A Review on Comb Shaped Amphiphilic Polymers for hydrophobic drug solubilisation

Abstract

Comb shaped amphiphilic polymers are rapidly emerging as an alternative approach to amphiphilic block copolymers for hydrophobic drug solubilisation. These polymers consist of a homopolymer or copolymer backbone to which hydrophobic and hydrophilic pendant groups can be grafted resulting in a comb like architecture. The hydrophobic pendants may consist of homopolymers, copolymers or other small molecular weight hydrophobic structures. In this review, we focus on hydrophobically modified pre-formed homopolymers. Comb shaped amphiphilic polymers possess reduced CAC values compared to traditional surfactant micelles indicating increased stability with decreased disruption experienced on dilution. They have been fabricated with diverse architectures and multifunctional properties such as site specific targeting and external stimuli responsive nature. The application of comb shaped amphiphilic polymers is expanding; here we report on the progress achieved so far in hydrophobic drug solubilisation for both intravenous and oral delivery.

Keywords: Graft amphiphilic polymer, comb shaped amphiphilic polymer, polymer architecture, oral delivery, anticancer delivery, hydrophobic drug

1.0 Introduction

Amphiphilic polymers were first reported in 1984 to form nano-sized polymeric self-assemblies and was indicated to exhibit the potential as hydrophobic drug solubilisers [1]. Since the beginning of 1990s, extensive research has been conducted to explore

the ability of these amphiphilic polymers to encapsulate hydrophobic drugs in particular for intravenous administration [2-6]. Today, a plethora of amphiphilic polymers have been designed such as block copolymers [7], comb shaped polymers [8], star shaped [9] or dendrimers [10] (Fig. 1) albeit all share the common polymer architecture consisting of both hydrophobic and hydrophilic segments within the same macromolecule [6]. Among these amphiphilic polymers, the most common type is block copolymers and they have been extensively reviewed [11-15]. Therefore, in this article, we focus on comb shaped polymers, the second most common type amphiphilic polymers. The polymer architecture of the comb shaped polymers, the physical-chemical properties of comb shaped polymers and their use in oral and intravenous delivery will be evaluated in this review.

2.0 Comb Shaped Amphiphilic Polymer

The interest in using comb shaped amphiphilic polymers for drug delivery was reported in mid 1990s where Chiu and colleagues first described the fabrication of an amphiphilic graft polymer comprising of stearyl methacrylate, methyl acrylate, acrylic acid and acrylate derivative of polyethylene glycol (PEG) monomer units [16]. This copolymer consists of hydrophobic (primarily stearyl groups) and hydrophilic (PEG part) side chains together with anionic carboxylate groups. Polymeric micelles with two size populations of 50nm and 389nm were formed in aqueous environments capable of encapsulating pyrene, a model hydrophobic compound. The authors attributed the different size population phenomenon was due to loose aggregates / large association complexes were in dynamic equilibrium with smaller more compact micelles in water [16]. Today, comb shaped polymers can be made up of either a water soluble homopolymer or copolymer backbone with hydrophobic pendant groups

conjugated or ‘grafted’ onto the backbone forming a comb shaped structure (Fig. 1B) [8,17,18]. The hydrophobic pendants may consist of homopolymers [19], copolymers [20] or other small molecular weight hydrophobic structures [21]. Often, hydrophilic groups such as quaternary ammonium moieties [22] or polymer such as polyethylene glycol (PEG) [23] are added to improve water solubility. Due to the diverse structure of amphiphilic graft polymers, in this review, we only focus on hydrophobically modified pre-formed homopolymer backbone. In aqueous solution, spontaneous nano aggregates are formed upon aggregation of the hydrophobic pendant groups. As a result, a hydrophobic core is formed and stabilised by the water soluble polymer backbone [24,25,26]. Comb shaped amphiphilic polymer can form polymeric micelles [17], nanoparticles [19], disc-like structures [27] and vesicles [28] in the aqueous environments. Adjusting the structural components such as the hydrophobic pendant group or the level of grafting has a direct impact on the physical properties of the self-assemblies formed [8,17,21,29]. Comb shaped polymers have previously been reported to successfully encapsulate hydrophobic drugs such as Cyclosporine A [8,19], doxorubicin [30] and paclitaxel [31,32] and other agents for other therapeutic agents such as proteins [8], peptides [33] and genes [32,34].

3.0 Types of water soluble pre-formed polymer backbones

3.1 Carbohydrate polymers

Carbohydrate polymers such as chitosan [22,35], dextran [36] (Fig. 2E) and hydroxypropylmethyl cellulose [37] have been widely used as polymer backbones for comb shaped amphiphilic polymers (Table 1). Prabaharan synthesised carboxymethylchitosan-g-phosphatidylethanol amine, CMC-PEA using chitosan as

the backbone [38]. Chitosan (Fig. 2D) is known for its non toxic, biodegradable and biocompatible nature. By grafting PEA hydrophobic pendant groups via the primary amine groups on the chitosan homopolymer backbone spontaneous self-assemblies were formed in aqueous environment where lipophilic drug, ketoprofen was encapsulated inside the hydrophobic core [38]. Yu *et al.* grafted chitosan (Chi) with biodegradable polyester poly(caprolactone) (PCL) forming PCL-g-Chi 40-60nm nano aggregates [39]. They reported that the physical properties of self-assemblies could be tailored by controlling the level of PCL grafting onto the chitosan backbone. The biodegradability of PCL-g-Chi makes it an ideal delivery system and further work is currently underway to investigate the potential of PCL-g-Chi as a drug solubilising agent [39]. Other biodegradable amphiphilic chitosan derivatives have been reported including chitooligosaccharide-graft-poly(ε-caprolactone) (COS-g-PCL) [40], chitosan-graft-poly(ethylenimine)-graft-poly(ethylene oxide) (chitosan-g-PEI-PEG-OH) [41] and hexanoyl chitosan-graft-poly(ethylene glycol) (PEG-g-hexanol chitosan) [42].

Like chitosan, dextran is a polysaccharide renowned for its attractive qualities such as biocompatibility [43], biodegradability [44] and versatility [45]. Francis and colleagues grafted dextran with a series of poly(ethyleneglycol) alkyl ether forming (DEX-g-PEG-C) [46]. The dextran derivatives were investigated for their ability to form polymeric micelles in aqueous environments. The results showed that CAC decreased with higher PEG-Cn units per dextran chain and also with decreased molecular weight of dextran [46]. Amphiphilic dextran derivatives have been extensively reported for use as drug solubilising agents (including Cyclosporin A and Paclitaxel) [36,43-48]. Recently modified dextrans with poly(N-isopropylacrylamide)

pendant groups have been reported possessing more sophisticated aggregates with stimuli responsive functionalities (pH or temperature) [49,50].

3.2 Synthetic polycations polymers

Most of the synthetic polycations (Table 1) such as polyethylenimine (PEI) [51,52] (Fig. 2A), poly-L-lysine (PLL) [42] (Fig. 2B) or polyallylamine (PAA) [53] (Fig. 2C) were first used as gene delivery systems due to the presence of amine functional groups for complexation with negatively charged DNA. PEI is known for its abundance of primary amines on the polymer backbone which provide extensive possibilities for grafting and further functionalisation [54-56]. PEI was discovered long before Bousiff *et al.* but these authors were first to introduce it as a non-viral vector for gene delivery [57]. However, due to the inherent cytotoxicity of PEI, attempts have been employed to improve the biocompatibility profile as well enhancing its efficiency as gene delivery system. One of the methods used is adding hydrophobic groups such as cholesteryl [52], hydrocarbon chains such as palmitoyl [51], cetyl [8], or polymer such as poly(caprolactone) [51]. Most of the research showed that grafting of hydrophobic moieties onto PEI improves the safety profile of PEI [8,58,59], which was attributed to the reduction of primary amines after hydrophobic conjugation [58,59]. In recent years, hydrophobically modified PEIs have been used for oral delivery of hydrophobic drugs such as cyclosporine A [8] or for targeting both hydrophobic drugs and gene to cancer cells [60].

Wang and colleagues substituted PLL with hydrophobic palmitoyl groups to form PLL amphiphiles [28]. They showed that addition of cholesterol is needed to form vesicles upon sonication in aqueous solution [28]. Recently, we reported on the use of polyallylamine (PAA) amphiphiles for hydrophobic drug solubilisation [21,61,62].

Previously PAA has been used clinically as an oral phosphate sequester [63], indicating a good degree of biocompatibility. PAA also possesses a high degree of primary amines making them amenable to simple modifications. The different types of hydrophobic pendant groups grafted onto PAA normally have an impact on the type of self-assemblies formed in aqueous solution [21].

3.3 Different pendant groups grafted to homopolymer

The hydrophobic pendant groups grafted onto the homopolymer backbone of comb shaped polymers are usually alkyl chains [17,51,64], acyl groups [65,66] or cholesterol moieties [17,21,62,67,68] (Fig. 3).

Wang grafted hydrophobic linear cetyl chains (Fig. 3A) onto a poly(ethylenimine) (PEI) backbone [69]. Interestingly it was reported that at low molar cetyl grafting (<23%) spontaneous aggregation in aqueous media formed micellar structures. At higher levels of cetyl grafting (23-42%) vesicle formation occurred, this phenomenon was also observed upon addition of hydrophobic cholesterol into the solution at lower grafting levels of 3-43% [69]. On further increasing the degree of cetylation (≥ 49 %) dense nanoparticles are formed. These results indicated that at varied levels of hydrophobicity these amphiphilic polymers resulted in a range of colloidal structures [58]. These findings have been observed across a large number of studies on different polymer amphiphiles [8,17,28,70].

Cholesterol (Fig. 3C) is a commonly occurring sterol in the body [71]. It regulates membrane fluidity and plays an important role in spontaneous association of molecules in biological pathways [86]. Yusa and colleagues reported the graft polymerization of sodium 2-(acrylamido)-2-methylpropanesulfonate with cholesteryl methacrylate (CholMA) and cholesteryl 6-methacryloyloxyhexanoate (Chol-C5-MA)

[72]. Even at low cholesterol grafting ratios, those polymers still possessed a strong tendency to form aggregates [72]. This phenomenon was also observed by Thompson on grafting cholesterol onto a PAA backbone where 2.5%-5% mole modification resulted in formation of nano-aggregates [17]. Unlike alkyl chains, it is thought that high number of carbon atoms in cholesteryl moieties may result in the observed phenomenon. Xu and colleagues also reported that the use of cholesterol as a hydrophobic cap on poly(2-methacryloyloxyethyl phosphorylcholine) improving the cytotoxicity [72]. Interestingly, when cholesterol is added into self-assemblies formed by amphiphilic graft polymers, very often it inserts into the forming bilayer vesicular structures [17,28,69], giving rise to the potential application of incorporating hydrophilic drug in the aqueous core.

Previously, fluorescent tags such as rhodamine [17,73] and fluorescein isothiocynate (FITC) [74-76] were conjugated onto amphiphilic polymers for *in vitro* and *in vivo* visualisation [17,73]. However, these dyes could affect the physical and chemical properties of the nano aggregates and hence altering the tissue interactions and cytotoxicity [77]. As such, amphiphilic polymers modified with aromatic fluorophores (e.g. pyrene, naphthalene, dansyl) have been developed (Fig. 4) [78]. Apart from simultaneous formation of nano-aggregation in aqueous solution for hydrophobic drug solubilisation [19], these polymers have a range of potential applications including *in vivo* tracers, fluorescent probes and cell labelling to mention a few [78].

Hydrophobic drug molecules can also be used as pendant groups in amphiphilic graft polymers [79-85]. Fan and colleagues created disulfide linked poly(amino amine) structure using methyl ether PEG (mPEG-g-SSPAA) [79]. To this compound

antitumor agent camptothecin was covalently conjugated as a secondary pendant group via hydrolysable bonds. Conjugation of camptothecin to the polymer backbone increased its aqueous solubility and stability. Upon incubation with mouse muscular cells (L929), the polymer-drug conjugate exhibited reduced cytotoxic effect on the cells compared to the free drug which was thought to be due to the controlled release of camptothecin from the micelle [79].

3.4 Types of hydrophilic moieties

Hydrophilic moieties including poly(ethylene glycol) (PEG) [23,83,84] and quaternary ammonium ions [17,22,85,86] are commonly attached to the homopolymer backbone (Fig. 5). PEG moieties improve the safety profile of the delivery system and can provide the nano aggregates with 'stealth' properties *in vivo* resulting in reduced phagocytotic clearance leading to increased drug circulation times [22,87,88]. In 2000 Brown and colleagues found that attachment of mPEG to their palmitoyl-PLL comb shaped amphiphiles reduced the surface charge, resulting in a neutral zeta potential leading to reduced cytotoxic effect on 4549 and A351 cell lines [89]. These results are consistent with the theory that addition of PEG both increases stability and decreases cytotoxicity of amphiphilic graft polymers [23,83,84,90,91].

Thompson and colleagues reported that addition of the quaternary ammonium ion onto PAA amphiphiles increased their aqueous solubility whilst reducing the size of their corresponding self-assemblies [17]. They also reported that hydrophilic substitution reduced the *in vitro* cytotoxic effect of the amphiphiles on human colorectal carcinoma (Caco-2 cells) [70,79,92]. It is thought permanently charged amines (i.e. quaternary ion) are less cytotoxic than protonable amines [92] and are in

agreement with Uchegbu and colleagues findings [24]. Table 1 shows the homopolymer backbones together with the hydrophobic and hydrophilic pendant constituents of some common graft amphiphilic polymers.

4.0 Comb Shaped amphiphilic polymer for site specific delivery

The versatility of the comb shaped amphiphilic polymers has enabled the design of stimuli responsive polymers which respond to external triggers such as temperature or pH [38,93-95] (Fig. 6). Prabaharan and colleagues fabricated a pH responsive chitosan derivative graft polymer of carboxymethyl chitosan-graft-phosphatidylethanolamine (CMC-g-PEA). The CMC-g-PEA possessed a pH-dependent swelling behaviour, which resulted in elevated levels of drug release. At physiological pH (7.4) 80% of ketoprofen release was observed after 70 h, however this was significantly decreased in acidic pH's (pH 1.4) where 80% drug loss was experienced after only 45 h [38]. Gu and colleagues synthesized a novel pH responsive poly(L-lysine) grafted with cholic acid and subsequently PEGylated via a benzoic imine linker (PEG-PLL-CA) [96]. This system was stable at physiological pH (7.4) but at lower pH values representative of endosomal conditions (pH 5.4) the benzoic imine linker degraded resulting in the release of the 'stealth' PEG, leaving the PLL-CA micelle which possessed much higher zeta potential values. Upon a decrease in the pH from 7.4 to 5.4 the zeta potential of PEG14-PLL-CA50 micelles raised from 0 to 36 mV. This increase in zeta potential resulted in a membrane disruptive property in porcine red blood cells. They concluded that this membrane disruptive behaviour at a pH lower than the extracellular pH of cells, can potentially be useful as a delivery system for intracellular transport into solid tumours [96].

Shi and Zhang synthesised an amphiphilic dextran derivative via conjugation of biocompatible and thermoresponsive poly(N-vinyl caprolactam) (PNVCL) pendant groups [97]. PNVCL is known to possess a lower critical solution temperature (LCST) at 32°C in aqueous solutions [97]. At temperatures above the LCST values spontaneous phase separation or precipitation occurs, this results in transition of the polymer solution from a well-dissolved coil structure to a less-soluble globular state and hence leading to drug release. Upon conjugation with the dextran backbone, Shi and Zhang showed that the new amphiphile also possessed an inherent thermoresponsive nature [97]. They showed that a temperature-dependent transmittance change occurred when PNVCL was grafted onto dextran with a LCST value of 31.9 °C. This value decreased from 35.4°C to 31.9°C upon dilution of the polymer from 0.87 g/L to 0.10 g/L respectively [97].

Similar to block-copolymer, ligands such as folate [98-100] galactose [101, 102] or antibodies [103] have been grafted onto comb shaped amphiphilic polymers. Folate receptors are commonly used for actively targeting cancerous tumour sites [98,100,103,104]. Morris and Sharma recently fabricated a site specific delivery system based on PEI amphiphiles for gene delivery [103]. The graft polymer consists of arginine modified oligo(alkylaminosiloxane) (P(SiDAAr)5) grafted onto a PEI backbone with poly(ethylene glycol)-folic acid (PEG-FA) conjugated forming P(SiDAAr)5FP2 [103]. Since folic acid is necessary for healthy proliferating cells, rapidly dividing cancerous cells experience and increased ‘appetite’ for such compound and hence over express folate receptors [104]. Morris and Sharma incubated their nanoparticulates with the ubiquitous keratin-forming tumor cell line HeLa (KB) cells, known for their high levels of folate receptors. They observed an

increase in cellular uptake of the P(SiDAAr)5FP2 compared to the P(SiDAAr)5 and PEI alone. This enhanced transfection indicating P(SiDAAr)5FP2 as potential future for gene therapy [103].

Table 1.

5.0 Micelle formation, drug loading and physicochemical properties

5.1 Critical Aggregation Concentration (CAC)

The lowest concentration required for polymeric aggregates to form in aqueous environments is known as the critical aggregation concentration (CAC). In general comb shaped amphiphiles have a higher CAC value than block copolymers which can be seen in Table 2. This can be attributed to the intramolecular aggregation mechanism by which they can spontaneously assemble forming looser larger aggregates [19].

Table 2.

Essa and colleagues synthesised a series of PEG modified poly(D-Lactide) (PLA) graft (PL-g-PEG) and multiblock copolymers (PLA-PEG-PEL) [126]. Their findings showed that when PEG was grafted onto the PLA backbone the polymer showed different physicochemical properties than when PEG was copolymerized to PLA. In graft polymer an increase in immiscibility was observed in solution, whereby more PEG moieties ‘separated’ from the hydrophobic PLA reaching the surface of the nanoparticles. This phenomenon resulted in a greater degree of hydrophobicity inside the nanoparticles core when compared to the copolymerised counterpart [126]. This

implies that graft or comb shaped polymers form more well defined aggregates, perhaps giving rise to greater drug encapsulation abilities [126].

It is well documented that at lower CAC values amphiphilic polymers experience increased stability resulting in less disruption upon dilution *in vivo* [2,3,4] (Fig. 7). There are generally 4 parameters affecting the CAC for amphiphilic graft polymers: a) the level of hydrophobic chain grafting [8,17,21] b) the length of hydrophobic pendant groups [17] c) the type of hydrophobic pendant groups [21] d) the molecular weight of the polymer backbone [28].

The major driving force behind polymeric self-assembly is the decrease in Gibbs free energy which results from the hydrophobic pendants being removed from the aqueous environment into the hydrophobic core as the nano aggregate forms [6]. Most of the studies to date has shown that higher levels of hydrophobic grafting results in lower CAC for amphiphilic graft polymers [8,17,21]. Thompson *et al.* reported that PAA amphiphiles with increased molar modification of hydrophobic pendant group possessed lower CAC values irrespective of the type of pendant group [17]. He also showed that cholesterol PAA amphiphiles (Ch) possessed lower CAC values compared with palmitoyl and cetyl chains due to the increased alkyl chain length in the cholesterol moiety. This resulted in greater hydrophobic interactions [17]. The stereochemistry of the hydrophobic pendant group also has a direct impact on the CAC value [8,21,70]. Recently we reported the fabrication of PAA amphiphiles with hydrophobic aromatic pendant groups [21]. Interestingly from that work we observed that PAA amphiphiles modified with both fmoc and naphthalene moieties possessed two CAC values [21]. It is proposed that the planar structure of the aromatic groups resulted in stacking at higher concentrations initiating excimer formation [21]. The

CAC values obtained from our PAA amphiphiles were larger than normally achieved using graft polymers, perhaps due to the aromatic rings exhibiting a lower degree of hydrophobicity or due to the aromatic groups being in closer proximity to the water molecules forming looser aggregates [26,68]. In 2006, Cheng and colleagues reported conjugation of alkyl chains onto poly(ethylenimine) (PEI) backbones of different molecular weight [8]. It was shown that by keeping the % molar grafting ratio constant between cetyl chains (5 % molar ratio) and PEI monomer unit, resulted in similar CAC values obtained across PEI with molecular weight of 1.8, 10 and 25 KDa [8,69].

5.2 Drug loading process

Two methods are traditionally used to incorporate hydrophobic drugs inside polymeric micelles formed by amphiphilic block copolymers or graft polymers [12,127-129] (Fig. 8A&B). The first method is solvent evaporation, where polymer and drug are dissolved in organic solvents [127,128]. After removal of the solvents under reduced pressure, the residue is reconstituted with water and spontaneous aggregation is initiated [127]. In the second method, dialysis is used where the polymer and drug are dissolved in water and miscible organic solvents. The solution is placed inside a dialysis membrane followed by exhaustive dialysis against water [12,129]. The exchange of solvent and water molecules in the dialysis bag drives the formation of self-assemblies with encapsulated drug [129]. Excess free drug is then filtered out of solution using a 0.45 micron syringe filter [129]. In this case the nano aggregates exceeded the pore size of the membrane and remained inside the dialysis tubing. However, the use of organic solvents might not be favourable due to the

possibility of residual organic solvents, which could be harmful upon administration *in vivo* [130].

Recently, a quick and simple technique has been adopted for loading hydrophobic drug into self-assemblies formed by graft polymers [8,17,21]. Probe sonication of the polymeric amphiphiles in aqueous solution encourages aggregation formation [8] (Fig. 8C). Hydrophobic drug is then added into the polymeric self-assemblies and upon sonication, the drug is encapsulated within the hydrophobic core of the self-assemblies [61]. Excess drug is then filtered via syringe filtration. The absence of organic solvents in this method eliminates the safety issue concerns associated with the aforementioned methods.

5.3 Factors affecting drug loading in polymeric self assemblies

The driving force for hydrophobic drug solubilisation inside polymeric self-assemblies is associated to basic energetic principle [2]. As the amphiphilic polymers spontaneously form their unique core–shell supramolecular structures in aqueous solution, the lipophilic drug molecules accumulate within the hydrophobic core. These molecules remain physically entrapped inside the core with the self-assemblies continuously and spontaneously disrupting and reforming in dynamic equilibrium. This phemonenon is only observed at concentrations above their CAC values [6,131,132]. A good polymeric solubiliser should have favourable and stronger interactions with solubilisate than the intermolecular interactions among the solubilisate molecules [133]. This is especially important for those solubilisates with high levels of crystalline structures [134,135]. A number of research groups have looked at the parameters affecting the drug solubilisation capacity of amphiphilic

block copolymers. The factors affecting the drug loading can be a) the ratio of hydrophobic block versus the hydrophilic blocks [136, 137], b) drug loading concentration [109] c) compatibility between the hydrophobic block with the drug [11,138] d) drug physicochemical properties [139] e) the glass transition temperature of the hydrophobic polymer segment [139]. In recent years a few experimental works have shown that attachment of drug molecules or functional groups with similar chemical structure of drugs onto the polymers could enhance the drug-polymer interaction [138,139,140]. For example, Mahmud and colleagues conjugated doxorubicin (DOX) to the hydrophobic block of poly(ethylene oxide)-blockpoly(ε-caprolactone) (PEO-b-PCL) which favoured DOX solubilisation [141],while the inclusion of cholesteryl groups in the PEO-b-PCL also resulted in a higher solubilisation of cucurbitacin I, a cholesterol drug, than the parent polymer [142].

Unlike those block copolymer micelles [141,142] the pendant group attached on a pre-formed water soluble polymer is the only hydrophobic moiety that will form the hydrophobic microdomains and contribute to the major interaction with the hydrophobic drug molecules. To date there are limited studies on the interaction between the hydrophobic drugs and the comb shaped polymers. We have conducted a number of systematic investigations on the impact of hydrophobic pendant groups of comb shaped amphiphilic polymers on the hydrophobic drug solubility enhancement. We have shown that poly-L-lysine (PLL) modified with sterol pendant groups (cholate) resulted in higher encapsulation of sterol drugs such as estradiol and prednisolone compared to PLL attached with alkyl chains such as palmitoyl pendant groups [143]. The structural compatibility between the drug molecules and hydrophobic pendant groups has resulted in better drug incorporation into the micellar

structures [143]. As shown in Table 1, most of the hydrophobic pendant groups used in hydrophobically modified pre-formed water soluble polymers consist of hydrocarbon chains despite most hydrophobic drugs consist of aromatic or cyclic ring systems. Therefore, our research has also focussed on the attachment of aromatic groups to PAA, where the aromatic groups serve as the only hydrophobic moiety. We investigated the ability of novel PPA modified with different types and levels of aromatic pendant groups (Fluorenylmethoxy carbonyl (Fmoc) and dimethylamino-1-naphthalenesulfonyl (Dansyl) (Fig. 9) on the enhancement of hydrophobic drug solubility of propofol, griseofulvin and prednisolone. Similar to studies on amphiphilic block copolymers, we have shown that increasing the level of hydrophobic modification would result in higher lipophilic content and thus causing stronger interaction with the drug molecules, which leads to higher drug encapsulation [144].

Interestingly, comparison among the aromatic modified PAAs reveals the poor solubilising capacity of Fmoc compared to Dansyl, which we hypothesised was due to the excimer formation of Fmoc at higher polymer concentrations [61]. The flat stereochemistry of aromatic structures allow π-π stacking and hence forming excimers, a known phenomenon supported by others [145]. This limits the expansion of the core to accommodate more drugs at higher concentrations, which in contrast to the presence of the N,N-dimethylamino side chain in the Dansyl moiety. The side chain gives rise to a 3D structure, thus hindering any stacking interactions of the aromatic rings [21]. As a result, the self-assemblies are able to enlarge its core to accommodate a larger amount of drug molecules. Another observation was the comb shaped amphiphilic polymers seem to have a much higher loading capacity (LC)

[54,61] (Dansyl grafted PAA exhibited LC > 100% [61]) compared to most of the reported block amphiphilic polymers, which is typically less than 20% [29,146-148].

In addition to the type and level of hydrophobic pendant groups, the molecular weight of the polymer backbone also plays an important role in drug encapsulation. Various molecular weight of PEI ranging from 1.2KDa, 10kDa and 20kDa were cetylated and subsequently quaternised and their ability to encapsulate cyclosporine was determined. It was found that the 10kDa polymer showed the highest encapsulation [8]. Other contributing factors such as initial drug loading ratios also played an important role. Qui and Bae grafted cationic PEI with various molecular weights of poly(ε-caprolactone) (PCL) 1800-5500 KDa. They found that increasing doxorubicin initial feed ratios resulted in higher amount of solubilised doxorubicin (DOX) irrespective of different molecular weight of PCL [60]. They concluded that the presence of the hydrophobic drug inside the polymeric nano aggregate core increased its hydrophobicity. The stronger hydrophobic interactions results in greater driving force for drug solubilisation and hence a higher drug loading capacity was observed [60].

6.0 Comb Shaped Amphiphilic Polymers for Anticancer Therapeutics

Polymeric self-assemblies have been extensively investigated for their ability to deliver anticancer agents to tumour sites [62,131,149-153]. Their unique size enables accumulation in tumour tissues due to the enhanced permeability and retention effect (EPR) [6,25,154-157]. Qui and Yan developed a graft amphiphilic polymer based on polyphosphazene derivatives for the delivery of DOX, an anticancer agent [30]. This was achieved by grafting hydrophobic ethyl tryptophan onto the polyphosphazene

followed by the addition of hydrophilic PEG (Fig. 10). After the addition of DOX to the grafted amphiphile formed a core-shell structure with the drug incorporated inside the hydrophobic core. When they exposed their formulation to HeLa cells for 24 h they discovered that the loaded aggregates had a higher IC_{50} value (0.88 $ugmL^{-1}$) when compared to the free DOX drug (0.22 $ugmL^{-1}$). However, the nature of the polymeric nano aggregates is such that they control the release over a long period of time, hence after 48 and 72 h, the DOX-loaded micelles exhibited comparable cytotoxicity with that of free drug. In the *in vitro* release studies they showed that after 50 h, the drug had not yet been fully released from the polymer nano aggregates [30]. These results indicate that the PEG/EtTry-PPPs micelles have great potential as sustained release vehicles; however, *in vivo* studies are needed to confirm this theory.

The synergistic effect whereby the presence of a cytotoxic drug inside a nontoxic carrier vehicle showing greater potency has been well documented using polymeric amphiphiles [158-161]. Westedt and colleagues reported such phenomenon when loading graft copolymer poly(vinyl alcohol)-g-poly(lactide-co-glycine) (PVA-g-PLGA) with paclitaxel [162]. Westedt observed that by tailoring the composition of the PVA and PLGA it is possible to control the drug release kinetics for desired clinical application. The cytotoxic effect of the unloaded and loaded nano aggregates was determined; the unloaded aggregates showed no toxic effect on primary rabbit vascular smooth muscle cells (RbVSMC) up to 370 μgmL^{-1} [162]. However, when the formulation was exposed to the RbVSMC, the polymer loaded paclitaxel possessed up to a 2-fold decrease in IC_{50} (concentration at which 50% of cell viability was achieved) compared to the free drug [162]. This effect can be attributed to the

polymer carrier vehicle not only protecting the drug but also, enabling the drug to enter the cell more efficiently [162-164].

Temperature responsive (thermo responsive) and pH responsive polymeric amphiphiles hold great potential for cancer therapy. Cancerous tissue proliferates at an increased rate compared to healthy tissue and as such at higher temperature (40 – 44 ºC) and a lower pH (<6.75) [165,166]. Thermo and pH responsive amphiphiles can be fabricated to release their payload upon slight change of temperature/pH making them ideal candidates as carrier vehicles for anti-cancer agents.

Graft polymers based on poly(amine) backbones are commonly studied for cancer therapy [167-169]. Poly(amine) polymers are stable in aqueous solution at physiological pH. Polymeric aggregates composing of histidine pendant groups on poly(amino acid) backbone (PHEA-g-C_{18}) possess inherent pH responsive nature [170]. Histidine is an endosomolytic compound causing rupture to endosomal membranes at lower pH (pH 5.4) [170,171] The release of DOX from the polymeric nano agregates has been shown to be accelerated at pH 5 due to swelling of the micellar structure [170]. Once the DOX is released from the endosome, it can diffuse into the nucleus to access its target resulting in a greater toxic effect when incubated with HeLa cells [170].

Poly(amine) graft amphiphiles have been created by grafting of poly(amino ester) with octadecyl acrylate forming PEA-g-ODA [168] (Fig. 11A). These polymeric nano structures achieved 35% DOX loading efficiency using the dialysis method. The increased potency on hepatoma (HepG2) cells shows potential as a therapeutic carrier vehicle. The polymer structure showed good buffering potential at physiological pH (7.4) with degradation occurring at lower pH’s representative of intracellular

environments (pH 5). At the lower pH DOX release occurred indicating the potential of PEA-g-ODA as a controlled release drug carrier [168]. Poly(amide amine) was grafted with a methyl ether PEG pendant group (PAA-g-mPEG) (Fig. 11B) [169]. Sun and colleagues reported that the micelles produced, were 'dis-assembled' in physiological environments and were less than 50nm. The self-assemblies were capable of loading 20%wt. DOX. HeLa and HepG2 cell lines were exposed to the formulation for 2 h, confocal microscopy was used to image the cells. Interestingly, the free DOX was only observed within the cell cytoplasm, however in the cells exposed to the DOX loaded PAA-g-mPEG, the DOX could be visualised within the cell nucleus. These result indicated that the polymeric nano carrier assisted in nuclear drug uptake [169].

Chitosan derivatives have also been studied for their use as drug carriers for therapeutic agents [172-174]. SN-38 is an antitumoral agent (a more potent form of camptothecin: 7-ethyl-10-hydroxy-camptothecin) which has been effectively solubilised in the chitosan grafted with poly(captrolactone) (CS-g-PCL) hydrophobic core [121]. The drug encapsulation resulted in an increased aqueous solubility with encapsulation efficiency up to 84% (similar to block amphiphilic polymers consisting of poly-lactide-co-glycolide-b-polyethylene glycol-folate (PLGA-PEG-FOL) with 89% encapsulation efficiency [175]). The CS-g-PCL chitosan derivatives also demonstrated improved stability and prolonged release [121]. Methyl ether PEG has been conjugated to a N-phthaloyl chitosan backbone forming PLC-g-mPEG [111]. When camptothecin was encapsulated inside the hydrophobic core of the self-assemblies, the drug was shielded from hydrolysis. This shielding effect was reported to increase the half-life of camptothecin from 94 min to 76 h which is a vast increase.

The longer the half-life of the drug, the greater therapeutic effect will occur over a sustained period of time [111]. These results are promising for the use of chitosan in chemotherapy, however *in vivo* studies must be carried out in order to determine the fate of the micellar carriers.

To date, limited *in vivo* work has been published using amphiphilic polymers based on a preformed water soluble backbone attached with small hydrophobic pendant groups in anticancer therapeutics. Recently, we reported on the use of PAA modified with cholesterol (5% molar ratio) known as Ch_5-PAA [21] (Fig. 9C). This amphiphile was used to encapsulate the novel hydrophobic anticancer agent bisnaphthalamidoproyldiamino octane (BNIPDaoct) [62]. A Ch_5-PAA, BNIPDaoct formulation (1 $mgKg^{-1}$) was administered weekly to tumour bearing nude mice over a 4 week period. Mice treated with the Ch_5, BNIPDaoct formulation experienced a significant reduction in tumour growth (compared to the Ch_5 control, tumour size: 0.26 cm^3) with the tumour volume on day 26 being only 0.1 cm^3 (after 6,9,12,16 days, p values= 0.028,0.01, 0.003, 0.028 respectively) [62]. The decrease in tumour size was similar with the clinically used gemcitabine, tumour size 0.125 cm^3 after the 26 days. The results strongly suggested that when BNIPDaoct was formulated with Ch_5 possessed a similar antitumoral effect to the clinically used gemcitabine, making it an ideal candidate for anticancer therapeutics [62].

Novel HPMA graft polymers conjugated with DOX have been reported to exhibit significantly increased blood circulation times of up to 96 h in mice bearing EL4T-cell lymphomal compared to their linear HPMA block copolymer counterparts [176]. A 6-fold and 50-fold increase in accumulation of drug in tumour sites was recorded

compared to the linear counterpart and free drug. Polymer–DOX conjugates also exhibited a significantly higher antitumoral effect *in vivo* than the conjugated or the free DOX (in mice with 38C13 B-cell or EL4 T-cell lymphoma). A significant number of long-term-surviving (LTS) mice with EL4 T-cell lymphoma treated were recorded after only one dosage of 15 mg DOX equiv./kg on day 10. These results suggest that the novel HPMA graft polymer-drug conjugates hold great potential as clinical chemotherapy agents [176].

7.0 Comb Shaped Amphiphilic Polymers for Oral Delivery

Similar to block copolymers, comb shaped amphiphilic polymers are mainly been studied for intravenous administration. However, recently a number of publications have emerged describing the use of comb shaped amphiphiles for oral administration of hydrophobic drugs such as griseofulvin, cyclosporine and camptothecin [25,92,177, 62,178] while the use of polymeric micelles based on block copolymers for oral drug delivery have been extensively reviewed in recent years [148,179]. Oral delivery using polymeric self-assemblies is more complicated than intravenous delivery due to the complexity of the physiological and biological barriers imposed by the gastrointestinal tract (GIT). For example, the pH in the GIT varies greatly from empty stomach (pH1.2) to the small intestine (5-7) and colon (6-7.5) [148]. In addition, the presence of proteolytic enzymes, bile salts, food in the GIT fluid might also affect the use of these nano-carriers for oral delivery. Drug absorption usually occurs in small intestine consisting of an epithelial layer of enterocytes covered with mucus, which constitutes a formidable physical barrier to drug absorption. In oral drug delivery, it is imperative that the therapeutic agent is not prematurely released in the upper GIT

before absorption occurs in small intestine. The polymeric self-assemblies must be stable and able to resist the dilution and the harsh environment in the GIT.

Winnik and colleagues were one of the first groups examined the potential use of comb shaped amphiphilic polymers (hydrophobically modified dextran and hydroxypropylcellulose) for oral delivery [37,46,112,177]. Cyclosporine A (CsA) was incorporated into the micelles formed by these hydrophobically modified polymers with relatively low loading capacity of 1.7-8.5%w/w [3.7,46,112]. They showed the ability of these polymeric micelles in resisting dilution in simulated gastric and intestinal fluids, demonstrating their potential for oral delivery [112]. Further work to improve the absorption of these polymeric micelles was achieved via attachment of vitamin B12 residues to dextran-g-polyethyleneoxide cetyl ether (DEX-g-PEO-C16). The CsA loaded VitB12 modified polymeric micelles led to a significantly higher transport across Caco-2 cell monolayer compared to CsA in unmodified micelles [177]. Despite the potential demonstrated in the *in vitro* assessment, *in vivo* experiments are needed to confirm the potential of these systems.

To date, there are limited *in vivo* studies on comb shaped amphiphilic polymers as oral delivery systems. Research led by Uchegbu and colleagues utilising cetylated polyethylenimine (PEI) has shown (Fig. 12) successful encapsulation of CsA with a 163-fold increase in aqueous solubility [8], which is significantly higher than hydrophobically modified polysaccharides designed by Winnik and colleagues [37] or block amphiphilic polymer methoxy poly(ethylene oxide)-b-poly(ε-caprolactone) (PEO-b-PCL) [180]. Uchegbu and colleagues showed in vivo efficacy of this system where the blood plasma CsA levels were shown to be comparable to the commercially available Neoral® formulation after oral administration to fasted rats

[8]. These findings indicated that the PEI amphiphiles have promising potential as oral delivery vehicles [8].

We have also recently demonstrated the promising potential of PAA modified with aromatic structures such as Fmoc and Dansyl groups as well as cholesteryl (Ch) pendant groups in enhancing oral bioavailability of a poorly soluble drug, griseofulvin [61]. Apart from exhibiting superior drug loading efficiency (>100%), we were able to demonstrate that both Dansyl and Ch formulations showed significantly higher plasma drug level compared to griseofulvin in water when administered via oral gavage to rats. Using similar dose as the clinical dose ($11.8 mgkg^{-1}$), Ch formulation had significantly higher drug plasma concentrations at all time points compared to Dansyl formulation and the maximum plasma drug concentration was achieved at 4h. To date, few *in vivo* studies using block copolymers for improving bioavailability of griseofulvin have been carried out. Pierri and colleagues attempted to use Poly(lactide)-poly(ethylene glycol) micelles as oral carriers for griseofulvin [181]. However, this work did not proceed to *in vivo* study due to the extremely poor drug loading capacity (4% w/w). Kano and colleagues reported the use of poly[2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate] (PMB) for enhancing the oral absorption of griseofulvin [182]. They found that PMB have similar C_{max}/Dose ratios with formulations such as niosomes, liposomes, self-emulsifying drug delivery systems and spray dried microparticles which range from 0.02 to 0.19 [182]. Interestingly our result showed a much higher C_{max}/Dose ratios of 1.44 (Ch_5) and 0.85 ($Dansyl_{10}$) [61]. Although direct comparison is not applicable, however the high plasma drug concentrations achieved in both PAA formulations and the differences observed between these formulations perhaps indicate there are other

contributing factors at play apart from solubilisation mechanism. More work needs to be done to understand the interaction of these self- assemblies with the gut enterocytes and subsequent absorption, the influence of bile salt, food, stomach acidity and other physiological factors when using these novel solubilisers for oral delivery.

8.0 Other applications for comb shaped amphiphilic polymers

Comb shaped amphiphilic polymers have also been developed as protein complexation agents, with the potential to facilitate oral delivery of proteins such as insulin whilst protecting them from enzymatic degradation throughout their journey [70]. One of the pioneering groups which utilises hydrophobically modified polyelectrolytes for oral protein delivery is Cheng and colleagues. They synthesised a range of poly(allylamine) amphiphiles with cetyl (Ce), cholesterol (Ch), and quaternary ammonium moieties (Q) grafted onto the backbone [70,76,92]. They systematically studied the effect of polymer architecture, mainly the type of hydrophobic pendant groups on complexation efficiency and degradation inhibition for oral insulin delivery. PAA amphiphiles were capable of forming a complex with insulin *via* hydrophobic and electrostatic interactions and the complexation efficiency is dependant on the type of hydrophobic pendant groups or the presence of quaternary ammonium moieties [70, 92]. Equally, polymer architecture also played a key role in the protection against enzymatic degradation *in vitro*. The level of protection against insulin degradation in the presence of proteases (tyrpsin, α-chymotrypsin and pepsin) is dependant on the type of enzymes, the presence of quaternary ammonium moieties and the type of the hydrophobic pendant groups. They concluded that in order to obtain effective protection from all these enzymes, it may be necessary to combine a

number of the PAA amphiphiles as the cholesterol, cetyl, palmitoyl and quaternary ammonium groups each possessed their own unique enzymatic protection profile [70].

Subsequent work by Cheng and colleagues looked at the complexation of quaternised palmitoyl poly(allylamine) (QPa) with salmon calcitonin (sCT) [33]. Calcitonin is a 32-residue calcium regulating peptide hormone produced by parafollicular cells in the thyroid, which has been used clinically as an adjunct treatment for osteoporosis and also as a second line treatment for Paget disease. Complexation generally takes place between oppositely charged polyelectrolyte and protein. However, in this work, Cheng and colleagues demonstrated that complexation can also occur between hydrophobically modified positively charged PAA with positively charged sCT at physiological pH [33]. When compared to free sCT, the complexes showed increased resistance to peptidases and serum and liver homogenates. Additionally, sCT complexed with QPa showed significant stability when stored at room temperature compared to free sCT. After IV administration on fasted Wistar rats (40 μgmL^{-1}), the complexes showed reduced serum concentrations after 120 minutes, demonstrating the bioactivity of complexed sCT was retained. Free and complexed sCT but not QPa also reduced serum calcium over 240 min following intra-jejunal administration. Cheng concluded that these nanocomplexes are stable, bioactive and resistant to a range of peptidases [33]. These enhanced features suggest that they may have the potential for improved efficacy when formulated for injected and oral delivery.

9.0 Future Prospects of comb shaped amphiphilic polymers

To date there has been no commercial success for comb shaped amphiphilic polymers while there are six polymeric micelles based on amphiphilic block copolymers in

various phases of clinical trials for chemotherapy [183]. However, with the recent plethora of literature on the use of comb shaped amphiphilic polymers for hydrophobic drug and protein delivery suggests that it is only a matter of time for comb shaped polymers to follow in the success of amphiphilic block copolymers amphiphiles. To date, numerous patents have been filed through both the intellectual property organization and the worldwide intellectual property organization for the use of a multitude of graft or comb shaped polymers for drug, gene and protein delivery vehicles [184,185]. However, boundaries such as the cross-over from synthetic organic chemistry to molecular cell biology and pharmacokinetic analysis coupled with industrial insight still to be fully exploited before the use of comb shaped amphiphilic polymers in clinics can be realised. Bearing this in mind, as we move forward, increased knowledge, understanding and expertise expand and so too does potential for success.

Executive Summary

- Polymer architecture such as type and level of hydrophobic pendant groups, the molecular weight and the type of polymer backbone, the presence of hydrophilic moieties have direct impact on physicochemical properties of self-assemblies.
- Drug loading is affected by a number of factors including the type and level of hydrophobic pendant groups, the structural compatibility of the hydrophobic pendant with the encapsulated drug and physicochemical properties of drug.
- Comb shaped amphiphilic polymers have been reported to solubilise a range of hydrophobic drugs such as doxorubicin, paclitaxcel, camptothecin and

griseofulvin and stimuli response comb shaped polymers are now being explored.

- Oral delivery using hydrophobically modified polymers show promising potential for hydrophobic drug and proteins and peptides.

Competing Interests

The authors declare that they have no competing interests.

References

1.Bader H, Ringsdorf H, Schmidt B. Water soluble polymers in medicine. *Angew. Chem. Int. Ed. Engl.* 123/124, 457-85 (1984)

2.Kwon G, Okano T. Polymeric micelles as new drug carriers. *Adv. Drug Deliver. Rev.* 21, 107-116 (1996).

3.Jagur-Grodzinski J. Biomedical application of functional polymers. *React. Funct. Polym*. 39, 99-138 (1999).

4.Letchford K, Burt H. A Review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur. J. Pharm. Biopharm*. 65, 259-269 (2007).

5.Branco MC, Schneider JP. Self-assembling materials for therapeutic delivery. *Acta. Biomaterialia*. 5, 817-813 (2009).

6.Nishiyama N, Kataoka K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol. Therapeut.* 112, 630-648 (2006).

7.Darling S B. Directing the self-assembly of block copolymers. *Prog. Polym. Sci*. 32, 1152-1204 (2007).

8.Cheng W-P, Gray AI, Tetley L *et al*. Polyelectrolyte Nanoparticles with High Drug Loading Enhance the Oral Uptake of Hydrophobic Compounds. *Biomacromolecules.* 7, 1509-1520 (2006).* * Demonstrates the potential of hydrophobically modified PEI as oral hydrophobic drug solubiliser *in vivo*.

9.Lapienis G. Star-shaped polymers having PEO arms. *Prog. Polym. Sci*. 34, 852-892 (2009).

10.Biricova V, Laznickova A: Dendrimers: Analytical characterization and applications. *Bioorg. Chem*. 37, 185-192 (2009).

11.Kwon G, Kataoka K. Block Copolymer micelles as long-circulating drug vehicles. *Adv. Drug Deliver. Rev.* 16, 295-309 (1995).

12.Yu BG, Okano T, Kataoka K, Kwon G. Polymeric micelles for drug delivery: solubilisation and haemolytic activity of amphotericin B. *J. Control. Release*. 53, 131-136 (1998).

13.Riess G. Micellization of block copolymers. *Prog. Polym. Sci*. 28, 1107-1170 (2003).

14.Nakashima K, Bahadur P. Aggregation of water-soluble block copolymers in aqueous solutions: Recent trends. *Adv. Colloid Interfac*. 123-126, 75-96 (2006).

15.Qui L, Zheng C, Jin Y, Zhu K. Polymeric micelles as nanocarriers for drug delivery. *Expert Opin. Ther. Pat*. 17, 819-30 (2007).

16.Chui H-C, Chern C-S, Lee C-K, Chang H-F. Synthesis and characterization of amphiphilic poly(ethylene glycol) graft copolymers and their potential application as drug carriers. *Polymer*. 39, 1609-1616 (1998).

17. Thompson C, Ding C, Qu X *et al*. The effect of polymer architecture on the nano self-assemblies based on novel comb-shaped amphiphilic poly(allylamine). *Colloid Polym. Sci*. 286, 1511-1526 (2008). * Demonstrates types of hydrophobic pendant groups have an impact on the formation of self-assemblies for hydrophobically modified polymer.
18. Bhattacharya A, Misra BN. Grafting: a versatile means to modify polymers Techniques, factors and applications. *Prog. Polym. Sci*. 29, 767-814 (2004).
19. Li G, Zhuang Y, Mu Q, Wang M, Fang Y. Preparation, characterization and aggregation behaviour of amphiphilic chitosan derivative having poly (L-lactic acid) side chains. *Carbohyd. Polym*. 72, 60-66 (2008).
20. Higa M, Yaguchi K, Kitani R. All solid-state polymer electrolytes prepared from a graft copolymer consisting of a polyimide main chain and poly(ethylene oxide) based side chains. *Electrochim. Acta*. 55, 1380-1384 (2010).
21. Hoskins C, Kong Thoo Lin P, Teltey L, Cheng W-P. Novel fluorescent amphiphilic poly(allylamine) and their supramacromolecular self-assemblies in aqueous media. *Polym. Adv. Technol*. 20 (2011).
22. Uchegbu IF, Sadiq L, Arastoo M *et al*. Quaternary ammonium palmitoyl glycol chitosan – a new polysoap for drug delivery. *Int. J. Pharm*. 224, 185-199 (2001).
23. Zhu S, Qian F, Zhang Y, Tang C, Yin C. Synthesis and characterization of PEG modified dimethylaminomethacrylate chitosan nanoparticles. *Eur. Polym. J.: Macromolecular Nanotech*. 43, 2244-2253 (2007).
24. Uchegbu IF. Pharmaceutical nanotechnology: polymer vesicles for drug and gene delivery. *Expert. Opin. Drug Deliver*. 3, 629-640 (2006).

25. Kedar U, Phutane P, Shidhaye S, Kadam V, Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine: Nanotechnology, Biology and Medicine*. 6, 714-729 (2010).

26. Ma Y, Cao T, Webber SE. Polymer micelles from poly(acrylic acid)-graft-polystyrene. *Macromolecules*. 31, 1773-1778 (1998).

27. Qu X, Omar L, Le TBH, Tetley L, Bolton K, Chooi KW, *et al*. Polymeric amphiphiles branching leads to rare nanodisc shaped planar self-assemblies. *Langmuir*. 24, 9997-10004 (2008). *Demonstrates branching of polymer backbone affect the morphology of self-assemblies.

28. Wang W, Tetley L, Uchegbu IF. The Level of Hydrophobic Substitution and the Molecular Weight of Amphiphilic Poly-L-lysine-Based Polymers Strongly Affects Their Assembly into Polymeric Bilayer Vesicles. *J. Colloid Interf. Sci*. 237, 200-207 (2001). *Demonstrates addition of cholesterol induces the formation of polymeric vesicles.

29. Cai G, Jiang H, Chen Z *et al*. Synthesis, characterization and self-assemble behaviour of chitosan-O-poly(ε-caprolactone). *Eur. Polym. J*. 45, 1674-1680 (2009).

30. Qui LY, Yan MQ. Constructing doxorubicin-loaded polymeric micelles through amphiphilic graft polyphosphazenes containing ethyl tryptophan and PEG segments. *Acta. Biomaterialia*. 5, 2132-2141 (2009).

31. Li H, Liu J, Ding S, Zhang C, Shen W, You Q. Synthesis of novel pH-sensitive chitosan graft copolymers and micellar solubilisation of paclitaxel. *Int. J. Bio. Macromolecules.* 44, 249-256 (2009).

32.Liu X-Q, Du J-Z, Zhang C-P *et al*. Brush-shaped polycation with poly(ehtylenimine)-b-poly(ethylene glycol) side chains as highly efficient gene delivery vector. *Int. J. Pharm*. 392, 118-126 (2010).

33.Cheng W-P, Thompson CJ, Ryan SM *et al*. In vitro and in vivo characterisation of a novel peptide delivery system: Amphiphilic polyelectrolyte-salmon calcitonin nanocomplexes. *J. Control. Release*. 147, 289-297 (2010). **Demonstrates the potential use of hydrophobically modified polymers for oral and intravenous delivery of protein/peptides.

34.Jeong JH, Kim SW, Park TG. Molecular design of functional polymers for gene therapy. *Prog. Polym. Sci*. 32, 1239-1274 (2007).

35.Uchegbu IF, Schältzen AG, Tetley L. Polymeric chitosan based vesicles for drug delivery. *J. Pharmaceut. Pharmacol*. 50, 453-458 (1998).

36.Wang L-Q, Tu K, Li Y *et al*. Synthesis and characterisation of temperature responsive graft copolymers of dextran with poly(N-isopropylacrylamide). *React. Funct. Polym*. 53, 19-27 (2002).

37.Francis MF, Piredda M, Winnik FM. Solubilization of poorly water soluble drugs in micelles of hydrophobically modified hydroxylpropylcellulose copolymers. *J. Control. Release*. 18, 59-68 (2003).

38.Prabaharan M, Reis RL, Mano JF. Carboxymethyl chitosan-graft-phosphatidylethanolamine: Amphiphilic matrices for controlled drug delivery. *React. Funct. Polym*. 67, 43-52 (2007).

39.Yu H, Wang W, Chen X, Deng C, Jing X. Synthesis and characterisation of the biodegradable polycaprolactone-graft-chitosan amphiphilic copolymers. *Biopolymers*. 83, 233-242 (2006).

40. Wang C, Li G, Tao S, Guo S, Yan Z. Crystalline and micellar properties of amphiphilic biodegradable chitooligosaccharide-graft-poly(ε-caprolactone) copolymers. *Carbohyd. Polym*. 64, 466-472 (2004).

41. Wu Y, Liu C, Zhao X, Xiang J. A new biodegradable polymer: PEGylated chitosan-g-PEI possessing a hydroxyl group at the PEG end. *J. Polym. Res*. 15, 181-185 (2008).

42. Cai G, Jiang H. pH-sensitive nanoparticles self-assembled from a novel class of biodegradable amphiphilic copolymers based on chitosan. *J. Mater. Sci.* 20, 1315-1320 (2009).

43. Abdurrahmangolu S, Firat Y. Synthesis and characterization of new dextran-acrylamide gels. *J. App. Polym. Sci.* 106, 3565-3570 (2007).

44. Bajgai MP, Parajuli DC, Ko JA, Kang HK, Khil M-S, Kim HY. Synthesis, characterisation and aqueous dispersion of dextran-g-poly(1,4-dioxan-2-one) copolymers. *Carbohyd. Polym*. 78, 833-840 (2009).

45. Krishnamoorthi S, Mal D, Singh RP. Characterization of graft copolymer based on polyacrylamide and dextran. *Carbohyd. Polym*. 69, 371-377 (2007).

46. Francis MF, Lavoie L, Winnik FM, Lerouxa J-C. Solubilization of cyclosporin A in dextran-g-polyethyleneglycolalkylether polymeric micelles. *Eur. J. Pharm. Biopharm*. 56, 337–346 (2003).

47. Shi R, Burt HM. Amphiphilic dextran-graft-poly(ε-caprolactone) films for the controlled release of paclitaxel. *Int. J. Pharm*. 271, 167-179 (2004).

48. Onishi Y, Eshita Y, Murashita A, Mizuno M, Yoshida J. Characteristics of DEAE-dextran-MMA graft copolymer as a nonviral gene carrier. *Nanomedicine: Nanotechnology, Biology and Medicine.* 3, 184-191 (2007).

49.Patrizi ML, Piatanida G, Coluzza C, Masci G. ATRP synthesis and association properties of temperature responsive dextran copolymers grafted with poly(N-isopropylacrylamide). *Eur. Polym. J*. 45, 2779-2787 (2009).

50.Tan S, Zhao D, Yuan D *et al*. Influence of indomethacin-loading on the micellation and drug release of thermosensitive dextran-graft-poly(N-iospropylacrylamide). *React. Funct. Polym*. 71, 820-827 (2011).

51.Browlie A, Uchegbu IF, Schätzein AG. PEI-based vesicle-polymer hybrid gene delivery system with improved biocompatibility. *Int. J. Pharm*. 274, 41-52 (2004).

52.Wang D-A, Narang AS, Kotb M, Gaber O, Miller DD, Kim SW *et al*. Novel branched poly(ehtylenimine)-cholesterol water-soluble lipopolymers for gene delivery. *Biomacromolecules*. 3, 1197-1207 (2002).

53.Boussif O, Delair T, Brua C, Veron L, Pavirani A, Kolbe HV. Synthesis of polyallylamine derivatives and their use as gene transfer vectors in vivo. *Bioconjugate Chem*. 10, 877-883 (1999).

54.Wang W, McConaghy AM, Tetley L, Uchegbu IF. Controls on polymer molecular weight may be used to control the size of palmitoyl glycol chitosan polymeric vesicles. *Langmuir*. 17, 631-636 (2001).

55.Hong JW, Park JH Huh KM *et al*. PEGylated polyethylenimine for in vivo local gene delivery based on lipiodolized emulsion system. *J. Control. Release*. 99, 167-176 (2004).

56.Nimesh S, Chandra R. Polyethylenimine nanoparticles as an efficient in vitro siRNA delivery system. *Eur. J. Pharm. Biopharm*. 73, 43-49 (2009).

57.Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B *et al*. A versatile vector for gene and oligonucleotide transfer into cells in

culture and in vivo: Polyethylenimine. *Proc. Natl. Acad. Sci. USA*. 92, 7297-7301 (1995).

58. Brownlie A, Uchegbu IF, Schätzein AG. PEI-based vesicle-polymer hybrid gene delivery system with improved biocompatibility. *Int. J. Pharm*. 274, 41-52 (2004).

59. Aravindan L, Bicknell KA, Brooks G, Khutoryanskiy VV, Williams AC. Effect of acyl chain length on transfection efficiency and toxicity of polyethylenime, *Int. J. Pharm*. 378, 201-210 (2009).

60. Qiu LY, Bae YH. Self-assembled polyethylenimine-graft-poly(ε-caprolactone) micelles as potential dual carriers of genes and anticancer drugs. *Biomaterials*. 28, 4132-4142 (2007).

61. Hoskins C, Kong Thoo Lin P, Qu X, Tetley J, Cheng WP. The Use of Nano Polymeric Self-Assemblies Based on Novel Amphiphilic Graft Polymers for Oral Hydrophobic Drug Delivery *Pharm. Res.* (2011). *Article in press.* DOI: 10.1007/s11095-011-0602-7. **First to demonstrate the in vivo performance of water soluble polymer modified with aromatic structures as oral hydrophobic drug solubilisers.

62. Hoskins C, Ouaissi M, Lima S *et al*. In vitro and In vivo anticancer activity of a novel nano-sized formulation based on self-assembling polymers against pancreatic cancer. *Pharmaceut Res*. 27, 2694-2703 (2010).

63. Nimesh S, Kumar R, Chandra.R. Novel polyallylamine-dextran sulfate-DNA nanoplexes: Highly efficient non-viral vector for gene delivery. *Int. J. Pharm.* 320, 143-149 (2006).

64. Malmstein M. Surfactants and Polymers in Drug Delivery. Basel NY. *Marcel Dekker* (2002).

65. Gao Y, Li H, Wang X. Synthesis and characterization of syndiotactic polystyrene-*graft*-poly(glycidyl methcrylate) copolymer by atom transfer radical polymerization. *Eur. Polym. J*. 43, 1258-1266 (2007).

66. Lai R, Guo H, Kamachi M. Synthesis of a graft polymer PVAc-g-[P(AN-r-BE)-b-PCHO] in "one step" by radical/cationic transformation polymerization and coupling reaction. *Polymer*. 50, 3582-3586 (2009).

67. Perry M, Cheng W-P. New Polyamine Based Amphiphilic polymers for Drug Delivery. *Proceedings of the23rd Annual Meeting & Exposition of the controlled Release Society in Vienna*. 22-27 July (2006).

68. Liu X-M, Pramoda KP, Yang Y-Y, Chow SY, He C. Cholesteryl-grafted functional amphiphilic poly(N-isopropylacrylamide-co-N hydroxyl methylacrylamide): synthesis, temperature sensitivity, self-assembly and encapsulation of a hydrophobic agent. *Biomaterials*. 25, 2619-2628 (2004).

69. Wang W, Qu X, Gray AI, Tetley L, Uchegbu IF. Self-Assembly of Cetyl Linear Polyethlenimine To Give Micelles, Vesicles and Dense Nanoparticles. *Macromolecules*. 37, 9114-9122 (2004).

70. Thompson C, Tetley L, Cheng W-P. The influence of polymer architecture on the protective effect of novel comb shaped amphiphilic poly(allylamine) against in vitro enzymatic degradation of insulin-Towards oral insulin delivery. *Int. J. Pharm*. 383, 216-227 (2010).

71. Xu J-P, Chen W-D, Shen J-C. Novel biomimetic polymersomes as polymer therapeutics for drug delivery. *J. Control. Release*. 107, 502-512 (2005).

72. Yusa S, Mikiharu K, Morishima Y. Hydrophobic Self-Association of Cholesterol Moities Covalentyl linked to Polyelectrolytes: Effect of Spacer Bond. *Langmuir*. 14, 6059-6067 (1998).

73.Limer AJ, Rullay AK, San Miguel V *et al*. Fluorescently tagged star polymers by living radical polymerisation for mucoadhesion and bioadhesion. *React. Funct. Polym*. 66, 51-64 (2006).

74.Kohle P, Khandare J, Pillai O *et al*. Preparation, cellular transport, and activity of polyamidoamine-based dendritic nanodevices with a high drug payload. *Biomaterials*. 27, 660-669 (2006).

75.Savíc R, Eisenberg A, Maysinger D. Block copolymer micelles as delivery vehicles of hydrophobic drugs: Micelle–cell interactions. *J. Drug. Target*. 14, 343 – 355 (2006).

76.Thompson C, Cheng W-P, Gadad P *et al*. Uptake and Transport of novel amphiphilic polyelectrolyte-insulin nanocomplexes by Caco-2 cells – Towards oral insulin. *Pharm. Res*. (2011). 28, 886–896

77.Ziang HL, Zhu KJ. Bioadhesive fluorescent microspheres as visible carriers for local delivery of drugs. I: preparation and characterization of insulin-loaded PCEFB/PLGA microspheres. *J. Microencapsulation*. 19, 451-461 (2002).

78.Laukkanen A, Winnik FM, Tenhu H, Pyrene-Labelled Graft Copolymers of N-Vinylcaprolactam: Synthesis and Solution Properties in Water. *Macromolecules*. 38, .2439-2448 (2005).

79.Fan H, Huang J, Li Y, Yu J, Chen J. Fabrication OF Reduction-degradable micelle based on disulfide-linked graft copolymer-camptothecin conjugate for enhancing solubility and stability of camptothecin. *Polymer*. 51, 5107-5114 (2010).

80.Khandare J, Minko T. Polymer-drug conjugates: Progress in polymeric prodrugs. *Prog. Polym. Sci*. 31, 359-397 (2006).

81. Hoffman AS, Stayton PS. Conjugates of stimuli-responsive polymers and proteins. *Prog. Polym. Sci*. 32, 922-939 (2007).

82. Chytil P, Etrych T, Kříž J, Šubr V, Ulrich K. N-(2-Hydroxyproply)methacrylamide-based polymer conjugates with pH-controlled activation of doxorubicin for cell-specific or passive tumour targeting. Synthesis by RAFT polymerisation and physicochemical characterisation. *Eur. J. Pharm*. 41, 473-482 (2010).

83. Maiti S, Jayachandran KN, Chatterji PR. Probing the association behaviour of poly(ethylene glycol) based amphiphilic comb-like polymer. *Polymer*. 42, 7801-7808 (2001).

84. Ryan SM, Wang X, Mantovani G *et al*. Conjugation of salmon calcitonin to a combed-shaped end functionalized poly (poly(ethylene glycol) methyl ether methacrylate) yields a bioactive stable conjugate. *J. Control. Release*. 135, 51–59 (2009).

85. Xie K-L, Hu K, Chen Y. Synthesis of amphiphilic polysiloxanes modified with multi-cationic groups to improve wettability of polyester materials. *Iran. Polym. J*. 19, 447-455 (2010).

86. Xu FJ, Ping Y, Ma J *et al*. Comb-shaped copolymers composed of hydroxypropyl cellulose backbones and cationic poly((2-dimethyl amino)ethyl methacrylate) side chains for gene delivery. *Bionconjugate Chem*. 20, 1449-1458 (2009).

87. Motornov M, Roiter Y, Tokarev I, Minko S. Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. *Prog. Polym. Sci*. 35, 174-211 (2010).

88.Chan P, Kurisawa M, Chung JE, Yang YY. Synthesis and characterization of chitosan-g-poly(ethylene glycol)-folate as a novel carrier for tumor-targeted gene delivery. *Biomaterials*. 28, 540-549 (2007).

89.Brown MD, Schätzlein A, Brownlie A *et al*. Preliminary characterization of novel amino acid based polymeric vesicles as gene and drug delivery agents. *Bionconjugate Chem*. 11, 880-891 (2000).

90.Parrish B, Breitenkamp RB, Emrick T. PEG- and peptide- grafted aliphatic polyesters by click chemistry. *JACS*. 127, 7404-7410 (2005).

91.Zhu L-P, Du C-H, Xu L et al. Amphiphilic PPESK-g-PEG graft copolymers for hydrophilic modification of PPESK microporous membranes. *Eur. Polym. J*. 43, 1383-1393 (2007).

92.Thompson CJ, Tetley L, Uchegbu IF, Cheng W-P. The complexation between novel comb-shaped amphiphilic polyallylamine and insulin- Towards insulin delivery. *Int. J. Pharm*. 376, 46-55 (2009).

93.Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. *Adv. Drug. Deliver. Rev*. 58, 1655-1670 (2005).

94.Jeong B, Gutowska A. Lessons from nature: stimuli responsive polymers and their biomedical applications. *Trends Biotechnol*. 7, 305-377 (2002).

95.Zhang L, Hu CH, Cheng SX, Zhuo RX. Hyperbranched amphiphilic polymer with folate mediated targeting property. *Colloids Surf. B Biointerfaces*. 79, 427-33 (2010).

96.Gu J, Cheng W-P, Liu J *et al.* pH-Triggered reversible 'stealth' polycationic micelles. *Biomacromolecules*. 9, 255-262 (2008).

97. Shi H-Y, Zhang L-M. Phase-transititon and aggregation characteristics of a thermoresponsive dextran derivative in aqueous solution. *Carbohyd. Res*. 341, 2414-2419 (2006).

98. Benns JM, Mahato RI, Kim SW. Optimization of factors influencing the transfection efficiency of folate-PEG-folate-graft polyethylenimine. *J. Control. Release*. 79, 255-269 (2002).

99. Ghiamazemi S, Amanzadeh A, Dinarvand R, Rafiee-Tehrant M, Amini M. Synthesis and characterisation and evaluation of cellular effects of FOL-PEG-g-PEI-GAL nanoparticles as a potential non-viral vector for gene delivery. *J Nanomater*. 63136, 1-10 (2010).

100. Zhu J, Gosen C, Marchant RE. Synthesis and Characterization of Poly(vinyl amine)-Based Amphiphilic Comb-Like Dextran Glycopolymers by a Two-Step Method. *J. Polym. Sci. A: Polym. Chem.* 44, 192-199 (2005).

101. Lin W-J, Chen MH. Synthesis of multifunctional chitosan with galactose as a targeting ligand for glycoprotein receptor. *Carbohyd. Polym.* 67, 474-480 (2007).

102. Iwata R, Satoh R, Iwasaki Y, Akiyoshi K. Covalent immobilization of antibody fragments on well defined polymer brushes via site-directed method. *Colloids. Surf. B: Biointerfaces*. 62 288-298 (2008).

103. Morris VB, Sharma CP. Folate mediated L-arginine modified oligo(alkylaminosiloxane) graft poly(ethyleneimine) for tumor targeted gene delivery. *Biomaterials*. (2011). *Article in press*

104. Wang Y, Wang Y, Xiang J, Yao K. Target-specific cellular uptake of Taxol-loaded heparin-PEG-folate nanoparticles. *Biomacromolecules*. 11, 3531-3538 (2010).

105. Jiang GB, Quan D, Liao K, Wang H. Novel polymer micelles prepared from chitosan grafted hydrophobic palmitoyl groups for drug delivery. *Mol Pharm*. 3, 152-160 (2006).

106. Zhao D, Sun D-X, Guo Y. Preparation of cetyl-chitosan nanoparticles as carriers for paracetamol. *Transactions of Tianjin University*. 8, 234-238 (2002).

107. Huang Y, Li L, Fang Y. Preparation of size-tunable, highly monodisperse particles by self-assembly of N-phthaloylchitosan-g-polycaprolactone molecular bottle brushes. *Mater. Lett*. 63, 1416-1418 (2009).

108. Du Y-Z, Wang L, Yuan H, Wei X-H, Hu F-Q. Preparation and characteristics of lineoleic acid-grafted chitosan oligosaccharide micelles as a carrier for doxorubicin. *Colloids. Surfac.e B*. 69, 257-263 (2009).

109. Qu X, Khutoryanskiy VV, Stewart A *et al*. Carbohydrate-based micelle clusters which enhance hydrophobic drug bioavailability by up to 1 order of magnitude. *Biomacromolecules*. 7, 3453-3459 (2006). **Demonstrates cluster formation of polymeric micelles based on hydrophobically modified chitosan and exhibits promising potential in vivo for brain and ocular delivery

110. Opanasopit P. Ngawhirunpat T, Rojanarata T, Choochottiros C, Chirachanchai S. N-Phthaloylchitosan-g-mPEG design for all-trans retinoic acid-loaded polymeric micelles. *Eur. J. Pharm. Sci*. 30, 424-431 (2007).

111. Opanasopit P, Ngawhirunpat T, Chaidedgumjorn A *et al*. Incorporation of camptothecin into N-phthaloyl chitosan-g-mPEG self-assembly micellar system. *Eur. J. Pharm. Biopharm*. 64, 269-276 (2006).

112. Francis MF, Cristea M, Yang Y, Winnik FM. Engineering polysaccharide-based polymeric micelles to enhance permeability of cyclosporine A across

Caco-2 cells. *Pharm. Res*. 22, 209-219 (2005). **In vitro* studies demonstrates the stability of polymeric micelles formed by hydrophobically modified polymers in simulated GIT fluids.

113. Yudovin-Farber I. Yanay C, Azzam T, Linial M, Domb AJ. Quaternary ammonium polysaccharides for gene delivery. *Bioconjugate Chem*. 16, 1196-1203 (2005).

114. Akiyoshi K, Kobayashi S, Shichibe S, Mix DM, Baudys M, Kim SW *et al*. Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. *J. Control. Release*. 54,313-320 (1998).

115. Jung S-W, Jeong Y-I, Kim S-H. Characterization of hydrophobized pullulan with various hydrophobicities. *Int. J. Pharm*. 254, 109-121 (2003).

116. Scomparin A, Salmaso S, Bersani S, Satchi-Fainaro R, Caliceti P. Novel folate and non-folated pullulan bioconjugates for anticancer drug delivery. *Eur. J. Pharm. Sci*. 42, 547-558 (2011).

117. Zhang L, Hu C-H, Cheng S-X, Zhou R-X. PEI grafted hyperbranched polymers with polyglycerol as a core for gene delivery. *Colloid. Surface. B*. 76, 427-433 (2010).

118. Wang W, Tetley L, Uchegbu IF. A new class of amphiphilic poly-Llysine based polymers forms nanoparticles on probe sonication in aqueous media. *Langmuir*. 16, 7859-7866 (2000).

119. Benns JM, Choi J-S, Mahato RI, Park J-S, Kim SW. PH sensitive cationic polymer gene delivery vechile: N-Ac-poly(L-histine)-graft-poly(L-lysine) comb shaped polymer. *Bioconjugate Chem*. 11, 637-645 (2000).

120.Jeong JH, Kang HS, Yang SR, Kim J-D. Polymer micelle-like aggregates of novel amphiphilic biodegradable poly(aspargine) grafted with poly(caprolactone). *Polymer*. 44, 583-591 (2003).

121.Duan K, Zhang X, Tang X *et al*. Fabrication of cationic nanomicelle from chitosan-graft-polycaprolactone as the carrier of 7-ethyl-10-hydroxy-camptothecin. *Collolid. Surface. B*. 76, 475-482 (2010).

122.Nam YS, Kang HS, Park JY *et al*. New micelle-like polymer aggregates made from PEI–PLGA diblock copolymers: micellar characteristics and cellular uptake. *Biomaterials*. 24, 2053–2059 (2003).

123.Lee SC, Kim C, Kwon IC, Chung H, Jeong SY. Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(ε-caprolactone) copolymer as a carrier for paclitaxel. *J. Control. Release*. 89, 437-446 (2003).

124.Lavasanifar A, Samuel J, Kwon GS. The effect of alkyl core structure on micellar properties of poly(ethylene oxide)-block-poly(L-aspartamide) derrivatives. *Colloid. Surface. B*. 22, 115-126 (2001).

125.Li Y, Ding L-J, Nakamura H, Nakashima K. Preparation and characterisation of nanoaggregates of poly(ethylene oxide)-b-polymethacrylate and poly-Llysine. *J. Colloid. Interf. Sci.* 264, 561-564 (2003).

126.Essa S, Rabanel JM, Hildgen P. Effect of polyethylene glycol (PEG) chain organization on the physicochemical properties of poly(D,L-lactide) (PLA) based nanoparticles. *Eur. J. Pharm. Biopharm*. 75, 96-106 (2010).

127.Aliabadi HM, Elhasi AS, Mahmud A *et al*. Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: The effect of solvent composition on micellar properties and drug loading. *Int. J. Pharm*. 329, 158-165 (2007).

128. Lavasanifar A, Samuel J, Kwon GS. Micelles self-assembled from poly(ethylene oxide)-*block*-poly(*N*-hexyl stearate L-aspartamide) by a solvent evaporation method: effect on the solubilisation and haemolytic activity of amphotericin B. *J. Control. Release* 77, 155-160 (2001).

129. Gong J, Huo M, Zhuo J *et al*. Synthesis, characterization , drug-loading capacity and safety of novel octyl modified serum albumin micelles. *Int. J. Pharm*. 376, 161-168 (2009).

130. Birnbaum DT, Kosmala JD, Henthorn DB, Peppas LB *et al*. Controlled release of β-estradiol from PLGA microparticles: The effect of organic phase solvent on encapsulation and release. *J. Control. Release*. 65, 375-387 (2000).

131. Park JH, Lee S, Kim J-H *et al*. Polymeric nanomedicine for cancer therapy. *Prog. Polym. Sci*. 33, 113-137 (2008).

132. Husseini GA, Pitt WG. Micelles and nanoparticles for ultrasonic drug and gene delivery. *Adv. Drug. Deliver. Rev*. 60, 1137-1152 (2008).

133. Huang J, Wigent RJ, Schwartz JB. Drug-polymer interaction and its significance on the physical stability of nifedipine amorphous dispersion in microparticles of an ammonio methacrylate copolymer and ethylcellulose binary blend. *J. Pharm. Sci*. 97, 251-261 (2008).

134. Soo PL, Luo L, Maysinger D, Eisenberg A. Incorporation and release of hydrophobic probes in biocompatible polycaprolactone-block-poly(ethylene oxide) micelles: Implications for drug delivery. *Langmuir*. 18, 9996-10004 (2002).

135. Marsac PJ, Shamblin SL, Taylor LS. Estimation of drug-polymer miscibility and solubility in amorphous solid dispersions using experimentally determined interaction parameters. *Pharm. Res*. 26, 139-151 (2009).

136. Gaucher G, Dufresne M-H, Sant VP *et al*. Block copolymer micelles: preparation, characterization and application in drug delivery. *J. Control. Release*. 109, 169-188 (2005).

137. Rekatas CJ, Mai SM, Crothers M *et al*. The effect of hydrophobic chemical structure and chain length on the solubilization of griseofulvin in aqueous micellar solutions of block copoly(oxyalkylene)s. *Phys. Chem. Chemic. Physic*. 21, 4769-4773 (2001).

138. Vassiliou AA, Papadimitriou SA, Bikiaris DN, Mattheolabakis G, Avgoustakis K. Facile synthesis of polyester-PEG triblock copolymers and preparation of amphiphilic nanoparticles as drug carriers. *J. Control. Release*. 148, 388-395 (2010).

139. Kataoka K, Matsumoto T, Yokoyama M *et al*. Doxorubicin-loaded poly(ethylene glycol)-poly(â-benzyl-l-aspartate) copolymer micelles: their pharmaceutical characteristics and biological significance. *J. Control. Release*. 64, 143-153 (2000).

140. Lavasanifar A, Samuel J, Sattari S, Kwon GS. Block copolymer micelles for the encapsulation and delivery of amphotericin B. *Pharm. Res*. 19, 418-422 (2002).

141. Mahmud A, Patel S, Molavi O *et al*. Self-associating poly(ethylene oxide)-b-poly(r-cholesteryl carboxylate-å-caprolactone) block copolymer for the solubilization of STAT-3 inhibitor cucurbitacin I. *Biomacromolecules*. 10, 471-478 (2009).

142. Mahmuda A, Xiong X, Lavasanifar A. Development of novel polymeric micellar drug conjugates and nano-containers with hydrolyzable core structure for doxorubicin delivery. *Eur. J. Pharm. Biopharm*. 69, 923-934 (2008).

143. Gu J, Cheng W-P, Hoskins C *et al*. Nano self-assemblies based on cholate grafted poly-L-lysine enhanced the solubility of sterol-like drugs. *J. Microencapsulation*. (2011) *Article in press*

144. Liu JB, Xiao YH, Allen C. Polymer-drug compatibility: A guide to the development of delivery systems for the anticancer agent Ellipticine. *J. Pharm. Sci*. 93, 132–143 (2004).

145. Benniston AC, Harriman A, Howell SL, Sams CA, Zhi YG. Intramolecular excimer formation and delayed fluorescence in sterically constrained pyrene dimers. *Chem. - Eur. J*. 13, 4665-4674 (2007).

146. Pierri E, Avgoustakis K. Poly(lactide)-poly(ethylene glycol) micelles as a carrier for griseofulvin. *J. Biomed. Mater. Res A*. 75, 639-647 (2005).

147. Zhang Y, Li X, Zhou Y *et al*. Preparation and evaluation of poly(ethylene glycol)- poly(lactide) micelles as nanocarriers for oral delivery of cyclosporine A. *Nanoscale Res. Lett*. 5, 917-925 (2010).

148. Gaucher G, Satturwar P, Jones M-C, Furtos A, Leroux J-C. Polymeric micelles for oral drug delivery. *Eur. J. Pharm. Biopharm*. 76, 147-158 (2010). * excellent review on amphiphilic block copolymer for oral drug delivery

149. Hu F-Q, Liu L-N, Du Y-Z, Yuan H. Synthesis and antitumor activity of doxorubicin conjugated stearic acid-g-chitosan oligosaccharide polymeric micelles. *Biomaterials*. 30, 6955-6963 (2009).

150. Zheng C, Qiu L, Yao X, Zhu K. Novel micelles from graft polyphosphazenes as potential anti-cancer drug delivery systems: drug encapsulation and in vitro evaluation. *Int. J. Pharm*. 373, 133-140 (2009).

151. Talelli M, Rijcken CJF, Oliveira S et al. Nanobody-Shell functionalized thermosensitive core-crosslinked polymeric micelles for active drug targeting. *J. Control. Release*. (2011). *Article in press*

152. Sabokatin MR, Tabatabaee RM, Maharramov A, Ramazanov A. Deseign and characterization of chitosan nanoparticles as delivery systems for paclitaxel. *Carbohyd. Polym*. 82, 466-471 (2010).

153. Oh KT, Bronich TK, Bromberg L, Hatton TA, Kabanov AV. Block ionomer complexes as prospective nanocantainers for drug delivery. *J. Control. Release*. 115, 9-17 (2006).

154. Maeda H, Bharate GY, Daruwalla J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. *Eur. J. Pharm. Biopharm.* 71, 409-419 (2009).

155. Matsumura Y. Poly(amino acid) micelle nanocarriers in preclinical and clinical studies. *Adv. Drug. Deliver. Rev*. 60, 899-914 (2008).

156. Rapoport N. Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. *Prog. Polym. Sci*. 32, 962-990 (2007).

157. Torchilin V. Tumor delivery of macromolecular drugs based on the EPR effect. *Adv. Drug. Deliver. Rev*. 63, 131-135 (2011).

158. Wang J, Mongayt DA, Lukyanov AN, Levchenko TS, Torchilin VP. Preparation and in vitro synergistic anticancer effect of vitamin K3 and 1,8-diazabicyclo[5,4,0]undec-7-ene in poly(ethylene glycol)-diacyllipid micelles. *Int. J. Pharm*. 272, 129-165 (2004).

159. Na HS, Lim YK, Jeong Y-I *et al*. Combination antitumor effects of micelle-loaded anticancer drugs in a CT-26 murine colorectal carcinoma model. *Int. J. Pharm*. 383, 192-200 (2010).

160.Zhang W, Chi Y, Chen Y *et al*. Multifunctional Pluronic P123/F127 mixed polymeric micelles loaded with paclitaxel for the treatment of multidrug resistant tumors. *Biomaterials*. 32, 2894-2906 (2011).

161.Wang Y, Yang T, Wang X *et al*. Materializing sequential killing of tumor vasculature and tumor cells via targeted polymeric micelle system. *J. Control. Release*. 149, 299-306 (2011).

162.Westedt U, Kalinowski M, Wittmar M *et al*. Poly(vinyl alcohol)-graft-poly(lactide-co-glycolide) nanoparticles for local delivery of paclitaxel for restenosis treatment. *J. Control. Release*. 119, 41-51 (2007).

163.Torchilin VP. Targeted polymeric micelles for delivery of poorly soluble drugs. *Cell. Molr. Life Sci*. 61, 2549-2559 (2004).

164.Wang X, Yang L, Chen Z, Shin DM. Application of nanotechnology in cancer therapy and imaging. *A Cancer. J. Clinicians*. 58, 97-110 (2008).

165.Wiradharma N, Zhang Y, Venkataraman S, Hedrick JL, Yang YY. Self-assembled nanostructures for delivery of anticancer therapeutics, *Nano Today*. 4, 302-317 (2009).

166.Li J, Wang B, Liu P. Possibility of active targeting tumor by local hyperthermia with temperature sensitive nanoparticles, *Med. Hypotheses*. 71, 249-251 (2008).

167.Osada K, Christine RJ, Kataoka K. Polymeric micelles from poly(ethylene glycol)-poly(amino acid) block copolymer for drug and gene delivery. *J. R. Soc. Interfac*. 6, 325-339 (2009).

168.Chen J, Xing MMQ, Zhong W. Degradable micelles based on hydrolytically degradable amphiphilic graft copolymers for doxorubicin delivery. *Polymer*. 52,933-941 (2011).

169. Sun Y, Yan X, Yuan T *et al*. Disassemblable micelles based on reduction-degradable amphiphilic copolymers for intracellular delivery of doxorubicin. *Biomaterials*. 31, 7124-7131 (2010).

170. Yang SR, Lee HJ, Kim J-D. Histidine-conjugated poly(amino acid) derivatives for the novel endosomolytic delivery carrier of doxorubicin. *J. Control. Release*. 114, 60-68 (2006).

171. Lo SL, Wang S. An endosomolytic Tat peptide produced by incorporation of histidine and cysteine residues as a nonviral vector for DNA transfection. *Biomaterials*. 29, 2408-2414 (2008).

172. Harish Prashanth KV, Tharanthan RN. Chitin/chitosan: modifications and their unlimited application potential-an overview. *Trends Food Sci. Tech*. 18, 117-131 (2007).

173. Zhao Y, Sun Y, Zang Z *et al*. Synthesis and characterization of graft copolymer of chitosan and poly ethylene glycol. *Molecular Biology Reports*. 38, 2455-2462 (2011).

174. Liu J, Li H, Jiang X, Zhang C, Ping Q. Novel pH-sensitive chitosan-derived micelles loaded with paclitaxel. *Carbohyd. Polym*. 82, 432-439 (2010).

175. Ebrahimnejad P, Rsssoul D, Sajadi A *et al*. Preparation and in vitro evaluation of actively targetable nanoparticles for SN-38 delivery against HT-29 cell lines. *Nanomedicine: Nanotechnology, Biology and Medicine*. 6, 478-485 (2010).

176. Etrych T, Chytil P, Mrkvan T. Conjugates of doxorubicin with graft HPMA copolymers for passive tumor targeting. *J. Control. Release*. 132, 184-192 (2008).

177.Francis MF, Cristea M, Winnik FM. Exploiting the vitamin B-12 pathway to enhance oral drug delivery via polymeric micelles. *Biomacromolecules.* 6, 2462–2467 (2005).* demonstrates addition of a ligand to hydrophobically modified polysaccharide can enhance the transport of drug loaded polymeric micelles across in vitro cell culture model

178.Aranaz I, Harris R, Heras A. Chitosan amphiphilic derivatives. Chemistry and Application. *Curr. Org. Chem*. 14, 308-330 (2010).

179.Bromberg, L. Polymeric micelles in oral chemotherapy. *J. Control. Release.* 128, 99-112 (2008).

180.Aliabadi HM, Mahmud A, Sharifabadi AD, Lavasanifar A. Micelles of methyoxy poly(ethylene)-b-poly(ε -caprolactone) as vechiles for the solubilisation and controlled delivery of cyclosporine A. *J Control. Release*. 104, 301-311 (2005).

181.Pierri E, Avgoustakis K. Poly(lactide)-poly(ethylene glycol) micelles as a carrier for griseofulvin. *J. Biomed. Mater. Res. A*. 75, 639-647 (2005).

182.Kano T, Kakinuma C, Wada S, Morimoto K, Ogihara T. Enhancement of drug solubility and absorption by copolymers of e-methacryloyloxyethyl phosphorylcholine and n-Butyl methacrylate. *Drg Metab. Pharmacokinet*. 26, 79-86 (2011).

183.Yokoyama, M. Polymeric micelles as a new drug carrier system and their required considerations for clinical trials. *Expert Opin.Drug Deliver*. 7, 145-158 (2010). **Excellent review on the connections between basic studies on polymeric micelles formed by block copolymers and clinical trials data.

184.www.iop.co.uk *accessed 22/02/11*

185.www.wipo.int *accessed 22/02/11*

Reference Annotations : * of interest; ** of considerable interest

List of Tables

List of Figures

Figure 10. Chemical structure of amphiphilic graft phosphazenes with ethyl tryptophan and PEG pendant groups [162].

Figure 11. Poly(amine) amphiphiles for DOX solubilisation. A) Poly(β-amino ester)-g-octadecyl acrylate (PAE-g-ODA) [174] and B) Polyamide amine-g-polyethylene glycol (PAA-g-PEG)[175].

Figure 12. Poly(ethylenimine) modified with cetyl chains and quaternary ammonium pendant groups for CsA solubilisation [5].

Table 1.

Polymer Backbone	Hydrophobic pendant groups	Hydrophilic moieties	Encapsulated drugs
Chitosan	**Palmitoyl [105,54]. cetyl [106], poly(caprolactone) [107], aminoacid[108]**	**Quaternary ammonium moieties [109] PEG [110]**	**All-trans retinoic acid [110], doxorubicin [108], camptothecin [111], prednisolone [109], propofol [109], Ketoprofen [38]**
Dextran	**cetyl [112], poly(caprolactone) [47]**	**Spermine [113], Quaternary ammonium [113],**	**Paclitaxel [47], Cyclosporin A [112],**
Pullulan	**Cholesterol [114], Acetyl [114]**		**Clonazepam [115] Doxorubicin [116]**
Methyl cellulose	**Cetyl [37] Octadecyl [37]**		**Cyclosporin A [37]**
Poly(allylamine)	**Cetyl [17], palmitoyl [17], cholestryl [17,21], aromatic [21]**	**Quaternary ammonium [17]**	**Propofol [61] Prednisolone [61] Griseofulvin [61] Gemcitabine [62]**
Poly(ethylenimine)	**Cetyl [8,69], Palmitoyl [58], Cholesterol [52], Poly(caprolactone) [60], Polyglycerol [117]**	**Quaternary ammonium [58], PEG [58]**	**Cyclosporin A[8], doxorubicin [60],**
Poly(L-lysine)	**Palmitoyl [28,118] Poly(L-Histine) [119]**	**PEG [28,118]**	

Table 2.

Amphiphilic polymer	Polymer Architecture	CAC value $mgmL^{-1}$
Poly(ethylenimine)-g-cetyl$_{1.8}$ ($C_{1.8}$) [8]	**Comb shaped**	**0.01000**
Poly(allylamine)-g-cholesterol$_5$ (Ch_5) [17]	**Comb shaped**	**0.02000**
Poly(aspargine)-g-poly (capro-lactone)$_6$ (PAsn-g-PCL6) [120]	**Comb Shaped**	**0.00250**
Chitosan-g-poly(caprolactone)$_{24}$ (CS-g-PCL_{24}) [121]	**Comb Shaped**	**0.00890**
Poly(D-L-lactide-co-glycolide) (PEI-PLGA) [122]	**Block**	**0.00154**
Poly(2-ethyl-2-oxazoline)-block-poly(ε-caprolactone) (PEtoz-PCLs) [123]	**Block**	**0.0018**
Poly(ethylene oxide)-block-poly (hydroxyethyl L-aspartamide) (PEO-b-$PHAA_{22}$) [124]	**Block**	**0.0090**
Poly(vinylpyrrolidone)-b-poly(D,L-lactine)-b-poly(vinyl pyrrolidone) (PVP-b-PDLLA-b-PVP) [93]	**Triblock**	**0.00510**
Poly(ethylene oxide)-b-poly (metha crylate)-b-poly(L-lysine) PEO-b-PMA-PLS [125]	**Triblock**	**0.01000**

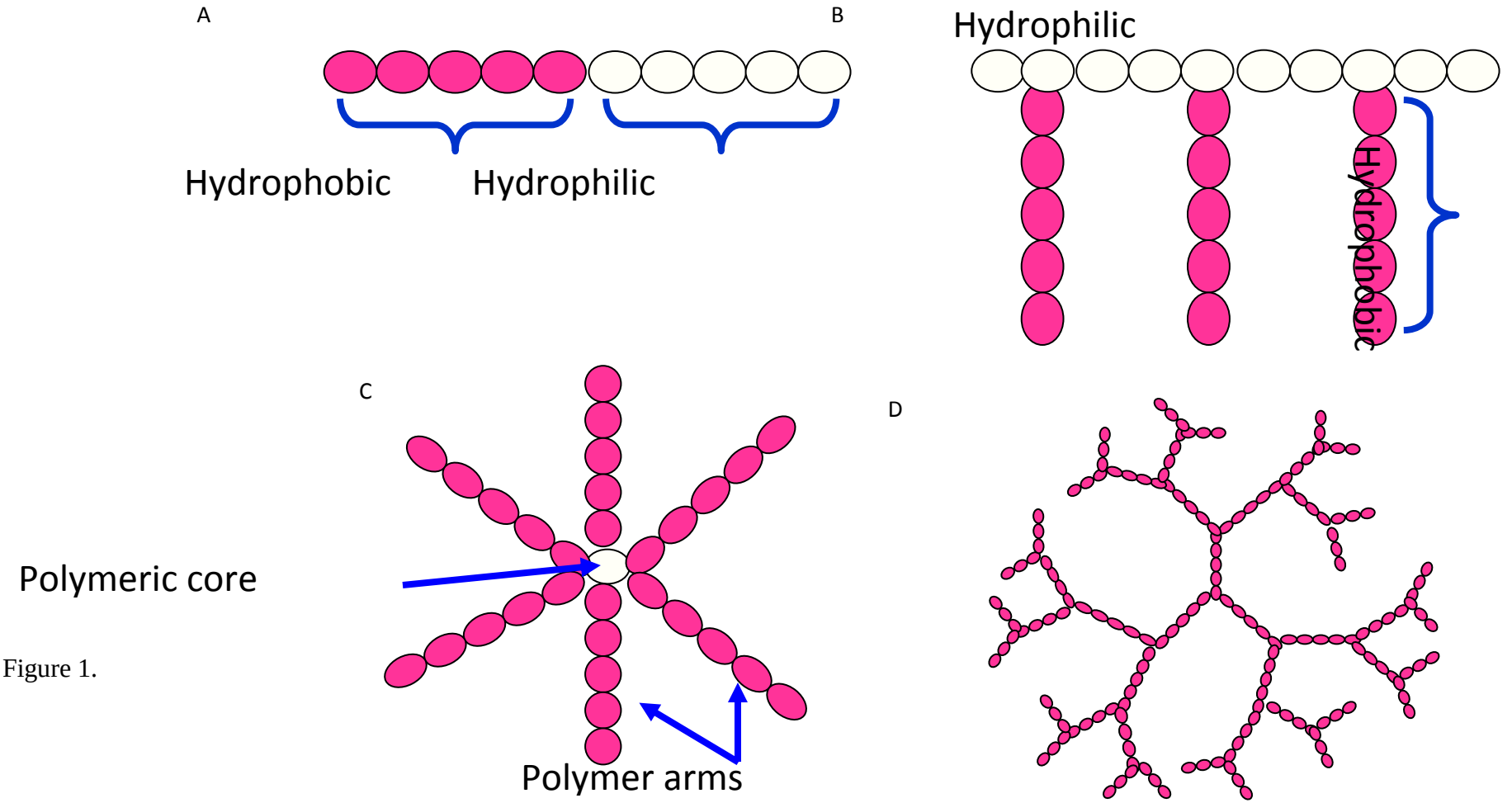

A
B
Hydrophobic
Hydrophilic
Hydrophilic
Hydrophobic
C
Polymeric core
Polymer arms
D

Figure 1.

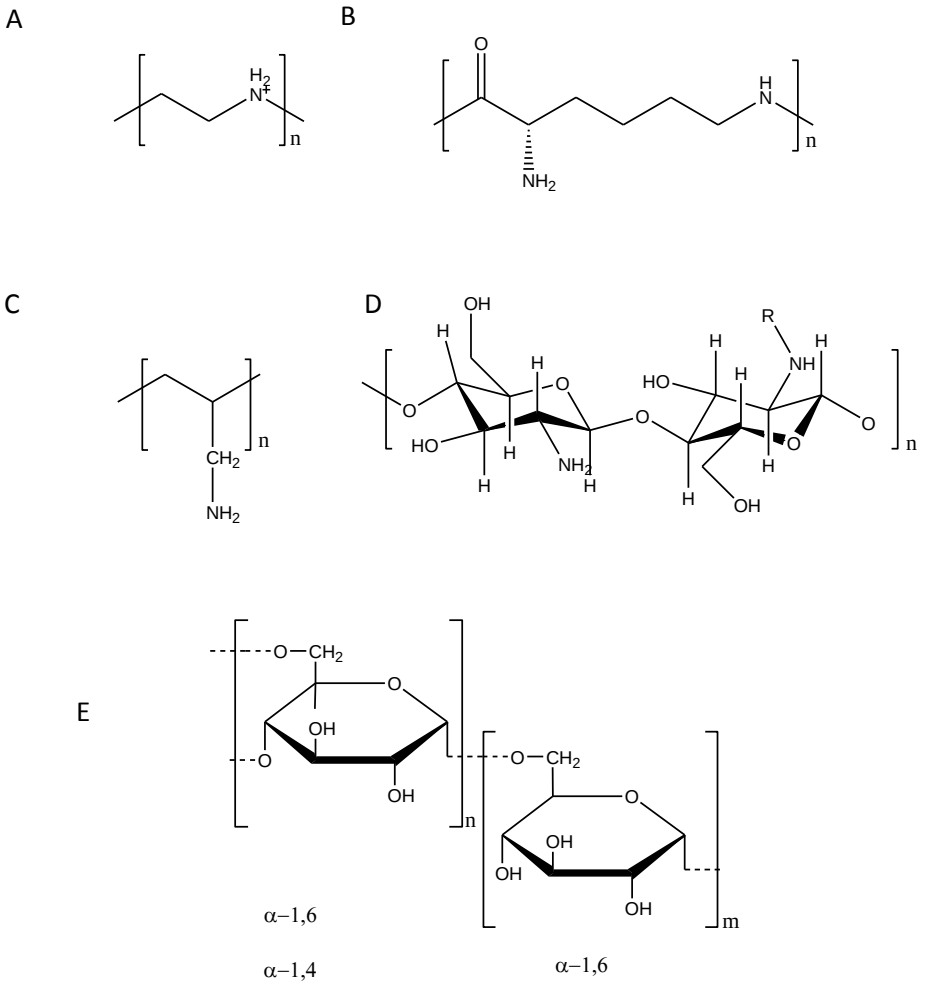


Figure 2.

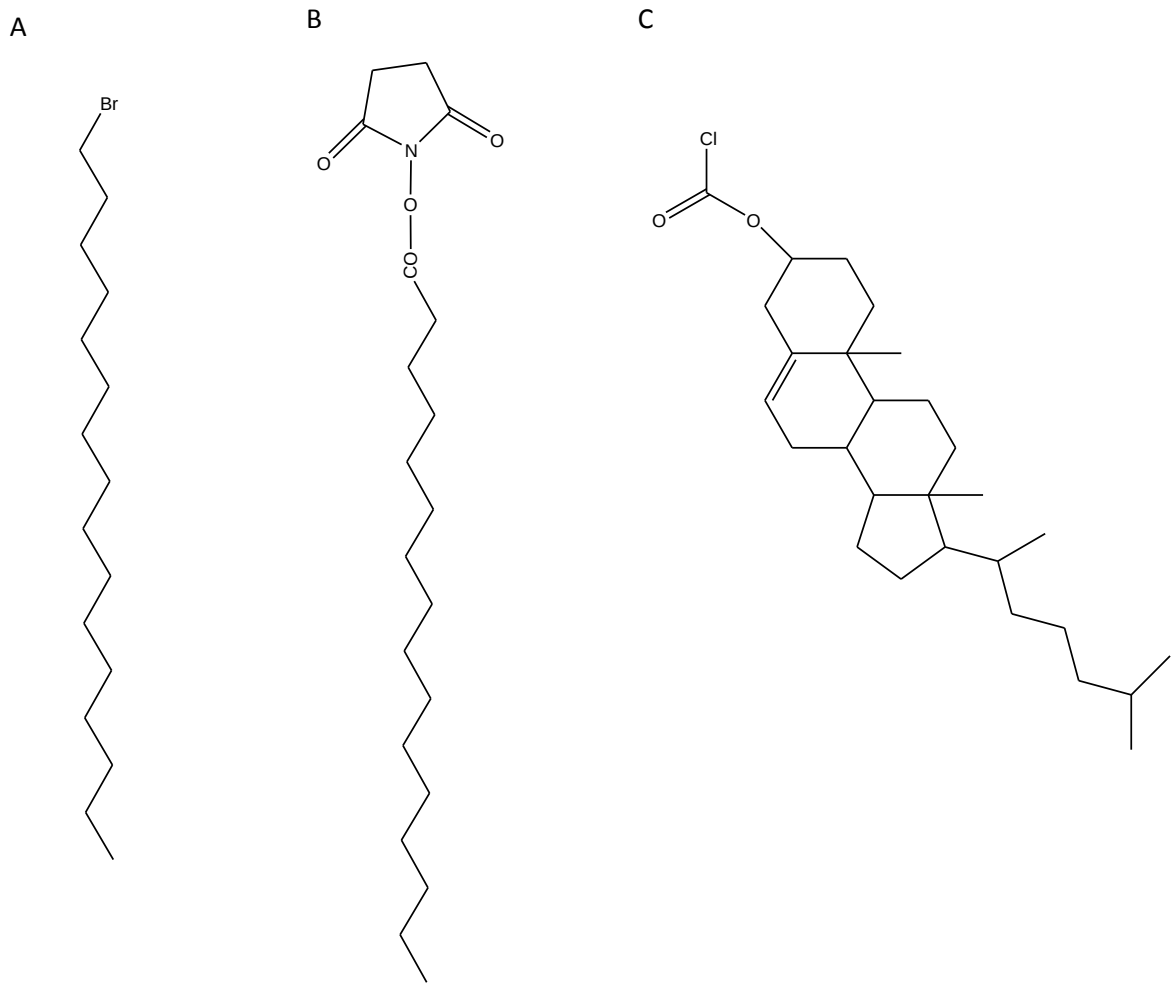


Figure 3.

A B C

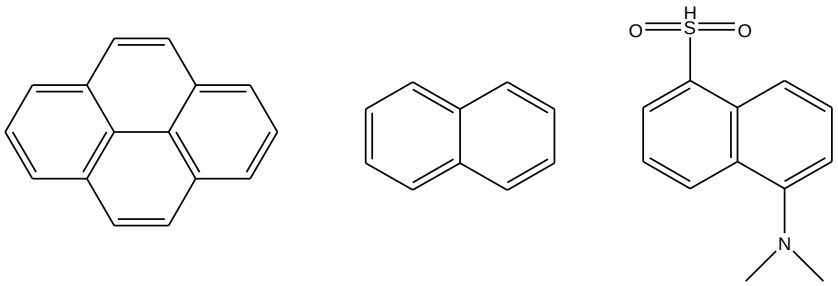


Figure 4.

A B

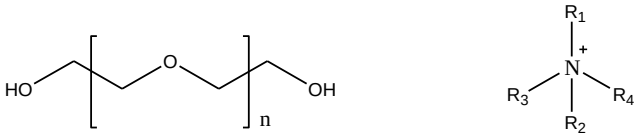


Figure 5.

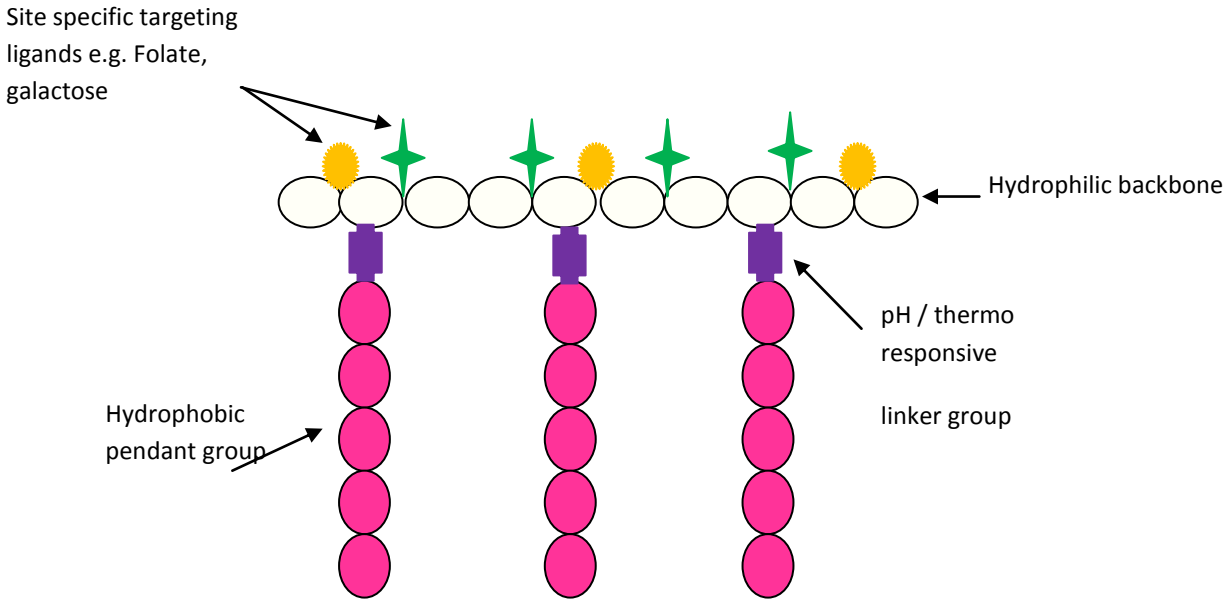


Figure 6.

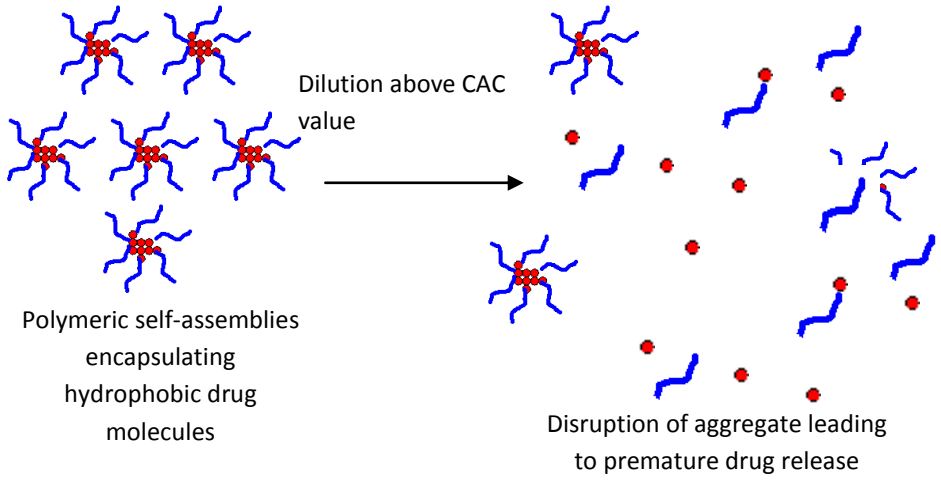
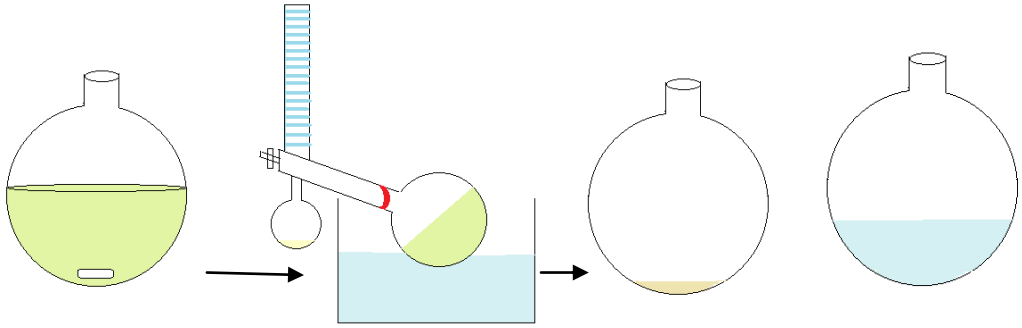


Figure 7.

A

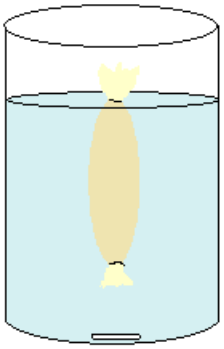


Polymer and drug dissolved in organic solvent

Solvent removed

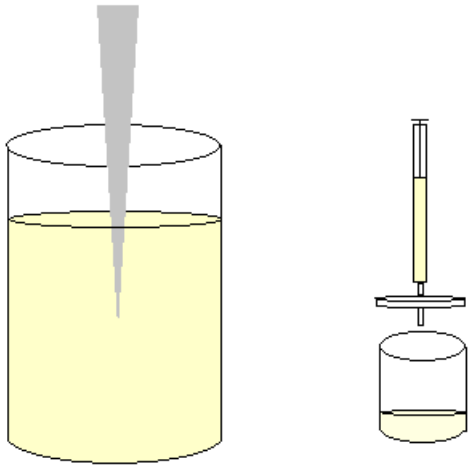
Residue formulation re-dissolved in water and filtered

B



Polymer and drug dissolved in organic solvent and dialysed against water. The exchange of solvent and water molecules in the dialysis bag drives the formation of self-assemblies with encapsulated drug

C

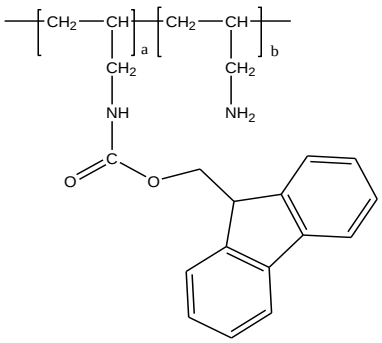


Polymer and drug probe sonicated in aqueous environment to form self- assemblies with encapsulated drug

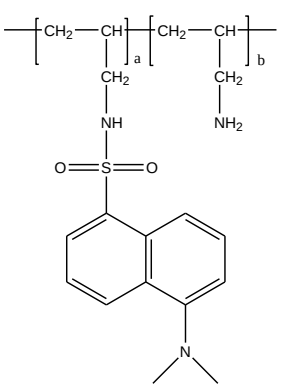
Resultant solution filtered to remove free drug

Figure 8.

A



B



C

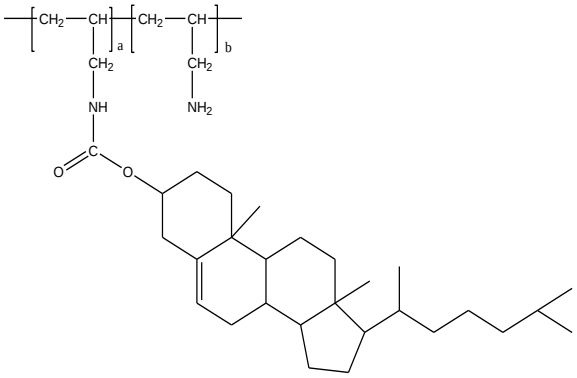


Figure 9.

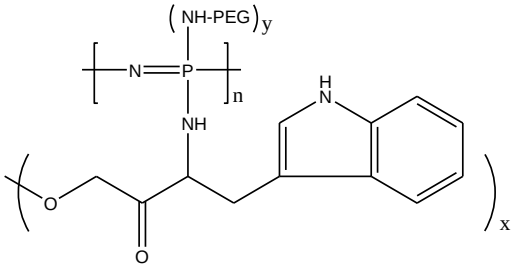
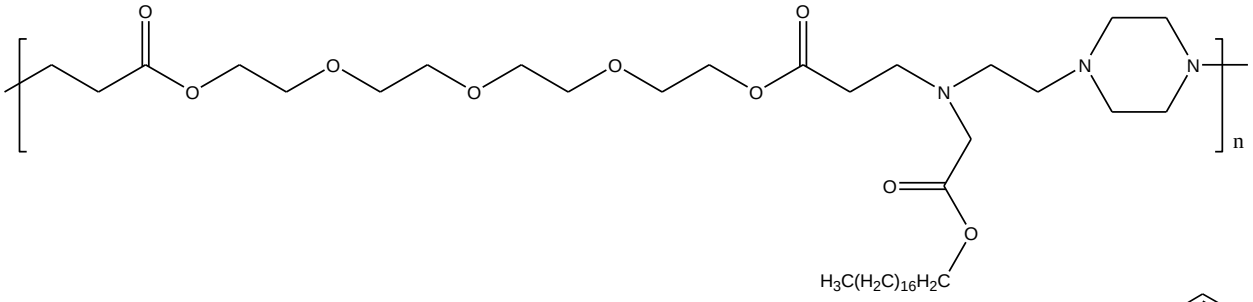


Figure 10.

A



B

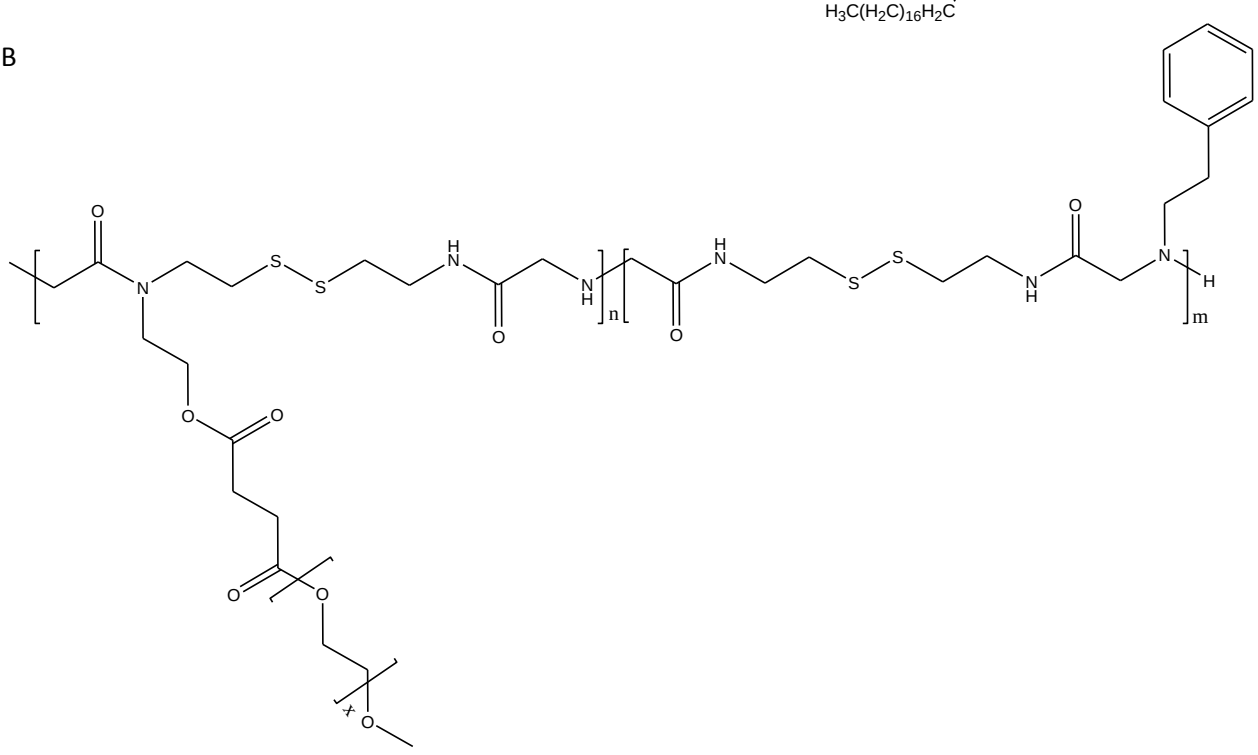


Figure 11.

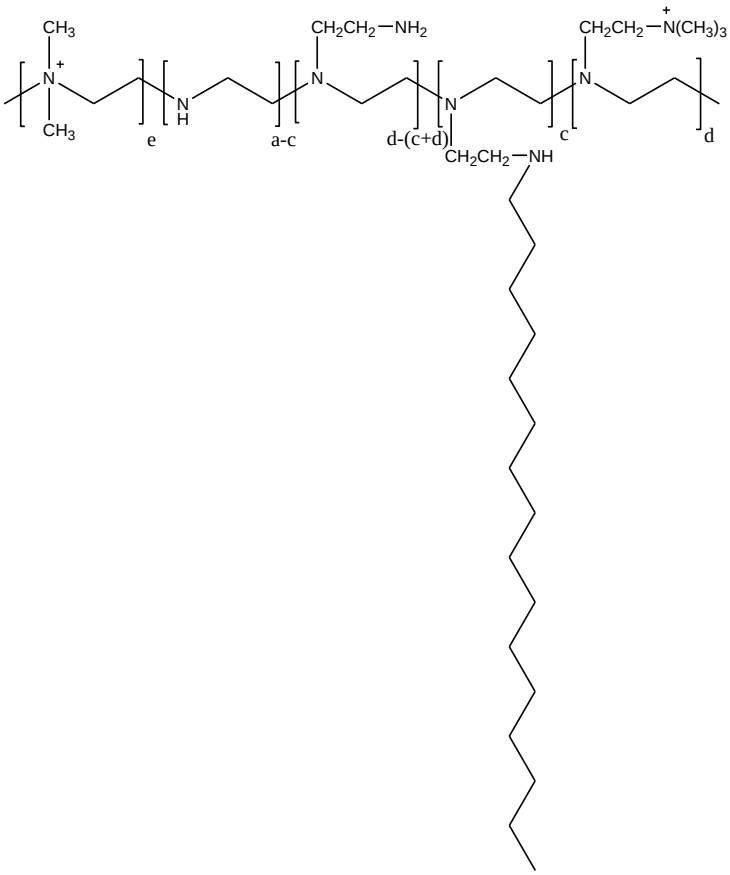


Figure 12.

174

175