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Review article

A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes

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Abstract

Amphiphilic block copolymers are able to form a range of different nanoparticulate structures. These include micelles, nanospheres, nanocapsules, and polymersomes. This review attempts to clarify some of the terminology used in the literature by providing an overview of the major features of each type of nanoparticle and the factors that influence the formation of particular nanoparticulate formulations.

Keywords: Amphiphilic block copolymer; Nanoparticle; Micelle; Nanosphere; Nanocapsule; Polymersome

1. Introduction

In recent decades there has been increased interest in the use of nanoparticles for drug delivery applications. Nanoparticles are colloidal-sized particles, possessing diameters ranging between 1 and 1000 nm, and drugs may be encapsulated, adsorbed or dispersed in them. A wide variety of nanoparticles composed of a range of materials including lipids, polymers and inorganic materials have been developed, resulting in delivery systems that vary in their physicochemical properties and thus their applications [1–4]. To date, an array of nanoparticulate drug delivery systems have been investigated including, but not limited to, liposomes, micelles, nanospheres, niosomes, nanocapsules, solid lipid nanoparticles, microemulsions and carbon nanotubes (Fig. 1).

The popularity of these systems is due in part to the several advantages they provide for delivering their drug payload. The nano-size range of these delivery systems allows them to be injected directly into the systemic circulation without the risk of blocking blood vessels [5,6]. It has been shown that the size of the nanoparticle is a major factor determining the in vivo fate of the particles. Researchers have demonstrated that opsonization and subsequent recognition and phagocytosis by macrophages is strongly correlated with the size of the particle [7,8]. It has been found that particles under 200 nm in diameter display a decreased rate of clearance and thus an extended circulation time as compared to those with a larger diameter [9]. This phenomenon may be explained by the fact that smaller particles display a surface with a high radius of curvature preventing the efficient binding of opsonins [10]. The circulation time of nanoparticles is further increased by the inclusion of surface bound hydrophilic molecules such as polyethylene glycol (PEG) [11–13]. PEG chains create a highly water bound barrier on the particle surface which blocks the adhesion of opsonins. The circulation time of nanoparticles is additionally prolonged by their reduced renal excretion due to their sufficiently large particle size which prevents glomerular filtration [14]. The extended circulation time combined with the small diameter of the

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particles has been shown to lead to increased accumulation of the entrapped drugs in tissues with increased vascular permeability and impaired lymphatic drainage such as tumours and inflamed tissues [15,16]. This phenomenon, referred to as the enhanced permeability and retention (EPR) effect, can be exploited as a way of passively targeting the encapsulated drug to its site of action, thus reducing the accumulation in healthy tissues and subsequent adverse effects. Commonly, nanoparticles composed of biodegradable polymers exhibit controlled release of their drug payload by diffusion, polymer degradation or micelle dissociation mechanisms [17–19]. These systems may provide prolonged exposure of the drug at their site of action once they have accumulated at their target. Nanoparticles composed of biocompatible materials have also been used to increase the aqueous solubility of several hydrophobic drugs via solubilization within the hydrophobic core of the nanoparticles [20]. This is a promising approach to solubilizing drugs and eliminating the use of excipients such as Cremophor EL™ which has been shown to cause hypersensitivity reactions [21–27].

Amphiphilic block copolymers have attracted a great deal of attention in terms of their ability to form various types of nanoparticles (see Fig. 1). These polymers are obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an aqueous solvent. These materials, when intended for use in drug delivery, are generally composed of biocompatible, biodegradable hydrophobic polymer blocks such as polyesters or poly(amino acids) covalently bonded to a biocompatible hydrophilic block, typically PEG. However, other hydrophilic blocks such as poly(*N*-vinyl-2-pyrrolidone), poly(2-ethyl-2-oxazoline), and poly(acrylic acid) have been investigated (Table 1) [26,28–32]. To date, numerous block copolymers have been synthesized, not only with a variety of block combinations, but also varying hydrophilic and hydrophobic block lengths. The literature abounds with studies using amphiphilic block copolymers of different compositions and various methods of preparation that produce nanoparticles referred to as micelles, nanospheres, core-shell nanoparticles, micelle-like nanoparticles, crew cut micelles, nanocapsules and polymersomes. In addition to the benefit of being able to custom synthesize these copolymers for their intended use, some amphiphilic copolymers have been shown to modulate the activity of the efflux pump, *P*-glycoprotein [33–37]. These materials may prove to be useful in the delivery of drugs across membranes expressing this protein such as the intestinal epithelia, drug resistant tumours and the blood–brain barrier. Although there are several excellent reviews of block copolymer micelles and their application in drug delivery [1,14,38–43], we believe it is worthwhile discussing the complete range of different nanoparticulate structures formed by amphiphilic block copolymers and to provide a perspective on the use of terminology in this field. There are examples of the interchangeable use of such terminology [17,24,44]. Thus, it is the objective of this review to discuss the major features of nanoparticles that are formed by amphiphilic copolymers, namely micelles, nanospheres, nanocapsules and polymersomes and to examine the factors that influence the physicochemical properties of the formed nanoparticles.

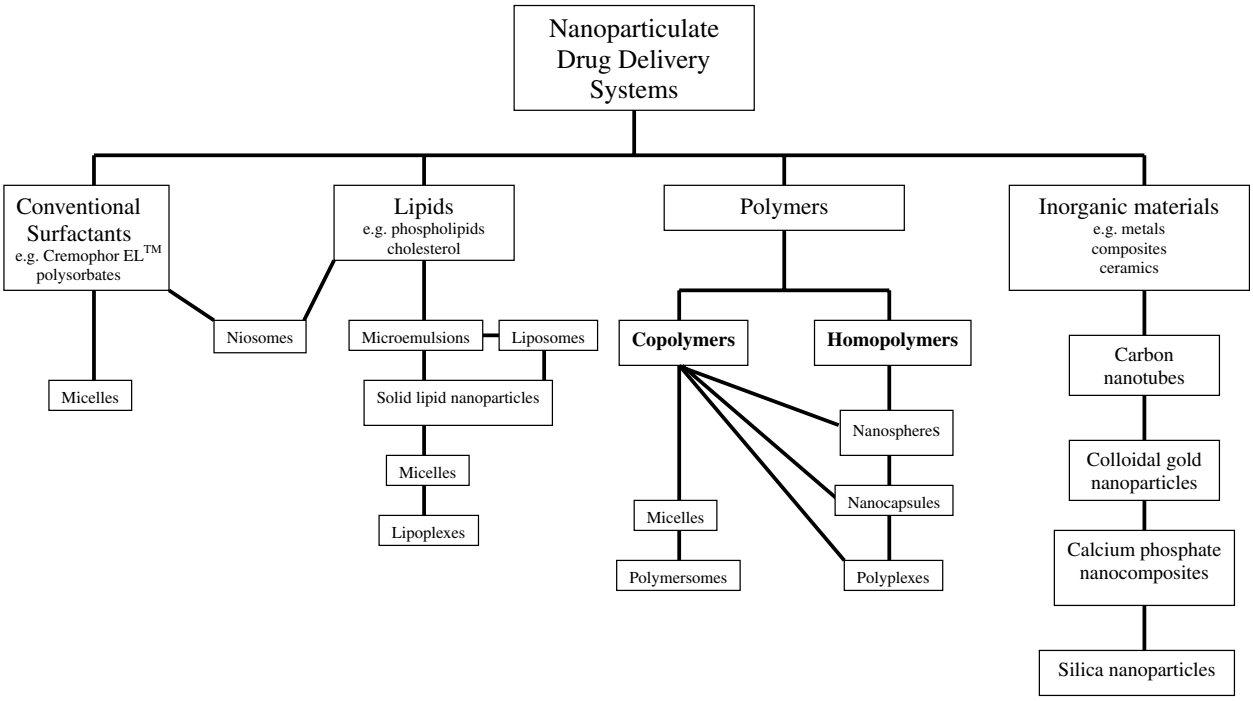

Fig. 1. Classification of nanoparticulate drug-delivery systems.

Table 1
Amphiphilic block copolymers commonly used for formation of drug delivery systems, their abbreviations and systems formed

Copolymer	Abbreviation	Delivery systems formed	Reference
MePEG-b-poly(D,L-lactic acid)	MePEG-b-PDLLA	Micelles, nanospheres, nanocapsules, polymersomes	[26,54,64,71]
MePEG-b-poly(caprolactone)	MePEG-b-PCL	Micelles, nanospheres, polymersomes, nanocapsules	[52,54,70,95]
MePEG-b-poly(lactic-co-glycolic acid)	MePEG-b-PLGA	Nanospheres, nanocapsules, micelles	[23,28,54]
PEO-poly(propylene oxide)-PEO	PEO-PPO-PEO Pluronic®	Micelles	[46]
PEG-poly(β-benzyl-L-aspartate)	PEG-b-PBLA	Micelles	[49]
Poly(acrylic acid)-b-polystyrene	PAA-b-PS	Micelles	[85]
PEO-b-polybutadiene	PEO-b-PBD	Polymersomes	[67]

MePEG, methoxypolyethylene glycol; PEO, polyethylene oxide; PEG, polyethylene glycol.

2. General characteristics of copolymer nanoparticles

2.1. Micelles

Due to the unique structure of amphiphilic molecules they have a tendency to accumulate at the boundary of two phases and thus are termed surfactants. In aqueous solutions, amphiphilic molecules orientate themselves so that the hydrophobic blocks are removed from the aqueous environment in order to achieve a state of minimum free energy. As the concentration of amphiphile in solution is increased, the free energy of the system begins to rise due to unfavourable interactions between water molecules and the hydrophobic region of the amphiphile resulting in structuring of the surrounding water and a subsequent decrease in entropy. At a specific and narrow concentration range of amphiphile in solution, termed the critical micelle concentration (CMC), several amphiphiles will self-assemble into colloidal-sized particles termed micelles (Fig. 2). The formation of micelles effectively removes the hydrophobic portion of the amphiphile from solution minimizing unfavourable interactions between the surrounding water molecules and the hydrophobic groups of the amphiphile. If the amphiphile concentration in solution remains above the CMC, micelles are thermodynamically stabilized against disassembly. Upon dilution below the CMC, micelles will disassemble with the rate of disassembly being largely dependent on the structure of the amphiphiles and interactions between the chains [41]. In this respect, amphiphilic copolymer micelles have a distinct advantage over those formed from conventional surfactants such as Cremophor EL™ or polysorbates, since they typically not only display lower CMCs, but also in some cases resist disassembly upon dilution due to physical interactions among chains in the micelle core. Due to their nanoscopic size and the nature by which they are formed, micelles are classified as association or amphiphilic colloids, but should not be considered solid particles [45]. It has been shown in several experiments using light scattering, sedimentation velocity and small angle X-ray scattering that the individual molecules or unimers that make up the micelle are in a dynamic equilibrium with the unimers in the bulk and can therefore obey what is termed a closed association model [41,46–48].

Micelles typically have diameters ranging from 10 to 100 nm and are characterized by a core-shell architecture

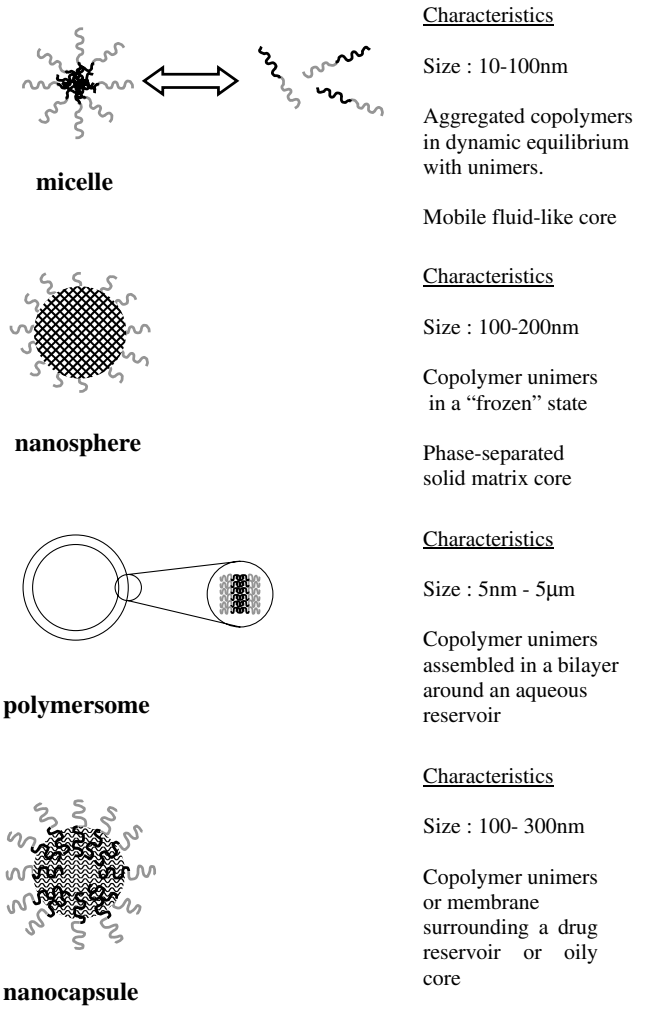

Fig. 2. Nanoparticulate drug delivery systems formed by amphiphilic block copolymers and their general characteristics.

in which the inner core is composed of the hydrophobic regions of the amphiphiles creating a cargo space for the solubilization of lipophilic drugs [14,38–41]. The core region is surrounded by a palisade or corona composed of the hydrophilic blocks of the amphiphiles. The hydrophilic blocks forming the corona region become highly water bound and adopt a "splayed" appearance, giving rise to different conformations such as a polymer "brush" [10]. These conformations sterically suppress opsonization by

blood components, thus resisting phagocytosis by macrophages and decreasing clearance by the reticuloendothelial system (RES), resulting in prolonged circulation times [10,49–52].

2.2. Nanospheres

A polymeric nanosphere may be defined as a matrix-type, solid colloidal particle in which drugs are dissolved, entrapped, encapsulated, chemically bound or adsorbed to the constituent polymer matrix [5,53,54]. These particles are typically larger than micelles having diameters between 100 and 200 nm and may also display considerably more polydispersity (Fig. 2) [39].

Even though elimination may be slowed by the submicron particle size of nanospheres, clearance is still inevitable due to capture by the RES, sequestering particles within organs such as the liver and spleen [55]. It has been shown that the hydrophobic surfaces of these particles are highly susceptible to opsonization and clearance by the RES. Hence, it became clear that in order to prolong the circulation of nanoparticles, the surfaces must be modified to "look like water" so that they appear to be invisible to the RES [56]. Attempts have been made to alter the surface of nanoparticles by adsorbing various surfactants to the particle surface including poloxamine, poloxamer and Brij [57–59]. Although surfactant coating reduced the total uptake by the RES organs over short periods of time, no difference between uncoated and coated particles was found over longer periods likely due to desorption of the surfactant [5,6,55,60]. Nanospheres prepared using amphiphilic copolymers such as MePEG-b-PLA with high molecular weight hydrophobic blocks provided conjugated PEG coatings with greater stability [12,61]. Diblock copolymer nanospheres show a phase-separated structure with a solid core [12].

As will be discussed in greater depth in Section 4 below, a clear distinction between micelles and nanospheres formed from diblock copolymers is not always possible, or desirable. Comprehensive studies using a series of MePEG-b-PDLLA copolymers by Riley et al. and Heald et al. investigated the effects of increasing hydrophobic block length on the physicochemical properties of nanoparticles formed [62,63]. They showed that aggregation behavior and copolymer architecture of MePEG-b-PDLLA were strongly dependent on copolymer composition. As the molecular weight of the PDLLA block increased, the central core of the nanoparticles became more solid-like, resembling nanospheres, whereas smaller PDLLA blocks produced nanoparticles that were termed micelle-like assemblies [62].

2.3. Nanocapsules and polymersomes

Polymeric nanocapsules and polymersomes are colloidal-sized, vesicular systems in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating [53] (Fig. 2). There are two variations possible, depending on the core and the structure of the surrounding polymer. Frequently, the core is an oily liquid, the surrounding polymer is a single layer of polymer, and the vesicle is referred to as a nanocapsule. These systems have found utility in the encapsulation and delivery of hydrophobic drugs including Ru 58668, methotrexate, xanthone and 3-methylxanthone [54,64,65]. Polymers used for the formation of nanocapsules have typically included polyester homopolymers such as PLA, PLGA and PCL. In recent years copolymers of PEG and PLA have been used to avoid opsonization of the particles, similar to nanospheres [54]. Nanocapsules composed of a copolymer of PEG and chitosan have recently been used for the oral delivery of salmon calcitonin. The PEG was found to increase the stability of the nanocapsules in gastrointestinal fluid while reducing their cytotoxicity [66].

Alternatively, if the core of the vesicle is an aqueous phase and the surrounding coating is a polymer bilayer, the particle is referred to as a polymersome [67]. These vesicles are analogous to liposomes and find utility in the encapsulation and delivery of water-soluble drugs which can be entrapped in their aqueous reservoir, but they differ from liposomes in that the external bilayer is composed of amphiphilic copolymers. The diblock copolymers PEG-b-PBD (polybutadiene) and PEG-b-PEE (polyethylethylene) are strong vesicle or polymersome formers [68,69]. These materials are bioinert but not biodegradable, and therefore investigations have focused on the development of polymersomes composed of pegylated polyesters such as PEG-b-PDLLA and PEG-b-PCL either as the sole constituent of the vesicle or blended with PEG-b-PBD [70,71]. Polymersomes generally possess a greater PEG surface density and longer circulation times compared to PEGylated liposomes [72].

3. General methods of preparation of polymeric nanoparticles

3.1. Micelles

The method chosen for the formation of block copolymer micelles is dependent on the solubility of the copolymer being used. If the copolymer is relatively water soluble, two methods may be employed for the formation of micelles. The first is the direct dissolution method, in which the copolymer is simply added to the aqueous media at a concentration above the CMC and drug is allowed to partition into the core of the micelles [73]. The second method is the film casting method, which involves the dissolution of the copolymer and drug in a volatile solvent which is then evaporated to leave a film in the bottom of a vial to be used. Warm buffer or water is then added with agitation to dissolve the polymer film [25,26]. If the copolymer is not readily soluble in water, a dialysis, or oil in water emulsion procedure can be utilized. In the dialysis method, the copolymer and drug are solubilized in a water-miscible

organic solvent and micelles are formed by the addition of water, or alternatively the addition of the copolymer/drug/solvent solution to stirring water, followed by dialysis against aqueous media to remove the solvent [18,19,74,75]. The oil in water emulsion procedure involves the addition of a solution consisting of the copolymer drug and a volatile, non-water-miscible organic solvent into rapidly stirring aqueous media, with or without a surfactant, and the evaporation of the solvent [76].

3.2. Nanospheres

Nanospheres can be prepared by two general methods depending on the polymer to be used. If the particles formed require polymerization, this can be achieved by either emulsion polymerization, for example in the case of poly(methylmethacrylate) and poly(ethylcyanoacrylate), or by interfacial polymerization as for poly(alkylcyanoacrylate). For preformed polymers such as biodegradable polyesters and their copolymers with PEG, nanosphere preparation can be achieved using emulsification/solvent evaporation, emulsification/solvent diffusion and salting out techniques. However, the most popular method is solvent displacement also referred to as nanoprecipitation [77,78]. This method involves the dissolution of the polymer in an organic, water-miscible solvent, which is then added to the aqueous phase in the presence or absence of a surfactant. Upon addition to the aqueous phase, the organic solvent immediately diffuses out leading to the precipitation of the polymer and formation of nanoparticles.

3.3. Nanocapsules

The most common method of producing nanocapsules is by the interfacial deposition of preformed polymers [79]. In this procedure, a solution of drug in a water-miscible organic solvent, such as acetone (with or without a lipophilic surfactant), is prepared. To this solution, an oil which is miscible with the solvent but immiscible with the mixture is added, and this solution is dispersed into the aqueous phase that frequently contains a hydrophilic surfactant (often poloxamer). Upon moderate agitation, the solvent diffuses into the aqueous phase and the polymer aggregates around the oil droplet. Nanocapsules may also be produced with a modification to the solvent displacement technique, in which an oil is added to the organic phase.

3.4. Polymersomes

Preparation of polymersomes is similar to that of liposomes, using a film rehydration technique. Briefly, the copolymer is dissolved in a volatile organic solvent such as chloroform in a glass vial. The solvent is evaporated under a stream of nitrogen gas to leave a thin film of polymer, which is rehydrated with the aqueous phase using vigorous stirring, sonication and extrusion to yield submicron vesicles with a narrow size distribution [68,70,72].

4. Factors influencing the formation and physicochemical properties of amphiphilic copolymer nanoparticle formulations

Fig. 3 attempts to summarize the different factors related to the block copolymer composition and the methods of preparation that may influence the types of nanoparticles formed. These are discussed in depth below.

4.1. Block length

In the literature it has been shown that as the ratio of the molecular weights of the hydrophilic and hydrophobic blocks changes, the method of preparation to obtain particular nanoparticle formulations needs to be correspondingly altered [53]. When the molecular weight of the hydrophilic block exceeds that of the hydrophobic block, the copolymer is easily dispersed in water and will self-assemble into small, relatively monodisperse micelles. However, when the molecular weight of the hydrophobic block approaches or exceeds the molecular weight of the hydrophilic block, the copolymer becomes progressively more water insoluble and therefore will not self-assemble into a nanoparticle through direct dissolution or film casting methods, but rather dialysis, emulsification or in some cases nanoprecipitation techniques must be employed.

The fundamental studies of Riley et al. and Heald et al. using MePEG-b-PDLLA copolymers with a range of PDLLA molecular weights and a fixed MePEG molecular weight of 5000 g/mol showed that if the PDLLA molecular weight was relatively low (2000–30,000 g/mol), the hydrodynamic radius (R_{hyd}) of the resulting particles was independent of the concentration of the polymer used during preparation and the polydispersity index was low, characteristic of block copolymer micelles [62,63]. It was determined that the R_{hyd} and aggregation number (N_{agg}) of nanoparticles are highly dependent on the length of the constituent blocks. Power laws have been developed to express the dependence of the R_{hyd} and N_{agg} of nanoparticles on the hydrophobic and hydrophilic block lengths, designated N_A and N_B, respectively [80–82]. In the case of star micelles in which $N_B \gg N_A$, the scaling relationships were found to be:

$$R_{hyd} \propto {N_A}^{4/25} {N_B}^{3/5}$$

and

$$N_{agg} \propto {N_A}^{4/5}$$

or for strongly segregated blocks

$$N_{agg} \propto {N_A}^{2}$$

For the other extreme, crew cut micelle-like aggregates in which $N_B \ll N_A$, the scaling relationships were found to be:

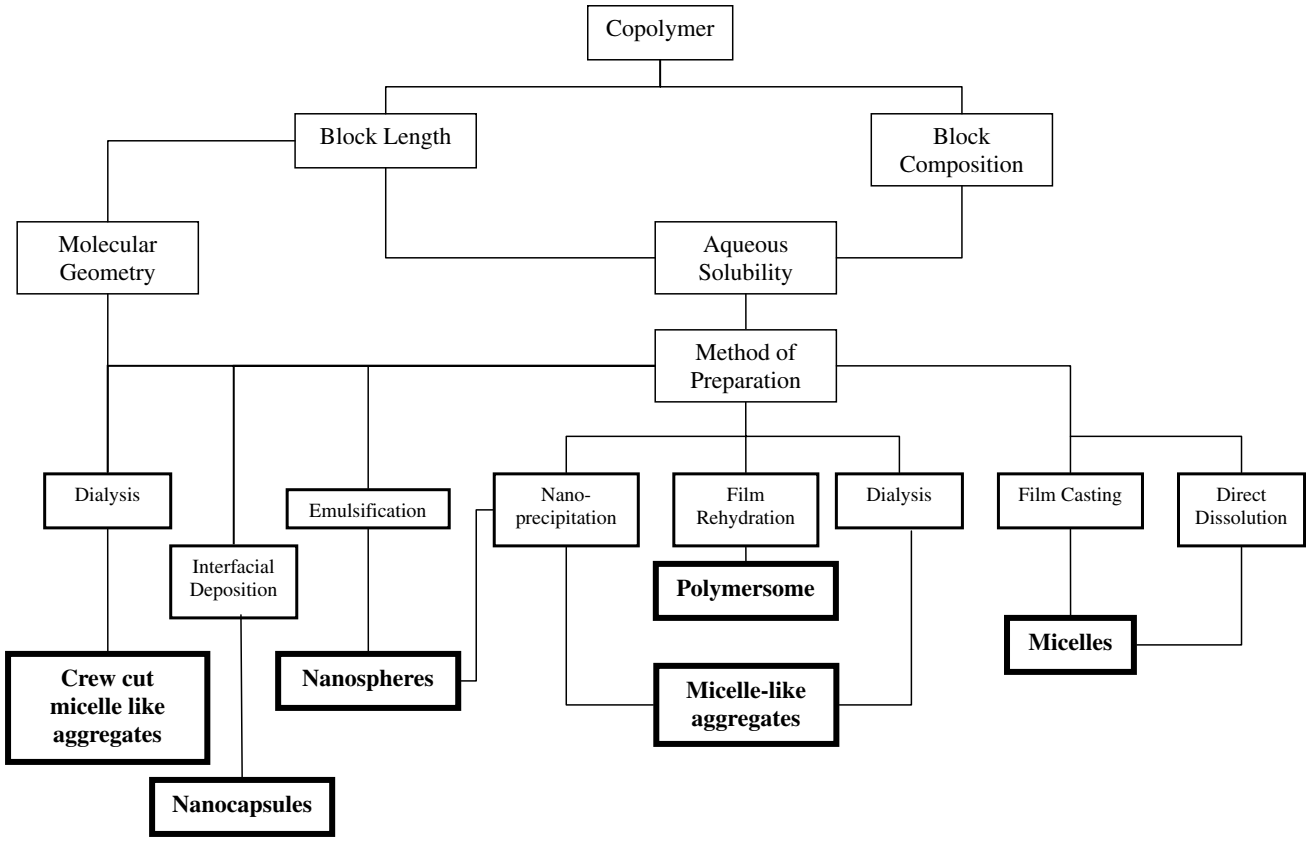

Fig. 3. Factors affecting the formation of amphiphilic block copolymer nanoparticles. Copolymer composition factors including block length composition and molecular geometry combine with different methods of preparation to produce varying types of nanoparticles.

$$R_{hyd} \propto {N_A}^{2/3}$$

and

$$N_{agg} \sim N_A$$

Riley et al. found that the hydrodynamic radius of particles formed by copolymers with PDLLA molecular weights between 2000 and 30,000 g/mol scaled linearly when plotted against ${N_A}^{1/3}$. This suggested a micellar regime somewhere between star micelles and crew cut micelle-like assemblies, and they settled on the term ''micelle-like'' assemblies. In contrast, nanoparticles formed from copolymers with higher PDLLA molecular weight segments (45,000–110,000 g/mol) displayed hydrodynamic radii that were dependent on the concentration of copolymer solution used during preparation. The nanoparticles produced were more ''particulate-like'' with a ''solid-like core'' [62,63], analogous to nanospheres shown in Fig. 2. The aggregation number of particles formed from diblocks with relatively low PDLLA molecular weights increased sharply, scaling in good agreement with micellar assemblies of strongly segregated blocks. Further increases in the PDLLA molecular weight from 9000 to 30,000 g/mol resulted in deviations from the scaling dependence of aggregation number on the PDLLA block length indicative of kinetically ''frozen'' micellar systems. Additional increases in PDLLA block length resulted in an aggregation number dependence on the concentration of copolymer used in preparation.

Heald et al. used a variety of NMR techniques including ^{1}H liquid state, T_1 relaxation and ^{13}C solid state to study the shift in the state of the copolymers comprising nanoparticles as the molecular weight of the PDLLA block was increased from 2000 to 25,000 g/mol while the MePEG molecular weight was held constant at 5000 g/mol [63]. It was found by ^{1}H liquid state NMR in D_2O that nanoparticles with a core shell structure were formed by all copolymers with almost complete disappearance of the signals from methyl protons. However, the presence of a doublet–doublet resonance indicated an intermediate more mobile phase at the interface between the MePEG and PDLLA. The ^{13}C solid-state NMR experiments confirmed the presence of two phases in the PDLLA core of the nanoparticles formed by all the copolymers regardless of the PDLLA molecular weight: a solid-like core and a more mobile interfacial region. Upon heating the nanoparticulate samples, it was found that the amount of methyl and methine protons detectable in the low molecular weight PDLLA samples (2000–4000 g/mol) increased indicating a more fluid core, whereas copolymers with PDLLA molecular weights above 6000 g/mol displayed little change indicating a solid core.

The relative ratio of the hydrophobic to hydrophilic block length not only has an effect on the physical state of the nanoparticle, but has also been shown to have profound effects on the nanoparticle morphology. The

morphology of prepared amphiphilic block copolymer nanoparticles is typically spherical, particularly if the molecular weight of the hydrophilic block exceeds that of the hydrophobic block thus forming aggregates in which the corona is larger than the core (so-called star micelles). However, if the copolymer is asymmetric in its relative block lengths (i.e., the hydrophobic block is considerably longer than the hydrophilic block) and the nanoparticles are carefully prepared by the slow addition of water to the polymer dissolved in a water-miscible organic solvent, varying morphologies can be obtained [63,83,84]. To date the majority of the work in this field has been done using diblock copolymers composed of hydrophobic polystyrene (PS) and hydrophilic poly(acrylic acid) (PAA) blocks. During the formation of these nanoparticles the copolymer is initially present as unimers in the organic solvent prior to the addition of water. As water is added to the polymer solvent mixture, the solvent becomes progressively worse for the hydrophobic block until a certain water concentration, termed the critical water content (CWC), is reached at which point the hydrophobic blocks begin to associate. It is worth noting that the CWC is highly dependent on the hydrophobic block length and copolymer concentration in the organic solvent such that the CWC decreases with an increase in the hydrophobic block length or concentration of the copolymer [85–87]. During this stage of the aggregation process, a true unimer/aggregate equilibrium is present. However, further addition of water locks unimers into the aggregated state due to the low mobility of the chains, as evidenced by the high glass transition temperature of polystyrene. After this point, the remaining organic solvent is removed by dialysis against water. The presence of copolymer chains frozen into the aggregate structure indicates that the thermodynamic equilibrium between the unimers and aggregates no longer exists and therefore these nanoparticles no longer fit the classical definition of a micelle. They are termed ''crew cut micelle-like aggregates'' [86].

As previously stated, these crew cut micelle-like aggregates can form multiple morphologies, which are dependent on, among other factors, the block lengths of the constituent copolymer. Morphologies found to date include, spheres, rods, vesicles, lamellar and compound micelles. Eisenberg et al. have shown that as the ratio of PAA to PS decreased, the morphologies of the formed nanoparticles changed from spherical to rod-like to vesicular or lamellar to large compound micelle-like aggregates consisting of reverse micelles contained within a large solid sphere [86].

The self-assembly of amphiphilic copolymers into polymersomes has been found to be dependent on the weight fraction of the hydrophilic block. Discher et al. have stated that in the case of PEG–PBD or PEG–PEE copolymers, if the fraction of PEG (f_{EO}) is between approximately 20% and 42% the copolymers will self-assemble into fluid-like, bilayer-forming vesicles. If the copolymer is considerably more hydrophobic with a $f_{EO} < 20\%$ the immobile hydrophobic blocks will be sequestered into solid-like particles (what we term nanospheres). For $f_{EO} > 42\%$ typically spherical micelles are formed [67]. Discher and Eisenberg and their groups have established that aggregate morphology is principally determined by the time-average molecular geometry of diblock copolymers, such that the balance of hydrophilic/hydrophobic blocks produces molecular shapes of cylinders, wedges or cones and this in turn, dictates whether membrane, rod-like or spherical morphologies will form [67,70].

It is evident that caution must be used when using terminology for amphiphilic copolymer nanoparticles, as it is apparent that not all core shell nanoparticles are appropriately defined as a micelle. There is some evidence that amphiphilic copolymer nanoparticles are occasionally termed micelles, even though the hydrophobic block length is exceptionally long and clearly not water soluble [19,88,89]. Typically these nanoparticles are formed by nanoprecipitation methods, which do not require self-assembly but rather the ''freezing'' of the copolymer chains into a kinetically stable structure upon removal of the solvent. The resulting structure is that of a core-shell nanoparticle with a solid-like core.

4.2. Methods of preparation

To date, most studies of the formation of copolymer nanoparticles have focused on how variations in preparation techniques have an influence on the loading of drugs, rather than on the physicochemical properties of the nanoparticles [19,90,91]. In studies conducted by Vangeyte et al. a systematic variation of the preparation technique and solvents used and their influence on the size of resulting particles was conducted [92]. It was found that direct dialysis of copolymer and solvent solutions led to the formation of large aggregates indicating a fast exchange of solvent, most likely due to the large porosity of the dialysis membrane used. Nanoparticulate formation by nanoprecipitation was also explored by varying the solvent used and the order of addition (i.e. organic phase added to aqueous phase or vice versa). There was very little difference found between nanoparticles formed from the various solvents with the exception of DMSO or THF, in which the particles were consistently larger. The lack of size difference was felt to be due to the rapid precipitation of the polymer, locking the polymer chains in a kinetically stable conformation and thus there was no clear dependence on the compatibility between the constitutive blocks and the solvents as calculated by Hildebrand solubility parameters. The increase in particle size when DMSO or THF was used was explained to be due to slower mixing rates of these solvents with water, caused by higher viscosity and a lower miscibility with water, respectively. When the nanoparticles were formed by adding the organic phase of DMSO or THF to the aqueous phase, larger particles resulted as compared to when the addition order was reversed. This was believed to be caused by the faster rate of precipitation

when the solvent was added to the aqueous phase. Interestingly in the preparation of nanoparticles of methacrylic acid copolymer (Eudragit L 100-55) with poly(vinyl alcohol) as a surfactant by nanoprecipitation, the mean particle size was clearly dependent on the compatibility of the solvent with water, however this trend was not seen with the PEG–PCL nanoparticles prepared by Vangeyte et al. [78].

Studies of the effect of other formulation parameters on the preparation of amphiphilic copolymer nanospheres and nanocapsules have shown that increases in the surfactant concentration, either the copolymer itself or an additional surfactant, resulted in a decrease in the diameter of the particles [5,64]. If additional surfactants were used, for example poly(vinyl alcohol) or cholic acid, it was shown that they may remain associated with the surface of the particle even after extensive washing, resulting in an alteration in the surface charge of the nanoparticle.

As noted previously, the rapid addition of organic phase to water or vice versa results in the almost instantaneous precipitation of the polymer and the kinetic locking of copolymer chains into the formed structure. However, Eisenberg et al. have shown that the dissolution of asymmetric crew cut aggregate-forming copolymers in organic solvents with small amounts of water (5.5–9.5 wt%) induced the aggregation of the copolymers. The dynamic equilibrium between unimers and aggregates is preserved provided the water content is not too high [85]. This chain mobility allows for the formation of thermodynamically stable structures with varying morphologies, which can then be ''frozen'' upon the addition of more water and subsequent dialysis of the remaining organic solvent. The various morphologies formed depend on the water content used for the initial dissolution of the polymer, the copolymer concentration in the organic solvent prior to addition of water and the presence of ions [93]. It was shown that the formed nanoparticles transitioned from morphologies such as spheres, rods, worms and bilayers reversibly with changes in the previously mentioned variables, provided the structures were not frozen and thus unimer equilibrium was preserved. The use of various solvents for the dissolution of the copolymer also affected the morphology of the resulting nanoparticles [94]. It was shown that the CWC was dependent on the nature of the solvent so that the CWC increased as the compatibility between the solvent- and core-forming block increased. Nanoparticle morphologies were found to be a result of a balance between interactions between the solvent and both the core- and corona-forming blocks, resulting in changes in aggregation number and degree of stretching of the core-forming block determining which morphology is most entropically favourable.

5. Conclusions

Nanoparticulate drug delivery systems composed of amphiphilic block copolymers have been extensively explored due to the many advantages they provide such as prolonged circulation, passive targeting and enhanced solubilization of hydrophobic drugs. Systems formed include micelles, nanospheres, nanocapsules and polymersomes which all display distinct structural and physicochemical properties. Formation of these various nanoparticles has been demonstrated to be highly dependent on the composition, molecular geometry and relative block lengths of the constitutive copolymers as well as the methods of preparation. Systematic experimentation has shown that subtle changes in these variables can lead to dramatic alterations in the physicochemical properties and morphologies of the resulting nanoparticles. The employment of suitable characterization methodology and the use of appropriate terminology in order to describe particular nanoparticulate formulations are recommended in this field of study.

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