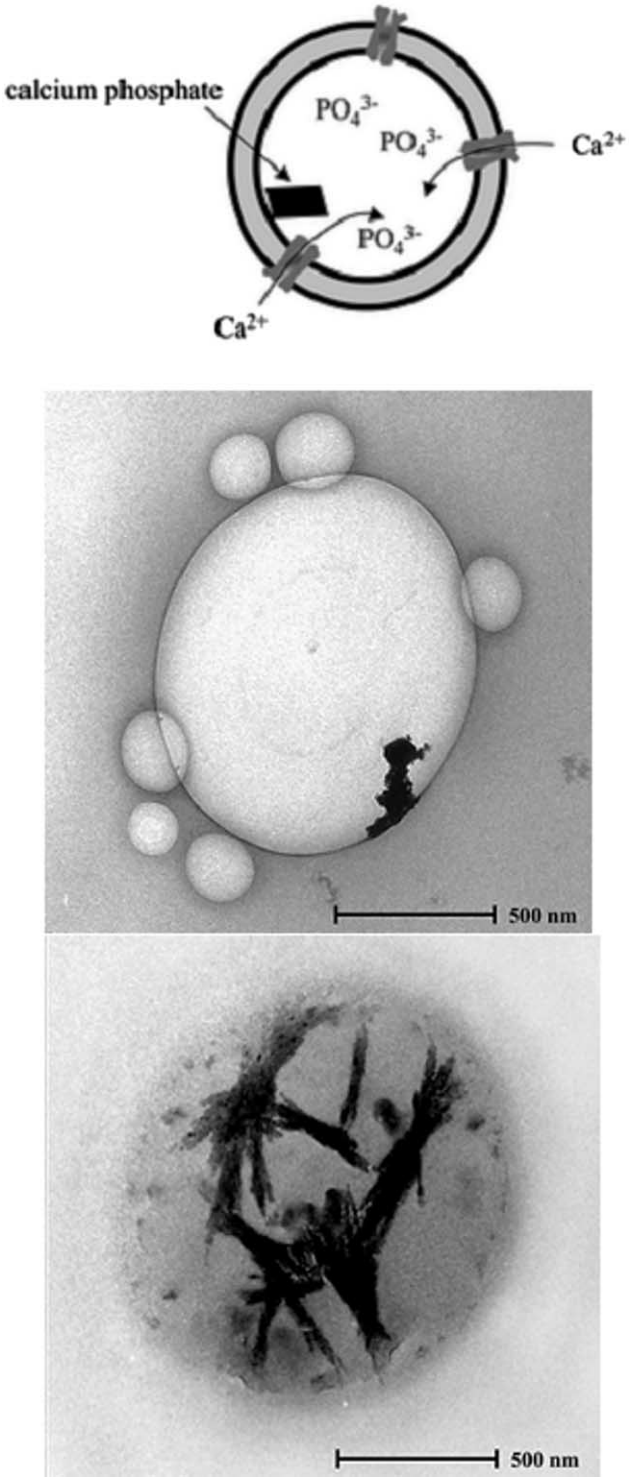

Fig. 20. A schematic representation of ion-channel controlled precipitation of calcium phosphate in block copolymer vesicles (upper drawing), and TEM micrographs (below) of phosphate-loaded PMOXA–PDMS–PMOXA triblock copolymer giant vesicles after 1 and 24 h of incubation with $CaCl_2$ solution in the presence of the ionophore; scale bar: 500 nm (from [3], with permission from The Royal Society of Chemistry).

Aquaporin 0 as the model protein. The control of the amount and orientation of protein molecules inserted in the membrane was possible by attaching a His-tag unit (ten histidine residues) to the protein's amino acid terminus, which, in physiological orientation, is directed to the inside of the (cell) membrane. Quantification of the protein insertion is possible using antibodies directed specifically against the His-tag sequence, previously labeled to allow for microscopic and spectroscopic measurements. This report proves that the symmetry or asymmetry of a vesicle membrane plays a role in protein insertion: when lipids or symmetric ABA polymers were used, the control experiments revealed a statistical distribution of protein orientations in the membrane, whereas ABC membranes always favor one particular orientation. Even though the detailed mechanism of such insertion, with a focus on the forces driving the process, is very complex and will be a motivation for further studies, the experimental findings offer a new possibility for polymer–protein complex materials and bio-devices. Additionally, it is important to note that the polymer membrane seems to stabilize and protect the protein macromolecules, which is crucial from the applications point of view.

More examples of successful applications of block copolymer vesicles have been reported. In material science, use can be made of giant vesicles, which can become conducting after the cross-linking of thiophene groups within the membrane using a chemical oxidant [159]. In the field of sensors, ion-binding block copolymers, which allow the formation of 'functionalized' vesicles could be of special interest. Additionally, to control a site of redox reaction, vesicles complexed with $FeCl_3$ have been employed [160].

Recently, amphiphilic block copolymers have also attracted attention as biomimetic materials, where their features are favorable for creation of composite materials of further use in biological and medical sciences. We already described above several examples of functional insertion of membrane proteins and biomineralization, yet more interesting reports exist in literature. For example, giant and stable worm-like micelles formed in water from a series of PEO-based diblock copolymer amphiphiles are able to mimic the flexibility of various cytoskeletal filaments [161].

One could notice a strong trend in studies aimed at applying vesicles as 'artificial cells'. This is not surprising, because their morphological features, with a membrane separating the core from the outside medium, are ideally suited for such developments. The construction of synthetic cells made from polymers with a particular focus on mimicking the structure and behavior of blood cells has been presented in [139].

Vesicles could also be used in the field of chemical applications. Some diblock copolymers form hairy vesicles in organic solvent mixture, consisting of a solvent-free spherical shell from one block and chains of the other block stretched into the solution phase. 'Shaving' of such hollow nanospheres takes place by degrading both the inner and outer chains using long ozonolysis times. Due to their poor solubility in common organic solvents, such vesicles may be useful as macro-porous resins in chemical separations [76].

8. Summary

Polymer vesicles, although being just one possible morphology in polymer solutions, comprise an extremely interesting system, from both theoretical and practical points of view. Their properties can be varied over an extremely wide range, thus allowing tailoring with respect to the desired applications. It has to be emphasized that during the recent years much work has already been devoted to the synthesis and characterization of vesicles from various polymers. However, the possibilities of polymer chemistry to vary chemical composition, molecular architecture etc. seem to be nearly unlimited.

In particular, incorporation of biological units in vesicular walls may lead to membranes that could serve as a bridging platform for controlling communication between synthetic and living matter. Once possible, they will have a huge impact on the development of new generations of composite materials, drug delivery systems, gene vectors or diagnostic tools.

The remaining challenge in this field is to develop tools to allow for exact spatial and temporal coordination of such structural units, in addition to a precise tuning of their mutual interactions. However, this seems possible in the near future with a fast development of investigation techniques and with increasing understanding of the physical chemistry of self-assembly processes.

Acknowledgements

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References

[1] Bangham AD. Chem Phys Lipids 1993;64:275–85.
[2] Najafi F, Sarbolouki MN. Biomaterials 2003;24:1175–82.
[3] Sauer M, Haefele T, Graff A, Nardin C, Meier W. Chem Commun 2001;(23):2452–3.
[4] Nardin C, Hirt T, Leukel J, Meier W. Langmuir 2000;16:1035–41.
[5] Lasic DD. Liposomes: from physics to applications. Amsterdam: Elsevier; 1993.
[6] Laughlin RG. The aqueous phase behaviour of surfactants. San Diego: Academic Press; 1994.

[7] Zhang L, Eisenberg A. Science 1995;268:1728–31.
[8] Ding J, Liu G, Yang M. Polymer 1997;38:5497–502.
[9] Holder SJ, Hiorns RC, Sommerdijk NAJM, Williams SJ, Jones RG, Nolte RJM. Chem Commun 1998;(14):1445–6.
[10] Discher BM, Won Y-Y, Ege DS, Lee JC-M, Bates FS, Discher DE, et al. Science 1999;284:1143–6.
[11] Regenbrecht M, Akari S, Förster S, Möhwald H. Surf Interf Anal 1999;27:418–21.
[12] Schillén K, Bryskhe K, Mel'nikova YS. Macromolecules 1999;32: 6885–8.
[13] Meier W, Nardin C, Winterhalter M. Angew Chem, Int Ed 2000;39: 4599–602.
[14] Discher DE, Eisenberg A. Science 2002;297:967–73.
[15] Hamley IW. Nanotechnology 2003;14:R39–R54.
[16] Simon J, Kühner M, Ringsdorf H, Sackmann E. Chem Phys Lipids 1995;76:241–58.
[17] Decher G, Kuchinka E, Ringsdorf H, Venzmer J, Bitter-Suermann D, Weisgerber C. Angew Makromol Chem 1989;166–167:71–80.
[18] Meng F, Hiemstra C, Engbers GHM, Feijen J. Macromolecules 2003;36:3004–6.
[19] Soo PL, Eisenberg A. J Polym Sci, Part B: Polym Phys 2004;42: 923–38.
[20] Napoli A, Boerakker MJ, Tirelli N, Nolte RJM, Sommerdijk NAJM, Hubbell JA. Langmuir 2004;20:3487–91.
[21] Lee JCM, Bermudez H, Discher BM, Sheehan MA, Won YY, Bates FS, et al. Biotechnol Bioeng 2001;73:135–45.
[22] Reeves JP, Dowben RM. J Cell Physiol 1969;73:49–60.
[23] Nikova AT, Gordon VD, Cristobal G, Talingting MR, Bell DC, Evans C, et al. Macromolecules 2004;37:2215–8.
[24] Photos PJ, Bacakova L, Discher BM, Bates FS, Discher DE. J Controlled Release 2003;90:323–34.
[25] Reinecke AA, Döbereiner H-G. Langmuir 2003;19:605–8.
[26] Dimova R, Seifert U, Pouligny B, Förster S, Döbereiner H-G. Eur Phys J E 2002;7:241–50.
[27] Evans E, Needham D. J Phys Chem 1987;91:4219–28.
[28] Angelova MI, Dimitrov DS. Faraday Discuss Chem Soc 1986;81: 303–11.
[29] Ahmed F, Discher DE. J Controlled Release 2004;96:37–53.
[30] Kukula H, Schlaad H, Antonietti M, Förster S. J Am Chem Soc 2002; 124:1658–63.
[31] Dimitrov DS, Angelova MI. Progr Colloid Polym Sci 1987;73: 48–56.
[32] Bucher P, Fischer A, Luisi PL, Oberholzer T, Walde P. Langmuir 1998;14:2712–21.
[33] Bagatolli LA, Gratton E. Biophys J 1999;77:2090–101.
[34] Taylor P, Xu C, Fletcher PDI, Paunov VN. Chem Commun 2003; (14):1732–3.
[35] Angelova MI, Dimitrov DS. Mol Cryst Liq Cryst 1987;152:89–104.
[36] Kremer JMH, Van der Esker MW, Pathmamanoharan C, Wiersema PH. Biochemistry 1977;16:3932–5.
[37] Szoka F, Papahadjopoulos D. Proc Natl Acad Sci USA 1978;75: 4194–8.
[38] Moscho A, Orwar O, Chiu DT, Modi BP, Zare RN. Proc Natl Acad Sci USA 1996;93:11443–7.
[39] Oku N, Scheerer JF, MacDonald RC. Biochim Biophys Acta 1982; 692:384–8.
[40] Liang YZ, Li ZC, Li FM. New J Chem 2000;24:323–8.
[41] Zumbühl O, Weder HG. Biochim Biophys Acta 1981;640:252–62.
[42] Murphy RM. Curr Opin Biotechnol 1997;8:25–30.
[43] Burchard W. Adv Polym Sci 1983;48:1–124.
[44] Sauer M, Meier W. Chem Commun 2001;(1):55–6.
[45] Norman AI, Cabral JT, Ho DL, Amis EJ, Karim A. Polym Mater Sci Eng 2004;90:339–40.
[46] Lindner P, Wignall G. MRS Bull 1999;24:34–9.
[47] Koizumi S, Hasegawa H, Suzuki J, Hashimoto T. Nipp Genshiryoku Kenkyusho, JAERI-M 1993;2:679–87.
[48] Zernike F. Z techn Physik 1935;16:454–5.
[49] Li Z-C, Shen Y, Liang Y-Z, Li F-M. Chin J Polym Sci 2001;19: 297–302.
[50] Chen L, Shen H, Eisenberg A. J Phys Chem B 1999;103:9488–97.
[51] Kapitza HG. Microscopy from the very beginning. 2nd ed. GmbH: Carl Zeiss; 1997.
[52] Nardin C, Meier W. Rev Mol Biotechnol 2002;90:17–26.
[53] Reichman J. Handbook of optical filters for fluorescence microscopy. Brattleboro: Chroma Technology Corp; 2000.
[54] Mulkey DK, Henderson A, Ritucci NA, Putnam RW, Dean JB. Am J Physiol 2004;286:C940–C51.
[55] Yip KP, Kurtz I. Methods Cell Biol 2002;70:417–27.
[56] Morgan CG, Mitchell AC. Chromosome Res 1996;4:261–3.
[57] Swanson JA. Methods Microbiol 2002;31:1–18.
[58] Koyama Y, Umehara M, Mizuno A, Itaba M, Yasukouchi T, Natsume K, et al. Bioconjugate Chem 1996;7:298–301.
[59] Ross ST. Am Lab 2002;22-24:27.
[60] Hinterdorfer P, Baber G, Tamm LK. J Biol Chem 1994;269: 20360–8.
[61] Buijs J, Britt DW, Hlady V. Langmuir 1998;14:335–41.
[62] Oheim M, Loerke D, Stuhmer W, Chow RH. Eur Biophys J 1998;27: 83–98.
[63] Wilson T, Sheppard CJR. Theory and practice of scanning optical microscopy. London: Academic Press; 1984.
[64] Beyer H. Handbuch der Mikroskopie. 2nd ed. Berlin: VEB Verlag Technik; 1985.
[65] Rawlings S, Byatt J. J Biophotonics Int 2002;9:44–8.
[66] Pawley J. Handbook of biological confocal microscopy. 2nd ed. New York: Kluwer Academic Publishers; 1997.
[67] Binnig G, Quate CF, Gerber C. Phys Rev Lett 1986;56:930–3.
[68] Ahmed F, Hategan A, Discher DE, Discher BM. Langmuir 2003;19: 6505–11.
[69] Luo L, Eisenberg A. Langmuir 2001;17:6804–11.
[70] Dean JM, Grubbs RB, Saad W, Cook RF, Bates FS. J Polym Sci B: Polym Phys 2003;41:2444–56.
[71] Won Y-Y, Brannan AK, Davis HT, Bates FS. J Phys Chem B 2002; 106:3354–64.
[72] Kickelbick G, Bauer J, Hüsing N, Andersson M, Palmqvist A. Langmuir 2003;19:3198–201.
[73] Harris JK, Rose GD, Bruening ML. Langmuir 2002;18:5337–42.
[74] Napoli A, Tirelli N, Wehrli E, Hubbell JA. Langmuir 2002;18: 8324–9.
[75] Ding J, Liu G. J Phys Chem B 1998;102:6107–13.
[76] Ding J, Liu G. Chem Mater 1998;10:537–42.
[77] Kabanov AV, Bronich TK, Kabanov VA, Yu K, Eisenberg A. J Am Chem Soc 1998;120:9941–2.
[78] Liu F, Eisenberg A. J Am Chem Soc 2003;125:15059–64.
[79] Luo L, Eisenberg A. Angew Chem, Int Ed 2002;41:1001–4.
[80] Peng H, Chen D, Jiang M. J Phys Chem B 2003;107:12461–4.
[81] Rusanov AI. Russ J Gen Chem 2002;72:495–511.
[82] Meier W. Chem Soc Rev 2000;29:295–303.
[83] Kawakatsu T, Kawasaki K, Andelman D. KEK Proc 1993;93:3–5.
[84] Huang L, Yuan H, Zhang D, Zhang Z, Guo J, Ma J. Appl Surf Sci 2004;225:39–46.
[85] Jenekhe SA, Chen XL. Abstr Pap Am Chem Soc 2000;220. PMSE-268.
[86] Di Marzio EA. Prog Polym Sci 1999;24:329–77.
[87] Klok H-A, Lecommandoux S. Adv Mater 2001;13:1217–29.
[88] Haluska CK, Goźdź WT, Döbereiner H-G, Förster S, Gompper G. Phys Rev Lett 2002;89:238302.
[89] Döbereiner H-G, Evans E, Kraus M, Seifert U, Wortis M. Phys Rev E 1997;55:4458–74.
[90] Lipowsky R, Döbereiner H-G. Europhys Lett 1998;43:219–25.
[91] Shillcock JC, Seifert U. Biophys J 1998;74:1754–66.
[92] Vila AO, Rodriguez-Flores C, Figueruelo J, Molina F. Colloids Surf A 2001;189:1–8.
[93] Rodriguez-Flores C, Vila AO, Figueruelo J, Molina F. Colloids Surf A 2001;191:233–40.

[94] Wang Z-G. Macromolecules 1992;25:3702–5.
[95] Luo L, Eisenberg A. J Am Chem Soc 2002;123:1012–3.
[96] Terreau O, Luo L, Eisenberg A. Langmuir 2003;19:5601–7.
[97] Terreau O, Bartels C, Eisenberg A. Langmuir 2004;20:637–45.
[98] Antonietti M, Förster S. Adv Mater 2003;15:1323–33.
[99] Lasic DD, Joannic R, Keller BC, Frederik PM, Auvray L. Adv Colloid Interf Sci 2001;89-90:337–49.
[100] Hyde ST. J Phys 1990;51:C7-209–C7-228.
[101] Israelachvili JN. Intermolecular and surface forces. London: Academic Press; 1992.
[102] Zhang L, Eisenberg A. J Am Chem Soc 1996;118:3168–81.
[103] Shen H, Eisenberg A. J Phys Chem B 1999;103:9473–87.
[104] Yu K, Eisenberg A. Macromolecules 1996;29:6359–61.
[105] Yu Y, Zhang L, Eisenberg A. Macromolecules 1998;31:1144–54.
[106] Bermudez H, Brannan AK, Hammer DA, Bates FS, Discher DE. Macromolecules 2002;35:8203–8.
[107] Shen H, Eisenberg A. Macromolecules 2000;33:2561–72.
[108] Liu F, Wu Q, Eisenberg A. Abstracts of Papers, 226th ACS National Meeting, New York, USA; 2003.
[109] Cornelissen JJLM, Fischer M, Sommerdijk NAJM, Nolte RJM. Science 1998;280:1427–30.
[110] Sommerdijk NAJM, Holder SJ, Hiorns RC, Jones RG, Nolte RJM. Polym Mater Sci Eng 1999;80:29.
[111] Borsali R, Minatti E, Putaux J-L, Schappacher M, Deffieux A, Viville P, et al. Langmuir 2003;19:6–9.
[112] Zhang L, Eisenberg A. Macromol Symp 1997;113:221–32.
[113] Yu Y, Eisenberg A. J Am Chem Soc 1997;119:8383–4.
[114] Yu K, Eisenberg A. Macromolecules 1998;31:3509–18.
[115] Schrage S, Sigel R, Schlaad H. Macromolecules 2003;36:1417–20.
[116] Zhang L, Eisenberg A. Macromolecules 1996;29:8805–15.
[117] Dawson BE. Kinetics and mechanisms of reactions. London: Educational Ltd; 1973.
[118] Burke SE, Eisenberg A. Polymer 2001;42:9111–20.
[119] Choucair AA, Kycia AH, Eisenberg A. Langmuir 2003;19:1001–8.
[120] Förster S, Platenberg T. Angew Chem, Int Ed 2001;41:688–714.
[121] Zhang L, Yu K, Eisenberg A. Science 1996;272:1777–9.
[122] Santore MM, Discher DE, Won Y-Y, Bates FS, Hammer DA. Langmuir 2002;18:7299–308.
[123] Yu K, Bartels C, Eisenberg A. Langmuir 1999;15:7157–67.
[124] Burke S, Shen H, Eisenberg A. Macromol Symp 2001;175:273–83.
[125] Peng H, Chen D, Jiang M. Langmuir 2003;19:10989–92.
[126] Shen H, Eisenberg A. Angew Chem, Int Ed 2000;39:3310–2.
[127] Hammer DA, Discher DE, Bates FS, Discher B, Won Y-Y, Lee CM, et al. NASA Conf Publ 2001;210827:293–4.
[128] Discher BM, Hammer DA, Bates FS, Discher DE. Curr Opin Colloid Interf Sci 2000;5:125–31.
[129] Jin R-h. Jpn Kokai Tokkyo Koho (Kawamura Institute of Chemical Research, Japan) 2003.
[130] Walther M, Faulhammer H, Finkelmann H. Macromol Chem Phys 1998;199:223–37.
[131] Grumelard J, Taubert A, Meier W. Chem Commun 2004;(13): 1462–3.
[132] Ahmed F, Omaswa I, Bates FS, Discher DE. Abstracts of Papers, 36th ACS Middle Atlantic Regional Meeting, Princeton, USA; 2003.
[133] Yu K, Bartels C, Eisenberg A. Macromolecules 1998;31:9399–402.
[134] Evans EA. Biophys J 1973;13:941–54.
[135] Evans E, Skalak R. Mechanics and thermodynamics of biomembranes. Boca Raton: CRC Press; 1980.
[136] Lenormand G, Hénon S, Richert A, Siméon J, Gallet F. Biophys J 2001;81:43–56.
[137] Dai J, Sheetz MP. Biophys J 1995;68:988–96.
[138] Engelhardt H, Sackmann E. Biophys J 1995;54:495–508.
[139] Hammer DA, Discher DE. Annu Rev Mater Res 2001;31:387–404.
[140] Discher BM, Bermudez H, Hammer DA, Discher DE, Won Y-Y, Bates FS. J Phys Chem B 2002;106:2848–54.
[141] Bermudez H, Hammer DA, Discher DE. Langmuir 2004;20:540–3.
[142] Aranda-Espinoza H, Bermudez H, Bates FS, Discher DE. Phys Rev Lett 2001;87:208301.
[143] Dalhaimer P, Bates FS, Aranda-Espinoza H, Discher D. C R Physique 2003;4:251–8.
[144] Nardin C, Winterhalter M, Meier W. Langmuir 2000;16:7708–12.
[145] Sauer M, Meier W. Aust J Chem 2001;54:149–51.
[146] Callisen TH. PCT Int Appl (Novozymes A/S, Denmark), WO 2002.
[147] Choucair A, Soo PL, Eisenberg A. Abstracts of Papers, 227th ACS National Meeting, Anaheim, USA; 2004.
[148] Brož P, Benito SM, Burger P, Saw CL, Heider H, Pfisterer M, et al. J Controlled Release 2005;102:475–88.
[149] Napoli A, Valentini M, Tirelli N, Muller M, Hubbell JA. Nature Mater 2004;3:183–9.
[150] Power-Billard KN, Spontak RJ, Manners I. Angew Chem, Int Ed 2004;43:1260–4.
[151] Checot F, Lecommandoux S, Gnanou Y, Klok H-A. Polym Prepr (Am Chem Soc Div Polym Chem) 2002;43:453–5.
[152] Graff A, Winterhalter M, Meier W. Langmuir 2002;17:919–23.
[153] Pata V, Dan N. Biophys J 2003;85:2111–8.
[154] Nardin C, Thoeni S, Widmer J, Winterhalter M, Meier W. Chem Commun 2000;(15):1433–4.
[155] Nardin C, Widmer J, Winterhalter M, Meier W. Eur Phys J E: Soft Matter 2001;4:403–10.
[156] Graff A, Sauer M, Van Gelder P, Meier W. Proc Nat Acad Sci USA 2002;99:5064–8.
[157] Stoenescu R, Meier W. Chem Commun 2002;(24):3016–7.
[158] Stoenescu R, Graff A, Meier W. Macromol Biosci 2004;4:930–5.
[159] Vriezema DM, Kros A, Hoogboom J, Rowan AE, Nolte RJM. Abstracts of Papers, 227th ACS National Meeting, Anaheim, USA; 2004.
[160] Smith TW, Luca DJ, Kaplan S, Abkowitz MA. Book of Abstracts, 215th ACS National Meeting, Dallas, USA; 1998.
[161] Dalhaimer P, Bermudez H, Discher DE, Abstracts of Papers, 227th ACS National Meeting, Anaheim, USA; 2004.

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