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Polymeric Micelles: General Considerations and their Applications

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ABSTRACT

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One of the most widely studied subjects in nanoscience technology is related to the creation of supramolecular architectures with well-defined structures and functionalities. These supramolecular structures are generated as a result of self-assemblage of amphiphilic block polymers. Self-assembly of block polymers via hydrophobic and hydrophilic effects, electrostatic interactions, hydrogen bonding, and metal complexation has shown tremendous potential for creating such supramolecular structures with a wide array of applications. Polymeric micelles have gathered considerable attention in the field of drug and gene delivery due to their excellent biocompatibility, low toxicity, enhanced blood circulation time, and ability to solubilize a large number of drugs in their micellar core.

In this article we have reviewed several aspects of polymeric micelles concerning their general properties, preparation and characterization techniques, and their applications in the areas of drug and gene delivery. Polymeric micelles can be used as 'smart drug carriers' for targeting certain areas of the body by making them stimuli-sensitive or by attachment of a specific ligand molecule onto their surface.

KEY WORDS: micellization, polymeric micelles, solubilization, targeting, stimuli-sensitivity

MICELLES

A sudden change in many physicochemical properties is seen in solutions of amphiphilic molecules or surfactant monomers that possess a polar head and a lipophilic tail. The change in physicochemical properties is associated with the orientation and association of amphiphilic molecules in solution resulting in the formation of structures called micelles. The micelles internally have a hydrophobic core and externally a hydrophilic surface. Micelles are generally made up of 50 to 200 monomers (an average number of monomers forming micelle at any given time is termed as the aggregation number). The radius of a spherical micelle is almost the same as the length of a fully extended surfactant monomer, which mostly is 1-3 nm, and thus micelles lie in the colloidal range.[1,2]

The major driving force behind self-association of amphiphilic molecules is the decrease of free energy of the system. The decrease in free energy is a result of removal of hydrophobic fragments from the aqueous surroundings with the formation of a micelle core stabilized with hydrophilic fragments exposed into water. The factors affecting the process of micelle formation are the size of the hydrophobic domain in the amphiphilic molecule, concentration of amphiphiles, temperature, and solvent. The assembly formation starts only when a certain minimum concentration is crossed by the amphiphilic molecules, called as critical micelle concentration (CMC). At low concentrations in medium, these amphiphilic molecules exist separately, and are so small that they appear to be subcolloidal. Below the CMC, the concentration of amphiphile undergoing adsorption at the air-water interface increases as the total concentration of the amphiphile is increased. Finally at CMC, the interface as well as the bulk phase is saturated with monomers. Any further amphiphile added in excess of CMC results in the aggregation of monomers in the bulk phase, such that the free energy of the system is reduced. The temperature below which amphiphilic molecules exist as unimers and above which as aggregates is the critical micellization temperature (CMT).[1,3,4]

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POLYMERIC MICELLES

Amphiphilic block or graft copolymers behave in the same manner as that of conventional amphiphiles and in aqueous solution, above CMC, these polymers form polymeric micelles. In contrast to the micelles of conventional surfactant monomers, in polymeric micelles there is a covalent linkage in individual surfactant molecules within the hydrophobic core. This linkage prevents dynamic exchange of monomers between free solution and the micellar pseudo-phase. This confers rigidity and stability to the polymeric micelles.[5] The aggregation number of polymeric micelles is of the magnitude of several hundreds and the diameter ranges from 10 to 100 nm. Factors controlling the size of the polymeric

micelles include molecular weight of the amphiphilic block copolymer, aggregation number of the amphiphiles, relative proportion of hydrophilic and hydrophobic chains, and the preparation process.[6]

In aqueous medium amphiphilic block copolymers can principally self assemble into spherical micelles, worm-like or cylindrical micelles, and polymer vesicles or polymersomes. Main factor governing the morphology of micelles is the hydrophilic–hydrophobic balance of the block copolymer defined by the hydrophilic volume fraction, f. For amphiphilic block copolymers with value of f nearly 35%, polymer vesicles are formed, whereas, for f value more than 45%, spherical micelles are formed from self-assembly.[7,8] By using amphiphiles of more complicated molecular design e.g., miktoarm star copolymers, or by varying the experimental conditions for self-assembly more complex morphologies such as that of crew-cut micelles, multicompartment micelles, toroids, etc. may be obtained.[9]

Why Polymeric Micelles are Attractive?

Suitable amphiphilic block copolymers are obtainable via controlled synthesis by varying the block ratio, the total molecular weight, and the chemical structure. By adjusting the structure of the amphiphilic copolymers, the size and morphology of the resulting polymeric micelles can be easily controlled. The micellar core produces a hydrophobic domain that can be used for solubilization of hydrophobic moieties. Most of the drugs being poorly water soluble can be easily incorporated into the core of polymeric micelles to overcome solubility problems. Solubility enhancement usually results in better oral bioavailability of the hydrophobic drugs.[10,11] Surfactant micelles tend to disintegrate upon dilution triggering lysis of cell membranes. Polymeric micelles are considerably more stable towards dilution than surfactant micelles and hence exhibit minimal cytotoxicity. The hydrophilic shell and the nanoscopic size prevent mechanical clearance of micelles by filtration or in the spleen.[12] This is beneficial for prolonging the blood circulation of drug. Also, the shell stabilizes the micelle, interacts with the plasma proteins and cell membranes and its nature controls biodistribution of the carrier. Nanoscopic size minimizes the risk of embolism in capillaries, contrary to larger drug carriers. It also favors the particular absorption in gastrointestinal system. Along with these features, low toxicity and faster rate of clearance of polymeric micelles from the body make them suitable for intravenously administered drug delivery systems. Additionally, there is no need of modification of chemical structure of the drugs.[13]

Polymeric micelles provide access to targeting because of the high drug-loading capacity of the inner core as well as the unique disposition characteristics in the body due to their size. End-functionalization of block copolymers with sugars and peptides on the periphery yield an array of micelles that have altered biological characteristics which can be used for the receptor-mediated targeted drug and gene delivery. Immunomicelles, another means of targeting, which are prepared by covalently attaching monoclonal antibody molecules to a surfactant or polymeric micelles demonstrate high binding specificity and targetability.[14] (Fig.1). Polymeric micelles may lead to the development of 'intelligent vehicles' by using stimuli-sensitive (pH, temperature sensitive) copolymers. Such intelligent vehicles are currently being explored for achieving controlled drug release.[15]

TYPES OF POLYMERIC MICELLES

On the basis of the type of intermolecular forces governing the segregation of the core segment from the aqueous environment, polymeric micelles can be classified in three main categories i.e., micelles formed by hydrophobic

Fig.1: Micellization and areas of a polymeric micelle important for drug delivery

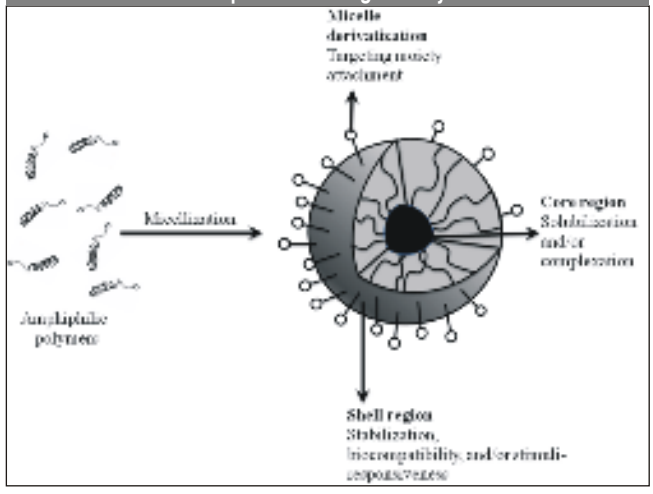
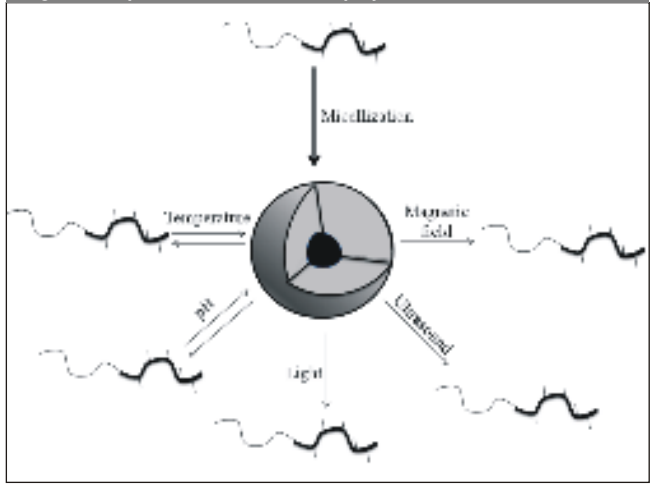


Fig.2: Examples of stimuli-sensitive polymeric micelle destabilization



interactions, those resulting from electrostatic interactions (polyion complex micelles), and micelles from metal complexation.

Conventional

These micelles are formed by hydrophobic interactions between the core segment and the corona region in the aqueous environment. One of the simplest amphiphilic block copolymer, poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide), forms micelles as a result of hydrophobic interactions.[16]

Polyion Complex Micelles

Electrostatic interactions between two oppositely charged moieties, such as polyelectrolytes, also allows for the formation of polymeric micelles. When oppositely charged polymers are added in the solution, they can penetrate in the corona of the micelle and give rise to polyionic micelle. Such formed micelles are termed polyion complex micelles (PICMs). The electrostatic forces and the van der Waals force of interaction control the structure and size of the charged micelle coronas. PICMs have some peculiar features such as simple synthetic route, easy self-assembly in aqueous medium, structural stability, high drug loading capacity, and prolonged circulation in the blood. The preparation of micelles is carried out in aqueous medium without involvement of any organic solvents, thus removing the associated side-effects produced by the residual organic solvents. The core of the PICMs can entrap many therapeutic agents such as hydrophobic compounds, hydrophilic compounds, metal complexes, and charged macromolecules through electrostatic, hydrophobic, hydrogen bonding interactions and release them after receiving suitable trigger. Because of these reasons, the PICMs have a great potential for drug release, especially for the delivery of charged drugs alongwith antisense oligonucleotides, DNA, and enzymes.[17,18]

Recently, Jung *et al.* prepared polymeric micelles of methoxy poly(ethylene glycol)-grafted-chitosan encapsulating all-trans retinoic acid through the formation of a polyion complex between the amine group of chitosan and the carboxylic acid group of all-trans retinoic acid. The PICMs were designed for drug delivery to the brain tumor. The sizes of PICMs were about 50 to 200 nm and the loading efficiency of micelle was higher than 80% (w/w).[19]

Noncovalently Connected Polymeric Micelles

A novel "block-copolymer-free" technique can also be used for preparing polymeric micelles. Here, polymeric micelles are obtained via self-assemblage of homopolymer, random copolymer, graft copolymer or oligomer for which interpolymer hydrogen bonding complexation serves as the driving force. Core and shell are non-covalently connected at their homopolymer chain end by specific intermolecular interactions such as H-bonding or metal-ligand interactions in the resultant structures and hence these are termed as non-covalently connected micelles.[20]

Jiang *et al*. prepared the intermolecular complexes with poly(4-vinylpyridine) as the backbone and carboxyl-terminated polybutadiene as the grafts due to hydrogen bonding in a common solvent, chloroform.[21]

TYPES OF POLYMER USED

Micelle-forming amphiphilic copolymers can be either block copolymers (di, tri, or tetra) or graft copolymers. A graft copolymer is one which comprises a polymer chain as a backbone and another polymer chain as side "grafted" parts. These copolymers usually demonstrate properties of both, i.e., polymeric backbones as well as of the graft. 'Click' reactions have emerged as a means to incorporate polymer chains onto polymeric backbones to result in well-defined graft copolymers.[22] Table 1 shows different possible structures of amphiphilic copolymers with representative example of each class.

Usually in aqueous solutions, spherical micelles are formed

Table 1: Structures of micelle-forming copolymers

Type of micelle-forming copolymers	Representation of structure*	Example of polymers	Ref
Block copolymers	di - block AAAAAAABBBBBB	Poly(styrene)-b-poly(ethylene oxide)	23
	tri - block AAAABBBBBAAAA	Poly(ethylene oxide)-b-poly (propylene oxide)-b-poly(ethylene oxide)	24
Graft copolymers	AAAAAAAAAAAA B B B B B B B	N-phthaloylchitosan-g-polycaprolactone	25

*A - hydrophilic unit; B - hydrophobic unit

Table 2: Various polymers used for micelle preparation

Examples of polymers	Ref
N-phthaloylcarboxymethylchitosan	12
Poly(2-ethylhexyl acrylate)-b-poly(acrylic acid)	26
Poly(tert-butyl acrylate)-b-poly(2-vinylpyridine)	27
Poly(ethylene oxide)-b-polycaprolactone	28
Poly(e-caprolactone)-b-poly(ethylene glycol)-b-poly(e-caprolactone)	29,30
Poly(e-caprolactone)-b-poly(methacrylic acid)	31
Poly(ethyleneglycol)-b-poly(e-caprolactone-co-trimethylenecarbonate)	32
Poly(aspartic acid)-b-polylactide	33
Poly(ethylene glycol)-block-poly(aspartate-hydrazide)	34
Poly(N-isopropyl acrylamide-co-methacryl acid)-g-poly(D,L-lactide)	35
Stearic acid-grafted chitosan oligosaccharide	36

from self-assemblage of amphiphilic diblock AB-type or triblock ABA-type copolymers with the length of a hydrophilic block exceeding to some extent that of a hydrophobic one. Whereas, if the length of a hydrophilic block is too large copolymers exist in water as individual molecules (unimers), and molecules with lengthy hydrophobic blocks develop various structures. Examples of different amphiphilic copolymers that have been investigated for producing micelles are given in Table 2.

PREPARATION PARAMETERS

Polymeric micelles are generally prepared by either of the two commonly used methods. Mostly, for block copolymers with low molecular weight and short length of the insoluble block, micelles are prepared by direct dissolution in a selective solvent for one of the blocks. To facilitate dissolution, stirring, thermal, or ultrasound treatments can be used. The micellar properties remain unchanged once the micelle is trapped in a solvent that is a strong nonsolvent for the core. Alternatively, molecularly dissolved chains of block copolymer can be obtained in a nonselective solvent. To trigger micellization in the molecularly dissolved chains a selective solvent for one of the blocks and precipitant for the other may be added, or temperature or pH variations may be used.[37]

Preparation of Drug-loaded Micelles

Drug-loaded polymeric micelles can be prepared mainly by three common approaches: direct dissolution, solvent evaporation, and dialysis. Direct dissolution of the amphiphilic copolymer and drug in water is the simplest technique of preparing drug-loaded polymeric micelles. At or above CMC, the copolymer and the drug self-assemble in water to form drug-loaded micelles. But this method usually is associated with low drug loading. To enhance drug loading, this technique can be combined with an increase in temperature or alternately a thin evaporated film of drug can be prepared before the addition of copolymer. In solvent evaporation or solution-casting technique, a volatile organic solvent is used to dissolve the copolymer and the drug. A thin film of copolymer and drug is obtained after the solvent is removed by evaporation. Drug-loaded polymeric micelles are obtained by reconstitution of film with water. When the core forming blocks are long and more hydrophobic, the two above-mentioned techniques are unsuitable. Micelles from such copolymers have more potential to solubilize large amounts of poorly water-soluble drugs. In these cases, the dialysis technique can be used to prepare drug-loaded micelles. Solutions of the drug and the polymer in organic solvent are placed in the dialysis bag, and the solvent is exchanged with water by immersing bag into water, inducing micelle assembly.[38,39] However, emulsification involving use of chlorinated solvents is not safer and dialysis process often requires more than 36 hours for efficient loading. Nevertheless, the above mentioned limitations can be overcome by employing a simple and cost-effective method in which water/tert-butanol mixture is used for dissolving drug as well as polymer and then the solution is lyophilized. Drug-loaded polymeric micelles are then obtained by redispersing the lyophilized product in a suitable vehicle.[40,41]

Owing to extreme dilutions by blood upon intravenous injections of micellar solution, polymeric micelles are prone to deformation and disassembly which may lead to leakage and burst release of loaded drugs. However, this limitation can now be overcome by improved interaction of the drug and polymer via chemical conjugation or by cross-linking of the shell.[42] The loss of hydrophilic and hydrophobic balance upon increased loading of hydrophobic moiety (drug) into the core

region also has been related to decreased stability of the polymeric micelles. Drugs or copolymers prone to hydrolytic cleavage in aqueous systems may as well pose stability problems. However, lyophilized polymeric micelle formulations have shown to possess improved long-term stability for intravenously administered preparations.[43]

CHARACTERIZATION OF POLYMERIC MICELLES

CMC

In aqueous media, amphiphilic polymers can exist in the form of micelles when the concentration is higher than CMC, and when diluted below this concentration, the micelles may collapse. Hence, CMC is the key parameter for the formation and the static stability of polymeric micelles. Some of the methods used for determination of CMC in aqueous dispersions of micelles include surface tension measurements, chromatography, light scattering, small angle neutron scattering, small angle X-ray scattering, differential scanning calorimetry, viscometry, and utilization of fluorescent probes. For easy practical determination, CMC is obtained from plots of the surface tension as a function of the logarithm of the concentration. The CMC is said to be attained when the surface tension stops decreasing and reaches a plateau value. Most of the researchers have relied upon use of pyrene as a fluorescent probe for estimating CMC.[44]

Size and Shape Determination

After the preparation of the micelles useful information regarding the polydispersity index of the prepared structures is obtained by examining the micellar solution with quasi-elastic light scattering technique. Monodisperse micelles produce blue color from light scattering which indicates good micellar preparation, as contrasted with the white color shown by aggregates.[37]

Size of polymeric micelles usually falls in the colloidal range. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques have been widely used past many years for the direct visualization, size and shape determination of block copolymer micelles. The more recently developed cryo-TEM technique has increasingly started gaining importance for characterization of block copolymer micelles in aqueous medium. SEM or atomic force microscopy (AFM) reveals information regarding size distribution when chemically attached micelles to surfaces are presented. Direct visualization of block copolymer micelles either in the dried state or directly "in situ" within a liquid cell can be achieved by AFM. Hydrodynamic diameters and polydispersity indices of micelles are obtained using photon correlation spectroscopy. Recently size characterization of drug-loaded polymeric micelles was done using asymmetrical flow field-flow fractionation and the structure of assemblies was determined by small angle neutron scattering.[45,46]

In Vitro Drug Release Behavior

In-vitro drug release behavior from micelles is easily studied by placing the micellar solution in a dialysis tube. The dialysis bag is immersed into a flask containing release medium, kept at a constant temperature. At predetermined time intervals, aliquots of the release medium are taken and replaced by fresh medium. The content of drug released in the medium can be measured by spectroscopic or other suitable method.[47]

APPLICATIONS

SOLUBILIZATION

The micellar core is a compatible micro-environment and a hub for incorporating water-insoluble guest molecules. The hydrophobic molecules can be covalently coupled to the block copolymers or physically incorporated into the hydrophobic core of micelles. The solubilization process leads to enhancement of their water solubility and thereby bioavailability.[48] It is often observed that the gastrointestinal (GI) uptake of particles is affected significantly by particle size. A 15 to 250-fold higher uptake efficiency of particles approximately 100 nm in diameter by the GI tract was noted than that of the micrometer-sized particles.[49] Thus, polymeric micelles (nanosized) elevate uptake and enhance bioavailability.

The extent of solubilization depends upon the micellization process, the compatibility between the drug and the core-forming block, chain length of the hydrophobic block, concentration of polymer, and temperature.[50] Above CMC, there is a sharp increase in the solubility of drug as it gets more space to occupy in the aggregates of the hydrophobic part of the micelle. The occupancy of the core region by drug leads to an increased Rc of the micelle. It is worth mentioning that the core region has limited capacity for accommodation, for instance, Pluronic P85 has a core region which is 13% of the whole micelle weight.[51] The influence on solubilization capacity of hydrophobic block length has been examined for griseofulvin in polyoxyethylene and polyoxybutylene copolymer micelles with varying number of hydrophobic block lengths and hydrophilic block lengths sufficient for formation of spherical micelles. It was found that the solubilization capacity was dependent on the hydrophobic block length upto a certain extent (15 units of hydrophobic block), after which the solubilization capacity became independent of the same.[52] Dong and coworkers also studied the effect of hydrophobic block length on solubilization of toluene in diblock and triblock polyurethane surfactants. It

was concluded that solubilization capacity of polyurethane surfactants increased with an increase in the hydrophobic segment for the same block chain structure.[53] Some noteworthy contributions for solubilization of drugs are noted in Table 3.

TARGETING

Targeting via polymeric micelles is usually achieved by one of the following approaches; the enhanced permeability and retention effect, stimuli-sensitivity, complexing specific targeting ligand molecules to the micelle surface, or by coupling monoclonal antibodies to the micelle corona, i.e. active targeting using immunomicelles.[58]

Enhanced Permeability and Retention Effect (EPR Effect)

Owing to their nanoscopic size, polymeric micelles passively accumulate at the interstitial spaces of various pathological sites by extravasating leaky capillaries (especially of solid tumors). They also have been shown to distribute to some of the cytoplasmic organelles, and infarct tissues, infected areas, inflammatory sites that have compromised barrier function.[59] As the polymeric micellar drug carriers cannot pass through walls of normal blood vessels, decreased side-effects of the drug are observed. In tumor neovasculature, there is a poorly developed lymphatic drainage system that leads to enhanced retention of polymeric micelles within the solid tumor as micelles are not efficiently cleared. This feature allows prolonged circulation of polymeric micelles in the circulatory system upon administration.[60] Due to these characteristics, it is possible to achieve passive drug targeting using polymeric micelles.

The hyperpermeability of tumors associated with the EPR effect is based on excessive production and secretion of vascular permeability factors stimulating extravasation within cancerous tissue. Commonly secreted chemicals are vascular endothelial growth factor bradykinins, nitric oxide, prostaglandins, enzyme collagenase, peroxynitrite.[61]

Vetvicka and his associates formulated a micellar drug delivery system designed to prolong the blood circulation time and maximize the efficiency of the EPR effect.[62] They prepared doxorubicin conjugated poly(ethylene oxide)-block-poly(allyl glycidyl ether) micellar system that circulated for long time and released doxorubicin efficiently at the tumor site because of the acidic pH prevailing at the tumor site. This also leads to destabilization and disruption of the micellar system generating free diblock unimers that could be excreted. Maitani *et al.* developed polymeric micelles composed of various poly(ethylene glycol)-poly(aspartate ester) block copolymers incorporating camptothecin, a naturally occurring cytotoxic alkaloid.[63] The micellar system solubilized the poorly water soluble drug and a stable formulation of camptothecin-loaded micelles was obtained. The stability of the formulation was found to strongly depend on the amount of benzyl esters and length of the PEG. The drug-loaded micelles were potentially delivered to tumor sites owing to the EPR effect.

Stimuli-Sensitivity

For ideal drug targeting, there should not be any drug release from the micelle during circulation. The drug should be released only after the polymeric micelles accumulate at the targeted tissue, by means of some internal trigger such as pH,

Table 3 - Examples of improvement in solubility of drugs using polymeric micellar system

Drug	Amphiphilic polymer	Comment	Ref
Camptothecin	Pluronic P105, d- α -tocopheryl polyethylene glycol 1000 succinate	increased micellar stability; increased cytotoxicity	54
Docetaxel	Poly(ethylene oxide)-block-poly(styrene oxide) (PEO-b- PSO) and PEO-b-poly(butylene oxide) (PEO-b-PBO)	PSO-based copolymers were associated with higher solubilizing capacities than PBO due to the aromatic structure of the core-forming polymer	55
Griseofulvin	E_mB_n copolymers (E = oxyethylene, B = oxybutylene, subscripts denote number-average block lengths in repeat units)	solubilization independent of B block length when it exceeds about 15B units	52
Paclitaxel	N-octyl-O-sulfate chitosan	improved bioavailability and reduced toxicity	56
Paclitaxel	mixed micelles of polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) and vitamin E	mixed micelles efficiently solubilized poorly soluble drug as compared to PEG-PE micelles	57

particular enzyme, etc. or by an external trigger including temperature, light, ultrasound or magnetic field (Fig. 2). Depending on the stimulus applied varied responses may be observed including disruption of the structure, changes in shape, volume, permeation rates, hydration state, swelling/collapsing, hydrophilic/hydrophobic surface, or conformational changes. Destabilization of micelles as a result of stimulation by either physiological or external trigger is termed as 'stimuli-sensitivity' or 'environmental sensitivity' of the micelles.[64] Release of drug from the micellar system is dependent on the exploitation of differences that exist in normal tissues and pathological tissues. Such a release mechanism from polymeric micelles is also termed as 'intelligent delivery' or 'smart delivery' by other researchers.

Acid-Sensitive Polymeric Micelles

There are a number of pH gradients that exist in normal and pathophysiological states inside the body. Acid-sensitive or pH-sensitive polymeric micelles exploit these differences in pH for drug targeting. In tumors and inflammatory tissues a mildly acidic pH is encountered (pH approx. 6.8). This is a slightly low value as compared with the pH of blood and normal tissues (pH approx. 7.4). Micelles can also be taken up into the cell by the process of endocytosis and may as well enter cell organelles as endosomes, lysosomes, etc. The pH value inside these organelles is nearly 5.5.[65] This has served as the basis for the development of pH-sensitive polymeric micelles. e.g., negatively charged oligo/poly(nucleic acids) can be delivered intracellularly by complexing them with cationic polymers. Once into endosomes, these are deprotonated causing disruption of endosomal membrane and releasing nucleic acids in the cytosol.[66]

Two main approaches that have been used for developing pH-sensitive systems are: involvement of a titrable group into the copolymer, and inclusion of labile linkages that are destabilized in acidic conditions. Incorporation of titrable groups such as amines, carboxylic acids into the backbone of the copolymer leads to an alteration of the solubility of the polymer upon protonation. This in effect may disrupt the micellar structure. Inclusion of acid-labile linkages, such as benzoic imine linkage, in polymeric structures has shown to cause change in micellar integrity or complete destruction of the micellar structure when these polymers encounter low-pH environment.[67]

Thermosensitive Polymeric Micelles

The thermosensitive micelles undergo a structural change as a response to temperature increase, resulting in the deposition of the drug and easier drug absorption by cells. Thermosensitive polymers at a certain temperature produce a volume phase transition associated with a sudden change in the solvation state. This transition temperature is termed as critical solution temperature. Polymers solubilized upon heating possess an upper critical solution temperature, and those which become insoluble possess lower critical solution temperature (LCST). With regard to the thermal targeting strategy, LCST is the most important parameter.[71]

Temperature changes can be internal, e.g. hyperthermia during inflammation, or can be external. Heat can be generated inside target tissues by locally applied ultrasound or by locally applied high frequency causing the oscillation of target-accumulated magneto-sensitive micelles.

Liu *et al*. demonstrated the use of poly(N-isopropylacrylamide-co-acrylamide)-b-poly(D,L-lactide) copolymer in tumor targeting of docetaxel.[68] They observed that hyperthermia greatly enhanced the targeting efficacy of drug-loaded micelles and also helped in reduction of toxicity of drug.

Complexing Targeting Ligand Molecules to Micelles

An impressive strategy to enhance cellular internalization of polymeric micelles at desired target tissue is attachment of cell-specific ligands on the surface of these nanocarriers. Thus, covalent attachment of cell specific ligands, e.g., sugars, peptides, and monoclonal antibodies, on the surface of polymeric micelles has been pursued to enhance drug delivery to various cells. For tumor targeting, cancer-specific peptides are more appropriate as peptides can easily be derivatized and engineered to achieve better *in vivo* stability and tissue specificity. In this context, Lavasanifar *et al.* conjugated an arginine-glycine-aspartic acid (RGD) containing peptide as a ligand, that can recognize adhesion molecules overexpressed on the surface of metastatic cancer cells, to the surface of poly(ethylene oxide)-block-poly(caprolactone) micelles.[69] It was found that micelles were good ligand-targeted carriers for enhanced drug delivery to metastatic tumor cells.

Torchilin *et al.* used the overexpression of Peripheral Benzodiazepine Receptor (PBR) in certain cancers for targeting such tissues.[70] Selective ligands to the PBR may induce apoptosis and cell cycle arrest. Thus, polyethylene glycol-phosphatidylethanolamine PBR-targeted micellar drug delivery system loaded with paclitaxel was prepared. They demonstrated the use of this system to reveal significantly enhanced toxicity against some cancerous cells.

Active Targeting using Immunomicelles

Attachment of antibodies to micelle surface provides the broadest opportunities in terms of diversity of targets. Thus, many researchers have tried to exploit this opportunity by covalently attaching an antibody to polymeric micelles for

generating the 'immunomicelles'.[71] To demonstrate the effectiveness of using immunomicelles in targeting of cancer, Torchillin *et al.* solubilized paclitaxel and camptothecin in mixed micelles of polyethylene glycol-phosphatidyl ethanolamine and vitamin E. These micelles were additionally modified with antinucleosome monoclonal antibody 2C5 (mAb 2C5), which can specifically bring micelles to tumor cells *in vitro*. These mixed micelles and mAb 2C5-immunomicelles demonstrated significantly higher *in vitro* cytotoxicity against various cancer cell lines.[62]

CONCLUSION

Polymeric micelles have emerged as important pharmaceutical carriers because of their attractive properties. Preparation of polymeric micelles appears to be relatively simple as compared with the other novel drug delivery systems. Polymeric micelles can be easily loaded with a wide variety of poorly soluble drugs, thus resulting in enhanced bioavailability of these drugs. Importantly, these can be effectively used to target certain pathological areas in the body with compromised vasculature such as tumors, infarcts because of their size and EPR effect. Targeting can also be achieved by attaching specific ligands or specific antibodies onto their surface. Thus polymeric micelles, as drug carriers, have a promising future.

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