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Polymeric micelles for drug delivery

Hamidreza Montazeri Aliabadi & Afsaneh Lavasanifar†
†*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada*

Polymeric micelles have been the subject of many studies in the field of drug delivery for the past two decades. The interest has specifically been focused on the potential application of polymeric micelles in three major areas in drug delivery: drug solubilisation, controlled drug release and drug targeting. In this context, polymeric micelles consisting of poly(ethylene oxide)-*b*-poly(propylene oxide), poly(ethylene oxide)-*b*-poly(ester)s and poly(ethylene oxide)-*b*-poly(amino acid)s have shown a great promise and are in the front line of development for various applications. The purpose of this manuscript is to provide an update on the current status of polymeric micelles for each application and highlight important parameters that may lead to the development of successful polymeric micellar systems for individual delivery requirements.

Keywords: block copolymer, drug delivery, drug targeting, polymeric micelles, solubilisation

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1. Introduction

The goal of drug delivery is to enhance drug performance. However, finding the optimal delivery technology for a given drug is often a matter of serendipity. In recent years, polymeric micelles have been the focus of much interest as novel colloidal delivery systems that can fulfil the requirements of an ideal and versatile drug carrier [1-18]. Polymeric micelles are formed through the self assembly of amphiphilic block copolymers in an aqueous environment. They have a nanoscopic, usually spherical, core/shell structure in which the hydrophobic core acts as a microreservoir for the encapsulation of hydrophobic drugs, proteins or DNA; and the hydrophilic shell interfaces the biological media. The unique feature that has made polymeric micelles superior to other colloidal delivery systems is the versatility of the core/shell structure. Chemical flexibility of the polymeric micellar structure allows the development of custom-made carriers, which may be designed individually with respect to the physicochemical properties of the incorporated drug, pathophysiology of the disease, site of drug action and proposed route of administration. Variations in the chemical structure of the core-forming block in polymeric micelles may be used to improve drug encapsulation, enhance micellar stability and control the rate of drug release from the carrier. The chemical structure of the micelle-forming block copolymer may also be modified to change the biological destination of the polymeric micellar carrier, enhance their specificity for an organ or tissue, or make them responsive to an external stimulus, thereby enhancing the targeting efficiency of the drug carrier.

To this end, polymeric micellar delivery systems have mostly been designed and used to refine three critical parameters in drug performance: solubility, release and biological distribution. In this paper, progress towards each goal will be followed and the level of success in polymeric micellar drug delivery will be evaluated. Emphasis will be placed on the polymeric micellar delivery systems consisting of poly(ethylene oxide)-*b*-poly(propylene oxide) (i.e., Pluronic®) poly(ethylene oxide)-*b*-poly(ester) and poly(ethylene oxide)-*b*-poly(amino acid) block copolymers (Figure 1).

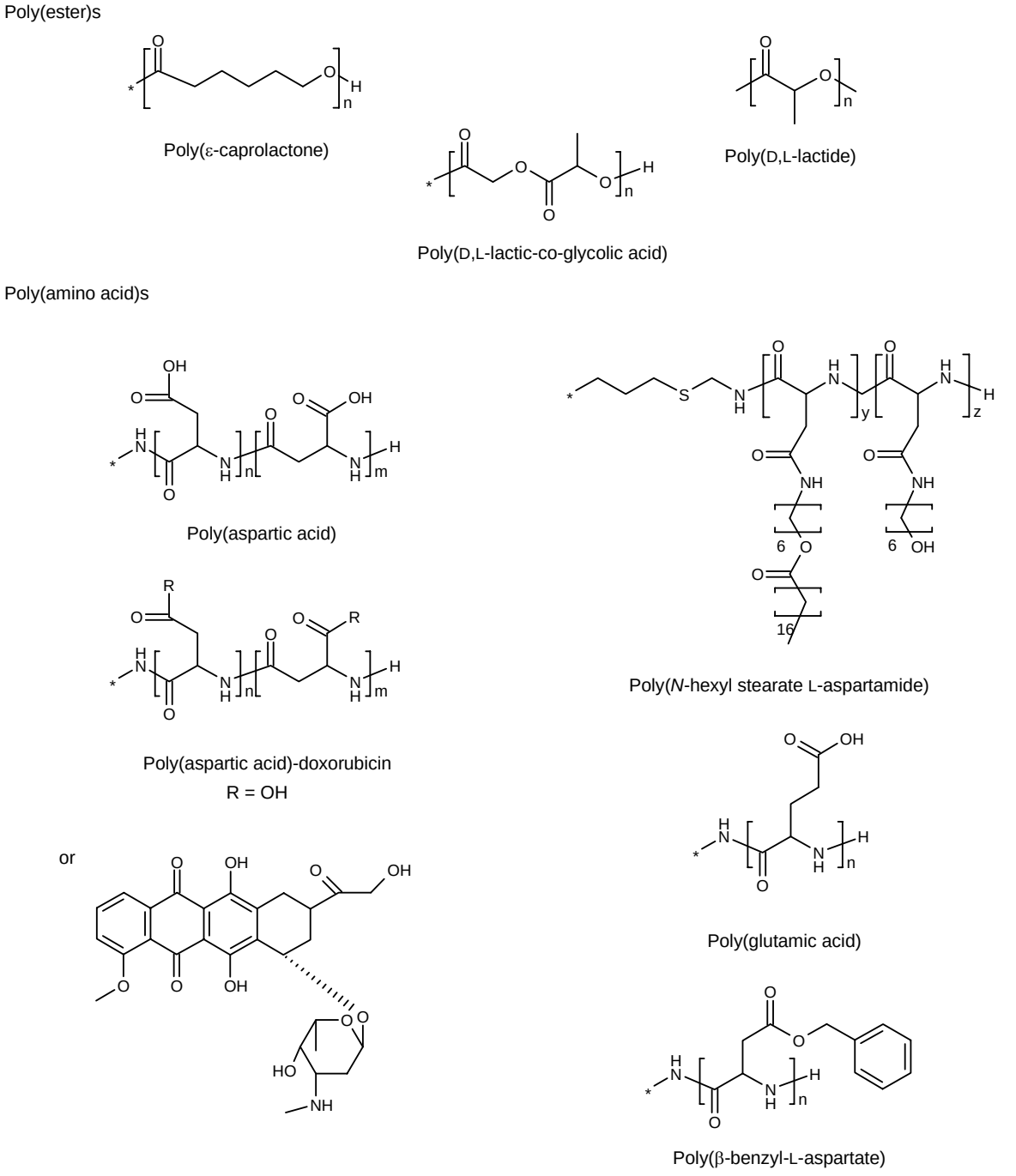

Figure 1. Chemical structure of the most commonly used poly(ester) and poly(amino acid) core-forming blocks in polymeric

2. The rational design in the development of polymeric micellar delivery systems

So far, the polymeric micellar carriers investigated for the purpose of drug delivery may be categorised under three distinct designs: micelle-forming polymer–drug conjugates, polymeric micellar nano-containers and polyion complex micelles.

2.1 Micelle-forming polymer–drug conjugates

In this approach the incorporation and stabilisation of drug within the micellar carrier is achieved through the formation of hydrolysable chemical bonds between the functional group(s) of the polymeric backbone and the drug (Figure 2A – D). Development of different micelle-forming drug conjugates based on poly(ethylene oxide)-*b*-

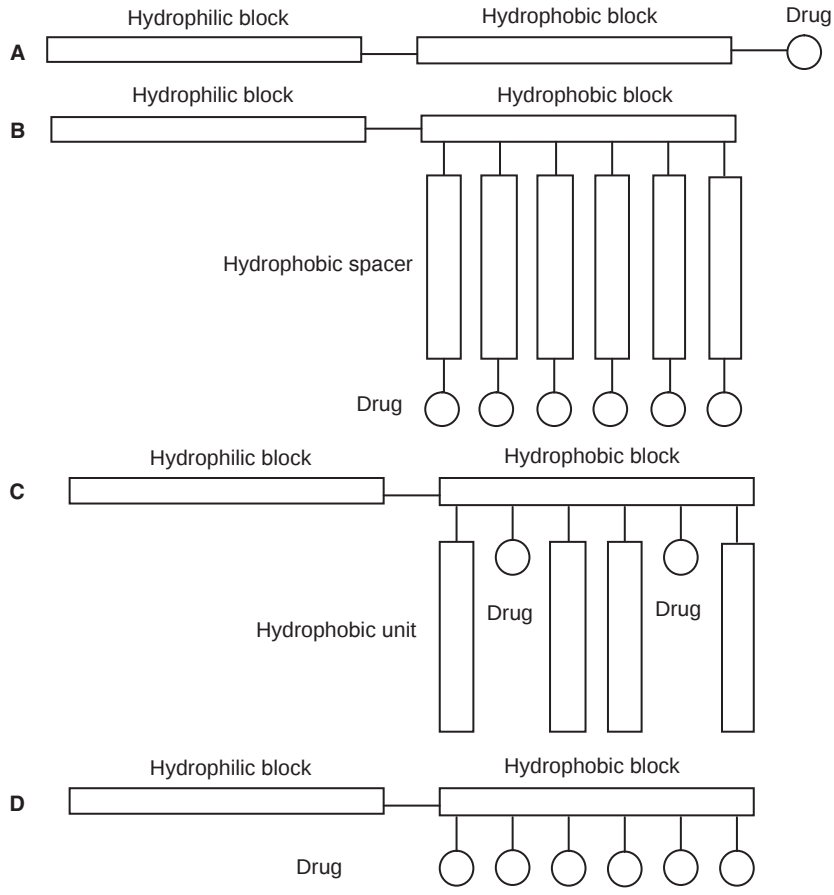

Figure 2. Different designs and models for micelle-forming drug–block copolymer conjugates.

poly(ester) and poly(ethylene oxide)-*b*-poly(amino acid) block copolymers have been the subject of several studies [19-27]. Drug conjugation to poly(ethylene oxide)-*b*-poly(ester)s is mostly carried out through the formation of covalent bonds between the activated terminal hydroxyl group of the poly(ester) section (Figure 1) and reactive groups on the drug molecule (Figure 2A) [28-30]. The poly(amino acid) block, however, has clear advantages over the poly(ester) block for drug conjugation. First, the poly(amino acid) segment bears several functional groups (Figure 1) providing several sites for the conjugation of a number of drug molecules to one polymeric chain (Figure 2B – D). This may lead to a lower dose of administration for the polymeric drug. On the other hand, the diversity of functional groups in a poly(amino acid) chain (amino, hydroxyl and carboxyl groups) allows the conjugation of different chemical entities to the polymeric backbone.

2.2 Polymeric micellar nano-containers

In this system, the formation of hydrophobic interactions or hydrogen bonds between the micelle-forming block copolymer and drug provides the basis for the solubilisation and stabilisation of drugs in the polymeric micelles.

Polymeric micellar nano-containers may be prepared by the direct addition and incubation of drug with block copolymers in an aqueous environment, only if the block copolymer and the drug are water soluble [31-33]. The method, however, is not very efficient in terms of drug-loading levels and not feasible for most block copolymer/drug structures. Instead, physical incorporation of drugs into polymeric micelles is usually accomplished through one of the following methods of encapsulation.

2.2.1 *Dialysis method*

The dialysis method (Figure 3A) is carried out through the dissolution of block copolymer and drug in a water-miscible organic solvent (such as *N*,*N*-dimethylformamide) followed by the dialysis of this solution against water [31-39]. In this method, gradual replacement of the organic solvent with water (i.e., the non-solvent for the core forming block) triggers the self-association of block copolymers and the entrapment of drug in the assembled structures. The semi-permeable membrane keeps the micelles inside the dialysis bag, but allows the removal of unloaded free drug from the polymeric micelles. This method has been extensively used for the preparation of polymeric micellar formulations in a

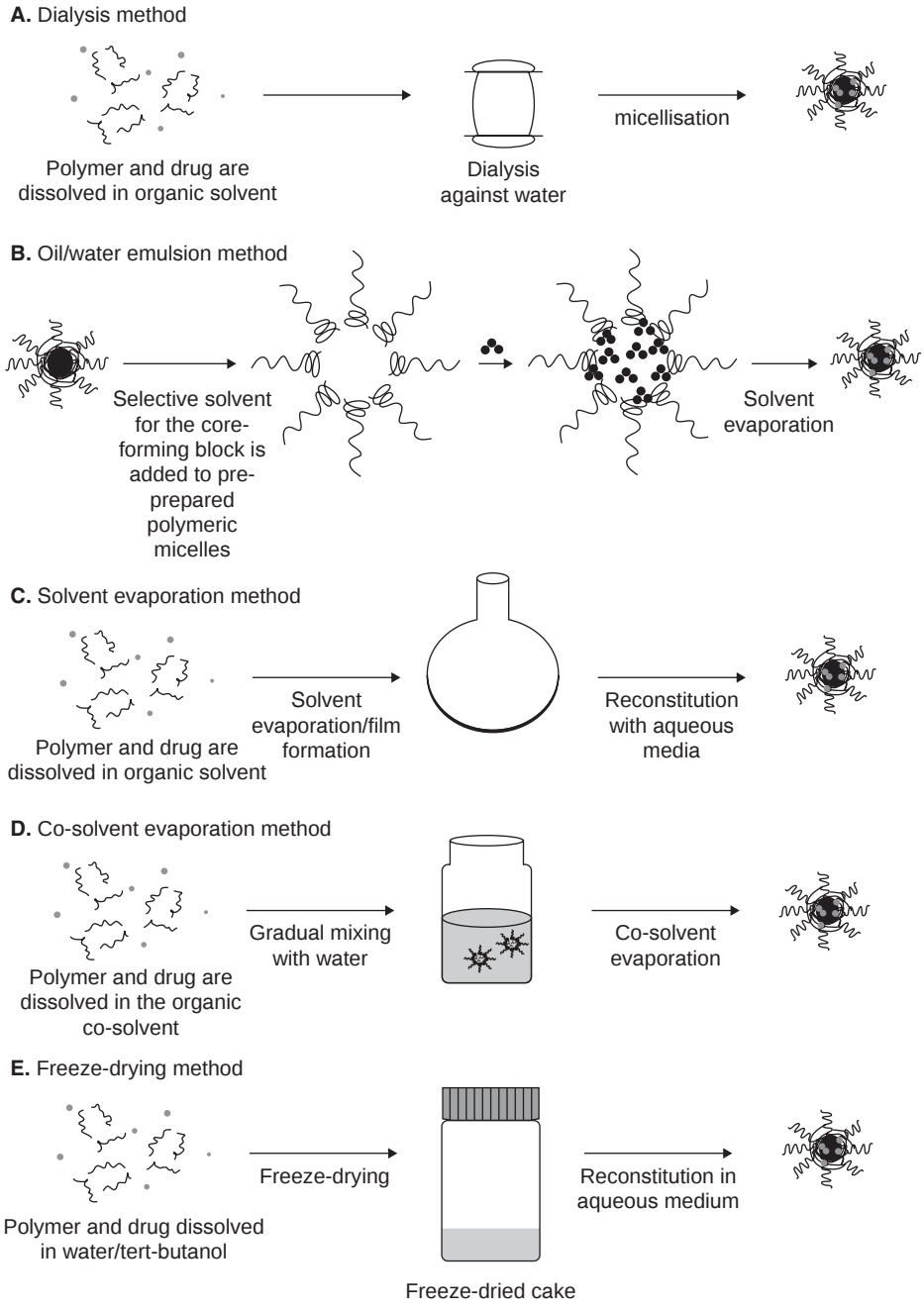

Figure 3. Physical methods of drug encapsulation in polymeric micelles: A) dialysis method; B) oil/water emulsion method; C) solvent evaporation method; D) co-solvent evaporation; E) freeze-drying method.

laboratory setting (Table 1), but may not suite large-scale production. Incomplete removal of the free drug from the polymeric micellar formulation is another drawback for this method of incorporation.

2.2.2 *Oil/water emulsion method*

The oil/water (o/w) emulsion method (Figure 3B) is accomplished by dissolving the drug in a water-immiscible organic solvent (such as chloroform or methylene chloride), followed by the addition of organic phase to aqueous phase under vigorous stirring. The polymer may be dissolved in either organic or aqueous phase. The organic solvent is then removed by evaporation. Solubilisation of doxorubicin (DOX) and indomethacin in poly(ethylene oxide)-*b*-poly(β-benzyl-L-aspartate) (PEO-*b*-PBLA; Figure 1) micelles by o/w emulsion method has been reported [40-42].

Table 1. Drug solubilisation by polymeric micelles

Drug	Initial water solubility	Polymer category	Block copolymer	Incorporation method	Final concentration	Loading level (%w/w)	Ref.
Doxorubicin	≤ 50 µg/ml	PEO-*b*-poly(amino acid)s	PEO-*b*-PBLA	Dialysis	NR	10	[34]
			PEO-*b*-PBLA	Oil/water emulsion	NR	12 – 20	[41,42]
			PEO-*b*-P(Asp)-DOX	Dialysis	NR	18 – 53	[27,81,97]
			PEO-*b*-PBLG	Dialysis	NR	33	[78]
		PEO-*b*-poly(ester)s	PEO-*b*-PDLLA		12 mg/ml		[68]
			PEO-*b*-PLGA	Dialysis	NR	0.51	[30,163]
			PLA-*b*-PEO-*b*-PLA	Co-solvent evaporation	NR	3.9	[48]
			PEO-*b*-PCL	Co-solvent evaporation	NR	4.3	[47]
		Mixed	PEO-*b*-P(His)/PEO-*b*-PLLA	Dialysis	NR	17	[86]
		Pluronic®	Pluronic® 85	Direct addition	NR	13	[33]
			Pluronic® 105	Direct addition	NR	NR	[31]
Paclitaxel	1 µg/ml	PEO-*b*-poly(ester)s	PEO-*b*-PLLA	Solvent evaporation	NR	0.5	[164]
			PEO-*b*-PDLLA	Solvent evaporation	50 mg/ml	25	[44,45,165]
			PEO-*b*-PDLLACL	Solvent evaporation	NR	25	[166]
			PEO-*b*-PDDLA	Solvent evaporation	5 mg/ml	25	[67]
			PEO-*b*-PCL	Dialysis	NR	21	[35]
			PEO-*b*-PCL	Co-solvent evaporation	NR	5.1	[46]
Paclitaxel	1 µg/ml	Miscellaneous	PEO-*b*-PE	Solvent evaporation	750 µg/ml	1.5	[167]
			PEtOz-*b*-PCL	Dialysis	NR	7.6	[64]
			PVP-*b*-PDLLA	Freeze drying	NR	20	[52]
			PEO-*b*-pHPMAmDL	Direct addition	2 mg/ml	22	[140]
Cisplatin	1.2 mg/ml	PEO-*b*-poly(amino acid)s	PEO-*b*-P(Lys)-S	Complexation	NR	5.5	[87]
			PEO-*b*-P(Glu)	Complexation	NR	31 – 39	[55,74]
			PEO-*b*-P(Asp)	Complexation	NR	49	[54]
			PEO-*b*-P(Asp)/P(Asp	Complexation	NR	61	[89]
DACHPt	0.25 mg/ml	PEO-*b*-poly(amino acid)s	PEO-*b*-P(Glu)	Complexation	NR	NR	[88]

BLA: β-Benzyl-L-aspartate; DACHPT: Dichloro(1,2-diaminocyclohexane)platinum(II); DBU: 1,8-Diazabicycloundec-7ene; DDB: Biphenyl dimethyl dicarboxylate; DHT: Dihydrotestosterone; DOX: Doxorubicin; DSPE: Distearoyl phosphoethanolamine; NR: Not reported; P(Asp): Poly (aspartic acid); P(C_{16}BLA): Partially cetyl ester substituted poly(ethylene oxide)-*b*-poly(β-benzyl-L-aspartate); PE: Phosphatidyl ethanolamine; P(Glu): Poly(glutamic acid); P(Lys): Poly(L-lysine); PBLA: Poly(β-benzyl-L-aspartate); PBLG: Poly(g-benzyl L-glutamate); PCL: Poly(caprolactone); PDGA: Poly(D,L-lactic-co-glycolic acid); PDLLA: Poly(D,L lactide); PDLLACL: Poly(D,L-lactide-co-caprolactone); PEO: Poly(ethylene oxide); PEtOz: Poly(ethyl-2-oxazoline); PHAZA: Poly(*N*-hexyl-L-aspartamide)-Z-fatty acid conjugates; P(His): Polyhistidine; pHPMAmDL: Poly(*N*-[2-hydroxypropyl] methacrylamide lactate; PHSA: Poly(Nl-hexyl stearate L-aspartamide); PLA: Polylactide; PLGA: Poly(D,L-lactic-co-glycolic acid); PLLA: Poly(L-lactic acid); POE: Hydroxypropylcellulose-g-polyoxyethylene alkyl ether; PPO: Poly(propylene oxide); PVP: Poly(*N*-vinylpyrrolidone); S: Succinate.

Table 1. Drug solubilisation by polymeric micelles (continued)

Drug	Initial water solubility	Polymer category	Block copolymer	Incorporation method	Final concentration	Loading level (%w/w)	Ref.
Amphotericin B	0.5 µg/ml	PEO-*b*-poly(amino acid)s	PEO-*b*-PBLA	Dialysis	30 – 141 µg/ml	0.6 – 2.8	[36,168]
			PEO-*b*-PHSA	Dialysis	332 µg/ml	1.7	[36,37,43,168]
			PEO-*b*-PHSA	Solvent evaporation	340 µg/ml	1.7	[43,72]
			PEO-*b*- PHAZA	Co-solvent evaporation	45 – 53 µg/ml	52 – 61	[169,170]
Indomethacin	35 µg/ml	PEO-*b*-poly(amino acid)s	PEO-*b*-PBLA	Dialysis/oil/ water emulsion	NR	20 – 22	[40]
		PEO-*b*-poly(ester)s	PEO-*b*-PCL	Dialysis	NR	42	[171,172]
		PVP-*b*-poly(ester)s	PVP-*b*-PDLLA	Dialysis	NR	22	[173]
KRN-5500		PEO-*b*-poly(amino acid)s	PEO-*b*-P(C_{16},BLA)	Dialysis	NR	3.9	[69]
Cyclosporin A	23 µg/ml	Miscellaneous	HPC-g-POE	Dialysis	NR	5.3	[134]
		PEO-*b*-poly(ester)s	PEO-*b*-PCL	Co-solvent evaporation	1.3 mg/ml	13	[50]
Fenofibrate	0.1 µg/ml	PEO-*b*-poly(ester)s	PEO-*b*-PCL	Co-solvent evaporation	90 µg/ml	10	[49]
Fluorecin	< 100 µg/ml	Pluronic®	Pluronic® 85	Direct addition	NR	34	[33]
Nimodipine	45 µg/ml	PEO-*b*-poly(ester)s	PEO-*b*-PCL	Co-solvent evaporation	NR	4.9	[174]
Daunorubicin	< 50 µg/ml	Pluronic®	Pluronic® 85	Direct addition	NR	61	[105]
DHT	28 µg/ml	PEO-*b*-poly(ester)s	PEO-*b*-PCL	Dialysis	1.3 mg/ml	39	[39]
Clonazepam		PEO-*b*-poly(ester)s	PCL-*b*-PEO–*b*-PCL	Dialysis	NR	12	[175]
DDB	2.5 µg/ml	PEO-*b*-poly(ester)s	PEO-*b*-PLLA	Solvent evaporation	13.29 mg/ml		[176]
Vitamin K3		PEO-*b*-phospholipid	PEO-*b*-DSPE	Solvent evaporation	2.2 mg/ml	NR	[177]
DBU		PEO-*b*-phospholipid	PEO-*b*-DSPE	Solvent evaporation	0.67 mg/ml	NR	[177]
Haloperidol	< 10 µg/ml	Pluronic®	Pluronic® 85	Direct addition	7 mg/ml	7	[32]
Docetaxel	1.25 µg/ml	Miscellaneous	PVP-*b*-PDLLA	Direct addition	NR	4	[179]

BLA: β-Benzyl-L-aspartate; DACHPT: Dichloro(1,2-diaminocyclohexane)platinum(II); DBU: 1,8-Diazabicycloundec-7ene; DDB: Biphenyl dimethyl dicarboxylate; DHT: Dihydrotestosterone; DOX: Doxorubicin; DSPE: Distearoyl phosphoethanolamine; NR: Not reported; P(Asp): Poly (aspartic acid); P(C_{16}BLA): Partially cetyl ester substituted poly(ethylene oxide)-*b*-poly(β-benzyl-L-aspartate); PE: Phosphatidyl ethanolamine; P(Glu): Poly(glutamic acid); P(Lys): Poly(L-lysine); PBLA: Poly(β-benzyl-L-aspartate); PBLG: Poly(g-benzyl L-glutamate); PCL: Poly(caprolactone); PDGA: Poly(D,L-lactic-co-glycolic acid); PDLLA: Poly(D,L lactide); PDLLACL: Poly(D,L-lactide-co-caprolactone); PEO: Poly(ethylene oxide); PEtOz: Poly(ethyl-2-oxazoline); PHAZA: Poly(*N*-hexyl-L-aspartamide)-Z-fatty acid conjugates; P(His): Polyhistidine; pHPMAmDL: Poly(*N*-[2-hydroxypropyl] methacrylamide lactate; PHSA: Poly(Nl-hexyl stearate L-aspartamide); PLA: Polylactide; PLGA: Poly(D,L-lactic-co-glycolic acid); PLLA: Poly(L-lactic acid); POE: Hydroxypropylcellulose-g-polyoxyethylene alkyl ether; PPO: Poly(propylene oxide); PVP: Poly(*N*-vinylpyrrolidone); S: Succinate.

2.2.3 *Solvent evaporation method*

The solvent evaporation method (Figure 3C) is based on dissolving the drug and polymer in a volatile organic solvent and the complete evaporation of the organic solvent leading to the formation of polymer/drug film. This film is then reconstituted in an aqueous phase by vigorous shaking. Successful encapsulation of paclitaxel (PTX) in PEO-*b*-poly(D,L-lactide) (PEO-*b*-PDLLA) micelles and amphotericin B in PEO-*b*-poly(N-hexyl stearate L-aspartamide) (PEO-*b*-PHSA) has been carried out by a solvent evaporation method [43-45]. Although the solvent evaporation method of drug loading may have advantages in terms of scale-up over the dialysis method, it can only be used for micelle-forming block copolymers with high hydrophilic lipophilic balance values for which the polymer film can be easily reconstituted in an aqueous media.

2.2.4 Co-solvent evaporation method

The co-solvent evaporation method (Figure 3D) involves the drug and polymer being dissolved in a volatile water-miscible organic solvent (co-solvent). Self-assembly and drug entrapment is then triggered by the addition of aqueous phase (non-solvent for the core-forming block) to the organic phase (or vice versa), followed by the evaporation of the organic co-solvent. Encapsulation of important therapeutic agents such as PTX [46], DOX [47,48], fenofibrate [49] and cyclosporin A (CsA) [50,51] in polymeric micelles composed of poly(ethylene oxide)-*b*-poly(ester) block copolymers through a co-solvent evaporation method have been reported.

2.2.5 Freeze-drying method

The freeze-drying method (Figure 3E) uses a freeze-dryable organic solvent such as *tert*-butanol to dissolve the polymer and drug. This solution is then mixed with water, freeze dried and reconstituted with isotonic aqueous media. This method may be pharmaceutically feasible for large-scale production, but its application is limited to block copolymers and drug structures that can be solubilised in *tert*-butanol. Due to the insolubility of PEO in *tert*-butanol, this method cannot be used for PEO-containing block copolymers. The freeze-drying method has been used for the encapsulation of PTX and its derivatives in poly(*N*-vinylpyrrolidone)-*b*-PDLLA (PVP-*b*-PDLLA) [52,53] (Table 1).

2.3 Polyion complex micelles

Polyion complex micelles can incorporate and deliver different therapeutic moieties that carry charge (e.g., small drugs [54,55], peptides [56] and DNA [56-59]). In this approach, drug incorporation is promoted through electrostatic interactions between oppositely charged polymer/drug combinations. Neutralisation of the charge on the core-forming segment of the block copolymer will then trigger self-assembly of the polyion complex and further stabilisation of the complex within the hydrophobic environment of the micellar core.

3. Polymeric micelles as solubilising agents for the delivery of water-insoluble drugs

Effective application of many existing potent therapeutic agents or future entities emerging from drug discovery efforts is restricted by their poor water solubility, whereas conventional solubilising agents currently in use for the formulation of such compounds are either ineffective or toxic. In this regard, the development of efficient solubilising agents that can be safely administered to human is of great importance.

In recent years, polymeric micelles have been the focus of much interest as alternative vehicles for the solubilisation of poorly water-soluble molecules (Table 1), rendering clear advantages over current solubilising agents in drug delivery [10]. An increasing body of evidence points to a better safety profile for polymeric micelles as alternative solubilising agents for the administration of hydrophobic drugs [36,47] (Table 2). Most solubilising agents currently in use are shown to be biologically active and not entirely inert; for example, Cremophor® EL, a major component for the solubilisation of potent water-insoluble drugs such as PTX and CsA, causes a range of side effects, including hypersensitivity reactions, hyperlipidaemia, neurotoxicity and the reversal of P-glycoprotein [60-63]. Tween 80 and sodium deoxycholate, used for the solubilisation of amiodarone and amphotericin B, respectively, are known to be haemolytic [36,64,65]. Moreover, polymeric micelles have shown enhanced loading capacity, higher thermodynamic and kinetic stability, and more control over the rate of drug release. As a result, they may act as depot drug delivery systems after intravenous administration and have a great potential in modifying the biological disposition of the incorporated drug in a favourable manner. This is in contrast to the low molecular weight surfactant micelles that release their drug content immediately after administration and dilution in the blood.

Polymeric micelles based on PEO-*b*-poly(propylene oxide), PEO-*b*-poly(ester)s and PEO-*b*-poly(amino acid)s have been shown to be successful in the solubilisation of a number of clinically important hydrophobic molecules such as DOX, PTX and amphotericin B (Table 1). Perhaps one of the most successful examples for the application of polymeric micellar formulations as alternative solubilising agents is the formulation of PTX in PEO-*b*-PDLLA micelles developed by Burt *et al.*, which have resulted in a 5000-fold increase in the solubilised PTX levels in an aqueous media [44,45,66,67]. Application of PEO-*b*-PDLLA micelles has also been reported to increase the water solubility of DOX up to 12,000-fold [68].

Compatibility between the micellar core and encapsulated drug molecule seems to be the most crucial factor to determine the final capacity of a polymeric micellar system for the solubilisation of a given therapeutic agent. With this in mind, chemical conjugation of DOX to the poly(aspartic acid) (P[Asp]) block of PEO-*b*-P(Asp) was pursued to increase the entrapment of DOX inside the hydrophobic core of PEO-*b*-P(Asp)-DOX micelles [27]. Further evidence for the importance of compatibility between core-forming block and solubilisate is provided in studies by Kwon *et al.* on the encapsulation of amphotericin B in polymeric micelles that consist of PEO-*b*-P(Asp) derivatives, in which the replacement of the aromatic core with aliphatic ones has resulted in an increase in the level of encapsulated drug [43]. Partial replacement of the benzyloxy group in PEO-*b*-PBLA with cetyl ester has also been used by Yokoyama *et al.* to achieve polymeric micellar nano-containers for KRN-5500; an aliphatic antineoplastic agent [69-71].

Encapsulation procedure may also play a significant role in determining the final solubilised levels of the encapsulated drug; for example, the application of a solvent evaporation method has resulted in enhanced and stabilised encapsulation of amphotericin B in PEO-*b*-PHSA micelles when compared with a dialysis method of drug incorporation [72]. The encapsulation of DOX in PEO-*b*-PBLA polymeric micelles was also shown to increase from 10 to 20 w/w% when an o/w emulsion method was used instead of a dialysis method [34,41].

Table 2. The result of preclinical studies for the development of tumour-targeted polymeric micellar delivery systems

Incorporated drug	Polymer	Animal model	Change in MTD compared with commercial formulation	Change in plasma AUC compared with commercial formulation	Change in tumour AUC/antitumour activity compared with commercial formulation	Ref.
Doxorubicin	PEO-*b*-P(Asp)-DOX (Chemical conjugate)					
		P388-bearing CDF1 mice	↑	-	-	[25]
		Healthy ddy mice	-	↑	NA	[22]
		Different tumours C57BL/6 mice	↑	-	-	[95]
		C26-bearing CDF1 mice	-	↑	Tumour AUC ↑	[96]
	PEO-*b*-P(Asp)-DOX (Physical encapsulation)					
		C26-bearing CDF1 mice	↑	↑	Tumour AUC ↑ (7.4 fold)/antitumour activity ↑	[81]
		C26-bearing CDF1 mice	-	↑ (28.9-fold)	Tumour AUC ↑ (3.4-fold)/ Antitumour activity ↑	[98]
	PEO-*b*-PPO-*b*-PEO					
		A2780-bearing nu/nu mice	-	-	Tumour AUC ↑	[143]
		Different tumours and mice	No	↑ (2-fold)	Tumour AUC ↑/ antitumour activity↑	[179]
	PEO-*b*-PBLA					
		C26-bearing CDF1 mice*	-	↑	Antitumour activity ↑	[41]
	PEO-*b*-PLGA					
		KB-bearing nu/nu mice	-	-	Tumour AUC ↑/ antitumour activity ↑	[163]
Paclitaxel	PEO-*b*-PDLLA					
		P388-bearing B6DF1 mice	↑	-	-	[44]
		MV-522-bearing nu/nu mice	↑	↓	-	[66]

*With dichloro(1,2-diaminocyclohexane)platinum(II).

AUC: Area under the concentration-time curve; DOX: Doxorubicin; HCPT: Hydroxylcampthotecin; MTD: Maximum tolerable dose; NA: Not applicable; P(Asp): Poly(aspartic acid; P(Glu): Poly(glutamic acid); P(Lys): Poly(L-lysine); PBLA: Poly(β-benzyl-L-aspartate); PCL: Poly(caprolactone); PDLLA: Poly(D,L lactide); PEO: Poly(ethylene oxide); PLGA: Poly(D,L-lactic-co-glycolic acid); PPBA: Poly(4-phenyl-1-butanoate) L-aspartamide; PVP: Poly(*N*-vinylpyrrolidone).

Table 2. The result of preclinical studies for the development of tumour-targeted polymeric micellar delivery systems (continued)

Incorporated drug	Polymer	Animal model	Change in MTD compared with commercial formulation	Change in plasma AUC compared with commercial formulation	Change in tumour AUC/antitumour activity compared with commercial formulation	Ref.
		LNCaP-bearing BALB/c mice	↑	-	Antitumour activity ↑	[67]
		Different tumours and mice	↑	No change	Tumour AUC ↑/ antitumour activity ↑	[35]
	PVP-*b*-PDLLA					
		C26-bearing BALB/c mice	↑	↓	No Change	[52]
	PEO-*b*-PPBA					
		C26-bearing CDF1 mice	-	↑ (86-fold)	Tumour AUC ↑ (25-fold)	[115]
Cisplatin	PEO-*b*-P(Asp)					
		LLC-bearing C57BL/6N mice	-	↑ (5.2-fold)	Tumour AUC ↑ (4.6 fold)	[116]
		LLC-bearing C57BL/6N mice	-	↑	Tumour AUC ↑/no change in antitumour activity	[117]
	PEO-*b*-P(Glu)					
		LLC- and C26-bearing mice	-	↑	Tumour AUC ↑/ antitumour activity ↑	[55]
		C26-bearing CDF1 mice*	-	↑	Tumour AUC ↑	[88]
	PEO-*b*- P(Lys)-succinate					
		Adenocarcinoma bearing rats	-	-	Tumour AUC ↑	[87]
Cyclosporin	PEO-*b*-PCL					
		Healthy Sprague-Dawley rats	-	↑ (6-fold)	NA	[51]
HCPT	PEO-*b*-PCL					
		S180-bearing mice	-	↑	Tumour AUC ↑	[180]

*With dichloro(1,2-diaminocyclohexane)platinum(II).

AUC: Area under the concentration-time curve; DOX: Doxorubicin; HCPT: Hydroxylcampthotecin; MTD: Maximum tolerable dose; NA: Not applicable; P(Asp): Poly(aspartic acid; P(Glu): Poly(glutamic acid); P(Lys): Poly(L-lysine); PBLA: Poly(β-benzyl-L-aspartate); PCL: Poly(caprolactone); PDLLA: Poly(D,L lactide); PEO: Poly(ethylene oxide); PLGA: Poly(D,L-lactic-co-glycolic acid); PPBA: Poly(4-phenyl-1-butanoate) L-aspartamide; PVP: Poly(*N*-vinylpyrrolidone).

4. Polymeric micelles as modified-release drug delivery systems

The mode of drug release from polymeric micelles mainly depends on the design used for the preparation of the polymeric micellar delivery system; the chemical structure of micelle-forming block copolymer and incorporated drug; their physicochemical properties; and the localisation of the incorporated drug in polymeric micelles.

Drug release from micelle-forming block copolymer–drug conjugates may proceed via two major pathways: micellar dissociation followed by drug cleavage from the polymeric unimers, or drug cleavage within the micellar structure and its further diffusion out of the micellar carrier

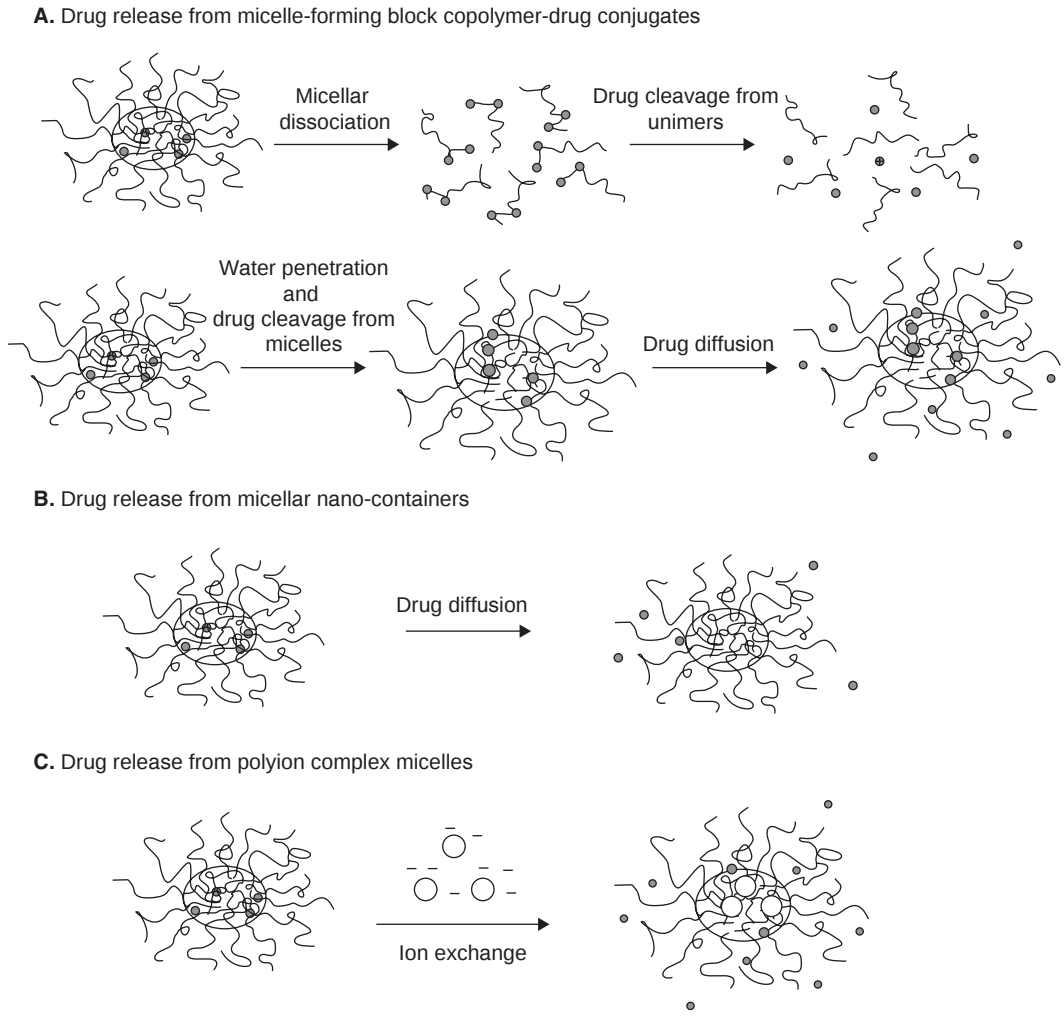

Figure 4. Modes of drug release from polymeric micelles.

(Figure 4A). Release of physically encapsulated drug from sufficiently stable micellar nanocontainers is usually proceeded by diffusion (Figure 4B), whereas drug exchange with free ions and proteins in the physiological media triggers drug release from polyion complex micelles (Figure 4C). In either case, it may be possible to tailor the chemical structure of the micelle-forming block copolymer and modify the physicochemical properties of the core/shell-forming blocks to adopt an instant, sustained, pulsed or delayed mode of drug release for specific delivery requirements. For example, hydrophobicity and rigidity of the micellar core may be enhanced to restrict the penetration of water and free ions to the micellar core in micelle-forming drug conjugates and polyion complex micelles, respectively. This may lead to a sustained or even delayed mode of drug release from the carrier [55,73,74]. The application of polymeric micelles that have glassy cores under physiological condition (37°C), crosslinking of the micellar core structure and the induction of strong hydrophobic interaction or hydrogen bonds between the core-forming block and solubilisate may be used to lower the rate of micellar dissociation, drug diffusion and the overall rate of drug release from the micellar carrier [46,56,75-77]. The introduction of hydrophilic or stimulus responsive groups to the core structure, on the other hand, may be used to provide an instant or pulsed mode of drug delivery. Finally, the method of drug incorporation in polymeric micelles may also be modified to improve the extent of drug loading, localisation or the physical state of the loaded drug, providing other means for controlling the rate of drug release from polymeric micelles [72]. To shed light on different strategies that have been used to modify the release profile of polymeric micellar delivery systems, specific examples from the literature will be discussed in the following section.

The *in vitro* rate of drug release from polymeric micelles has been assessed through different methods. The most popular method is dialysis of the polymeric micellar formulation against a physiological recipient phase. The molecular

weight cutoff of the dialysis membrane in this experiment is chosen in a way to assure the restriction of micellar structures inside the bag, but free diffusion of the released portion of drug to the recipient phase [30,34,41,47,48,78]. An alternative method relies on the separation of the released from the encapsulated drug by gel permeation chromatography after direct incubation of the polymeric micellar formulation with the recipient phase [73]. In case of DOX, quenching of the fluorescence for the entrapped drug has been followed to investigate the *in vitro* rate of drug release from its polymeric micellar formulation [34].

Although these methods provide an approach to assess the relative effect of formulation parameters on drug release, they may not offer a realistic picture of drug release in a biological system. First, because the required driving force for the release of poorly soluble compounds may not be reached under such conditions. Second, polymeric micelles are subject to sink condition in the biological system and extreme dilution after administration, which may bring the final concentration of the polymeric micellar system below its critical micellar concentration. In this case, the kinetic stability of the polymeric micellar system plays an important role in determining the final rate of drug release from these systems. The application of biomimetic recipients (e.g., lipid vesicles or serum albumin) may provide a better insight into the rate and duration of drug release for these nanoscopic drug delivery systems [34,43,50]. Nevertheless, the possibility of micellar dissociation following dilution, which may lead to a faster drug release after *in vivo* administration, has to be accounted for.

Evidence gathered from *in vitro* experiments points to a sustained mode of drug release for many polymeric micellar delivery systems developed so far. DOX is one of the most studied drugs incorporated into polymeric micelles of different structures by chemical and physical means. Micelle-forming DOX conjugates of PEO-*b*-P(Asp) were designed (the chemical structure is shown in Figure 1) to provide a delayed mode of DOX cleavage from the PEO-*b*-P(Asp), which is only triggered in the acidic condition of endosomes inside tumour cells [79]. PEO-*b*-P(Asp)-DOX micelles showed sufficient micellar stability evidenced by a slow rate of dissociation even in the presence of rabbit serum *in vitro*, but the amide linkage formed between the carboxylic group of the aspartic acid and the amino group of the glycosidyl residue on DOX was found to be too stable for any drug release *in vivo*. With the same goal, Yoo *et al.* studied the chemical conjugation of DOX to PEO-*b*-poly(D,L-lactic-co-glycolic acid) (PEO-*b*-PLGA) forming an amide bond between DOX and *p*-nitrophenyl chloroformate preactivated polymer (model shown in Figure 2A) [30]. In PBS, PEO-*b*-PLGA-micelles released 50% of their drug content in a sustained manner over 2 weeks, whereas release from physically encapsulated DOX in PEO-*b*-PLGA only lasted for 3 days. The same research group have reported on the conjugation of DOX to PEO-*b*-poly(L-lactic acid) (PEO-*b*-PLLA) diblock copolymer via two acid cleavable linkages: a hydrazone linkage between amino-terminated PEO-*b*-PLLA and the ketone group of DOX or a *cis*-aconityl linkage between amino-terminated PEO-*b*-PLLA and the amino group of the glycosidyl residue on DOX [28]. For conjugates with hydrazone linkage, the release of DOX was found to be five-times faster than PEO-*b*-PLGA-DOX conjugates and pH dependent. The dependence of drug release from conjugates with *cis*-aconityl linkage on pH was more noticeable. Finally, DOX-conjugated micelles with a hydrazone linkage have shown higher toxicity against human lymphoblast cells (HSB-2) after 48-h incubation when compared with free DOX, *in vitro*. The observation has been contributed to the more efficient uptake of DOX in the micellar carrier by endocytosis and the further cleavage of the drug–polymer bond in the acidic pH of endosomes.

To modify the release pattern of PEO-*b*-P(Asp)-based drug conjugates, Kwon *et al.* have used a different approach. They reported on the formation of ester bonds (that are more sensitive to hydrolysis than amide bonds) between the carboxylic acid groups of methotrexate (MTX) and hydroxyl groups of PEO-*b*-poly(hydroxyl alkyl-L-aspartamide) (PEO-*b*-PHAA; model shown in Figure 2B) [73,80]. The results of release studies showed a 5 – 20% of MTX release from the polymeric micellar carrier within 21 days in PBS. An increase in the hydrophobicity of the core-forming block, achieved through a raise in the level of conjugated MTX, was found to be a crucial factor in sustaining the rate of drug release in this system.

Drug release from polymeric micellar nano-containers is expected to be faster than micelle-forming drug conjugates. A slow mode of release for DOX from PEO-*b*-PBLA nano-containers into phosphate buffer (pH = 7.4) and bovine serum albumin containing recipient phases has been shown by Kataoka *et al.*, where 80% of the drug content remained in the micellar carrier after 4 days of incubation [42]. The release profile was found to be rapid in the initial stages and sustained in later stages. A decrease in the pH of the recipient phase from 7.4 to 5.0 accelerated the release of DOX from the micellar carrier, pointing to a possibility for accelerated drug release from the carrier in the acidic environment of tumours [41]. Similar results were reported for DOX encapsulated in PEO-*b*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles [47]. The application of micelle-forming star block copolymers of PEO and poly(γ-benzyl L-glutamate) (PEO-*b*-PBLG) for DOX encapsulation, on the other hand, has resulted in 80 – 100% of DOX being released within 4 days in PBS (pH 7.4) [78].

The application of drug-compatible moieties in the micellar core is shown to lower the rate of drug release from the carrier. The presence of DOX dimers in block copolymer nano-carriers of DOX, for example, is shown to reduce the rate of DOX release from PEO-*b*-P(Asp)-DOX nano-containers [81]. The same concept has been used in studies for the development of modified-release polymeric micelles for aliphatic drugs [12,37,43,72,82]. Through the development of PEO-*b*-P(Asp) derivatives having saturated fatty acid substitutes on their core-forming block, Kwon *et al.* have made

the core of polymeric micelles more compatible with amphotericin B. A reduced haemolytic activity as a result of reduced amphotericin B release from polymeric micelles bearing more drug-compatible moieties in their core (> 50% of stearic acid substitution) has been achieved.

The formation of hydrogen bonds between drug and core-forming block has been suggested as an alternative strategy to lower the rate of drug release in polymeric micelles. Lee *et al.* reported on the preparation of functionalised PEO-*b*-PDLLA-bearing free carboxylic groups on the PDLLA block [76], and studied the loading and release of papaverine. With an increase in the level of free carboxylic groups on the polymeric backbone, a significant increase in drug loading and reduction in drug release was observed, which was attributed to increased interactions between the loaded drug and the core-forming block.

Several groups have reported on an effect for the level of drug loading on the *in vitro* drug-release rate from polymeric micelles [83,84]. In micelles formed from the assembly of PEO-*b*-PBLG star block copolymers, drug crystallisation at higher DOX-loading levels was correlated with slower drug-release rates from this system. At 1 and 3% DOX-loading levels in PLLA-*b*-PEO-*b*-PLLA micelles, 50 and 17% of encapsulated drug was released within 6 and 8 days, respectively [48]. Similarly, for DOX encapsulated at a 20 µM level in PEO-*b*-P(Asp)-DOX micelles, drug release continued for 72 h in saline, but at the encapsulation level of 2 µM almost all the loaded drug was released after 3 h [85].

Formation of mixed micelles of PEO-*b*-PLLA and PEO-*b*-poly(L-histidine) has been used by Lee *et al.* to trigger a pH-dependent mode of DOX release from polymeric micelles [86]. Drug-release pattern followed first-order kinetics and reached plateau in 24 h, releasing 30% of loaded DOX at pH 8.0. By manipulating the combination of two block copolymers, the pH-dependent release profile of this system has been modified. The mixed micelles containing 25 wt% PEO-*b*-PLLA showed a desirable pH-dependency, where 32, 70 and 82% of the incorporated drug was released at pH 7.0, 6.8 and 5.0, respectively.

Release from polyion complexes is triggered by the presence of high concentrations of salt in the medium, which will replace the complexed drug. The hydrophobic core of polyion complex micelles may stabilise the electrostatic interaction between the polymer and drug against ion replacement, leading to a slower rate of drug release from such a system. Cisplatin (CDDP) is an example of a charged drug, which has been extensively studied for the formation of polyion complex micelles. In 1996, Bogdanov *et al.* reported on the incorporation of CDDP in PEO-*b*-poly(L-lysine)-succinate [87]. A slow rate of drug release in saline (half-life of 63 h) has been reported for this system. In the presence of serum albumin, 20% of the drug was released within 2 h, but the remaining drug was released slowly with a rate similar to that observed in protein-free saline media.

Formation of polyion complex micelles of CDDP and PEO-*b*-P(Asp) has been reported by Kataoka *et al.* [54]. Micelles were prepared through the complexation of CDDP with PEO-*b*-P(Asp) in an aqueous medium. The results of *in vitro* release studies on this system in normal saline at physiological temperature have shown a sustained-release profile. For 50% drug release > 20 h was required. Elongation of the P(Asp) segment slowed the rate of drug release even further. Polyion complex micelles of CDDP and PEO-*b*-poly(glutamic acid) (PEO-*b*-P[Glu]), which is more hydrophobic than PEO-*b*-P(Asp), showed a slower rate of drug release in physiological conditions [55,74]. Platinum was released over a period of > 150 h in a sustained manner, with no initial burst. To lower the rate of drug release further, a more hydrophobic derivative of CDDP, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt), was loaded into PEO-*b*-P(Glu) micelles [88]. This system showed a 15-h delay in the release of platinum. After the 15-h lag time, DACHPt was released in a sustained manner similar to that of CDDP-loaded micelles.

The same research group have used a mixture of the PEO-*b*-P(Asp) block copolymers and P(Asp) homopolymers (with different feed ratios) to incorporate CDDP [89]. An increase in the homopolymer ratio in this system raised the micellar size and encapsulation efficiency. Independent from the homopolymer ratio, an 8.6-h delay in the release of CDDP was observed. This delay was attributed to the slow diffusion of chloride ions into the dense core of polymeric micelles. Release rate was increased in the presence of higher salt concentrations.

5. Polymeric micelles for passive drug targeting

Polymeric micelles are considered to be one of the most promising delivery systems for drug targeting in cancer for several reasons: the hydrophobicity of the micellar core provides an excellent host for the incorporation and stabilisation of anticancer agents, which are mostly hydrophobic. Nanoscopic dimension and stealth properties induced by the hydrophilic shell of polymeric micelles may lead to the efficient accumulation of the micellar carrier at the tumour site as a result of the enhanced permeation and retention (EPR) effect [90]. The small size of polymeric micelles may also facilitate the deep penetration of carrier to tumour tissue and ease their uptake by tumour cells. At the same time, the flexibility of the core/shell structure in polymeric micelles provides opportunities for the attachment of targeting ligands [58,91,92] as well as fine tuning the colloidal carrier for optimal properties.

The results of investigations on the development of polymeric micellar delivery systems for the purpose of drug targeting are summarised in Table 2. The first attempt for the design of polymeric micellar systems for targeted delivery of anticancer drugs has been made by Ringsdorf *et al.*, in which micelle-forming block copolymer–drug conjugates of cyclophosphamide (CP) sulfide and PEO-*b*-poly(L-lysine) with a similar design to what is shown in Figure 2B and C were prepared [93].

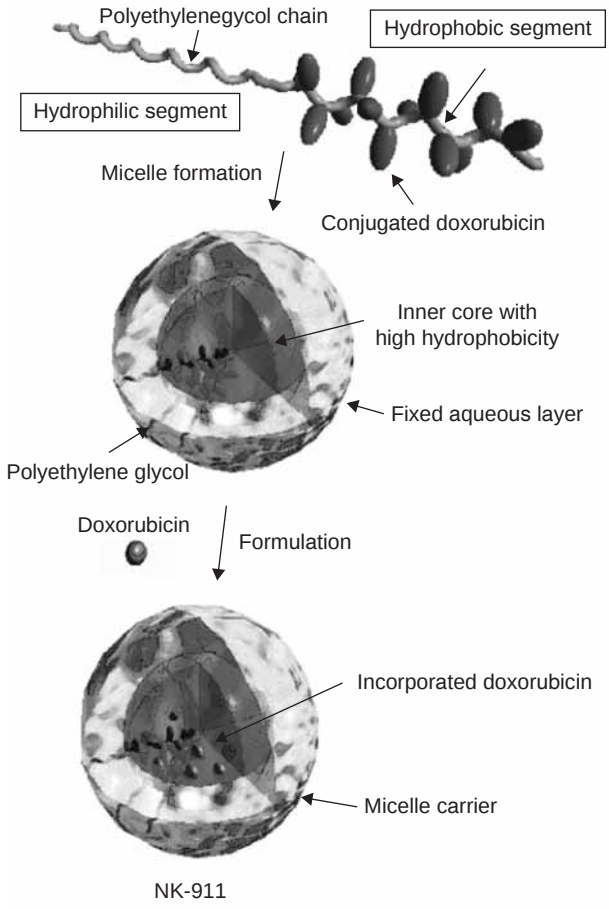

Figure 5. Schematic model of NK-911. Reprinted from NAKANISHI T, FUKUSHIMA S, OKAMOTO K *et al.*: Development of the polymer micelle carrier system for doxorubicin. *J. Control. Release* (2001) 74(1-3):295-302, copyright (2001), with permission from Elsevier. Chemical structure of core-forming block used for doxorubicin encapsulation in this system (Poly[aspartic acid]-doxorubicin) is shown in Figure 1.

This formulation was found to be efficient in the stabilisation of the active CP metabolite and caused a fivefold increase in the lifespan of L1210 tumour-bearing mice even at a reduced CP-equivalent dose.

In 1987, Kataoka *et al.* reported on the preparation of micelle-forming conjugates of DOX and PEO-*b*-P(Asp) (chemical structure and model shown in Figure 1 and 2D, respectively) [24,94]. Drug level measurements 1 h after its intravenous administration in healthy mice models revealed the presence of 17.1 versus 2.9% of ^{125}I-labelled PEO-*b*-P(Asp)-DOX micelles per gram blood and heart (site of DOX toxicity), respectively [95]. An increase in the molecular weight of the PEO block to 12,000 g.mol^{-1} in this system resulted in a further raise in its blood levels in healthy mice (68% of dose remained in blood 4 h after intravenous injection) [22]. In biodistribution studies in solid tumour (C26)-bearing mice a tumour/heart concentration ratio of 12, 8.1 and 0.9 was demonstrated for PEO-*b*-P(Asp)-DOX with PEO chains of 12,000 and 5000 g.mol^{-1}, and free DOX, respectively, 24 h after intravenous injection [96]. Compared with free drug, higher levels of conjugated DOX were, however, required for an equal antitumour activity. The lower toxicity of micelle-forming polymer–DOX conjugates in comparison to free drug allowed the administration of higher drug levels (almost 20-times increase in maximum tolerable dose [MTD]), leading to an apparent superior therapeutic index for PEO-*b*-P(Asp)-DOX in haematological [26] or solid tumour-bearing animal models [95].

In fact, careful characterisation of PEO-*b*-P(Asp)-DOX micelles revealed that it is the unconjugated physically entrapped DOX that plays a significant role in the antitumour activity of this system [27]. This has led to the application of PEO-*b*-P(Asp)-DOX micelles as nano-containers for physically encapsulated DOX [97]. Optimisation of this system in a laboratory setting showed the presence of DOX dimers within the micellar core to stabilise the physically encapsulated DOX unimers, and delayed its release from the carrier in a biological environment [81]. Physically encapsulated DOX in PEO-*b*-P(Asp)-DOX micelles (Figure 5), namely NK-911, entered clinical trials in Japan in 2001 [98]. Because lyophilised micelles that contained DOX dimers were non-reconstitutable in water after long periods of storage, the dimer form of DOX has been removed from NK-911.

NK-911 is one of the few polymeric micellar formulations that have shown a favourable change in the normal pharmacokinetic parameters and biodistribution pattern of the incorporated drug in animal models [98]. Compared with free drug, NK-911 showed 28.9-fold higher area under the concentration–time curve (AUC) in plasma (within 24 h), higher tumour drug levels, less toxicity and superior *in vivo* activity in solid and haematological cancers in mice (Table 2). After 24 h, the pharmacokinetics of encapsulated and free DOX became similar, indicating drug release from the micellar carrier.

The results of Phase I clinical trials and human pharmacokinetic studies on NK-911 showed a similar spectrum in the side effects and recommended dose for NK-911 and free DOX [99]. In pharmacokinetic evaluations in humans, NK-911 exhibited 2.5-fold increase in half-life, 2.2-fold decrease in plasma clearance (CL), 1.6-fold decrease in volume of distribution (Vd) and twofold increase in plasma AUC. A comparison between pharmacokinetic parameters for NK-911 and the liposomal formulation of DOX indicated a lower stability for NK-911 in the bloodstream. However, infusion-related reactions that occur after the administration of liposomal DOX were not seen for NK-911. NK-911 is currently under Phase II clinical trial for the treatment of metastatic pancreatic cancer.

Pluronic micelles have also been suggested for potential use in DOX delivery, especially in drug-resistant cancers. Pluronic copolymers were shown to enhance the delivery of drugs across the blood–brain barrier [32,33,100,101], hyper-sensitise drug resistance carcinoma cells and enhance the response of

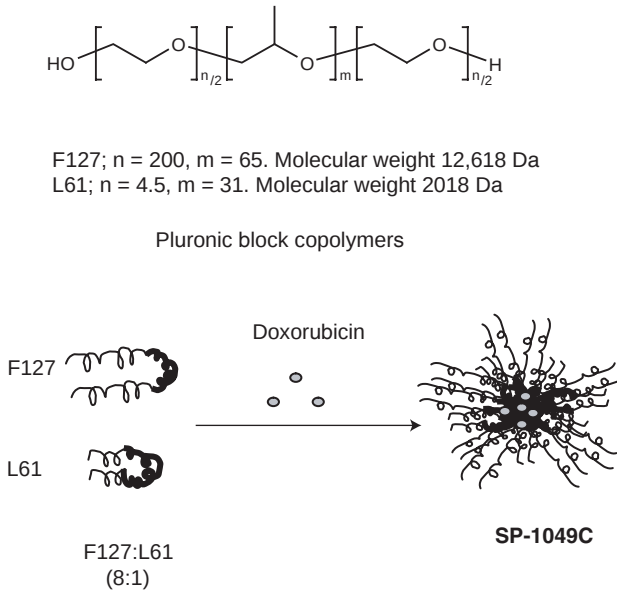

Figure 6. Chemical structure and schematic model of SP-1049C.

resistant tumours to chemotherapeutic agents such as DOX [102-110]. The reason behind this observation is not completely clear, but it has been related to an increase in drug uptake, a reduction in drug efflux possibly due to ATP depletion and changes in the intracellular trafficking of DOX in resistant tumour cells by block copolymer unimers [111]. Among the different chemical structures, pluronics in which PEO block constitutes ≤ 50% of the total molecular weight were shown to have the highest modulating effect.

Pluronic formulation of DOX, known as SP-1049C, entered preclinical and clinical trials in Canada in 1999 [112]. In the final formulation, the hydrophobic copolymer (i.e., L61) is combined with more hydrophilic pluronics (i.e., F127) at a ratio of 1:8 to avoid micellar aggregation (Figure 6). The results of pharmacokinetic and biodistribution experiments on SP-1049C in healthy mice showed a 2.1-fold increase in plasma AUC, and a respective 2.1- and 1.5-fold decrease in CL and Vd for this formulation compared with free DOX (Table 2). The same trend was observed in Lewis lung carcinoma (LLC)-bearing C57Bl/6 mice, although the changes were not as significant (1.2-fold increase in plasma AUC, 1.2-fold decrease in CL and 1.4-fold decrease in Vd). Biodistribution experiments showed an increase in AUC for SP-1049C in tumour and brain. The toxic level of SP-1049C was shown to be similar to that of free drug in animal models (an MTD of 15 mg/kg in mouse and 7.5 mg/kg in rat for both SP-1049C and DOX). The toxicity profile of free drug and pluronic formulation were identical, except for the histopathological changes in skin and thymus, which was less in rats dosed with SP-1049C. At an equal dose of 5 mg/kg, SP-1049C was shown to be more effective than DOX in haematological and solid tumour models under *in vivo* anti-tumour efficacy studies [109].

In 2004, Danson *et al.* reported on the results of the Phase I clinical trials of SP-1049C [112]. In this study, SP-1049C was given to patients with histologically proven cancer refractory to conventional treatments with escalating doses of 5 – 90 mg/m^2 as an intravenous injection. The dose-limiting toxicity in human (i.e., myelosuppression) was reached at SP-1049C-equivalent DOX dose of 90 mg/m^2; therefore, a maximum tolerated dose of 70 mg/m^2 was recommended for future trials. The pharmacokinetic profile of SP-1049C was similar to conventional DOX, but with the exception of a slower terminal clearance. SP-1049C was shown to be effective at inducing partial responses in several patients with advanced solid tumours, although its effects were mostly temporary. The results were considered to be promising, and a Phase II stage was planned. An update on the results of Phase II trials on this system was reported by Valle *et al.* in 2004 [113]. This time the effect of SP-1049C in patients with less severe forms of cancer were evaluated. Patients received 75 mg/m^2 of SP-1049C (30-min intravenous infusion) four times a week for up to six cycles. Authors have reported partial responses in some patients after four to six cycles of treatment. However, data shows the appearance of haematological and nonhaematological signs of toxicity and a significant fall in left ventricular ejection fraction, a measure of cardiac function, in some patients. Nevertheless, it seems too early to draw any conclusion on the efficacy of SP-1049C.

PTX is another potential drug candidate for targeted delivery by polymeric micelles. It is a highly hydrophobic molecule that is solubilised with the aid of a significant amount of Cremophor EL (in Taxol®, Bristol-Mayer Squibb) for clinical use [60]. The hydrophobic nature of PTX and absence of safe carriers, which can reduce the toxicity and enhance the efficacy of injectable PTX, has driven a tremendous amount of attention to the application of polymeric micellar carriers for this drug. Polymeric micellar formulations of PTX were mostly successful in replacing the toxic solubilising agent in its commercial formulation (i.e., Cremophor EL), thus allowing the administration of higher doses of PTX. However, except for the Japanese formulation, known as NK-105, other polymeric micellar formulations of PTX failed to show any benefit over its commercial formulation in terms of passive targeting.

In 1996, Burt *et al.* reported on the application of PEO-*b*-PDLLA micelles for the physical encapsulation of PTX by a solvent evaporation method and a 5000-fold increase in solubilised PTX levels by this formulation [45]. Results of further studies on the biodistribution of PEO-*b*-PDLLA in comparison with Cremophor EL formulation showed a 5.5-fold decrease in the AUC of polymeric micellar PTX in blood after its intravenous administration [66]. This is in contrast to the expected trend in pharmacokinetic parameters for carriers with stealth properties. The results of biodistribution experiments

Table 3. Polymeric micellar delivery systems in clinical trials.

Trade name	Incorporated drug	Polymer	Company	Progress	Ref.
NK-911	Doxorubicin	PEO-*b*-P(Asp)-DOX	Nippon Kayaku Co., Japan	Phase II	[99]
SP-1049C	Doxorubicin	PEO-*b*-PPO-*b*-PEO	Supratek Pharma, Inc., Canada	Phase II	[112]
PAXCEED®	Paclitaxel	PEO-*b*-PDLLA	Angiotech	Phase I/II	[201]
Genexol®-PM	Paclitaxel	PEO-*b*-PDLLA	Samyang Corp., South Korea	Phase II	[114]
NK-105	Paclitaxel	PEO-*b*-PPBA	Nippon Kayaku Co., Japan	Phase I	[115]

PEO: Poly(ethylene oxide); P(Asp): Poly (aspartic acid); PDLLA: Poly(D,L lactide); PPBA: Poly(4-phenyl-1-butanoate) l-aspartamide.

in healthy rats using radiolabelled PTX demonstrated a rapid loss of drug from the micellar carrier. This formulation was evaluated further in athymic male nude mice bearing LNCaP prostate tumour [67]. In the group that received Cremophor EL, all animals died within 1 day after injection because of lethal anaphylaxis caused by Cremophor EL. The polymeric micellar group, on the other hand, did not show any significant side effects or signs of mortality. Administration of polymeric micellar PTX caused a 96 and 91% decrease in serum prostate specific antigen levels and tumour volume after three cycles of treatment, respectively. This formulation (PAXCEED®, Angiotech) is now in Phase II clinical trials for the treatment of rheumatoid arthritis, and is also evaluated for the therapy of psoriasis by Angiotech Pharmaceuticals in Canada (Table 3).

In 2001, Kim *et al.* reported on the toxicity profile, pharmacokinetics and biodistribution of PTX loaded in a similar carrier (i.e., PEO-*b*-PDLLA micelles prepared by an identical solvent evaporation technique, known as Genexol®-PM, Samyang Corp.) (Table 3) [35]. The MTDs of Genexol-PM and Cremophor EL in intravenous administration in nude mice were determined to be 60 and 20 mg/kg, respectively. Despite a 2.5-fold increase in the administered dose, maximum concentration and AUC were found to be lower for the polymeric micellar formulation. Instead, the CL and Vd at steady state (Vdss) values were higher. At an equal dose of 20 mg/kg, the distribution was comparable for both formulations in all organs except for plasma, which showed 10- to 20-fold lower AUC for Genexol-PM. At a threefold higher dose of administration, Genexol-PM showed better antitumour effects in ovarian and breast cancer mice models when compared with Cremophor EL formulation of PTX. The administration of Genexol-PM allowed the administration of higher drug doses in Phase I clinical trials studies in humans, but resulted in a lower AUC in plasma and a shorter half-life for PTX [114].

Encapsulation of PTX in micelles consisting of PDLLA as the core-forming block and poly(*N*-vinylpyrrolidone) (PVP) as the shell-forming block via a freeze-drying method was reported in 2004 [53]. *In vivo* evaluation of PTX-loaded PVP-*b*-PDLLA micelles in comparison to Cremophor EL revealed similar results to Genexol-PM: a decline in PTX plasma levels and an increase in Vdss and CL (Table 2) [52]. The inverse relationship between the extent of drug loading and AUC seen in the mice model was attributed to the drug loss from the carrier at higher drug encapsulation levels. The results of biodistribution studies showed a decrease in the AUC of PTX in liver, kidney, spleen and heart for the micellar formulation, but the AUC in tumour did not change significantly. For the polymeric micellar formulation, MTD was not reached even at 100 mg/kg, whereas the MTD of Cremophor EL formulation of PTX was established at 20 mg/kg. This allowed a threefold increase in the therapeutic dose of PTX in its polymeric micellar formulation leading to a significant increase in antitumour activity for this system over Cremophor EL formulation of PTX.

Recently, Hamaguchi *et al.* reported on the development of the only polymeric micellar formulation that has shown stealth and passive targeting properties for PTX delivery, so far: NK-105, which consists of micelle-forming block copolymers of PEO-*b*-poly(4-phenyl-1-butanoate) L-aspartamide (PEO-*b*-PPBA) (Figure 7). NK-105 has shown an 86-fold increase in its AUC in plasma, an 86-fold decrease in CL and a 15-fold decrease in Vdss compared with Cremophor EL after intravenous injection (Table 2) [115]. This has in turn resulted in a 25-fold increase in drug AUC in tumour and stronger antitumour activity in C-26 tumour bearing mice model. At a PTX-equivalent dose of 100 mg/kg, a single administration of NK-105 resulted in the disappearance of tumours and all mice remained tumour-free thereafter. This formulation is currently in Phase I clinical trials in Japan (Table 3).

Another example of nano-engineered polymeric micellar carriers developed for the purpose of passive drug targeting is cisplatin-loaded PEO-*b*-P(Asp) (PEO-*b*-P(Asp)-CDDP) micelles [116]. Pharmacokinetic evaluation of this formulation in a LLC model showed a 5.2- and 4.6-fold increase in the plasma and tumour AUC (calculated based on total platinum content) for CDDP-loaded micelles in comparison to the free drug (Table 2) [116,117]. Liver and spleen platinum levels in animals receiving polymeric micellar CDDP increased, but kidney drug levels stayed the same. Despite an elevated administration dose (8 – 15 mg/kg for polymeric micellar CDDP versus 6 mg/kg for free drug), polymeric micellar CDDP did not show any significant benefit in antitumour

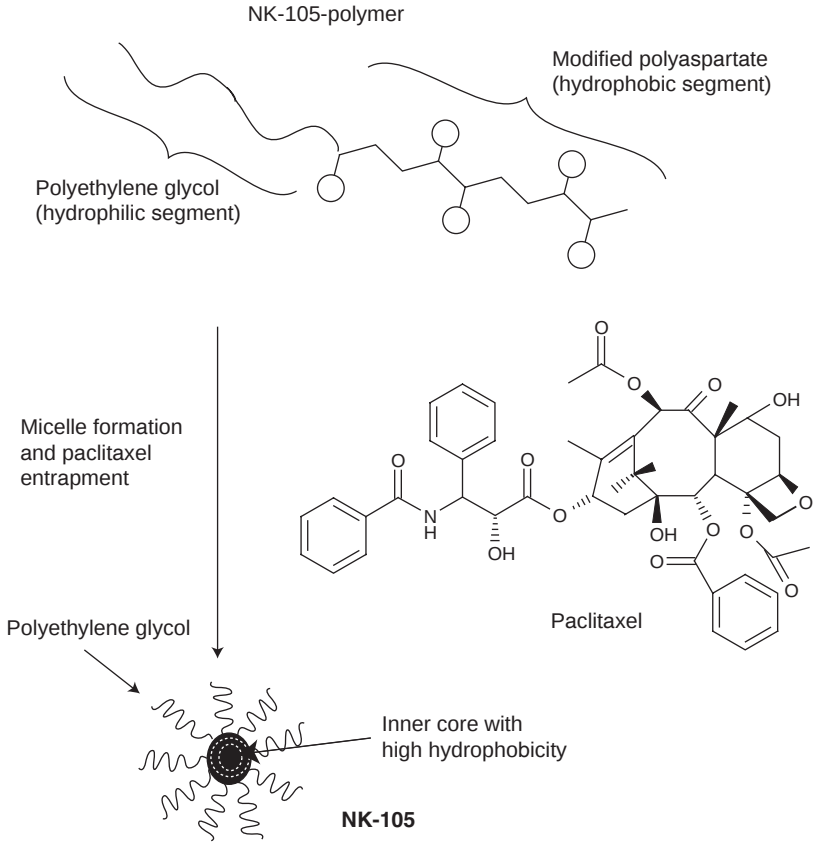

Figure 7. Schematic model of NK-105. Reprinted by permission from the *British Journal of Cancer (*HAMAGUCHI T, MATSUMURA Y, SUZUKI M *et al.*: NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumour activity and reduce the neurotoxicity of paclitaxel. *Br. J. Cancer* (2005) 92(7):1240-1246), copyright (2005) Macmillan Publishers Ltd.

activity beyond 4 days. The poly(Asp) core was later replaced with poly(Glu), which is more hydrophobic, to improve micellar stability and the drug release profile. A comparison between the results of the pharmacokinetic and biodistribution studies for the two polymeric micellar systems showed elevated platinum plasma and tumour levels for CDDP in PEO-*b*-P(Glu) micelles [55]. *In vivo* antitumour activity studies in CDF1 mice-bearing C26 tumours, showed that 4 out of 10 mice receiving the polymeric micellar formulation showed complete cure, whereas with free drug no mice showed complete tumour regression.

The incorporation of a less toxic analogue of cisplatin (i.e., DACHPt) in the PEO-*b*-P(Glu) micellar system has recently been reported [88]. In comparison to CDDP, DACHPt was expected to stabilise micelles of PEO-*b*-P(Glu)–drug complex to a greater extent because its structure is more hydrophobic and bulky. In fact, biodistribution studies after intravenous administration of the PEO-*b*-P(Glu)-DACHPt system revealed a slightly higher platinum plasma levels for this system compared with PEO-*b*-P(Glu)-CDDP, but tumour accumulation was similar for both drugs.

The development of polymeric micellar carriers for encapsulation of CsA has also been pursued by Lavasanifar *et al.* to avoid CsA distribution in sites of drug toxicity (kidneys) [50,51]. CsA is a neutral, lipophilic cyclic endecapeptide with very low water solubility (23 µg/ml). It is a potent immunosuppressive agent used primarily to reduce the incidence of graft rejection in recipients of transplanted organs. CsA is also one of the most effective inhibitors of P-glycoprotein, modulation of which has been a focus for improving antineoplastic therapy by overcoming drug resistance. Severe toxic side effects caused by CsA itself or the solubilising agent used in its commercial formulation (i.e., Cremophor EL) have jeopardised the clinical benefit of CsA administration for either immunosuppression or as an inhibitor of tumourogenic P-glycoprotein [118,119]. Preliminary data indicated that PEO-*b*-PCL can effectively solubilise CsA and cause a favourable shift in the pharmacokinetics and biodistribution of this drug [50]. Pharmacokinetic studies in a healthy rat model showed a significant increase in plasma AUC (from 32.7 to 199 µg.h/ml), a decrease in Vdss (from 2.33 to 0.232 l/kg) and a decrease in CL (from 0.195 to 0.0255 l/kg/h) for the polymeric micellar formulation in

comparison to commercial formulation of CsA (i.e., Sandimmune®, Novartis) (Table 2) [51]. The polymeric micellar formulation elevated CsA levels in blood and plasma, but restricted the rapid distribution of CsA to the kidney and liver. This is a significant finding since liposomal and phospholipids micellar carriers of CsA have shown similar pharmacokinetics to the commercial formulation in animal models pointing to premature CsA release from those systems [120-122].

6. Conclusions

Clearly, polymeric micelles offer viable means for the solubilisation of water-insoluble drugs. So far, however, only a few polymeric micellar formulations have demonstrated success in targeted drug delivery in a biological system (Table 2). Micelle-forming drug conjugates developed to date are mostly found to be too stable and incapable of providing adequate drug release in the diseased site, whereas polymeric micellar nanocontainers and polyion complex micelles may release their drug content prematurely. The challenge is to find the right polymeric micellar carrier architecture and drug–block copolymer combination that can withstand the destabilising effect of the biological environment and provide a proper balance between carrier size, micellar stability and drug release in the biological system. Polymer chemistry provides an infinite opportunity for nano-engineering of the polymeric micellar carrier with respect to these requirements.

7. Expert opinion

The unique properties offered by the special architecture of polymeric micellar carriers has made them the closest colloidal delivery system developed so far, to the definition of an ideal carrier for targeted drug delivery, the so-called 'magic bullet'. The presence of a dense hydrophilic polymeric brush on the micellar surface and their small size (10 – 100 nm) is expected to provide a better protection for polymeric micellar delivery system against uptake by RES, which will, in turn, result in prolonged circulation times and higher accumulation of the carrier in selective tissues that have leaky vasculature (e.g., tumour or inflammation sites). A capacity for the stabilised encapsulation of hydrophobic compounds offered by polymeric micelles is considered to be one of their other advantages over lipid-based colloidal delivery systems that have mostly failed in retaining their hydrophobic drug content in the biological system. Finally, chemical flexibility of the core/shell structure makes nano-engineering of the carrier a feasible approach in case of polymeric micelles. Nano-engineering of polymeric micellar formulations is mostly pursued to enhance the stability of micellar structure or to modify drug-release properties. Chemical modification of the polymer structure in the micellar core (e.g., attachment of hydrophobic structures [56], introduction of drug compatible moieties [43,69,98], chemical core crosslinking [123,124] and partial crystallisation of the micellar core through stereocomplex formation [125]) are among the successful approaches taken to address the issue of micellar instability and/or premature drug release.

So far, five polymeric micellar formulations have successfully passed the phase of bench-top development and advanced to the stage of clinical evaluations in a relatively short period of time (Table 3). The progress in the field of selective drug delivery by polymeric micelles has been expanded to other hydrophobic antineoplastic drugs and prodrugs and it is only a matter of time before other polymeric micellar formulations enter the stage of clinical evaluations (Table 2). In this context, the potential therapeutic benefit of polymeric micellar carriers for the delivery of different hydrophobic agents has not been fully explored and deserves more attention. Limited information on the therapeutic efficacy of polymeric micellar formulations for therapeutic agents other than anticancer drugs is available [126-128].

Research for the development of targeted polymeric micelles has been expanded to the area of active drug targeting. The second generation of polymeric micellar carriers (i.e., nano-carriers decorated with different ligands, such as sugars, peptides and antibodies, or covered with bioadhesive polymers on their surface) are also developed to enhance the targeting efficiency of the colloidal carrier or increase the oral bioavailability of the incorporated drugs [15,58,91,92,129-136]. Surface-modified polymeric micelles have shown benefit in enhancing the recognition of carrier by selective cells leading to improved drug/gene delivery at a cellular level [129,130,132].

A novel approach in increasing the specificity of the polymeric micellar formulations involves the application of external stimulus (e.g., temperature, ultrasound) that can trigger either drug release or polymeric micelle–cell interaction only at the desired site of drug action. Dehydration of the thermo-responsive shell-forming blocks in polymeric micelles above the specific transition temperature of the polymer leads to an increase in the hydrophobicity of the shell-forming block enhancing the interaction of the micellar carrier with hydrophobic components of cells. A localised raise in temperature will switch on the response leading to an enhanced drug delivery, only to desired cells [137-141]. Local application of focused ultrasound is an alternative approach, which has shown success in enhancing drug release and delivery by polymeric micelles to desired cells in both *in vitro* and *in vivo* models [31,77,142-151].

On the other hand, pH-responsive polymeric micelles are designed that can respond to localised changes in pH and release their drug content in specific organs or intracellular compartments. For example, the lower pH of the tumour interstitium and acidic condition of intracellular organelles (e.g., endosomes/lysosomes) can trigger drug cleavage form micelle-forming polymer–drug conjugates or induce micellar disruption leading to localised drug/DNA release in acidic environment [86,155-162]. The chemical structure of the core-forming block can be tailored to respond to the higher pH of the jejunum and improve drug delivery from an oral route of administration. In this case, the higher pH of the jejunum acts as an internal stimulus to induce micellar core

ionisation, micellar disruption and drug release in specific regions of the gastrointestinal tract [160-162].

Finally, the flexible polymer chemistry has provided a tremendous potential for polymeric micelles in targeted delivery of diagnostic agents and a great advantage for them in the delivery of future drugs (i.e., therapeutic genes, proteins and vaccines [5,57-59,123,126-129,132]). This has sparked a new line of investigation in the development of new polymeric micellar systems that can not only target specific cells but can trigger release and interaction with specific intracellular targets. Finding the right carrier architecture and optimum polymer chemistry that can improve the delivery of these sophisticated and complex diagnostic/therapeutic agents to their cellular and intracellular targets is expected to be the demanding challenge of the future research in this field.

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Angiotech Pharmaceuticals, Inc. website.

Affiliation

Hamidreza Montazeri Aliabadi[1] PharmD & Afsaneh Lavasanifar[†2] PharmD, PhD
[†]Author for correspondence
[1]Graduate student, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada
[2]Associate Professor, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2N8, Canada
Tel: +1 780 492 2742; Fax: +1 780 492 1217;
E-mail: alavasanifar@pharmacy.ualberta.ca