



Amphiphilic Block Copolymer Micelles for Drug Delivery Vehicles

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 July 1st, 2010*

Abstract

Amphiphilic block copolymer micelles have been the focus of research for the last decades because of the promising properties as nano size drug delivery vehicles. In polar aqueous milieu, copolymers self assemble into micelle structures which consist of hydrophilic corona and hydrophobic core regions. Space created inside the micelle core can be used for transportation of drug molecules of hydrophobic nature, low solubility in blood, and with numerous side effects to human body, especially those for cancer treatments. In this paper, I present an overview for use of amphiphilic block copolymers as drug delivery systems by addressing the formation theory of self assembled micelles, factors affecting the efficacy of drug delivery vehicles and a number of methods to improve it. Two main functionalities of drug delivery vehicles with temporal and distribution controls will be discussed. Former term refers to protection of drug molecules from the enzymatic degradation during the circulation in bloodstream and latter refers to accumulation of vehicles around the target site.

Keywords: Amphiphilic block copolymer micelles, drug delivery vehicles, temporal and distribution controls, core and corona cross-linking, surface functionalized micelles.

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1. Introduction

In order to maximize therapeutic effects of drugs and to minimize the negative side effects, drug delivery vehicles have been developed in past decades. One of the most widely used drug delivery systems is the self assembly of amphiphilic block copolymer carriers in micelle forms in aqueous environment. Distinctive property of micelles for the delivery applications stems from its unique structural composition which consists of two or more hydrophilic and hydrophobic blocks with different solubility ratios in aqueous milieu. The hydrophobic polymer block has a low solubility in polar solvents and forms the core of the micelle whereas the hydrophilic segment has the reverse solubility and forms the corona, in other words, the outer shell of the micelle. Formation theory of the micelles concerning the thermodynamic parameters will be discussed in section 2 of this review paper.

Amphiphilic block copolymer micelles for drug delivery applications are significant mainly in two aspects. First of all, hydrophobic drug molecules can be loaded to the micelle core to be carried to the target with a higher solubility than its intrinsic solubility in bloodstream which shows hydrophilic properties. This would bring the advantage of accumulating higher amount of drug molecules around the target site and thereby rapid and intensive healing. Secondly, the corona part of the micelle which is usually poly ethylene oxide (PEO) provides an impermeable shell for proteins and enzymes; hence prevents hydrolysis and enzymatic degradation of the drug molecules during the transport to the target. In blood circulation, an important obstacle is the recognition of the drug molecules by the reticuloendothelial system (RES) which is a part of the immune system and consists of the phagocytic cells located in reticular connective tissue, primarily monocytes and macrophages. The PEO corona of the micelle blocks this system identifying the drug molecules and causing prolonged circulation time of drug molecules in the bloodstream [1].

Two primary factors influencing the efficacy of drug delivery system by block copolymer aggregates are temporal and distribution controls [2]. The former term refers to the required time and process to release the drug molecules from the inside of micelle core to the target. The latter term means is associated to the drug molecules are distributed to the target site and accumulated in these special regions without getting lost in other parts of the circulation system. In many examples of mechanism for temporal control, lower critical solution temperature (LCST) property of the block copolymers has a significant role. Variation to above or below LCST breaks up the structure of the micelles and release of the drug molecules is triggered. As an alternative to temperature which is based on LCST, in some systems start of the drug release could be prompted by magnetic field variations. However distribution control, particularly for cancer treatments, is achieved by exploiting the enhanced permeability and retention effect. Increase in the accumulation of drug molecules around the target is rationalized by combination of the increased permeability of tumor blood vessels and the decreased rate of clearance caused by the lack of functional lymphatic vessels in the tumor.[3]

In this paper, formation theory of micelles, effects on size and distribution of micelles for drug delivery applications, modifications made on core or corona regions of the micelles to improve the temporal and distribution control will be covered.

2. Micelle as a Whole

2.1 Micelle Formation Theory

Self assembly of soft molecules in aqueous environments can be driven by the weak van der Waals, hydrophobic, hydrogen-bonding and screened electrostatic forces between the molecules. Since these forces are not as strong as covalent bonding, bonds are readily formed and broken up by small changes in environment. For this reason the thermodynamic viewpoint of self assembly of these molecules, micelles in particular, will be considered first in this paper. Chapter 16 and 17 of Intermolecular and Surface Force by Jacob Israelachvili investigate these relations for aggregates in detail and following derivations were taken from that book [4].

For systems to be in equilibrium chemical potential of the constituents should be equal to each other. Otherwise, constituents undergo a change which will end up satisfying this equality if the driving forces are present in the system. For micelles to be formed and kept in equilibrium, this condition can be formulized as follows:

$$\mu = \mu_1^0 + kT \log X_1 = \mu_2^0 + \frac{1}{2} kT \log \frac{1}{2} X_2 = \mu_3^0 + \frac{1}{3} kT \log \frac{1}{3} X_3$$

monomers dimers trimers

or

$$\mu = \mu_N = \mu_N^0 + \frac{kT}{N} \log \left(\frac{X_N}{N} \right) = \text{constant}, \quad N = 1, 2, 3, \dots,$$

where μ is the mean chemical potential of a molecule in an aggregate of aggregation number N , μ_N^0 the standart part of the chemical potential (the mean interaction free energy per molecule) and X_N the concentration (strictly saying, the activity) of molecules.

From these calculations following relation can be obtained:

$$X_N = N \{ X_1 \exp[(\mu_1^0 - \mu_N^0)/kT] \}^N$$

conservation of concentration requires that

$$C = X_1 + X_2 + X_3 + \dots = \sum_{N=1}^{\infty} X_N$$

The mean interaction free energy per molecule can be expressed as:

$$\mu_N^0 = \mu_{\infty}^0 + \frac{\alpha kT}{N^p}$$

where α is a positive constant depending on the strength of the intermolecular interactions and p is a number that depends on the shape or dimensionality of aggregates.

For micelles to be formed, the concentration of block copolymer should exceed the certain amount which is calculated as following by combining equations given above:

$$X_N = N\{X_1 \exp[(\mu_1^0 - \mu_N^0)/kT]\}^N = N\{X_1 \exp[\alpha(1 - 1/N^p)]\}^N \approx N[X_1 e^\alpha]^N$$

From this equation it can be read that at very low monomer concentration, i.e. when $X_1 e^\alpha$ is much less than unity, X_N is very low. It means that $X_1 \sim C$. Keeping in mind the fact that X_N can never exceed unity, the equation above restricts the monomer concentration X_1 by maximum value of $e^{-\alpha}$. This point is defined as critical aggregate concentration (CAC) or more commonly used as critical micelle concentration (CMC). Definition of the critical point is represented in Fig 1 clearly.

$$(X_1)_{critical} = CMC \approx e^{-\alpha}$$

In order to understand the structural aspects of self assembly, not only thermodynamic viewpoint is enough but the forces between the amphiphilic molecules within the aggregates and how these are affected by solution condition also need to be known.

Driving forces for the formation of micelles are those attractive interactions between the hydrocarbon molecules at the hydrocarbon-water interface and those repulsive interactions which are due to hydrophilic, ionic or steric forces in the headgroup. The tradeoff between the attractive forces which favors the formation of aggregates and repulsive forces which try to keep the monomers apart determines the final structure and shape of the micelle by minimizing the total interfacial free energy which can be formulized as in the equation below.

$$\mu_N^0 = \gamma\alpha + K/\alpha,$$

where a is area per molecule and γ is free energy per unit area which is specific to hydrocarbon-water interface. K is a constant and K/a represents the repulsive forces. By taking the derivative of this equation and make it equal to zero, the optimal head area a_0 can be calculated.

$$\mu_N^0(\min) = 2\gamma\alpha_0, \text{ and } \alpha_0 = \sqrt{K/\gamma}$$

$$\mu_N^0 = 2\gamma\alpha_0 + \frac{\gamma}{\alpha}(\alpha - \alpha_0)^2$$

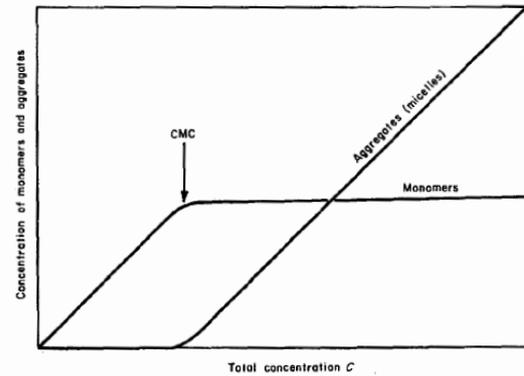


Figure 1 CMC analysis

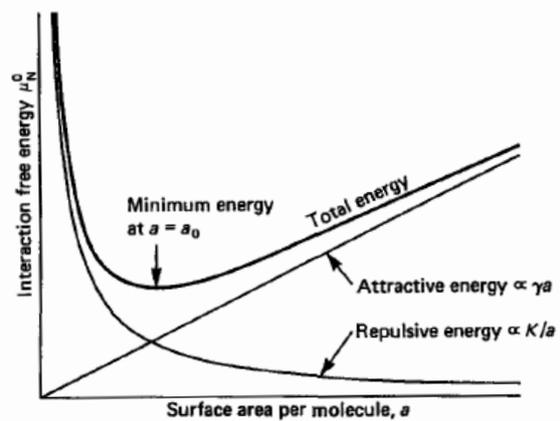


Figure 2 Energy balance in micelle formation

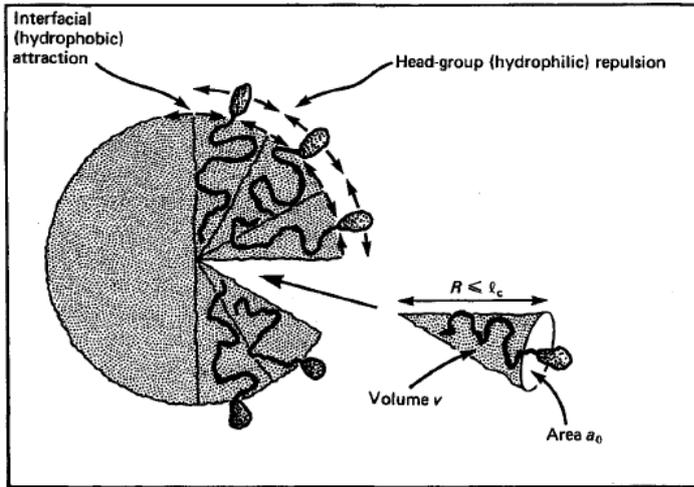


Figure 3 Micelle structure

By taking into account the optimal head area, critical length and volume of the molecules, shape of the anticipated micelle can be figured out. The relation between these parameters and the micelle structure was derived by Israelachvili and summarized in the table below.

Table 1 Parameters determining shape of the micelle

Lipid	Critical packing parameter v/a_0l_c	Critical packing shape	Structures formed
Single-chained lipids (surfactants) with large head-group areas: <i>SDS in low salt</i>	$< 1/3$	Cone 	Spherical micelles
Single-chained lipids with small head-group areas: <i>SDS and CTAB in high salt, nonionic lipids</i>	$1/3-1/2$	Truncated cone 	Cylindrical micelles
Double-chained lipids with large head-group areas, fluid chains: <i>Phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, sphingomyelin, DGDG^a, dihexadecyl phosphate, dialkyl dimethyl ammonium salts</i>	$1/2-1$	Truncated cone 	Flexible bilayers, vesicles
Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains: <i>phosphatidyl ethanolamine, phosphatidyl serine + Ca²⁺</i>	~ 1	Cylinder 	Planar bilayers
Double-chained lipids with small head-group areas, nonionic lipids, poly (<i>cis</i>) unsaturated chains, high <i>T</i> : <i>unsat. phosphatidyl ethanolamine, cardiolipin + Ca²⁺, phosphatidic acid + Ca²⁺, cholesterol, MGDG^b</i>	> 1	Inverted truncated cone or wedge 	Inverted micelles

^a DGDG, digalactosyl diglyceride, diglucosyl diglyceride.

^b MGDG, monogalactosyl diglyceride, monoglucosyl diglyceride.

In a nutshell, formation of micelles is driven by the competing attractive and repulsive forces which arise from the interactions between the solvent and the different blocks of the copolymer. If a polar solvent used, the hydrophilic segment of the copolymer forms the outer shell whereas hydrophobic segment forms the core region. In a non polar solvent, the structure would be reversed. Since the blood is a polar solvent, hydrophobic block forms the core of the micelle and creates a good space for hydrophobic drugs to be carried in.

2.2 Micelle Preparation

Two methods are exploited in order to prepare block copolymer micelles [1]. The first method is called direct dissolution and includes addition of the copolymer to water or another aqueous environment. The dialysis technique, the second method, requires dissolution of the copolymer first in an organic compound that is miscible with water such as dimethylformamide, tetrahydrofuran, or dimethylacetamide and then solution is mixed with water at controlled rate and eventually evaporation of the organic solvent remains copolymer molecules in micelle form in water solvent. The choice of which method to be used depends on the solubility of the copolymer in water or aqueous environment. If solubility of the copolymer in water is marginal, direct dissolution method is preferred whereas for low solubility dialysis method is. Amphiphilic molecules consist of two different segments which differ especially in solubility and the overall solubility is determined by the length of the blocks.

In dialysis method, the size and size population distribution of the micelles are influenced by the chosen organic solvent and the mixing rate of organic solvent with water. At a certain critical water concentration, formation of micelles initiated and after this point the number of micelles forming continues to grow. La et al. [13] have exploited the dialysis method in their micelle systems and found out how the solvent affects the size of the micelles. The use of DMSO as an organic solvent gave rise to formation of poly-(ethylene oxide)-poly(α -benzyl L-aspartate) (PEO-b-PBLA) micelles around 17 nm in size with 6% yield. However, when the organic compound was chosen as N,N-dimethylacetamide (DMAc), micelles were produced in high yield and also average 19 nm size was obtained with very narrow distribution. In this respect, dialysis method can be used to tailor the micelle properties based on the requirements of the application.

Loading of drug molecules into the core of the empty micelles depend on the way how the micelles are prepared. If the direct dissolution method is employed, drug stock solution could be prepared with an organic solvent like acetone in a veil. Then mixing of the micelle solution and drug solution could incorporate the drug molecules into micelle core after evaporating the drug stock solvent. While preparing the micelle solution with dialysis method, drug molecules can be dissolved in the solvent in which amphiphilic block copolymer already has been dissolved. The reason of this solvent being appropriate for the drug molecules is that the solvent was already chosen such that it can dissolve the hydrophobic part of the copolymer and thereby drug molecules which are hydrophobic too. During the addition of the organic solvent containing copolymer and drug molecules to water, micelles are formed and drugs are incorporated into the micelle core at the same time.

Having micelles formed and drug molecules incorporated into the core, the next step is to transport the drug vehicles without getting any damage during the circulation in bloodstream. From thermodynamic point of view, stability of micelles is measured by critical micelle concentration. As discussed in the previous section, opposite forces created by hydrophobic and hydrophilic parts of the micelle requires a lower limit to keep the structure of drug delivery vehicle steady. This lowest limit becomes important upon intravenous administration because the concentration of drug vehicles gets lower immediately and it might cause dissociation of micelles by ending up with the release of drug molecules. For this reason CMC value of the drug vehicles should be controlled very well and considered while designing a new micelle system for therapeutic applications.

CMC value depends on the ratio of length of the hydrophobic part to that of hydrophilic part. Low ratio copolymer micelles have higher CMC value compared to high ratio ones because attractive forces which arise from hydrophobic segment drive self assembly of the micelles and lowering the ratio of hydrophobic chain brings the CMC to a higher point. Many studies have been conducted so far to understand how the chain length ratio affects the CMC. Astafieva's [5] group carried out a study in which effects of polymer chain length on CMC was investigated for the copolymer polystyrene-b-poly(sodium acrylate) (PS-b-PANa) micelles. In water as solvent, micelles form of insoluble and hydrophobic PS blocks in the core region and hydrophilic PAN blocks in the corona region. It was reported that increase in the ratio of hydrophobic/hydrophilic segments from 6/400 to 110/380 monomer units resulted in dramatic decrease of CMC from 4.2×10^{-5} to 8×10^{-8} mol/L. In 2008, a more recent study was done by Wang et al.[3] in which CMC was determined by using a fluorescence spectroscopic method based on the preferential partition of pyrene probe in hydrophobic core against an aqueous environment and concluded that the increase from 27 to 186 units in degree of polymerization of hydrophobic block, PCL in triblock copolymer poly(ethyl ethylene phosphate) and poly(-caprolactone) (PEEP-PCL-PEEP) caused a sharp decrease in CMC from 10.2 to 0.55×10^{-3} gL⁻¹. [3] As these studies show and as it is expected, the increase in the hydrophobic segment of the copolymer lowers the CMC value which enhances their potential to be used in actual treatments by preventing dissociation of drug vehicles upon intravenous administration.

While considering the thermodynamic aspect, kinetics point of view also should not be skipped because it might create cases which can overrule thermodynamics. For instance, drug vehicles can still survive in bloodstream even below CMC, if the glass transition temperature of core forming polymer block is high, i.e. higher than body temperature of 37 Celsius, core forming segment can behave like a solid which enhances the required energy and time to distort micelle structure. Hence by controlling the glass transition temperature, copolymers can be utilized below their CMC values.

Other crucial properties which should be considered while preparing drug delivery vehicles are the size and size population distribution of micelles. Those micelles which are smaller than 200 nm in size are less susceptible to RES clearance and those smaller than 5 μ m are able to enter small capillaries. Therefore size of the micelles is one of the determining factors of drug vehicle efficacy and thereby parameters affecting the micelle size are required to be

revealed. Size of the micelles formed from block copolymers primarily depends on the chain length of the segments. According to Halperin's [6] study which was carried out in 1987 based on the Daoud Cotton model for star like polymers in which A and B blocks refer to the soluble and insoluble segments with polymerization degree N_A and N_B respectively, it was figured out that core radius of the micelle varies by $N_B^{3/5}$ and overall micelle radius scales as $N_B^{4/15}N_A^{3/5}$.

In addition to micelle size factor, population distribution of the micelle size is also an important issue in drug delivery vehicles. It should be apparent that micelles with size of 10 and 200 nm are expected to have different circulation and disposal start time. In order to gain more power on the control of injection and disposal of drug vehicles, size distribution of micelles should be uniform as much as possible. One of the acceptable explanations for why some micelles are much larger than the others, even though they are formed in the same solvent, is that large size clusters might be formed by agglomeration of small size micelles due to the secondary attractive forces between hydrophobic micelle cores. In order to avoid formation of these large aggregates, concentration of solution can be lowered by dilution. This solution was exploited by Allen et al. [1] and their results showed that population of large aggregates were decreased in PCL-b-PEO micelles by reducing the concentration from 0.2% (w/w) to 0.01% (w/w).

2.3 The micelle corona

The micelle corona acts as a stabilizing interface between the insoluble micelle core and the external medium. The properties of the corona mostly influence the biodistribution of drug vehicles and thereby that of incorporated drug as well as its pharmacokinetic parameters. Predominantly used molecule for outer shell segment of the micelle is poly ethylene oxide (PEO) which has unique features for drug delivery applications.

2.3.1 PEO as a corona component

In drug delivery vehicles, PEO with a molecular weight of 1000 to 12000 g mol^{-1} and with a length which is greater than or equal to that of micelle core chain is usually employed. The bulk PEO is a non ionic, crystalline, thermoplastic polymer at room temperature. The unique feature of PEO comes along with its solubility in water which has been reported as unlimited at room temperature for all degrees of polymerization.

The steric stabilization of colloidal particles is provided by the repulsive forces arising from hydrophilic block and on the contrary attractive van der Waals forces resulting from interaction between the hydrophobic parts. In this respect, solution properties of hydrophilic PEO provide a good stabilizer for drug delivery vehicles.

Another important functionality of PEO corona, in addition to protecting hydrophobic segment, is that protecting the incorporated drug molecules by creating an impermeable shell against proteins and enzymes which are present in blood. If the vehicles were destroyed by the enzymes, drug molecules would be blocked by the immune system too and accumulation of drug molecules on the target site would be very inefficient and not knowing how much of drug molecules reacting on the tumor site would lower the power of control on the treatment.

Steric stabilization effect of PEO depends on the surface density and thickness of the outer shell. As stated before, secondary attractive forces between micelle cores are responsible for the aggregation of small micelles to form large cluster. However surface density and thickness of the shell take a role to screen these attractive forces by insulating the interior from the outside medium. Surface density of PEO is measured by the aggregation number which is defined as number of chains per micelle. The higher aggregation number possessed, the higher surface density is obtained.

In order to understand the effect of surface density on biodistribution of drug incorporated vehicles, Hagan et al. [7] carried out a set of experiments in which micelles formed from polylactide-b-poly(oxyethylene) (PLA-b-PEO) copolymer were investigated with two different composition ratios. It was shown that PLA-b-PEO with ratio 1.5:2 transported higher amount of drug to the target site than the one with a ratio 2:5 by comparing the amount of collected vehicles in the livers.

2.3.2 Other corona components

PEO has been studied many times for drug delivery vehicles because of its superior properties compared to other copolymers. However it is not the only one and there are some other copolymers developed to be used in the corona region of the micelles such as oligo(methyl methacrylate) (oMMA) and poly(acrylic acid) (PAAc) which have been reported by Inoue et al. [8] These polymers create charged surfaces in the micelle which brings another unknown parameter to be figured out how these charges affect the transport of vehicles to the target sites. In order to understand the effect of negatively charged groups on the vehicle surface, Gregoriadis et al. [9] worked on liposomes and found out that accumulation of vehicles in livers were enhanced. On the other hand, while positively charged surfaces are expected to provide better biodistribution, it was shown that positive charge enhances the uptake of vehicles by both the lungs and the liver.

Many other studies gave rise to the idea of evaluating the any charged surface vehicle in its own system because in some cases positively charged vehicles give better results whereas for some other systems negatively charged vehicles do. It is worth to emphasize that if the target site is on the liver, negatively charged drug delivery vehicles could be used.

2.4 The Micelle Core

In drug delivery vehicles, the micelle core is employed as a space where the drug molecules are carried to the target site. Most important parameter determining efficacy of the vehicle is its loading capacity, as an example, 5 μ l in a 1 ml of PCL-b-PEO 1% (w/w) micelle solution [1]. For this reason, it is significant to know which factors affect the loading capacity and efficiency. Nature of the solute and core forming block, the length of the core and total copolymer molecular weight are some parameters affecting loading capacity as well as the nature and length of the corona forming block. Overriding effect is the compatibility between the solute and nature of core forming block.

2.4.1 Compatibility between the solubilize and the core forming block

Solubilization refers to enhancement in solubility of compounds (solubilizes) by addition of solubilizers. In 1987, Nagarajan et al.[10] studied the solubilization by block copolymers in which 10 wt % poly(ethylene oxide-propylene oxide) or 20 wt % poly(N-vinylpyrrolidone-styrene) were used as solubilizers. The aim of the work was figuring out how block copolymer solubilizers respond to aromatic and aliphatic hydrocarbons. In the work, solubilization of some solubilizes in block copolymer solubilizers were figured out by comparing their solubilization in surfactants sodium dodecylsulfate (SDS) and cetylpyridinium chloride (CPC) which were used as reference solubilizers. The extent of solubilization differs by 2 or 3 times in the reference solubilizers whereas the magnitude of the solubility in block copolymer solubilizers increased to the 20-50 times higher. As a result, it was realized that aromatics hydrocarbons which have better compatibility with block copolymers show higher solubility than aliphatic solubilizes. Table 2 shows the result of the experiment.

Table 2 Extent of Solubilization of Hydrocarbons

solubilize	mol solubilized/g of polymer or surfactant			
	poly(ethylene oxide-propylene oxide)	poly(N-vinylpyrrolidone-styrene)	SDS	CPC
benzene	3.5×10^{-3}	1.8×10^{-2}	5.8×10^{-3}	8.8×10^{-3}
o-xylene	1.2×10^{-3}	1.9×10^{-2}	2.6×10^{-3}	3.5×10^{-3}
ethylbenzene	1.7×10^{-3}	1.6×10^{-2}		
toluene	1.9×10^{-3}	8.9×10^{-3}	4.7×10^{-3}	6.6×10^{-3}
cyclohexane	5.9×10^{-4}	1.8×10^{-3}	2.7×10^{-3}	3.4×10^{-3}
n-hexane	2.0×10^{-4}	4.6×10^{-4}	1.4×10^{-3}	2.3×10^{-3}
n-heptane	1.7×10^{-4}	2.8×10^{-4}		
n-octane	1.5×10^{-4}	1.1×10^{-4}		
n-decane	9.2×10^{-5}	4.3×10^{-5}	6.9×10^{-4}	9.4×10^{-4}

The solubility difference of benzene and hexane solubilizes is almost 4 fold in the conventional surfactant solvents whereas it is almost 17 fold in block copolymer solubilizer. In SDS, solubility values are 5.8×10^{-3} and 1.4×10^{-3} mol solubilized per gram of polymer for benzene and hexane respectively whereas in poly(ethylene oxide-propylene oxide) the values are 3.5×10^{-3} and 2×10^{-4} mol solubilized per gram of polymer in the same order.

The relation between molecular volume and extent of solubilization was investigated in the same study and results were represented in Figure 4. At first glance, the graph can be interpreted as increase in molecular volume reduces the solubilization. Formation of micelles with solubilizes was studied by the same group before this study and no influence of solubilizes was found out on size of the micelles. For this reason, it is reasonable to argue that larger size solubilizes have less solubilizations in micelles

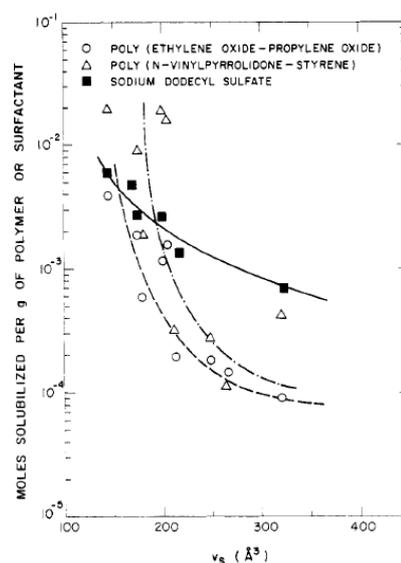


Figure 4 Dependence of the amount of hydrocarbon solubilized on the molecular volume of the solubilize. The solubilizes involved are those of Table I: (-) SDS; (- - -) poly(ethylene oxide-propylene oxide); (· · ·) poly(N-vinylpyrrolidone-styrene).

because it will require higher energy compared to the small size solubilizates.

On the other hand, molecular volume explanation is not enough to elucidate the solubilization differences for comparable size solubilizates such as toluene and cyclohexane pair or ethylbenzene and n-hexane pair. The molecular volume of toluene and cyclohexane are 107 and 108 mL/mol whereas the solubilization values are 1.9×10^{-3} and 5.9×10^{-4} in poly(ethylene oxide-propylene oxide) respectively and differs 4 fold. The reason lying behind this phenomenon comes from the interaction at micelle-water interface. Aromatic molecules create lower tension against water and thereby the free energy of formation per unit area of the micelle core-water interface becomes smaller. Consequently, solubilization of aromatic hydrocarbons turns out to be greater than that of aliphatic compounds. In general, compatibility between the core forming block and the solubilizates is modeled as in the following equation:

$$\chi_{sp} = (\delta_s - \delta_p)^2 \frac{V_s}{RT}$$

where χ_{sp} is the interaction parameter between solubilizate (s) and core-forming polymer block (p). δ_s =the Scatchard–Hildebrand solubility parameter of the solubilizate, δ_p =the Scatchard–Hildebrand solubility parameter of the core-forming polymer block and V_s =the molar volume of the solubilizate.

The lower positive value of the interaction parameter, the greater compatibility between the solubilizate and the core forming polymer block obtained. The higher compatibility between the core forming block and the drug molecules, the higher loading capacity achieved for that vehicle. Since nature of all drug molecules is different from each other, the interaction parameter changes based on polymer used in the micelle core. For this reason, there cannot be a universal drug delivery vehicle which would serve for all types of drugs. In the same way, it is not possible to expect that a specific drug can be delivered by all block copolymer systems with equal efficacy. As a rule of thumb, it is a much known fact that polar molecules like the polar ones whereas non polar likes non polar. For instance, the partition coefficient of a hydrophobic molecule pyrene in different block copolymer micelle system was studied and obtained results confirmed that pyrene molecule is much more compatible with less polar core forming polymer blocks. It was noted that partition coefficient for PEO-b-PPO-b-PEO block copolymer systems is 10^2 whereas is 10^5 for PS-PEO system [1].

2.4.2 Other factors affecting drug loading

2.4.2.1 Length of the core forming block

For a block copolymer drug delivery vehicle system with a constant length of hydrophilic segment, it is expected that increase in the length of core forming block raises up the partition coefficient for the solubilizate. In other words, increase in the length of core forming segment results in higher drug loading capacity.

2.4.2.2 Length of the corona forming block

The increase in the corona forming hydrophilic segment results in an increase in CMC and thereby a decrease in number of micelles present in the solution. It directly reduces the total volume of hydrophobic regions in the solution and total amount of transported drug molecule.

2.4.2.3 Nature of the corona forming block

The relation between the solubilizate and the corona forming polymer block depends on the interaction parameter χ_{sp} as in the case of nature of core forming segment. If the interaction parameter between the corona forming polymer and the solubilizate is lower than that of core forming, the drug molecules might prefer to sit in the outer shell. In studies by Gadelle et al. [11] PEO-b-PPO-b-PEO micelles were found to have more affinity for chlorobenzene than for benzene although the interaction parameter between PPO and benzene is lower than that of PPO and chlorobenzene. The reason is that the interaction parameter for PEO-chlorobenzene is lower than for PEO-benzene. For amphiphilic block copolymers in which both segments have a comparable and low interaction parameter with the solute, drug molecules can be even found on the interface between the core and corona.

2.4.2.4 Copolymer Concentration

Theoretical studies by Xing et al.[1] showed that solubilization capacity ascends by increase in the copolymer concentration up to a saturation point. If the interaction between micelle cores is strong, saturation is reached at a low copolymer concentration. Experimental results demonstrated that solubilization capacity can increase by copolymer concentration as well as stay constant. Hurter and Hatton [1] discovered that for PEO-b-PPO-b-PEO block copolymer system, the solubilization of naphtalane into micelles which contains bigger hydrophobic segment is independent of copolymer concentration. On the other hand, for micelles which have larger hydrophilic segment, extent of solubilization of naphtalane increases by concentration until reaching to a saturation point.

2.4.2.5 Solute Concentration

Mattice et al.[12] performed a Monte Carlo simulation with triblock copolymers and argued that the presence of solubilizate can enhance the aggregation of copolymers. It means that micelles can form at lower concentration and thereby number of micelles formed in the solvent increases. Moreover, the solubilizate increases the aggregation number of the micelles which results in growing in the size and capacity of the micelle. For this reason, increase in the solute concentration can make a positive contribution to the loading capacity of the vehicle.

As discussed above, there are many factors influencing the loading capacity and efficacy of the drug delivery vehicles. However the most important parameter is compatibility between the drug molecules and the core forming polymer block. While designing a vehicle for a specific drug, first of all nature of the solubilizate and core forming polymer block should be considered and then other factors could be improved.

2.4.3 Release Kinetics

Release of drug molecules from the micellar vehicle is as important as loading of the micelles. The release rate can be considered as diffusion and affected from the micelle stability and biodegradability of the copolymer micelle. If the micelle is stable enough and biodegradability rate is low, release rate of drug molecules from the micelle core influenced by many factors such as the strength of the interaction between the core forming block and drug molecules, the physical state of the micelle core, the amount of drug molecules loaded, the molecular volume of drug molecules, the length of the core forming block and the localization of drug molecules within the micelle. Most of these factors also affect the loading capacity of the micelle and could have reverse effect on it. For this reason, the optimum value for these factors should be figured out while designing a new drug delivery vehicle.

2.4.3.1 Polymer-drug interactions

Increase in the interaction between the core forming block polymer and the drug molecules lowers the release rate of the drug from the micelle. In studies by La et al. [13] it was shown that the release rate of indomethacin (IMC) from PEO-b-PBLA block copolymer micelle depends on the pH of the medium. Since the increase in pH of environment makes more carboxylic groups ionized and hydrophobic-hydrophobic interaction lowered, it enhances the release rate of drug molecules from the micelle core. The results are represented in Figure 5.

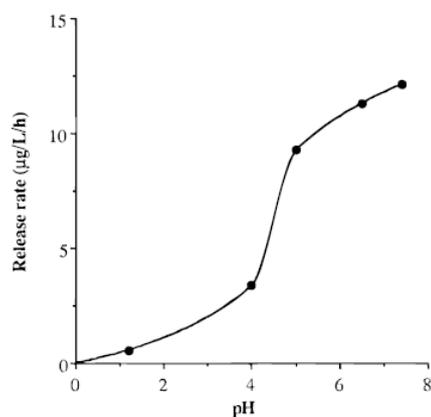


Figure 5 Release rate profile of IMC from IMC/PEO-PBLA micelles in different

Strong polymer drug interaction improves the loading capacity of the micelle while decreasing the release rate. Therefore compatibility between the core forming polymer and the drug molecules should be considered with respect to both loading capacity and release rate consequences.

2.4.3.2 Localization of the drug molecules within the micelle

The incorporated drug molecules may lie within the micelle core as well as the interface between core and corona, even within the corona depending on the solubility of drug molecules. Since blood is a polar solvent and drug molecules transported by drug delivery vehicles are hydrophobic, it is expected that drug molecules diffuse into micelle core and stays there during circulation. However use of less hydrophobic drug molecules can increase the probability of residing the molecules at the interface or within the corona due to the enhanced hydrophilic interactions. It is obvious that drug molecules carried within the corona or at the interface will be released more rapidly in comparison to those carried within the micelle core. This type of unloading is called ‘burst release’.

2.4.3.3 Physical state of the micelle core

Glass transition temperature is defined as the temperature at which transition from solid like behavior to liquid like behavior occurs in polymers while the temperature increases. Rapid

changes in physical properties are observed at this transition point and one of the apparent features is viscosity. If T_g of the core forming block polymer is above the operating temperature of the drug delivery vehicle i.e. 37° Celsius, the polymer is in solid like phase and has a high viscosity which reduces the release rate of the drug. Studies by Teng et al.[14] showed that diffusion constant of pyrene in poly(styrene) is less than it is in poly(tert-butyl acrylate). The result is expected since the T_g of poly(styrene) is higher than that of poly(tert-butyl acrylate) which are 100° and 42° Celsius, respectively. The results of the study are demonstrated in Table 3.

Table 3 Release Kinetic Results

sample ^c	probe	$\lambda_{\text{buffer}}(0)$	$\Delta\tau_{\text{expt}} = r_{\text{core}}^2/D$ (s) ^b	r_{core} (nm)	D (cm ² /s) ^b
PS-PMA	pyrene	0.064 (±0.04)	$2.2 (\pm 0.8) \times 10^6$	14.4 ^a	$9.4 (\pm 3.5) \times 10^{-19}$
	Phen	0.37 (±0.05)	$2.0 (\pm 0.5) \times 10^5$		$1.0 (\pm 0.3) \times 10^{-17}$
PS latex	pyrene	0.18 (±0.05)	$7.8 (\pm 2.6) \times 10^6$	32	$1.3 (\pm 0.5) \times 10^{-18}$
	Phen	0.37 (±0.05)	$8.6 (\pm 3.0) \times 10^5$		$1.2 (\pm 0.5) \times 10^{-17}$
PBA-PVPH ⁺	pyrene	0.59 (±0.07)	$2.9 (\pm 0.5) \times 10^4$	14.3 ^a	$7.0 (\pm 1.3) \times 10^{-17}$
	Phen	0.64 (±0.03)	$6.8 (\pm 0.9) \times 10^3$		$3.0 (\pm 0.4) \times 10^{-16}$
PBA-PVP/PVP-PEO	pyrene	0.62 (±0.03)	$3.2 (\pm 1.1) \times 10^4$	14.3 ^d	$6.4 (\pm 2.5) \times 10^{-17}$
	Phen	0.78 (±0.05)	$9.3 (\pm 1.8) \times 10^3$		$2.2 (\pm 0.4) \times 10^{-16}$

^a Estimate of the micelle core radius, r_{core} , based on eq 21. ^b See text, eq 20. ^c Dilution factor same as Table 2. ^d See text; this assumes the core of the onion micelle to be the same as the PBA-PVPH⁺ micelle.

2.4.3.4 The length of the core forming block

The length of the core forming polymer block affects the release rate in negative way if the drug molecules reside within the micelle core. In case of burst release in a vehicle, the length of core forming block lose its influence on the release rate.

2.4.3.5 Molecular volume of the drug

The larger size drug molecules incorporated into micelle core is, the slower the release rate is obtained. It is quite evident due to dependence of the diffusion constant on the molecular volume of the diffusing molecule. However, as stated before, if drug molecules reside within micelle corona, molecular volume would not affect the unloading rate because of corona segment is soluble in the solvent and has high mobility which would not make the release difficult.

2.4.3.6 The physical state of drug molecules in the micelle

Physical state of drug molecules determines its interaction with the polymer. In this aspect it can have significant effects on the release rate. If the incorporated drug molecules are dissolved in the core forming block polymer, it might decrease the glass transition temperature of the polymer and make the release faster. Instead of drug molecules being in dissolved phase, crystallization could happen on the micelle core for some systems. In this case, drug molecules act as reinforcing filler and enhance the T_g of the polymer which would cause a decrease in the release rate.

2.4.3.7 Temperature of the environment

In 2008, Cheng et al. [15] published an article in which effect of temperature on release rate was shown. In this study, drug delivery vehicle micelles were formed from the block copolymer of biotin-poly(ethylene glycol)-block-poly(N-isopropylacrylamide-co-N-hydroxy methylcryla- mide) (biotin-PEG-b-P(NIPAAm-co-HMAAm)) which were incorporated with a

poorly water soluble anticancer drug, MTX. Experiments were done in two different temperature conditions and it was demonstrated that at 43° Celsius, 66 percent of drug molecules were released in 96h and at 37° Celsius 90 percent were achieved in the same time interval. Drastic increase in the drug release rate was ascribed to acting of the core segment with hydrophilic nature upon drop in temperature from 43° to 37° Celsius and hence deforming the micelle structure and resulting in quick diffusion of drug molecules.

2.4.3.8 Additives used for trigger mechanism

Release rate of drug molecules from the micellar vehicle happens through diffusion and depends on the micelle stability remarkably. Therefore triggering the dissociation of micelles when they reach to the target site increases the release rate and efficacy of the vehicle significantly. One of the potent trigger mechanisms is use of light illumination which creates stimulation on photo sensitive molecules. As an example, azobenzene groups have been used studied a lot as the photo active molecules and the conversion between *cis* and *trans* conformations were exploited as the source of micelle dissociation upon illuminating with light. The planar *trans* (visible light) form of such micelles are more hydrophobic than the nonpolar *cis* (UV light) isomer of the micelle. Since the stability is mostly ascribed with the hydrophobic nature, *trans* form is more stable as compared to *cis* conformation. It means illuminating light to stable *trans* conformation micelle can convert it into *cis* and triggers the release of drug molecules by deforming the vehicle structure. [16]

3. Modifications on Micelles to improve efficacy

During the circulation of drug carrying micellar vehicles in bloodstream, two important factors which should be considered to increase the efficacy are temporal and distribution control. Loaded drug molecules are aimed to be delivered to a specific target site in the body and there should be mechanism developed to fulfill this need. In other words, control of how the target site recognizes the drug delivery vehicle more efficiently and increases the amount of accumulation on it also should be taken into account while designing a new vehicle. Improvements in efficiency of the drug delivery vehicles could be considered in two separate parts namely functionalization of block copolymers and auxiliary agents. Core and corona forming block copolymers can be modified with

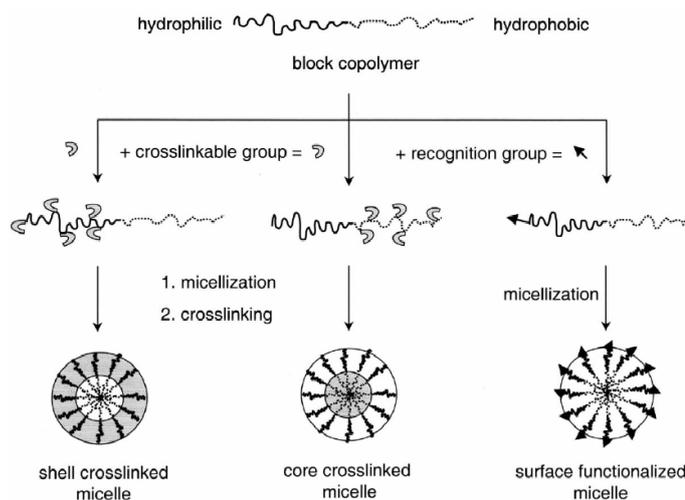


Figure 6 Schematic representation of the three different classes of functional amphiphilic block copolymers discussed. Depending on the type

specific chemical reactions by adding ligands which aims mainly increase in the temporal control as well as in the distribution control. Auxiliary agents are widely used to develop binding between the target site and the drug vehicle.

3.1 Functional Block Copolymers

Functional block copolymers designed to improve properties of the self assembled drug vehicles could be classified in three categories as depicted in Fig 6. Substituents can be attached to both hydrophobic and hydrophilic segments in order to start crosslinking to happen in core or corona region. The second use of substituents might be to enhance the functionality of micelle surface.

Chemical fixation of micelles by crosslinking of either core or the corona is of interest for a number of reasons. Crosslinking usually increases stability of the micelles and some of them could survive in the solvent below CMC. For this reason, crosslinked micelles can be isolated and redissolved as stable nanoparticles and less likely to collapse. In the case of crosslinked outer shell, chemical fixation enables to control of permeability of the corona as well as the stability. Permeability of the corona directly affects release rate of the drug from the micelle core. Increase in the stability leads to prolonged circulation time of the micelles in bloodstream. By this way, recognition of the vehicle by RES clearance during the transportation is reduced.

3.1.1 Core Crosslinkable Micelles

There are various ways of cross-linking the core forming segment of the copolymer and the most suitable way is carried out by adding polymerizable group to the hydrophobic chain end. Polymerization achieved by these new groups increases the micelle stability while keeping the loading capacity constant. Other ways of cross-linking are addition of polymerizable groups along the hydrophobic segment or encapsulation and polymerization of low molecular weight monomer in the hydrophobic core of the micelle. The last two methods are a bit inefficient compared to previous method because cross-linking reaction will reduce the free volume of the hydrophobic core and thereby decrease loading capacity.

Kim et al.[17] examined the effects of cross-linking in poly(D,L-lactide)- b-poly(ethylene glycol) (PLA-PEG) copolymer with MeO groups at the PEG chain end and methacryloyl groups at the PLA chain end. Spherical shape micelles were prepared by using dialysis method which generated particles with the size of diameter 30 nm and low polydispersity factor. After the formation of micelles, the polymerization of methacryloyl group was carried out by both thermally and by light illumination. Thermal stimulation was triggered by addition of initiator Azobis-2,4-dimethylvaleronitrile in a DMAc solvent during the dialysis of micelles. Polymerization reaction took place at 60° Celsius for 20h. UV radiation induced polymerization was also exploited in the study by use of photoinitiator 2,2-dimethoxy-1,2-diphenylethane-1-one (BDMK) during the dialysis of micelles. Then exposure to irradiation with a high pressure mercury lamp, IR light range filtered out, started the polymerization at the core chain. Enhancement in the micelle stability by crosslinking of core polymer was confirmed by spectroscopic and light scattering techniques. Upon the improvement in the micelle stability, they performed a set of experiments to investigate how the crosslinking

affects the loading capacity of the micelles by the anti-cancer drug, Taxol which ended up by no change in the capacity.

In the study by Rösler et al.[2] heterobifunctional PLA-b-PEG copolymers in which crosslinkable groups and aldehyde groups incorporated to PLA and PEG chains respectively were used. Coating of these micelles on an amine functionalized surface in the presence of NaCNBH_3 resulted in fixation of the PEG chain end on the surface. Results showed that when crosslinked micelles used, micelle core/corona structure survived on the substrate whereas non crosslinked micelles were disrupted upon dispersion on the surface. Fig 7 depicts the schematic for formation of layers by using this method. The motivation of this study was to prepare the vehicles in the multilayer structure which prevents the adsorption of proteins and can release hydrophobic reagents in a controlled manner.

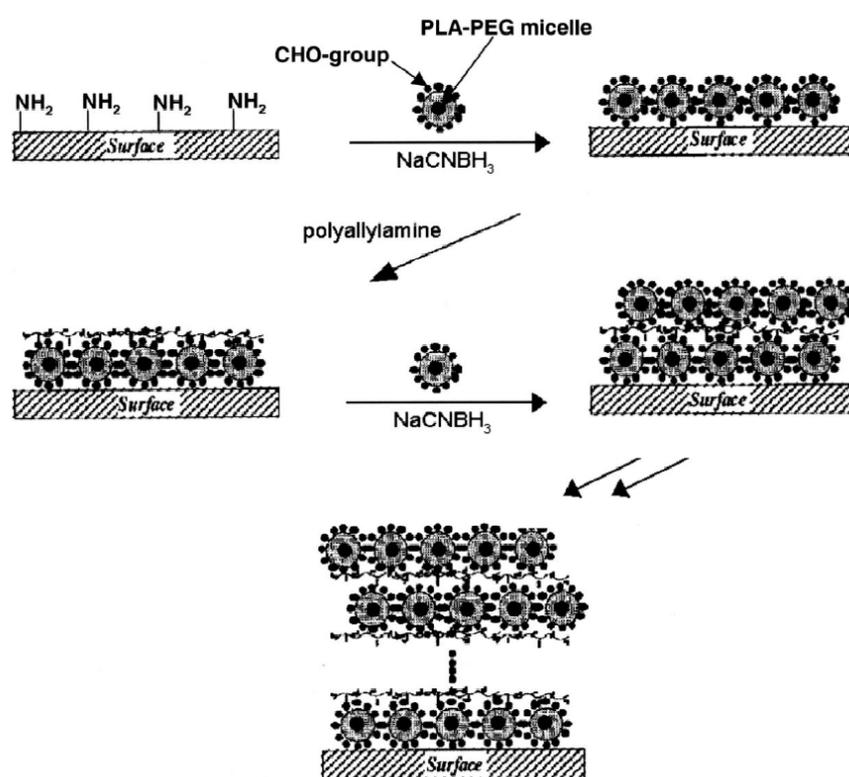


Figure 7 The preparation of well-defined layers of surface-bound PEG–PLA micelles via alternate deposition of aldehyde functionalized

In 2008, Zhang et al.[18] studied the core crosslinking on the copolymer poly(polyethylene glycol methyl ether methacrylate)-block-poly(5-O-methacryloyluridine) (PPEGMEMMA30-b-PMAU80) which was self assembled in aqueous environment. Then polymerization took place at 60° Celsius by RAFT process using bis(2-methacryloyloxyethyl)disulfide agent as the initiator. Dissociation of core crosslinked micelles were promoted by addition of dithiothreitol (DTT), depending on concentration of reducing agent and the amount of cross linker in the micelle. In order to rationalize the application of the vehicles to the actual treatments, vitamin B₂ was loaded into the micelles and increase in the loading capacity was

observed by cross-linking degree. It was reported that 60-70% of the drug molecules was released after 7h in the presence of DTT.

Remarkable improvements in the efficacy of drug delivery vehicles by cross linking of core forming segment was demonstrated in the study of Rijcken et al.[19] They have used the copolymer mPEG5000 and N-(2-hydroxyethyl)methacrylamide)-oligolactates (mPEG-b-p(HEMAm-Lac_n)) which forms thermosensitive biodegradable micelles by heating up above the critical micelle temperature. Exposure to irradiation in presence of photoinitiator resulted in cross-linked micelles. Experiments showed that cross-linked micelles kept their integrity upon drop in the temperature below critical value whereas non cross-linked micelles collapsed rapidly. In addition to that fate of micelles were kept track after intravenous administration and observed that circulation kinetics were much better for cross linked micelles such as liver uptake was 10% of injection dose for cross-linked and 24% for non cross linked micelles. Amount of accumulated vehicles around a tumor was 6 times higher for cross linked micelles than that of non cross-linked micelles.

Kataoka et al. in 2009 [20] released the results of a work in which they have studied efficacy of siRNA transfection by iminothiolane-modified poly(ethylene glycol)-block-poly(L-lysine) (PEG-b-(PLL-IM)) micellar vehicles. Under physiological ionic strength conditions, disulfide crosslinked core containing micelles maintain the stability and their structure whereas non-crosslinked micelles cannot self assemble into micelle structures. Therefore siRNA transfection with non-crosslinked micellar vehicles is 100 times lower than crosslinked micellar vehicles.

3.1.2 Shell Cross-linked Micelles

As mentioned before, one of the problems drug vehicles encounter is that sudden drop in the vehicle concentration upon intravenous injection and if it goes down below CMC, might result in dissociation of self assembled micelles, thereby release of drug molecules. One efficient way of overcoming this problem was proposed by Wooley and her coworkers in 1996 [21]. The idea was that cross-linking of the shell forming segment in a copolymer might give rise to more stable micelles which can even stand against high dilution. Their study was done on the diblock copolymer based on hydrophobic polystyrene (PS) and hydrophilic 4-(chloromethyl)styrene-quaternized poly(4-vinyl-pyridine) (QP4VP). Cross-linking of the shell region was carried out via radical oligomerisation of the pendent styrenyl groups on the coronal P4VP blocks in a THF-water mixture.

Discovery of the idea above opened a promising way for other curious chemists to apply many known cross-linking reactions to the micelle systems in order to design less toxic, low cost and high efficient drug delivery vehicles. Cross linking of a polymer is

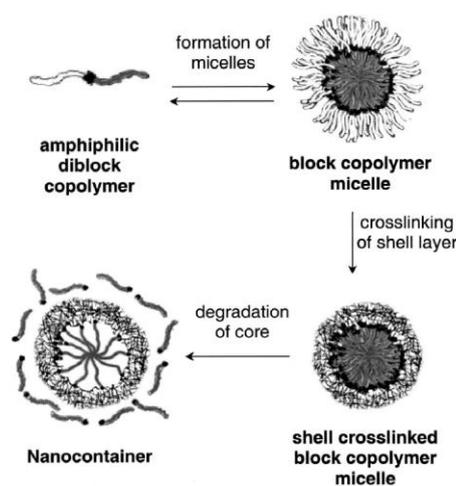


Figure 8 The formation of shell-crosslinked block copolymer micelles via the self-assembly of amphiphilic block copolymers. Degradation

nothing but creating new bonds between the polymer chains, which might be done by covalent bonds, ionic bonds or combination of both. There are number of ways to create these kinds of interactions and some of them can be given as an example. Poly(acrylic acid)-based systems are typically cross linked by the addition of a diamine under certain conditions which are also required in peptide chemistry [22]. 2-(Di methylaminoethyl)methacrylate containing shells have been crosslinked via quaternization with 1,2- bis(2-iodoethoxy)ethane [23]. Crosslinked poly (isoprene)- or poly(styrene)-b-poly(2-cinnamoyl ethyl methacrylate) shells have been prepared by UV irradiation in the presence of photoinitiator [24]. Micellar coronas composed of 4-vinylpyridine have been crosslinked by quaternization with p-chloromethylstyrene, followed by photo induced radical polymerization of the styrene double bonds [21]. Even though the mechanisms are varying too much, the aimed result is to get the shell region of the micelle stronger so that it can stand its structure upon changes in the external medium. Formation steps of crosslinked corona micelle are depicted in the scheme below.

Maintaining the micelle structure integrated below CMC is not the only advantage brought by the enhancement in stability due to crosslinking in the corona region but it also comes along with the possibility of removing the core forming segment so that increase in the inside volume of vehicle core increases the loading capacity. The empty corona turns out to be a nanocontainer for various types of drug molecules particularly for hydrophilic ones due to polar nature of the corona forming segment. Removal of the core of shell-crosslinked micelles has been accomplished in a number of ways, including ozonolysis of poly(isoprene). [25] UV photolysis of poly(silanes), [26] acid catalyzed hydrolysis of poly(ϵ -caprolactone) [27] and thermolysis of a C–ON bond connecting the poly(styrene) and poly(acrylic acid) segments of a block copolymer obtained via a combination of nitroxide mediated and atom transfer radical polymerization. [28]

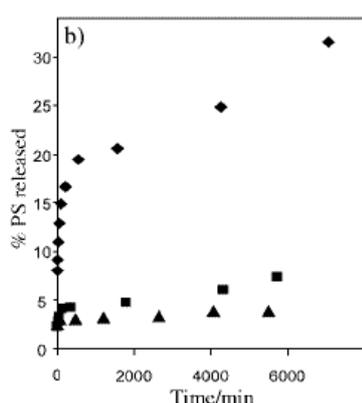


Figure 9 Release kinetics of PS from the core of SCKs crosslinked to different extents (20%, -50% and :100%)

Another impact of crosslinking is on the release kinetics of the vehicle. It is not difficult to estimate that release rate of drug molecules will be lowered by increase in the crosslinking composition of the corona region. Murthy et al. investigated this hypothesis in their group and confirmed its validity by depicting the results in Fig. 9. By changing and modifying the structure of corona forming polymer, control of release rate could be improved as well as efficiency of delivering to the target site.

Based on the improvements by crosslinking of core or corona forming polymers, fantastic studies started to come out in the last decade. An impressive one was published in 2007 by Liu et al. [29] This research group studied a double hydrophilic ABC triblock copolymer, poly(2-(diethylamino)ethyl methacrylate)-b-poly(2-(dimethylamino)-ethyl methacrylate)-b-poly(N-isopropylacrylamide) (PDEA-b-PDMA-b-PNIPAM). The unique property of this triblock copolymer comes from the pH-responsive PDEA block and thermoresponsive PNIPAM

block. The copolymer in aqueous milieu showed “schizophrenic” micelle formation which means supramolecular self assembly of three layers in onion-like shape. At acidic pHs and elevated temperatures, outer shell of the micelle formed by PNIPAM whereas at alkaline pH’s and room temperature formed by PDEA, so called inverted structure. Dynamic laser light scattering (LLS) and optical transmittance results demonstrated the fully reversible transition between the normal and inverted structure upon changes in the external medium. In both structures, the middle shell formed by PDMA which was crosslinked by using 1,2-bis(2-iodoethoxy)- ethane as the initiator. After the formation of crosslinked structure between the pH sensitive and thermoresponsive blocks, drug release kinetics were investigated by using the same methods and transmission electron microscopy. Results of the experiments were promising for use of this copolymer in real application because of its capability to answer both types of stimulations, change in pH and temperature.

3.1.3 Surface Functionalized Micelles

Cross-linking of the micelle core and corona mostly enhances the stability of drug delivery vehicle and results in an increase in the temporal control. On the other hand, there is another factor which influences the efficacy and should not be ignored during the vehicle design is the distribution control. Delivery of the drug to the target site can be divided into two, namely, passive and active targeting. An example of passive targeting was given in the introduction section; enhanced vascular permeability of solid tumors triggers the accumulation of drug vehicles on the target site more efficiently. However, there are some specific molecules and proteins located on the cell surface and act as a receptor and determine which molecules should be getting in and out of the cell. Exploiting this functionality of the receptors allows use of a ligand molecule by binding it to the periphery of the drug delivery. Apparently this would increase the accumulation of the drug delivery vehicles around the target site and decrease the negative side effects of the drug molecules to other parts of the body.

Saccharides have an important role in the interaction between the protein-cell and cell-cell pairs and hence can be strong candidates for drug delivery vehicles to be used as ligands which provide active targeting. Kataoka et al. [30] succeeded to prepare micelles in which galactose and glucose were attached to the micelle surface as ligands by ring opening polymerization of successively ethylene oxide and D,L-lactide, using protected sugars as the initiator. The binding ability of sugar-functionalized micelles was checked by means of affinity chromatography, using a RCA-1 lectin functionalized column. RCA-1 is a plant lectin well known to bind b-D-galactoseresidues. As shown in Figure 10 the unsubstituted and glucose functionalized micelles were not recognized by the lectin and were rapidly eluted, giving a sharp, single peak in the chromatogram. The galactose- bearing micelles, in contrast, were found to interact strongly with the column, indicating recognition and binding by RCA-1.

In order to find a treatment for cancer diseases, enormous research has been conducted so far. It was reported that for cancer cells on ovarian, breast, brain and lungs, tumor tissues contain

a protein 100-300% higher in dose compared to normal tissue. This protein glycosyl-phosphatidylinositol-anchored glycoprotein was well known to be having high affinity to bind

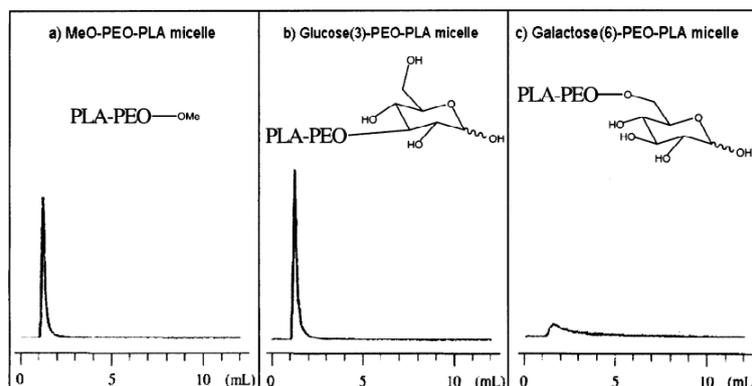


Figure 10 Chemical structures of three differently end-functionalized diblock copolymers (top) and the elution profiles that were recorded after passing micellar solutions of these block copolymers through an RCA-1 functionalized affinity column (bottom)

with folic acid. Keeping this in mind, Yoo and Park et al. [31] came up with the idea of enriching the surface of DOX loaded, PEG-PLGA micelles with folic acid to increase the efficiency of uptake of drug delivery vehicles around the tumor tissue. Results of in vitro studies demonstrated a slight increase in the cytotoxicity of drug delivery vehicles. However targeted micelles caused 2 times decrease in the growth rate of tumor tissue as compared to nontargeted micelle vehicles. Researchers made a further step to increase loading capacity of vehicles by removing PLGA segment of the copolymer and created self assembled folate-PEG-DOX systems [32]. Thereby they achieved 57% increase in the loading capacity and 40% decrease in the volume of tumor tissue.

In 2007, Sutton et al. [33] published a comprehensive review paper on the functionalization of micelle surfaces for targeted drug delivery systems. In the paper, they covered different ways of binding a specific ligand to micelle surface and presented the Table below to give an idea of which ligand can be used in which micelle system.

Table 4 Ligand-targeted Micelle Formulations

Ligand Type	Ligand	Polymer Composition	Micelle Size (nm)	Drug	<i>In Vitro</i> Model	Animal Model
Small Organic Molecule	Folic Acid	PEG-PLGA	105	DOX	KB cell	KB in nu/nu mouse
	Folic Acid	PEG-Dox	200	DOX	KB cell, A549	KB in nu/nu mouse
Peptide	Folic Acid	PEG-PCL	50-130	Paclitaxel	MCF-7, HeLa	
	cRGD peptide	PEG-PCL	20-40	DOX	SLK	
Carbohydrate	Galactose	poly(L-benzyl l-glutamate)-PEG	104	Paclitaxel	P388, SK-Hep 01, HepG2	
	Lactose, Galactose, Mannose, Glucose	PEG-PLA	38-42			
	Galactose	PEG-PLA	32			
Antibody	Lactose	PEG-PLA	20-40			
	Anti-GFA Ab	Pluronic	Not reported	Haloperidol		Mouse
RNA Aptamer	mAb 2C5	PEG-PE	20	Paclitaxel	LLC, EL4 T, BT20	LLC in C57BL/6J mice
	Anti-PSMA aptamer	PEG-PLA	168	Docetaxel	LNCaP	LNCaP in BALB/c mouse

A recent and interesting study for the surface functionalized micelles was published in 2010 by Wu et al. [34]. They used aptamer micelle vehicles which were constructed by attaching lipid tail phosphonidite with diacyl chains onto the end of an aptamer inserted with a poly(ethylene glycol) (PEG) linker. The focus of the study was to recognition of micellar vehicles by cancer tumors and loading/release rate properties were not reported. However they claim that aptamers at physiological conditions were detected and recognized in the micelle structure much better than it was in individual monomer conditions by making the measurements in artificially created human blood circulation sample.

Another work modifying the micelle surface to improve the efficacy was done by Prabakaran et al. in 2009 [35]. In their study, amphiphilic hyperbranched block copolymer micelles with the structure of hydrophobic poly(L-lactide) (PLA) inner shell and a hydrophilic methoxy poly(ethylene glycol) (MPEG) and folate-conjugated poly(ethylene glycol) (PEG-FA) outer shell (H40-PLA-b-MPEG/PEG-FA) were synthesized. Incorporated DTX drug molecules were exposed to initial burst release after 4h and significant release was observed up to 40h. It was demonstrated that cellular uptake of folate conjugated micelles is higher than that of not conjugated systems. The reason was attributed to folate mediated endocytosis.

3.2 Auxiliary Agents

Another modification way of micellar drug delivery vehicles to improve efficacy is binding an auxiliary agent to the micelle surface. Main purpose of involving auxiliary agents in delivery vehicles is to remedy the temporal control and be able to manage release of drug molecules in a controlled manner. Auxiliary agents could have an influence either on micelle forming copolymer or on the encapsulated drug molecules i.e. in some cases by transforming the lipophilic precursor into active hydrophilic drug molecule.

3.2.1 Channel Proteins

Meier et al. [36] worked on the channel proteins which were incorporated into self assembled vesicles or membranes obtained from triblock copolymers without any change in the hydrophobic segment. They preferred to use OmpF channel protein which can be found on the cell wall of Gram negative bacteria because of its profound properties. First of all, size selectivity of the OmpF channel protein allows only for small size molecules to diffuse passively through the channel while sterically prohibiting the pass of molecules which are larger than 400 Da. In drug delivery aspect, size selectivity of OmpF is significant during the circulation because it prevents the possible leakage of enzymes from blood to vehicle core which would result in degradation of drug molecules. Another reason of OmpF being a good candidate as an auxiliary agent for drug delivery system is its ability to act as a gate for small size molecules. Upon application of Donnan potential [37] across the membrane in which channel protein resided, the OmpF turns off and stops the transfer of drug molecules. Exploiting this functionality enables one to control opening and closing time of the micelle gate for drug molecules. When the drug delivery vehicle reaches to the target site, Donnan potential can be removed by adding low molecular weight electrolyte or by simple dilution and then drug molecules would be released from the micelle core.

In studies by Meier et al. stimuli sensitive nanocontainers were produced by mixing solution of amphiphilic poly(2-methyloxazoline)-b-poly(dimethylsiloxane)-b-poly(2-methyloxazoline) (PMOXA– PDMS–PMOXA) with a solution of the channel proteins and the enzyme β -lactamase. After equilibrium was reached vesicles with OmpF protein bridging the hydrophobic PDMS shell and the enzyme encapsulated in the nanocontainer was produced as shown in Fig11.

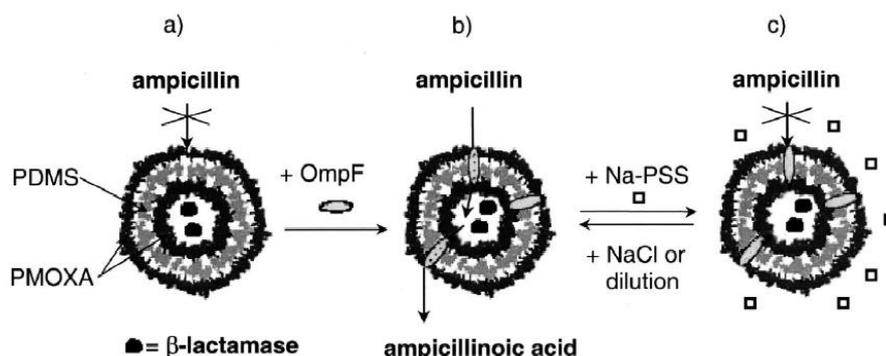


Figure 11 Formation of OmpF incorporated micelles

The enzyme β -lactamase was encapsulated in the micelle core in order to demonstrate that OmpF protein can be reversibly activated and deactivated with external stimuli. Under normal circumstances OmpF channel is found open for ampicillin enzyme and can carry it into the container which is realized by detecting the produced ampicillinoic acid after the reaction and release from the core. However applying Donnan potential by adding electrolyte such as NaCl or simple dilution turns the channel off and no molecule transfer occurs.

3.2.2 Metal (nano)particles

The use of thermosensitive micelles was already mentioned in the Introduction. The mechanism which triggers the release of the drug from the micelle was explained based on the LCST behavior. Change in the temperature of the environment leads to distortion in the structure of the micellar vehicle and let the drug molecules free. Although the system seems to be clinically feasible, there are still some challenges faced in this technique. Individual micelles cannot be addressed by an external stimulus which is the local heating of the body. Local heat treatment can fail because the body itself has an autonomous control the temperature constant at 37° Celsius and even one can manage the heating up local regions on the skin, it is going to lose its influence as it goes deeper and may not even reach to micelles circulating in the blood vessel.

In order to improve the temporal and distribution control, metal nanoparticle auxiliary agents can be used. Especially Au and γ -Fe₂O₃ are appropriate for drug delivery purposes because they can be thermally excited readily by irradiation with IR light or by exposing to an alternating magnetic field, respectively. Since IR light can pass largely without being absorbed through the skin and alternating magnetic field do not create unspecified heat increases in the body, they can be used as stimulators to activate the drug release from the micelles by creating a temperature change.

Au and $\gamma\text{-Fe}_2\text{O}_3$ have not been investigated for thermosensitive micellar drug delivery systems actually but West et al.[38] and Takahashi et al. [39] have studied these metals for hydrogels composing of N-isopropylacrylamide and acrylamide. Application of alternating magnetic field led to a 10° Celsius increase in the temperature of $\gamma\text{-Fe}_2\text{O}_3$. Raising up the temperature above LCST initiates the collapse of hydrogels and burst like release takes place. The reaction is represented in Fig.12 showing that repetitive application of IR radiation can be used to create multiple bursts of bovine serum albumin (BSA) from an acrylamide based hydrogel. The application of triggering pulsatile release is delivery of insulin in the treatment of diabetes mellitus.

Despite the fact that metal auxiliary agents have been only investigated for hydrogels, they could have played an important role on the improvement of thermosensitive micellar drug delivery vehicles.

4. Conclusion

Use of block copolymer micelles for drug delivery applications is really one of the hot topics in science especially in the field of biomaterials. Amphiphilic nature of the block copolymers results in self assembled micelle structures. In aqueous medium, the hydrophilic segment forms the shell/corona region and hydrophobic segment forms the core region where the drug molecules are carried. Formation theory of micelles was covered in Section 2 and the discussion of importance of CMC value was given in the same section. CMC should be kept as low as possible to reduce the likelihood of micelle collapse upon intravenous administration.

Efficacy determining parameters for a drug delivery system are loading capacity, release kinetics of drug molecules from the micelle core and cytotoxicity to the human body. In this review, mostly the attention was given to factors affecting loading capacity and release kinetics rather than cytotoxicity.

In order to increase the temporal and distribution controls, modifications on micellar vehicles have been done extensively. Some of those ways were considered in this paper such as cross-linking of micelle core and corona regions, functionalization of micelle surfaces and use of auxiliary agents. Design of drug delivery vehicles requires taking care of all these aspects and then something useful can come out for commercial use which is still the driving force in this field.

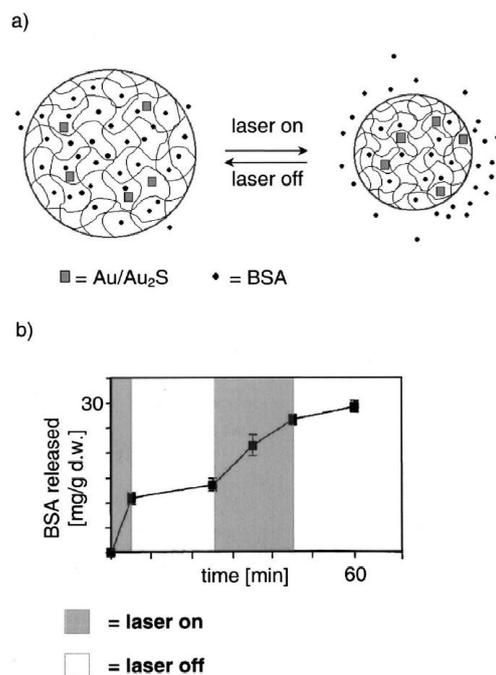


Figure 12(a) IR light-induced thermal activation of Au nanoparticles can cause collapse of acrylamide-based hydrogels. (b) Repetitive exposure of a hydrogel to IR laser light can be used to create multiple bursts of encapsulated molecules.

5. References

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