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Recent progress in designing the shell cross-linked polymer capsules for a drug delivery.

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Abstract. This tutorial review highlights the progress made during recent years in the development of the shell cross-linked (SCL) polymer nanocapsules and the impact of the most important scientific ideas on this field of knowledge. More precisely, it provides some guidelines towards the innovative design of SCL polymer capsules and presents different methods that have been used so far. It also includes the discussion on the major topics of stimuli-responsive moieties incorporated into spherical hollow polymer nanostructures. A wide variety of light and pH-responsive compounds together with synthetic strategies which enable introducing them in defined location of macromolecular architectures are illustrated in this article. Moreover, the tutorial explains diverse methodologies for classifying the manifold formations of vesicular systems that allow for the preparation of SCL polymer capsules based on the general approach concerning the organization of polymers into well-defined vesicular assemblies followed by stabilization through covalent cross-linking.

Outline

1. Introduction
   A) Layer-by layer (LbL) ionic complexation.
   B) The emulsion polymerization by using simple surfactants.
   C) The dispersion of oil droplets into water by amphiphilic copolymers.
   D) The self-assembly method using common phospholipids.
2. SCL vesicles obtained in result of ionic complexation using LbL deposition
3. SCL vesicles obtained from block copolymers
4. SCL vesicles obtained in emulsion polymerization from surfactants
4.1. SCL vesicles from simple ammonium surfactants
4.2. SCL vesicles from phospholipid assemblies
5. Conclusions

1. Introduction

Design and working strategies for multifunctional nanocarriers involve selected coupling reaction and engineering.[1,2] These constructions represent a subtle equilibrium between organic chemistry, macromolecular synthesis, and physico-chemistry science.[3-6] The formation of nanoscopic structures with unique morphologies and controlled dimensions is of critical importance for various technological applications such as nanodevices, sensing, and drug delivery. In this context, self-assembly of macromolecules (so-called 'soft nanotechnology') forming nanoscale structures such as spherical micelles or vesicles is a particularly promising strategy.[7] The soft vesicles like liposomes consist of the lipid molecules (phospholipids, surfactants) which can spontaneously aggregate into spherically closed bilayer membranes in water. However, vesicles with reticulated membranes with elastic properties after suspending in an external flowing fluid deform in a complex model and may eventually destroy. Thus, they should be transferred to the hard nanocapsules due to the shell cross linking. Simplified, capsules are made of an extensible polymer shell which is like a solid envelope. The role of this system is the protection the encapsulated substances to avoid its dispersion in the ambient environment or its degradation in contact with it. The characterization of their mechanical properties (shape, size, degree of reticulation, membrane mechanical properties) is difficult owing to their small size and fragility. Controlling the membrane properties is essential to optimize the design and production of specific polymeric capsules for each application. This publication provides the opportunity to confront the various approaches used to study these hollow polymeric capsules and helps to establish some guidelines for future research. The aim of this article is to give an overview of the current state of the development in the field of hollow polymer nanocapsules with the cross-linked shells obtained by using a variety of different techniques.[8,9] The reason for cross-linking is simple and directly linked with the fact that the

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thermodynamically stable nanostructures in bulk will become thermodynamically unstable in solution due to the interaction of solvents and polymers. Thus, conventional capsules exist in solution only at concentrations higher than their CMC (critical micelles concentration), below which become thermodynamically unstable and spontaneously disintegrate, leading to premature drug release. This represents a major obstacle for intravenous drugs delivery applications. The cross-linking polymerizations can substantially enhance the chemical and thermal stability of polymer capsules with respect to dilution and the other external stimuli as a surfactant challenge, the addition of a good solvent, changes in the pH, or the temperature. Stability and structural integrity of the hollow nanostructures can be achieved by varying the degree of crosslinking of the polymeric shell structures. The ultimate goal is to produce hollow polymeric capsules with substantially improved shape stability, structural robustness, and 'smart' or stimuli responsive properties.

Polymer capsules can be created by using different methods including: ***a)*** layer-by-layer (LbL) ionic complexation, ***b)*** the emulsion polymerization by using simple surfactants, ***c)*** the dispersion of oil droplets into water by amphiphilic copolymers, ***d)*** the self-assembly method using common phospholipids.

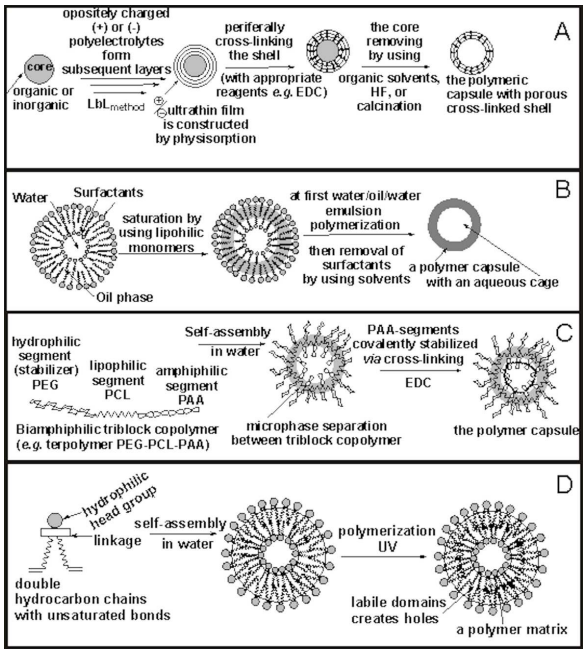
Ad. A) The LbL method depends on ionic complexation and deposition of polyelectrolytes with different charges onto a colloidal template which is then decomposed.[10] Templating[11] introduces covalent stabilization by binding together the shell. Then the polymer chains that constitute the core can be degraded and extracted to leave hollow nanocage networks. The shell cross-links enable complete removal of the core in order to generate hollow (solvent-filled) nanocapsules.

Ad. B) In the mini-emulsion polymerization the monomers are polymerized inside each monomer-containing droplet.[12,13] The membrane of capsules is formed by judiciously choosing the oil phase or monomer so that the produced polymer is insoluble in either the oil or the water phase thus, the polymer then migrates to the oil-water interface to generate the shell.

Ad. C) The preparation of capsules from the direct dispersion of oil droplets into water by ordinary amphiphilic copolymer[14-16] requires a dispersant with at least three components. While one component is soluble in water and another is soluble in oil, the third is soluble in neither oil nor water. Thus, the two former components reduce the interfacial tension between water and oil, and the third component forms a polymer membrane at the interface. Ternary copolymers used for this purpose have so far included linear triblock terpolymers[17] and ternary graft copolymers,[18,19] which consist of a polymer backbone bearing three types of side chains. Capsules made from the self-assembly of block copolymer have been widely investigated, and became the subject of cross-linking treatment.[20-23]

Ad. D) The self-assembly method generates more scientific interest due to the use of common surfactants. It is considered that an amphiphile molecule should be made of the following structural elements which determine the aggregate morphology: (1) flexible tail, (2) rigid segment, (3) hydrophilic head group, (4) spacer group, and (5) additional interacting group.[24] Such features are useful for developing morphology-related functions in artificial molecular assemblies. However, such self-assembled structures (e.g. phospholipids) are often unstable on dilution to below the critical micelle concentration (CMC) indeed, cross-linking is an appropriate method to fix the self-assembled morphologies and modify their properties, such as their permeability or strength.

Table 1. Schematic illustration of four methods for the preparation of polymer capsules: A) layer-by-layer (LbL), B) emulsion polymerization, C) amphiphilic triblock terpolymer, D) self-assembly of phospholipids (surfactants) with unsaturated bonds.



The cross-linking can be obtained by different approaches that have been recently reviewed,[25] with radical cross-linking polymerization and chemical reaction between two functional groups being mainly used. The most common strategy involves cross-linking within the vesicle membrane. Depending on the polymer structure, cross-linking strategies may include: 1) quaternization of poly(vinyl pyridine),[26,27] 2) UV photo-induced cyclization of cinnamates,[28,29] 3) a polymerizable residue as an isoprenyl, diacetylenyl or vinyl groups located along polymer chains,[30-34] 4) moieties containing a polymerizable isocyano function by combination with "click" chemistry,[35,36] 5) gelation of silicates,[37] 6) ring-opening of epoxy groups,[38-42] 7) disulfide formation using thiole bearing compounds,[43] and 8) covalent amide bondings between carboxylic and amine groups according with a standard carbodiimide method.[44] Various dispersed nanostructures have been created from the reactive block copolymers with different cross-linkable

domains, such as the copolymers with poly(2-cinnamoyloxyethyl methacrylate),[45] polybutadiene,[46] polyisoprene,[47] poly(3-(triethoxysilyl)propyl methacrylate)[48] and poly(glycidyl methacrylate).[49] Polymer capsules have attracted much attention because of their sensitivity to the external stimuli such as pH, temperature, light, ionic strength, and electrical field. They have been widely utilized as chemical or biological sensors and nanoreactors[50] due to their changes of property and structure upon exposure to stimuli, but the most remarkable applications of theses capsules are visible in the controlled release and delivery of drugs,[51-53] enzymes,[54-56] hormones,[57] and DNA.[58.59] The detailed understanding diversity in supramolecular structure of the shell cross-linked polymer vesicles equipped with the certain domains could develop innovative vehicles which carried out the special function. Each domain has functional importance, e.g. membrane fusion, transport, recognition, and catalysis. The rational design of well-defined hollow nanospheres with controlled dimensions has become increasingly important, especially the one concerning shell cross-linked nanocapsules, which are covalently stabilized supramolecular structures. The relationships within biological membranes represent one of the most important challenges presently facing chemists and biologists.

2. SCL vesicles obtained in result of ionic complexation using LbL deposition

The layer-by-layer (LbL) technique[60] led to the formation of hollow structures replicating the templating particles in terms of size and shape. The greatest advantage of the LbL protocol is that striking simplicity with which the shell thickness can be tuned to nanometric precision by controlling the physical chemistry and the number of adsorbed molecular layers. The strategies of deposition developed by Decher and co-workers[59] have been explored based on many intermolecular interactions, such as hydrogen bonding,[61-64] charge-transfer interaction,[65] coordination bonding,[66,67] and covalent bonding[68.69] and is now used in order to obtain polyelectrolyte composite capsules.[70,71] These systems exhibit switchable layer/wall permeability and are of particular interest for both loading/release and microreactor applications. The permeability of LbL capsules can be controlled by a number of factors, such as the choice of polymer, number of polymer layers, and surface modification. The diverse template particles such as melamine formaldehyde,[72*a,b,c*] polystyrene,[72*b*] silica oxides,[73,61] gold,[74] and calcium carbonate[75] of different size for layer build up can be used as well. Thus, the cores from decomposable supports could be eliminated by dissolution (dissolved at low pH or in a water-miscible organic solvent such as dimethylsulfoxide) or decomposition (in calcination process during heating to 450 ºC).[76]

The synthetic polymers were used to render vesicle membranes structurally stable and/or environmentally sensitive thus, a pH-dependent complexation of several poly(acrylic acid) or (PAA) derivatives with phospholipid vesicles yielded properties sensitive to pH.[77] The complexation was provided by the formation of hydrogen bonds between un-ionized carboxyl groups of the poly(carboxylic acids) and the phosphodiester functions of the lipid-bilayer surface. The sub-micrometer-sized reactors based on liposomes (from dipalmitoyldiphosphatidylcholine) composed of alternating layers of poly(L-Lysine) or (PLL) with PAA is depicted in the Figure 1. However, such ionically cross-linked vesicles are presumably rather sensitive to the presence of salt, due to screening of the electrostatic attractive forces. The PLL/PAA deposition with the multilayer polyelectrolyte shell was further stabilized by covalently crosslinking the carboxylic groups of PAA and *ε*-amine groups of PLL generating amide bonds using *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (EDC)-coupling chemistry. A number of approaches can be established by using covalent cross-linking to stabilize polyelectrolyte[78] films or self-supported membranes of hollow capsules by water soluble EDC, in combination with *N*-hydrosulfosuccinimide, coupling *via* short difunctional oligomeric cross-linker (poly(ethylene oxide); PEO-NH_2),[79*b*] or cross-linked through amidation reactions with a unique pH-sensitive cross-linker (relied upon an acetal-aldehyde conversion) containing a labile central acetal linkage.[80]

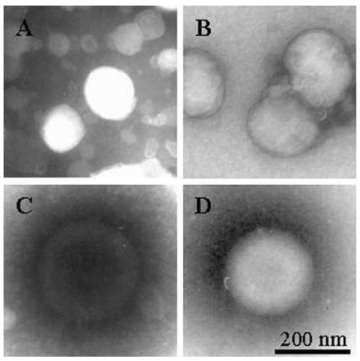


Figure 1. TEM images of liposomes (A) uncoated, (B) coated with 25 polyelectrolyte layers $(PAA/PLL)_{25}$, (C) coated with $(PAA/PLLA)_{25}$ cross-linked layers, (D) coated with $(PAA/PLL)_{25}$ cross-linked layers and 0,1% TX-100. *Reprinted with permission (Adv. Mater. 2006, (18), 2868).*

An alternative route reported by Schaaf utilized to the stabilization of vesicles made from negatively charge phospholipids (2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidyl-DL-glycerol (POPG)) the polycation adsorption, e.g. poly(D-lysine) (PDL) that covered the surface in a very homogeneous and compact way without vesicle disruption.[81] These structures served a different purpose: the liposome shell provided a biologically compatible pH and ion concentration for the encapsulated protein, while the cross-linked multilayer polyelectrolyte shell provided a robust physical structure to protect it. Such supported vesicles were more stable, but due to interactions with the underlying polyelectrolyte skeleton, their shells may have defects, i.e., not all of them were completely tight.[82] Winterhalter[83] encapsultated superparamagnetic nanoparticles in liposomes (from L-*α*-phosphatidylcholine) to the

stepwise adsorption of polyelectrolytes of opposite charges using poly(allylamine hydrochloride) or (PAH) as the polycation and poly(sodium 4-styrenesulfonate) or (PSS) as the polyanion. The four layers of $(PAH/PSS)_4$ which were absorbed alternately on the surface of magnetic liposomes were sufficient to protect the lipid shell from membrane disruption by the detergent (Triton TX-100). Their structures are illustrated in the Figure 2.

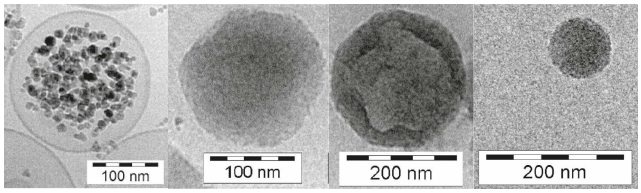


Figure 2. Cryo-TEM images of magnetic capsules in the order: uncoated liposomes, then (PAH/PSS)-coated, after that (PAH/PSS/PAH)-coated and the last $(PAH/PSS)_2$-coated. *Reprinted with permission from (Langmuir 2009, (25), 6793). Copyright (2009) American Chemical Society.*

In a similar method Moya[82] obtained polyelectrolyte capsules by stepwise assembly of PSS and poly(diallyldimethylammonium chloride) instead of PAH onto melamine resin particles as a template followed by the decomposition of the cores by hydrochloride. Also Caruso et.al.[72*b*] formed the polyelectrolyte particles by LbL assembly of poly(diallyldimethylammonium chloride) and PSS onto colloidal melamine formaldehyde seeds through electrostatic interaction, but he used the inorganic-based synthetic lipid, *N*-[*N*-(3-triethoxysilyl)propylsuccinamoyl] dihexadecylamine (Si-lipid) for membrane formation which covered with the polyelectrolyte particles. Finally, it is necessary to demonstrate the versatility of LbL method (formation of the polyelectrolyte complex PSS/PAH) being applicable to other template particles as calcium carbonate ($CaCO_3$).[75*c*] The core of calcium readily degradated in the acetate buffers or was dissolved by extraction and complexation with a chelating agent such as ethylenediaminetetraacetic acid (EDTA). Such a method extends the application in the presence of therapeutics.[75*a*,*b*] These structures are illustrated in the Figure 3.

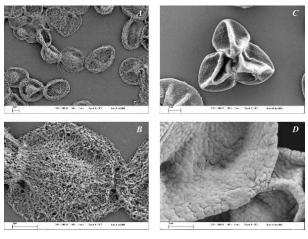
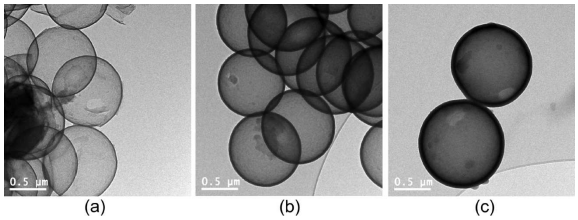


Figure 3. SEM images of $(PAH/PSS)_5$ microcapsules prepared using $CaCO_3$ microparticles template with FITC-BSA (fluorescein isothiocyanate-serum albumin) loaded by physical adsorption in performed particles (A,B) and by capture of protein from solution during growing $CaCO_3$ particles (C, D). *Reprinted with permission (Biotechnol. Prog., 2005, 21, 918).*

The ability to create uniform, mechanically robust nanoshells is templating silica (anionic tetraethoxysilane) onto a sacrificial polymer latex core from a cationic polystyrene (PS). Each subsequent silica layer was deposited after adsorbing cationic poly(allylamine)hydrochloride (PAH). The polystyrene-silica/core-shell particles were calcined at 500 ^{O}C to remove the PS latex core.[84*a*] The example of spherical silica nanoshells is illustrated in the Figure 4. Moreover, the template of the decomposable polyester microparticulate template of biodegradable nature poly(DL-lactic acid) and poly(DL-lactic-*co*-glycolic acid) was covered with biocompatible polyelectrolyte multilayers using PSS and PAH as shell components.[84]



(a) (b) (c)

Figure 4. TEM images of silica nanocapsules made from (a) one, (b) two, (c) three sequential deposition of silica on polystyrene (PS) latex template. subsequent silica layer was deposited after adsorbing cationic poly(allylamine)hydrochloride (PAH). PS cores were removed from sample by dissolution in THF (a) or by calcination (b and c). Scale bars are 0.5μm. *Reprinted with permission from (Langmuir 2014, (30), 584). Copyright (2014) American Chemical Society.*

These hollow structures obtained by core dissolution revealed complete coating with oppositely charged polyelectrolyte pairs. Following on the LbL method from weak polyelectrolytes - biopolymers have been increasingly used since they enable one to obtain biodegradable films (with properties that depend on the pH) or films that can mimic the extracellular matrix, by using polysaccharides[85] (hyaluronan,[86] chitosan[87]), proteins (biotin/streptavidin),[88] alginate/polylysine,[89] and enzymes.[90] So liposomes loaded with macromolecules such as nucleic acids, proteins and enzymes represent a very suitable matrix for polyelectrolyte shells nanocapsule formation.[91] The SCL polymer capsules that were studied used a system of cationic poly(L-lysine) (PLL) with hyaluronan (HA) or (PLL/HA),[44] poly(acrylic acid)/PHA or (PAA/PHA),[92] and poly(L-glutamic acid/poly(L-lysine) or (PGA/PLL).[93*a*,*b*] This chemical cross-linking led to the formation of amide bonds between primary amines in PLL and carboxylate groups in HA. Moreover, chitosan is polycation at low pH, thus it has been used to fabricate capsules with other polyectrolytes (e.g. PAA) that were cross-linked using gluteraldehyde.[87*b*] The example of such capsules containing doxorubicin[73] is presented in the Figure 5. Torres and co-workers[94*a*] developed of hyaluronan (HA) nanocapsules composed of a negatively charged oily lipids core (from Miglyol 812 or Epikuron 170) with encapsulated hydrophobic anticancer drug (docetaxel). The cationic surfactant (hexadecyltrimethylammonium bromide (CTAB), benzalkonium chloride (BKC) or cetylpyridinium chloride) using as an interphase

bridge coated the oily core was surrounded by the shell of negatively charged hydrophilic polymer from hyaluronic acid.

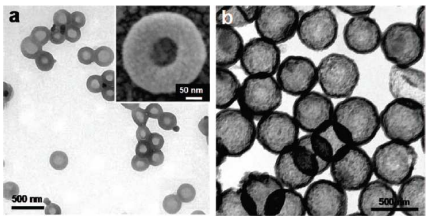


Figure 5. TEM images of PLL nanocapsules formed using silica particle templates, follow by cross-linking and removal of cores. The cross-linking agents used are (a) glutaraldehyde, or (b) 3,3'-dithiopropionimidate dihydrochloride. Scale bars are 500 nm. *Reprinted with permission from (Nano Letters 2008, (8), 1741). Copyright (2008) American Chemical Society*.

Also Alonso et. al. [94*b*] obtained polyglutamic nanocapsules for oncological therapy with an encapsulated drug (plitidepsin) in similar way, by using a modified solvent displacement technique, where the polyamino acid was electrostatically deposited as a coat onto the lipid core. An alternative type of capsules they obtained using polyarginine,[94*c*] as well as the system of hyaluronan (HA)/chitosan (CS) as a model of gen delivery vehicles, in which CS were cross-linked due to the electrostatic interaction between the CS and the HA or the crosslinking of CS was induced by tripolyphosphate.[94*d,e*] Since, in principle, vesicle cross-linking can be achieved by ionic complexation[95*e*] thus, Kataoka and co-workers used a binary mixture of block-ionomer poly(ethylene glycol): PEG-polyacid with a PEG-polybase to produce a vesicular polyion complex by modulating the chain length of PEG and ionomers segments.[95*a*] These structures are shown in the Figure 6. Such polyion complex capsules, which are self-assembled from a pair of oppositely charged PEG-based block aniomers [PEG_{45}-poly(α,β-aspartic acid)$_{75}$] and homocatiomers poly([5-aminopentyl]-α,β-aspartamide)$_{82}$[95*b*] can be cross-linked in a controlled manner using EDC to tune the stability and the permeability, which leads to a controlled release of payloads in physiological environments.[95*c*] Alternatively, cationic steric stabilizer chains of the block copolymer (PEG-*b*-PLL) with selectively introduced thiol groups to a fraction of the lysine repeat units formed a unimer polyion complex by addition of an anionic homopolyelectrolyte comprising a single siRNA molecules or gold nanoparticles.[95*d*] Their structures are illustrated in the Figure 6.

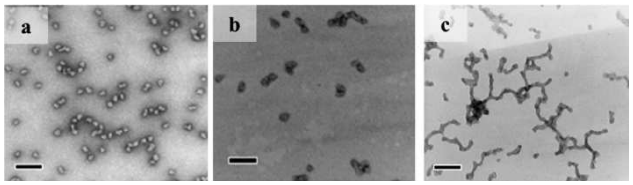


Figure 6. TEM images of polyion complex capsules obtained at room temperatures, and polymer concentration 1 mg/mL with f_{PEG} : (a) 12.1%, (b) 11.1%, and (c) 10.0%. Scale bars represents 100 nm. *Reprinted with permission from (Macromolecules 2014, (47), 3086). Copyright (2014) American Chemical Society.*

Also Eisenberg and co-workers prepared self-assembled materials from block ionomers PEG-*b*-poly(sodium methacrylate) or (PEG-*b*-PMA) reacting with single tail cationic surfactants. The surfactant ionic groups formed ion pairs with the PMA chain, while the surfactant tails segregated into hydrophobic clusters. PEG segments prevented macroscopic phase separation and stabilized complexes in aqueous dispersions. Resulting complexes spontaneously arranged into capsules composed of closed bilayers from PMA-bound surfactant, with corona from PEO chains.[96,97] Zhang and co-workers[68] reported the use of the negatively charged sulfonate-stabilized polystyrene core covered with positively charged *N*-methyl-2-nitro-diphenylamine-4-diazoresin as a template for deposition of *m*-methylphenol-formaldehyde or (MPR) resin in the synthesis of hollow capsules. The process *in situ* coupling reaction was repeated until the desired number of bilayers was reached, followed by the core from polystyrene was dissolved in tetrahydrofuran (THF). Hydrogen-bonding self-assembly is much weaker than the electrostatic interaction; consequently, the formation and destruction of capsules can be controlled easily by external stimuli, such as pH. Zhang[61] also prepared hollow capsules with successful procedures by removing the sacrificial core (spherical template from polystyrene or SiO_2 particles) without rupturing the multilayer shell, obtained by repeating the alternate deposition process, from poly(vinylpyrrolidone) (PVP) and by using MPR resin as a hydrogen acceptor and donor respectively. The surface coating as the stable PVP/MPR membrane after removing a core (using THF or HF) formed capsules that were stable in neutral or acidic aqueous solutions, but become unstable in alkali solutions due to the break-up of hydrogen bonding as a result of the ionization of the phenolic hydroxyl groups. These structures are shown in the Figure 7. Also Caruso and co-workers[98*a*] designed "smart" nanocapsules from hydrogen bonded polymer multilayers that are cross-linked through disulfide (S-S) bonds, which respond to specific stimuli for therapeutic delivery applications and changes in their environments. He used poly(methacrylic acid) or (PMA) as a hydrogen bonding donor with different ratios of alkyne and protected thiol functionalities ($PMA_{Alk,SH}$). The alternate deposition $PMA_{Alk,SH}$ and PVP as a hydrogen bonding acceptor was prepared on the SiO_2 particle templates yielding stable capsules with the combination of copper-catalyzed azide-alkyne cycloaddition 'click chemistry', followed by removal the core using hydrogen fluoride (HF).

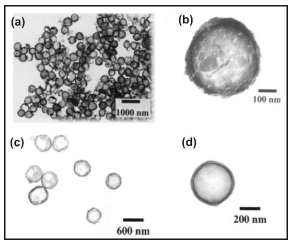


Figure 7. TEM images of five-bilayer hollow PVP/MPR capsules (a, b) and $(PVP/MPR)_{11}$/PVP/PAA/PAH capsules (c, d). *Reprinted with permission from (Adv. Mater. 2003, (15), 832).*

Capsule pH responsive behavior and permeability in the "closed" state (disulfide cross-linking intact) and "open" state (free thiol groups) demonstrated the potential of these gateable systems.[98*b*] The polymer capsules with redox-responsive behavior and intracellular degradability was reported by Caruso.[76*c*] He used an oligo(2-ethyl-2-oxazoline)methacrylate (OEtOxMA) macromonomer which copolymerized with glycidyl methacrylate (GMA) by atom transfer radical polymerization (ATRP) and yielded poly(oligo(2-ethyl-2-oxazoline methacrylate)-*stat*-glycidyl methacrylate) ($PEtOxMA_{GMA}$). The synthesized polymer which was modified with 2-(pyridylthio)-ethylamine (PDA) *via* a ring-opening reaction of the glycidyl groups to introduce redox-sensitive disulfide linkers, after treatment with dithiothreitol revealed deprotected thiol groups in polymer ($PEtOxMA_{SH}$). Sequential deposition of $PEtOxMA_{SH}$/poly(methacrylic acid) (PMA) multilayers onto silica (SiO_2) particle templates and crosslinks through disulfide formation using chloramine T has helped to obtain stable capsules after the removal of the core by buffered hydrofluoric acid. The redox-responsive nature of the disulfide crosslinking groups enables the degradation of these capsules under stimulated intracellular conditions at pH 5.9 and glutathione (GSH). The attractive method of one-pot route to obtain the shell cross-linked hollow nanospheres from photocrosslinkable chitosan[99] i.e. carboxymethyl chitosan (CMCS) mixing with hydrophobic moieties azidobenzaldehyde (Az) was developed by Yin and co-workers.[100] Such amphiphatic biocopolymers self-assemble in aqueous media into nanocapsules in which the Az groups formed hydrophobic microdomains by contrast the carboxymethyl chitosan backbones coil created the photo-cross-linking shells. In other words, inter- and/or intramolecular hydrogen bonds among tightly packet carboxymethyl chitosan backbones will promote self-aggregation of Az-CMCS molecules thus, this phenomenon creates non-covalently cross-linked hollow spherical nanostructures by association of hydrophobic Az molecules. The UV light (at λ = 253.7 nm) induces *in situ* cross-linking of hydrophobic photoreactive phenyl azide functional groups in the shells by elimination of N_2 and forms hollow nanocapsules. The interior and exterior regions of the shell were stabilized by the hydrophilic carboxyl groups forming a void spherical architecture. Thus, carboxylic acid groups located at the surface of the nanocapsules could be seen as a conjugation side of various ligands. This strategy avoiding a process of encapsulation integrated the synergistic effect of self-assembly with drug-loading which is achieved by hydrogen-bonding between hydrophilic polymer as the shell with hydrophobic anti-cancer drug (melphalan) loading as the core for constructing pH-sensitive micelles. Their structures are illustrated in the Figure 8. Moreover, the polymer hydrophilic carboxymethyl chitosan by Schiff base reaction with 4-pyridinealdehyde formed "amphiphilic graft-like" complex with melphalan *via* hydrogen bonding with pyridine groups.[101]

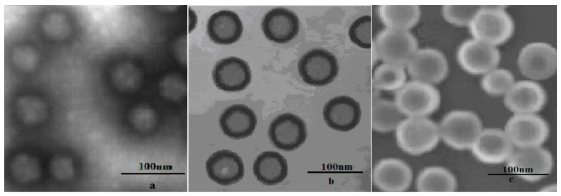


Figure 8. TEM images of Az-CMCS nanocapsules before (a) and after cross-linking (b). SEM image (c) proves the stability of the cross-linked capsules. Scale bars are 100 nm. *Reprinted with permission from (Int. J. Pharm. 2014, 463, 108).*

Couvreur and co-workers[102] reported poly(*iso*-butylcyanoacrylate) aqueous core nanocapsules with entrapped hydrophilic molecules like oligonucleotides (antiviral nucleotide analogues azidothymidine-triphosphate and cidofovir) for clinical use, but it is limited due to hydrophilicity and highly restriction their diffusion into the target cells.[103] The presence of cationic polymers (i.e. poly(ethyleneimine, PEI) or chitosan) in the nanocapsule aqueous compartment allowed successful encapsulation of oligonucleotides. The combined strategy based on the entrapment of azidotymidine-triphosphate/PEI complexes into hybrid poly(*iso*-butylcyanoacrylate) nanocapsules improves the delivery to macrophages. Their structures are presented in the Figure 9.

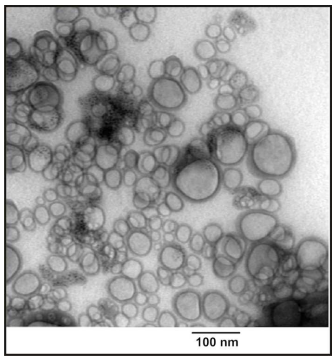


Figure 9. The poly(*iso*-butylcyanoacrylate) nanocapsules observed in TEM. The scale bar is 100 nm. *Reprinted with permission from (Int. J. Pharm. 2007, (331), 148).*

Thus, to sum up, the crosslinking is essential to retain conformational reorganization of the polyelectrolyte chains induced by a change in the solvent medium, which in turn leads to precipitation. Such counterion cross-linked vesicular systems also have an added advantage; they may retain the fluidity of the lipid bilayer while at the same time possessing enhanced stability. Notabene, even though many shell cross-linked capsules have been made from synthetic block copolymers through a strategy of self-assembly of block copolymers with selective cross-linking on the shell block, it is still not possible to synthesize well-defined block copolymers from natural polysaccharides.

3. SCL vesicles obtained from block copolymers

Compared to conventional surfactant-based liposomes, polymer-based vesicles have much thicker membrane walls (offering a greater barrier to diffusion), are more durable/robust, and offer the intriguing possibility of tunable physicochemical and biological properties by simply varying the block copolymer structure. In a block-selective solvent, which is good for one but poor for the other block, a diblock copolymer may form vesicles with the soluble block in contact with the solvent to stabilize the collapsed insoluble block. The preparation of nanocages appears to be significant synthetic challenges, and such difficulties to obtain cavity-containing nanostructures encourage looking for the further development of self-assembly and templating approaches.[104] This technique relies upon the micellization of diblock copolymers into spherical particles of core-shell morphology, followed by the network formation selectively throughout the shell layer, and then degradation and extraction of the core material to leave the membrane-like shell as the hollow nanosphere (nanocage) product. Wooley with co-workers[26] designed a pioneering method that involves a combination of linear polymer synthesis with self-assembly, and as a result the shell-cross-linked (SCL) knedel-like structures were achieved. In her work, a linear block copolymer of poly(styrene-*b*-(4-vinyl pyridine)) or (PS-*b*-P4VP) was synthesized in which PS served the hydrophobic block and P4VP was quaternized to generate the hydrophilic block for the introduction of the cross-linkable functional groups. Due to the reaction of the copolymer with *p*-(chloromethyl)styrene using 4,4'-azobis(4-cyanovaleric acid) as a water-soluble radical photoinitiator (at UV irradiation λ = 254 nm), stabilization of the micelles was accomplished. Thus, cross-linking took place within P4VP block through polymerization of the styrenyl side chain groups, which occurred in the peripheral aqueous layer – exterior layer of the micelles, that formed outer P4VP shell and PS mobility core. The structures are composed of an immobile but permeable cross-linked coat and mobile non-cross-linked core region. More importantly, condensation reactions between diamino cross-linkers and pendant carboxylic acid groups along the poly(acrylic acid) (PAA) segments located in the periphery of the polymer micelles composed of a poly(*cis*-1,4-isoprene) hydrophobic core domain and acrylic acid diblock copolymers (PI-*co*-PAA) yielded the amphiphilic shell cross-linked knedel-like nanostructures.[105*a*] In order to obtain the hollow nanostructures the double bonds presented along the backbone of the *cis*-1,4-polyisoprene formed cores underwent oxidative scission upon exposure to ozone, followed by reduction of the ozonides by reaction with sodium sulfite. Hollow nanospheres with a stable network were achieved after removal of the nucleating core domain as small molecule fragmentation products which were extracted by diffusion through the cross-linked shell in a dialysis step. In this way a robust capsule with a polymer membrane surrounding a nanoscale cavity was obtained.[105*a,b*] These nanocapsules are illustrated in the Figure 10.

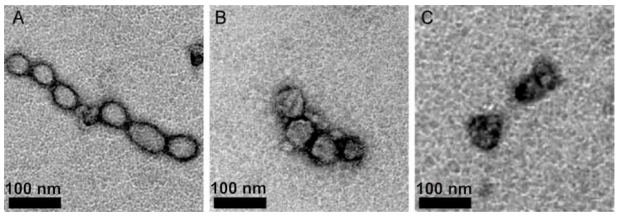


Figure 10. TEM images of PI_{97}-PAA_{78} capsules (A) nanocage, (B) Shiff-base functionalization nanocage-lipid construct, and (C) carbodiimide functionalized nanocage-lipid construct. Scale bars denotes 100 nm. *Reprinted with permission from (J. Contr. Release 2005, (109), 189).*

Likewise, Eisenberg with co-workers[46] investigated the self-assembly of polybutadiene-*block*-poly(2-vinylpyridine) or (PB-*b*-P2VP) diblock copolymers into vesicles in aqueous media. The formed structures with the chelating properties of P2VP were easily accomplished by the ability to cross-link the butadiene block with a UV photoinitiator (Lucirin TPO). He also, explored amphiphilic diblock copolymers PS-*b*-PAA which formed "crew-cut" aggregates with various morphology and studied of ionic polystyrene-*b*-poly(4-vinylpyridinium methyl iodide) or (PS-*b*-P4VMeI).[27] Their structures are illustrated in the Figure 11.

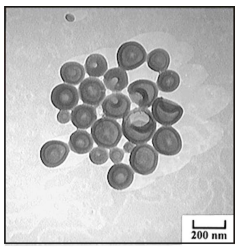


Figure 11. The TEM image of PS_{195}-$P4VPMeI_{18}$ vesicles from 50 wt % THF and 50 wt % dioxane. *Reprinted with permission from (J. Am. Chem, Soc. 1997, (119), 8383). Copyright (1997) American Chemical Society.*

Furthermore, Liu with co-workers[22] examined a polyisoprene-*block*-poly(2-cinnamoylethyl methacrylate) (PI-*b*-PCEMA) sample that formed vesicles in THF/hexane solutions. In such vesicles, PCEMA made up the essentially solvent-free spherical shell and the PI chains stretched into the solution phase from both the inner and outer PCEMA surfaces.[29] Hairy hollow nanosheres were prepared by photo-cross-linking the PCEMA skeleton-shell due to photoinduced cycloaddition and the dimerization among CEMA groups of different chains. Treating the cross-linked vesicles with ozone for a short period selectively degraded the outer PI chains to yield "semi-shaved" hollow nanospheres. "Fully shaved" hollow nanospheres were prepared by degrading both the inner and outher PI chains using long ozonolysis times. Moreover, PI-*b*-PCEMA hollow nanospheres were made water-soluble by converting the PI chains with hydroxylation (by using formic acid and hydrogen peroxide) to a hydroxyformoxy derivative, which after hydrolysis of the formyl ester in aqueous sodium hydroxide yields poly(2,3-dihydroxyl-2-methyl-butane) PHI chains.[47] Such capsules prepared

from tailor-made biocompatible and degradable polymers may be used in drug carriers applications (Figure 12).

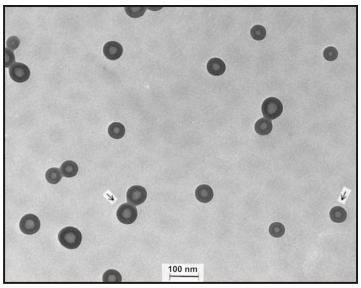


Figure 12. TEM images of PI-*b*-PCEMA vesicles prepared in hexanees/THF (20 %). b) The schematic structure of diblock polymer polyisoprene-block-poly(2-cinnamoylethyl methacrylate) or PI-*b*-PCMA. The scale bar is 100 nm. *Reprinted with permission from (Chemistry of Materials 1998, (10), 537). Copyright (1998) American Chemical Society.*

Wooley transferred the effect of the cross-linking on the properties of knedel-like nanostructures from biodegradable copolymers possessing a hydrolytically degradable core domain. Poly(*ε*-caprolactone)-*b*-poly(acrylic acid) or (PCL-*b*-PAA) amphiphilic diblock copolymers was used in the process of self-assembly into polymer micelles followed by intramicellar cross-linking of the hydrophilic shell layer *via* condensation reactions between the carboxylic acid functionalities of PAA and the amine groups of 2,2'-(ethylenedioxy)bis(ethylamine) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide as the coupling reagent. The further modification of micelles by hydrolytically degradation of PCL core domain due to acidic or basic hydrolysis gave transformation to nanoscale cage-like membranes.[105*c,d*] Liu reported copolymers with poly(*tert*-butyl acrylate) or P*t*-BA components which can readily hydrolyze (using a formic or trifluoroacetic acid mixture) to PAA. He found, that long PAA chains are water-soluble under basic conditions due to deprotonation, but become insoluble in water under acidic conditions (the apparent pK_a value of PAA is rather small at 4.7).[106] On the other hand, acrylic acid (AA) groups that have been protonated can undergo H-bonding with amine group (units) and performed complexes which have poor solubility in water even at pH = 7.0. Moreover, PAA, P(*t*-BA), and poly(methacrylic acid) can produce pores as templating porogens *via* the expansion of the polyelectrolyte chains upon their neutralization with a base.[107*a,b*] Membranes containing PAA-linked nanochannels may facilitate water or water vapor transport. The pH-dependent conformation change of the PAA chains may enable gated water flow as has been demonstrated for other types of PAA-containing membranes.[107*c,d,e,f,g;*108,109] These membranes with "chemical valves" should be useful in controlled drug release or chemical sensing. Chen with co-workers reported the reactive self-assemblies prepared from block copolymers poly(*t*-butyl acrylate)-*block*-poly(glycidyl methacrylate) or P(*t*-BA)-*b*-P(Gly) synthesized by the atom transfer radical polymerization (ATRP) method. The P(*t*-BA)-segment was selected because in the process of hydrolysis *t*-Bu groups into the PAA domains (under HF or CF_3COOH) other ester bonds were well-preserved.[37-39] Glycidyl methacrylate (Gly), instead, is a functional monomer with both vinyl group and epoxy group in its molecular structure, and it can be polymerized by both radical polymerization and ring-opening polymerization.[110,111] The radical polymerized P(Gly) bears pendant epoxy groups which are reactive for nucleophilic attack of various chemicals and readily cross-linked with a variety of amines. At the same time, the amines that react with epoxy can bear various functional groups; therefore, they not only can act as cross-linking agents but also may endow the cross-linked domains with a variety of functionalities. Chen showed that after cross-linking of P(Gly) blocks by addition of primary amines (e.g. propargylamine, PA) bearing functional groups can endow the nanoobjects with more functionalities simultaneously. When PA was used to cross-link in P(Gly) blocks, the alkyne groups were introduced to conduct click chemistry of cycloaddition with the azide groups. Also, the azide groups could be introduced into the partial cross-linked nanoobjects by reaction between the remained epoxy groups and NaN_3.[38*a*] Chen also reported a reactive unilamellar vesicle by self-assembly of poly(ethylene oxide)-*block*-poly(glycidyl methacrylate) copolymer or (PEO-*b*-PGly).[38*b*] The vesicular morphology was fixed by cross-linking *via* the primary amines (3-aminopropyl trimethoxysilane (APS), hexamethylenediamine (HD), dodecylamine (DA)) which reacted with the two epoxys to generate a tertiary amines. In the present system, the epoxys were grafted along the polymer chain, and the cross-linking reaction would have occurred if the reacted epoxys came from different copolymers. These capsules are illustrated in the Figure 13.

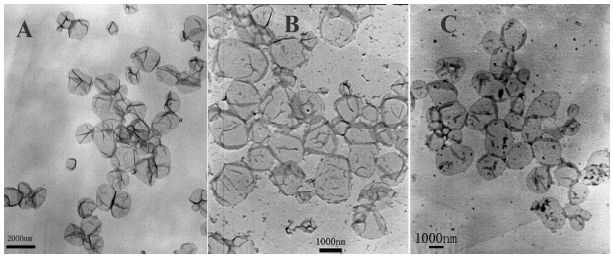


Figure 13. TEM images of PEO_{43}-*b*-$PGMA_{121}$ vesicles in the presence of cross-linking agents: (A) HAD (0.091 wt %), the scale bar is 2000 nm; (B) DA (0.14 wt %), and (C) the vesicles in (B) were treated with THF. Scale bars are 1000 nm. *Reprinted with permission from (Langmuir 2007, (23), 790). Copyright (2007) American Chemical Society.*

Moreover, the addition of 3-aminopropyl trimethoxysilane induced sol-gel process originated from trimethoxysilyl groups. Thus, both reactions would result in a cross-linking network in the vesicles. Armes with co-workers obtained block copolymers with cross-linkable and/or degradable blocks by using ATRP. The copolymer poly(solketal methacrylate)-*b*-poly((N,N,-dimethylamino)ethyl methacrylate) or ($PSMA_{105}$-*b*-$PDMAEMA_{122}$) was used to form hollow nanospheres. This diblock was targeted because PDMAEMA coronas block could be cross-linked by 1,2-bis(2'-iodoethoxyl)ethane, which transformed the tertiary amine groups

into quaternary as demonstrated in the literature.[112] The acetonide groups of the PSMA core block could be hydrolyzed under acidic condition to yield poly(glyceryl methacrylate).[113] Thus, the coronas of micelles from PSMA-*b*-PDMAEMA was cross-linked, and the hydrophobic block - formed cores of the nanospheres were made hydrophilic by hydrolyzing the acetonide groups of PSMA.[114] The spherical structures are illustrate in the Figure 14.

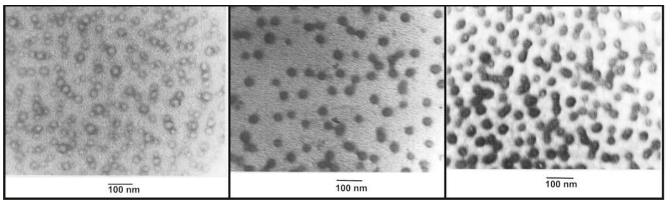


Figure 14. TEM images of block copolymer PSMA-*b*-PDMAEMA micelles: the first illustrates objects before the cross-linking, the second after cross-linking, and the last depicts cross-linked objects after acetonide group removal. Scale bars are 100 nm. *Reprinted with permission from (Macromolecules 2000, (33), 7577). Copyright (2000) American Chemical Society.*

Armes has also developed a self-assembly formulation by using living free radical polymerization with reversible addition-fragmentation chain transfer (RAFT) to explore diblock copolymers in an aqueous dispersion polymerization. The diblock copolymer comprised biomimetic poly(2-(methacryloyloxy)ethyl phosphorylcholine) or (PMPC) chain transfer agent served as the solvated "A" block and as a chain extension of the hydrophobic zwitterionic "B" block with poly(2-hydroxypropyl methacrylate) or (PHPMA) as a core-forming. In the presence of varying levels (zero or relatively low) of ethylene glycol dimethacrylate (EGDMA) cross-linked spherical morphologies of vesicles or micelles were observed.[115*b*] These structures are illustrated in the Figure 15.

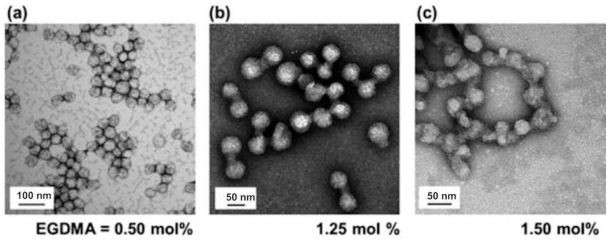


Figure 15. TEM images obtained for: (a) $PMPC_{50}$-($PHPMA_{400}$-stat- $EGDMA_2$), the scale bar is 100 nm, (b) $PMPC_{50}$-($PHPMA_{400}$-stat-$EGDMA_5$), the scale bar is 50 nm, (c) $PMPC_{50}$-($PHPMA_{400}$-stat- $EGDMA_6$) prepared by RAFT aqueous dispersion polymerization at 70 ^{o}C using $PMPC_{50}$ as a chain transfer agent, and the scale bar is 50 nm. *Reprinted with permission from (Soft Matter 2011, (7), 10787).*

He also prepared the zwitterionic diblock copolymers comprising pH-responsive poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) or a poly(2-(dimethyamino)ethylmethacrylate) (PDMA) with highly biocompatible poly[2-(methacryloyloxy)ethyl phosphorylcholine] (PMPC).[116] These copolymers were evaluated for their potential use as nontoxic intracellular delivery vehicles because they contained the biomimetic phosphorylcholine motif. Thus, such vesicles can be considered as very close polymeric analogues of conventional surfactant-based liposomes. PDPA homopolymer dissolves in water below pH 6 as a weak cationic polyelectrolyte, but becomes insoluble above approximately pH 6 due to deprotonation of its tertiary amine groups (its pK_a is around 6.3).[117] The vesicles were stained with gold nanoparticles by incorporation of $AuCl_4$ as a counterion at pH 6.4-7.0 in diluted aqueous $HAuCl_4$ solution, which partially protonated the PDPA chains. These vesicles are presented in the Figure 16.

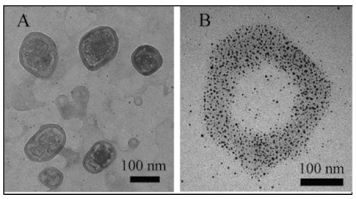


Figure 16. TEM images of (A) vesicles prepared from $PMPC_{25}$–$PDPA_{120}$ copolymer at pH 7, and (B) vesicles decorated with gold nanoparticles located within the vesicle walls after in situ reduction of $HAuCl_4$ (pH 6.4). Scale bars are 100 nm. *Reprinted with permission from (J. Am. Chem. Soc., (2005, (127), 17982). Copyright (2005) American Chemical Society.*

Polymerization-induced self-assembly offers an efficient and highly attractive route for the direct synthesis of a range of diblock copolymer nano-objects. For example, polymerization of benzyl methacrylate (BzMA) using a macromolecular chain transfer agent as poly(methacrylic acid) PMAA *via* RAFT chemistry produced a diblock copolymer that becomes highly anionic at (or above) neutral pH due to extensively ionization of the PMAA chains. Cross-linking was conducted for strengthening vesicular morphology by addition of EGDMA as a third short block after most of the BzMA comonomer had been consumed, so a desired copolymer morphology had been achieved for $PMAA_{70}$-$PBzMA_{200}$-$PEGDMA_{20}$ triblock copolymers. Moreover, semi-fluorinated triblock polymer was synthesized from these diblock (PMAA-*b*-BzMA) precursor using 2,2,2-trifluoroethyl methacrylate (TFEMA) as the second hydrophobic block produced $PMAA_{70}$-$PBzMA_{191}$-$PTFEMA_{223}$ vesicles with phase-separated binary membranes. Their structures are illustrated in the Figure 17.

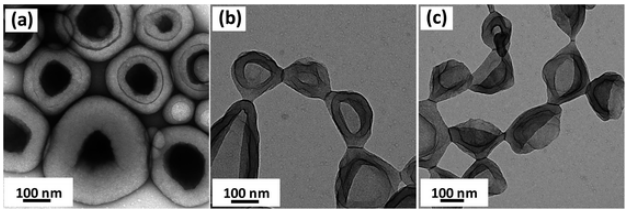


Figure 17. TEM images of $PMAA_{70}$-$PBzMA_{200}$ vesicles (a) before cross-linking, (b) $PMAA_{70}$-$PBzMA_{200}$-$PEGDMA_{20}$ after cross-linking, (c) cross-linked vesicles at pH 9. *Reprinted with permission from* (*Polym. Chem. 2014, (5), 3466).*

Sakurai with co-workers[118a] reported the polysilane with an ability to self-assemble from an amphiphilic diblock copolymer poly(1,1-dimethyl-2,2-dihexyldisilane)-*b*-poly(methacrylic acid). Although polysilanes vesicles from amphiphilic block copolymer (poly(ethylene oxide)-*b*-poly(methylphenylsilane), PEO-PMPS)[119] formed well-defined structures, the recent efforts have focused on the synthesis nanoscale cavity spheres, which have been prepared by chemical degradation of the core-forming block. The reactive methacrylic acid which formed the hydrophilic side chains along the block allowed to compose the cross-linking shell due to the reaction with 1,10-diaza-4,7-dioxadecane and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. Thus, the micelles were obtained due to polysilanes-based encapsulated as the core that was protected by shell cross-linked solvated corona. The preparation of hollow particles from polysilane shell cross-linked micelles was done by photochemical degradation under UV irradiation (λ ≥ 280 nm)[118b] and dialysis against water. Their structures are illustrated in the Figure 18.

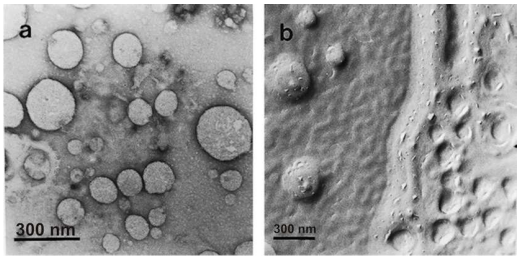
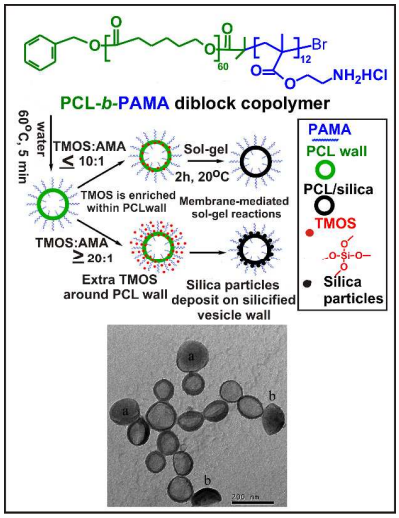


Figure 18. Transmission electron micrographs (TEM) of PEO-PMPS vesicles observed for copolymer dispersions obtained by (a) direct addition to water, (b) ultrafiltration/dilution method (freeze-fracture). Bars represent 300 nm. . *Reprinted with permission from (Chem. Commun. 1998, 1445).*

In a different method which was known as "vesicle membrane-mediated sol-gel reaction Armes et.al.[120a] obtained the organic/inorganic hybrid capsules. The role of vesicles depends on the "template" for a hydrophobic membrane destined for silicification. The silica precursor tetramethyl orthosilicate (TMOS) was chosen because of its poor water solubilty and therefore preferentially solubilization within the hydrophobic domains. A direct dissolution of the diblock copolymer poly(ε-caprolactone)-*block*-poly(2-aminoethyl methacrylate hydrochloride) (PCL_{60}-*b*-$PAMA_{17}$) in water at 60 °C caused the spontaneous formation of vesicles with the hydrophobic PCL membrane and the hydrophilic PAMA chains expressed at both the interior/exterior walls. The copolymer was synthesized by (i) ring-opening polymerization of ε-caprolactone in anhydrous toluene, (ii) end group modification by esterification with 2-bromoisobutyryl bromide producing the desired macroinitiator (PCL_{60}-Br), and (iii) ATRP polymerization of 2-aminoethyl methacrylate hydrochloride. These vesicles were stabilized by aqueous sol-gel reactions using TMSO accumulated within PCL. Moreover polycondensation of the TMSO was catalyzed by the cationic PAMA chains, particularly at the membrane/solution interface. Calcination of the silicified vesicles at 800 °C led to the creation of hollow purely silica particles (Scheme 1).



Scheme 1. Formation of PCL-*b*-PAMA diblock copolymer vesicles by direct dissolution and their subsequent silicification using TMSO according to vesicle membrane-mediated sol-gel reaction mechanism. At the bottom there is a TEM image obtained for PCL_{60}-*b*-$PAMA_{17}$ vesicles after membrane-mediated silicification. The scale bar is 200 nm. *Reprinted with permission from (Langmuir 2008, (24), 13710). Copyright (2008) American Chemical Society.*

A similar method was used in the case of amphiphilic biocompatible zwitterionic diblock copolymer poly(ε-caprolactone)-*block*-poly[2-(methacryloyloxy)ethyl phosphorylcholine], PCL_{60}-*b*-$PMPC_{37}$.[120b] The PMPC block was highly biocompatible and clinically proven to reduce both cell adhesion and protein adsorption. The neutral PMPC chains remained well-solvated and mobile, which suggested that they did not act as the locus for silica deposition. The water-immiscible TMSO precursor was initially solubilized within the hydrophobic membrane prior to its *in situ* transformation into silica.

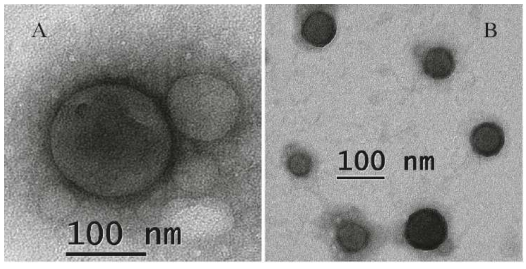


Figure 19. TEM images obtained for PCL_{60}-*b*-$PMPC_{37}$ vesicles after membrane mediated silicification. The TMOS/MPC molar ratio was 10:1 and the reaction time for silicification was (A) 40 h and (B) 4 days. *Reprinted with permission from (Langmuir 2009, (25), 9564). Copyright (2009) American Chemical Society.*

These hybrid organic/inorganic capsules (Figure 19) provided a more robust barrier towards diffusion than conventional surfactant-based liposomes and offered the intriguing possibility of tunable physicochemical and biological properties. Moreover, the hollow interior was suitable for encapsulation of hydrophilic guest

molecules, which could be very useful for biomedical applications. Chen with co-workers also proved the study of organic/inorganic hollow hybrid vesicles based on a reactive amphiphilic diblock copolymer, poly(ethylene oxide)-*block*-poly[3-(trimethoxysilyl)propyl methacrylate], (PEO_{45}-*b*-$PTMSPMA_{59}$) which was synthesized by the ATRP method. Self-assembly of block copolymers with the hydrophilic PEO and the hydrophobic PTMSPMA side formed capsules in a methanol/water solvent mixture. The structure was strengthened using a gelation process involving $–Si(OCH_3)_3$ groups which easily hydrolyzed into $–Si(OH)_3$ with a base catalyst trimethylamine (TEA). These groups were transferred into cross-linked polysilsesquioxane by polycondensation and played a critical role in the morphological fixation.[37a,b,c,d] Such nanocapsules with a cross-linked polysilsesquioxane wall were shape-persistent against environmental changes even after calcination at 450 °C which caused decomposition of the organic PEO chains. Moreover, 3-mercaptopropyltrialkoxysilane (MMS) as the functional silane agent with a –SH group was fused into vesicle walls from poly(ethylene oxide-*b*-poly[3-(triethoxysilyl)propyl methacrylate] (PEO-*b*-PTESPMA) *via* Si-O-Si networks by co-self-assembly of a gelable diblock copolymer and that allowed sulphydryl groups to capture Au nanoparticles.[38] This was a facile way to stabilize and functionalize the block copolymer vesicles that can be generalized to other silanes. Their structures are illustrated in the Figure 20.

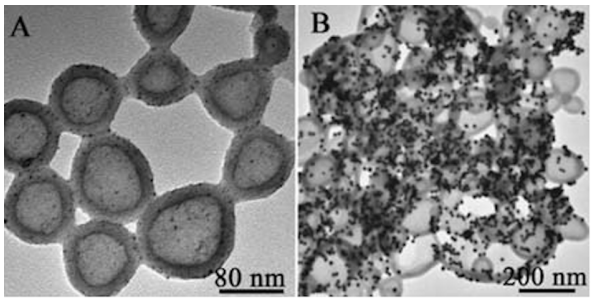
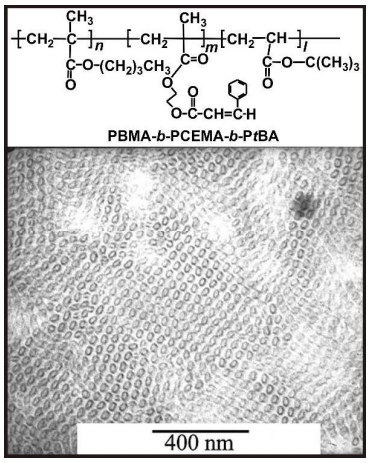


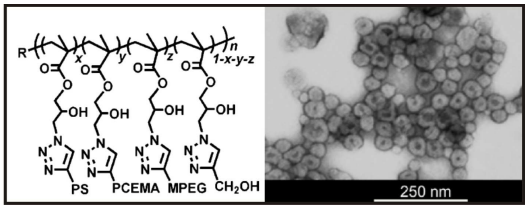
Figure 20. TEM images of vesicles from PEO_{45}-b-$PTESPMA_{90}$ and MMS decorated with Au nanoparticles by (A) reducing the Au^{+3} ions on vesicles and by (B) absorbing the performed Au nanoparticles. *Reprinted with permission from (Macroml. Rapid Commun. 2008, (29), 1368).*

Linear triblocks $A_nB_mC_1$ are far richer in block segregation patterns than diblocks, as the patterns are governed not only by the volume fractions of the different blocks, as in the diblock case, but also by the linking sequence of the blocks and the relative strength of block-block interactions. Thus, the triblocks of the poly(butyl methacrylate)-*block*-poly(2-cinnamolyloxyethyl methacrylate)-*block*-poly(*tert*-butyl acrylate) or (PBMA-*b*-PCEMA-*b*-P*t*-BA) were used to obtain the self-assembly of cylindrical structures, which were locked in by photo-cross-linking the PCEMA shells (with a focused UV beam at 310 nm). Then nanotubes with PAA-line cores were obtained after cleaving the *t*-Bu groups by trimethylsilyl iodide to hydrolize them selectively in methanol.[45]



Scheme 2. The schematic structure of triblock polymer $PBMA_{630}$-*b*-$PCEMA_{180}$-*b*-$PtBA_{210}$ and TEM image of (PBMA-*b*-PCEMA-*b*-PAA) ellipses with dark shells and light cores. *Reprinted with permission from (Macromolecules 2001, (34), 9112). Copyright (2001) American Chemical Society.*

Liu synthesized the ternary graft copolymer incorporating alkyne end-capped polystyrene (PS), poly(ethylene glycol)methyl ether (MPEG), and poly(acrylic acid) (PAA) as the side chains obtained *via* poly(*tert*-butyl acrylate) (P*t*-BA) hydrolysis, which were randomly grafted onto an azide-bearing poly(2-hydroxy-3-azidopropyl methacrylate) (PGMA-N_3) back-bone *via* "click" chemistry.[18] The resulting nanocapsules has partially cross-linked coronas because of using a diamine 2,2'-(ethylenedioxy)bis(ethylamine) as mentioned Wooley,[79a,b,c,d] that reacted with the PAA chains distributed among MPEG chains of graft copolymers PGMA-*g*-(MPEG-*r*-P*t*BA-*r*-PS). Thus, controlled radical polymerization, in combination with 'click chemistry', plays an important role in the design and synthesis of precursor polymers and copolymers with precisely controlled architecture and tailored properties to impart novel functionalities to the hollow nanostructures (Scheme 3).



Scheme 3. Structure of ternary graft PGMA-*g*-(PS-*r*-PCEMA-*r*-MPEG) polymer and a TEM image of cross-linked capsules. *Reprinted with permission from (Macromolecules 2013, (46), 2646). Copyright (2013) American Chemical Society.*

Chen reported also thermoresponsive organic/inorganic hybrid vesicles, which were prepared by self-assembly of poly(ethylene oxide)-*block*-poly[*N*-isopropylacrylamide-random-3-(trimethoxysilyl)propyl methacrylate] or [PEO_{45}-*b*-P($NIPAM_{63-69}$-*r*-$TMSPM_{9-28}$)] in DMF/water mixture.[121] The copolymers were

synthesized by RAFT mediated radical polymerization using a PEO macromolecular chain transfer agent. The sol-gel reaction of the reactive PTMSPM block was performed to stabilize capsules by cross-linking using trimethylamine (TEA). The membranes of the as formed capsules had generated pores due to the crosslinking-induced tensile force and offered possibilities for exchanging small compounds through the holes for a drug delivery system. The higher cross-linking density of a membrane kept almost the same size of capsules under different temperatures while capsules with lower cross-linking density displayed obviously thermoresponsive size transitions between 22 – 36 °C and exhibited enhanced permeability with temperature elevation.[121] Their structures are illustrated in the Figure 21.

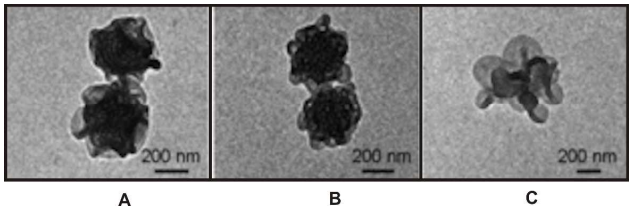
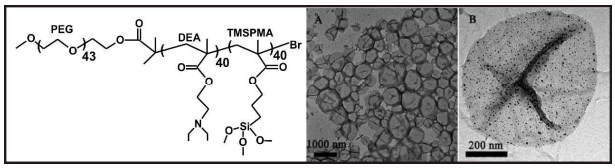


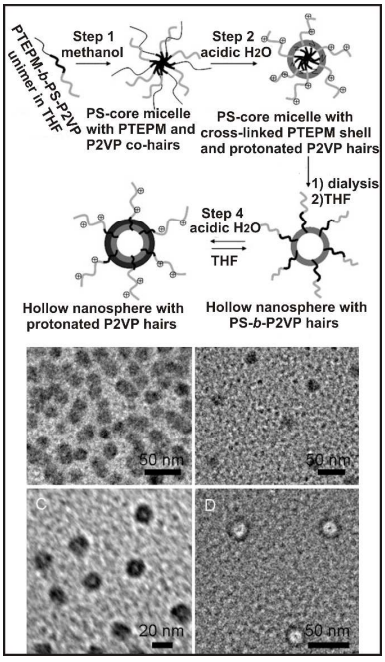
Figure 21. TEM images of gelated large-compound vesicles prepared from (A) PEO_{45}-*b*-P($NIPAM_{63}$-*r*-$THPM_{28}$), (B) PEO_{45}-*b*-P($NIPAM_{69}$-*r*-$THPM_{17}$), (C) PEO_{45}-*b*-P($NIPAM_{66}$-*r*-$THPM_9$). Scale bars are 200 nm. *Reprinted with permission from* (*Macromol. Rapid Commun. 2013, 34, 1169).*

Armes[122] reported the shape-persistent polymeric capsules with pH-tunable membrane permeability, that formed by self-assembly of a pH-responsive hydrolytically self-cross-linkable copolymer poly(ethylene oxide)-*block*-poly[2-(diethylamino)ethyl methacrylate-*stat*-3-(trimethoxysilyl)propyl methacrylate] or [PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$)], in THF/water mixtures. This block copolymer (Scheme 4) was synthesized by statistical copolymerization DEA and TMSPMA in methanol at room temperature, using a standard ATRP method with PEO_{45}-based macro-initiator.



Scheme 4. Structure of the copolymer chain and (A) TEM images of capsules prepared using PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) in 1:2 v/v THF/water. The scale bar is 1000 nm. (B) the same capsules decorated with gold nanoparticles located solely within their walls. The scale bar is 200 nm. *Reprinted with permission from (J. Am. Chem. Soc. 2005, (127), 12800). Copyright (2005) American Chemical Society.*

Because the pH-responsive PDEA block was dissolved in water at low pH as a weak cationic polyelectrolyte, but was insoluble above pH 7 due to deprotonation of its tertiary amine groups, the capsules dissociate below pH 6 due to protonation of PDEA block. The basic DEA residues in the copolymer catalyzed the rates of hydrolysis and capsules cross-linking. The addition of trimethylamine to capsules accelerates the rate of cross-linking due to *in situ* hydrolysis of the $Si(OCH_3)_3$ groups to $–Si(OH)_3$. This led to the deprotonation of the DEA residues, which caused some degree of vesicle reorganization prior to cross-linking. Chen[48] reported the core extractable nano-objects (Scheme 5) that formed by manipulating triblock copolymer micelles from poly(3-(triethoxysilyl)propyl methacrylate)-*block*-polystyrene-*block*-poly(2-vinylpyridine) or (PTEPM-*b*-PS-*b*-P2VP) by changing solvents through chemically crosslinking. In methanol, which is a common solvent of PTEPM and P2VP but a poor solvent of PS, the copolymer formed micelles with a PS core. When changing the medium into acidic water, the PTEPM segments further collapse and gelate to form a cross-linked shell outside of the PS core. When the particles are re-dispersed into tetrahydrofuran (THF), the PS segments are extracted out, producing a uniform small cavity of few nanometers in each particle. The gelable PTEPM block was employed to cross-link the assemblies *via* an *in situ* sol-gel reaction. By changing the dispersing solvent, the structure transformed from solid to hollow spheres, which was attributed to the migration of core-block chains across the cross-linked PTEPM shells of the micelles. These structures are illustrated in the Scheme 5.



Scheme 5. The illustration of preparation of the nano-objects from $PTEPM_{55}$-*b*-PS_{249}-*b*-$P2VP_{432}$ triblock copolymers and the structure transformation under different condition. Follow by TEM images for: (A) micelles of $PTEPM_{55}$-*b*-PS_{249}-*b*-$P2VP_{432}$ formed in the mixture of THF and methanol, the scale bar is 50 nm., (B) cross-linked micelles in acidic water (pH = 2), the scale bar is 50 nm., (C) hollow nanospheres by re-dispersing the cross-linked micelles in pure THF, the scale bar is 20 nm., and (D) hollow spheres by dispersing in acidic water (pH = 2), the scale bar is 50 nm. *Reprinted with permission from (J. Polym. Sci. Part B: Polym. Phys.* 2012, (50), 323).

Meier with co-workers synthesized poly(2-methyloxazoline)-*b*-poly(dimethylsiloxane)-*b*-poly(2-methyloxazoline) or (PMOXA-*b*-PDMS-*b*-PMOXA) triblock copolymers carrying polymerizable methacrylate groups at both chains ends to form capsules.[123*a,b*] These 'macromonomers' were polymerized within the capsules using a UV-induced free radical polymerization and formed hollow polymer nanospheres.[123*c,d*] The polymer chain reaction occurs mainly intravesicularly thus, the polymerization process led to the formation of covalently cross-linked polymer network structures. As a result, the particles possess solid state properties like shape persistence.[123*e,f,g*] The physical and chemical stability of polymer capsules was of great advantage (e.g., nanoreactor applications), but at the same time provided a disadvantage due to the difficulty to exchange substances between the interior and the exterior through the polymer membrane barrier. Thus, Meier pioneered reconstitution of channel proteins (e.g., OmpF, aquaporins) within membranes of PMOXA-*b*-PDMS-*b*-PMOXA copolymers[123*h,i,j,k,l*] introducing permeability into polymersomes to those molecules that can pass through the porins and therefore enable the effective design of nanoreactors. Another way to increase the permeability of the membranes toward hydrophilic molecules was the addition of photoinitiators: *α*-hydroxyalkylphenone and 2-hydroxy-4'-2-(hydroxyethoxy)2-methylpropiophenone (PP-OH), to solution of polymeric capsules. The photoinitiators reacted with the polymer membranes under UV-irradiation causing chemical modification of the polymers with the hydrophilic PP-OH.[123*b*] Their structures are illustrated in the Figure 22.

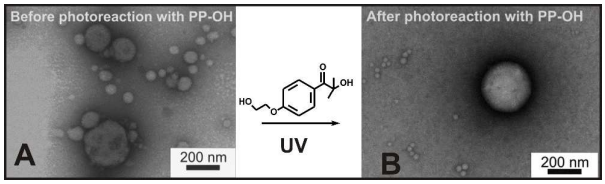


Figure 22. TEM micrographs of polymersomes PMOXA-*b*-PDMS-*b*-PMOXA before (A) and after (B) photoreaction with PP-OH. *Reprinted with permission from (J. Am. Chem. Soc. 2013, (135), 9204). Copyright (2013) American Chemical Society.*

Armes and co-workers[115] synthesized in one-pot route the shell cross-linked nanocages avoiding chemical degradation of the core-forming blocks. In the synthesis, triblock poly(PEO-*b*-PDMA-*b*-PMPC) copolymers were prepared according to the ATRP method with a poly(ethylene oxide) macroinitiator (PEO), which was used to polymerize first 2-(dimethylamino)ethyl methacrylate (DMA) and then 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) using the sequential monomer addition method in 9:1 *i*PrOH/water mixture. Since these types of capsules contain the biomimetic phosphorylcholine motif, they can be considered to be very close polymeric analogues of conventional liposomes. The resulting triblock copolymers underwent *in situ* spontaneous self-assembly to form PMPC-core micelles as a result of the well-known salts effect in the cononsolvency of PMPC chains in response to the mixture consisting of water and alcohol. Such triblock copolymer micelles formed a three-layer "onionlike" structure, with the PMPC block occupying the micelle core and the PDMA and PEO blocks forming the inner shell and micelle corona layers, respectively. The shell cross-linked micelles were obtained by adding a bifunctional quaternizing agent, 1,2-bis(2'-iodoethoxy)ethane, directly *in situ* during the polymerization solution, hence cross-linking the inner shell. The PEG block acted as a steric stabilizer and minimized any intermicelle cross-linking. The low target degree (30%) of cross-linking allowed for the production of shell-cross-linked nanocages due to the PMPC chains' sufficient mobility to migrate into the coronal shells. Thus, these SCL micelles formed "nanocages" in aqueous solution and micelle cores became "hollowed out".[115*a*] Their structures are illustrated in the Figure 23. The self-assembled linear poly(glycerol monomethacrylate-*b*-2(hydroxypropylmethacrylate)) or ($PGMA_{55}$-*b*-$PHMA_{330}$) diblock copolymer capsules conveniently prepared *via* RAFT aqueous dispersion polymerization formulation were easily disrupted by the addition of small molecule surfactants, which caused their rapid dissolution.[115*b,c*] To address this problem a $PGMA_{55}$ macro-chain transfer ($PGMA_{55}$macro-CTA) agent was synthesized using 2-cyano-2-propyl dithiobenzoate (macro-CTA) for statistical copolymerization of HPMA and glycidyl methacrylate (GlyMA) using the 4,4'-azobis(4-cyanopentanoic acid) radical initiator. In this case the GlyMA units were presented as statistically distributed along a non-reactive polymer chain.

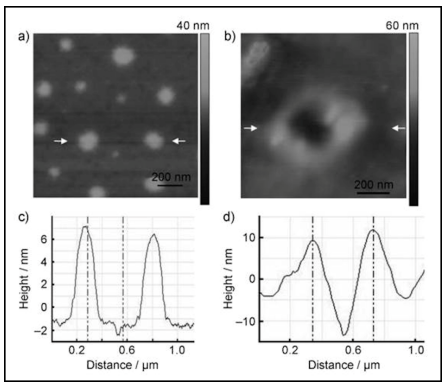


Figure 23. Tapping mode AFM (height images) (a) the capsules at 80% cross-linking in water and (b) the hollow nanocages obtained at 30% target cross-linking in water. Cross-sectional profiles c) and d) were obtained from the images shown in a) and b), respectively. *Repirinted with permission from (Angew. Chem. Int. Ed. 2010, (49), 3500).*

As a result of polymerization induced self-assembly, the capsules obtained from epoxy-fuctional block copolymer poly(glycerol monomethacrylate-*b*-2-hydroxypropyl methacrylate-*stat*-glycidyl methacrylate) or ($PGMA_{55}$-P($HPMA_{247}$-*stat*-$GlyMA_{82}$) were readily cross-linked in aqueous solution by using an addition of polymeric diamines (Jeffamines D-230, D-400, D-2000).[115d] These epoxy-functionalized copolymer capsules reacted rapidly with nucleophilic compounds, such as primary amines, in aqueous solution under mild conditions. The diamine cross-linker was added after capsule formation so as to minimize side-reactions. The treated capsules

were covalently stabilized since they were colloidally stable in the presence of an ionic surfactant. This diamine cross-linking methodology not only produces unique morphologies and excellent surfactant resistance, but also allows amine groups to be conveniently introduced into the vesicles. Thus, they may be unreacted pendant primary amines (if just one primary amine group on the diamine cross-linker reacts with either one or two epoxy groups) or secondary or tertiary amines, respectively. In all cases, these amine groups are readily protonated at low pH and hence should confer cationic character on the copolymer vesicles. Moreover, pendant unreacted amine groups yielded cationic character on these cross-linked capsules offering further opportunities for functionalization. Their structures are illustrated in the Figure 24.

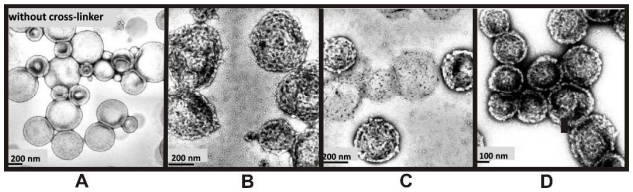
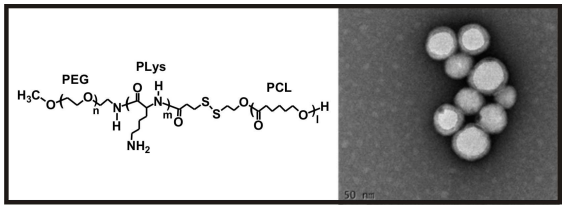


Figure 24. TEM images obtained for (A) noncross-linked $PGMA_{55}$-*b*-($PHPMA_{247}$-*stat*-$Gly(Ma_{82})$ copolymer capsules, and the same capsules after epoxy-amine cross-linking using (B) Jeffamine D-230, (C) Jeffamine D-400, or (D) PEG_{34} diamine. *Reprinted with permission from (Langmuir 2012, (28), 1196). Copyright (2009) American Chemical Society.*

Park with co-workers designed biostable and bioreducible polymersomes for intracellular delivery of doxorubicin and camptothecin.[124*a*] Polymersomes from a biocompatible triblock copolymer were prepared with the use of poly(ethylene glycol)-*b*-poly(lysine)-*b*-poly(caprolactone) bearing a disulfide bond (PEG-*b*-PLys(Z)-SS-PCL). The block copolymers were synthesized by ring-opening polymerization of lysine *N*-carboxy anhydride in the presence of a PEG macroinitiator (CH_3-PEG-NH_2). The obtained diblock copolymer was modified using 3-(2-pyridyldithio)propionic acid *N*-hydroxysuccinimide ester to introduce a pyridyl disulfide group to the chain end of the polymer. Finally, a bioreducible disulfide bond was achieved in subsequent thiol exchange reaction with thiolated poly(caprolactone) (PCL-SH). Then deprotection of the benzyl group produced the triblock copolymers PEG-*b*-PLys(Z)-SS-PCL. The PEG hydrophilic shell protected the polymersomes from intermolecular bridge formation during the cross-linking process, PCL acted as the biodegradable hydrophobic membrane that separated the aqueous core and enabled encapsulation of hydrophobic drugs, and the PLys middle block was used for disulfide cross-linking of the polymersomes. As a consequence, polymersomes were highly stable in extracellular environments, whereas they could be reduced by a high concentration of glutathione (GSH) in the cytoplasm upon endocytosis, causing rapid release of payloads. Also polymer capsules from bioreducible block copolymers based on poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate)s bearing the disulfide bond (PEG-SS-PBLGs) were prepared for intracellular delivery of camptothecin.[124*b*] Their structures are illustrated in the Scheme 6.



Scheme 6. The molecular structure of PEG-*b*-PLys-SS-PCL triblock copolymer and the TEM image of obtained polymersomes. The scale bar is 50 nm. *Reprinted with permission from (J. Mater. Chem. 2012, 22, 22028).*

In addition, Wooley prepared supramolecular assembly of the block graft terpolymer poly(2-ethylbutoxy phospholane)-*block*-poly(2-butynyl phospholane)-*g*-PEG or (PEBP-*b*-PBYP-*g*-PEG) into micelles, followed by shell cross-linking *via* thiol-yne click chemistry[125*a*,*b*] with hexa(ethylene glycol)dithiol and UV irradiation at 365nm.[125*c*] Because polyphosphoesters[125*d*,*e*] due to their biocompatibility and structural similarity to naturally occuring biomacromolecules are biodegradeable, paclitaxel was loaded in polyphosphoester-based amphiphilic *block*-graft terpolymers.

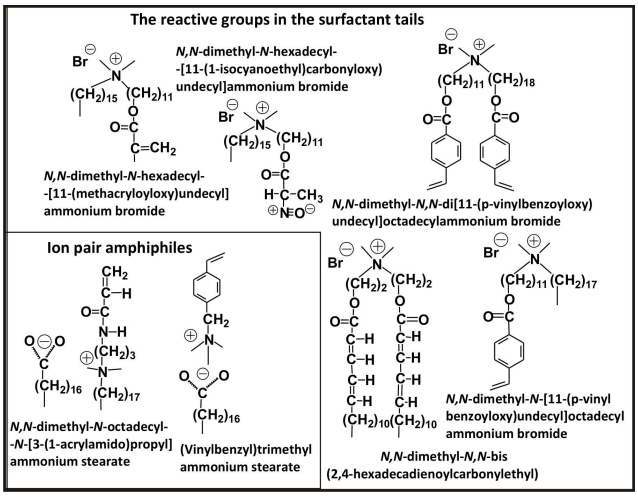
Thus, to sum up, the synthesis of well-defined copolymers have been facilitated by recent development of controlled living radical polymerization and this approach does not involve the removal templates. It is clear that one of the key applications of SCL polymer capsules is their use as nanocarriers for controlled drug delivery. In this respect, the required biocompatible and biodegradable characteristic features are accomplished by terpolymers.

4. SCL vesicles obtained in emulsion polymerization from surfactants

4.1. SCL vesicles from simple ammonium surfactants

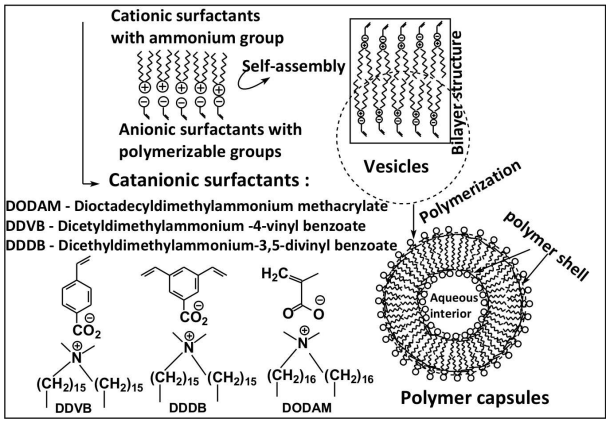
Emulsion polymerization involves the propagation reaction of free radicals with monomers molecules dispersed in a continuous aqueous phase has been widely used in the preparation of SCL hollow nanospheres from the water/oil/water (W/O/W) bilayer emulsion system. Owing to their amphiphilic nature and unique molecular geometry, emulsifier molecules can aggregate in diluted solution into spherical closed W/O/W bilayer structures. These droplets are thermodynamically stable and can be used to prepare SCL vesicles *via* polymerization in the oil phase in W/O/W droplets, which form polymer layer. The notable examples mention the use of polymerizable surfactants, the incorporation of polymers during the formation of vesicles, and surface grafting with water-soluble polymers.[126] The target of interest an amphiphilic systems containing both cationic and anionic surfactants was to capture or template the vesicles structure in order to form hollow polymer capsules. Some commonly used combinations included e.g. didodecyldimethylammonium bromide with sodium dodecyl sulfate (SDS), or cetyltrimethylammonium bromide (CTAB) with sodium octylsulfate.[127-131] Polymerized vesicles have been reported from surfactants containing a polymerizable residue as an isopropenyl, vinyl, or diacetylenyl function.[30*c*,32-34] Their examples are illustrated in the Scheme 7. Enhanced stability, controllable size, rigidities, and

permeabilities have prompted the development of polymerized surfactant aggregates. The ammonium surfactant (e.g. dimethyl-*n*-hexadecyl[11-(methacryloyloxy)undecyl]ammonium bromide) polymerized in vesical form, and resulting capsules retain their spherical nature and aqueous interior while exhibiting enhanced stability.[132*a,b*] Also, amphiphile dimethyl-*n*-hexadecyl[11-((1-isocyanoethyl)carbonyloxy)undecyl]ammonium bromide containing polymerizable properties due to the isocyano function formed capsules by cross-linking presented between the halves of the bilayer of vesicles.[30*c*] The examples of commonly used surfactants for the preparation of SCL capsules are illustrated in the Scheme 7. Moreover, Regen showed that CTAB, a common micelle-forming single-chain surfactant, can be converted into a bilayer-forming polyelectrolyte, when the bromide ion was replaced by a polymeric counterion (i.e. poly(acrylate) or palmitic acid) as in "ion-pair amphiphiles". In principle, ionically paired single chain surfactants could provide a means for "bridging the supramolecular gap" between single-and double-chain amphiphiles.[132*c,d,e*] The only report by Regen describes a potentially crosslinkable diallylammonium group as a counter-ion for dihexadecylphosphate, which formed a "ghost vesicle" [133*a*] stabilized by a polymer coat that supports membranes. There have been only a few reports on nonpolymerized vesicles with the polymerizable group as a counterion[133*b,c*] using the surfactant monomer as a hybrid dioctadecyldimethylammonium methacrylate[133*d*] or choline methacrylate counterion for dihexadecyl phosphate.[134]



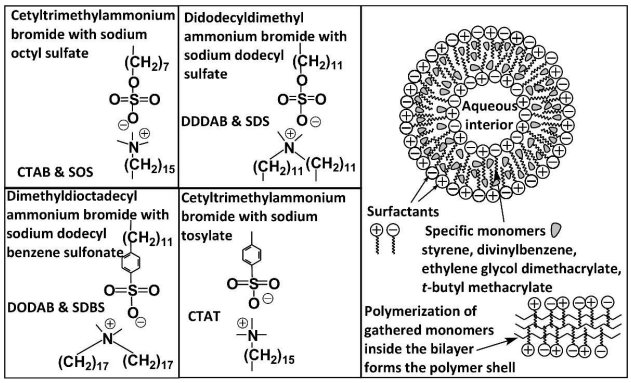
Scheme 7. The examples of commonly used surfactants for preparation of SCL capsules.

The "polymer-encased vesicles" formed the lipid bilayer not covalently linked together but, instead, ionically encased within two concentric poly(methacrylate) monolayers.[133*b*] Also, Jung with co-workers studied hollow polymer spheres with final polymer of styrene and divinylbenzene cross-linking density, which templated from "catanionic" equilibrium vesicles formed spontaneously by mixing cationic and anionic surfactants, e.g. cetyltrimethylammonium tosylate (CTAT) and sodium dodecylbenzenesulfonate (SDBS) or CTAB and sodium octyl sulfate.[137*a,b*] The plausible structure is presented in the Scheme 8.



Scheme 8. The plausible structure of vesicles formed spontaneously by mixing cationic and anionic surfactants.

Moreover, the combination of cationic surfactant CTAB with the anionic surfactant-like ionic liquid 1-butyl-3-methylimidazolinum octylsulfate enhanced solvatophobic interactions yielded vesicles with incorporated in this system moieties of coumarin-153, which induced the fluorescent properties of the shell.[138] The investigation of the polymerization of unsaturated amphiphilic building blocks of a vesicle by immobilization within the aggregate in order to improve vesicle stability and thus increase its lifetime was suggested by Murtagh and others.[135*a,b,c*] The radical polymerization of styrene in the hydrophobic part of dioctadecyldimethylammonium bromide vesicles was performed by German,[136*a*] who described that the polymer particle developed within the midplane of the surfactant bilayer, thus splitting the two halves.[136*b*] Furthermore, Pinkhassik also obtained polymer vesicles from CTAB and SDS associated with hydrophobic monomers using butyl methacrylate and ethylene glycol dimethacrylate[139*a,b*] as well as from CTAT with SDBS using *tert*-butyl methacrylate and 4-*tert*-butyl styrene.[139*c*] The Scheme 9 illustrates the accumulation of monomers inside the bilayer of vesicles. The reactive stabilization of vesicles from single-tail surfactant, isothiuroniumethylhexadecyldimethylammonium bromide (C_{16}SU) cations, that dimerize upon cleavage of isothiuronium group at elevated temperature or alkali pH was also developed.[140-142] Meier and co-workers[143] conducted a cross-linking polymerization of a mixture of hydrophobic methacrylate monomers (1-methacryloyloxybutane, and 1,2-bis(methacryloyloxy) ethane) within the interior of the surfactant bilayer of dimethyldioctadecyl ammonium chloride vesicles that led to the formation of a quasi-two-dimensional polymer network.



Scheme 9. The examples of surfactants which were used to obtain SCL vesicles by saturation of their bilayer with hydrophobic monomers.

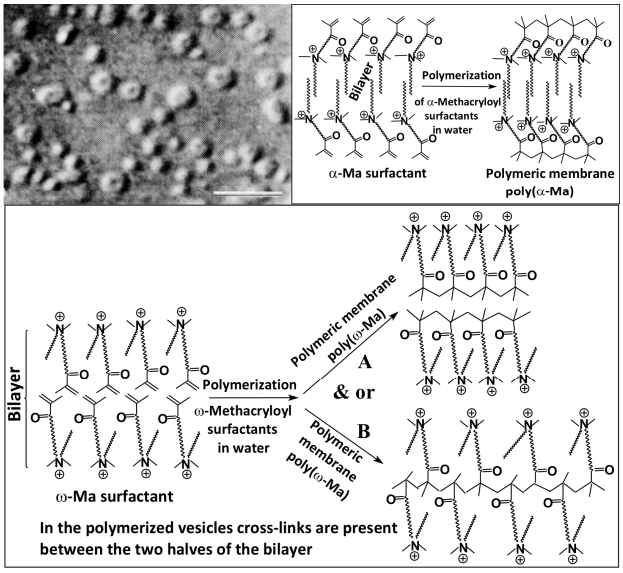
Yuasa and co-workers[144] prepared divinylbenzene bilayer vesicles using vinylbenzyltrimethylammonium chloride and SDS as surfactant. Recently, Forcada and co-workers[145] synthesized biocompatible and thermo-responsive cross-linked nanocapsules through a cationic surfactant dimethyldioctadecylammonium bromide (DODAB) as vesicle templating. For this, random anionic copolymers poly(*N*-vinylcaprolactam-*block*-acrylic acid) or P(VCL-*b*-AA) obtained by RAFT polymerization with dibenzyltrithiocarbonate (as the RAFT agent) were adsorbed onto the cationic vesicles. Then, the cross-linked polymer capsules were synthesized by a semicontinuous emulsion polymerization under monomer-starved conditions for both the main monomer (VCL) and the cross-linker (*N,N'*-methylenebisacrylamide, MBA or ethylene glycol dimethacrylate, EGDMA) in order to obtain a polymer shell around the vesicles, and in this way, to produce biocompatible and thermo-responsive nanocapsules. The similar method was described by Herk[146] who reported the synthesis of pH-responsive polymeric nanocapsules by templating unilamellar cationic vesicles of DODAB using a RAFT-based a short-chain living anionic copolymer from acrylic acid and butyl acrylate units. The chains were further extended to form a thick polymeric shell by feeding a monomer mixture comprising methyl methacrylate and tertiary butyl acrylate (*t*-BA) in combination with the divinyl crosslinker EGDMA under starved feed condition. Subsequent acid hydrolysis of the *t*-BA ester groups of the cross-linked polymeric shell resulted in the formation of pH-responsive nanocapsules.

To sum up, nowadays the use of soft templates, such as emulsion droplets and surfactant vesicles is gaining interest because surfactants are very useful due to their easy preparation with a controllable size. Their advantage is the possibility of pre-encapsulation of different substances before the formation of the nanocapsules. In an emulsion polymerization the polymer shells of nanocapsules were fully formed with no significant pinhole defects thus, it was a successful example of synergistic self-assembly, where components of a temporary supramolecular scaffold and building blocks acted together to form soft nanoscale aggregates. Polymerization of monomers with cross-linkers in the interior of surfactant vesicles resulted in the formation of hollow polymer nanocapsules. Emulsion polymerization is a convenient and economical means for the preparation of polymeric nanocapsules but the selection of emulsifiers is critical to the process.

4.2. SCL vesicles from phospholipid assemblies

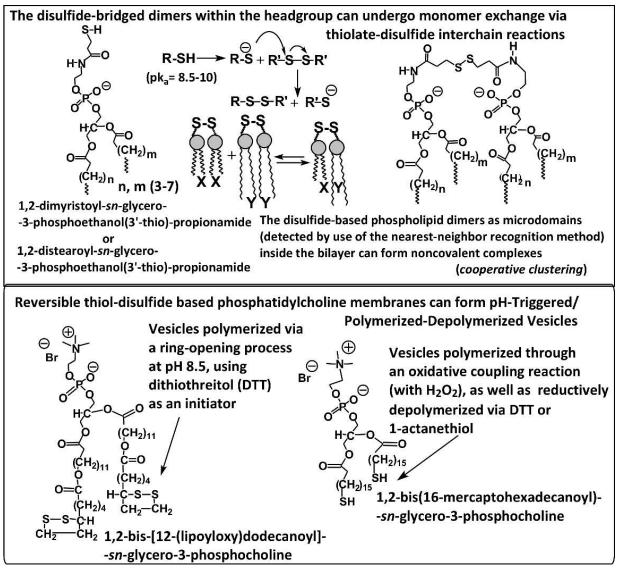
Liposomes are not suited for many material applications because of their sensitivity toward environmental factors such as pH changes, osmotic stress, lipases, and the presence of detergents.[147*a*] Liposomes have two distinct regions: the aqueous core and the hydrophobic bilayer interior for templating of nanocapsules. Thus they can be used as the scaffold for the synthesis of hollow polymer nanospheres.[147*b*] In addition to synthetic liposomes used as membrane models, vesicles are of interest because of their ability to sequester or encapsulate reagents, for the separation of charges and charged species, and for the effect of the lipid bilayer organization on chemical reactions.[147*c*] Increased stability of lipids might be expected to result from a "polymerization" of the bilayer through association of the polymer with charged or polar sites of the amphiphile head group. The polymerization of vesicles produced a more robust polymer membrane that should resist degradation. The obtained membranes are patchy with nanoscale segregated regions of structure and function (nanodomains formation) and also the lipid regions vary in thickness and composition. Many different approaches have been devised to fortify liposomes.[148] Regen with co-workers[133*e*] introduced the concept of polymerized vesicles and suggested that phosphatidylcholine analogues (e.g. bis[12-(methacryloyloxy)dodecanoyl]-L-*α*-phosphstidylcholine) might constitute an important new class of materials which exhibit greater stability than natural liposomes. Although a large variety of polymerized liposomes have been synthesized,[149] only one specific system has been carefully examined with regard to its permeability characteristics; i.e., poly(butadiene phosphocholine) vesicles in comparison with polymeric liposomes from the methacryloyl ammonium lipids (with the methacryl amide attached to the hydrophilic head group *α*-MA, or one of the hydrophobic chain *ω*-MA)[31] like is it illustrated in the Scheme 10. Such polymerized liposomes designed with controllable permeability could reveal a practical value as drug delivery devices having "tunable" release rates.[133*f*] Regen modulated bilayer permeability by controlling the cross-link density of membranes from polymerized liposomes derived from 1,2-bis[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine[30*a,b*] and a non crosslinkable analogue, 1-palmitoyl-2-[12-(lipoyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine, by controlling of the relative packing efficiency of both of them.[30*c*] He also examined a vesicles membrane based on disulfide formation using the thiol-bearing lipid 1,2-bis(11-mercaptoundecanoyl)-*sn*-glycero-3-phosphocholine that can be "swichted on" (polymerized) and "switched off" (depolymerized) *via* oxidation and reduction respectively, in the thiol-disulfide redox cycle.[43*a,c,d,e*] The essential criterion for polymerization was that the oxidation process led *via*

interlipid coupling that one phospholipid unit was a covalently attached to nearest neighbor of another in the bilayer state.[43*h*]



Scheme 10. Schematic representation of *α*-Ma and *ω*-MA lipids in a bilayer membrane before and after formation of poly(*α*-Ma)and poly(*ω*-MA). *Reprinted with permission from (J. Am. Chem. Soc. 1984, (106), 4279). Copyright (2009) American Chemical Society.*

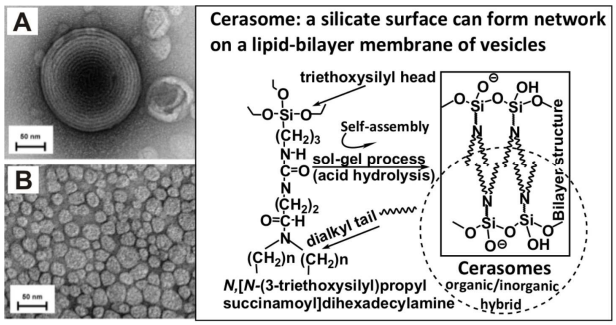
Moreover, this ability provides a method for obtaining a continuum of polymeric/monomeric states within the same vesicle (a preference as nearest-neighbor recognition)[43*b,f*] that can be polymerized, or made to lie in a dormant state, by pH adjustment.[43*g*] If polymerized vesicles could be depolymerized, in a reversible manner, their utility as a membrane "on-off" model would be significantly increased in biochemical studies since they would be suitable as the time-release carriers of drugs. Moreover, the two halves of the bilayer can "cross talk" to each other, because the perturbation of the lateral organization of one leaflet by an external agent also alters the lateral organization of the adjoining leaflet.[133*g,h,i*] like it is illustrated in the Scheme 11. Photo-polymerizable diacetylenic lipids combine the plasticity of lipids with the robustness of polymers, but do not disturb the spontaneous self-assembly of the lipid architecture and retain the biomimetic potential of polymerized lipids. The lipid-based scaffolds, once polymerized, form extremely stable structures which may be used in surface coating for biocompatible materials, supporting matrices for bio-sensing molecules, and carrier vehicle for drugs.[150] Phospholipids molecules with polymerizable groups (diacetylene) inhibit the lipid chain rearrangement within the bilayer leaflet by setting up a covalent bond between lipid moieties during the process of polymerization. The strategy depends on the introduction of photoactivable bonds that utilize the principle of photopolymerization (photo-cross-linking). The self-assembled structures can have diacetylenic groups in the fatty acyl chains[151] but also modifiable reagions of the photoreactive headgroup[152] as synthesized by Ramakrishnan.[153] He introduced a counterion with reactive vinyl groups dicetyldimethylammonium-4-vinyl benzoate (DDVB) or dicetyldimethylammonium-3,5-divinyl benzoate (DDDB).



Scheme 11. Basis of the nearest - neighbor recognition method.

The vinyl functionality was not covalently bonded to the lipid; however, the vinyl groups were electrostatically associated with the lipid polar head. Polymerization of the liposomes counterion shell provided a durable coating around the lipid bilayer, and the bilayer components retained their monomeric state due to their non-covalent interaction. The cross-linked rigid network prevented lipid chain reorganization and the semirigid cross-linked vesicles did not aggregate and remained suspended in solution. In this case of counterion cross-linked systems, the conformational flexibility is greatly constricted. An important advantage of such counterion cross-linked vesicles is that the vesicular bilayer is only electrostatically bound to the cross-linked network and is therefore likely to maintain its fluid nature. Blumenthal and co-workers[154] constructed component liposomes with a photo-polymerizable phospholipid 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine) ($DC_{8,9}PC$) which bears highly reactive diacetylenic groups, that can be polymerized under UV (λ = 254 nm) irradiation to form chains of covalently linked lipid molecules in the bilayer. Diacetylenic (1,2-bis(trideca-12-ynoyl)-*sn*-glycero-3-phosphocholine) or butadienic lipids (e.g. dioctadecadienoylammonium bromide or dimethylbis[2(tetradeca-2,4-dienoyl)oxyethyl] ammonium bromide) were classical candidates for use in the polymerization in fluid phase of the membrane.[155] A stabilization of the liposomes by polymerization of reactive lipid molecules is feasible but destroys the lateral mobility of the lipids required for the functionality of certain membrane proteins. The use of different types of polymerizable groups placed at different locations within the bilayer and forming an integral part of either their hydrophobic hydrocarbon tails[156] or glycerol

backbone near their hydrophilic headgroup was reported by O'Brien et al.[157a] Lipids that contain a single reactive moiety in either of the hydrophobic tails or associated with the hydrophilic head group yielded linear polymers, but polymerization of lipids with reactive groups in each hydrophobic tail yielded a cross-linked polymeric network.[157b] The degree of polymerization was generally increasing with the number of dienoyl groups (bis-substituted lipids) per monomer and depended on the initiation chemistry. Thus, photoirradiation yielded oligomers with insufficient length to create a polymeric network in vesicles, whereas redox-initiated radical polymerization afforded cross-linked polymeric vesicles if the spacer length was 7, 9, or 11 atoms.[156a] In the case where the two diene groups were separated by only six methylene groups, the polymerization did not yield the cross-linked shell vesicles, because lipids with a short spacer preferred to react with the same neighboring lipid in a linear, ladderlike fashion.[147c,d] The differences between the two modes of polymerization offer opportunities to control the material features of polymerized lipid assemblies. Kikuchi with co-workers[158] put forward cerasomes having a lipid-like structure, which formed a liposomal bilayer with a silicate framework on its surface by adopting the sol-gel process for an organoalkoxysilane. These authors used vesicles made from *N,N*-dihexadecyl-N^{α}-{6-[3-triethoxysilylpropyl)dimethylammonio] hexanoyl}alaninamide bromide which were stabilized by the formation of a polymerized silica layer at the vesicle surface by spontaneous condensation of triethoxysilyl groups in water. The cationic and anionic cerasomes belongs to a bioinspired organic-inorganic hybrid composed of a liposomal membrane with a ceramic surface. They obtained magnetic cerosomes with ultrathin magnetic metal layers on the vesicle surface, which allowed to increase various functional coatings by using many kinds of surface modification.[158j,k,] Their structures are presented in the Scheme 12.



Scheme 12. Molecular structures and schematic drawing of the cerasome forming lipids. TEM images of cerasomes prepared by (a) vortex mixing and followed by (b) ultrasonication. Scale bars are 50 nm. *Reprinted with permission from (Chem. Eur. J. 2007, (13), 5272).*

An alternative to surfactant polymerization was the use of the vesicle architecture as a template for the further preparation of polymer capsules. In this case, common hydrophobic monomers were added to swell the bilayers of a vesicle solution and were subsequently polymerized. Using hydrophobic monomers (styrene, acrylates), subsequent polymerization led to the formation of polymer chains entrapped in the hydrophobic part of the membrane to stabilize structures. Winterhalter with co-workers[159] created hollow nanometer-sized polymer capsules serving liposomes as a two-dimentional template for the cross-linking of hydrophobic monomers (butyl methacrylate or hydroxyethyl methacrylate) with the bifunctional ethyleneglycol dimethacrylate. The incorporation of hydrophobic functional constituents that are apt to undergo radical polymerization inside the bilayer was a step toward the development of stable nanocapsules with functionalized walls. In this way polymer capsules with the channel proteins, e.g. magnetic nanoparticles, anchored inside the 2D polymer network was obtained. Moreover, Pinkhassik with co-workers[160] prepared a polymer fabrication within the well-defined environment of a temporary self-assembly scaffold loaded with building blocks to arrange them into a desire shape before forming covalent bonds. Bilayers act as 2D solvents and limit the thickness of polymerized materials. The polymerization of hydrophobic monomers (e.g. styrene, *tert*-butyl styrene, divinylbenzene or *tert*-butylmethacrylate) within the bilayer under UV irradiation was carried out to form nanometer-thin polymer walls. This distribution of monomers within the bilayer corroborates the successful imprinting of nanopores using templating molecules that do not span the membrane. Thus, hollow polymer nanocapsules that have shells containing nanopores with a programmed size and chemical environment with narrow size distribution can be prepared by polymerization of monomers in the presence of pore-forming templates in the interior of liposomal bilayer.[161] This was achieved by locating nonpolymerizable, molecular-size pore forming templates (porogens) in the bilayer along with monomers before polymerization. Thus, the fairly symmetric hydrophobic molecules of pentaesters (glucose pentaacetate (0.8 ± 0.2 nm size) or pentabenzoate (1.3 ± 0.2 nm size)) and their analogues with styrene or methacrylate moieties[162a] were chosen to dissolve them in the bilayer interior. The derivatives of a glucose containing polymerizable groups were copolymerized with monomers and cross-linkers forming a polymer shell inside the bilayer and in this way carboxylic groups were imprinted by using pore-forming templates containing multiple ester groups. Upon hydrolysis in alkaline medium, they formed small hydrophilic molecules of glucose and either acetic or benzoic acid that diffused from the pore into the aqueous solution. Thereby, polymer capsules containing multiple carboxylic groups in the pore orifice were obtained. The bulk of hydrophobic monomers and cross-linkers was concentrated in the interstitial layer in the middle of the lipid leaflet but also throughout the bilayer. Size-selective pores in the shells of hollow polymer nanocapsules enabled a combination of self-assembly and entrapment of molecules. Polymer nanocapsules with the required number of carboxylic groups per pore and number of pores per capsule enabled fine-tuning of the pH threshold and/or kinetics of the release of encapsulated cargo. Thus, nanometre-thick porous walls provide communication with the external environment through imprinted pores which controlled the size-

selective permeability and permit ultrafast transport of species smaller than the pore size.[162b,c] A similar strategy but modified by the incorporation of hydrophobic coumarin derivatives (Coumarin 6) into the lipids bilayer allowed the production of polymer capsules with a high fluorescent wall[163] used for imaging. Moreover, copolymer poly(2-ethylhexyl methacrylate-*co*-7-(4-trifluoromethyl)coumarin acrylamide) formed the cross-linked wall of capsules with encapsulated 5-fluorouracil. These structures were designed for a drug delivery.[163b] Because of a reversible photocyctoaddition of coumarin moieties upon UV irradiation the shell of polymeric capsules was cross-linked under exposure at UV λ > 310 nm but after irradiation at UV λ < 254 nm capsules became cleavage. Thus, the system in which light is able to cause the formation/disruption of polymer capsules was distinguished. These capsules are illustrated in the Figure 25.

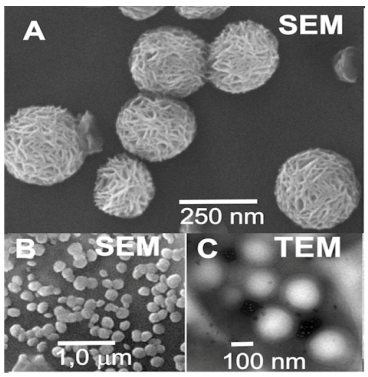


Figure 25. SEM (A, B) and TEM (C) images of polymer nanocapsules generated due to dimerization of the coumarin moieties. The copolymer poly (2-ethylhexyl methacrylate-*co*-7-(4-trifluoromethyl)coumarin acrylamide) formed cross-linked wall. *Reprinted with permission from (New J. Chem. 2015, (39), 1506.)*.

During the past years, chemical lipidology has led to the creation of a large variety of synthetic phospholipids, which formulated "smart" liposomes that reacted to different external stimuli and significantly enhancing the potential for drug delivery.

Table 2. Selective samples of drugs encapsulated in the shell cross-linked polymer capsules.

Drug	Model	Ref.
Doxorubicin	LbL	73
Plitidepsin	LbL	94b
Melphalan	LbL	101
Cidofavir	LbL	103
Camptothecin	Block-copolymer	124b
Doxorubicin	Block-copolymer	124a
5-Fluorouracil	Liposomes template	163b

5. Conclusions

In summary, this article has demonstrated convenient methods to produce the shell cross-linked stable capsules suitable to drug delivery. Thus, the greatest advantage of the LbL method is the simplicity to obtain the shell thickness with the nanometric precision by controlling the physical chemistry and the number of adsorbed molecular layer. However, the flexibility of using a broad variety of colloidal species as templates is strongly limited by the usability of decomposable cores which should be eliminated by dissolution without hazardous hydrofluoric acid or organic solvents. Therefore, the universal template for LbL technology is still searching. Controlling the thickness and composition of the capsules wall should allow selective and switchable permeation for the encapsulation and release of various substances. In the case of block copolymers the inherent flexibility and long-chain nature of most synthetic polymers, as well as the specific interactions between complementary polymers which usually occur in an uncontrollable way lead to irregular structures. It has been a great challenge to construct regular capsules with a monomodal distribution from copolymer building blocks in solution. In turn, biomimetic properties of polymerized lipids remains essential in case of the designing a cross-linked network of polymer capsules. The polymerization of self-assembled dienoyl-lipids structures is a strategy that results in robust biocompatible capsules with diverse reactive functional groups. However, the intensive irradiation by using UV light during the photopolymerization of liposomes can potentially deactivate encapsulated biological molecules. Additionally, the preparation of asymmetric bilayer with different lipids compositions in the inner and outer layers (leaflets), represents a significant challenge. The cross-linked a polylipid bilayer formed stable capsules, but also nonpolymerized lipids can be coated by a cross-linkable wall from polyacrylates, which implement regular interactions with the liposomes surface to protect capsules. Thus, this review demonstrates the development of the shell cross-linked polymer nanocapsules and illustrates their potential use as novel nanomedicines. Engineering of SCL polymer nanocapsules or combined therapeutic and diagnostic applications (as theranostic devices) requires knowledge about their material, chemical, physical, biochemical, and toxicological nature. Their surface can be modified by the incorporation of the special domains for smart drug delivery and improved biocompatibility. Incorporation of fluorescent moieties into SCL layers has resulted in a new paradigm for imaging effects for clinical diagnostic and therapy. SCL polymer capsules as carrier systems have the potential to prolong the half-life of the encapsulated drug in the body through enhanced permeation and retention (EPR) effects. Such systems may find application in diverse fields including templates, catalysis, and sensors, but the main motivation remains their potential use for drug delivery applications. Chemical cross-linking of capsules systems and polymer-drug chemical conjugation can also be exploited for further stabilization of macromolecular therapeutics during systemic circulation, but the use of stimulus-responsive interactions is preferred. The supramolecular self-

assembly together with polymer chemistry led to receive the intended effect in the form of the shell cross-linked polymer capsules. The polymer capsules promise to be an ideal system for combining different approaches for targeting, and, therefore, for a controlled drug delivery and there is still much room in this field for future improvement and development.

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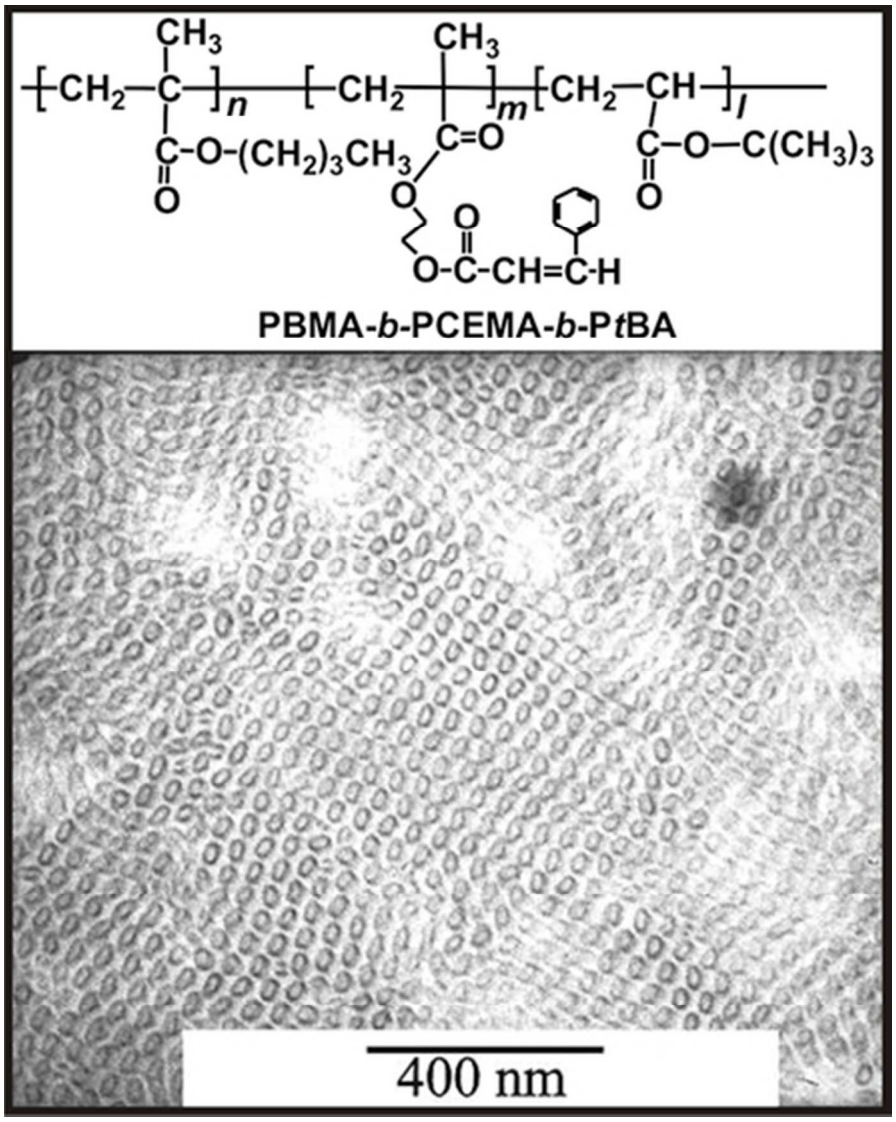
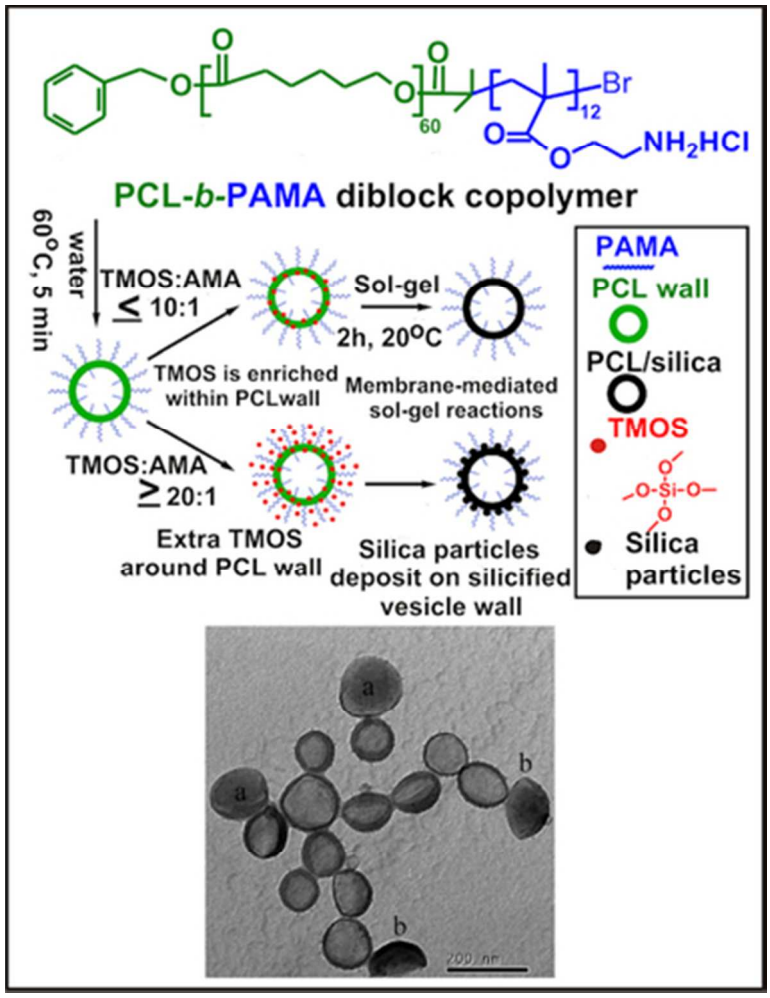
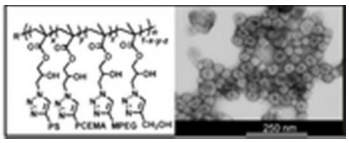


The schematic structure of triblock polymer $PBMA_{630}$-b-$PCEMA_{180}$-b-$PtBA_{210}$ and TEM image of (PBMA-b-PCEMA-b-PAA) ellipses with dark shells and light cores. . Reprinted with permission from (Macromolecules 2001, (34), 9112). Copyright (2001) American Chemical Society.
37x46mm (300 x 300 DPI)



Formation of PCL-*b-PAMA diblock copolymer vesicles by direct dissolution and their subsequent silicification using TMSO according to vesicle membrane-mediated sol-gel reaction mechanism. At the bottom there is a TEM image obtained for* PCL_{60}*-b-*$PAMA_{17}$ *vesicles after membrane-mediated silicification. The scale bar is 200 nm. Reprinted with permission from (Langmuir 2008, (24), 13710). Copyright (2008) American Chemical Society.*
32x41mm (300 x 300 DPI)



Structure of ternary graft PGMA-*g*-(PS-*r*-PCEMA-*r*-MPEG) polymer and a TEM image of cross-linked capsules.
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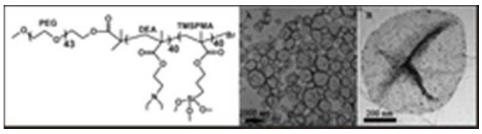
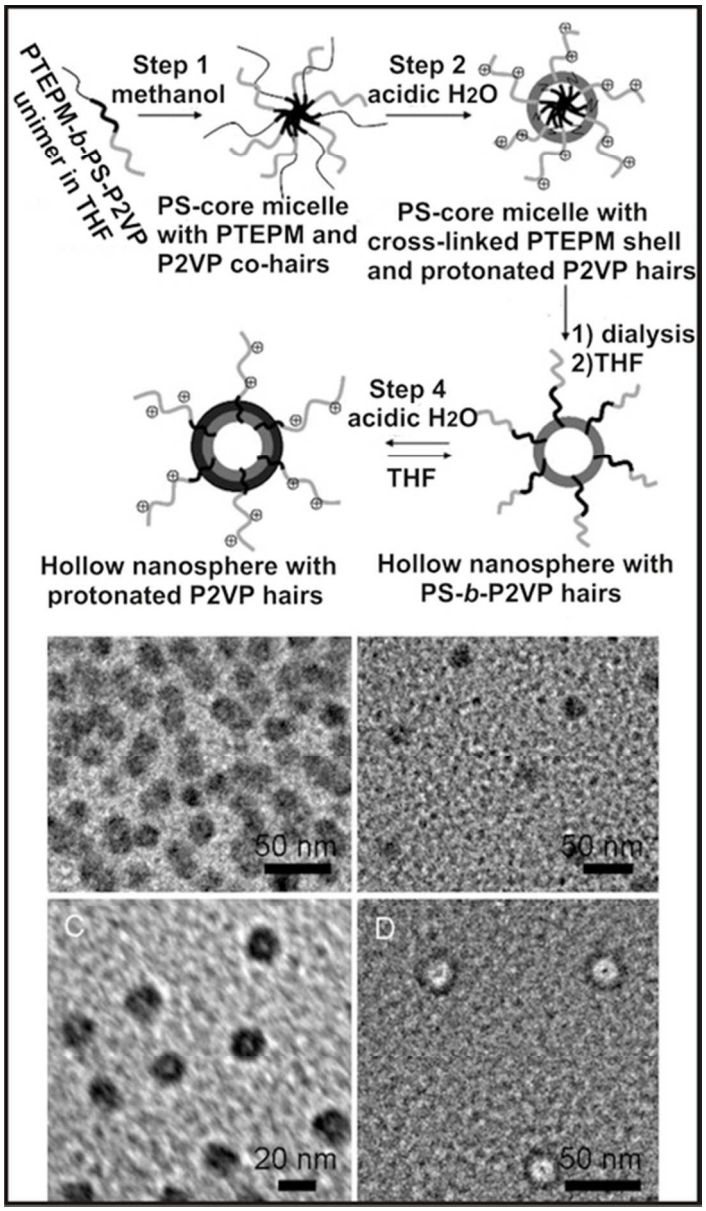
14x5mm (300 x 300 DPI)

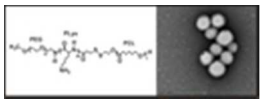


Structure of the copolymer chain and (A) TEM images of vesicles prepared using PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) in 1:2 v/v THF/water. The scale bar is 1000 nm. (B) the same vesicles decorated with gold nanoparticles located solely within the vesicles walls. The scale bar is 200 nm. *Reprinted with permission from (J. Am. Chem. Soc. 2005, (127), 12800). Copyright (2005) American Chemical Society.*
20x5mm (300 x 300 DPI)

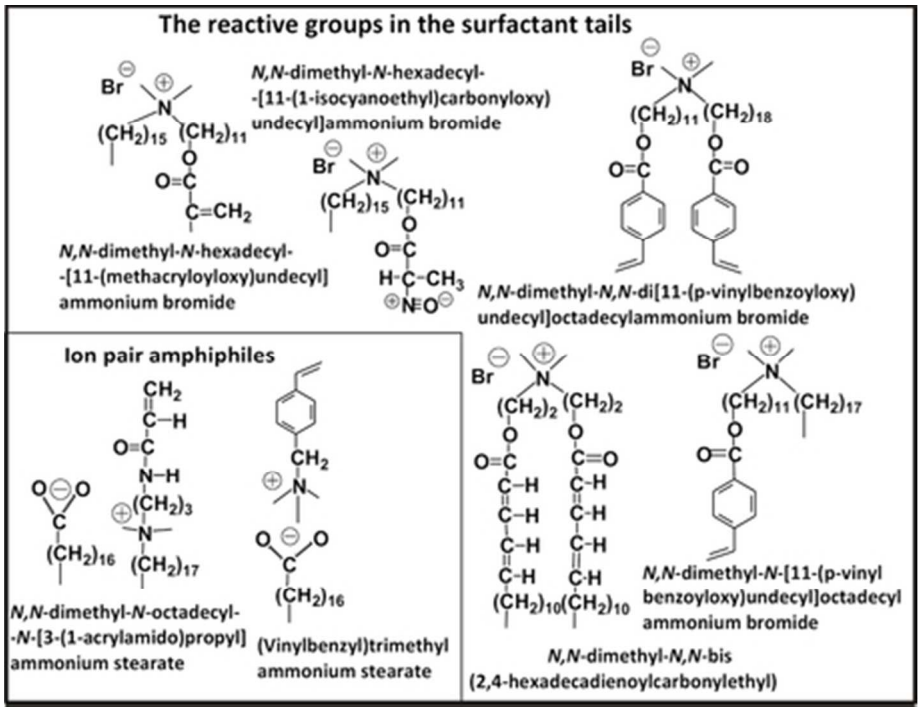


The illustration of preparation of the nano-objects from $PTEPM_{55}$-*b*-PS_{249}-*b*-$P2VP_{432}$ triblock copolymers and the structure transformation under different condition. Follow by TEM images for: (A) micelles of $PTEPM_{55}$-*b*-PS_{249}-*b*-$P2VP_{432}$ formed in the mixture of THF and methanol, the scale bar is 50 nm., (B) cross-linked micelles in acidic water (pH = 2), the scale bar is 50 nm., (C) hollow nanospheres by re-dispersing the cross-linked micelles in pure THF, the scale bar is 20 nm., and (D) hollow spheres by dispersing in acidic water (pH = 2), the scale bar is 50 nm. *Reprinted with permission from (J. Polym. Sci. Part B: Polym. Phys. 2012, (50), 323).*

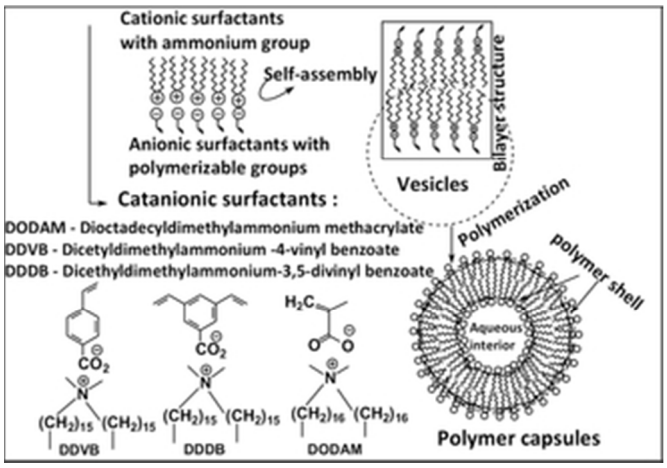
41x70mm (300 x 300 DPI)



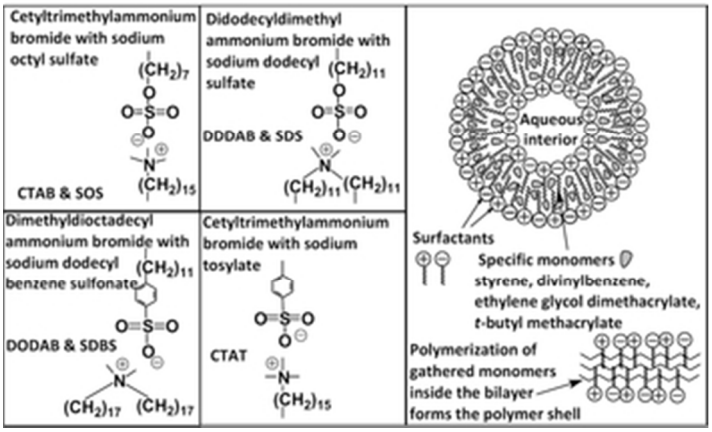
The molecular structure of PEG-*b*-PLys-SS-PCL triblock copolymer and the TEM image of obtained polymersomes. The scale bar is 50 nm. *Reprinted with permission from (J. Mater. Chem. 2012, 22, 22028).*
10x3mm (300 x 300 DPI)



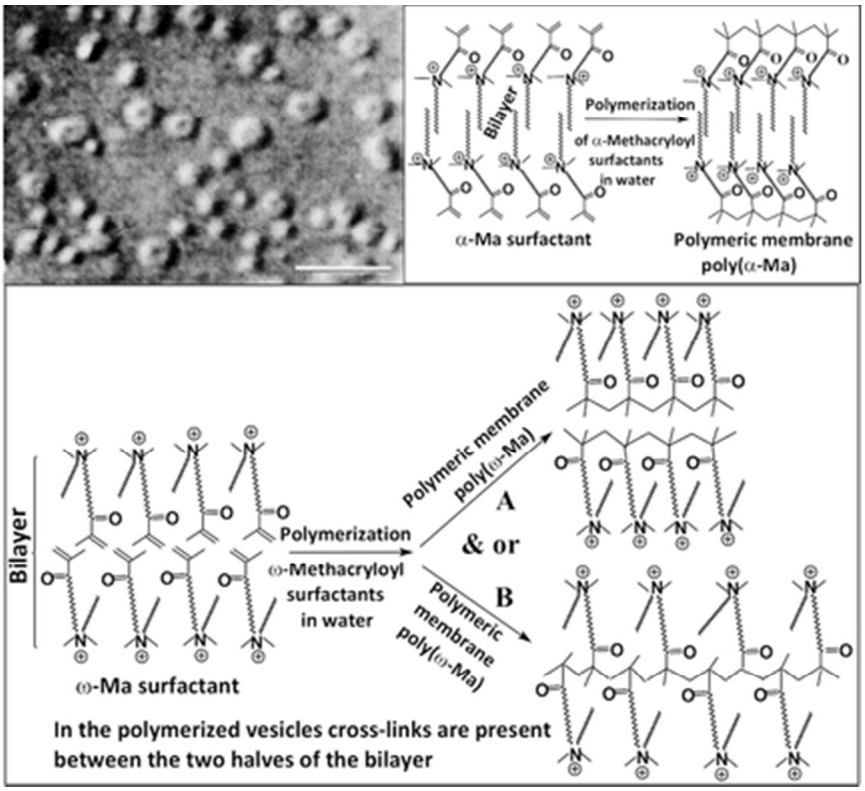
The examples of commonly used surfactants for preparation of SCL vesicles.
38x29mm (300 x 300 DPI)



The plausible structure of vesicles formed spontaneously by mixing cationic and anionic surfactants.
27x19mm (300 x 300 DPI)



The examples of surfactants which were used to obtain SCL vesicles by saturation of their bilayer with hydrophobic monomers.
29x18mm (300 x 300 DPI)



Schematic representation of *a*-Ma and *ω*-MA lipids in a bilayer membrane before and after formation of poly(*a*-Ma)and poly(*ω*-MA).

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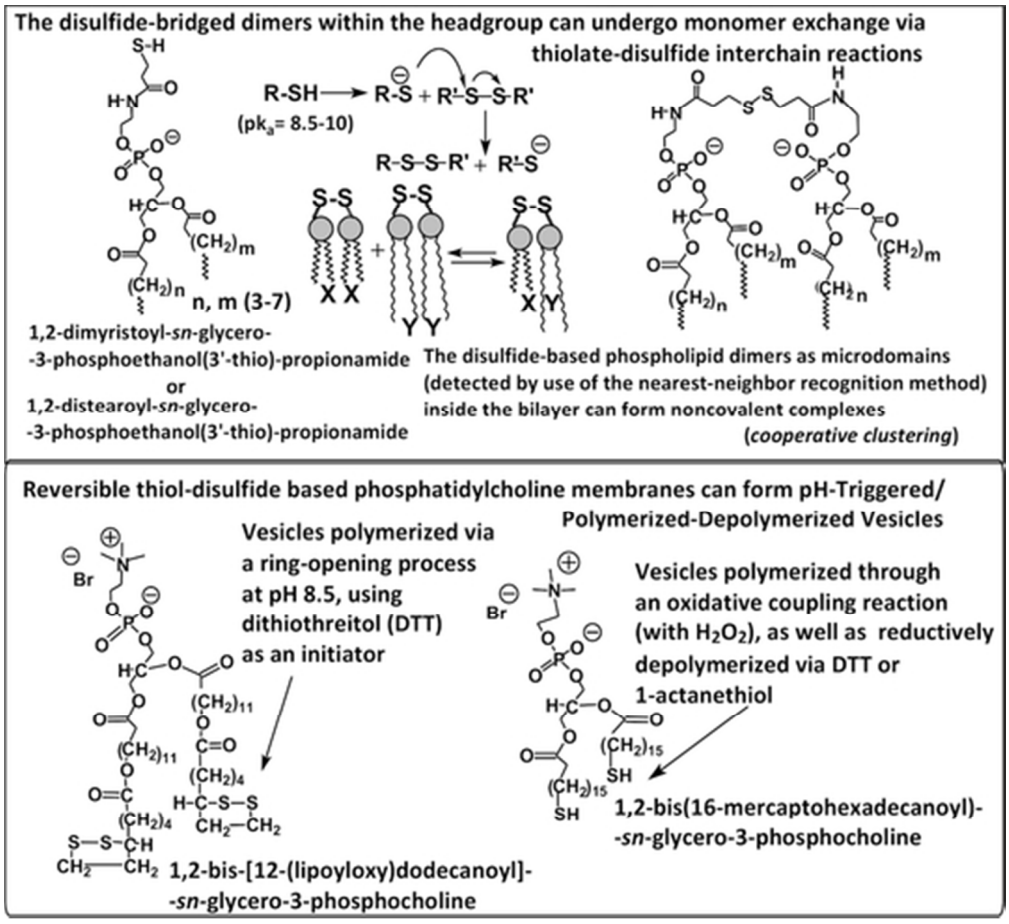
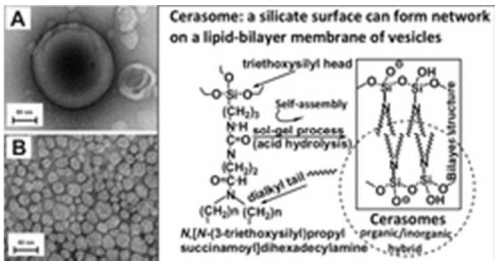
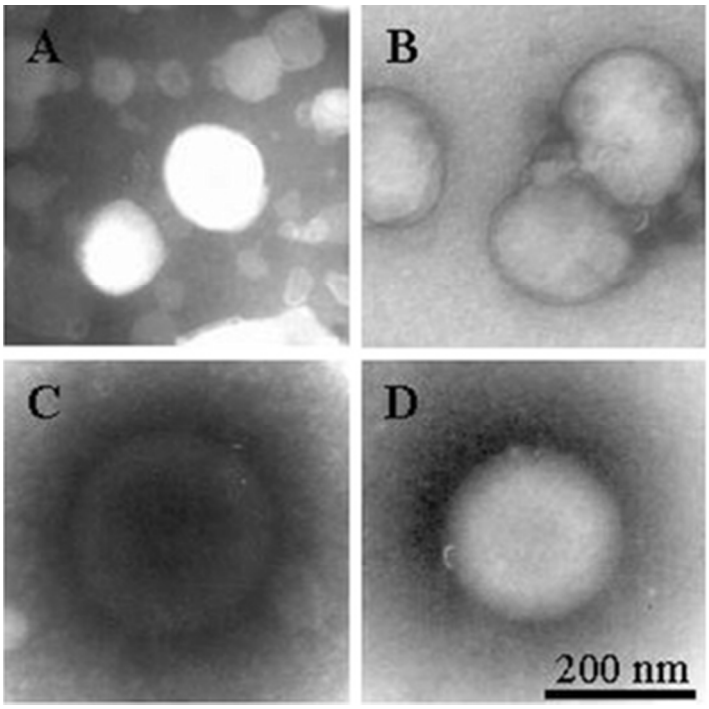
36x33mm (300 x 300 DPI)



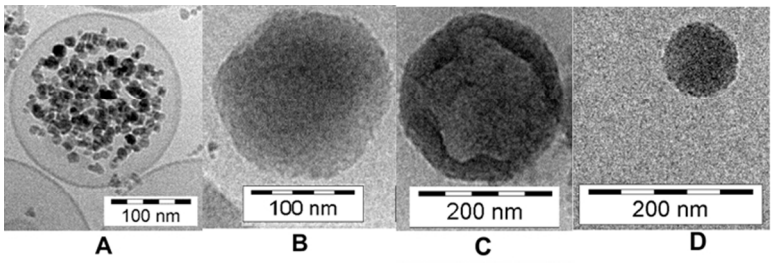
Basis of the nearest - neighbor recognition method.
45x41mm (300 x 300 DPI)



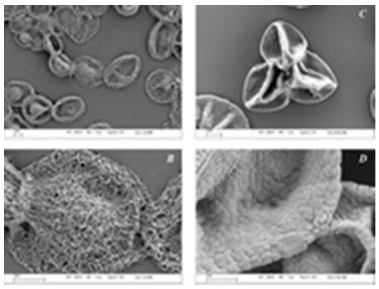
Molecular structures and schematic drawing of the cerasome forming lipids. TEM images of cerasomes prepared by (a) vortex mixing and followed by (b) ultrasonication. Scale bars are 50 nm. *Reprinted with permission from (Chem. Eur. J. 2007, (13), 5272).*
20x10mm (300 x 300 DPI)



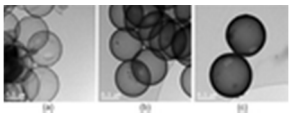
TEM images of liposomes (A) uncoated, (B) coated with 25 polyelectrolyte layers $(PAA/PLL)_{25}$, (C) coated with $(PAA/PLLA)_{25}$ cross-linked layers, (D) coated with $(PAA/PLL)_{25}$ cross-linked layers and 0,1% TX-100. (Ref. 77*b*). *Reprinted with permission (Adv. Mater. 2006, (18), 2868).*
29x29mm (300 x 300 DPI)



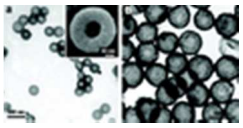
Cryo-TEM images of magnetic capsules in the order: uncoated liposomes, then (PAH/PSS)-coated, after that (PAH/PSS/PAH)-coated and the last $(PAH/PSS)_2$-coated. *Reprinted with permission from (Langmuir 2009, (25), 6793). Copyright (2009) American Chemical Society.*
203x114mm (120 x 120 DPI)



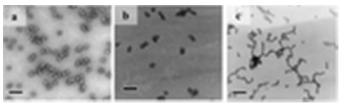
SEM images of $(PAH/PSS)_5$ microcapsules prepared using $CaCO_3$ microparticles template with FITC-BSA (fluorescein isothiocyanate-serum albumin) loaded by physical adsorption in performed particles (A,B) and by capture of protein from solution during growing $CaCO_3$ particles (C, D). *Reprinted with permission (Biotechnol. Prog., 2005, 21, 918).*
15x11mm (300 x 300 DPI)



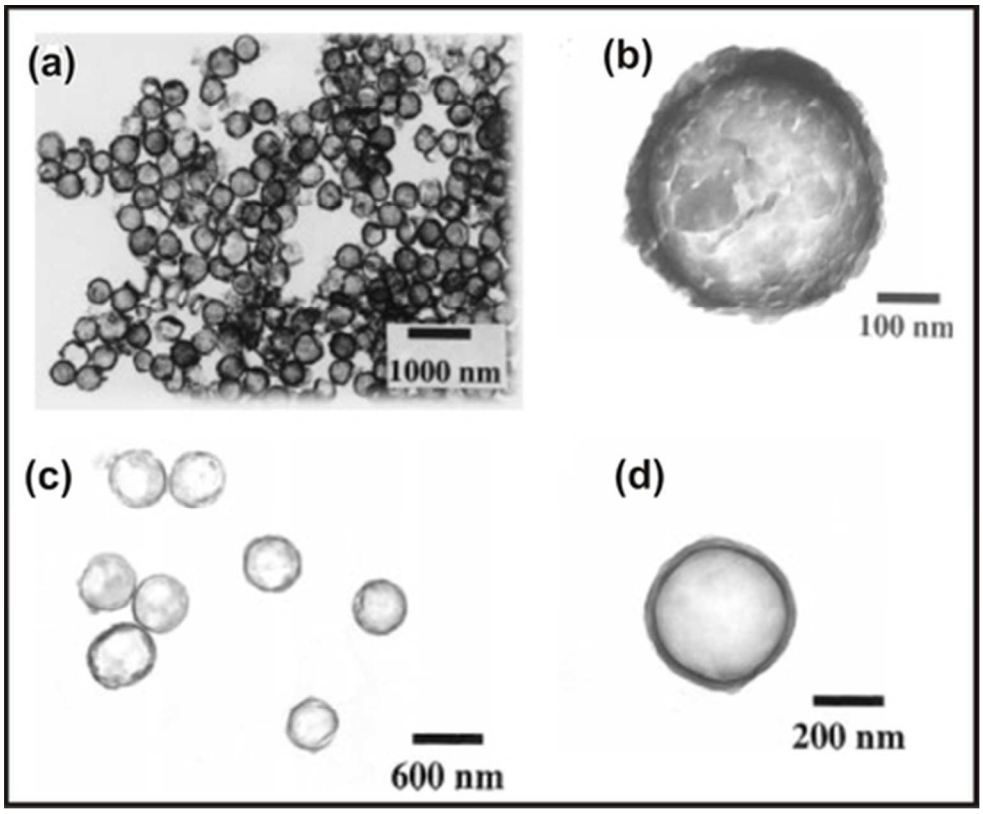
TEM images of silica nanocapsules made from (a) one, (b) two, (c) three sequential deposition of silica on polystyrene (PS) latex template. subsequent silica layer was deposited after adsorbing cationic poly(allylamine)hydrochloride (PAH). PS cores were removed from sample by dissolution in THF (a) or by calcination (b and c). Scale bars are 0.5μm. *Reprinted with permission from (Langmuir 2014, (30), 584). Copyright (2014) American Chemical Society.*
12x4mm (300 x 300 DPI)



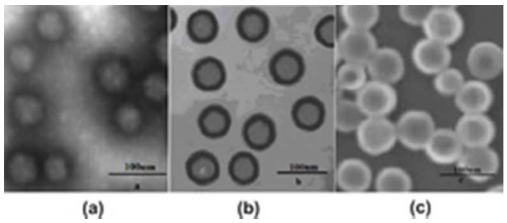
TEM images of PLL nanocapsules formed using silica particle templates, follow by cross-linking and removal of cores. The cross-linking agents used are (a) glutaraldehyde, or (b) 3,3'-dithiopropionimidate dihydrochloride. Scale bars are 500 nm. *Reprinted with permission from (Nano Letters 2008, (8), 1741). Copyright (2008) American Chemical Society.*
9x4mm (300 x 300 DPI)



TEM images of polyion complex capsules obtained at room temperatures, and polymer concentration 1 mg/mL with fPEG : (a) 12.1%, (b) 11.1%, and (c) 10.0%. Scale bars represents 100 nm. *Reprinted with permission from (Macromolecules 2014, (47), 3086). Copyright (2014) American Chemical Society.*
14x4mm (300 x 300 DPI)

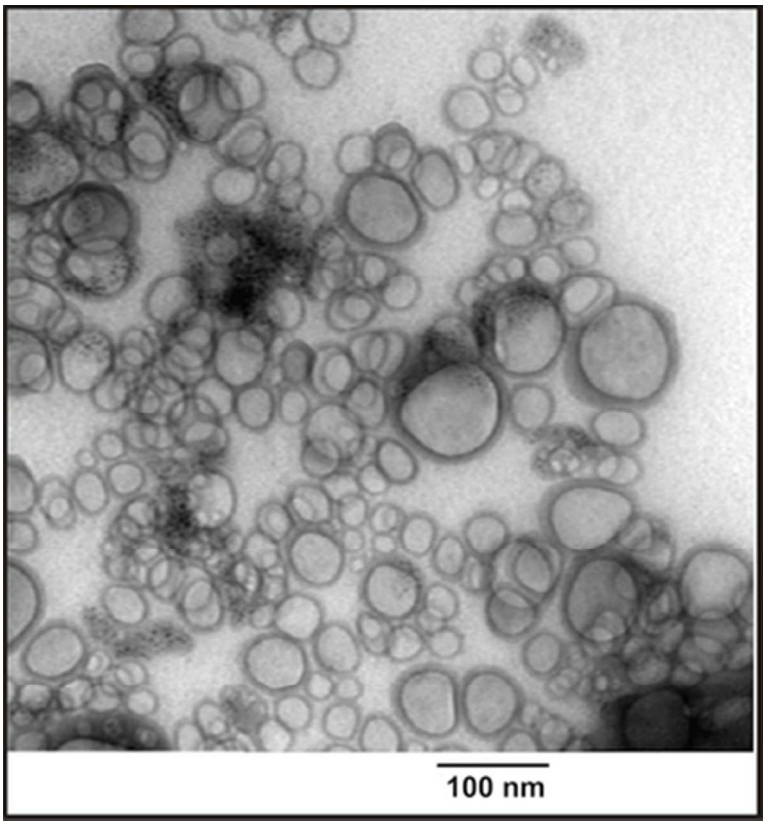


TEM images of five-bilayer hollow PVP/MPR capsules (a, b) and (PVP/MPR)11/PVP/PAA/PAH capsules (c, d).
Reprinted with permission from (Adv. Mater. 2003, (15), 832).
41x33mm (300 x 300 DPI)

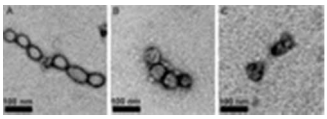


TEM images of Az-CMCS nanocapsules before (a) and after cross-linking (b). SEM image (c) proves the stability of the cross-linked capsules. Scale bars are 100 nm. *Reprinted with permission from (Int. J. Pharm. 2014, 463, 108).*

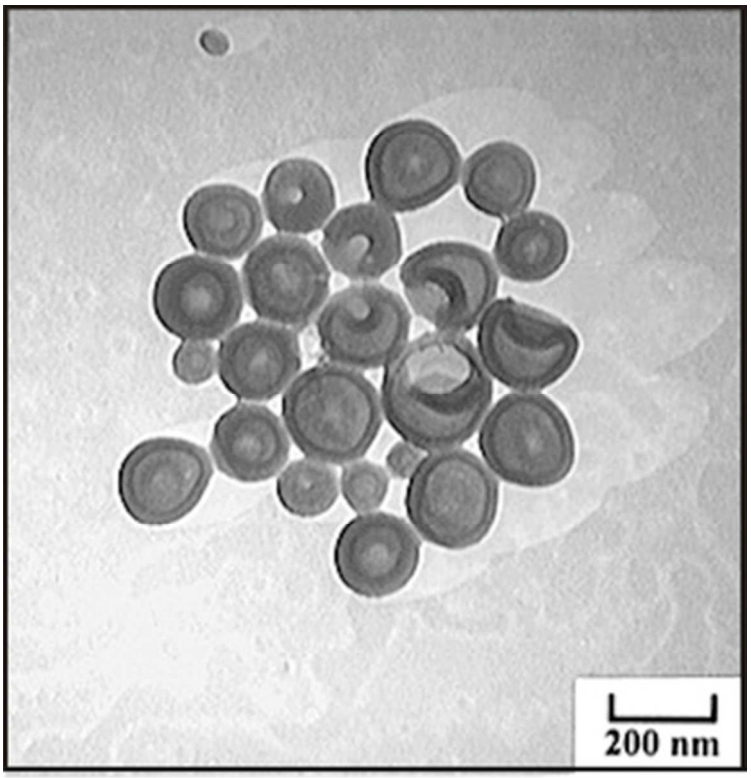
21x8mm (300 x 300 DPI)



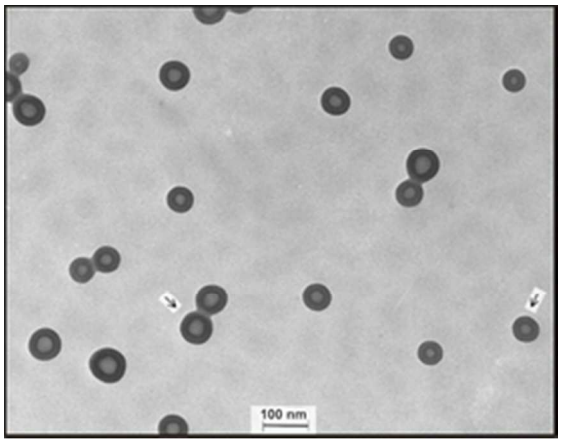
The poly(iso-butylcyanoacrylate) nanocapsules observed in TEM. The scale bar is 100 nm. *Reprinted with permission from (Int. J. Pharm. 2007, (331), 148).*
32x34mm (300 x 300 DPI)



TEM images of PI_{97}-PAA_{78} capsules (A) nanocage, (B) Shiff-base functionalization nanocage-lipid construct, and (C) carbodiimide functionalized nanocage-lipid construct. Scale bars denotes 100 nm. *Reprinted with permission from (J. Contr. Release 2005, (109), 189).*
13x4mm (300 x 300 DPI)

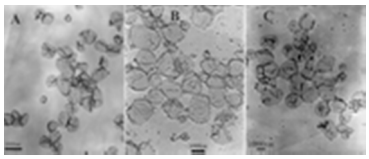


The TEM image of PS_{195}-P4VPMeI_{18} vesicles from 50 wt % THF and 50 wt % dioxane. *Reprinted with permission from (J. Am. Chem, Soc. 1997, (119), 8383). Copyright (1997) American Chemical Society.*
31x32mm (300 x 300 DPI)



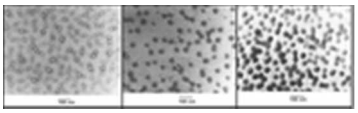
TEM images of PI-*b*-PCEMA vesicles prepared in hexanees/THF (20 %). b) The schematic structure of diblock polymer polyisoprene-block-poly(2-cinnamoylethyl methacrylate) or PI-b-PCMA. The scale bar is 100 nm. *Reprinted with permission from (Chemistry of Materials 1998, (10), 537). Copyright (1998) American Chemical Society.*

23x18mm (300 x 300 DPI)

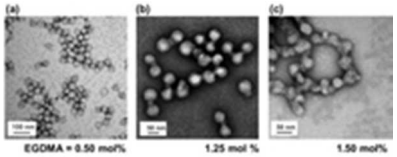


TEM images of PEO_{43}-*b*-$PGMA_{121}$ vesicles in the presence of cross-linking agents: (A) HAD (0.091 wt %), the scale bar is 2000 nm; (B) DA (0.14 wt %), and (C) the vesicles in (B) were treated with THF. Scale bars are 1000 nm. *Reprinted with permission from (Langmuir 2007, (23), 790). Copyright (2007) American Chemical Society.*

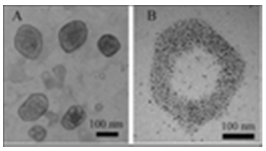
15x6mm (300 x 300 DPI)



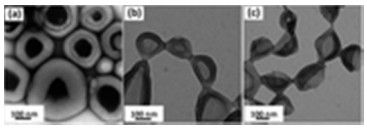
TEM images of block copolymer PSMA-*b*-PDMAEMA micelles: the first illustrates objects before the cross-linking, the second after cross-linking, and the last depicts cross-linked objects after acetonide group removal. Scale bars are 100 nm. *Reprinted with permission from (Macromolecules 2000, (33), 7577). Copyright (2000) American Chemical Society.*
14x4mm (300 x 300 DPI)



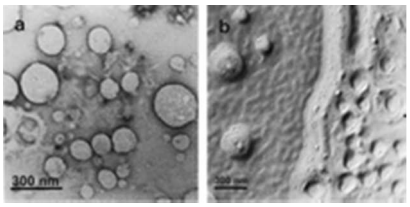
TEM images obtained for: (a) $PMPC_{50}$-($PHPMA_{400}$-*stat*- $EGDMA_2$), the scale bar is 100 nm, (b) PMPC50-($PHPMA_{400}$-*stat*-$EGDMA_5$), the scale bar is 50 nm, (c) $PMPC_{50}$-($PHPMA_{400}$-*stat*- $EGDMA_6$) prepared by RAFT aqueous dispersion polymerization at 70 ^{0}C using $PMPC_{50}$ as a chain transfer agent, and the scale bar is 50 nm. *Reprinted with permission from (Soft Matter 2011, (7), 10787).*
16x6mm (300 x 300 DPI)



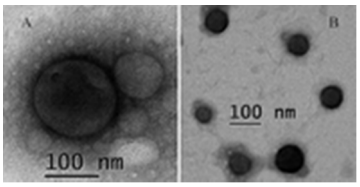
TEM images of (A) vesicles prepared from $PMPC_{25}$–$PDPA_{120}$ copolymer at pH 7, and (B) vesicles decorated with gold nanoparticles located within the vesicle walls after in situ reduction of $HAuCl_4$ (pH 6.4). Scale bars are 100 nm. *Reprinted with permission from (J. Am. Chem. Soc., (2005, (127), 17982). Copyright (2005) American Chemical Society.*
10x5mm (300 x 300 DPI)



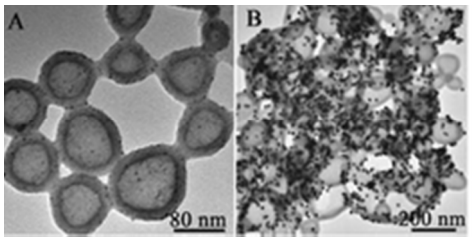
TEM images of $PMAA_{70}$-$PBzMA_{200}$ vesicles (a) before cross-linking, (b) $PMAA_{70}$-$PBzMA_{200}$-$PEGDMA_{20}$ after cross-linking, (c) cross-linked vesicles at pH 9. *Reprinted with permission from (Polym. Chem. 2014, (5), 3466).*
15x4mm (300 x 300 DPI)



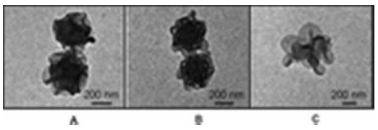
Transmission electron micrographs (TEM) of PEO-PMPS vesicles observed for copolymer dispersions obtained by (a) direct addition to water, (b) ultrafiltration/dilution method (freeze-fracture). Bars represent 300 nm. .
Reprinted with permission from (Chem. Commun. 1998, 1445).
17x8mm (300 x 300 DPI)



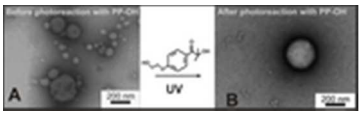
TEM images obtained for PCL_{60}-*b*-$PMPC_{37}$ vesicles after membrane mediated silicification. The TMOS/MPC molar ratio was 10:1 and the reaction time for silicification was (A) 40 h and (B) 4 days. *Reprinted with permission from (Langmuir 2009, (25), 9564). Copyright (2009) American Chemical Society.*
14x7mm (300 x 300 DPI)



TEM images of vesicles from PEO_{45}-*b*-$PTESPMA_{90}$ and MMS decorated with Au nanoparticles by (A) reducing the Au^{+3} ions on vesicles and by (B) absorbing the performed Au nanoparticles. *Reprinted with permission from (Macroml. Rapid Commun. 2008, (29), 1368).*
19x9mm (300 x 300 DPI)

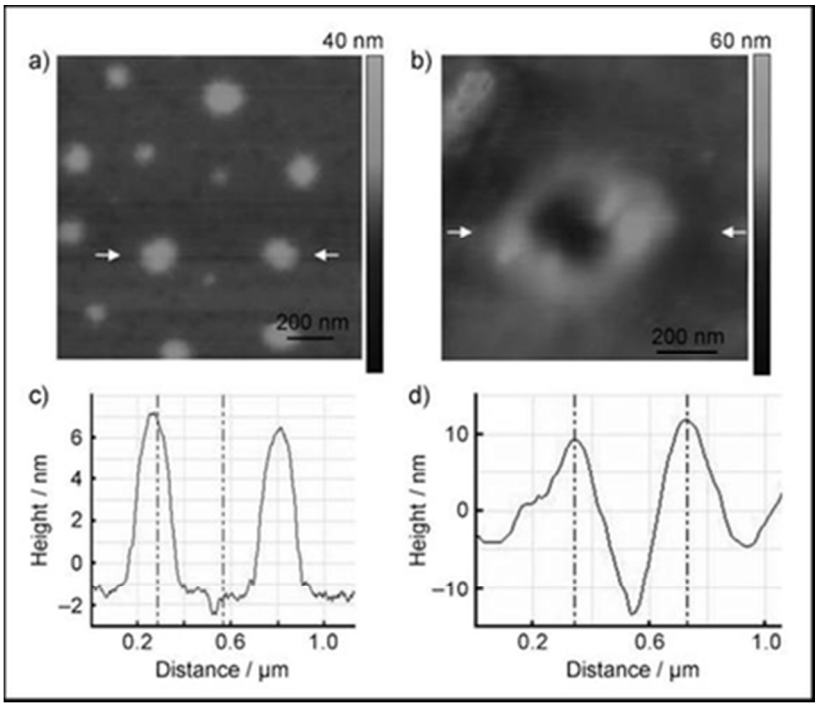


TEM images of gelated large-compound vesicles prepared from (A) PEO_{45}-*b*-P($NIPAM_{63}$-*r*-$THPM_{28}$), (B) PEO_{45}-*b*-P($NIPAM_{69}$-*r*-$THPM_{17}$), (C) PEO_{45}-*b*-P($NIPAM_{66}$-*r*-$THPM_{9}$). Scale bars are 200 nm. *Reprinted with permission from (Macromol. Rapid Commun. 2013, 34, 1169).*
15x4mm (300 x 300 DPI)

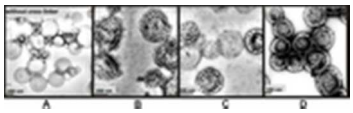


TEM micrographs of polymersomes PMOXA-*b*-PDMS-*b*-PMOXA before (A) and after (B) photoreaction with PP-OH. *Reprinted with permission from (J. Am. Chem. Soc. 2013, (135), 9204). Copyright (2013) American Chemical Society.*

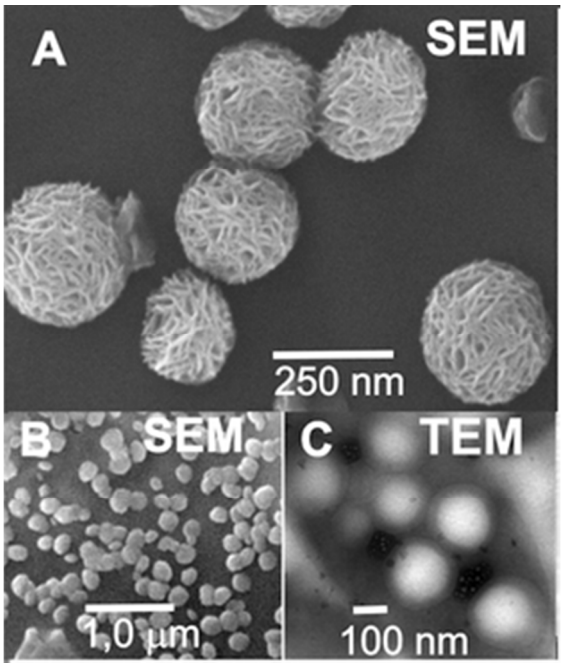
14x4mm (300 x 300 DPI)



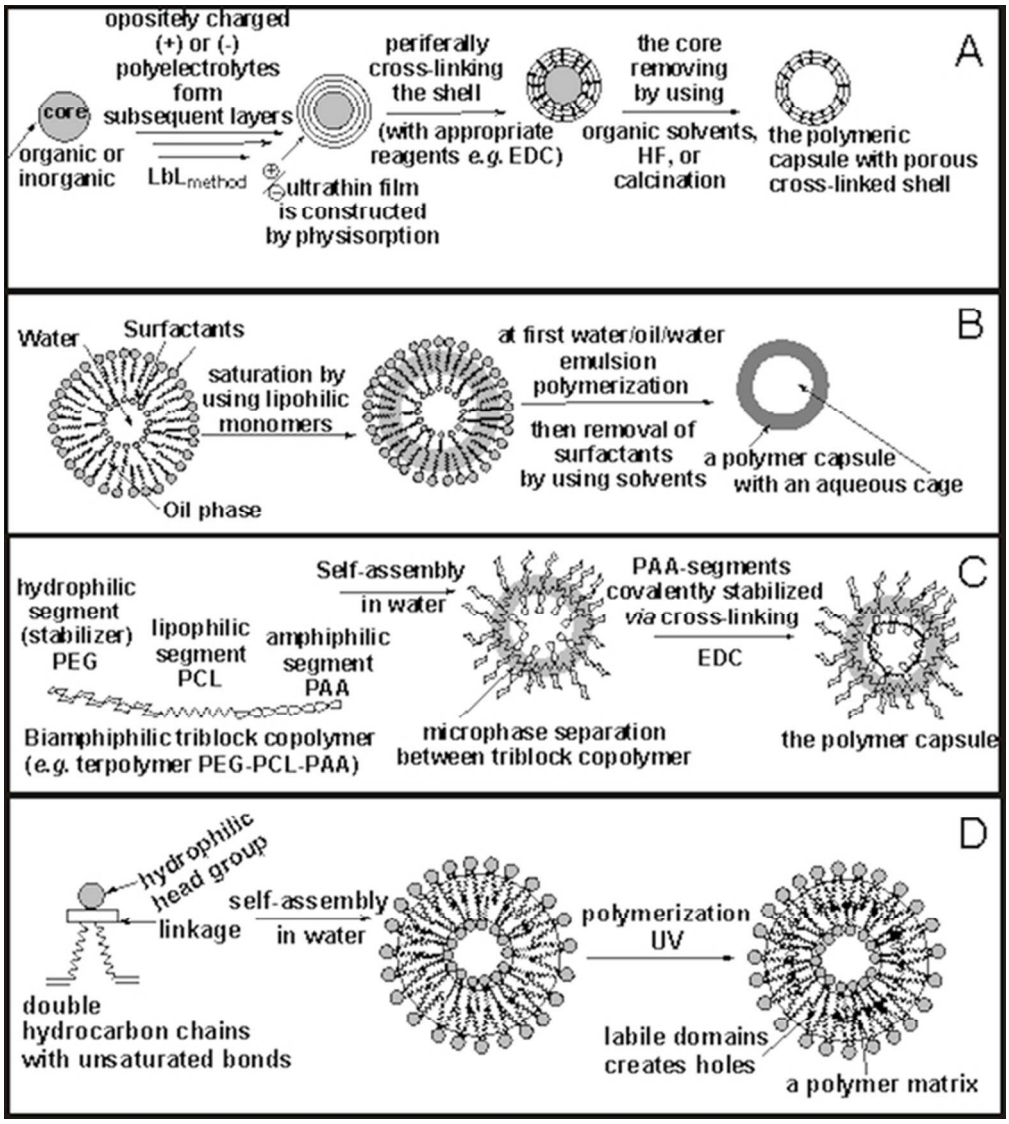
Tapping mode AFM (height images) (a) the spherical micelles at 80% cross-linking in water and (b) the hollow nanocages obtained at 30% target cross-linking in water. Cross-sectional profiles c) and d) were obtained from the images shown in a) and b), respectively. *Repirinted with permission from (Angew. Chem. Int. Ed. 2010, (49), 3500).*
34x29mm (300 x 300 DPI)



TEM images obtained for (A) noncross-linked $PGMA_{55}$-*b*-($PHPMA_{247}$-*stat*-Gly(Ma_{82}) copolymer vesicles, and the same vesicles after epoxy-amine cross-linking using (B) Jeffamine D-230, (C) Jeffamine D-400, or (D) PEG34 diamine. *Reprinted with permission from (Langmuir 2012, (28), 1196). Copyright (2009) American Chemical Society.*
14x4mm (300 x 300 DPI)



SEM (A, B) and TEM (C) images of polymer nanocapsules generated due to dimerization of the coumarin moieties. The copolymer poly (2-ethylhexyl methacrylate-*co*-7-(4-trifluoromethyl)coumarin acrylamide) formed cross-linked wall. *Reprinted with permission from (New J. Chem. 2015, (39), 1506.).*
23x27mm (300 x 300 DPI)



Schematic illustration of four methods for the preparation of polimer capsules: A) layer-by-layer (LbL), B) emulsion polymerization, C) amphiphilic triblock terpolymer, D) self-assembly of phospholipids (surfactants) with unsaturated bonds.
55x61mm (300 x 300 DPI)

Drug	Model	Ref.
Doxorubicin	LbL	73
Plitidepsin	LbL	94b
Melphalan	LbL	101
Cidofavir	LbL	103
Camptothecin	Block-copolymer	124b
Doxorubicin	Block-copolymer	124a
5-Fluorouracil	Liposomes template	163b