Expert Opinion

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Pharmaceutical nanotechnology: polymeric vesicles for drug and gene delivery

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Improving the therapeutic index of medicines is a goal of drug delivery. Employing nanosystems that control drug biodistribution is one way of achieving therapeutic improvements, and polymeric bilayer vesicles are one such nanosystem. Polymeric vesicles, with the ability to transport drugs or genes, are prepared in one of two ways: i) the self-assembly of amphiphilic polymers and ii) the polymerisation of monomers, following self-assembly (polymerised vesicles). There are two types of self-assembling amphiphilic polymers: water-soluble polymers derivatised with hydrophobic pendant groups and amphiphilic block copolymers. Amphiphilic alkenes and alkynes are the main compounds that are used to make polymerised vesicles. This review discusses polymer architecture fundamentals that govern the self-assembly of polymers into vesicles, the fine control on vesicle size that is achievable with polymeric vesicles and the application of the vesicles to drug delivery.

Keywords: block copolymers, chitosan, gene delivery, polyethylenimine, poly(L-lysine), polymeric vesicles, polymers, polymersomes

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1. Introduction

Drug delivery is the application of chemical and biological principles to control the *in vivo* temporal and spatial location of drug molecules for clinical benefit. By improving the locoregional specificity of drug molecules, drug delivery scientists seek to maximise drug activity and minimise side effects. By employing well-defined nanosystems, the distribution of drugs may be controlled and a common example of this approach is the use of liposomes, as exemplified by the anticancer doxorubicin liposomal chemotherapeutic Caelyx® (ALZA Corp.) [1]. Liposomes direct the biodistribution of anticancer chemotherapeutics such that, at any point in time, a greater proportion of the drug is located within the tumour and a lower proportion of the drug is located in healthy tissue [2]. The nanosystem that is considered in this review is the polymeric vesicle (Figure 1). As well as controlling biodistribution, drug delivery systems of the future should be able to respond directly to the local environment of the host pathology in order to achieve a beneficial interaction with the host pathology/physiology. This latter goal is not yet achievable, but such an objective should guide our design of drug delivery elements.

Polymeric vesicles are closed bilayer spherical structures, usually in the nanometre size domain (Figure 1), which are able to encapsulate both hydrophilic and hydrophobic solutes. However, this definition is not entirely true, as there are two ways that polymeric vesicles may be prepared: i) following polymerisation of self-assembled monomers [3], or ii) following the self-assembly of polymeric amphiphiles [3-8]. In the latter case, it is not always clear that the fluidity of the bilayer has been maintained after the polymerisation step, as it is conceivable that a more solid polymer shell may result.

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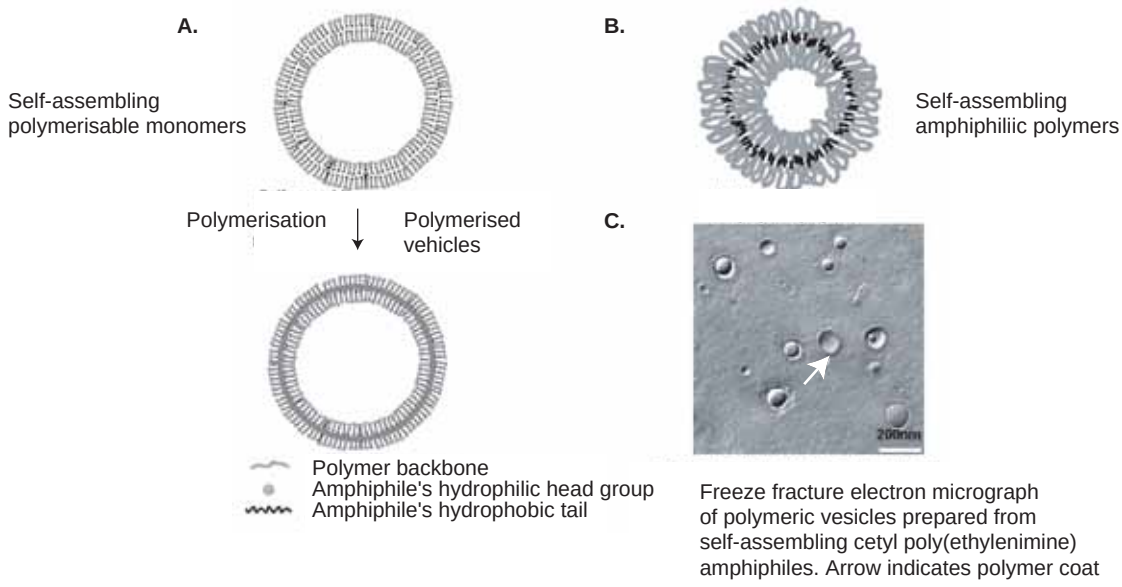

Figure 1. Schematic of polymeric vesicles. Polymeric vesicles may arise from two methods (**A** and **B**). **A.** The self-assembly of polymerisable monomers that are subsequently polymerised. **B** and **C.** The self-assembly of amphiphilic polymers in which polymers bear random hydrophobic pendant groups or the self-assembly of block copolymers.

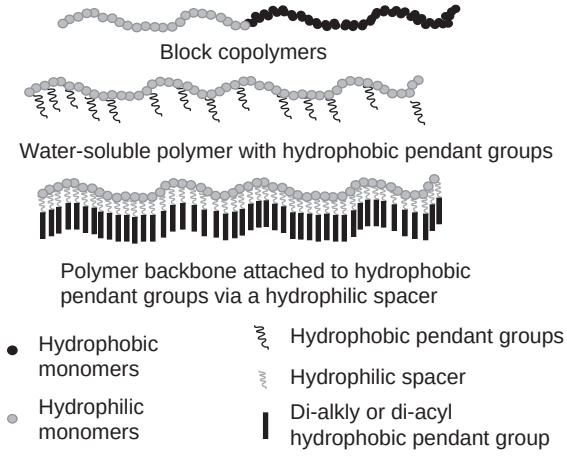

Figure 2. Schematic representation of self-assembling vesicle-forming polymers.

2. Self-assembling polymers

The self-assembly of certain amphiphilic polymers in aqueous media results in the production of polymeric vesicles (closed bilayer membrane vesicles) [3-8]. Closed bilayer membrane vesicles also result when low molecular weight amphiphiles self-assemble in various solvents [9]. It is known that vesicle formation from low molecular weight amphiphiles in aqueous media is the result of the amphiphiles' attempts to reduce the high interfacial energy between the hydrophobic portion of the amphiphile and the aqueous disperse phase, and to maximise the low energy interaction between the hydrophilic head group and the disperse phase [9]. Attaining a minimal interfacial energy is thus served by the formation of a closed spherical bilayer. Excellent reviews exist on the self-assembly of low molecular weight amphiphiles [9] and, hence, this topic will not be discussed in great detail here.

There are two classes of polymer amphiphiles that have been reported to form polymeric vesicles: polymers bearing lipid pendant groups that are attached either directly or via a hydrophobic spacer to a polymer backbone [3,6-8], and block copolymers [4,5] (Figure 2). There are some common aspects governing the self-assembly of these two types of polymers into vesicles. These common features will be discussed first before a more detailed treatment of the different polymer types.

Both types of polymers give rise to polymeric vesicular self-assemblies that reside in the nanometre to micrometre size domain [6,10,11]. Polymer self-assembly into vesicles is not a spontaneous process, and normally requires an input of energy (e.g., in the form of extrusion [12] or probe sonication [8]). Also, in a similar manner to that reported for low molecular weight amphiphiles, which form micelles from the more hydrophilic molecular variants and vesicles from amphiphiles of intermediate hydrophobicity [9], the self-assembly of polymers into vesicles is governed by the hydrophilic–lipophilic balance of the polymers [5,8] (e.g., Figure 3). With both the block copolymers and pendant-type polymers, low levels of hydrophobicity

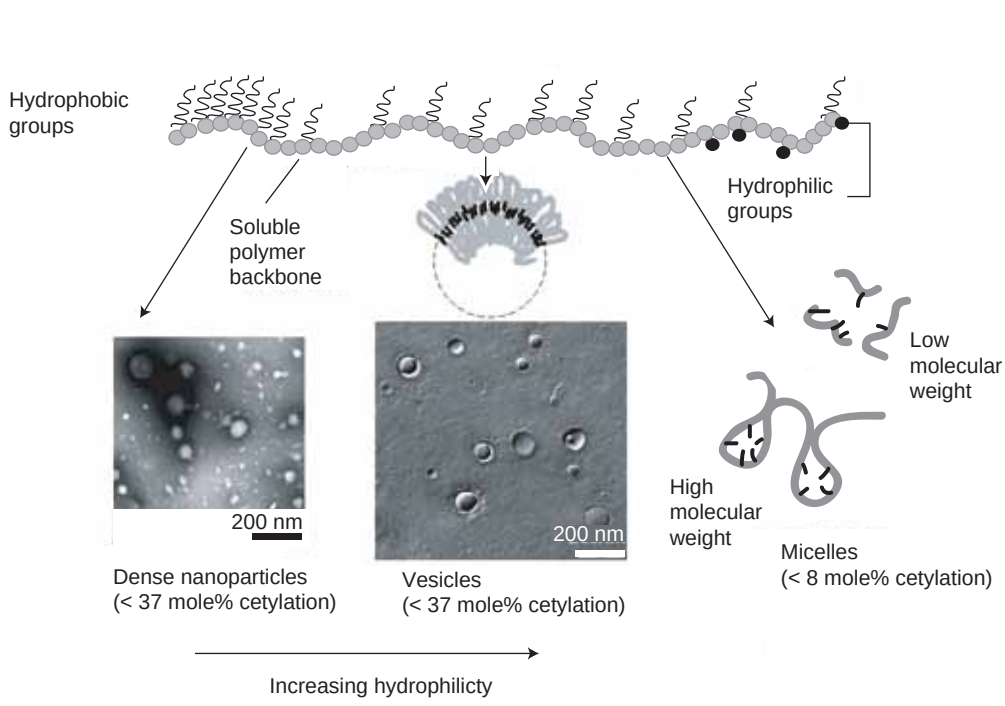

Figure 3. The self-assembly of polymeric amphiphiles (cetyl linear polyethylenimine) into vesicles and other nanostructures. As the level of hydrophobic substitution decreases, polymers self-assemble into dense nanoparticles, then into vesicles and finally into polymeric micelles.

(< 40% by weight of the polymer molecule consisting of hydrophobic moieties) favours the formation of micelles [10,13]; intermediate levels of hydrophobicity (~ 40 – 80% by weight of the polymer molecule consisting of hydrophobic moieties) favours the formation of bilayer vesicles [10,13-15]; and higher levels of hydrophobicity (> 60 – 80% by weight of the polymer molecule consisting of hydrophobic moieties) favours the formation of dense nanoparticles [10].

Although the preceding narrative highlights the common features governing the self-assembly of block copolymers and pendant-type amphiphilic polymers, as well as similarities between the self-assembly of low molecular weight and polymer amphiphiles, it must be noted that there are some polymer-specific factors that impact on vesicle-forming ability. For example, the degree of polymerisation is critical to vesicle forming ability and, in general, very high degrees of polymerisation prevent vesicle formation [8,16]. The interplay between polymer hydrophilic/lipophilic character and molecular weight has been captured for polyamino acids in a vesicle-formation index (F; Equation 1).

$$F = \frac{H}{L\sqrt{DP}} \qquad (1)$$

H is the mole% unreacted L-lysine units, L is the mole% L-lysine units substituted with palmitic acid, and DP is the the degree of polymerisation of the polymer. An F value in excess of 0.168 was found to be necessary for vesicle formation [8].

Polymeric vesicles are largely unilamellar [6,11] and studies have shown that unilamellarity is favoured when the molecular weight of the amphiphile increases [10]. Generally, polymeric vesicles also possess superior mechanical stability [5], are less susceptible to degradation by organic solvents and soluble surfactants [5] and are less permeable to low molecular weight solutes when compared with vesicles formed from low molecular weight amphiphiles [3,17].

2.1 Polymers bearing hydrophobic pendant groups

2.1.1 Nature of the polymer

The first report on the use of preformed polymers to prepare polymeric bilayer vesicles was presented in 1981 by Kunitake *et al*. [18]. In this report, bilayer vesicles were prepared from polymer 1 (Figure 4). This polymer comprises a hydrophilic polyacrylamide backbone and dialkyl hydrophobic pendant groups separated from the polymer backbone by hydrophilic oligo-ethylene oxide spacers (shown schematically in Figure 2). The hydrophilic spacer group, which allows the decoupling of the polymer motion from the ordering of the bilayer [17], is essential for vesicle formation for these polyacrylamide-type polymers [3].

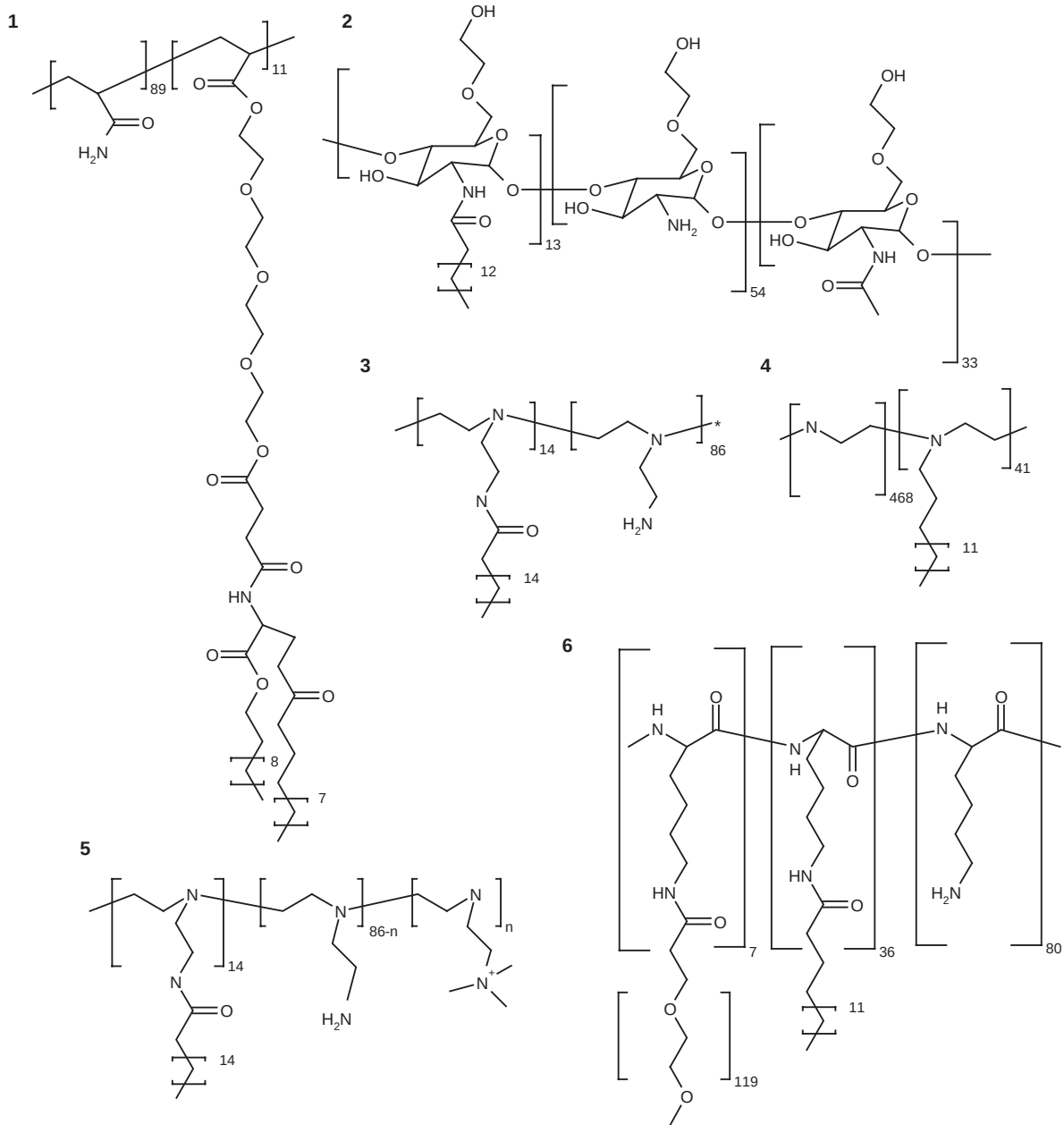

Figure 4. Representative vesicle-forming polymers from the polyacrylamide, chitosan, polyamine and polyamino acid classes of polymers bearing hydrophobic pendant groups. Polyacrylamide: polymer **1**; polyacrylamide-polyacroyl-(4-[3′6′9′12′-tetraoxadodecyl]-*N*-[dodecyl-2′-amido-5′-oxo-heptadecanoate]4-oxobutanamide) [18]. Chitosan: polymer **2**; palmitoyl glycol chitosan [6,19]. Polyamine: polymers **3** and **5**; palmitoyl branched polyethylenimine; polymer **4**; cetyl linear polyethylenimine [10,48]. Polyamino acid: polymer **6**; palmitoyl poly(L-lysine)-graft-poly(ethylene oxide) [8].

The introduction of carbohydrate (e.g., polymer 2 [6,19]; polyamine (e.g., polymers 3 – 5 [10]) and polyamino acid (e.g., polymer 6 [8]) vesicle-forming amphiphiles is a recent development. Cholesterol is usually added at 33% by weight to form these vesicles. The bilayer arrangement is as depicted in Figure 5, and the thick polymer coat is clearly visible on micrographs (Figure 1).

2.1.2 *Polymer architecture and vesicle properties*

Polyethylenimine (PEI)-based (polymer 5 [10]) and poly(L-lysine)-based (polymer 6 [8,20]) amphiphiles have been studied in some detail. The formation of vesicles from these amphiphiles is dependent on the level of lipid pendant groups (Figure 3). For example, hydrophobically modified PEIs form dense nanoparticles, bilayer vesicles or micellar

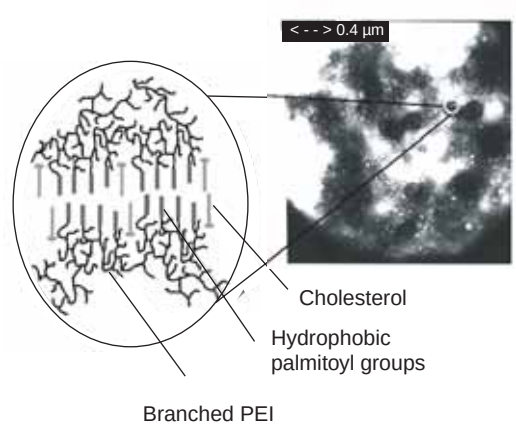

Figure 5. The branched PEI polymers are arranged in the bilayer as shown. Polymer 3 (palmitoyl branched PEI) and polymer 5 (*N*-trimethyl quaternary ammonium palmitoyl PEI; Figure 4). Micrograph shows a negatively stained transmission electron microscopy image of vesicles prepared from polymer 3 (palmitoyl branched PEI; Figure 4), cholesterol (2:1 g/g).
PEI: Polyethylenimine.

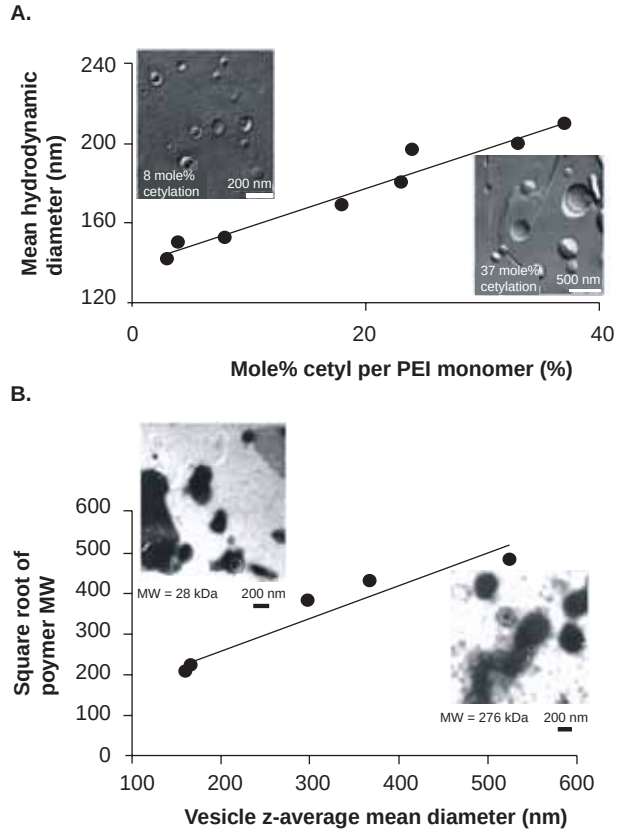

Figure 6. Graphs depicting how vesicle size is dependent on polymer MW and polymer hydrophobicity. A. Polymer hydrophobicity (exemplified by variants of cetyl linear PEI; polymer 4) may be used to control vesicle size. Freeze fracture micrographs show vesicles prepared from variants of cetyl linear PEI. Mole% cetylation are as shown on the micrographs and a higher level of cetylation yields larger vesicles [10]. **B.** Polymer molecular weight (exemplified by variants of palmitoyl glycol chitosan [Polymer 2]) may be used to control polymeric vesicle size. Negatively stained transmission electron micrographs show vesicles prepared from variants of palmitoyl glycol chitosan. Polymer molecular weights are as shown on the micrographs, and an increase in molecular weight leads to an increase in vesicle size [19].
MW: Molecular weight; PEI: Polyethylenimine.

self-assemblies, depending on their hydrophobic content [10]. PEI amphiphiles in which the polymers have a hydrophobic content ≥ 58% by weight favour dense nanoparticle self-assemblies, but a hydrophobic content of 43 – 58% favours bilayer vesicle assemblies, and a hydrophobic content of < 43% favours the formation of micellar assemblies [10]. Polymer molecular weight also affects the ability of polymers to form vesicles. The importance of this parameter has been demonstrated with the poly(L-lysine) vesicle system [8] (e.g., polymer 6), where an increase in molecular weight diminishes the possibility of vesicle formation (Equation 1).

With polymeric vesicles, unlike vesicles formed from low molecular weight amphiphiles, a precise level of control of vesicle size is possible by specific controls on either polymer hydrophobicity or molecular weight [10,19]. When vesicle size is measured using photon correlation spectroscopy, vesicle size within a homologous series of polymers is dependent on polymer levels of hydrophobic modification (mole% cetylation), exemplified for cetyl PEI amphiphiles in Equation 2 and Figure 6A [10].

$$d_v = 1.95Ct + 139 \qquad (2)$$

d_v is the z-average mean vesicle hydrodynamic diameter and Ct is the mole% cetylation. Vesicle size is also dependent on polymer molecular weight (Equation 3 and Figure 6B) [19].

$$\sqrt{MW} = 0.78d_v + 107 \qquad (3)$$

MW is the polymer molecular weight and d_v is the vesicle z-average mean hydrodynamic diameter. Although the influence of polymer molecular weight polydispersity on vesicle size has not been specifically studied, the relationship detailed in Equation 3 was established from polymers with polydispersities of 1.24 – 1.78, indicating that polymer molecular weight heterogeneity has a minimal effect on vesicle formation.

This ability to control particle size by altering polymer architecture is important, as particle size is a crucial determinant of particle biodistribution [21-23].

2.2 Block copolymers

Block copolymer vesicles, termed polymersomes were first reported in the 1990s [24]. Polymersomes have been prepared from a variety of block copolymers; some examples of which

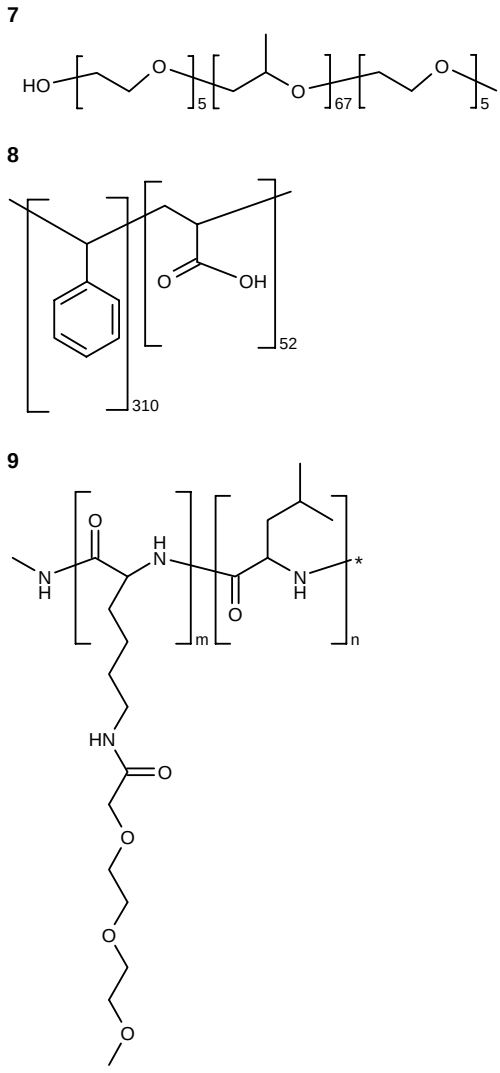

Figure 7. Examples of some vesicle-forming block copolymers. Polymer **7**: poly(ethylene oxide)-*b*-poly(oxypropylene)-*b*-poly(ethylene oxide) copolymer [12], polymer **8**: polystyrene-*b*-poly(acrylic acid) [52] and polymer **9**: poly(*N*ε-2-[2-{2-methoxy ethoxy}ethoxy]acetyl-L-lysine-*b*-poly[L-leucine]) [16].

are given in Figure 7. Other vesicle-forming block copolymers include: poly(ethylene oxide)-*b*-poly(ethylethylene) [11], poly(ethylene oxide)-*b*-polycaprolactone [14], poly(ethylene oxide)-*b*-poly([3-{trimethyoxy-silyl}propyl methacrylate]) [25,26], poly(ethylene oxide)-*b*-polybutadiene, [27-29], poly(ethylene oxide)-*b*-poly(propylene sulfide) [30] and poly(ethylene oxide)-*b*-polystyrene [24].

With block copolymers (Figure 2), it has been established that the surfactant parameter shown in Equation 4 should approach unity for vesicular self-assemblies to be obtained [5]:

$$\left(\frac{v}{aI}\right) \qquad (4)$$

where v is the molecular volume of the hydrophobic block, l is the molecular length of the hydrophobic block and a is the molecular area of the hydrophilic block. This is similar to the situation with low molecular weight amphiphiles [9]. However, once again, polymer-specific factors also govern the self-assembly of block copolymers. The flexibility of the hydrophobic block determines which self-assemblies will be formed, with more flexible hydrophobic portions of the polymer able to form vesicles and the more rigid polymers unable to self-assemble into three-dimensional structures [5].

Block copolymer vesicle sizes are more varied than those of the pendant-type polymers, and range from tens of nanometres [31] to tens of microns [32]. In a similar manner to the pendant-type polymer vesicle membranes [10], polymersome membranes are also thicker than conventional vesicle membranes, ranging from 8 – 21 nm in thickness; 2 – 5 times thicker than the 4-nm hydrophobic cores displayed by conventional low molecular weight amphiphile membranes [9,11,14,15,31]. Polymersome membrane thickness is determined by the degree of polymerisation in the hydrophobic block [15]. These extra-thick membranes confer exceptional stability to organic solvents and water-soluble surfactants, as well as superior mechanical stability [5,11,33,34]. Polymersomes are thus less likely to rupture on perturbation [11] and their mechanical stability increases with polymer molecular weight [15].

3. Polymerisation after self-assembly

Some examples of polymerisable vesicle-forming monomers are shown in Figure 8. A polymerisation step follows self-assembly with these vesicles. This method of polymeric vesicle production is one of the earliest methods of producing polymeric vesicles [35,36]. However, it must be stated that vesicles produced from polymerised self-assembling monomers are essentially polymer shells, and it is unclear how much of the bilayer assembly actually survives the polymerisation step.

The main advantage of this technology is the ability to produce extremely stable carriers that resist degradation from detergents [37,38] and organic solvents [38-40]. They are also less leaky [39], are thermostable [41], and, because the vesicle-forming components are kinetically trapped by the polymerisation process, they have improved colloidal stability [40]. The resulting nanosystems may be isolated as dry powders that are readily dispersible in water to give 50- to 100-nm particles [38], potentially allowing the formulation of nanosystem-based solid dosage forms. However, as polymerisation involves reactive species, this technology is best applied prior to drug loading, and, thus, has some limitations.

4. Stability

Vesicle stability is a desirable characteristic for vesicle-containing medicines, and, as such, a great deal of scientific effort has been expended on producing ever more stable systems. As the drive for nanomedicines (medicines incorporating functional

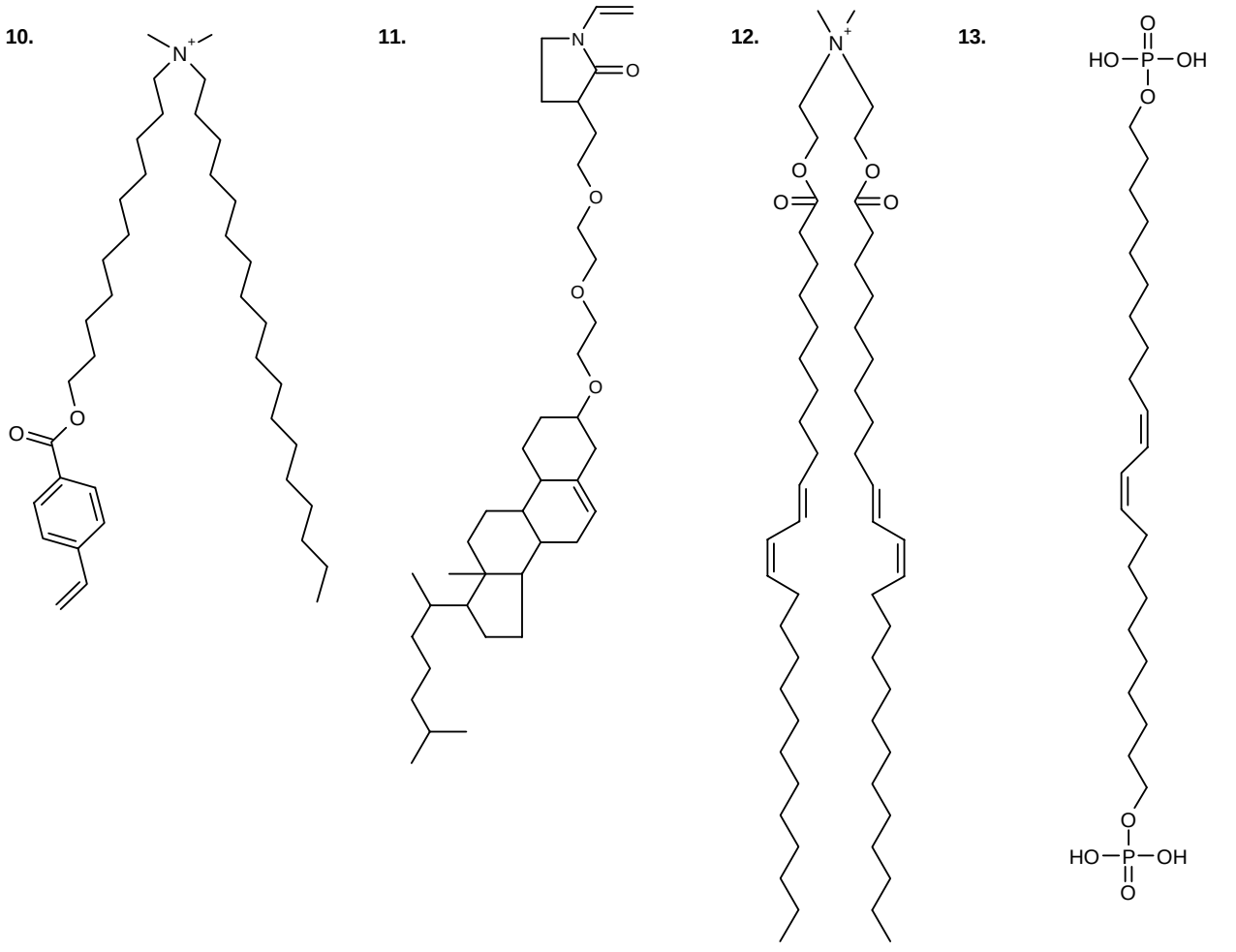

Figure 8. Polymerisable vesicle-forming monomers that are used to make polymerised vesicles. Monomer **10**: *N*,*N*-dimethyl-*N*-(11-[p-vinyl benzoyl oxy]undecyl)octadecyl ammonium bromide [53]. Monomer **11**: 1-ethenyl-3-(8-[cholest-5-en-3β-yloxy]-3,6-dioxaoctyl)-2-pyrrolidone [40]. Monomer **12**: *N*,*N*-dimethyl-2-(pentacosa-10,12-diynoyloxy)-*N*-(2-[pentacosa-10,12-diynoyl oxy]ethyl) ethanaminium bromide [35]. Monomer **13**: 10,12-docosadiyne-1,22-diphosphate [54].

nanoparticles) grows, stability issues will need to be adequately addressed to ensure the widespread adoption of such systems. In fact, the early workers in the polymeric vesicle field were primarily driven by this need to produce stable versions of the then promising liposomal drug carriers. Although carbohydrate vesicles have been reported to be morphologically stable for at least 2 months [19], extremely stable systems are possible on polymerisation of block copolymers after self-assembly. Poly(ethylene oxide)-*b*-poly(3-[trimethoxysilyl]propyl methacrylate) copolymer vesicles in water, methanol, triethylamine mixtures produced polymerised polymersomes that are colloidally stable for up to 1 year [25]. Triethylamine hydrolyses the trimethoxysilyl groups and then catalyses their polycondensation to yield an extremely stable hydrophobic polysilsesquioxane core [25,26].

5. Drug delivery applications

5.1 Prolonged circulation and the potential for drug targeting

Polymersomes composed of poly(ethylene oxide)-*b*-polybutadiene or poly(ethylene oxide)-*b*-polyethylethylene, in which the entire vesicle surface is covered with a poly(ethylene oxide) coat, have been studied as long-circulating nanocarriers for drug delivery [42]. These studies have been prompted by the observation that prolonged circulation of certain poly(ethylene oxide)-coated nano-sized (~ 50 – 250 nm) drug-carrying particulates facilitates tumour targeting on intravenous administration [1,43,44]. The circulation time of poly(ethylene oxide)-coated polymersomes is directly dependent on the length of the poly(ethylene oxide) block, and polymersome

Table 1. Biological activity of PEI- and poly(L-lysine)-based vesicles.

Polymer	A431 cells		A549 cells	
	IC_{50} (μg/ml)	Gene transfer relative to parent polymer	IC_{50} (μg/ml)	Gene transfer relative to parent polymer
PEI	1.9	1.0	5.2	1.00
Palmitoyl PEI (polymer 3)	16.9	0.2	12.6	0.08
Palmitoyl PEI (polymer 3), cholesterol 2:1 (g/g) vesicles	15.9	0.2	11.0	0.08
Poly(L-lysine)	7.0	1.0	7.0	1.00
Palmitoyl poly(L-lysine)-graft-poly(ethylene oxide) (polymer 6), cholesterol 2:1 (g/g) vesicles.	74.0	7.8	63.0	2.30

PEI: Polyethylenimine.
Information from [46,48].

half-lifes of up to 28 h have been recorded in rats with a poly(ethylene oxide) degree of polymerisation of 50 [42]. This half-life compares favourably with a half-life of 14 h recorded for poly(ethylene oxide)-coated liposomes [45]. It is assumed that the 100% surface coverage of the polymeric vesicles is responsible for the reduced clearance of these polymersomes from the blood [34]. The long half-life of these polymersomes makes them possible candidates for the development of anti-tumour medicines.

Furthermore, drug release may be controlled in the polymersomes by controlling the hydrolysis rate of the hydrophobic blocks [14]. This has been demonstrated with poly(L-lactic acid)-*b*-poly(ethylene oxide) and polycaprolactone-*b*-poly(ethylene oxide) vesicles [14]. Hydrolysis of the hydrophobic block causes the polymer to move from a vesicular to a micellar assembly as the overall level of hydrophobic content diminishes, and this, in turn, leads to drug release [14]. Hydrolysis rates and implicit release rates may be controlled by varying the relative level of the hydrophobic blocks. It is conceivable that an optimum combination of prolonged circulation and programmed release rates may yield nanosystems that are able to deliver high numbers of drug molecules to a target site at precise times after dosing, thus yielding extreme levels of both temporal and locoregional control of drug activity.

5.2 Cationic polymeric vesicles in drug and gene delivery

Poly(L-lysine)-based vesicles, prepared from polymer 6, have been used for gene delivery [46,47] as these vesicles are less toxic than unmodified poly(L-lysine) and produced higher levels of gene transfer (Table 1) [46]. The production of polymeric vesicles and the resultant reduction in cytotoxicity enables poly(L-lysine) derivatives to be used as *in vivo* gene carriers: the unmodified polymer is too toxic to use as an *in vivo* gene transfer system. When the targeting ligand–galactose was bound to the distal ends of the poly(ethylene oxide) chains, gene expression was increased in HepG2 cells *in vitro* [47]. However, *in vivo* targeting to the liver hepatocytes was not achieved with these systems [47].

A similar procedure with the PEI vesicles prepared using polymer 3 (Figure 4) also resulted in a reduction in the cytotoxicity of the polymer (Table 1) [48], although in this case the PEI vesicles were not as efficient gene transfer agents as the free polymer.

Carbohydrate polymeric vesicles may also be used as drug-targeting agents. Vesicles prepared from glycol chitosan amphiphiles improve the intracellular delivery of hydrophilic macromolecules [49] and anticancer drugs [50]; the latter is achieved with the help of a transferrin ligand attached to the surface of the vesicle. These carbohydrate vesicles improve the *in vivo* tolerability of the cytotoxic agent, doxorubicin. However, they are no more active than the drug alone against subcutaneously implanted A431 tumours [50].

5.3 Responsive release

The ultimate goal of all drug delivery efforts is the simple fabrication of responsive systems that are capable of delivering precise quantities of their payload in response to physiological or, more commonly, pathological stimuli. Preprogrammable and intelligently responsive pills, implants and injectables are so far merely the unobtainable ideal. However, polymeric systems have been fabricated with responsive capability and it is possible that, in the future, these may be fine-tuned to achieve truly intelligent and dynamic drug delivery.

The various environmental stimuli that may be used to trigger the release of encapsulated drug are outlined in the following subsections, and examples are provided regarding existing developments in the area. However, in addition to the areas covered below, it may be possible in the future for pathology-specific molecules to interact with polymeric vesicles and trigger release.

5.3.1 *pH*

Diblock polypeptides, in which the hydrophilic block consists of ethylene oxide-derivatised amino acids (L-lysine) and the

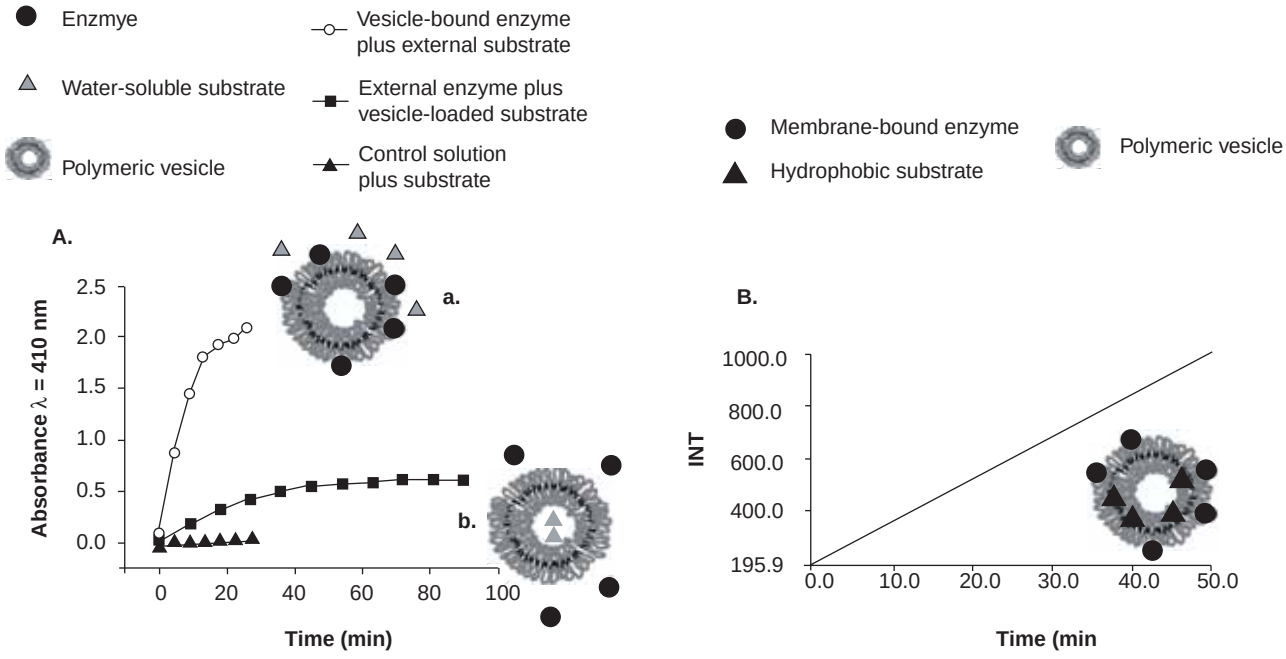

Figure 9. Enzyme-activated polymeric vesicles. A. Vesicles bearing membrane-bound enzyme (a) were formed by: probe sonicating palmitoyl glycol chitosan (polymer 2), cholesterol, *N*-biotinylated dipalmitoyl phosphatidyl ethanolamine (8:4:1 g/g) in sodium phosphate monobasic (0.1 M, 2 ml); isolation of the vesicles by ultracentrifugation (150,000 g); redispersion in a similar volume of sodium phosphate monobasic (0.1 M); and incubation of the vesicles with β-galactosidase streptavidin (3 U). Membrane-bound enzyme (0.2 ml) was then incubated with *O*-nitrophenyl-β-D-galactoside (2.1 mM, 2 ml-substrate) and the absorbance monitored (λ = 410 nm). The control solution contained similar levels of substrate, but no enzyme. Vesicles encapsulating *O*-nitrophenyl-β-D-galactoside (b) were prepared by: probe sonicating palmitoyl glycol chitosan, cholesterol (8:4 g/g) in the presence of *O*-nitrophenyl-β-D-galactoside solution (34 mM, 2 ml); and isolation of the vesicles by ultracentrifugation and redispersion in sodium phosphate monobasic (0.1 M). These latter vesicles (0.4 ml) were then incubated with β-D-galactosidase (2 U/ml, 0.1 ml) and the absorbance once again monitored. **B.** Vesicles bearing membrane-bound enzyme and containing the hydrophobic substrate fluorescein di-β-D-galactospyranoside were formed by: probe sonicating palmitoyl glycol chitosan (polymer 2), cholesterol, *N*-biotinylated dipalmitoyl phosphatidyl ethanolamine, fluorescein di-β-D-galactospyranoside (8:4:1:0.0005 g/g) in sodium phosphate monobasic (0.1 M, 2 ml); and incubation of the resulting vesicles with β-galactosidase streptavidin (0.3 U). The fluorescence of the enzyme-hydrolysed substrate was monitored (excitation wavelength = 490 nm, emission wavelength = 514 nm).
INT: Fluorescence intensity.

hydrophobic block consists of poly(L-leucine) (polymer 9), form pH-responsive vesicles that disaggregate at low pH (3.0), providing that the level of L-leucine and polymer chain length is maintained within defined limits of ~ 12 – 25 mole%, and the polymer has a degree of polymerisation of < 200 [16]. It is possible that such L-lysine systems, if suitably developed, may be applied to facilitate release in intracellular acidic compartments, such as the endosome, where a pH gradient of ~ 2 exist between the cytosol and the endosome.

5.3.2 *Enzymatic*

Vesicles that release their contents in the presence of an enzyme may be formed by loading polymeric vesicles with an enzyme-activated prodrug (Figure 9). The particulate nature of the drug delivery system should allow the drug to accumulate; for example, in tumours where the particulates may be activated by an externally applied enzyme in a similar manner to the antibody-directed enzyme prodrug therapeutic strategy. This strategy enables an enzyme to be targeted to tumours using antibodies, followed by the application of an enzyme-activated prodrug [51]. Alternatively, a membrane-bound enzyme may be used to control and ultimately prolong the activity of either an entrapped hydrophilic drug (entrapped in the vesicle aqueous core) or an entrapped hydrophobic drug (entrapped in the vesicle membrane), as demonstrated in Figure 9. It is possible that the enzyme may be chosen such that it is activated in the presence of pathology-specific molecules, thus achieving pathology-responsive and -localised drug activity.

5.3.3 *Magnetic*

Magnetically responsive polymerised liposomes composed of 1,2-di-(2,4-octadecadienoyl)-sn-glycerol-3-phosphoryl choline, loaded with ferric oxide and subsequently polymerised, may be localised by an external magnetic field to the small intestine and, specifically, to the Payer's patches [37].

These polymerised vesicles are stable to the degradative influence of solubilising surfactants, such as triton-X 100 [37], and, hence, should not suffer excessive bile salt-mediated degradation during gut transit. These magnetically responsive polymeric vesicles may be used to improve the absorption of drugs via the oral route, and the authors of this report found higher levels of a radioactive marker in the liver of magnetically treated mice, compared with control animals that had not been subjected to magnetic treatment [37].

5.3.4 *Oxygen*

Block copolymer vesicles that are destabilised by oxidative mechanisms have been constructed from poly(ethylene oxide)-*b*-poly(propylene sulfide)-*b*-poly(ethylene oxide) ABA block copolymers (in which two identical peripheral polymer blocks are situated at either end of a third differing polymer block) [30]. These polymeric vesicles are destabilised on oxidation of the central sulfide block to give sulphoxides and, ultimately, sulphones [30]. On oxidation, vesicles are transformed to worm-like micelles and, finally, spherical micelles, eventually releasing their contents. Such vesicles may find uses in the treatment of conditions that are characterised by inflammation.

6. Conclusions

In summary, polymeric vesicles may be fabricated from a variety of macromolecular amphiphile architectures, which include block copolymers, random graft copolymers and polymers bearing hydrophobic low molecular weight pendant groups. The size of the vesicles prepared from pendant-type polymers may be finely controlled by the polymer architecture, unlike vesicles prepared from low molecular weight amphiphiles. Additionally, polymeric vesicles may be prepared from the polymerisation of self-assembled polymerisable monomers. These high-stability particles, which reside in the nanometre and micrometer size domains, are ripe for exploitation in the areas of drug targeting and responsive delivery.

7. Expert opinion

For polymeric vesicles to make the transition from laboratory curiosities, albeit with interesting and demonstrably beneficial drug delivery properties, to clinically relevant technologies, issues such as biocompatibility of the constituent materials will need to be addressed. Ultimately, as drug developers seek to extend further benefits from off-patent molecules, technologies such as the ones described in this paper may gradually edge towards the mainstream, and biocompatibility issues will then be systematically dealt with for the more promising of their number. A further issue that also needs to be addressed is the complexity of the technologies and the knock-on effect that this may have on the large-scale manufacture and, ultimately, accessibility of the technology to patients. Colloidal systems that add demonstrable benefits to drugs, by enabling their bioavailability via non-traditional routes or substantially diminishing their side effects, will only be commercially exploited if their large-scale manufacture is not associated with considerable cost. In summary, the position of a technology on a sliding scale of rewards versus cost of manufacture/biocompatibility will ultimately determine the success of the technology in discussion.

Bibliography

1. GABIZON AA: Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. *Cancer Invest.* (2001) 19:424-436.
2. ALLEN T, CULLIS P: Drug delivery systems: entering the mainstream. *Science* (2004) 303:1818-1822.
3. RINGSDORF H, SCHLARB B, VENZMER J: Molecular architecture and function of polymeric oriented systems: models for the study of organisation, surface recognition, and dynamics of biomembranes. *Angew. Chem. Int. Ed. Engl.* (1988) 27:113-158.
4. DISCHER DE, EISENBERG A: Polymer vesicles. *Science* (2002) 297:967-973.
5. ANTONIETTI M, FORSTER S: Vesicles and liposomes: a self-assembly principle beyond lipids. *Adv. Mater.* (2003) 15:1323-1333.
6. UCHEGBU IF, SCHATZLEIN AG, TETLEY L *et al.*: Polymeric chitosan-based vesicles for drug delivery. *J. Pharm. Pharmacol.* (1998) 50:453-458.
7. WAKITA M, HASHIMOTO M: Bilayer vesicle formation of *N*-octadecylchitosan. *Kobun. Ronbun.* (1995) 52:589-593.
8. WANG W, TETLEY L, UCHEGBU IF: The level of hydrophobic substitution and the molecular weight of amphiphilic poly-L-lysine-based polymers strongly affects their assembly into polymeric bilayer vesicles. *J. Coll. Interf. Sci.* (2001) 237:200-207.
9. ISRAELACHVILI J: *Intermolecular & Surface Forces*. Academic Press, London, UK (1991).
10. WANG W, QU X, GRAY AI, TETLEY L, UCHEGBU IF: Self assembly of cetyl linear polyethylenimine to give micelles, vesicles and nanoparticles is controlled by the hydrophobicity of the polymer. *Macromolecules* (2004) 37:9114-9122.
11. DISCHER B, WON YY, EGE JC *et al.*: Polymersomes: tough vesicles made from diblock copolymers. *Science* (1999) 284:1143-1146.
12. SCHILLEN K, BRYSKHE K, MEL'NIKOVA YS: Vesicles formed form poly(oxyethylene)-poly(oxypropylene)-poly (oxyethylene) triblock copolymers in dilute aqueous solution. *Macromolecules* (1999) 32:6885-6888.
13. KUKULA H, SCHLAAD H, ANTONIETTI M, FORSTER S: The formation of polymer vesicles or 'peptosomes' by polybutadiene-*block*-poly(L-glutamate)s in dilute aqueous solution. *J. Am. Chem. Soc.* (2002) 124:1658-1663.
14. AHMED F, DISCHER DE: Self-porating polymersomes of PEG-PLA and PEG-PCL: hydrolysis-triggered controlled release vesicles. *J. Control. Release* (2004) 96:37-53.

15. BERMUDEZ H, BRANNAN AK, HAMMER DA, BATES FS, DISCHER DE: Molecular weight dependence of polymerosome membrane structure, elasticity, and stability. *Macromolecules* (2002) 35:8203-8208.
16. BELLOMO EG, WYRSTA MD, PAKSTIS L, POCHAN DJ, DEMING TJ: Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nature Mater.* (2004) 3:244-248.
17. ELBERT R, LASHEWSKY A, RINGSDORF H: Hydrophilic spacer groups in polymerizable lipids: formation of biomembrane models from bulk polymerised lipids. *J. Am. Chem. Soc.* (1985) 107:4134-4141.
18. KUNITAKE T, NAKASHIMA K, TAKARABE M *et al.*: Vesicles of polymeric bilayer and monolayer membranes. *J. Am. Chem. Soc.* (1981) 103:5945-5947.
19. WANG W, MCCONAGHY AM, TETLEY L, UCHEGBU IF: Controls on polymer molecular weight may be used to control the size of palmitoyl glycol chitosan polymeric vesicles. *Langmuir* (2001) 17:631-636.
20. WANG W, TETLEY L, UCHEGBU IF: A new class of amphiphilic poly-L-lysine based polymers forms nanoparticles on probe sonication in aqueous media. *Langmuir* (2000) 16:7859-7866.
21. CHARROIS GJR, ALLEN TM: Rate of biodistribution of STEALTH® liposomes to tumor and skin: influence of liposome diameter and implications for toxicity and therapeutic activity. *Biochim. Biophys. Acta* (2003) 1609:102-108.
22. FLORENCE AT, HILLERY AM, HUSSAIN N, JANI PU: Factors affecting the oral uptake and translocation of polystyrene nanoparticles – histological and analytical evidence. *J. Drug Target.* (1995) 3:65-70.
23. LITZINGER DC, BUITING AMJ, VANROOIJEN N, HUANG L: Effect of liposome size on the circulation time and intraorgan distribution of amphipathic poly(ethylene glycol)-containing liposomes. *Biochim. Biophys. Acta* (1994) 1190:99-107.
24. YU KH, EISENBERG A: Bilayer morphologies of self-assembled crew-cut aggregates of amphiphilic PS-*b*-PEO copolymers in solution. *Macromolecules* (1998) 31:3509-3518.
25. DU JZ, CHEN YM: Preparation of organic/inorganic hybrid hollow particles based on gelation of polymer vesicles. *Macromolecules* (2004) 37:5710-5716.
26. DU JZ, CHEN YC, ZHANG Y *et al.*: Organic/inorganic hybrid vesicles based on a reactive block copolymer. *J. Am. Chem. Soc.* (2003) 125:14710-14711.
27. LIN JJ, SILAS JA, BERMUDEZ H *et al.*: The effect of polymer chain length and surface density on the adhesiveness of functionalized polymersomes. *Langmuir* (2004) 20:5493-5500.
28. AHMED F, HATEGAN A, DISCHER DE, DISCHER BM: Block copolymer assemblies with cross-link stabilization: from single-component monolayers to bilayer blends with PEO-PLA. *Langmuir* (2003) 19:6505-6511.
29. BERMUDEZ H, ARANDA-ESPINOZA H, HAMMER DA, DISCHER DE: Pore stability and dynamics in polymer membranes. *Europhys. Lett.* (2003) 64:550-556.
30. NAPOLI A, VALENTINI M, TIRELLI N, MULLER M, HUBBELL JA: Oxidation-responsive polymeric vesicles. *Nature Mater.* (2004) 3:183-189.
31. DISCHER BM, HAMMER DA, BATES FS, DISCHER DE: Polymer vesicles in various media. *Curr. Opin. Coll. Interf. Sci.* (2000) 5:125-131.
32. HALUSKA CK, GOZDZ WT, DOBEREINER HG, FORSTER S, GOMPPER G: Giant hexagonal superstructures in diblock-copolymer membranes. *Phys. Rev. Lett.* (2002) 89:238302.
33. DIMOVA R, SEIFERT U, POULIGNY B, FORSTER S, DOBEREINER HG: Hyperviscous diblock copolymer vesicles. *Eur. Phys. J E Soft Matter* (2002) 7:241-250.
34. LEE JCM, BERMUDEZ H, DISCHER BM *et al.*: Preparation, stability, and *in vitro* performance of vesicles made with diblock copolymers. *Biotechnol. Bioengineer.* (2001) 73:135-145.
35. HUB HH, HUPFER B, KOCH H, RINGSDORF H: Polymerizable phospholipid analogues – new stable biomembrane cell models. *Angew. Chem. Int. Ed. Engl.* (1980) 19:938-940.
36. EATON PE, JOBE PG, KAYSON N: Polymerised vesicles. *J. Am. Chem. Soc.* (1980) 102:6638-6640.
37. CHEN HM, LANGER R: Magnetically-responsive polymerized liposomes as potential oral delivery vehicles. *Pharm. Res.* (1997) 14:537-540.
38. CHO I, CHUNG KC: Cholesterol-containing polymeric vesicles – syntheses, characterization, and separation as a solid powder. *Macromolecules* (1988) 21:565-571.
39. CHO I, KIM YD: Synthesis and properties of tocopherol-containing polymeric vesicle systems. *Macromol. Symp.* (1997) 118:631-640.
40. CHO I, DONG S, JEONG SW: Vesicle formation by nonionic polymerizable cholesterol-based amphiphiles. *Polymer* (1995) 36:1513-1515.
41. CHO I, KIM YD: Formation of stable polymeric vesicles by tocopherol-containing amphiphiles. *Macromol. Rapid Commun.* (1998) 19:27-30.
42. PHOTOS PJ, BACAKOVA L, DISCHER B, BATES FS, DISCHER DE: Polymer vesicles *in vivo*: correlations with PEG molecular weight. *J. Control. Release* (2003) 90:323-334.
43. UNEZAKI S, MARUYAMA K, ISHIDA O *et al.*: Enhanced tumour targeting and improved antitumour activity of doxorubicin by long-circulating liposomes containing amphipathic poly(ethylene glycol). *Int. J. Pharm.* (1995) 126:41-48.
44. UCHEGBU IF, FLORENCE AT: Nonionic surfactant vesicles (niosomes) – physical and pharmaceutical chemistry. *Adv. Coll. Interf. Sci.* (1995) 58:1-55.
45. BLUME G, CEVC G: Molecular mechanism of the lipid vesicle longevity *in vivo*. *Biochim. Biophys. Acta* (1993) 1146:157-168.
46. BROWN MD, SCHATZLEIN A, BROWNLIE A *et al.*: Preliminary characterization of novel amino acid based polymeric vesicles as gene and drug delivery agents. *Bioconjug. Chem.* (2000) 11:880-891.
47. BROWN MD, GRAY AI, TETLEY L *et al.*: *In vitro* and *in vivo* gene transfer with polyamino acid vesicles. *J. Control. Release* (2003) 93:193-211.
48. BROWNLIE A, UCHEGBU IF, SCHATZLEIN AG: PEI based vesicle-polymer hybrid gene delivery system with improved biocompatibility. *Int. J. Pharm.* (2004) 274:41-52.

49. DUFES C, SCHATZLEIN AG, TETLEY L *et al.*: Niosomes and polymeric chitosan based vesicles bearing transferrin and glucose ligands for drug targeting. *Pharm. Res.* (2000) 17:1250-1258.

50. DUFES C, MULLER J-M, COUET W *et al.*: Anticancer drug delivery with transferrin targeted polymeric chitosan vesicles. *Pharm. Res.* (2004) 21:101-107.

51. BAGSHAWE KD, SHARMA SK, SPRINGER CJ *et al.*: Antibody directed enzyme prodrug therapy (ADEPT): clinical report. *Dis. Markers* (1991) 9:233-238.

52. BURKE SE, EISENBERG A: Kinetic and mechanistic details of the vesicle-to-rod transition in aggregates of PS310-*b*-PAA(52) in dioxane-water mixtures. *Polymer* (2001) 42:9111-9120.

53. JUNG M, DEN OUDEN I, MONTOYA-GONI A *et al.*: Polymerization in polymerizable vesicle bilayer membranes. *Langmuir* (2000) 16:4185-4195.

54. BADER H, RINGSDORF H: Liposomes from α, ω-dipolar amphiphiles with a polymerizable diyne moiety in the hydrophobic chain. *J. Polymer Sci. Polymer Chem. Ed.* (1982) 20:1623-1628.

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