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Review article

Polymeric micelles – a new generation of colloidal drug carriers

Marie-Christine Jones, Jean-Christophe Leroux*

Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada

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Abstract

Polymeric micelles have recently emerged as a novel promising colloidal carrier for the targeting of poorly water soluble and amphiphilic drugs. Polymeric micelles are considerably more stable than surfactant micelles and can solubilize substantial amounts of hydrophobic compounds in their inner core. Due to their hydrophilic shell and small size they sometimes exhibit prolonged circulation times in vivo and can accumulate in tumoral tissues. This review examines the chemical nature of polymeric micelles as well as the methods used to characterize them with regard to drug delivery. Special emphasis is put on the determination of critical micelle concentration and on drug loading procedures. Potential medical applications, especially in cancer chemotherapy, are described and discussed. © 1999 Elsevier Science Ireland B.V. All rights reserved.

Keywords: Polymeric micelles; Review; Drug targeting; Anticancer agents; Polymers; Colloids

1. Introduction

In order to improve the specific delivery of drugs with low therapeutic index several drug carriers such as liposomes [1], microparticles [2], nano-associates [3], nanoparticle [4], drug polymer-conjugates [5] and polymeric micelles [6], have been developed. In recent years, polymeric micelles have been the object of growing scientific attention. They have emerged as a potential carrier for poorly water soluble drugs because they can solubilize those drugs in their inner core and they offer attractive characteristics such as a generally small size (<100 nm) and a propensity to evade scavenging by the mononuclear phagocyte system (MPS) [7]. They were first proposed as drug carriers by Bader et al. in 1984 [8]. Micelles are often compared to natural occurring carriers such as viruses or lipoproteins [9,10]. All three carriers demonstrate a similar core-shell structure that allows for their content to be protected while it is transported to the target cell, whether it is DNA for viruses or water-insoluble drugs for lipoproteins and micelles.

Lipoproteins were proposed as a vehicle for the targeting of antitumor compounds to cancer cells because tumors express an enhanced need for low density lipoproteins [11]. However, their efficiency as carriers has been questioned, mainly because drug-incorporated lipoproteins would also be recognized by healthy cells and because they would have to compete with natural lipoproteins for receptor sites on tumors [12]. On the other hand, viral carriers are mainly used for the delivery of genetic material and may have optimal use in applications that do not require repeated application of the delivery vehicle, since they are likely to elicit an immune response [13].

At the present time, polymeric micelles seem to be one of the most advantageous carriers for the delivery of water-insoluble drugs, although some questions may arise regarding their stability in plasma. The present work briefly reviews the preparation, characterization and potential applications of polymeric micelles as drug carriers.

2. Chemical nature of polymeric micelles

Polymeric micelles are characterized by a core-shell structure. Pharmaceutical research on polymeric micelles has been mainly focused on copolymers having an A-B diblock structure with A, the hydrophilic (shell) and B, the hydrophobic polymers (core), respectively (Fig. 1, left part). Multiblock copolymers such as poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) (A–B–A) can also self organize in micelles [14,15] and have been described as potential drug carriers [16]. The hydrophobic core which generally consists of a biodegradable polymer such as poly(β-benzyl-L-aspartate) (PBLA) [17], poly(DL-lactic acid) (PDLLA) [18] or poly(ϵ-caprolac-

* Corresponding author. Faculty of Pharmacy, University of Montreal, C.P. 6128 Succ. Centre-ville, Montreal, Quebec H3C 3J7, Canada. Tel.: + 1-514-343-6455; fax: +1-514-343-2102.

E-mail address: leroujea@pharm.umontreal.ca (J.C. Leroux)

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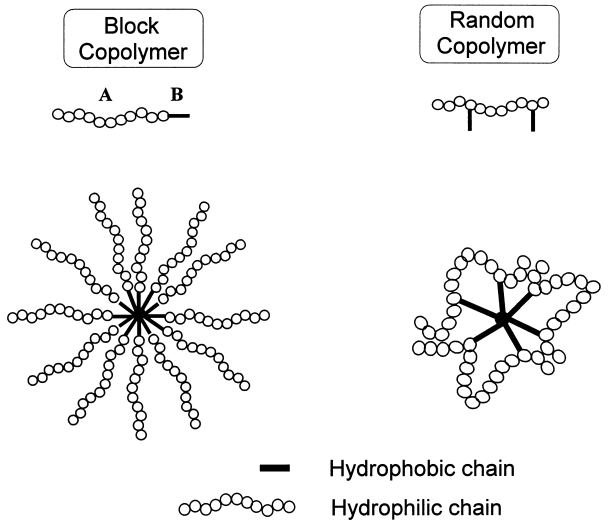

Fig. 1. Schematic representation of block and random copolymer micelles.

tone) (PCL) [19], serves as a reservoir for an insoluble drug, protecting it from contact with the aqueous environment. The core may also consist of a water-soluble polymer (e.g. poly(aspartic acid; P(Asp)) which is rendered hydrophobic by the chemical conjugation of a hydrophobic drug [20–22], or is formed through the association of two oppositely charged polyions (polyion complex micelles) [23–25]. Several studies describe the use of non-or poorly biodegradable polymers such as polystyrene (Pst) [26,27] or poly(methyl methacrylate) (PMMA) [28] as constituents of the inner core. These polymers offer interesting properties such as a glassy state which confers remarkable stability to the micelle core. It has to be pointed out that, in order to be considered as clinically relevant drug carriers, non-biodegradable polymers must be non-toxic and have a molecular weight sufficiently low to be excreted via the renal route [29]. The hydrophobic inner core can also consist of a highly hydrophobic small chain such as an alkyl chain [30–32] or a diacyllipid (e.g. distearoyl phosphatidyl ethanolamine (DSPE)) [33]. The hydrophobic chain can be either attached to one end of a polymer [34] or randomly distributed within the polymeric structure [30,35] (Fig. 1, right part).

The shell is responsible for micelle stabilization and interactions with plasmatic proteins and cell membranes. It usually consists of chains of hydrophilic non-biodegradable, biocompatible polymers such as PEO. The biodistribution of the carrier is mainly dictated by the nature of the hydrophilic shell [6]. Other polymers such as poly(*N*-isopropylacrylamide) (PNIPA) [36–38] and poly(alkylacrylic acid) [39] impart temperature or pH-sensitivity to the micelles, and could eventually be used to confer bioadhesive properties [28]. Micelles presenting functional groups at their surface for conjugation with a targeting moiety have also been described [40–43].

3. Characterization

The use of polymeric micelles as drug carriers requires the determination of several important parameters. In this section the micellization process is briefly described and some methods commonly used for the characterization of polymeric micelles are reviewed.

3.1. Mechanism of micelle formation

Micelle formation occurs as a result of two forces. One is an attractive force that leads to the association of molecules while the other one, a repulsive force, prevents unlimited growth of the micelles to a distinct macroscopic phase [44,45]. Amphiphilic copolymers self-associate when placed in a solvent that is selective for either the hydrophilic or hydrophobic polymer.

The micellization process of amphiphilic copolymers is similar to the process described for low molecular weight surfactants. At very low concentrations, the polymers only exist as single chains. As the concentration increases to reach a critical value called the critical micelle concentration (cmc), polymer chains start to associate to form micelles in such a way that the hydrophobic part of the copolymer will avoid contact with the aqueous media in which the polymer is diluted. At the cmc, an important quantity of solvent can be found inside the micellar core and micelles are described as loose aggregates which exhibit larger size than micelles formed at higher concentrations [46]. At those concentrations, the equilibrium will favor micelle formation; micelles will adopt their low energy state configuration and the remaining solvent will gradually be released from the hydrophobic core resulting in a decrease in micellar size**.** Amphiphilic copolymers usually exhibit a cmc much lower than that of low molecular weight surfactants. For instance, the cmc of PEO-PBLA and PNIPA-PSt are between 0.0005–0.002% [17,36]. However some amphiphilic copolymers exhibit much higher cmc, reaching values up to 0.01–10% in the case of poloxamers [15,41,47]. Amphiphiles with high cmc may not be suitable as drug targeting devices since they are unstable in an aqueous environment and easily dissociate upon dilution.

The micellization of amphiphilic copolymers can result in two different types of micelles depending on whether the hydrophobic chain is randomly bound to the hydrophilic polymer or grafted to one end of the hydrophilic chain. Micelles from randomly modified polymers are smaller than end-modified polymers [48]. The micellar size is mainly determined by the hydrophobic forces which sequester the hydrophobic chains in the core and by the excluded volume repulsion between the chains which limits their size [48], a difference in the balance of these two forces in random and end-modified copolymers may account for their different size. When terminal hydrophobic groups associate to form micelles, the water clusters immobilized around the hydrophobic segments are excluded from the

core and no direct interaction exists between the core and the hydrophilic shell which remains as mobile linear chains in the micellar structure [38,48]. Randomly modified polymers, however, associate in such a manner that hydrophobic and hydrophilic parts of the polymer are entangled together allowing possible contact between the core and the aqueous media. In this case, the hydrophilic chains forming the shell are less mobile [38]. This is an important issue since exposed hydrophobic cores may result in secondary aggregation of polymeric micelles [17,46,49]. Secondary aggregation has also been proposed as an hypothesis to explain the presence of large particles (>100 nm) in micellar systems of PEO–P(Asp) bearing conjugated doxorubicin (DOX) [49].

3.2. Determination of critical micelle concentration

The cmc can be determined by several methods and the reader is referred to specialized textbooks for further information on this topic [50,51]. Theoretically, any physical property (e.g. interfacial tension, conductivity, osmotic pressure) that shows sudden changes at or near the cmc could be used [52]. Usually, variation in the plot of such properties as a function of concentration is used as an indicator of the onset of micellization [53]. However, for polymeric micelles, the cmc is generally too low to be determined by such methods. Light scattering is widely used for the determination of the molecular weight and aggregation number of micelles. However the onset of micellization can be detected only if the cmc falls within the sensitivity of the scattering method which is rarely the case for polymers in water [44]. Gel permeation chromatography (GPC) under aqueous conditions can be employed since single chains and micellar chain fractions of copolymers exhibit different elution volumes [54]. It is also possible to simultaneously determine by GPC the micelles' molecular weight and aggregation number. It is important that the integrity of polymeric micelles during their elution through the size exclusion column is maintained. Adsorption of the polymer on the column may prove to be a problem [55], especially at concentrations close to the cmc, where micelles consist of large loose aggregates.

A preferred method to determine the cmc involves the use of fluorescent probes [44,47,56], among which pyrene is the most widely used. Pyrene is a condensed aromatic hydrocarbon that is highly hydrophobic and sensitive to the polarity of the surrounding environment [57]. Below the cmc, pyrene is solubilized in water, a medium of high polarity. When micelles are formed, pyrene partitions preferentially toward the hydrophobic domain afforded by the micellar core and thus, experiences a non-polar environment [57]. Consequently, numerous changes such as an increase in the fluorescence intensity, a change in the vibrational fine structure of the emission spectra and a red shift of the (0,0) band in the excitation spectra, are observed. The apparent cmc can be obtained from the plot of the fluorescence of pyrene, the I_1/I_3 ratio from emission spectra or the I_{333}/I_{338} ratio from excitation spectra, against concentration: a major change in the slope indicates the onset of micellization [57] (Fig. 2). The I_1/I_3 ratio is the intensity ratio between the first and third highest energy emission peaks and is measured at a constant excitation wavelength and variable emission wavelengths corresponding to I_1 and I_3. Some claim that I_1/I_3 ratio should be reserved for evaluation of polarity since it is affected by the wavelength of excitation and may result in an erroneous cmc [44]. Thus, cmc may be better ascertained by the I_{333}/I_{338} ratio [19,44]. The cmc determined with fluorescence techniques needs to be carefully interpreted for two reasons. First, the concentration of pyrene should be kept extremely low (10^{-7} M) so that a change in slope can be precisely detected as micellization occurs. Second, a gradual change in the fluorescence spectrum can sometimes be attributed to the presence of hydrophobic impurities or association of the probe with individual polymer chains or premicellar aggregates [39]. Changes in anisotropy of fluorescent probes have also been associated with the onset of micellization [58,59].

It has been demonstrated for several copolymers that the onset of micellization is mainly dependent upon the length of the hydrophobic polymer chains, while the effect of the hydrophilic chain length on the cmc is less pronounced [19,44,46]. The effect of the medium composition or the loaded drug on the cmc may be difficult to predict. Zhang et al. [58] showed that the cmc of methoxy (Me) PEO-PDLLA micelles remained the same in water, 0.9% saline solution and 5% dextrose solutions. This is not surprising considering the non-ionic nature of the polymer. However, we recently found that the cmc of PNIPA copolymerized with octadecylacrylate and methacrylic acid was not significantly different in water and phosphate buffered saline (PBS), although these micelles have a negative zeta potential [60]. In this case, the increased ionization of the copo-

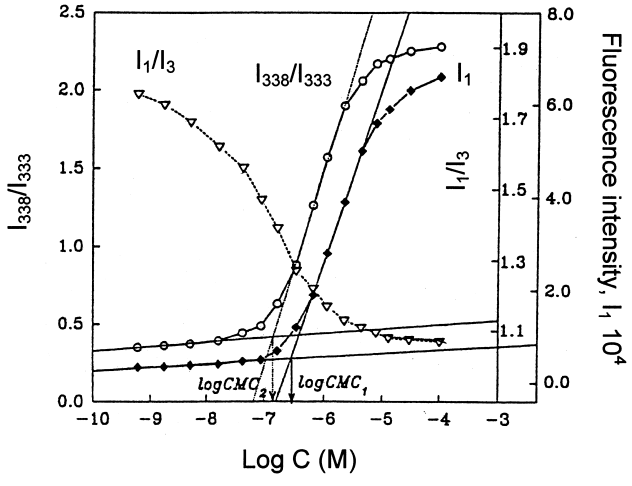

Fig. 2. Plots of the fluorescence intensity I_1 and intensity ratios I_1/I_3 (from pyrene emission spectra) and I_{338}/I_{333} as a function of PSt-b-poly(sodium acrylate) concentration. Values of cmc_{app} are indicated by arrows. Reproduced from [44]with permission from the American Chemical Society.

lymer in PBS versus water may compensate for the potential lowering effect of electrolytes on the cmc [53]. It was also found that the incorporation of 10% paclitaxel into MePEO-PDLLA micelles did not cause the cmc to change significantly [58].This is an important observation, since in this example, a substantial amount of drug could be loaded into polymeric micelles without compromising the carrier's stability.

3.3. Intrinsic viscosity of the micellar core

The viscosity of the micellar core may influence the physical stability of the micelles as well as drug release. The intrinsic viscosity of the hydrophobic core, or microviscosity, can be determined by using fluorescent probes such as bis(1-pyrenyl-methyl)ether (dipyme) [48], 1,2-(1,1'-dipyrenyl)propane [61] or 1,6-diphenyl-1,3,5-hexatriene (DPH) [31]. Dipyme is sensitive to both polarity and viscosity changes in its local environment. The extent of intramolecular excimer emission depends upon the rate of conformational change of the chain linking the two pyrenyl groups. The local friction in the environment causes resistance to motion [48]. Thus, the intensity ratio of excimer to monomer (I_E/I_M) can be used to estimate the fluidity of the environment surrounding dipyme. A small ratio correlates with a low mobility and a solid-like core. Winnik et al. [48], showed with dipyme that the microviscosity of the inner core of PNIPA micelles was dependent on the position of the hydrophobic moiety (random versus end-grafted chain). Internal viscosity can also be obtained from the depolarisation of DPH [31,58]. Anisotropy values are directly related to the rotational freedom of DPH: the higher the local viscosity of the associated DPH region, the higher the anisotropy values will be [31].

^{1}H-nuclear magnetic resonance (NMR) also provides some information on the viscosity of the micellar core. The copolymers are usually dissolved in D_2O and in a solvent where micelle formation is not expected and where all the peaks proper to the hydrophilic and hydrophobic part of the polymer can be detected (e.g. $CDCl_3$). In D_2O, the presence of micelles with a highly inner viscous state results in a restricted motion of the protons within the micellar core as demonstrated by the weak signals associated with the hydrophobic part of the copolymer [62,63]. Highly viscous states were found to exist in PEO-PDLLA [58] and PEO-PBLA [61] micelles.

3.4. Micelle size and size distribution

Small size (10–100 nm) is one of the most interesting features of polymeric micelles. Besides allowing the extravasation of the carriers, it permits the sterilization of the preparation to be done simply by filtration and minimizes the risks of embolism in capillaries, contrary to larger drug carriers [7]. Micellar size seldom exceeds 100 nm, but depends on several factors including copolymer molecular weight, relative proportion of hydrophilic and hydrophobic chains and aggregation number [10,19,21]. The size of micelles prepared by dialysis can be affected by the organic solvent used to dissolve the polymer [17,64]. It was shown that PEO–PBLA micelles prepared by first dissolving the block copolymer in DMF and dialyzing the resulting solution against water, yielded larger micelles than micelles directly prepared in water [17]. Size measurements can be done to study the interaction of polymeric micelles with biological media. For instance, PEO–PPO–PEO micelles were found to maintain their initial size in the presence of antibodies and bovine serum albumin, suggesting the apparent absence of interaction with plasma proteins [41].

Determination of micelle size is particularly useful for the characterization of thermo-responsive micelles. Polymers used to prepare such micelles exhibit a lower critical solution temperature (LCST) which can be defined as the temperature at which the polymer phase separates [65]. Below the LCST the polymer is soluble, but it precipitates at temperatures above the LCST. The diameter of these micelles rapidly rises at temperatures above the LCST, due to hydrophobic interactions that result in the aggregation of the micelles (Fig. 3) [66]. This effect of temperature on size was shown to be reversible, since the micellar architecture was maintained after lowering the temperature below the LCST.

Micellar diameter and size polydispersity can be obtained directly in water or in an isotonic buffer by dynamic light scattering (DLS). DLS can also provide some information on the sphericity of polymeric micelles [24,42]. By DLS, it was shown that the addition of a low molecular weight surfactant such as sodium dodecyl sulfate (1% w/v) can destroy the polymeric micelle structure and brings about a complete shift of the mean diameter from approximately 50 to 3 nm [20].

Micellar size can also be estimated by atomic force microscopy (AFM) [36,66], transmission electron microscopy [67], scanning electron microscopy (SEM) [68].

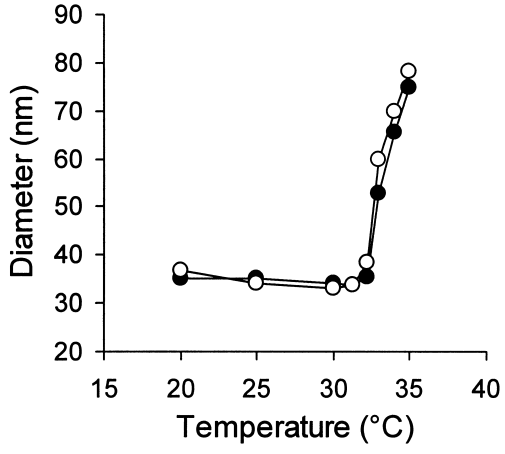

Fig. 3. Size of PNIPA–PDLLA micelles as a function of temperature. Heating plot from 20 to 35°C (●) and cooling plot from 35 to 20°C (○). Reproduced from [66] with permission from Elsevier Science.

These methods allow the characterization of the micelle shape and size dispersity. Conventional SEM is widely used in the field of colloidal carriers since it has high resolution and the sample preparation is relatively easy. However, to be analyzed, the samples must withstand high vacuum. Furthermore, the visualization of the particles requires them to be conductive, which is achieved by coating their surface with gold. The thickness of the coating, which can reach several nanometers, has to be taken into account in the size determination. New imaging tools such as AFM enable the visualization of polymeric micelles at atmospheric pressure without gold coating [69]. By AFM, Cammas et al. [36] showed that micelles of PNIPA–b–PSt had a discus shape with a 5 nm height and a 20 nm diameter, which was close to the 24 nm size measured by DLS. Finally, ultracentrifugation velocity studies are sometimes performed to assess the polydispersity of polymeric micelles [49,70].

3.5. Stability of polymeric micelles

The rate of dissociation of the polymeric micelles into single chains and their interaction with plasma components is as important as the cmc and the micellar size. Once injected polymeric micelles should maintain their integrity for a sufficient period of time in order to deliver the drug to its site of action. High in vitro stability of DOX-loaded polymeric micelles was correlated with efficient in vivo antitumor activity against murine colon adenocarcinoma 26 [71]. Conversely, unstable conjugates were not effectively delivered to the tumor. Physical stability is often assessed by GPC. Yokoyama et al. [55] showed that PEO–P(Asp/DOX) micelles had a very slow rate of dissociation in water and PBS. Dissociation was accelerated in a 1:1 mixture of serum rabbit and PBS but was still below 30% after 6 h. In this case, the stability could be modulated by varying the amount of DOX and the length of the P(Asp) and PEO chains. Polymers containing long hydrophobic chains of P(Asp/DOX) were less stable, while longer hydrophilic chains of PEO yielded higher in vitro stability [55,71]. Lipid moieties can impart good stability to polymeric micelles since the presence of two fatty acid acyls increases the hydrophobic interactions between polymeric chains in the micelle core. Indeed, no dissociation into individual polymeric chain was observed upon the chromatography of serial dilutions of diacyllipid-PEO conjugates [33].

4. Drug incorporation

4.1. Drug loading procedures

Insoluble drugs can be incorporated in micelles by chemical conjugation or by physical entrapment through dialysis or emulsification techniques (Fig. 4). The simple equilibration of the drug and micelles in water may not result in high levels of incorporated drug [72,73]. Chemical conjugation implies the formation of a covalent bond, such as an amide

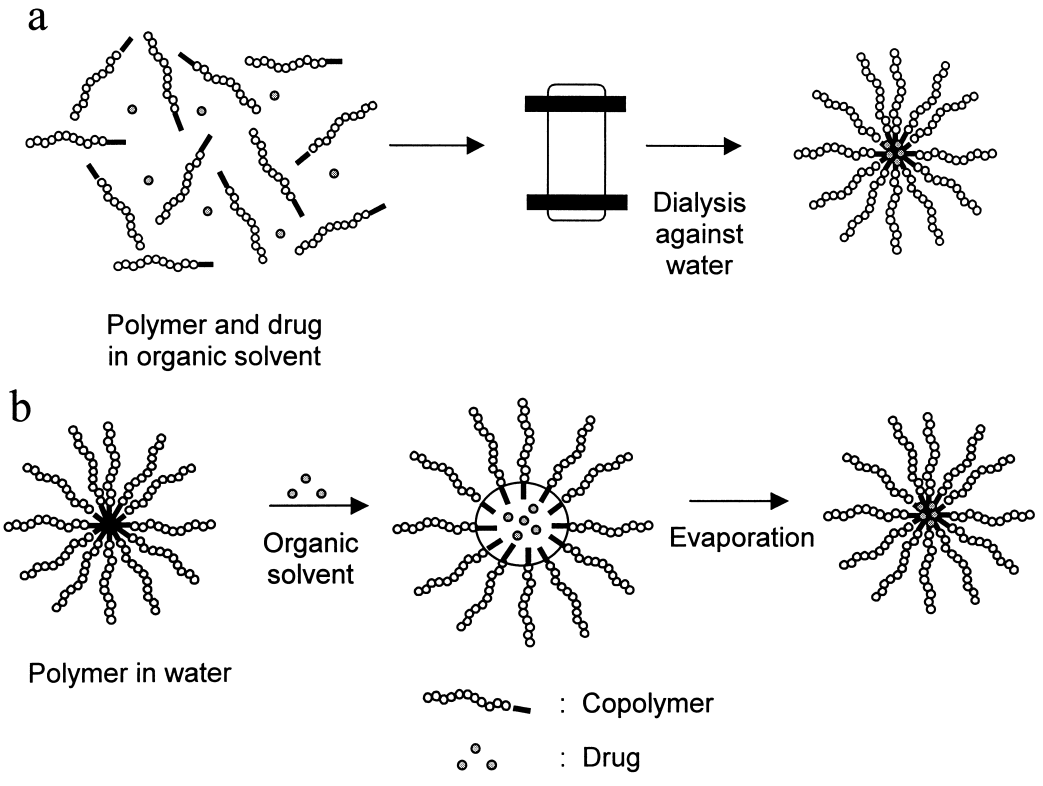

Fig. 4. Drug loading of polymeric micelles by the dialysis (a) and the oil-in-water methods (b). Adapted from [17].

bond, between specific groups on the drug and the hydrophobic polymer of the core. Such bonds are resistant to enzymatic cleavage mainly because of steric hindrance and cannot be readily hydrolyzed unless spacer groups are introduced [74]. When possible, the incorporation of a drug by a physical procedure should be preferred. However, the insertion of hydrophilic compounds such as proteins may require the chemical hydrophobization of the molecule [10,75]. Polyionic compounds can be incorporated through the formation of polyion complex micelles [24,76].

Physical entrapment of drugs is generally done by the dialysis (Fig. 4a) or oil-in-water emulsion procedure (Fig. 4b). The dialysis method consists in bringing the drug and copolymer from a solvent in which they are both soluble (e.g. ethanol, *N-N*-dimethylformamide) to a solvent that is selective only for the hydrophilic part of the polymer (e.g. water). As the good solvent is replaced by the selective one, the hydrophobic portion of the polymer associates to form the micellar core incorporating the insoluble drug during the process. Extending the dialysis over several days may ensure the complete removal of the organic solvent. The oil-in-water emulsion method consists in preparing an aqueous solution of the copolymer to which a solution of the drug in a water-insoluble volatile solvent (e.g. chloroform) is added in order to form an oil-in-water emulsion. The micelle-drug conjugate is formed as the solvent evaporates. The main advantage of the dialysis procedure over the latter method is that the use of potentially toxic solvents such as chlorinated solvents can be avoided. Both dialysis and oil-in-water emulsion methods were compared for the incorporation of DOX in PEO–PBLA micelles [72]. The emulsification method was more efficient since the DOX content of the micelles was estimated to be 12% (w/w) [72] compared to 8% (w/w) for the dialysis technique [73].

The drug loading procedure may affect the distribution of a drug within the micelle. Cao et al. [77] showed that pyrene incorporated in micelles as they were forming was not protected from the aqueous environment as well as pyrene incorporated after micelles were formed, although the first method yielded a drug loading three times higher than the second method [77]. Protection the from aqueous environment may explain the improved chemical stability of DOX incorporated into polymeric micelles [72] and the increased resistance of plasmid DNA in polyion complex micelles against enzymatic degradation [76].

Entrapment efficiency depends on the initial amount of drug added. Going over the maximum loading capacity results in precipitation of the drug and lower yield [64,72]. Drug loading efficiency was also found to be dependent on the aggregation number of the copolymer [70]. Micelles showing a higher aggregation number allow a greater amount of drug to be solubilized in their inner core.

4.2. Examples of drug-loaded polymeric micelles

Examples of compounds loaded into polymeric micelles as well as the corresponding drug loading procedure are given in Table 1. Although polymeric micelles have mostly been studied as delivery systems for anticancer drugs they could be used to transport plasmid DNA [76,78], antisense oligonucleotides [24] or for the delivery of diagnostic agents to a specific organ in the body [10,79].

Evidence of drug incorporation can be obtained by GPC or DLS since both methods can detect a change in micellar size which usually increases in the presence of drugs [67,72]. The location of a drug inside the micelle core is sometimes demonstrated by quenching experiments [72,73]. For instance, iodide (I^-) which is a water soluble quencher of DOX, does not affect the fluorescence of the micelle-incorporated drug but quenches the fluorescence of the free drug. Such experiments showed that DOX was retained in PEO–PBLA after freeze drying and reconstitution in water [72]. In the case of DOX, the self-association of the drug in the micelle core also results in a decrease in the fluorescence intensity of the drug [73]. More recently, the retention and slow release of amphotericin B from polymeric micelles was indirectly ascertained by measuring the decrease of its hemolytic activity after incorporation into PEO–PBLA micelles [67].

5. Pharmaceutical applications

Theoretically, polymeric micelles may find practical applications in a variety of pharmaceutical fields, from oral delivery to sustained release and site-specific drug targeting. However, until now polymeric micelles have been almost exclusively evaluated for the parenteral administration of anticancer drugs. This section briefly analyses the recent advances in the delivery of drugs using polymeric micelles, and the reader is referred to the recent review by Yokoyama [6] for a more comprehensive discussion on this topic.

5.1. Passive drug targeting

Polymeric micelles serve mainly for the transport of water-insoluble drugs. They can increase drug efficiency by targeting specific cells or organs, therefore lowering the accumulation of the drug in healthy tissues and minimizing its toxicity, sometimes allowing higher doses to be administered. Theoretically, following intravenous administration, polymeric micelles should have a prolonged systemic circulation time due to their small size and hydrophilic shell which minimize uptake by the MPS, and to their high molecular weight which prevents renal excretion (Fig. 5). Indeed, intact polymeric micelles have been recovered from plasma several hours after intravenous injection [84,85]. However, liposomes with similar surface characteristics seem to have a longer circulation time than micelles, possibly because extravasation of liposomes from the vasculature is more difficult due to their larger size [75]. The capacity of polymeric micelles to reach regions of the

Table 1
Examples of drugs and tracers loaded into polymeric micelles[a]

Drug	Polymer	Incorporation mode	Micelle size with drug (nm)	Year	Ref.
Amphotericin B	PEO–PBLA	P	26	1998	[67]
Antisense oligonucleotide	PEO–P(Lys)	EA	50	1996	[24]
Cisplatin	PEO-P(Asp)	C	16	1996	[22]
Cyclophosphamide	PEO–P(Lys)	C	Na	1984	[8]
Dequalinium	PEO–PE	P	15	1998	[54]
DOX	PEO–P(Asp)	C	50	1990	[20]
DOX	PEO–P(Asp)	C	14–131	1992	[21]
DOX	PEO–P(Asp)	C	17–42	1994	[49]
DOX	PEO–PBLA	P	30	1995	[73]
DOX	PEO–PDLLA	P	Na	1995	[80]
DOX	PEO–PBLA	P	37	1997	[72]
DOX	PEO–P(Asp)	P + C	Na	1998	[81]
DOX	PNIPA–PBMA	P	Na	1998	[82]
DOX	PAA–PMMA	P	Na	1998	[28]
Gd-DTPA-PE ^{111}In-DTPA-SA	PEO–PE	P	20	1996	[10]
Haloperidol	PEO–PPO-PEO	P	Na	1989	[16]
Haloperidol	PEO–PPO-PEO	P	15	1992	[41]
Indomethacin	PEO–PBLA	P	25–29	1996	[17]
Indomethacin	PEO–PCL	P	145–165	1998	[68]
Indomethacin	PEO–PCL	P	114–156	1998	[19]
Iodine derivative of benzoic acid	PEO–P(Lys)	C	80	1997	[79]
KRN-5500	PEO–PBLA PEO–(C_{16},BLA) PEO–P(Asp,BLA)	P	71[b]	1998	[64]
Paclitaxel	PEO–PDLLA	P	Na	1996	[58]
Paclitaxel	LCC	P	< 100	1998	[83]
Plasmid DNA	PEO–P(Lys)	EA	140–150	1998	[76]
Soybean trypsin inhibitor	PEO–PE	P	15	1998	[75]
Testosterone	PEO–PDLLA	P	Na	1996	[70]
Topoisomerase II inhibitor ellipticine	PEO–PE	P	Na	1995	[33]

[a] Na, not available; P, physical entrapment; C, chemical bonding; EA, electrostatic association; DTPA, diethylenetriamine pentaacetic acid; PEO–P(Lys), poly(ethylene oxide)–poly(L-lysine); SA, stearylamine; LCC, *N*-laurel-carboxymethyl-chitosan; PE, phosphatidyl ethanolamine; PAA, poly(acrylic acid).
[b] After the sonication of PEO(C_{16},BLA) aggregates.

body that are poorly accessible to liposomes has been exemplified by Trubetskoy and Torchilin [10]. They showed that after subcutaneous injection in the dorsum of rabbit hindpaw, polymeric micelles exhibited higher accumulation in the primary lymph node than liposomes and reached the systemic circulation after massage of the lymph node. As for other drug carriers, plasmatic half-life and uptake of polymeric micelles by the MPS depend on the molecular weight [84] and density of the hydrophilic shell [70].

Polymeric micelle-incorporated drugs may accumulate to a greater extent than free drug into tumors and show a reduced distribution in non-targeted areas such as the heart [84]. Accumulation of polymeric micelles in malignant or inflamed tissues may be due to an increased vascular permeability and impaired lymphatic drainage (enhanced permeability and retention (EPR) effect) [87,88]. The tumor vessels are more leaky and less permselective than normal vessels (Fig. 5). Large pores exist and may account for the perivascular accumulation of macromolecules and colloidal drug carriers [89,90]. However, differences in the biodistribution pattern cannot be always evidenced. Indeed, Zhang et al. [91] were not able to demonstrate any difference between the biodistribution of paclitaxel loaded into MePEO–PDLLA micelles versus paclitaxel solubilized in Cremophor EL. These two formulations also showed similar

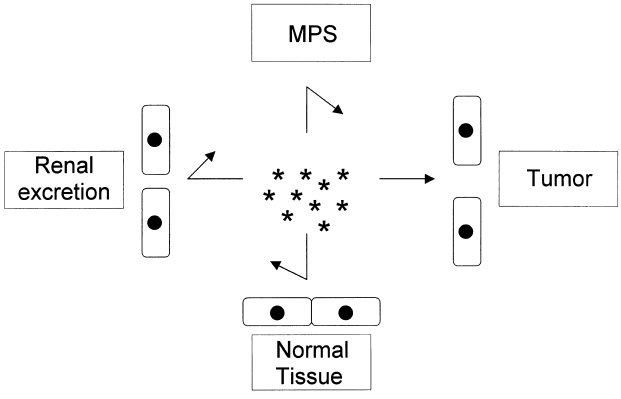

Fig. 5. Accumulation of polymeric micelles in tumors. Reproduced from [86] with permission from Marcel Dekker Inc.

in vitro distribution between the lipoprotein and lipoprotein-deficient fraction of plasma [92].

Several in vivo studies showed that polymeric micelles were able to improve the efficiency of anticancer drugs against leukemia [20,93] and solid tumors [91,94]. Strict comparisons between the activity of free *vs.* incorporated drug are sometimes difficult to make because efficacy experiments have often been carried out at the maximum tolerated dose (MTD) which may be different for the two formulations [20,81,91]. The mechanism that governs the pharmaceutical activity of drug loaded polymeric micelles may be more complicated than a simple accumulation of the carrier in the target area. For instance, an early study by Kabanov et al. [16] showed that the neuroleptic activity of intraperitoneally administered haloperidol was increased by more than two orders of magnitude after its incorporation into PEO–PPO–PEO micelles coupled to brain specific antibodies. In this particular case, the enhancement of drug efficacy may be due to specific targeting [41] and/or increased permeability of the drug through biological membranes given by the polymeric amphiphile [6]. Recently, Yu et al. [95] were able to increase the in vitro antifungal efficiency of amphotericin B while at the same time decreasing its hemolytic activity by loading the drug into polymeric micelles. It was suggested that polymeric micelles could stabilize amphotericin B against auto-oxidation and/or enhance membrane perturbation of fungal cells.

Prolonged exposure due to slow drug release may also be involved in the mechanism of action of polymeric micelles. Drugs can be released directly from micelles by diffusion or consequently to the dissociation of the micelle into free polymeric chains [9]. Ideally, insoluble drugs must be slowly released from polymeric micelles because uncontrolled release due to weak micellar stability, may result in intra-vascular precipitation of the drug. Controlled released patterns have been demonstrated for several micelle preparations [28,68,80].

Polymeric micelle formulations are generally associated with a lower toxicity which allows the administration of doses higher than those found to be toxic for the free drug. For instance, the activity of DOX on tumors is limited by its toxicity. In C26 tumor bearing mice, the administration of doses of 20 mg/kg of doxorubicin resulted in toxic deaths, while doses of 5mg/kg were not efficient in inhibiting tumor growth. Thus, the MTD was estimated at 10 mg/kg. However, incorporation in PEO–P(Asp) micelles permitted the administration of doses as high as 50 mg/kg (MTD) [81]. Interestingly, the antitumor activity against subcutaneous mouse colon adenocarcinoma 26 was shown to be a result of the physical entrapment of the drug in the micelle, since chemically bound DOX showed no significant anti-tumor effect (Fig. 6) [81], probably because chemically-attached DOX is not released due to the absence of hydrolysable link between the drug and the hydrophobic chains of the core.

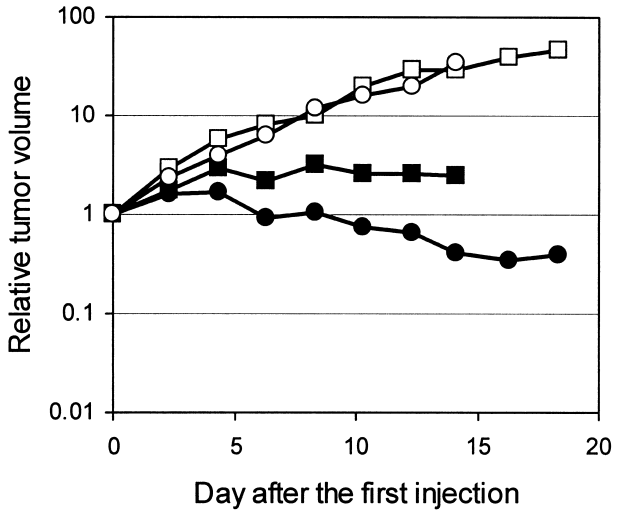

Fig. 6. In vivo antitumor activity of DOX-loaded polymeric micelles against subcutaneous mouse colon adenocarcinoma 26 in mice. Plot symbols: control (□), free DOX 10 mg/kg (■), DOX chemically bound to polymeric micelles 50 mg/kg (○), DOX physically entrapped and chemically bound to polymeric micelles 50 mg/kg (●). Adapted from [81].

5.2. Active drug targeting

The EPR effect is considered as a passive targeting method, but drug targeting could be further increased by binding pilot molecules such as antibodies [16] or sugars [96] or by introducing a polymer sensitive to variation in temperature [36,38] or pH [60,97]. Thermo-response may be utilized to enhance drug release and/or vascular transport by local temperature change. An in vitro study on DOX incorporated into PNIPA-poly(butylmethacrylate) (PBMA) micelles showed that below the LCST (33°C), the micelle formulation expressed lower cytotoxicity than free DOX towards bovine aorta endothelial cells. However, at temperatures above the LCST, the activity of the micelle-drug conjugate was greater than that of free DOX [82]. Also, the release of DOX from the micelles reached 80% after 15 h at 37 and 40°C, while it remained under 20% at temperatures under 33°C [82]. pH-sensitive micelles could serve for the delivery of drugs to tumors, inflamed tissues or endosomal compartments, since they are all associated with a lower pH than normal tissue [98–100]. Indeed, it has been hypothesized that polymeric micelles could enter the cell via the endocytic pathway [101]. Release of drugs in the endosomes might prevent inactivation of the latter in the lysosomes.

6. Conclusion

Because of their distinct advantages, such as small size, high solubility, simple sterilization, controlled release of drugs, polymeric micelles seem to be the prototype of an ideal carrier for poorly water soluble drugs. However, the physical stability of this carrier is a critical issue since

rapid release of the incorporated drug may occur in vivo. Still little is known about the interaction of polymeric micelles with plasmatic and cellular components, and much work remains to be done in order to design micelles which will be able to deliver efficiently a drug to its site of action. Until now, only a limited number of polymeric micelle formulations have been tested as drug delivery systems and it will be necessary to systematically investigate the influence of micelle composition, structure and zeta potential on the pharmacokinetics, biodistribution and activity of the carried drug. Recently, Zhang and Eisenberg [27,102] have described a variety of polymeric micelles with different morphologies, opening very interesting perspectives in drug delivery.

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