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Polymeric Micelles and Alternative Nanonized Delivery Vehicles for Poorly Soluble Drugs

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Abstract

Poorly soluble drugs often encounter low bioavailability and erratic absorption patterns in the clinical setting. Due to the rising number of compounds having solubility issues, finding ways to enhance the solubility of drugs is one of the major challenges in the pharmaceutical industry today. Polymeric micelles, which form upon self-assembly of amphiphilic macromolecules, can act as solubilizing agents for delivery of poorly soluble drugs. This manuscript examines the fundamentals of polymeric micelles through reviews of representative literature and demonstrates possible applications through recent examples of clinical trial developments. In particular, the potential of polymeric micelles for delivery of poorly water-soluble drugs, especially in the areas of oral delivery and in cancer therapy, is discussed. Key considerations in utilizing polymeric micelles' advantages and overcoming potential disadvantages have been highlighted. Lastly, other possible strategies related to particle size reduction for enhancing solubilization of poorly water-soluble drugs are introduced.

Keywords

Polymeric micelle; oral delivery; anticancer; drug solubilisation; clinical trial; micelle stability; nanocrystal; nanoemulsion

1. INTRODUCTION

Over the past ten years, the number of drug candidates with solubility problems has steadily increased as a result of using combinatory chemistry and high-throughput screening in drug discovery. At present it is estimated that approximately 70% of new chemical entities are poorly soluble in aqueous and many even in organic media, and approximately 40% of currently marketed immediate-release oral drugs are considered practically insoluble (solubility less than 100 µg/ml) in water^{1, 2}. Poor solubility leads to a variety of issues. Low solubility limits the drug dissolution rate, which frequently results in low bioavailability of the orally administered drug³. In such a case the therapeutic drug concentration in the blood

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can be achieved by dose escalation. However, dose escalation is often undesirable for the following reasons: 1) possibility of increased toxicity and therefore decreased patient compliance; 2) difficulty in designing formulations for drug product with high drug load; and 3) increase in manufacturing costs associated with higher consumption of active pharmaceutical ingredients (API). Low solubility also may result in erratic absorption patterns, which detract from the clinical efficacy of the drugs. Consequently one of the major challenges of the pharmaceutical industry is developing strategies to enhance the aqueous solubility of drugs. This is particularly pertinent to drugs within class II and IV of the biopharmaceutical classification system (BCS), where dissolution velocity is a rate limiting step for absorption.

Various methods to overcome the poor aqueous solubility of drug candidates have been investigated in the research and development of oral formulations. These methods include changing the chemical structure of drug candidate in lead optimization phase and utilizing pro-drug approaches whereby a polar functional group is introduced into the structure of the drug molecule⁴. The most often used approach is to enhance the dissolution of these poorly water-soluble drugs, especially in the case of BCS class II and IV drugs. According to the Noyes – Whitney equation, the rate of dissolution is affected by the effective surface area, diffusion coefficient, diffusion layer thickness, saturation solubility, the amount of dissolved drug as well as volume of dissolution media³. Among them, effective surface area, diffusion layer thickness and saturation solubility are factors that can be modified by formulation efforts. Ways to modify these factors include crystal modification (e.g., metastable polymorphs, cocrystal and salt formation), particle size reduction (e.g. micronization, nanocrystals), amorphization, pH modification and self-emulsification.

Particle size reduction to the nanometer range is one of the most widely investigated approaches to enhance dissolution. Nanonization, i.e., production of drug nanocrystals, reduces the drug particle size to the sub-micron range via either bottom-up methods such as precipitation and self-assembly or top-down technologies such as milling and high pressure homogenization⁵⁻⁹. Nanocrystals dramatically increase the drug particle surface area, thereby enhancing the dissolution rate of poorly soluble drugs. In addition, an increase in saturation solubility is also expected as described by Ostwald-Freundlich's equation¹⁰. To stabilize nanocrystal formulations, hydrophilic polymers and/or surfactants are usually added to the nanocrystal suspensions. These formulations have been found to demonstrate 1.7-60folds and 2-30 folds enhancements in C_{max} and AUC, respectively, when compared with crystalline formulations with particle sizes in the micrometer range¹¹⁻¹³.

In addition to the nanocrystal approach, other types of nanonization strategies have emerged as new nanopatforms for the delivery of poorly soluble drugs. Typical examples of these nanopatforms include nanoemulsions and polymeric micelles. A common feature of these nanopatforms is the ability to solubilize poorly water-soluble drugs in a hydrophobic reservoir or core. Poorly water-soluble drugs are encapsulated within the reservoir or core in a dissolved state, and the reservoir or core is often stabilized by surfactants or polymeric shell to prevent rapid diffusion of encapsulated drug from the reservoir or core. More specifically, for nanoemulsions nanoscopic oil droplets (typical size 20 – 200 nm) are suspended in aqueous phase. The oil droplets are the reservoirs for hydrophobic drugs. Widely used oil molecules include saturated and unsaturated fatty acids, fatty acid esters and soybean oils. Although nanoemulsions have tendency to phase separate and flocculate, kinetically stable nanoemulsions with sufficient shelf life stability can be achieved by using common surfactants such as poloxamers, lecithin and Tween 80. Combinations of various surfactants have also been explored for controlling particle size and improving nanoemulsion stability. Commercially available nanoemulsion-based formulations include

Estrasorb[®] (estradiol, Novavax/Graceway), Flexogan[®] (camphor, menthol and methyl salicylate, AlphaRX, Canada) and Restasis[®] (cyclosporine, Allergan).

Polymeric micelles have gained considerable attention in the last two decades as a multifunctional nanotechnology-based delivery system for poorly water soluble drugs. Typically polymeric micelles are formed from self-aggregation of amphiphilic polymers with the hydrophobic part of the polymer on the inside (core) and hydrophilic on the outside (shell). As a result of this characteristic, the advantages of polymeric micelles as delivery vehicles are two-fold: first, the hydrophobic core serves as a solubilisation depot for drugs with poor aqueous solubility; second, the hydrophilic shell provides some protection in limiting opsonin adsorption, which contributes towards a longer blood circulation time or better blood stability. The small size of polymeric micelles also contributes towards longer blood circulation time by evading scavenging by the mononuclear phagocytic system in the liver and bypassing the filtration of inter-endothelial cells in the spleen. Ultimately longer circulation time leads to improved accumulation at tissue sites with vascular abnormalities. This last particular characteristic provides one of the strongest arguments for using polymeric micelles for delivering anti-cancer drugs, most of which also have very low aqueous solubility.

Despite increasing attention in polymer micelles as drug delivery vehicles for poorly soluble drugs, so far much of the work has been conducted in a laboratory setting with very few in clinical trial studies. Those currently in clinical trials include phase II and phase IV studies of paclitaxel-loaded polymer micelles for non-small cell lung cancer (Samyang Biopharmaceuticals Corporation) and recurrent breast cancer (Korean Breast Cancer Study Group), respectively. While polymeric micelle delivery systems have remained promising, thus far significant problems have impeded their progress and limited their applications. The fundamentals of polymer micelle drug delivery systems (PMDDS) is reviewed with a focus on application of PMDDS in oral delivery and anti-cancer therapy, two of the most widely investigated applications for PMDDS. The scope is limited to polymeric micelles that encapsulate poorly soluble drugs by purely physical interactions rather than via chemical linkages, which describe a group of micelles formed by self-assembly of hydrophilic polymer-drug conjugates. A priority is placed on PMDDS that form by physical drug loading because of the preference for intact drug molecules during drug development. Alternative nanotechnology-based delivery methods for poorly water soluble compounds are proposed at the end.

2. STRUCTURE AND COMPOSITION

Amphiphilic polymers self-assemble in aqueous environment to form supramolecular core-shell structures, either with a solid core or a more fluid structure. In the former case, nanospheres are formed and in the latter structures called polymeric micelles are formed (Fig. 1). The core of polymeric micelle is a dense region consisting of the hydrophobic part of the amphiphilic polymer. In PMDDS the core serves as a reservoir for drugs with low aqueous solubility due to the tendency of these drugs to partition into the core as a result of hydrophobic interactions. Due to the non-covalent nature of interaction, it is unlikely that drugs will encounter chemical stability issues as a result of encapsulation inside micelle cores. The shell of polymeric micelles is composed of the hydrophilic part of the amphiphilic polymer. The shell is a physical shield that stretches away from the core and limits micelle-micelle or micelle-protein (opsonin) interactions. Typically, average hydrodynamic diameter of polymer micelles is within the 20-80 nm range¹⁴. Primary methods used to study micelle dimensions are dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM) and atomic force microscopy

(AFM). TEM and AFM also provide direct images of micelles and insight into shape, which is generally spherical in nature.

2.1. Core of Polymeric Micelles

The hydrophobic core is a key component in determining the micelle's capacity to solubilize a poorly water-soluble compound. The ability of the core to encapsulate drug is largely dependent upon the compatibility between the hydrophobic core and the drug molecule¹⁵. Generally, a good indication of compatibility is structural similarity between drug molecule and the hydrophobic part or hydrophobic side chain of core-forming amphiphilic polymer. Compatibility can also be estimated by comparing the polarity of the poorly water-soluble drug compound and the hydrophobic segment of polymer. Somewhat similar to the 'like dissolves like' rule (though in the case of polymer micelles the core does not really dissolve the drug), a general rule of thumb is drug and core-forming block with similar polarities are more compatible than a combination with larger differences in polarity. To quantify this interaction, a commonly used parameter to estimate compatibility using polarity is the Flory-Huggins interaction parameter, χ_{sp} . The equation to calculate χ_{sp} is given below:

$$\chi_{sp} = \frac{(\delta_s - \delta_p)^2 v_s}{kT}$$

where δ_s and δ_p are solubility parameters for drug and core-forming polymer segment respectively, v_s is the molar volume of the drug, k is the Boltzmann constant, and T is the temperature in Kelvins. The solubility parameters of drug (small molecule) and polymer can be calculated using group contribution method, and the solubility parameter of drug can also be determined experimentally by measuring mole fraction solubility of drug in different solvent systems. Theoretically, minimization of χ_{sp} leads to better compatibility and therefore better core encapsulation of the poorly soluble drug, though more studies involving wide range of compounds and polymers are needed to strongly validate this conclusion.

Although polymer-drug miscibility is apparently one of the most important parameters to govern drug encapsulation within the micelle core, factors such as hydrophilic-lipophilic balance (HLB) of block copolymers and polymer – drug ratio (P/D ratio) are also worth noting. For example, block copolymer PEO-*b*-PCL with longer PCL hydrophobic segment demonstrated better drug loading than one shorter PCL segment¹⁶. Furthermore, the encapsulation capacity was shown to increase with increasing initial concentration of drug in the preparation (decrease in P/D ratio). In this case, the drug displayed good compatibility with the core polymer, which was confirmed by dilution and drug content determination studies. In such situations, relatively low P/D ratio enables drug solubilisation. However, when compatibility between drug and core polymer is low, P/D ratio generally should be increased to achieve sufficient solubilisation. It should be noted that changes in P/D ratio not only affects drug encapsulation but also other properties of the formulation such as absorption and elimination. In the example given here, lowering P/D ratio to achieve higher encapsulation efficiency also resulted in increased hemolytic activity of the formulation compared with the commercial formulation.

In terms of the composition of the hydrophobic core, biocompatibility and non-toxicity are key prerequisites in selecting the appropriate hydrophobic segment. Commonly used core-forming hydrophobic polymers for drug delivery can be classified into the following groups: poly(propylene oxide) (PPO) as in Pluronic^{®17}; poly(esters) such as poly(lactic acid) (PLA)¹⁸ and poly(ϵ -caprolactone) (PCL)^{19, 20}; poly(L-amino acids) such as poly(L-lysine)²¹; and phospholipids and lipid-derivatives such as phosphatidyl ethanolamine²². In addition, core-forming polymers such as polystyrene have been used in both in drug

delivery systems²³ as well as fundamental research regarding polymer micelles²⁴. These core-forming constituents cover a wide range of structural diversity and polarity for solubilizing a wide range of poorly water-soluble drugs. The encapsulation of drug within hydrophobic cores constructed from these polymers occurs via hydrophobic interactions that are thermodynamically driven. Besides hydrophobic interactions, micelles can also take up bioactive compounds by electrostatic interactions such as in the case of PEGylated gene nanocarriers based on block cationomers with ethylenediamine repeating units²⁵, but such polyion complex micelles and interactions are not within the scope of this article. Polymeric micelle core can also take up drug through metal complexation, though this approach is less commonly employed than the previous two approaches.

2.2 Shell of Polymeric Micelles

As previously mentioned, the shell of polymeric micelles is composed of hydrophilic portion of amphiphilic polymer. In almost all cases studied, poly(ethylene glycol) (PEG) is invariably the shell-forming polymer of choice. There are several reasons for using PEG in PMDDS. First, it's non-toxic and one of the few synthetic polymers already approved by FDA for use in the drug products. Second, in aqueous environment, PEG is highly hydrated and can move rapidly to sweep out a large exclusion volume. In micelles, PEG forms a dense, brush-like shell that stretches away from the core. These characteristics act to limit micelle interaction with other micelles (leading to aggregation) and proteins (opsonin), which promote uptake and removal by the mononuclear phagocytic system. Third, PEG can be easily functionalized to tether ligands for targeted drug delivery. This particular property has generated a lot of excitement in delivery of highly potent compounds such as anti-cancer agents, which would benefit immensely both in terms of efficacy and safety profiles. The above mentioned reasons all contribute to the large number of studies on polymer micelles involving PEG.

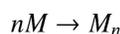
Despite the obvious advantages outlined above for using PEG, it is important to note that there are several major drawbacks in the use of PEG, especially in the clinical setting. The potentially unfavorable effects of using PEG can be attributed to several causes: (i) immunological response due to the polymer itself or side products during synthesis; (ii) unexpected changes in the pharmacokinetic profile of PEGylated nanocarriers and (iii) non-biodegradability of PEG and relatively easy degradation upon exposure to oxygen. Adverse reactions of intravenously administered PEG occur through complement activation, which causes hypersensitivity reactions that can lead to anaphylactic shock^{26, 27}. In addition, accelerated blood clearance phenomenon was seen with the use of PEG^{28, 29}. This phenomenon not only affects the drug bioavailability, but also the blood circulation and extravasation process^{29, 30}. The third drawback of PEG is its non-biodegradability. Therefore, lower molecular weight PEG would be preferable. In drug formulation lower molecular weight PEG is generally used as a solvent, and higher molecular weight PEG is used as component of micelles, possibly because oxidative degradation significantly decreases with increasing molar mass. However, care should be taken not to exceed the renal clearance threshold molar mass to allow complete excretion of the polymer. These considerations have important impact on polymeric micelle design and development.

Besides PEG, several other hydrophilic shell-forming polymers have been used in polymer micelle formation. Poly(N-vinyl-2-pyrrolidone) (PVP) is a frequently used PEG alternative³¹. Another alternative is the hydrophilic, non-immunogenic and biocompatible polymer poly[N-(2-hydroxypropyl) methacrylamide] (pHPMA)³². pHPMA has been investigated for use as the building block for hydrophilic shell. An advantage of pHPMA over PEG is greater multi-functionality, which allows multiple drugs or targeting ligands to be conjugated to the same polymer chain. Examples of pHPMA as the shell-forming block include A-B-A triblock copolymers of pHPMA (A block) with PCL (B block)³³ as well as

star-shaped PCL-*b*-pHPMA³⁴. These pHPMA-based copolymers self-assemble at concentrations above CMC in aqueous solutions to form micelles with pHPMA shell and PCL core. Poly(N-isopropylacrylamide) (pNIPAAm) is a temperature sensitive polymer that has been investigated to prepare thermo-sensitive polymeric micelles³⁵. pNIPAAm exhibits a lower critical solution temperature (LCST) of approximately 33 °C in aqueous solution, above which it is water insoluble and below which it becomes water soluble. This unique property allows pNIPAAm to be used either as the hydrophilic shell-forming segment at temperatures below LCST, or the core-forming segment at temperatures above LCST. Examples of block-copolymers used in micelle formation with pNIPAAm as the shell segment include pNIPAAm-*b*-PLA, p(NIPAAm-*co*-methacrylic acid-*co*-octadecyl acrylate), p(NIPAAm-*co*-N,N-dimethylacrylamide-*co*-10-undecenoic acid) among many other examples³⁶⁻³⁸.

2.3. Thermodynamic and Kinetic Stability

The major driving force behind self-assembling of amphiphilic polymers is hydrophobic interactions that lower the free energy of the system by removing the hydrophobic segments from the aqueous environment. The threshold at which unimers (non-assembled amphiphilic polymer molecule) start to assemble into polymeric micelles is called the critical micelle concentration (CMC). Below the CMC in aqueous environment, amphiphilic molecules exist separately; above the CMC unimers exist in equilibrium with polymer micelles. One of the best models to describe micellar colloidal solutions is the closed association model. In this model, we assume that each micelle is composed of n amphiphilic unimers (M) and that each micelle is formed in a single step. That is:



The equilibrium constant for this pathway is therefore:

$$K_{micellation} = \frac{[M_n]}{[M]^n}$$

From the above equation it is easy to see that the rate of micellation is heavily dependent upon the concentration of unimer M. Perhaps less obvious from the equation but more intuitive is the dependence of K on temperature. The effect of temperature on micellation can be derived from the following equations, which describe the standard free energy ΔG^0 associated with micelle formation:

$$\Delta G^0 = RT \ln(CMC)$$

where CMC is expressed in mole fraction, R is the gas constant and T is the temperature in kelvins. Since:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0,$$

where ΔH^0 and ΔS^0 represent standard enthalpy and entropy changes, respectively, substituting into the first equation and solving for $\ln(CMC)$ would give the following expression:

$$\ln(\text{CMC}) = \frac{\Delta H^0}{RT} - \frac{\Delta S^0}{R}$$

If we plot $\ln(\text{CMC})$ versus $1/T$ we would obtain a straight line with slope equals $\Delta H^0/R$ and intercept of $-\Delta S^0/R$, assuming ΔH^0 is independent of temperature. The above equation implies that for certain polymers such as Pluronics[®], where ΔH^0 is positive, the value of $\ln(\text{CMC})$, and therefore CMC, would decrease with increasing T . The practical implication is that the increase in temperature lowers CMC, which allows CMC to form at a lower concentration of unimers. As applied in drug delivery, a lower CMC means a greater resistance to dissociation by dilution when the PMDDS is introduced into the physiological environment. The opposite effect of temperature on CMC can be said for polymers with negative ΔH^0 , where CMC increases with increasing T .

Besides thermodynamic stability, kinetic stability also has several important implications for drug delivery. At equilibrium, polymeric micelles exhibit inordinate kinetic stability with regards to the dissociation and exchange of unimers between different polymeric micelles. Numerous studies involving polymeric micelles with a poly(styrene) core show that exchange of unimers between micelles in water at ambient temperature is imperceptibly low to none^{24, 39}, but the exchange rate can be modified by changes in temperature³⁹ and presence of co-solvents or co-surfactants⁴⁰. The implications for polymeric micelle-based drug delivery are: (i) preparation of polymeric micelles may not be possible for some polymers by direct dissolution in water at ambient temperature; and (ii) blood components may modify the extent of exchange between micelles and promote dissociation of polymeric micelles⁴¹, even when they are administered far beyond the CMC. In regards to the first implication, it should be noted that for some moderately hydrophobic copolymers such as poloxamers with low PPO content the direct dissolution approach can be employed to prepare drug-loaded polymeric micelles⁴². Direct dissolution was used to prepare PLA / PEG micelles containing paclitaxel⁴³. In addition, liquid copolymers such as low molecular weight PEG-b-poly(CL-co-trimethylenecarbonate) can be easily mix with hydrophobic drug in the absence of organic solvents to prepare micelles by direct dissolution⁴⁴.

3. POLYMERIC MICELLES FOR ORAL DELIVERY

The oral route of drug delivery remains the most preferred route of drug administration. From the drug developer's point of view, the oral route of drug administration is widely accepted by the authorities, is well studied and understood. From the patient's point of view, it is easy and painless to administer, and allows for self-medication, which is especially convenient for chronic therapy. However, even though it is a widely utilized approach and well-understood, the formulation of drugs for oral delivery remains an intricate process, especially for the poorly water-soluble drugs. In order for absorption of orally administered drug to take place, it must first dissolve into its molecular form. For a poorly soluble drug, the rate of dissolution may be so slow or the saturation solubility so low that there is incomplete or inadequate release of drug, which ultimately leads to poor bioavailability and low drug efficacy. In this regard, polymeric micelles can positively impact bioavailability by solubilizing the poorly water-soluble drug which otherwise would precipitate in the aqueous fluids of the GI tract. In addition, encapsulation of drug inside the core of polymeric micelle may protect against rapid clearance from circulation, which can lead to reduced amount of drug available for absorption. The following sections provide some practical considerations in the formulation of polymeric micelles for oral delivery.

3.1. Maintaining Micelle Stability

All orally administered drugs must pass through the gastrointestinal (GI) environment, and conditions in the GI vary depending on location. The most obvious example is the pH value, which ranges from 1-2 in the stomach to 5-7 in the small intestine⁴⁵. The fluid volumes inside the GI tract also vary depending on location as well as fasted or fed state. In the fasted state, total fluid volume in stomach and small intestine is approximately 130 ml whereas in the fed state, the total volume increases to 740 ml⁴⁶. The implication for PMDDS development is that the micelle carriers must be able to resist rapid and premature dissociation upon dilution and exposure to the harsh and changing conditions of the GI tract.

Generally speaking, lower CMC values denote more resistance to effects of dilution and therefore greater stability⁴⁷. As an indicative guide, a CMC value of less than 135 mg/ml should be resistant to rapid dissociation by dilution in orally administered PMDDS. A relatively low CMC value is usually conferred by the presence of highly hydrophobic regions within the micelle core. In order to achieve a lower CMC, we can keep the shell-forming polymer at the same chain length but increase the chain length of the core-forming polymer. For example, Peng, Liu and Tong demonstrated that increasing hydrophobic polystyrene (PS) or poly(methyl methacrylate) (PMMA) block length decreased CMC of micelles formed from triblock co-polymers of PS-*b*-PEG-*b*-PS or PMMA-*b*-PEG-*b*-PMMA⁴⁸. Conversely we can also keep the core-forming polymer at the same chain length but decrease chain length of hydrophilic shell-forming polymer block, though the effect of this change on CMC is less dramatic than the previous approach. For example, Ashok et al. examined the effect of various PEG chain lengths (2000, 3000 and 5000) on CMC of PEGylated phospholipid micelles and found that the CMC was higher for micelles made from longer PEG chain lengths⁴⁹.

An examination of CMC alone is not sufficient to ensure polymeric micelle stability within the GI tract. As previously mentioned, from the point of administration to absorption polymeric micelles may encounter a range of pH values. In addition, the effect of various digestive enzymes and bile salts must also be taken into consideration. The most straightforward *in vitro* studies involve investigating drug release from micelles upon exposure to both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Francis et al. examined cyclosporine A (cyA) release from micelles in SGF (pH 1.2) and SIF (pH 6.8) and found that in both cases, the drug release reached a plateau within 4 hours with less than 12% cyA release, indicating good micelle integrity under these conditions⁵⁰. Elsewhere in another study, less than 50% of griseofulvin was released from PEG-*b*-PLA micelles in phosphate buffered saline (PBS, pH 7.4), SGF and SIF, though such a release may be too slow for oral drug delivery purposes⁵¹. Although these studies seem to indicate good micelle stability in GI, the results should be interpreted with caution. The major limitation of the above studies and indeed many other *in vitro* studies carried out to predict polymeric micelle stability in GI *in vivo* is that no enzymes or bile salts added in SIF, which is different from the actual situation where these components abound in the small intestinal environment. In a study that did include bile salts in SIF a decrease in micelle size was detected compared with micelles in SIF without bile salts, indicating partial destabilization⁵². The effect may be more pronounced *in vivo* since the presence of enzymes must also be accounted for. Drug retention within polymeric micelles is a prerequisite to successful delivery of poorly soluble drugs to the absorption site, but the retention should not so extended that it hinders the absorption of drug molecules through the GI mucosa.

Interestingly, several studies have been carried out to investigate the effect of using pH sensitive polymeric micelles on drug release and oral bioavailability of poorly water-soluble drugs. Satturwar et al. constructed pH-sensitive polymeric micelles using PEG-*b*-

poly(alkyl(meth)acrylate-*co*-methacrylic acid) and the poorly water-soluble drug candesartan cilexetil was encapsulated within the micelles in amorphous form⁵³. The release of candesartan cilexetil was monitored *in vitro* as a function of pH. Results show release of drug from micelles was triggered when pH increased from 1.2 to 7.2. In another study, Sant et al. synthesized the pH sensitive block copolymer PEG-*b*-poly(alkyl acrylate-*co*-methacrylic acid) by atom transfer radical polymerization. This ionizable block copolymer formed self-assembled micelles at pH below 4.7 and dissociated partially or completely above this pH⁵⁴. It was hypothesized that these polymeric micelles can enhance bioavailability of orally administered poorly water-soluble compounds by preventing drug release and subsequent phase separation in the low pH environment of the stomach, but releases the drug in molecularly dispersed form upon the more basic environment of the small intestine. The poorly water-soluble model drugs fenofibrate and progesterone were encapsulated by oil-in-water emulsion or film-casting methods. One important result of the study demonstrated that relative bioavailability of fenofibrate incorporated in pH-sensitive micelles increased by 156% and 15% compared with fenofibrate coarse dispersion and commercial formulation. The increase in relative bioavailability is attributed to enhanced solubility of drug in GI track as well as reduction of leakage and precipitation in stomach. These studies show that use of pH-sensitive block copolymers to construct polymer micelles can alter the stability profile of micelles under different pH environment, a fact that can be utilized for controlled drug release and as a possible way to increase bioavailability of poorly water-soluble drugs.

3.2. Interactions with Intestinal Mucosa

Different experimental methods have been employed to study the interaction of polymeric micelles with intestinal membrane, mostly using Caco-2 monolayer as the model membrane. In general, polymeric micelles are not known to interact extensively with cell membranes, probably due to steric hindrance from shell-forming polymer segments. Therefore, indicators of paracellular permeability such as the often used trans-epithelial electrical resistance (TEER) usually remain unaltered in the presence of polymeric micelles. Instead most of the *in vitro* studies carried out assess the effect of micelle encapsulation on drug permeability compared with un-encapsulated drug permeability. Theoretically the encapsulation of BCS class II drugs in polymeric micelles should bring about an increase in absorption, but cell permeability studies have been known to give contradicting results. For example, cyA loaded in hydrophobically-modified dextran or hydroxypropyl cellulose micelles demonstrated increases of 1.5- and 3-fold respectively in permeability⁵⁰, but risperidone loaded in PEG-*b*-P(CL-*co*-TMC) micelles did not show any improvement in permeability⁵⁵. However, caution should be used in the interpretation of these results, because the *in vitro* cell permeability studies do not accurately represent the conditions that lead to micelle dissociation and drug release *in vivo*. In fact, the correlation between cell permeability studies and *in vivo* pharmacokinetics is still a subject of much discussion in current literature.

As mentioned previously, the intestinal mucosa is normally relatively impermeable to polymeric micelles. However, there are other pathways that allow the transport of micellar carrier systems across the membrane (Fig. 2). First, in the absence of targeting moieties on its surface, polymeric micelle can be absorbed in its intact form by enterocytes or M cells through an endocytotic pathway triggered by non-specific interactions such as hydrogen bonding or van der Waal interactions between the micelle surface and the cell⁵⁶. Second, the micelles can be absorbed through the process of pinocytosis, in which the cell surface forms invagination that engulfs the micelle carrier. Third, polymeric micelles can be absorbed through receptor-mediated pathway, which is an approach widely investigated in parenteral drug delivery but rarely researched on in oral drug delivery. Still, the ability to

enhance oral absorption through increased receptor-mediated endocytotic pathways remains an attractive prospect. Francis et al.⁵⁷ studied the permeability of cyA encapsulated in dextran-g-PEO-C16 micelles decorated with vitamin B12 (VB12) on the surface. VB12 promotes receptor-mediated endocytosis by binding to intrinsic factor, and together the complex is transported across the mucosa. The permeation coefficient of cyA transported by VB12-decorated micelles was 3.3 cm/sec compared to 1.4 cm/sec for undecorated micelles.

Besides uptake, drugs can be and often are pumped out of enterocytes by efflux transporters on the surface of intestinal mucosa. The extent of absorption for poorly water-soluble drugs (and indeed all orally administered drugs in general) is affected by these efflux pathways. Among the efflux transporters, the most well-known and widely studied is the P-glycoprotein (Pgp) efflux transporters. Pgp is thought to be one of the most significant causes of decreased permeability and therefore oral bioavailability. Consequently modulation of its activity is seen as a way to improve absorption for orally administered drugs. Inhibition of Pgp has been demonstrated most extensively with the use of d-alpha-tocopheryl polyethylene glycol (TPGS) and poloxamers, though exact mechanisms are still unclear. In studies involving TPGS and poloxamers, improvement in permeability was seen at polymer concentrations below CMC and maximal at concentrations just below CMC⁵⁸⁻⁶⁰. These findings seem to suggest that while amphiphilic unimers are able to influence Pgp activity, the formation of micelles will likely negatively impact drug permeability by rendering the encapsulated drug impermeable to the intestinal mucosa. Rather interestingly, when Zastre et al.⁶¹ carried out a comprehensive study of the inhibition of Pgp activity by PEG-b-PCL micelles of various compositions (different hydrophobic and hydrophilic block lengths), they found that maximal permeability rhodamine-123 (R-123) was seen at concentrations 8 – 100 times over CMC of PEG-b-PCL. However, even at these polymer concentrations, less than 20% of the dye was encapsulated inside the micelles, so the increased permeability could be ascribed to either or both the Pgp inhibitory action of PEG-b-PCL or the high concentration of free dye in the system. It's important to point out that not all polymers used for polymeric micelles have an effect on Pgp, despite some being able to alter membrane fluidity⁶². The criteria that make a polymer good inhibitor of efflux transporters remain unclear.

3.3. *In vivo* Investigation of Polymeric Micelles

Very limited studies have been carried out to investigate the pharmacokinetics of orally administered polymeric micelles for delivery of poorly water-soluble drugs. A polymeric micelle formulation of paclitaxel (aqueous solubility < 0.1 µg/ml)⁶³ was administered intravenously or orally to cannulated rats⁶⁴. Data from oral administration indicated an estimated bioavailability of 12.4%, which is significantly higher than reported 6.5%⁶⁵ for Cremophor EL micelle formulation of paclitaxel (Taxol[®]) given orally. Interestingly, the polymeric micelle formulation administered via the portal vein showed a 50% reduction in AUC compared to the i.v. infusion, indicating high metabolism of paclitaxel despite encapsulation in polymeric micelle. In an *in vivo* study involving risperidone (aqueous solubility ~ 103 µg/ml)⁶⁶ bioavailability was not improved by formulating into polymeric micelles⁵⁵. Another study of polymeric micelles containing itraconazole (aqueous solubility 1.8 µg/ml)⁶⁷ showed similar performance between micelle formulation and commercial formulation using cyclodextrin as solubilizing agent⁶⁸. Ould-Ouali et al. studied oral delivery of risperidone encapsulated in self-assembling PEG- α (CL-*co*-trimethylene carbonate) structures in male Wistar rats⁶⁹. The micellar solution was compared to an aqueous solution of risperidone in tartaric acid. Results show no statistically significant differences between the plasma concentration time profiles of the two formulations, though C_{max} of risperidone was lower in micelle formulation (162 ± 12 ng/ml vs. 256 ± 56 ng/ml for aqueous risperidone solution, p=0.16), which was thought to indicate a more sustained

drug absorption, but the authors cautioned that additional data will be required to validate this observation. The apparent lack of statistically significant enhancement in bioavailability in some of these studies between micelle formulation and non-micelle formulation suggest that although micelles may enhance solubilisation of poorly water-soluble drugs, absorption is not necessarily increased, possibly due to the low availability of drug in its readily absorbable form within its absorption window in the gastrointestinal tract.

This issue can be potentially overcome using pH-sensitive micelles as delivery vehicles instead of non-sensitive micelles. As previously mentioned, Sant et al. incorporated fenofibrate into pH-responsive micelles and administered these micelles to male Sprague-Dawley rats that were fasted overnight by oral gavage⁵⁴. Relative bioavailability was enhanced compared to coarse drug dispersion and commercial formulation. While these studies have greatly advanced our understanding of polymeric micelle delivery systems for poorly water-soluble drugs, there remain many unanswered questions. For one, in almost all such studies the micelle formulations were administered after fasting or infused directly into the duodenum, which do not provide sufficient information regarding the effect of stomach conditions or release of bile salts and enzymes (as occurs during digestion) on micelle performance, especially non-pH responsive micelles. It suffices to say that designing PMDDS for poorly water-soluble compounds is a complex procedure, and many more fundamental studies need to be carried out in order to fully understand these processes.

4. POLYMERIC MICELLES IN ONCOLOGY

Polymeric micelles are perhaps most extensively explored for use in anti-cancer treatments for several reasons. First, the hydrophobic cores of polymeric micelles help to solubilize anti-cancer drugs, which are often poorly water-soluble. Second, encapsulation of anti-cancer drugs inside polymer micelles may minimize drug degradation and loss. As previously mentioned in this manuscript, the hydrophilic shell provides some protection in limiting opsonin adsorption. The small size of polymeric micelles also enables avoidance of scavenging by the mononuclear phagocytic system in the liver and filtration of inter-endothelial cells in the spleen. Both factors contribute towards a longer blood circulation time, which allows drug-loaded PMDDS sufficient time to travel to tumor site. Third, once the micelles are in the tumor vicinity, their small size allows them to escape into the affected tissue area via the leaky vasculature found at tumor sites, and because of the lack of lymphatic drainage in these areas, the micelles can be retained there for an extended period of time. Such an effect is known as the enhanced permeability and retention effect (EPR). Last but not least, we can modify the shell of polymer micelles by attaching specific ligands to promote PMDDS-cell specific interactions, which is especially useful for preventing harmful side-effects stemming from highly potent anti-cancer agents acting on normal cells. Because of the above reasons, the potential benefit of using polymeric micelles in cancer therapy is great. This section will focus on research in using PMDDS for oncology.

4.1. Improvements in Solubility

Solubility enhancement of several commonly studied anti-cancer drugs by incorporation into polymeric micelles will be discussed in this section. Paclitaxel, an anti-cancer agent with an aqueous solubility of approximately 0.3 µg/ml, was loaded into 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(PEG)/TPGS (PEG-DSPE/TPGS) micelles⁵². The aqueous solubility of paclitaxel was enhanced by up to 5000 times to achieve an aqueous solubility of approximately 5 mg/ml. The most impressive enhancement in paclitaxel solubility was achieved by the work of Kim et al⁷⁰, in which it was reported that the aqueous solubility of paclitaxel was as high as 38.9 mg/ml through encapsulation in micelles. In this study, nicotinamide derivatives, i.e. N,N-diethylnicotinamide and N-picolyl nicotinamide were shown to be powerful hydrotropes for paclitaxel. Copolymers with

a segment containing such nicotinamide derivatives could be used to produce polymeric micelles with hydrotropic properties toward paclitaxel. In addition, micelles composed of PEG-b-poly(vinylbenzyloxy)-N,N-diethylnicotinamide (PEG-b-PVBODENA) could achieve a remarkably high drug loading (37.4% w/w) for micelle-based delivery systems. The drug loading increased proportionally to the length of the hydrotropic DENA segment. As a comparison, PEG-b-PLA micelles could only load up to 27.6 % w/w of paclitaxel under similar conditions. The difference in drug loading capacity is very likely due to the extent of polymer-drug compatibility, as explained in more detail in section 2.1 'core of polymeric micelles'.

Camptothecin is an inhibitor of topoisomerase I – an enzyme involved in the replication of DNA. As such it is a widely investigated possible anti-cancer agent for several forms of cancer^{71, 72} with an aqueous solubility of approximately 1.3 $\mu\text{g/ml}$ ⁷³. Camptothecin was loaded into polymeric micelles consisting of Pluronics[®] (PEO-PPO-PEO) covalently conjugated to poly(acrylic acid) (Pluronic-PAA)⁷⁴. This micellar formulation demonstrated an approximately 3- to 4-fold enhancement in the aqueous solubility of camptothecin at pH5, a pH at which the lactone form (or the therapeutically active form) of camptothecin is stable but still insoluble. Rather interestingly, the amount of camptothecin solubilized per unit PPO was considerably greater in Pluronic-PAA formulation than in the Pluronic-alone formulation, which seems to suggest solubilisation not only by the hydrophobic core but also by the hydrophilic PEO-PAA micelle shells of the micelles.

Tamoxifen is another anticancer hydrophobic drug with extremely low aqueous solubility ($\sim 0.24 \mu\text{g/ml}$)⁷⁵. Tamoxifen was incorporated into micelles consisting of a new self-assembling polyaspartylhydrazide co-polymer, which is synthesized by grafting both PEG(2000) chains and hydrophobic palmitic acid (C(16)) moieties on the the hydrosoluble polyaspartylhydrazide (PAHy) backbone⁷⁶. The PAHy-PEG2000-C16 micelles were able to achieve 4% w/w drug loading with tamoxifen, which was three times more efficient than previously studied systems containing similar polyaspartic copolymers⁷⁷. The solubility enhancement of tamoxifen after incorporation into PAHy-PEG2000-C16 micelles was approximately 500-fold, reaching an aqueous solubility of 0.12 mg/ml. The enhancement in solubility is substantial and rather remarkable. Using the polymeric micelle approach, other anticancer agents besides the three examples given above have also achieved increases in solubility.

4.2. Improvements in Stability

Polymeric micelles improve drug stability by inhibiting drug degradation. For example, the therapeutically active lactone form of camptothecin was physically incorporated into hydrophobic core of N-phthaloylchitosan-grafted PEG methyl ether (PLC-g-MPEG) micelles by the dialysis method⁷⁸ and analyzed for *in vitro* release behaviors as well as stability. The *in vitro* release profile of camptothecin-loaded PLC-g-MPEG micelles showed sustained release of over 96 hours when the drug loading was around 10% w/w for the micelles. More importantly, when compared to the unprotected camptothecin, camptothecin loaded PLC-g-MPEG micelles were able to protect the lactone form of the drug from being hydrolyzed. The prevention of lactone hydrolysis is crucial for camptothecin formulation development to prevent severe systemic toxicity and poor tumor response efficacy associated with lactone form hydrolysis. Furthermore, camptothecin-loaded PLC-g-MPEG micelles showed an increased half-life in the presence of human serum albumin (HSA) and fetal bovine serum (FBS) from 94 minutes to 76.15 hours compared to un-encapsulated drug. When camptothecin was loaded into micelles composed from Pluronic-PAA⁷⁴ hydrolysis of the lactone form of the drug was prevented for up to 2 hours at pH 8 in water. When comparison was made between the encapsulated formulation and unprotected drug, it was found that drug hydrolysis in human serum was approximately 10-fold slower for the

Pluronic-PAA formulation. The half-lives of unprotected camptothecin versus micelle-encapsulated camptothecin were 0.16 and 1.1 – 1.7 hours respectively.

Another substantial advantage of encapsulation in polymeric micelles is that non-specific interactions, including RES, may be reduced due to steric repulsion by the hydrophilic polymers surrounding the drug-encapsulated hydrophobic core. The steric hindrance effect provides a possibility for designing PMDDS with prolonged circulation time in the blood, which is ultimately beneficial for accumulation at tumor site. So far, experimental results have been promising. Doxorubicin is one of the most powerful and widely investigated anticancer drugs in the clinical field. However, dose-limiting toxicity often occurs with doxorubicin therapy because of the drug lacks sufficient selectivity against tumor cells. Protection from pre-mature drug release and prolonged circulation until reaching tumor site would improve clinical usefulness of doxorubicin immensely. Yu et al.⁷⁹ incorporated doxorubicin into self-assembling aggregates consisting of cholesterol-modified glycol chitosan (CHGC) using a dialysis method. In rat pharmacokinetic studies, doxorubicin-CHGC formulation demonstrated significant increase in mean residence time (2.477 ± 0.297 hours) compared with free doxorubicin (0.125 ± 0.016 hours), indicating a remarkably delayed blood clearance. The area under the plasma concentration-time curve (AUC) of the encapsulated formulation was approximately 6.61 times higher than free doxorubicin. A previous paper reported the detection of self-assembled glycol chitosan in the blood for three days⁸⁰; thus it was postulated that the glycol chitosan offers steric hindrance to plasma opsonin that contributed to the delayed blood clearance. When doxorubicin was physically loaded into PEG-poly(beta-benzyl-L-aspartate) (PEG-PBLA) block copolymer micelles by an o/w emulsion method, approximately 77.5% of PEG-PBLA dose was cleared from blood circulation after 1 hour compared to almost 100% clearance of un-encapsulated doxorubicin⁸¹.

One particular aspect that is important to note is the effect of specific surface area on the ability of the micelles to stabilize encapsulated drug. Elsabahy et al. synthesized PEO-*b*-poly(styrene oxide) (EO-SO) and PEO-*b*-poly(butylene oxide) (EO-BO) of different chain lengths and studied their self-assembling properties in water as well as the resulting polymeric micelles' ability to solubilize and protect docetaxel from degradation *in vitro*⁸². The size and shape of micelles are controlled by various factors such as length and nature of core and shell-forming segments. In the present study, micelles composed of EO-BO with number-average block lengths of 45 and 15 for PEO and poly(butylene oxide) units, respectively, (denoted EO₄₅-BO₁₅) had the smallest diameter measured by dynamic light scattering compared with EO₄₅-BO₂₄, EO₄₅-SO₁₅ and EO₄₅-SO₂₅ but EO₄₅-BO₂₄ micelles yielded the lowest specific surface area based on calculations. When chemical stability of docetaxel was investigated in water over a period of 24 hours at 50 °C, only EO₄₅-BO₂₄ was able to preserve most of the docetaxel chemical integrity. The vastly different protective effect was partially attributed to the lower specific surface area of EO₄₅-BO₂₄ micelles, which decreases interaction of drug and aqueous medium at the water-micelle interface.

4.3. Clinical Trials

Clinical trials of polymeric micelles containing anti-cancer agents are few in comparison to the large number of research conducted in laboratory settings. A summary of PMDDS-based formulations in clinical trials can be found in Table 1. In this section, we will examine each of these formulations, with a concentration on formulation and important results of clinical trials.

4.3.1. Genexol[®]-PM—Genexol[®]-PM is a polymeric micelle-based formulation of paclitaxel encapsulated in monomethoxy-PEG-*b*-poly(D,L-lactide) (MPEG-PDLLA). The

amphiphilic polymer was synthesized by a ring-opening polymerization reaction with MPEG molecular weight of 2000 g/mol⁸³. Physical encapsulation of paclitaxel was carried by using a solid dispersion technique. The final formulation contained PMDDS less than 50 nm in diameter with a drug loading of approximately 16.7%⁸⁴.

MPEG-PDLLA was shown to be non-toxic and biocompatible in both *in vitro* and *in vivo* studies⁸⁵. In comparison to Taxol[®] (paclitaxel solubilized by Cremophore EL), Genexol[®] PM displayed similar cytotoxicity against various human cancer cells, including breast, colon, ovarian and non-small cell lung cancer (NSCLC) cells⁸⁶. However, unlike usual studies involving micelle-based formulations, Genexol[®] PM showed an 82% decrease in AUC after IV administration when compared with Taxol[®] given in equivalent dose⁸⁶. It was postulated that such dramatic decrease was likely due to rapid dissociation of MPEG-PDLLA micelles in presence of α - and β -globulin in the blood, resulting in the rather rapid release of paclitaxel from the micelles⁸⁷. Despite the decrease in AUC, the formulation was deemed superior to Taxol[®] for its higher efficacies in subsequent clinical studies. In Phase II and III trials comparing Genexol[®], Abraxane[®] (an albumin nanoparticle formulation of paclitaxel) and Taxol[®] for metastatic breast cancer, the response rate to Genexol[®] (administrated over 3 hours every 3 weeks at 300 mg/m²) was higher than both Abraxane[®] and Taxol[®]^{88, 89}. For NSCLC, Genexol[®] PM combined with cisplatin was more effective than Abraxane[®] alone⁹⁰. Due to its superior efficacies and lower adverse reactions, Genexol[®] PM is currently available commercially for treatment of NSCLC, ovarian cancer, breast cancer and gastric cancer in some countries. Current phase III and IV clinical trials are ongoing.

4.3.2. NK105—NK105 is a formulation consisting of paclitaxel physically incorporated into polymeric micelles self-assembled from PEG-poly(aspartic acid) (PEG-P(Asp)) modified with 4-phenyl-1-butanol to increase the hydrophobicity⁹¹. The drug loading obtained for this formulation was approximately 23% w/w. Prior to clinical injections, the lyophilized powder was dissolved in 5% glucose solution, and average particle size was approximately 85 nm, with a rather wide size distribution ranging from 20 nm to 430 nm.

Phase I clinical trials of NK105 began in 2004 in 19 patients with pancreatic, bile duct, gastric or colonic cancers⁹². In the typical dose escalation study (10 mg/m² to 180 mg/m²), NK105 demonstrated reduced toxicity and lowered adverse reactions compared to Taxol[®] treatments. Neutropenia was the only grade-4 toxicity observed, and neuropathy, the most common adverse reaction associated with Taxol[®] treatments, was only grade 1 or 2. Allergic reactions were not observed except for one patient who had grade-2 hypersensitivity at dose of 180 mg/m². Amongst the 19 patients, partial response was observed for 1 (out of 11) patient with metastatic pancreatic cancer; colon (1 patient) and gastric (2 patients) patients experienced stable disease state lasting for ten and seven courses of treatments respectively. In pharmacokinetics studies, AUC and total clearance of NK105 administered at 150 mg/m² were 32- fold higher and 72-fold lower respectively compared with that of Genexol[®] PMat 300 mg/m², indicating higher blood stability of NK105⁸⁴. Phase II clinical studies were conducted in Japan in 2007 and completed in 2010, and Phase III trials are in preparation.

4.3.3. SP1049C—SP1049C is a Pluronic[®] based polymeric micelle formulation of doxorubicin. This formulation is prepared by reconstituting doxorubicin with a 0.9% sodium chloride solution containing 0.25% w/v Pluronic[®] L61 and 2% w/v of F127 to a final concentration of 2 mg/ml⁹³. The rationale for using Pluronic[®] is the discovery that such non-ionic surfactants can reduce drug resistance considerably, which is expected to improve effectiveness of clinical treatments by decreasing multidrug resistance (MDR) in cancer cells. L61 was found to be the most effective Pluronic[®] modulator of doxorubicin activity

against various MDR cell lines, but L61 with doxorubicin alone would not produce stable formulation due to liquid phase separation⁹⁴. Therefore, F127 was added as a stabilizer. The average particle size in SP1049C is approximately 30 nm with 8.2% drug loading.

Phase I clinical trials of SP1049C was conducted in Canada in 1999⁹³. In initial dose escalation studies (5 mg/m² to 90 mg/m²), SP1049C showed similar spectrum of toxicities as conventional Doxil treatment at doses of 35 mg/m² and above. Neutropenia was the primary toxicity observed. Unlike Doxil treatment, hand-foot syndrome was not observed for SP1049C. Amongst the patients enrolled in the clinical trial, 3 (corresponding to 11.5%) had a complete or partial response during treatment, and 8 (30.8%) had stable disease with time to progression ranging from 9 to 24 weeks with median of 17.5 weeks. Phase II clinical trials began in 2002 in patients with advanced adenocarcinoma of the esophagus and gastro esophageal junction⁹⁵. The dosing regimen consisted of 30 minute IV infusion at dose of 75 mg/m² given once every 3 weeks for up to 6 cycles. The results showed some unexpected toxicities associated with this treatment. 61.9% of patients experienced grade 3/4 neutropenia, with 1 patient requiring granulocyte colony-stimulating factor treatment for grade 4 neutropenia and fever. 1 patient experienced grade 3/4 mucositis. Gradual absolute decrements in left ventricular ejection fraction (LVEF, a measure of how much blood is pumped out of the left ventricle with each contraction) were also observed with cumulative treatment. These adverse responses were likely due to micelle instability and subsequent degradation issues. However, the encouraging results are that out of 19 patients evaluated, 9 had partial response and 8 had stable disease. The median overall survival and progression-free survival were longer than for formulation consisting of free doxorubicin combined with cisplatin and 5-FU, reaching 10 and 6.6 months respectively. The drug is currently in Phase III clinical studies and was designated an orphan drug by the FDA in 2008.

4.3.4 Docetaxel-loaded targeted polymeric nanoparticle (DTXL-TNP)—A drug delivery system that allows targeted delivery of therapeutic agents to the disease location is a particularly desirable strategy in cancer treatments, because the therapeutic agents are often cytotoxic and cause damage to normal cells and tissues. Hrkach et al. developed targeted polymeric micelles containing the chemotherapeutic agent docetaxel (DTXL) for the treatment of patients with advanced and metastatic solid tumors⁹⁶. The micelles were targeted to the extracellular domain of prostate-specific membrane antigen (PSMA) using a PSMA substrate analog inhibitor S,S-2-[3-[5-amino-1-carboxylpentyl]-ureido]-pentanedioic acid (ACUPA). A combinatory library of DTXL-TNPs was prepared by self-assembly of particles from varied proportions of PLA-PEG polymer conjugated to ACUPA, DTXL, and PLA, PLGA, PLA-PEG, and PLGA-PEG copolymers of varying PLA, PLGA and PEG block lengths and PLGA ratio of glycolic to lactic acid units. The micelles formed thus consisted of a hydrophobic core with encapsulated DTXL, and a hydrophilic shell with PEG and PEG-ACUPA. The suspension was diluted with aqueous polysorbate 80 solution. After being purified and concentrated, the final formulation was stored as a frozen suspension in a 10% aqueous sucrose solution. The formulations were screened to optimize drug loading, consistency of particle size distribution across different batches, stability and drug release properties. The most promising formulations were evaluated in healthy Sprague Dawley rats. On the basis of their findings, the final formulation selected for clinical evaluation consisted of 10 wt% DTXL encapsulated in 100 nm particles composed of PLA-PEG (16-kD PLA, 5-kD-PEG), and PLA-PEG-ACUPA (also 16-kD PLA, 5-kD PEG), with PLA-PEG and PLA-PEG-ACUPA representing 97.5% and 2.5% of polymer mass respectively.

In Phase I clinical trial (NCT01300533), DTXL-TNP was given intravenously every 3 weeks in patients with advanced or metastatic cancer. The study is currently ongoing and full results have yet to be published. In interim analysis of patients receiving doses up to 75 mg/m² DTXL-TNP displayed pharmacological properties different from commercially

available, solvent-based DTXL formulation sb-DTXL that is consistent with results from the animal models. In the PK profile for patients receiving dose of 30 mg/m², DTXL-NP showed higher plasma levels for all time points greater than 1 hour post-administration. The plasma levels were at least two orders of magnitude higher compared to equivalent dose of sb-DTXL. Furthermore, the high plasma concentration was maintained for at least 48 hours. In dose-ranging studies of 3.5 to 75 mg/m², PK for DTXL-TNP was essentially dose proportional, with correlation coefficients of 0.87 and 0.79 for C_{max} and AUC versus dose respectively (Figure 3). In terms of efficacy, 2 patients exhibit stable disease at dose below 30 mg/m². The computed tomography images of a 51-year-old male patient with metastatic cholangiocarcinoma showed disappearance or shrinkage of multiple lung metastases after 2 treatment cycles of 15 mg/m² DTXL-TNP. A 63-year-old patient with tonsillar cancer showed 25% shrinkage of a target tonsillar lesion after 2 dosing cycles at 30 mg/m². These results are highly promising at this early stage of clinical development.

4.3.5. Non-physically Incorporated Formulations—The above mentioned formulations all incorporate drugs into the micelle through physical interaction. There are also a few formulations in clinical trials that load cancer drugs into polymeric micelles via chemical conjugation or metal complexation. Since the focus of this manuscript is on PMDDS that form by physical drug loading, we will only briefly examine the non-physically loaded formulations in clinical trials.

NC-6004 is a cis-dichlorodiammineplatinum(II) (CDDP) loaded polymer micelle formulation consisting of PEG and polymer-metal complex between poly(glutamic acid) (P(Glu)) and CDDP. The average particle size was approximately 30 nm with a drug loading of 39%⁹⁷. Clinical trials of NC-6004 began in the UK in 2006, with a dosing regimen of IV administration over 1 hour every 3 week at a dosing range of 10 mg/m² to 120 mg/m². Minor nephrotoxicity was observed with no significant myelosuppression, ototoxicity or neurotoxicity. However, unexpectedly hypersensitivity reactions occurred more frequently than CDDP alone regardless of dosing level. In terms of anti-tumor efficacy, the best response was stable disease in 7 out of total of 17 patients. Currently, clinical Phase I/II studies are taking place in Singapore and Taiwan in patients with advanced or metastatic pancreatic cancer.

NK012 contains 7-ethyl-10-hydroxy-camptothecin (SN38) (aqueous solubility < 5 µg/ml)⁹⁸, which is an active metabolite of irinotecan hydrochloride (CPT-11) with powerful cytotoxic effects against various cancerous cell lines *in vitro*. NK012 is formed by covalently conjugating SN38 with the P(Glu) segment of PEG-P(Glu) copolymer followed by self-assembling of the amphiphilic copolymer PEG-P(Glu)(SN38) in aqueous media⁹⁹. Average particle size in NK012 formulation is approximately 20 nm with a drug loading of 20% w/w. Phase I clinical trials were conducted in both Japan¹⁰⁰ and the US¹⁰¹ with different dosing regimens. NK012 was administered IV for 30 minutes every 3 weeks with an SN38 equivalent dose range of 2 – 28 mg/m² and 9 – 28 mg/m² in Japan and US respectively. Prior to infusion, NK012 was diluted to total volume of 250 ml with 5% glucose solution. No dose limiting toxicity (DLT) was observed for both until 28 mg/m² with the exception of one elevated γ-glutamyl transpeptidase at 20 mg/m² in the Japanese trial. Non-hematologic toxicities were minimal. In comparison to CPT-11 trials, cholinergic reactions appeared less frequently. NK012 also exhibited a higher systemic exposure and slower elimination than CPT-11. 8 total partial responses were reported in Japanese and US trials. Phase II clinical trials are currently underway in Japan and US¹⁰².

NK911 is yet another polymer micelle-based formulation in clinical trials involving chemical conjugation of drug to hydrophobic segment of amphiphilic block polymer. NK911 contains doxorubicin (DOX) conjugated to P(Asp) with PEG (molecular weight

5000 g/mol) as the hydrophilic segment. Upon reconstitution, the product contains particles with average size of 40 nm. Drug loading was dictated by the extent of DOX substitution, which for NK911 was approximately 45% substituted¹⁰³. Clinical trials began in 2001 in Japan 23 patients. Dose escalation studies showed similar toxicity spectrum as that of free DOX. Grade 3/4 hematological toxicity was observed until dose reached 50 mg/m², at which 3 patients had grade 3 leucocytopenia, 5 had grade 3 neutropenia and 2 had grade 4 neutropenia. No DLT was observed for non-hematological toxicity. Common side effects associated with Doxil administration were rare and mild. However, AUC of NK911 was more than 1429-fold lower than Doxil at the same dose of 50 mg/m², indicating the lower stability of NK911 than Doxil. Nevertheless, 1 patient had a partial response and 8 had stable disease. Phase II clinical trials are underway for NK911.

5. EVALUATION OF POLYMERIC MICELLES AS DELIVERY VEHICLES

Early clinical trials and abundant laboratory research have revealed several advantages of using polymeric micelles as solubilizing vehicles for delivery of poorly water-soluble drugs. These advantages include their small size, lower toxicity and advent of adverse reactions, and potentially long blood circulation times. However, several aspects of polymeric micelle-based delivery systems remain to be elucidated. Without a clearer understanding of these issues the potential of polymeric micelles as solubilizing vehicles may not be fully realized.

One of the key issues of polymeric micelles is their stability in the physiological environment. From the clinical studies mentioned previously, we have seen how insufficient stability may lead to unexpected side effects and adverse reactions. Therefore, it is critical that polymeric micelles should have enough structural integrity to remain stable in the body after administration or until they have arrived at their destination site in the case of cancer therapy. However, polymeric micelles are liable to dissociate, especially upon administration when they are diluted to a concentration below the CMC. The kinetic stability of polymeric micelles is also important to consider when evaluating PMDDS stability, because blood components can alter the kinetic stability of micelles and cause dissociation. Usefulness of PMDDS would be drastically reduced if the micelle carrier system cannot maintain the poorly water-soluble drug for the desired period of time. Chemical crosslinking of micelles is one way to prevent micelle dissociation and preserve drug inside the hydrophobic core^{104, 105}. However, a concern is that these crosslinked micelles are too stable and may not release sufficient amounts of drug to achieve therapeutic efficacy, or that their prolonged circulation may result in unpredictable physiological disruption⁴⁴. Biodegradable or physical crosslinking may be more suitable for drug delivery purposes by introducing reversible crosslink bonds, but so far only limited data have been made available on studies *in vivo*. Undoubtedly, the issue of micelle stability has to be resolved before PMDDS can achieve more clinical significance.

Biocompatibility and cytotoxicity are highly important in the development of effective PMDDS. Consequently, it is critical to gain a more comprehensive understanding of the fate of amphiphilic copolymers after administration into the body. However, this is rather challenging to do, so most of the studies on PMDDS choose to use amphiphilic copolymers with well characterized biocompatibility and minimal side effects, for example PEG-PLA, PEG-PCL or PEG-poly(Asp). As a result, the application of PMDDS becomes severely limited by the few copolymers already in use. On the other hand, studies that report novel copolymer strands for PMDDS are often focused on drug loading efficiency, drug release behavior, targeting capabilities and stability, with little attention (if any at all) devoted to biocompatibility or cytotoxicity. This approach has stunted the development of more suitable copolymers for PMDDS application. More research in this area may lead to increasing number of PMDDS with clinical significance.

6. ALTERNATIVES TO POLYMERIC MICELLES

As briefly mentioned in the beginning of this manuscript, there are currently many ways to overcome the poor aqueous solubility of BCS Class II and IV drugs, including crystal modification, amorphization, pH modification and self-emulsification. Particle size reduction to the micro- and often nanometer range is a widely explored option to solubilize poorly soluble drugs. The use of polymeric micelles is one possible approach to achieve particle size reduction. Alternatively, we can also achieve particle size reduction through the nanocrystal approach or nanoemulsions approach. In this section, we will examine these two alternative approaches separately and as a comparison to polymeric micelles.

6.1. Drug Nanocrystals

Drug nanocrystals are essentially nanoscopic crystals of the parent compound. By definition, the dimensions of nanocrystals are less than 1 μm but for practical purposes they are often less than 500 nm in dimensions. Preparation of nanocrystals takes place either via top-down or bottom-up techniques. Top-down techniques generally involve physically breaking up larger particles of the parent compound into smaller particles via high shear, high pressure or a combination of both. Typically used methods are milling and high pressure homogenization. Disadvantages associated with these methods include contamination issues from beads and equipment, and long processing times required to physically grind particles down to the nanometer range. On the other hand, bottom-up techniques such as nano-precipitation form nanocrystals by nucleation events followed by growth of drug crystals. The major problem with this approach is difficulty in controlling crystal growth and preventing further growth beyond target size. Nevertheless, a number of nanocrystal-based drug products have made it to the market and this information is summarized in Table 2.

The basis for using nanocrystals (and indeed other particle size reduction techniques) as a solubilisation strategy for poorly soluble drugs can be explained by the Noyes-Whitney and Ostwald-Freundlich equation. According to the Noyes-Whitney equation dissolution velocity dC/dt is proportional to the concentration gradient $A(C_s - C_x)/h$, where A is the surface area of the solid, C_s is the concentration of the solid in the diffusion layer, C_x is the bulk concentration of solid and h is the diffusion layer thickness¹⁰⁶. The nanonization of drug particles leads to great enhancements in solid surface area, which the equation predicts will lead to increased dissolution velocity. An additional effect of nanonization is reduced diffusion layer thickness, which also contributes towards the increase in dissolution rate. The higher saturation solubility of nanocrystals can be explained by Ostwald-Freundlich equation, which states that $\log(C_s/C_\infty)$ is proportional to the inverse of r , where C_s is the saturation solubility, C_∞ the solubility of bulk and r is the radius of drug particles¹⁰⁷. Thus smaller particles are also expected to demonstrate higher saturation solubility than parent compound.

After nanonization, drug nanocrystals are often formulated into conventional dosage forms such as tablets, capsules, pellets and suspensions for IV administration. Prior to formulation, extra steps should be taken to remove any residual organic solvents below maximum acceptable concentrations and concentrate the drug nanocrystals without compromising the physical and chemical properties of these crystals¹⁰⁸. Techniques often used to achieve such results include freeze drying, spray drying, centrifugation and ultrafiltration. For solid dosage forms, the final product may also contain excipients such as fillers, binders, humectants, disintegration agents and lubricants to ensure that the drug nanocrystals maintain their physical, chemical and pharmaceutical properties both during storage and when administered into the body.

6.2. Nanoemulsions

Nanoemulsions consist of two immiscible liquids (usually an oil phase and an aqueous phase) where one liquid is dispersed as droplets in the other liquid. The nanoscopic droplets typically have dimensions ranging from 20 – 200 nm¹⁰⁹. In a broad sense, the term ‘nanoemulsions’ consist of two closely related systems termed ‘microemulsions’ and ‘submicron emulsions’¹¹⁰. The defining hallmark of microemulsions from submicron emulsions is thermodynamic stability¹¹¹. Microemulsions are described as thermodynamically stable, whereas submicron emulsions are described as approaching thermodynamic stability¹¹². Nanoemulsions used for solubilisation of poorly water-soluble drugs usually consist of oil phase as the dispersed phase and aqueous phase the dispersing medium, because the oil droplets can serve as reservoirs for hydrophobic drugs¹¹³. The most widely used components of oil phase are saturated and unsaturated fatty acids, fatty acid esters and soybean oils¹⁰⁹. In order to stabilize these drug-loaded oil droplets, nonionic or amphoteric surfactants such as poloxamers, lecithin and Tween 80 are commonly used. Nanoemulsion-based products that are commercially available are listed in Table 2.

Theoretically, the arrangement of emulsifier molecules occurs spontaneously, possibly with the aid of co-surfactants. This low energy emulsification method or so-called self-emulsifying method enables formation of nanoemulsions spontaneously when an oil/surfactant mixture is added to water or when a water/surfactant mixture is diluted with oil, and mixing of all the components occur in the final composition. Low energy emulsification method is mainly adopted for preparation of microemulsions, and hence microemulsions typically exist as microemulsion concentrate or so-called self-microemulsifying drug delivery systems (SMEDDS). The major disadvantage associated with this method is the lack of control over droplet size, and quite often a large size distribution is seen. In contrast to this low energy method, in some cases energy is input into the system to accelerate the re-arrangement of the surfactant molecules or to overcome a small kinetic energy barrier. Methods such as high pressure homogenization, microfluidization and ultrasonication are included in this category¹¹⁴. High pressure homogenization, microfluidization and ultrasonication are similar in that they all form nanoemulsions by high disruptive forces that essentially break apart the oil droplets into smaller ones. In high pressure homogenization, the disruptive forces are created by high pressure (as the name implies); in microfluidization the parent emulsion is forced through many microchannels in the central chamber of the microfluidizer; in ultrasonication, the disruptive force is supplied by ultrasonic energy. Generally, microfluidization produces nanoemulsions with the most narrow size distribution^{115, 116}.

Nanoemulsions have been shown to increase bioavailability compared with conventional drug suspensions. Ezetimibe, a BCS class II molecule with lipid-lowering effects, was formulated into nanoemulsions form with various surfactants and subjected to *in vitro* and *in vivo* testing¹¹⁷. Plasma concentration profile of ezetimibe nanoemulsions formulation in rats showed greater improvement in drug absorption than the marketed formulation and simple drug suspension, with approximate C_{max} and AUC values of 69.53 ng/ml and 948 ng hr/ml respectively for nanoemulsions formulation, 43.74 ng/ml and 222 ng hr/ml for marketed tablet formulation and 47.42 ng/ml and 294 ng hr/ml for simple drug suspension. The relative bioavailability of nanoemulsions formulation with respect to marketed tablet formulation was 477.09%, whereas with respect to simple drug suspension relative bioavailability was found to be 323.02%. The remarkable improvement in bioavailability of nanoemulsions-based formulation was attributed to increase in ezetimibe solubility and immediate dispersion in the GI tract.

6.3. Comparison of Nano-formulation Strategies

Table 3 summarizes and compares the three major nano-formulation strategies discussed in this manuscript: polymeric micelles, nanocrystals and nanoemulsions. Currently, the most established and widely used technique (especially in the industry setting) is the nanocrystal approach. This approach has also resulted in more clinically approved pharmaceutical products compared with the other two strategies. The major reasons for its popularity in industry are its excellent reproducibility and applicability to wide range of drugs with various solubility profiles, including those drugs that are poorly soluble in both water and oils. However, nanocrystal approach sometimes requires high energy input which drives up the cost of production. Moreover, the nanocrystals formed usually require extra steps to ensure stability. Unmodified nanocrystals are not suitable for cytotoxic drugs with small therapeutic indices such as anticancer agents due to rapid dissolution kinetics and lack of controlled release mechanism.

Nanoemulsions offer several advantages over nanocrystals. First of all, nanoemulsions can be administered *via* various routes of administration with several clinically approved products for topical and ophthalmic use already on the market. High drug loading is easily achievable using this approach. Moreover, there is the potential to enhance bioavailability further by increasing permeability in GI tract (in the case of oral delivery) or mucous layer (in the case of sublingual or intranasal delivery), which would be particularly beneficial to BCS class IV compounds. The major disadvantage of nanoemulsions is lack of stability, with flocculation and coalescence often taking place during storage. The lack of controlled release mechanism is also a limitation if delivering cytotoxic compounds such as anticancer drugs.

We have discussed in depth the advantages and disadvantages of the third nano-formulation approach, polymeric micelles, in the rest of the manuscript; here we will focus on those improvements or lack thereof as compared to nanocrystals or nanoemulsions. The major advantage of polymeric micelles is its improved controlled release properties, which makes possible to deliver a variety of poorly water-soluble cytotoxic drugs for chemotherapy. In addition, it is relatively easy to design a multifunctional particle for polymeric micelles, incorporating imaging, targeting and therapeutic agents all into one vehicle, whereas this multifunctional design is rather difficult to implement for nanocrystal or nanoemulsions approach. However, major disadvantages of polymeric micelles include lack of stability, limited polymers for use and lack of suitable methods for large-scale production.

7. CONCLUSION

This manuscript has attempted to explain the fundamentals of polymeric micelles through reviews of representative literature and recent clinical trial developments. The primary purpose of this manuscript is to illustrate the potential of polymeric micelles for delivery of poorly water-soluble drugs, especially in the areas of oral delivery and in cancer therapy, which would benefit most from using polymeric micelles. Key considerations to utilize polymeric micelles' advantages and overcome potential disadvantages have been highlighted. Lastly, other possible particle size reduction related strategies to solubilize poorly water-soluble drugs were introduced.

When designing an appropriate PMDDS for solubilisation, it is crucial to consider compatibility between core and drug. The Flory-Huggins interaction parameter is one possible way to gauge core polymer-drug compatibility. The shell of polymeric micelle acts as a physical barrier to protect the drug-loaded core. At present the most commonly used component is PEG and its various conjugates. However, use of PEG may be limited by immunological responses, unpredictable pharmacokinetics profile and lack of

biodegradability. Therefore, in order to choose the appropriate amphiphilic copolymer, biocompatibility and cytotoxicity issues must be taken into consideration. Currently understanding of non-PEG based amphiphilic copolymers is limited, which prevents development of clinically significant PMDDS. Another important issue in designing PMDDS is the stability of the polymeric micelle, which is dependent on both thermodynamic and kinetic factors.

Polymeric micelles have been investigated for both oral and IV administration of poorly soluble compounds. Although oral delivery of drugs using polymeric micelles is an attractive approach, few studies have been carried out *in vivo*. One possible reason is the availability of other well-understood methods such as drug nanocrystals to formulate poorly soluble drugs into orally delivered formulations, while polymeric micelles are still not yet well-understood for applications in this field. As a result, most recent progress in polymeric micelles for drug delivery has been almost exclusively in the field of intravenous delivery of anticancer agents. Several polymeric micelle-based formulations for anticancer agents have made it to clinical trials and a few are commercially available. Still, in order to fully realize the potential of polymeric micelles as a solubilisation strategy for poorly water-soluble drugs, more fundamental research promoting deeper understanding of amphiphilic copolymer degradation mechanisms and micelle stability characterization *in vivo* is needed.

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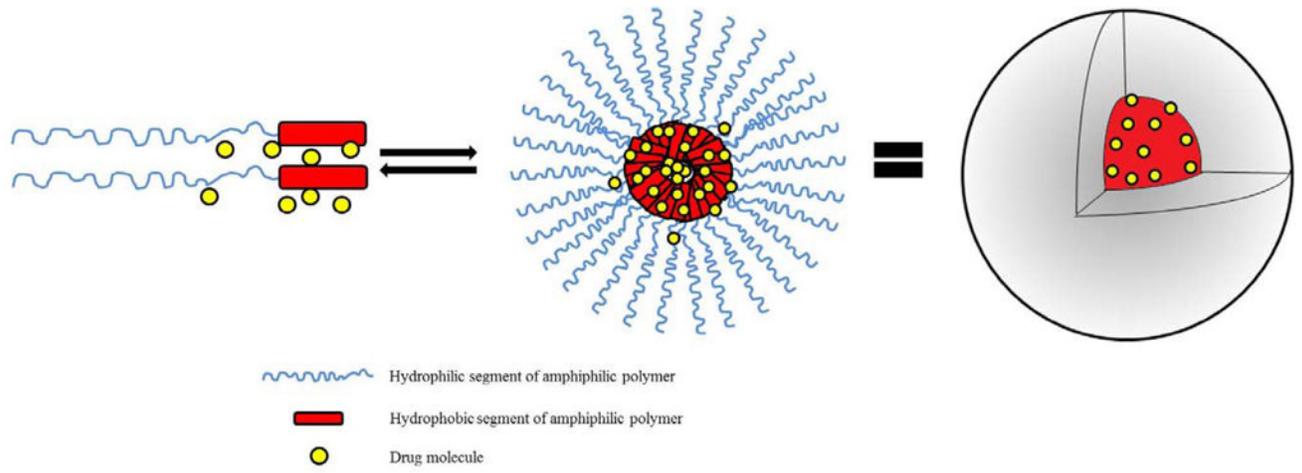


Figure 1. Schematic representation of supramolecular structure of polymeric micelles.

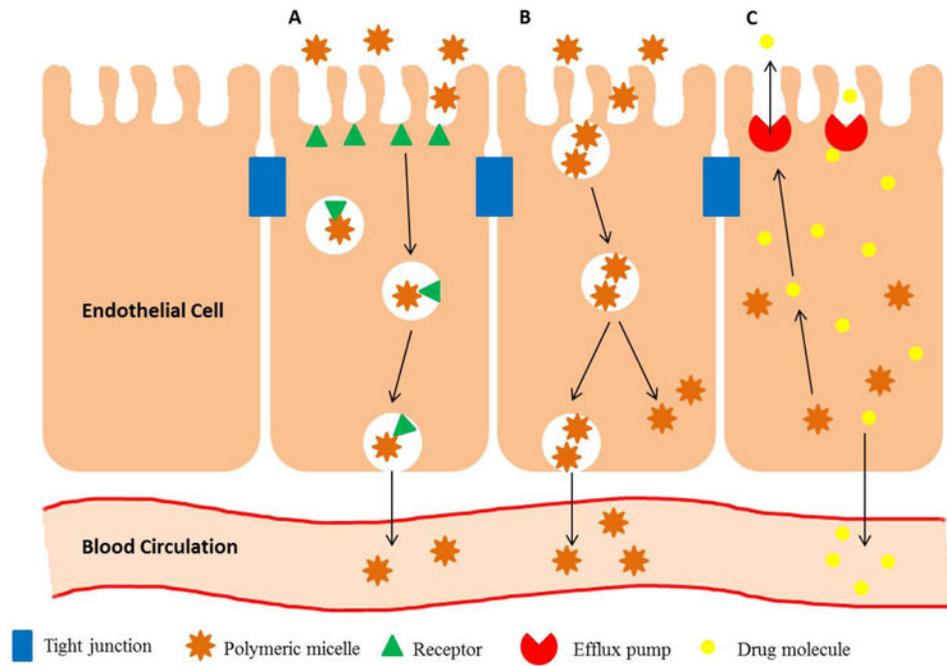


Figure 2. Schematic summary of the pathways by which polymeric micelles may interact with the intestinal mucosa. A) receptor-mediated endocytosis; B) pinocytosis; C) Efflux of drug molecules

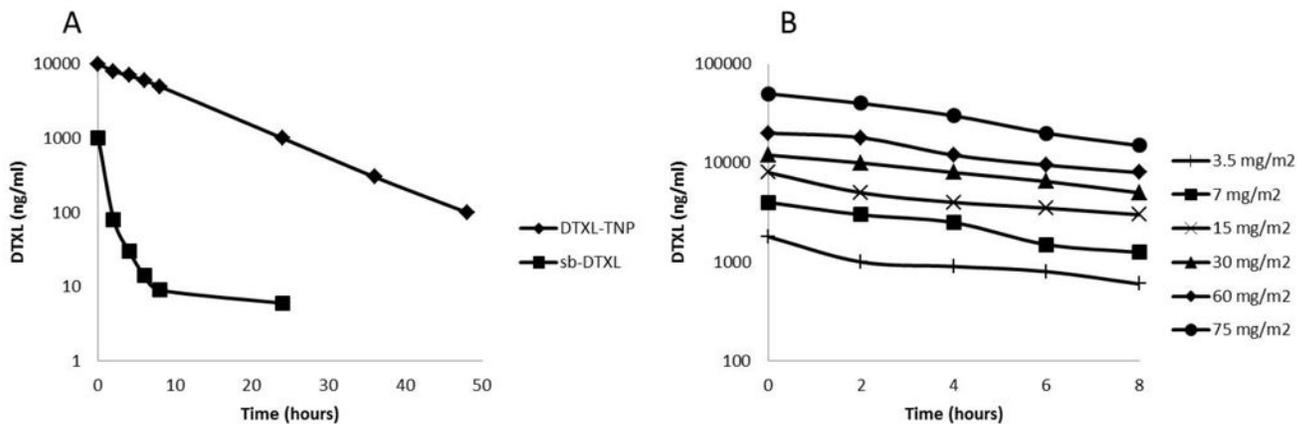


Figure 3. (A) Pharmacokinetic profile of DTXL-TNP in humans: A) comparison of plasma concentration time profile of DTXL-TNP at a dose of 30 mg/m² compared to published sb-DTXL data at the same dose in patients with advanced solid tumors (n=3). (B) Plasma concentration time profile in dose-ranging studies over the first 8 hours after single dose administration of DTXL-TNP. (Figures adapted from Reference 96).

Table 1

Summary of polymeric micelle-based formulations containing anticancer agents in clinical trials

Formulation	Drug	Polymer	Particle Size (nm)	Drug loading (%)	PK parameters (fold change over free drug)			Phase	Company
					t _{1/2}	AUC _{blood}	AUC _{tumor}		
Genexol-PM ^{83, 84, 118, 119}	Paclitaxel	mPEG-PDLLA	< 50	16.7	0.62	0.74	1.74	III, IV	Sanyang, Korea
NK105 ¹²⁰	Paclitaxel	PEG-P(Asp)	85	23.0	6.11 ^a , 3.71 ^b	86.11 ^a , 50.40 ^b	24.00 ^a , 24.06 ^b	II, III	Nanocarrier/Nippon Kayaku, Japan
SP1049C ^{93, 94}	Doxorubicin	Pluronic L61, F127	30	8.2	1.38 ^c , 1.05 ^d	2.06 ^c , 1.20 ^d	1.69	III	Suprateck, Canada
DTXL-TNP ⁹⁶	Docetaxel	PLA-PEG, PLA-PEG-ACUPA	100	10	n.a	n.a	n.a	I	BIND Biosciences
NC6004 ^{97, 121, 122}	Cisplatin	PEG-P(Glu)-Cisplatin	30	39.0	0.19	64.77	3.59	I, II	Nanocarrier, Japan
NK012 ^{99, 100}	SN-38	PEG-P(Glu)-SN38	20	20.0	16.41 ^e	14.09 ^e	9.53 ^e	II	Nippon Kayaku, Japan
NK911 ^{91, 103}	Doxorubicin	PEG-P(Asp)-Dox	40	N/A	2.62	28.88	3.46	II	Nippon Kayaku, Japan

^aDose: 50 mg/kg

^bDose: 100 mg/kg

^cData gathered in normal mice

^dData gathered in tumor-bearing mice

^eMarketed in South Korea in 2007

Table 2

Representative nanocrystal or nanoemulsion based formulations of poorly soluble drugs that are currently available commercially

Formulation approach	Commercial name	Drug	Free drug solubility (µg/ml)	Major indication	Dosage form
Nanocrystal	Triglide®	Fenofibrate	0.3 ¹²³	Hypercholesterolemia	Tablet
	Rapamune®	Sirolimus	2.6 ¹²⁴	Immunosuppression	Tablet
	Emend®	Aprepitant	3 - 7 ¹²⁵	Antiemetic	Capsule
	Tricor®	Fenofibrate	0.3	Hypercholesterolemia	Tablet
	Megace ES®	Megestrol		Antianorexia	Suspension (oral)
	Invenga™	Paliperidonepalmitate	11 - 30 ¹²⁶	Schizophrenia	Suspension (intramuscular)
Nanoemulsion	Estrasorb®	Estradiol	2 ¹²⁷	Menopausal vasomotor symptoms	Emulsion (topical)
	Restasis®	Cyclosporine	20 ¹²⁸	Chronic dry eye	Emulsion (ophthalmic)
	SandimmunNeoral®	Cyclosporine	20 ¹²⁸	Prophylaxis of organ rejection following organ transplant	SMEDDS
	Norvir®	Ritonavir	1 ¹²⁹	HIV infection	SMEDDS
	Fortovase®*	Saquinavir	29 ¹³⁰	HIV infection	SMEDDS

SMEDDS: self-microemulsifying drug delivery system

* Currently discontinued in the US

A comparison of nanocrystal-, nanoemulsion- and polymeric micelle-based nanonization approaches for delivery of poorly water-soluble compounds

Table 3

Approach	Advantages	Disadvantages
Polymeric micelles	<ul style="list-style-type: none"> Improved controlled release functions Multifunctional design possible Suitable for intravenous administration 	<ul style="list-style-type: none"> Lack of stability in blood Limited number of polymers for use Lack of suitable methods for scale-up
Nanocrystal	<ul style="list-style-type: none"> Well-understood and established manufacturing techniques Excellent reproducibility Applicable to drugs with different solubility profiles Suitable for oral administration 	<ul style="list-style-type: none"> Requires high energy input that drives up cost Needs further modification to ensure stability Lack of controlled release functions
Nanoemulsion	<ul style="list-style-type: none"> Low cost of production Suitable for various routes of administration High drug loading achievable 	<ul style="list-style-type: none"> Tendency to flocculate and coalescent Lack of stability in blood Lack of controlled release functions