3 Passive elements of active drug targeting may even become the major event when large doses of a drug are introduced by an actively targetable carrier. For example, in antibody–drug conjugates targeted to tumors, physicochemical properties of the drug carrier may reduce the carrier's antibody specificity by steric hindrance, and reduce delivery to the targeted tumor site due to strong reticuloendothelial (RES) capture.

Another important issue in the design of drug delivery systems is that they impose two opposite requirements– drug incorporation (association or binding) and drug release (dissociation or detachment). In most cases, the drug can express its pharmacological activity after its release from the carrier. If the drug is released during circulation in the bloodstream, targeting efficiency is correspondingly reduced. Thus, the incorporated drug must remain associated with carrier while in blood circulation, and be released rapidly at the target site. There are two possible solutions to this problem.

First, drug release can be designed to occur at an optimum sustained rate so as to maximize the targeting efficiency. In this approach, drug release in the bloodstream is inevitable, but favorable targeting conditions can be achieved by balancing various release parameters to reduce unwanted release in the bloodstream. Optimum drug release rate is dependent on the time period required for delivery to the target. In general drug release must be slower for carriers requiring longer time to reach their targets. The second solution is selective drug release at the target. This is achieved with carriers that release the drug at the target site by specific internal stimuli such as pH, high level of certain enzymes, or by externally applied physical triggers such as hyperthermia or sonication. These carrier systems include polymer–drug conjugates with a spacer that is specifically cleaved by hydrolytic enzymes, trigger sensitive nano- and microcapsules, liposomes, and thermoresponsive polymeric assemblies discussed in this chapter.

Polymeric Assemblies as Drug Carriers

Polymer micelles have been studied as drug carriers since 1984 [3], and more actively since 1990s [4–7]. Polymeric micelles are macromolecular assemblies of block (and graft) copolymers with core and shell structures [8]. Figure 2 shows an AB type block copolymer that can form a micellar structure if one segment of the block copolymer can provide enough interchain interactions to create the core of the micelle. Most studies of polymeric micelles, both basic and applied, have been done with AB or ABA

type block copolymers, because the relationship between the polymer structure and its micelle forming behavior can be more easily examined than that of graft or multi-segmented block copolymers.

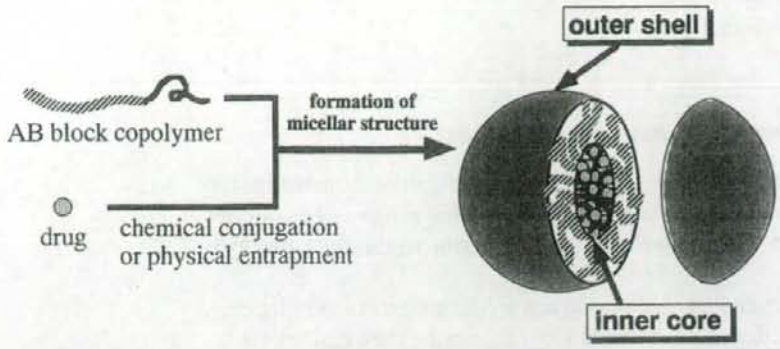


Figure 2: Formation of drug loaded polymeric micelles from amphiphilic block copolymers.

Drugs can be incorporated into polymeric micelles by both chemical conjugation and physical entrapment, and their core, shell or both. But the core is usually considered more suitable for drug incorporation. If the drug is incorporated into the shell, interactions between the incorporated drug molecules, or between the drug and the shell segment of the block copolymer may lead to micelle aggregation. Since stability of the micelles is essential, their aggregation must be avoided.

Interaction between the polymer chains that serve as the driving force for micelle formation include hydrophobic, electrostatic, π–π interactions and hydrogen bonding. Hydrophobic drugs and hydrophobic polymer blocks are the most common combinations studied, since many drugs contain hydrophobic moieties, even when they are water soluble. In fact, most reported examples of polymeric micelle drug carriers belong to this combination [9–21] [22]. Electrostatic (ionic) interactions may also be applicable to macromolecules with electric charges in combination with, for example, DNA and RNA [23]. Proteins with many charged groups such as aspartic acid and lysine residues are also suitable for this combination [24]. Hydrogen bonding and π–π interactions usually act cooperatively with other interactions. For drugs with aromatic rings, π–π interactions are considered to act cooperatively with hydrophobic interactions.

Main features of polymeric micelles used for drug delivery are as follows.

1 Small particle size (10–100 nm)
2 High structural stability
3 High water dispersibility
4 Dual core–shell functionality
5 Low toxicity

1.1 Particle size of Polymeric Micelles

Polymer micelles are formed typically in a diameter range from 10 to 100 nm with relatively narrow size distribution. This size range is considered ideal for stable long circulation in the bloodstream due to the following two reasons.

1 Carrier systems larger than about 300 nm in diameter are rapidly captured by the reticuloendothelial systems [25], therefore they cannot circulate in the bloodstream for a long enough time to deliver the drug efficiently to the target.

2 Macromolecules with molecular weights less than about 40,000 are excreted through the renal filtration system [26], and they, too, cannot remain sufficiently long in the bloodstream.

Furthermore, the small size of polymer micelles is a major benefit in sterilization of drug formulation. Polymer micelles can be readily sterilized by filtration with typical sterilization filters with 0.45 or 0.22 μm pore size.

1.2 Stability of Polymeric Micelles

Polymeric micelles possess sufficient structural stability usually due to entanglement of their hydrophobic chain segments in the core. This stability comprises two components– static and dynamic [27–30]. Static stability of polymeric micelles is defined by their critical micelle concentration (cmc). Generally, polymeric micelles have very low cmc values, much smaller than typical cmc of micelles from low molecular weight surfactants.

The second component, dynamic stability, of polymeric micelles is defined by their low dissociation rate, which may be more important than static stability for in vivo drug delivery which is necessarily under non-equilibrium conditions. When the micelle core is completely solid, dissociation of micellar structures is kinetically frozen. This high structural stability of polymer micelles is a key factor in their in vivo delivery, so as to avoid the presence of dissociated polymer chains in the formulation.

1.3 Dispersibility of Polymeric Micelles

Another important feature of polymeric micelles is their high water dispersibility. Poor water solubility or dispersibility is a general problem in drug delivery because most drug molecules are hydrophobic. In polymer–drug conjugates, for example, conjugation of the drugs with a polymer may lead to precipitation because of the highly hydrophobic drug molecules attached along the polymer chain. This problem has been reported by several research groups for the synthesis of drug–polymer conjugates [31–33] or during injection into the blood stream [34]. Therefore, conventional polymer–drug conjugates must be designed with relatively low drug contents to avoid the risk of precipitation.

But polymeric micelles maintain their water dispersibility by inhibiting intermicelle aggregation of the hydrophobic cores with their hydrophilic shell layer. Thus, polymer micelles can carry even more strongly hydrophobic drugs than do conventional polymeric carriers. For example, the maximum possible weight of adriamycin and daunomycin (adriamycin derivative) is reportedly between 10 and 35% in conventional polymer–drug conjugates [31,32,35,36], while we have reported 60% adriamycin in polymer micelles [37].

Polymeric micelles are also not subject to renal filtration due to their much larger size than the critical renal filtration size, even if molecular weight of the constituting block copolymers is usually lower than the critical molecular weight for renal filtration.

1.4 In Vivo Fate and Toxicity of Polymeric Micelles

Polymeric micelles are formed by intermolecular noncovalent interactions. This means that all polymer chains are gradually dissociated as single polymer chains, hence complete excretion from the renal route if the polymer chains are designed with a lower molecular weight than the critical value for the renal filtration. This is an important advantage of polymeric micelles over polymer–drug conjugates, and is possibly one reason for the low toxicity of polymeric micelles.

The fifth feature of polymer micelles listed above is their two-phase core–shell structure, and each phase plays one specific role or function in drug delivery. As shown in Figure 3, micelle core furnish its drug reservoir, while the shell is responsible for interaction with the biological environment, e.g. proteins and cells which determine their pharmacokinetic behavior and biodistribution. Therefore, in vivo delivery of drugs may be controlled by the micelle shell independently of its core which is designed

for drug loading and release. This two-phase structure is more favorable for constructing highly functional carrier systems than conventional polymeric carriers, since properties of each phase can be independently controlled by appropriate choice of the two chain segments in the block copolymer.

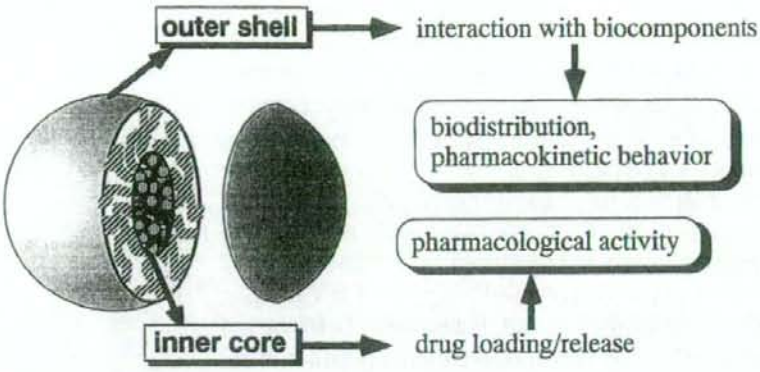


Figure 3:
Dual functionality of polymeric micelles based on a core of drug reservoir and a stabilizing shell.

2 Passive Targeting of Polymeric Micelles

Passive targeting of polymers and nanoparticulates (e.g. polymeric micelles, liposomes, and nanospheres) to solid tumors is subject to enhanced permeability and retention (epr), as proposed by Maeda et al. [26,38,39]. Figure 4 illustrates the relatively high vascular permeability of tumor tissues due to actions of secreted factors such as kinin. As a result of this increased vascular permeability, macromolecules more readily permeate the vasculature of solid tumors. Furthermore, lymphatic drainage does not operate effectively in tumor tissues. Therefore, macromolecules are retained in the tumor interstitial space for correspondingly longer periods of time. As shown in Figure 5, intravenously injected Evans blue-modified albumin is found in transplanted tumor S-180 at higher concentrations than in normal skin for up to 6 days. Epr has also been confirmed in a chemically induced tumor [40].

In principle, epr must be seen in all solid tumors generally, and hyperpermeability of solid tumors to macromolecules has been reported by many groups since 1950s [41–43]. Although there are different opinions

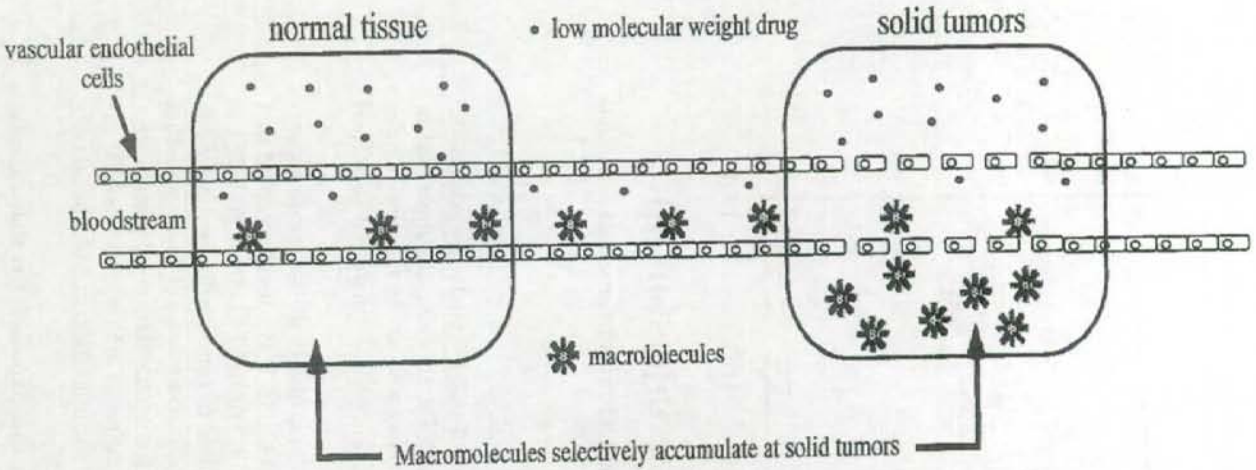


Figure 4: Schematic illustration of passive targeting of macromolecules to solid tumors by enhanced permeability and retention.

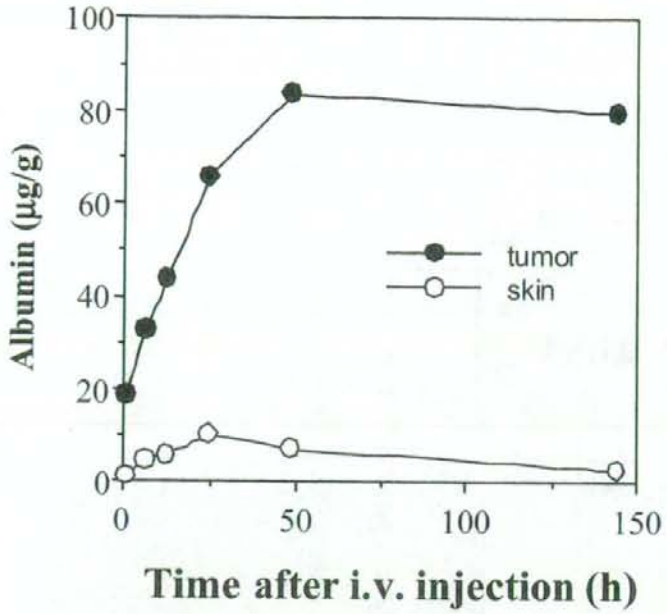


Figure 5: Selective accumulation of albumin in solid tumor by enhanced permeability and retention (modified from ref 38).

about the routes through which macromolecules translocate within the vascular endothelial cells, whether through intracellular channels [44] or intercellular junctions [45,46], there is a consensus that permeation of macromolecules through solid tumors is 3–10 times higher than that in normal tissues.

A hypothesis in support of general applicability of the epr in tumors has also been presented by Dvorak et al. [47,48]. It is widely accepted that angiogenesis (new vasculature formation) is essential to tumor growth, because sufficient nutrients cannot be supplied to tumor cells merely by diffusion from distant blood vessels [49,50]. Therefore, it is argued that vascular hyper-permeability is required for accumulation of plasma proteins in the interstitial space for the formation of new and provisional extravascular matrix, which in turn permits the migration of endothelial cells and fibroblasts for new vasculature formation.

One strong supporting evidence for this hypothesis is that vascular endothelial growth factor (VEGF) acts also as a vascular permeability factor

(VPF). In fact this specific protein for angiogenesis was first discovered as VPF [51], and its VEGF function was established later [52]. Thus, the combination of these two activities in this particular protein may indicate a link between growth and hyper-permeability in vascular endothelial cells. Whatever the exact mechanism of hyper-permeability, epr enables targeted drug delivery to tumors even without specific targeting moieties such as antibodies.

However, although many synthetic and natural macromolecules may preferentially accumulate in solid tumors, the polymer still must fulfill the following two requirements to avoid excretion or nonspecific capture in nontumor sites. Both of these two requirements can usually be met by polymeric micelles.

1 Appropriate size or molecular weight. Diameter of the carrier must be smaller than about 200 nm to evade the reticuloendothelial system [25], and molecular weight higher than a critical value of about 40,000 to evade renal filtration,
2 No strong interactions with, or uptake by, healthy organs, especially the reticuloendothelial system (RES). Cationic [53] and strongly hydrophobic [54] polymers are typically known to be captured by the RES [25]. This means that the polymer must be hydrophilic and neutral or have weakly negative charge, with no other chemical structures recognizable by normal tissues.

We have reported passive targeting of the anticancer drug, adriamycin (ADR = doxorubicin, DOX) to solid tumors with polymeric micelles based on the amphiphilic block copolymer of poly(ethylene glycol)–poly(aspartic acid) (PEG–PAsp) (Figure 6) [55–64]. For this work, first DOX was chemically conjugated to aspartic acid residues of the PAsp segment by amide bond formation. The resulting conjugate, PEG–PAsp–DOX is amphiphilic–hydrophilic PEG and hydrophobic PAsp–DOX segments, and hence forms the micelle core. In the second step, DOX was also incorporated into the micelle core by physical entrapment. This physical entrapment is assumed to involve hydrophobic and π–π interactions between the free and the chemically conjugated DOX molecules. As a result, polymeric micelles containing both chemically conjugated and physically entrapped DOX in the core, with PEG shells, were obtained.

As shown in Figure 7, the physically entrapped DOX is delivered to solid tumor in mice at much higher concentrations than free DOX. On the other hand, accumulation of the physically entrapped DOX in polymeric micelles in normal organs and tissues is similar to, or lower than, that of free DOX. The observed time profile with a peak concentration at 24 h post intravenous injection, and retention of such a high concentration of DOX

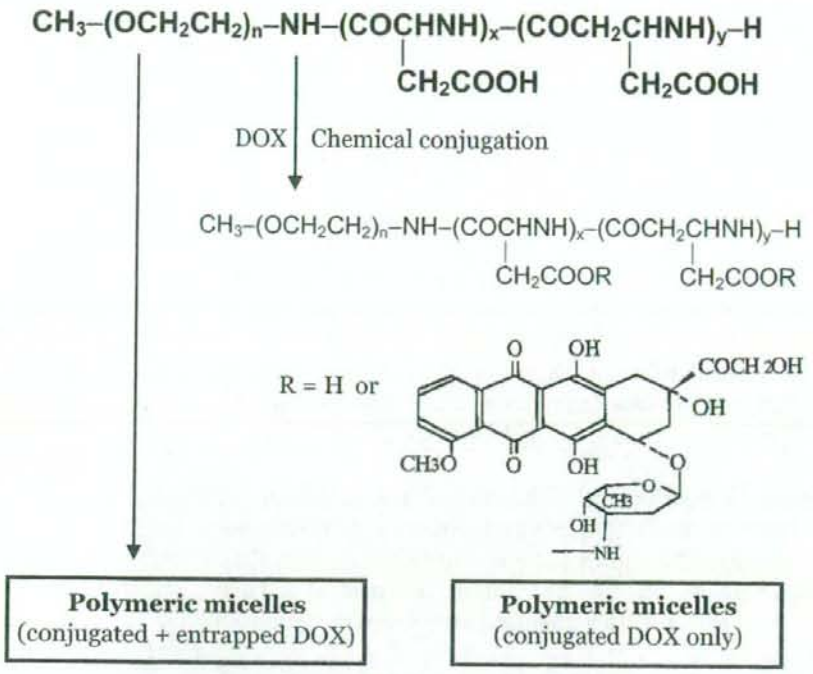


Figure 6: Chemical structure of block copolymers used for preparation of polymeric micelles containing adriamycin (= doxorubicin, DOX).

For relatively long periods post injection is consistent with the epr mechanism indicated in Figure 5.

As expected, this highly selective delivery to solid tumor resulted in marked enhancement of antitumor activity against murine colon adenocarcinoma 26, as shown in Figure 8. For free DOX, only the maximum tolerated dose (10 mg/kg body weight) provided noticeable inhibition of tumor growth, but without any tumor volume reduction. For the polymeric micelles, on the other hand, tumor completely disappeared for both 20 and 10 mg/kg doses. All the mice treated with the polymeric micelles with these two doses survived over 60 days, while control mice died after about day 20. Such large enhancement of antitumor activity is rarely reported for other types of drug carriers. These results clearly prove the strong passive targeting of DOX to solid tumors by polymeric micelles described here, and the

micelles have now passed Phase 1 clinical trials, and entered Phase 2 trial in the National Cancer Hospital, Japan, in autumn of 2003.

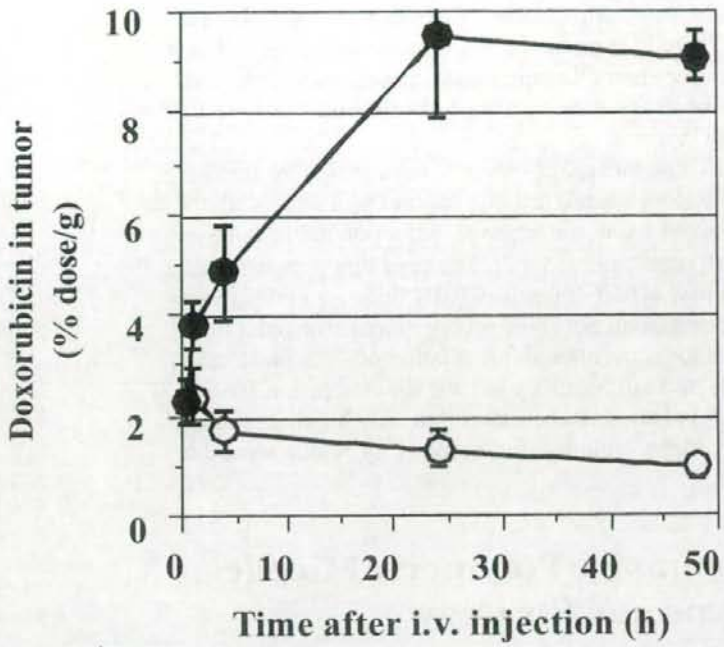


Figure 7: Selective delivery of doxorubicin (DOX) with polymeric micelles. C26 tumor-transplanted mice were injected intravenously with free DOX (○) or DOX (●) in polymeric micelles. Dose was 10 mg DOX/kg.

Interestingly little inhibition of tumor growth was observed for the polymeric micelle containing only the chemically conjugated DOX, thus indicating that the antitumor activity of the micelles is almost totally due to the physically entrapped DOX [63].

An important factor in antitumor activity in passive targeting (and generally) is optimum drug release rate from micelles. A release rate that matches well with a time period during extravasation to the endothelial cells near the tumor cells should provide a favorable drug action on tumors with minimum unwanted drug release in the bloodstream. Thus, although much enhanced in vivo antitumor activity is obtained with conventional

polymeric micelles described above, stimuli responsive and completely selective drug release at tumor site is still highly desirable. To this end, we have also developed thermoresponsive polymeric micelles, as discussed in the next two sections.

Although DOX is relatively hydrophobic, it is still water soluble, and can be used on its own. There is at present great interest in delivery of water insoluble drugs in cancer chemotherapy, since many newly developed and very potent anticancer drugs such as camptothecin and taxol are insoluble or scarcely soluble in water.

Thus, we have studied the incorporation of a water insoluble anticancer agent, KRN-5500 (KRN5500, a semisynthetic protein synthesis inhibitor and antitumor agent derived from spicamycin), into polymeric micelles based on PEG–PAsp block copolymer [65–67]. The resulting polymeric micelles show higher antitumor activity than free KRN injected in a conventional formulation. The micelles do not show severe vascular or pulmonary toxic side effects observed for conventional formulation of KRN due to toxicities of organic solvents and surfactants used for its dissolution [66,67]. These results indicate that polymeric micelles are not only highly useful for drug targeting, but also highly suitable for delivery of water insoluble drugs.

3 Thermoresponsive Polymeric Micelles: Polymer Type and Strategy

3.1 Multi-targeting Systems

As discussed above, passive targeting of anticancer drugs with polymeric micelles is achievable by combining sustained drug release with epr. However, undesirable drug release in the bloodstream cannot be totally avoided by passive targeting. Thus the potential of polymeric micelles can be better utilized by designing active targeting for selective delivery to diseased sites.

Figure 8 (opposite page): In vivo antitumor activity of free doxorubicin (DOX, top) and polymeric micelle containing DOX (bottom). C26 tumor transplanted mice were injected intravenously with free DOX or polymeric micelles. Drug injection started on day 8 past tumor transplantation. Drug injection was repeated three times every 4 days. Dose is expressed only for DOX physically entrapped polymeric micelles. The chemically bond DOX is used for its binding effect, and is not released from the polymer. Arrows indicate days of injection.

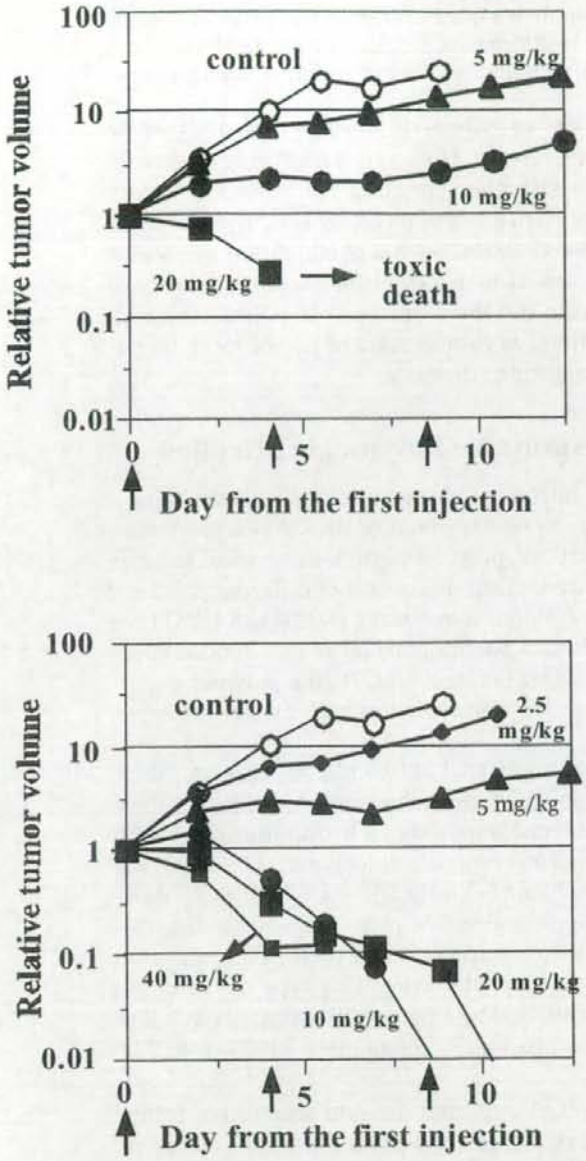


Figure 8: See caption on opposite page.

To this end, various stimuli responsive drug carriers responsive to specific internal or external triggers, including local pH, enzymes, electric and magnetic fields and temperature change are being widely studied, as covered in this book.

For example, thermoresponsive polymeric micelles are designed to achieve temperature driven drug release. However, when using thermoresponsive polymeric micelles, even higher targeting efficiency can be obtained by combining thermoresponsive active targeting (i.e. hyperthermia) with passive targeting as discussed above. Such a combination provides a multi-targeting system, as illustrated in Figure 9 for targeted delivery of anticancer drugs with amphiphilic and thermoresponsive polymeric micelles. Multi-targeting is, thus, defined as combination of two or more targeting methodologies to increase targeting efficiency.

3.2 Type of Thermoresponsive Polymeric Micelles

Thermal responsiveness can be introduced to polymeric micelles by using a temperature responsive polymer as one segment of the diblock copolymer to form the micelles. For this purpose, polymers with lower critical solution temperature (LCST) are often used. For a discussion of different polymers with lower and upper critical solution temperatures (LCST and UCST) see ref [69]. In LCST polymers, below LCST the polymer is in extended chain form, hydrophilic and water soluble, but above LCST, the polymer undergoes a reversible phase transition, it becomes hydrophobic, water insoluble, and forms aggregates.

Since polymeric micelles are composed of two phases, core and shell, two types of thermoresponsive polymeric micelles can be designed as illustrated in Figure 9. Block copolymers composed of a hydrophilic block such as poly(ethylene glycol) and a thermoresponsive block with LCST, are water soluble as extended polymer chains below LCST. Upon heating to above LCST, the thermoresponsive block undergoes phase transition, becomes water insoluble, the polymer chains aggregate with each other, and thus form a micellar structure with hydrophobic core. This type of thermoresponsive polymeric micelles was studied by Feijen et al. using poly(ethylene glycol)–poly(N-isopropylacrylamide) block copolymers (PEG–PNIPAM, LCST 32 °C) [68,70].

In another study Hennink et al. reported the synthesis of poly(ethylene glycol)–poly[N-isopropylacrylamide–(N-2-hydroxypropyl methacrylate)]–g-poly(lactic acid). This polymer has an LCST below 32 °C due to the effect of the hydrophobic poly(lactic acid) grafts, and forms polymeric micelles at 37 °C. After hydrolysis of the poly(lactic acid) grafts, the resulting

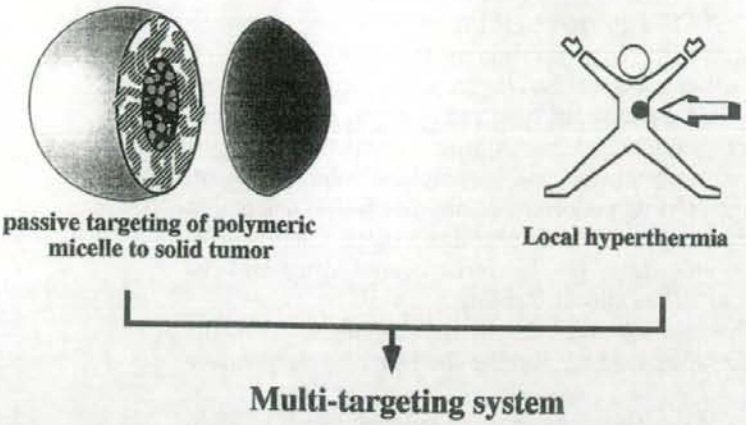


Figure 9: Schematic illustration of a multi-targeting system based on thermoresponsive polymeric micelles and hyperthermia.

block copolymer, poly(ethylene glycol)–poly[N-isopropylacrylamide–(N-2-hydroxypropyl methacrylate)] does not form micelles at 37 °C because its LCST increases to above 37 °C due to contribution of the hydrophilic poly(N-2-hydroxypropyl methacrylate) segment [70a].

Thermoresponsive polymeric micelles can also be prepared from block copolymers with a hydrophobic segment and a thermoresponsive hydrophilic segment, and hence forming micelles in which thermal responsiveness resides in the micelle shell. Below the LCST, micelles with hydrophobic core and hydrated shell are formed. Upon heating above LCST, the shells shrink and become hydrophobic, leading to smaller micelles with shrunken shells. The shrunken micelles may also aggregate, depending on micelle concentration and the strength of hydrophobic interactions between the shrunken shells. Examples of this type of micelles are discussed in the last section.

In comparing the two types of thermoresponsive polymeric micelles, a possible advantage of the micelles with thermoresponsive core is faster drug release upon thermal stimuli, because the hydrophobic core that acts as drug reservoir breaks up upon temperature change to below LCST. But these micelles have also two main disadvantages.

1 Temperature must be lowered to induce drug release. In medical applications, temperature can only be lowered in limited body regions such as skin and blood vessels that can be accessed from outside the body or with medical catheters. On the other hand, when using upper critical

solution temperature (UCST) polymers [25] many body organs can be heated to different depths with hyperthermia instruments.

2 When micelle core is thermoresponsive, its scope for drug loading becomes necessarily limited, because the type and strength of interactions required for drug incorporation, maximum drug content, incorporation efficiency, stability, or drug release rate may not be compatible with thermal responsiveness. If drug molecules do not match the characteristics required for thermal responsiveness above the LCST, the drug cannot be incorporated efficiently, or the incorporated drug may be released too quickly due to low micelle stability.

In contrast, in polymeric micelles with thermoresponsive shell, the core provides flexibility for drug loading, and the shell enables temperature control for drug release.

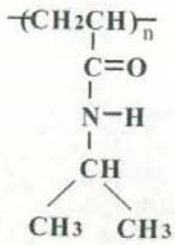
Chemical structures of two thermoresponsive polymers with LCST in aqueous media are shown in Figure 10. PNIPAM is the most widely used synthetic temperature responsive polymer not only in drug delivery but also in biomaterial studies. One major reason for the frequent use of this polymer is that its LCST is near the body temperature. PNIPAM has a phase transition temperature of 32 °C in water, and this phase transition temperature can be modified to a more suitable temperature of about 39 °C for multi-targeting micelles by incorporation of hydrophilic comonomers such as N,N-dimethylacrylamide.

Alternatively, an elastin-like peptide (Figure 10) has been studied for biomedical applications by Chilkoti et al. [69,70]. This pentapeptide has an LCST of 40 °C, and because it is produced by genetic engineering, is monodisperse, a feature that is not available in synthetic polymers. Chilkoti et al. reported that accumulation of this polymer in a solid tumor can be enhanced by hyperthermia application [69].

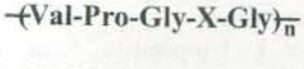
4 Thermoresponsive Polymeric Micelles for Drug Targeting

We have studied PNIPAM for preparation of polymeric micelles with thermoresponsive shells [71–76], and poly-D,L-lactide (PLA) and poly(butyl methacrylate) (PBMA) for their hydrophobic polymer segments that form the hydrophobic cores. PLA was chosen for its biodegradability, and PBMA for its chain flexibility required for structural changes on temperature change.

In PNIPAM–PBMA, LCST of thermoresponsive block, PNIPAM, is 32 °C. But for combination of PNIPAM with PLA, a hydrophilic monomer, N,N-dimethylacrylamide, was copolymerized with N-isopropylacrylamide



Poly(N-isopropylacrylamide)

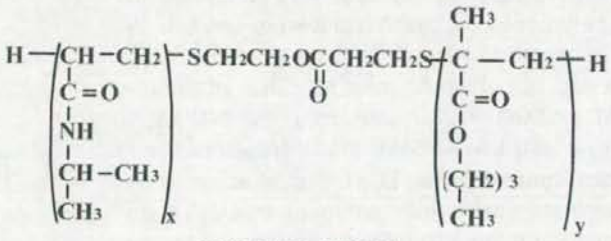


X : Val : Gly : Ala = 5 : 3 : 2

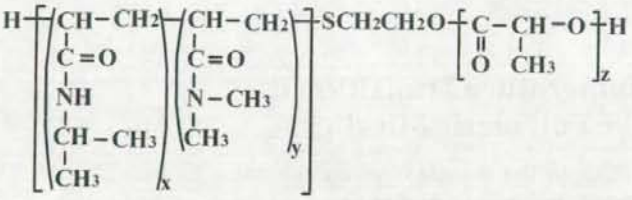
Elastin-like polypeptide (ELP)

Figure 10: Chemical structures of two early examples of thermoresponsive polymers reported for preparation of thermosensitive micelles.

to adjust the phase transition temperature of the thermoresponsive segment to 39–40 °C, which is considered more suitable for therapeutic hyperthermia. Chemical structures of both these block copolymers are shown in Figure 11.



PNIPAM–PBMA



P(NIPAM–DMA)–PLA

Figure 11: Chemical structure of block copolymers for thermoresponsive polymeric micelles. PBMA, poly(butyl methacrylate); PLA, poly(lactic acid)

Preparation of Thermoresponsive Polymeric Micelles

Polymeric micelles were prepared, as follows. The polymers were dissolved in a water miscible organic solvent such as N,N-dimethylacetamide, followed by dialysis against water at a temperature below their LCSTs. Micelles containing the anticancer drug, DOX, were also prepared similarly by mixing DOX with the block copolymers in organic solvent, followed by the same dialysis procedure.

Thermoresponsive character of the polymeric micelles was confirmed by turbidity measurements as shown in Figure 12. Thus, aqueous dispersion of PNIPAM–PBMA micelles (molecular weights of PNIPAM 6100 and PBMA 8900, 0.5 wt% micelles) was heated with a circular water jacket at a heating rate of 0.1 °C/min until the dispersion suddenly lost its transparency in a very narrow temperature range around 32 °C. It became visibly turbid due to aggregation of the polymeric micelles by intermicelle hydrophobic interaction of the dehydrated shells. Interestingly the observed phase transition temperature of the micelles was identical to that of PNIPAM.

Although the thermoresponsive polymer segment was connected to the hydrophobic core-forming polymer block, the thermoresponsive behavior was not influenced by the connected hydrophobic segment. This is an important advantage in the design of polymeric micelles. This advantage was confirmed for P(NIPAM–DMAA)–PLA (mole ratio of NIPAM/-DMAA/LA = 43/13/43) micelles which had an ideal phase transition temperature of 40 °C in physiological buffers. Thus, LCST can be adjusted by copolymerization of NIPAM with the hydrophilic comonomer DMAA (in this case to 40 °C), but the transition temperature is not influenced by the connected PLA segment. Nor did the indicated phase transition temperatures of the two types of polymeric micelles we studied changed by incorporation of DOX.

4.1 Mechanism of Temperature Transition in Thermoresponsive Polymeric Micelles

We studied the changes occurring in the micelle core upon heating by incorporating pyrene in the micelles, and measuring its emission spectrum to determine the ratio I_1/I_3 of its first and third vibrational bands. This ratio is larger when pyrene is in more hydrophilic environments. Figure 13 shows changes in I_1/I_3 of PNIPAM–PBMA micelles against temperature, and indicates that the micelle core is more hydrophilic above the transition

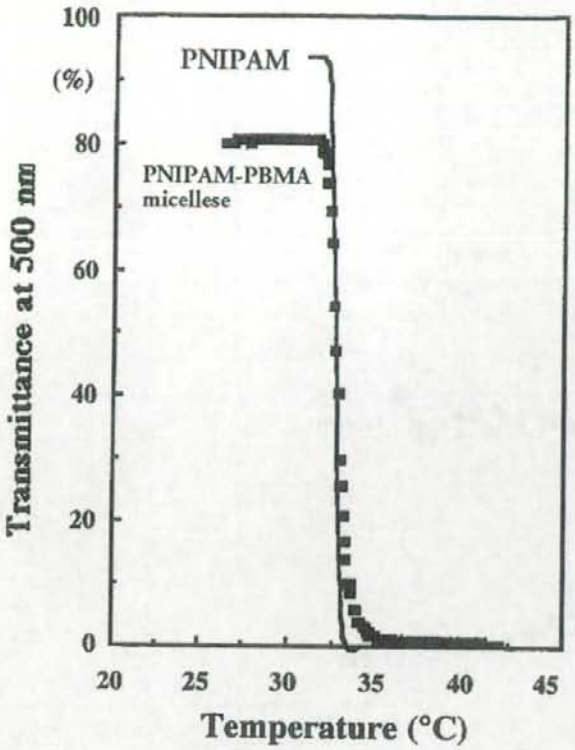


Figure 12: Phase transition behavior of PNIPAM–PBMA micelles measured by turbidimetry of its aqueous dispersion.

temperature during both heating and cooling. This change is in opposite direction to that of the hydrophilic–hydrophobic transition in PNIPAM shell.

As illustrated in Figure 14(1), hydrophobicity of PBMA core decreases when mixed with the dehydrated shell above its transition temperature, but when the shell becomes hydrophobic below LCST, it is phase separated. Upon heating above the transition temperature, the shell changes to hydrophobic and becomes miscible with the core. But the dehydrated shell segments still have lower hydrophobicity than the core-forming segments. Accordingly, the hydrophobicity of the core is reduced by this phase mixing with the less hydrophobic PNIPAM block. In fact, the I_1/I_3 ratio in the

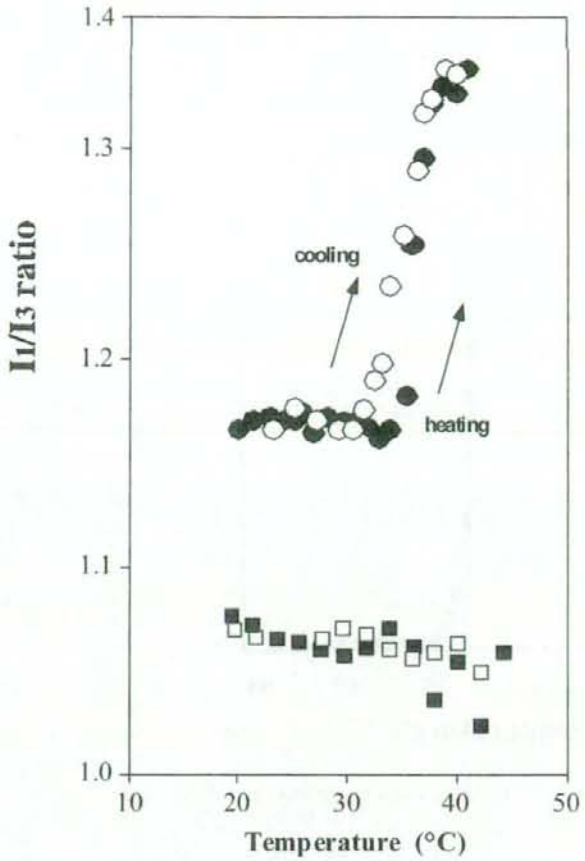


Figure 13: Changes in pyrene fluorescence as a measure of hydrophobicity of micelle core in PNIPAM–PBMA and PNIPAM–PS micelles.

○,●, PNIPAM–PBMA; □,■, PNIPAM–PS; ■,● heating cycle; □,○, cooling cycle. PBMA, poly(butyl methacrylate); PS, polystyrene.

dehydrated PNIPAM homopolymer is 1.38, which is larger than that of PNIPAM–PBMA micelles above LCST, i.e. the homopolymer is less hydrophobic than the copolymer.

These considerations are important, because the hydrophobic drug is released more rapidly from less hydrophobic polymer matrixes due to

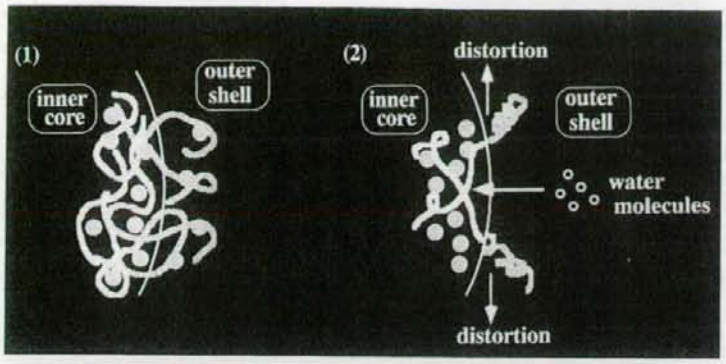


Figure 14: Two possible mechanisms of enhanced drug release from polymeric micelles above their LCST.

larger diffusion coefficients, and smaller partition coefficients, of the drug in hydrophilic matrixes. Therefore, enhanced drug release is expected at temperatures above LCST.

Alternatively, the shell may aggregate upon heating above the transition temperature. This aggregation may distort some polymer segments in the core because they are chemically connected to the aggregated shell segments. Due to this distortion, water molecules penetrate the core, and the core may become less hydrophobic by penetration of water, as illustrated in Figure 14(2). Consequently, water channels provide a conduit for drug release, as the core becomes more hydrophilic. However, it has not been experimentally established which of these or other mechanisms can best describe the changes in the micelle core upon heating above the transition temperature.

In case of PNIPAM–PS micelles, on the other hand, the fluorescence probe did not show any changes, as shown in Figure 13 (the lower plots). PNIPAM–polystyrene micelles showed a phase transition of 32 °C by turbidity measurement. A possible reason for this difference in the two micelle types in Figure 13 may be the stronger hydrophobicity and rigidity of PS relative to PBMA. In fact, PS core is more hydrophobic than PBMA core, as revealed by its lower I_1/I_3 ratio. It is also more rigid than PBMA core, as evident from fluorescence measurement of the incorporated 1,3-bis(1-pyrenyl)propane that was used as a flexibility–rigidity marker. Although it