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NANO MICELLES A NOVEL APPROACH - A REVIEW

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ABSTRACT

Emerging nanotechnology has already developed various innovative nanomedicines. Nanomicelles, self-assemblies of block copolymers, are promising nanomedicines for targeted drug delivery and imaging. Stimulus-responsive targeted nanomicelles are designed to release drugs based on stimuli such as pH, temperature, redox potential, magnetism and ultrasound. This article will focus on recent advancements in the design of stimulus-responsive targeted nanomicelles loaded with anticancer drugs to fulfill the challenges associated with cancer cells (e.g., multidrug resistance) for the effective treatment of cancer. The significant toxicity issues and a possible future perspective associated with nanomicelles are also discussed here.

INTRODUCTION

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Particles are further classified according to size, in terms of diameter coarse particles cover a range between 10,000 and 2,500 nanometers. Fine particles are sized between 2,500 and 100 nanometers. Ultrafine particles, or nanoparticles are sized between 100 and 1 nanometers. The reason for this double name of the same object is that, during the 1970-80's, when the first thorough fundamental studies were running with "nanoparticles" in the USA (by Granqvist and Buhrman) and Japan, (within an ERATO Project) they were called "ultrafine particles" (UFP). During the 1990s before the National Nanotechnology Initiative was launched in the USA, the new name, "nanoparticle" had become fashionable. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles.

Emergence of nanotechnology in 1980s was caused by the convergence of experimental advances such as the invention of scanning tunneling microscope in 1981 and the discovery of fullerenes in 1985, with the elucidation and popularization of a conceptual framework for the goals of nanotechnology beginning with the 1986 publication of the book "*Engines of Creation*". The scanning tunneling microscope, an instrument for imaging surfaces at the atomic level, was developed in 1981 by Gerd Binnig and Heinrich Rohrer at IBM Zurich Research Laboratory, for which they received the Nobel Prize in Physics in 1986. Fullerenes were discovered in 1985 by Harry Kroto, Richard Smalley, and Robert Curl, who together won the 1996 Nobel Prize in Chemistry.

Use of nanotechnology

As of August21, 2008, the Project on Emerging Nanotechnologies estimates that over 800 manufacturer identified nanotech products is publicly available, with new ones hitting the market at a pace of 3–4 per week. The project lists all of the products in a publicly accessible online database. Most applications are limited to the use of "first generation" passive nanomaterials which includes titanium dioxide in sunscreen, cosmetics, surface coatings, and some food products; Carbon allotropes used to produce gecko tape; silver in food packaging, clothing, disinfectants and household appliances; zinc oxide in sunscreens and cosmetics, surface coatings, paints and

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outdoor furniture varnishes; and cerium oxide as a fuel catalyst.

Further applications allow tennis balls to last longer, golf balls to fly straighter, and even bowling balls to become more durable and have a harder surface. Trousers and socks have been infused with nanotechnology so that they will last longer and keep people cool in the summer. Bandages are being infused with silver nanoparticles to heal cuts faster. Cars are being manufactured with nanomaterials so they may need fewer metals and less fuel to operate in the future. Video game consoles and personal computers may become cheaper, faster, and contain more memory thanks to nanotechnology. Nanotechnology may have the ability to make existing medical applications cheaper and easier to use in places like the general practitioner's office and at home. The National Science Foundation (a major distributor for nanotechnology research in the United States) funded researcher David Berube to study the field of nanotechnology. His findings are published in the monograph Nano-Hype: The Truth behind the Nanotechnology Buzz. This study concludes that much of what is sold as "nanotechnology" is in fact a recasting of straightforward materials science, which is leading to a "nanotech industry built solely on selling nanotubes, nanowires, and the like" which will "end up with a few suppliers selling low margin products in huge volumes." Further applications which require actual manipulation or arrangement of nanoscale components await further research. Though technologies branded with the term 'nano' are sometimes little related to and fall far short of the most ambitious and transformative technological goals of the sort in molecular manufacturing proposals, the term still connotes such ideas.

Medicinal use of nanotechnology

Fast developing nanotechnology, among other areas, is expected to have a dramatic impact on medicine. The application of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems has recently been determined. Among the approaches for exploiting nanotechnology developments in medicine, various nanoparticulates offer some unique advantages as pharmaceutical delivery systems and image enhancement agents. Several varieties of nanoparticles are available, different polymeric and metal nanoparticles, liposomes, micelles, quantum dots, dendrimers, microcapsules, cells, cell ghosts, lipoproteins, and many different nano assemblies a major role in diagnosis and therapy. Among particulate drug carriers, liposomes, micelles and polymeric nanoparticles are the most extensively studied and possess the most suitable characteristics for encapsulation of many drugs and diagnostic (imaging) agents. Among many possible applications of nanotechnology in medicine, the use of various nanomaterials as pharmaceutical delivery systems for drugs, DNA, and imaging agents has gained increasing attention. Two forms of nanomedicine that have already been tested in mice and are awaiting human trials are using gold nanoshells to help diagnose and treat cancer, and using liposomes as vaccine adjuvants and as vehicles for drug transport. Similarly, drug detoxification is also another application for nanomedicine which has shown promising results in rats. A benefit of using nanoscale for medical technologies is that smaller devices are less invasive and can possibly be implanted inside the body, plus biochemical reaction times are much shorter. These devices are faster and more sensitive than typical drug delivery.

Nanomedical approaches to drug delivery centre on developing nanoscale particles or molecules to improve drug bioavailability. Bioavailability refers to the presence of drug molecules where they are needed in the body and where they will do the most good. Drug delivery focuses on maximizing bioavailability both at specific places in the body and over a period of time. This can potentially be achieved by molecular targeting by nanoengineered devices. It is all about targeting the molecules and delivering drugs with cell precision. More than $65 billion are wasted each year due to poor bioavailability. *In vivo* imaging is another area where tools and devices are being developed. Using nanoparticle contrast agents, images such as ultrasound and MRI have a favorable distribution and improved contrast. The new methods of nanoengineered materials that are being developed might be effective in treating illnesses and diseases such as cancer.

Drug delivery systems, lipid or polymer based nanoparticles, can be designed to improve the pharmacological and therapeutic properties of drugs. The strength of drug delivery systems is their ability to alter the pharmacokinetics and biodistribution of the drug. When designed to avoid the body's defence mechanisms, nanoparticles have beneficial properties that can be used to improve drug delivery. Where larger particles would have been cleared from the body, cells take up these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, including the ability to get drugs through cell membranes and into cell cytoplasm. Efficiency is important because many diseases depend upon processes within the cell and can only be impeded by drugs that make their way into the cell. Triggered response is one way for drug molecules to be used more efficiently. Drugs are placed in the body and only activate on encountering a particular signal. For example, a drug with poor solubility will be replaced by a drug delivery system where both hydrophilic and hydrophobic environments exist, improving the solubility. Also, a drug may cause tissue damage, but with drug delivery, regulated drug release can eliminate the problem. If a drug is cleared too quickly from the body, this could force a patient to use high doses, but with drug

delivery systems clearance can be reduced by altering the pharmacokinetics of the drug. Poor biodistribution is a problem that can affect normal tissues through widespread distribution, but the particulates from drug delivery systems lower the volume of distribution and reduce the effect on non-target tissue. Potential nanodrugs will work by very specific and well-understood mechanisms; one of the major impacts of nanotechnology and nanoscience will be in leading development of completely new drugs with more useful behaviour and less side effects.

MICELLES [2]

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. 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.

POLYMERIC MICELLES [3]

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. 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. 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. 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.

ADVANTAGES OF POLYMERIC MICELLES [4]

Very small size

The first physico-chemical characteristic is the polymeric micelle's very small size. Polymeric micelles are formed typically in a diameter range from 10 nm to 100 nm with a substantial narrow distribution. This size range is considered ideal for the attainment of stable, long-term circulation of the carrier system in the bloodstream. Alternatively, the small size of polymeric micelles is a big benefit in the sterilization processes in pharmaceutical productions. Polymeric micelles are easily (without micron-sized particle's clogging) and inexpensively (without another separation process) sterilized by filtration using typical sterilization filters with 0.45-mm or 0.22-mm pores owing to a fact that polymeric micelles are essentially free of micro-sized particle's contamination. This is a good contrast to other typical pharmaceutical nanosized carrier systems (e.g., nanoparticles, liposomes) which need a removal process of contaminated micron-sized particles.

High structural stability

The second physicochemical characteristic is high structural stability. It is known that polymeric micelles possess high structural stability provided by the entanglement of polymer chains in the inner core. This

stability has two aspects: static and dynamic. Static stability is described by a critical micelle concentration (CMC). Generally, polymeric micelles show very low CMC values in a range from 1 mg/ml to 10 mg/ml. These values are much smaller than typical CMC values of micelles forming from low-molecular weight surfactants. The second aspect, dynamic stability, is described by the low dissociation rates of micelles, and this aspect may be more important than the static one for in vivo drug delivery in physiological environments that are in non equilibrium conditions. The high structural stability of polymeric micelles stated earlier is an important key to in vivo delivery in micellar forms and simultaneously eliminates the possible contribution of single polymer chains to drug delivery. Therefore, although they share the root word "micelle," polymeric micelles are very different from low-molecular-weight-surfactant micelles in their physicochemical properties. This difference is critical in the applications for drug carriers.

Large amount of drug loading

The third advantage of the polymeric micelle carrier system as a drug carrier is its high water solubility even when it incorporates a large amount of hydrophobic drugs. Accordingly, "large amount of drug loading" is listed as the forth advantage. Generally, in conventional synthetic polymer-drug conjugate systems and antibody drug conjugate systems, a loss of the carrier's water solubility resulting from the conjugation of a hydrophobic drug creates a serious problem. Several research groups reported this problem of the polymer-drug conjugates in synthesis and in their intravenous injections. Polymeric micelles can incorporate a large number of hydrophobic drug molecules in the micelles' inner core, and simultaneously, the micelles can maintain their water solubility by inhibiting intermicellar aggregation of the hydrophobic cores with a hydrophilic outer shell layer that works as a barrier against inter micellar aggregation. This is a great advantage because many potent drugs that have been developed in recent years are very hydrophobic and are, therefore, water insoluble.

Low toxicity

Generally, polymeric surfactants are known to be less toxic than low-molecular-weight surfactants, such as sodium dodecyl sulfate. Furthermore, in theory, polymeric micelles are considered very safe in relation to chronic toxicity. Possessing a much larger size than that for critical filtration in the kidney, polymeric micelles can evade renal filtration, even if the molecular weight of the constituting block copolymer is lower than the critical molecular weight for renal filtration. On the other hand, all polymer chains can be dissociated (as single polymer chains) from the micelles over a long time period. This phenomenon results in the complete excretion of the block copolymers from the renal route if the polymer chains are designed with a lower molecular weight than the critical value for renal filtration. Such a result constitutes an advantage of polymeric micelles over the conventional (nanomicelle forming) and nonbiodegradable polymeric drug carrier systems.

Incorporation of various chemical species

As explained previously, the most commonly examined chemical species are hydrophobic low-molecular-weight organic compound drugs. These drugs can be incorporated into the micelle inner core either by chemical conjugation to the inner core forming polymer block or by physical entrapment owing to hydrophobic interactions between the entrapped drug molecules and the hydrophobic inner core forming polymer block. Hydrophobic interactions also work as a driving force for micelle formation. On the other hand, polymeric micelles are formed through ionic interactions between charged polymer chains. For example, polymeric micelles form from poly(ethylene glycol) (PEG)-b-poly(lysine) block copolymers and poly(aspartic acid) (ASP) homopolymers where the poly(lysine) chain is positively charged and the poly(ASP) chain is negatively charged. If negatively charged polypeptides or nucleic acid are used in place of poly (ASP), these pharmacologically active macromolecules are incorporated into polymeric micelles for protein, gene, and small interfering RNA delivery purposes. Furthermore, metal ions or metal ions' chelates can be incorporated into polymeric micelles through coordination bonds or ionic interactions. A platinum chelate cisplatin, which is a widely used anticancer drug, was successfully incorporated into polymeric micelles forming from PEG-b-poly (ASP) through a ligand exchange reaction between a carboxylic acid residue of the poly (ASP) chain and a chloride ion of cisplatin. Alternatively, gadolinium (Gd) ions, which can work as a magnetic resonance imaging (MRI) contrast agent, were incorporated into polymeric micelles by the use of a chelatemoiety conjugated block copolymer. As stated above, various pharmaceutical drugs, genes, and contrast agents can be incorporated into polymeric micelles with appropriate choices of block copolymer structures.

DISADVANTAGES [4]

It is worthwhile to explain the disadvantages of the polymeric micelle systems and the advantages described above. Two of them are polymeric micelle-specific ones, whereas the other two disadvantages are common for polymeric carriers including non-micelle-forming systems.

Difficult polymer synthesis

The first disadvantage is a fact that relatively high levels of polymer chemistry are needed in the polymeric micelle studies. An AB type of block copolymer is one of the most favourable structures for polymeric micelle

carriers. The architecture of the AB block copolymer is very simple, however, its synthesis is more difficult than that of random polymers, where different units are aligned on a polymer chain in a random manner. Furthermore, researchers may encounter a problem in a synthesis of the block copolymer of a large industrial scale in a highly reproducible manner.

Immature drug incorporation technology

The second disadvantage, specifically, for the polymeric micelle systems is the immature technology for drug incorporation in a physical manner. Yokoyama et al reported that physical incorporation efficiencies were dependent on various factors in drug-incorporation processes. Presently, there seem to be no universal incorporation method applicable to any polymer. Furthermore, in some methods the drug incorporation may be difficult on a large industrial scale, whereas the drug incorporation is easy and efficient on a small laboratory scale.

Slow extravazation

The third disadvantage is much slower extravazation of polymeric carrier systems than that of low-molecular weight drugs. This results from a difference in extravazation mechanisms between polymeric carrier systems and low molecular weight drugs. The polymeric systems translocate from the bloodstream to the interstitial space of organs and tissues through intra-cellular channels and inter-cellular junctions, whereas the drugs permeate directly through lipid bilayer cell membranes. Therefore, a long circulation character of the polymeric systems is an essential requirement for delivery of a therapeutic amount owing to compensation of the slow extravazation with a large Area under the Curve value that results from the long circulation.

Chronic liver toxicity

Drugs conjugated or incorporated in the polymeric carrier systems are metabolized in liver in a slower manner than free drug, since access of metabolic enzymes to drugs is inhibited because of the conjugation and incorporation. Therefore, toxic side effects of the conjugated and incorporated drug may be exhibited for a longer period than a case of free drug whose toxic effects can be lowered through metabolism in a short period.

WHY POLYMERIC MICELLES ARE ATTRACTIVE [2]

Suitable amphiphilic block copolymers are obtainable via controlled synthesis by varying the block ratio, the total molecular weight, and the chemical structure. By adjusting 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. 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. 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 favours 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. 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. 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.

TYPES OF POLYMERIC MICELLES [2]

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

Conventional micelles

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)-bpoly(ethylene oxide), forms micelles as a result of 16 hydrophobic interactions.

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 vander 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 along with antisense oligonucleotides, DNA, and enzymes. 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).

Non-covalently 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. 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.

PREPARATION PARAMETERS [2]

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.

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. 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. 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. 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.

APPROACHES TO SUSTAIN DRUG DELIVERY FROM MICELLES [5]

Prodrugs

Synthesizing a prodrug of the drug of interest and encapsulating in a micelle is useful for sustaining drug release. In this approach a prodrug that is most compatible with the micelle-forming amphiphilic molecule is desirable. Prodrug release from the micelles and prodrug conversion to drug are the two limiting processes controlling drug release in this approach. One such example is paclitaxel palmitate, a paclitaxel prodrug, which was synthesized by Forrest et al. and encapsulated in PEG-b-polycaprolactone (PEG-b-PCL) (Mw of PEG: 5000, Mw of PCL: 10,500) micelles. The mean diameter of these micelles was about 27–44 nm. The prodrug micelles released the prodrug over 14 days compared with 1 day release with the plain drug. However, this study did not assess the release of the drug from the prodrug by itself. The prodrug micelles also showed better antiproliferative effects in breast cancer cells when compared with the unencapsulated prodrug or paclitaxel itself, although the improvements were marginal (~10–20% greater inhibition of cell growth). Further, the micelles sustained serum drug as well as prodrug levels for prolonged periods in Sprague Dawley rats. Encapsulation of paclitaxel in micelles increased the time for which the drug was detected in the serum from less than 10 to 25 h. For prodrug, micelles retained drug levels up to 50 h when compared with approximately 11 h for plain prodrug. Thus, the prodrug approach in conjunction with micelles prolongs drug release and hence, potentially its effects as well. Apart from increasing the release time and effect, this approach may lead to an increase in tolerability of the drugs among drug recipients. Another study by Xiong et al. showed an increase in tolerability towards geldanamycin prodrug in rats when formulated in methoxy PEG-b-PCL (5000:9200) micelles. Free prodrug was administered to Sprague Dawley rats at 10, 20 and 40 mg/kg doses and prodrug encapsulated in micelles was administered at 10, 20, 40, and 200 mg/kg doses. Blinded observers made observations of nose bleeding, diarrhoea and visible behavioural changes associated with geldanamycin prodrug toxicity. The encapsulated prodrug did not show any signs of toxicity for 24 h at 40 mg/kg dose while the free prodrug showed signs of toxicity within 12 h of administration of 10 mg/kg and higher doses. Thus, micellar formulation improves the tolerability of geldanamycin prodrug. However, a disadvantage for this approach is that the drug has to be modified to suit the delivery system and the chemistry of the drug may not always be amenable for suitable manipulations.

Drug polymer conjugates

This is one of the most effective ways to sustain drug release from a micellar delivery system. This approach typically involves forming a conjugate of the drug with the hydrophobic part of an amphiphilic polymer and then forming micelles out of this conjugate. Such a formulation will add two steps for the release of the drug. First, the drug is released from the polymer through enzyme hydrolysis or other means of breakdown and second, the drug is released via diffusion of the drug out of the micelles, with the former typically being the rate-limiting step. A major advantage of this method is that the drug remains in the micelle for a long period of time due to conjugation. However, this method needs some complex chemistry in forming a drug–polymer conjugate. Yoo and Park conjugated doxorubicin (DOX) with PLGA portion of PLGA-PEG (Mn = 13000, Mw = 23000), wherein the molecular weight of the PEG used to prepare the copolymer was 2000. In this preparation, PLGA–DOX formed the core and PEG formed the shell of the micelle. The reported size of the micelles was approximately 61.4 nm. The CMC of the micelles with or without DOX was found to be 0.1 μg/ml, indicating that the conjugated DOX did not affect the micelle-forming properties of the polymer. The drug-loading efficiency of the conjugate in micelles was approximately 99% while that of the physically entrapped drug was about 23%. In vitro drug release studies indicated approximately 60% drug release over 16 days for conjugate micelles as opposed to physical entrapment micelles, which released the entire dose in about 4 days . Comparison of drug-conjugated micelles with the plain drug indicated that the micelles were 10-fold more cytotoxic than the free drug in HepG2 cells. Jeong and Park synthesized a conjugate of an oligodeoxynucleotide (ODN) and PLGA polymer using carbodiimide chemistry. The ODN acted as the hydrophilic portion and the PLGA acted as the hydrophobic portion of the conjugate. Micelles formulated using this conjugate had a mean diameter of approximately 65.2 nm and sustained in vitro release of the ODN up to 50 days. The CMC of the ODN conjugated micelles was found to be 7.5 μg/ml, which was higher than that of the PEG–PLA (<2 μg/ml). Higher CMC was attributed to the negative charge of the ODN and the associated charge repulsion. However, the CMC was lower than that of oligomethyl methacrylateacrylic acid polymers (> 100 μg/ml) the ODN uptake in mouse fibroblasts was higher for the conjugated micellar formulation compared with the plain ODN. A PLGA-DOX conjugate shows an in vitro release for at least 16 days compared to 4 days when the drug is physically entrapped in the micelles. On the other hand, an oligodinucleotide–PLGA conjugate sustained in vitro drug release up to 50 days. ODN–PLGA and PEG–PLGA–DOX represent the micelles prepared by the same strategy – polymer–drug conjugates. Hence, it is informative to compare the in vitro release from both these types of micelles.

Drug release was assessed in phosphate buffer saline (PBS) at 37°C. (A) Triangles show the in vitro release in PBS of DOX from PEG–PLGA–DOX micelles. The micelles showed 60% in vitro release of the drug on day 15. (B) Squares show the in vitro release of oligodeoxynucleotide (ODN) from ODN-PLGA micelles in phosphate buffered saline. ODN was conjugated to PLGA forming an amphiphilic polymer. The micelles showed a full release in approximately 50 days. DOX: Doxorubicin; ODN: Oligodeoxynucleotide; PEG: Polyethylene glycol.

NK012 micelles

NK012 polymeric micelles with a 20 nm diameter were developed by Kuroda et al. to encapsulate 7-ethyl 10-hydroxy camptothecin (SN38) and was compared with its prodrug irinotecan hydrochloride (CPT11). The formulation and the prodrug were tested using five human glioblastoma cell lines. The IC50 of plain drug SN38 was found to be 0.052 μmol/l, while that of the micellar formulation was found to be 0.069 μmol/l. The IC50 of the prodrug CPT11 was found to be 13 μmol/l, which is significantly higher than both the plain drug and the micellar formulation. The micelle formulation and the plain drug were also tested in orthotopic glioblastoma xenografts in nude mice. The micellar formulation of SN-38 demonstrated a significant (almost six times) decrease in the relative tumour volume until day 25 compared with the prodrug CPT11. The relative tumour volume was maintained near zero until 80 days with the micellar formulation. The relative body weight change in the mice during treatment was also about 10% less for the micellar formulation than the prodrug, which indicates that the micellar formulation was relatively better tolerated than the prodrug. Thus, conjugating the drug with the polymer and then forming micelles out of the conjugate leads to a sustained release of the drug.

Novel polymers

This is the most common approach used to prepare sustained release micelles. Polymers with very low CMC (< 0.1 μg/ml) can be used for prolonging the circulation time before the micelle degrades. Upon intravenous injection, the micelles undergo dilution in the body. If the CMC of the micelles is high, the concentration of the polymer or surfactant falls below the CMC upon dilution and hence, the micelles dissociate. Therefore, a higher concentration of the polymer or surfactant has to be used to prepare the micelles so that they withstand the dilution up to 5 l in the blood. However, the use of high concentrations might not be feasible due to toxicity related dose limitations. If the polymer or surfactant has a CMC lower than 0.1 μg/ml, concentrations as low as 5 mg/ml may be used to prepare a micelle formulation in order to counter the dilution effects in the blood. A variety of polymers including diblock copolymers, triblock copolymers and graft copolymers have been synthesized for this purpose. Figure 3A–C show the shapes and arrangement of the micelles based on such polymers. The structures shown in Figure 3A–C do not always have a low CMC. The CMC depends on the polymer that is used to prepare the micelles. Diblock copolymers have two different blocks of different polymers while triblock co polymers have three different blocks of polymers. A graft polymer is comprised of one polymer that is attached to the backbone of another polymer.

(A) Diblock copolymer micelles prepared from polymers with two different blocks (e.g., PEG–PLGA, PEG–PLA, PEO–PPO micelles). (B) Triblock copolymer micelles prepared from polymers with three different blocks, for example PLA–PEO–PLA micelles in aqueous medium (flower-like micelles). (C) Graft copolymer micelles prepared from polymers where a side chain is grafted onto a main polymer (e.g., cellulose–PLLA micelles in aqueous medium, pthaloyl chitosan–mPEG micelles in aqueous medium). (D) Flower-like micelle prepared from triblock copolymers (e.g., PLA–PEO–PLA micelles). (E) Formation of supramolecular micelles: α-CD and urea in water and PCL in THF were gently mixed at 60°C. Urea facilitates the initialization of polymerization of α-CD and PCL and on dialysis against water, the supramolecular micelles are obtained. This concept is called one pot chemistry. PEO: Polyethylene oxide; PCL: Polycaprolactone; PEG: Polyethylene glycol; PLGA: Poly(lactide-co-glycolide); PLLA: Poly-l-lactic acid.

Block copolymers with lipids

Block copolymers between a polymer and a lipid is one useful approach in preparing micelles. It has been shown that increasing the length of the hydrophobic portion of a micelle will lead to a decrease in its CMC. Lipids are more hydrophobic than most polymers and hence, a micelle made with a lipid as its hydrophobic part might lower the CMC. Hence, using fatty acyl chains as hydrophobic segments in an amphiphilic copolymer might be a useful approach. Distearoyl phosphatidyl ethanolamine (DSPE) has been used as the hydrophobic block in a diblock copolymer with hydrophilic polyethylene oxide (PEO) to form 22 nm micelles. These micelles sustained release of lipophilic beclomethasone dipropionate (partition coefficient [logD] = 3.49) for up to 6 days. Lavasanifar et al. prepared micelles of polyethylene oxide-poly[N-(6-hexyl stearate-l-aspartamide)] (PEO-PHSA) to encapsulate amphotericin B (an antifungal). The plain drug was released within 10 mins while the encapsulated drug was only 20% released in 1 h. The release depended inversely on the degree of fatty acid substitution in the core. A higher substitution leads to a slower release of the drug from the micelles. The slow release was attributed to the favourable interactions between the drug and the micellar core consisting of fatty acids. Slower release also protected the red blood cells

from haemolysis, a side effect of the drug. However, the sustained release was only maintained up to a few hours.

Block copolymers with cyclodextrins

Another approach for drug delivery is supramolecular polymeric micelles. This involves non-covalent interactions between a macromolecular polymer, which works as a host, and a small polymer molecule, which works as a guest. One such attempt was made using α-cyclodextrins (α-CDs) as the hydrophilic macromolecular host and PCL (Mn = 37,000) as the hydrophobic guest molecule. Using this approach, supramolecular polymeric micelles with a mean diameter of 30 nm were made. These micelles resulted in sustained release of an anti-inflammatory drug up to 700 h. One pot chemistry was used to synthesize the micelles. Urea was used in the formation of the supramolecular micelles to facilitate formation of the copolymer. Urea is protonated before addition to the mixture. When it is added to the mixture, at a pH below its acid dissociation constant (pKa), deprotonation of urea occurs leading to the release of a proton from urea. The deprotonation leads to a weakening of the strong intermolecular H-bonds between the CDs, thereby, allowing it to interact with PCL leading to the formation of PCL–α-CD copolymer.

Diblock copolymer micelles

Using a polymer that physically interacts with the drug can result in drug retention and sustained release of the drug from such polymer micelles. If the drug can form hydrogen bonds with the core of the micelle, then the release obtained from the micelle will be much more sustained. For example, Yang et al. prepared micelles from PEG-b-poly-l-lactic acid (PEG-b-PLLA; Mw: 8500 Da) and PEG-b-PCL (Mw: 10,050 Da) block copolymers and studied the in vitro release of the hydrophobic drug quercetin from these micelles. The release of quercetin was sustained from PEG–PLLA and PEG–PCL micelles for approximately 160 h. The in vitro release studies also showed that the total amount of drug released in 160 h was less for the PEG–PCL micelles than the PEG–PLLA micelles. The sustained release was attributed to the H-bonds formed between the drug and the hydrophobic core of the micelle. The lower amount of drug released by the PEG-b-PCL micelle was attributed to a higher degree of H-bonding between quercetin and PCL than quercetin and PLLA.

Using a polymer that participates in hydro-phobic interactions with the drug can also sustain the release of the drug from the micelle. If the polymer hydrophobically interacts with the drug, then the hydrophobic core of the micelle resists the migration of the drug from the core to the media, thus resulting in sustained drug release. This means that the release is affected not only by micelle properties but also by polymer and drug properties. An attempt to form such micelles was made by Xiangyang et al., who synthesized micelles of N-succinyl, N′-octyl chitosan (chitosan Mw: 100,000 Da) and loaded DOX. The mean diameter of the micelles was 100–200 nm and the CMC ranged from 2.4 to 5.9 μg/ml, depending on the percentage octyl content. The release inversely depended on the number of octyl chains, indicating that the octyl chain participates in the hydrophobic interactions with the drug. The cyto toxicity of the micelles was tested on HepG2, A549, BGC and K562 cancer cell lines and was compared with free DOX. The IC50 for the drug and the micelles was compared and the IC50 for the micelles was found to be lower than the free drug by two- to six-fold.

Polymeric micelles made from PEG-poly(benzyl aspartate) were used to encapsulate synthetic retinoids Am80 and LE540. In vitro release studies at 37°C in phosphate buffered saline (PBS) showed that only 10% of the highly hydrophobic retinoid LE540 was released in 4 days while the less hydrophobic retinoid Am80 was approximately 100% released in 4 days. This again shows that hydrophobic interactions between the drug and the polymer may play a role in sustaining the release of the encapsulated drug.

A highly hydrophobic drug, griseofulvin, was entrapped in PLA–PEG (Mn = 11,800; Mw = 14,000) micelles. The mean diameter of the micelles was 26.9 nm and the CMC of the polymer was 0.07–0.09 mg/ml. These micelles exhibited biphasic drug release, with 66% released within 20 days, and the rest by 30 days. On the other hand, the unentrapped drug was released completely within 24 h. The slow release may be due to hydrophobic interactions between the drug and the core polymer.

Zhang et al. prepared micelles from amphiphilic graft polyphosphazenes with poly(N-isopropylacrylamide) (PNIPAAm) as the hydrophilic segment and ethyl 4-aminobenzoate (EAB) as the hydrophobic group. PNIPAAm oligomer with a number average molecular weight of 1800 Da were synthesized by radical polymerization, which was used to synthesize amphiphilic polyphosphazene. A copolymer with a PNIPAAm/EtGly (Ethyl Glycinate) ratio of 1:5 was synthesized. The Mw and Mn of this copolymer was 26,000 Da and 14,000 Da, respectively. The CMCs obtained for the polymers were 0.089, 0.087, 0.083, 0.072 and 0.047 g/l at 15, 20, 25, 30 and 35°C, respectively. The average particle size of plain micelles was 85.2–389.7 nm. Loading 10.4% indomethacin resulted in micelles of 65.0–359.7 nm. Increasing the drug loading to 25.3% resulted in micelles of 201–412.4 nm. Further increase in indomethacin content led to a decrease in particle size to 96.6 nm. These changes in size might occur because of the strong hydrogen bonding between the amide group of the PNIPAAm group of the polymer and the carboxylic group of the drug. This interaction might lead to the formation of a pseudo-hydrophobic amphiphilic copolymer compared with the copolymer itself.

In vitro release profiles showed drug release for at least 40 h and indicated an increase in the release times

with a decrease in pH. The micelles and the drug were injected subcutaneously in male Sprague Dawley rats and the pharmacokinetic profiles were compared. The Tmax increased from 0.5 h for the free drug to 2 h for the micelles. The area under the curve (AUC) increased from 279.4 to 551.3 μg h/ml. The efficacy of the formulation was tested in a carrageenan-induced rat paw edema model. The micellar formulation showed a consistently higher decrease in the degree of rat paw edema over a period of 6 h. At 6 h, the edema volume of the control group was 0.61 ml, edema in plain indomethacin oral administration group was 0.52 ml while that in the micelle formulation was 0.36 ml. The same group of investigators reported that polyphosphazene micelles were also more effective in Complete Freunds Adjuvant (CFA)-induced ankle arthritis model of Sprague Dawley rats. The swelling degree after 7 h for indomethacin micelle formulation decreased to 0.5 compared with 0.62 for the control. Indomethacin by oral administration also showed a swelling decrease from 0.62 to 0.52 after 7 h. However, the dose of indomethacin was 5 mg/kg compared with 1.5 mg/kg for micelles. This means that the micelle formulation was helpful in achieving a similar therapeutic effect at lower doses.

The micelle formulation also showed no ulceration in the rats compared with higher dose free indomethacin oral administration, which showed considerable gastric ulceration (degree of ulceration: 2.75). Pegylated poly(l-lactide) (PLL), polyvalerolactone (PVL) and PCL (Mw = 15,000–31,000 g/mol) were used to prepare polymeric micelles. The CMC of the micelles ranged from 10-7 to 10-8 M. The particle size ranged from 159 to 206 nm and the release of the encapsulated indomethacin lasted at least until 14 days. Drug was not released fully by this time. In vivo pharmacokinetics after subcutaneous injections in rats showed a statistically significant increase in the AUC of the drug from 518.9 μg h/ml for plain drug to 721.32 μg h/ml for the micellar formulation. Reduced clearance of the micelles (and thus the drug) by the liver and the kidneys is the proposed mechanism for sustained drug release by these pegylated lactone micelles. This was shown by a statistically significant decrease in the levels of drug found in kidney and liver in the micelle formulation compared with the free drug. The plasma clearance values were not statistically significant for micelles and free drug. The advantage of this strategy is the low CMC of the polymers, which makes the micelles very stable to dilution. However, the large size of the micelles might hinder their delivery.

Triblock copolymer micelles

Flower-like micelles can be formed with a triblock copolymer with small hydrophobic ends and a long hydrophilic midsection. When dissolved in water, such polymer molecules assemble to form flower-like micellar structure. These flower-like micelles can dissolve the drug in the hydrophobic core and sustain drug release for long periods of time. Sustained, zero-order release has been reported using PLA–PEO–PLA (Mw of PEO = 8900 Da; Mw of PLA= 4100–6500 Da) triblock flower-like micelles (mean diameter of approximately 7–13 nm) for sulindac (20 days) and tetracaine (10 days). Figure 3D shows the formation of flower-like micelles. The hydrophobic interactions between the micellar core and the drugs was proposed to be responsible for the sustained release of the drugs. Drug release was faster with crystalline PLA blocks than amorphous PLA blocks, possibly because crystalline PLA stacks together, leaving the drug largely at the periphery while amorphous PLA might better integrate/disperse the drug within the polymer matrix. Most micelle-forming polymers are first dissolved in organic solvent followed by addition to an aqueous medium to form micelles. The use of organic solvents can be avoided for some triblock copolymer micelles. Furthermore, through suitable selection of polymers, greater drug loading as well as sustained drug release can be achieved. For example, PCL–PEG–PCL (Mw of PEG = 4000 Da; Mw of copolymer = 6000 Da) micelles were formed by Wei et al.. This polymer can be thermally induced to self-assemble when the polymer is added to water at 50°C. The mean diameter of these micelles was 61 nm. Furthermore, the freeze-dried micelles were easily redispersable. These micelles sustained the release of honokiol, an anticancer herbal drug from magnolia leaves, for at least 144 h. During this period, about 50% of the loaded drug was released from the micelles. On the other hand, the plain drug was released completely by 24 h. The cytotoxicity of the free drug and the drug encapsulated in micelles was also compared in lung cancer cells and both were found equally effective, which implies that the encapsulation in micelles did not improve the cytotoxic potency of the drug. However, the micelles might be advantageous in vivo due to prolonged circulation and/or drug targeting to the tumor sites.

Pluronics

Paclitaxel-loaded pluronic micelles of 150 nm in diameter were prepared from pluronic P105 polymer. The pharmacokinetics and biodistribution of paclitaxel was studied in rats following intravenous administration. The half-life and AUC of the drug in micelle formulation were 4.0- and 2.2-fold higher, respectively, when compared with plain taxol.

Unimolecular micelles [5]

Formation of a unimolecular micelle also helps sustain release of very fast-acting drugs. The unimolecular micelle is made out of a polymer that has several hydrophilic and hydrophobic portions in itself and forms a single molecular micelle. Hence, by definition, unimolecular micelles do not have a clear CMC. Figure 1C shows a unimolecular micelle. Lipids and PEG-like hydrophilic polymers can be conjugated to form such

unimolecular micelles. One such polymer is core(laur) PEG, which, when formed into unimolecular micelles, prolongs the release of lidocaine to about 20 h from less than 10 h observed for the plain drug. The core(laur)PEG polymer consists of a core of lauroyl ester of mucic acid (19,000 Da) and a shell of mPEG-5000. The unimolecular micelles formed had a mean diameter of 50 nm. Kainthan et al. prepared unimolecular micelles with a mean diameter of less than 10 nm from hyper branched polyglycerols conjugated to PEG. These micelles released paclitaxel up to 15 days in a sustained manner. However, the CMC of the surfactant (polyglycerol–PEG) was found to be > 1mM, which is very high when compared with the CMC of the block copolymers. Unimolecular micelles were also prepared from hyper branched glycerol-block-PEG. The micelles had a hydrodynamic diameter of less than 10 nm and loading of paclitaxel did not affect the particle size of the micelles. The micelles were compared for their efficacy in four human bladder cancer cell lines and in vivo in athymic nude mice. These micelles had mucoadhesive properties and showed significantly greater reduction in orthotropic tumor growth and they were better tolerated when compared with taxol.

Multiarm coblock polymers

Synthesizing multiarm block copolymers can also be useful to overcome the stability problem of regular micelles. For instance star-shaped or multiarmed micelles can be formed with an amphiphilic block copolymer with multiple hydrophilic blocks and a single hydrophobic block. These polymers can form micelles if the number of arms is high enough. One such polymer is H40–PLA–mPEG (Mn = 108,516 Da; Mw = 148,678 Da). H40 is a polyol that contains 64 hydroxyl groups and is hydrophilic. This means that the multi-arm copolymer has two hydrophilic portions and one hydrophobic region. This polymer was used to form micelles containing 5-FU (5-fluorouracil). The micelles sustained 5-FU release for up to 80 h, unlike plain drug, which was released completely in 4 h. The CMC of this polymer was found to be 4.5 μg/ml and the mean diameter of the micelles was 74 nm. Neither the polymer (400 μg/ml) nor the micelle (up to 400 μg/ml) exposure up to 24 h showed any cytotoxicity in cultured human endothelial cells. Such a polymer with some stabilizing strategies (discussed later) might result in more prolonged release of the drug.

Graft polymers

Graft polymers have recently attracted significant attention in preparing micelles. Figure 3C shows a graft polymer. Cellulose graft polymers can be used to form micelles for sustained drug release. The cellulose portion of the polymer can be the hydrophilic part, with any hydrophobic segment conjugated to it to form an amphiphilic graft polymer. Such polymers are claimed to be biodegradable. Cellulose-g-PLLA (Mn of cellulose = 1.2 × 105 g/mol; Mn of PLLA = 11,000 g/mol) polymer has been used for the sustained delivery of prednisone acetate. Delivery of prednisone acetate was sustained up to more than a week with the use of these micelles. However, drug release for plain drug was not reported. The polymer had a CMC of 47.1–58.1 μg/ml and the mean diameter of the micelles was 30–80 nm. Similarly, graft polymer micelles of pthaloyl chitosan (Mw = 5.78 × 105 Da) and mPEG-2000 sustained the release of camptothecin for 96 h. Moreover, this polymer was synthesized in such a way that the release rate and the percentage yield depended on the degree of deacetylation of chitosan. The CMC of the polymer was 28 μg/ml and the mean diameters ranged from 100–250 nm. The diameter increased with an increase in the degree of deacetylation. Further, higher amounts of drug were incorporated with an increase in the degree of deacetylation of chitosan. The cytotoxicity in HeLa cells also increased with the degree of deacetylation, most likely due to a greater amount of drug incorporated in the micelles.

Oligopeptides

Polymers have some degree of toxicity even if they are biocompatible. Therefore, there is a need to synthesize materials that are more biocompatible for the preparation of micelles and incorporation of drugs. Oligopeptides can be very useful amphiphilic molecules for the preparation of micelles. Hydrophobic residues, such as alanine, can be used to synthesize the hydrophobic block and hydrophilic residues like histidine or lysine can be used to synthesize the hydrophilic block. Such molecules can be used as amphiphilic molecules to formulate micelles. Histidine residues can facilitate endosomal escape and lysine residues can facilitate DNA binding. Using other similar amino acids, the peptide sequences can be tailored to meet the requirements for the drug to be incorporated and therefore, such micelles may have the potential to deliver genes or drugs. For instance, Ac-(AF)6-H5-K15-NH2 peptide (Mw = 3977.92 Da) was synthesized and used for the delivery of DOX. The peptide is made of three blocks: one hydrophobic block of 6 alanine residues, one hydrophilic block of 5 histidine residues and one hydrophilic block of 15 lysine residues. The CMC of the oligopeptide was 42 μg/ml and the reported mean diameter was 102 nm. The uptake of DOX was studied in HepG2 cells by confocal microscopy, which showed a higher uptake of DOX from micelles. This system released approximately 35% of DOX in 40 h. Interestingly, in this study, the plain drug was more cytotoxic than the micellar drug. Okuda et al. prepared 173 nm polymeric micelles using a PEG–polyaspartic acid co polymer. They encapsulated the anti-tumour drug fenretidine in the polymeric micelles and demonstrated a significant increase in the AUC and half-life in mice, when compared with O/W and pegylated O/W emulsion formulations. Reduced drug clearance was considered to be the reason for the

observed increase in AUC. The AUC values were 197, 225, and 4717 μg/h/ml, for O/W emulsion, pegylated O/W emulsion and polymeric micelle, respectively. The corresponding clearance values were 382, 333 and 15.9 ml/h/kg, respectively.

Combination of polymer and amino acid

A combination of polymer and polyamino acid can form an amphiphilic polymer. PEG–polyglutamic acid copolymer was used to prepare micelles for the delivery of cisplatin. The mean diameter of the micelles was 28 nm. The micelles showed a consistent and sustained release of the drug during a 150 h release study. Only 60% of the drug was released during 150 h. The micelles were injected in vivo in mice and compared with the free drug. After 25 h, approximately 10% of the injected dose was found in plasma with micelles compared with 0.1% with the plain drug. This increase was attributed to the decrease in clearance of the micelles compared with the plain drug. This corresponded to less accumulation of the drug in liver, kidney and spleen compared with the tumor with the micelles. Wei et al. reported the synthesis of a polyglutamic acid–poly(propylene oxide) (PPO)–poly glutamic acid polymer (PPO-4000) that is pH sensitive. At high pH, the polyglutamic acid residues form a coil conformation. But at low pH, it transforms to an α-helix conformation. Therefore, at low pH, the polyglutamic acid chain shrinks and creates a stress on the core and hence, results in the distortion of the core of the micelles, which causes the entrapped drug to leak out. DOX showed release up to 168 h with this system. Further, this system can be dispersed in a temperature-sensitive gel and hence, a very sustained release dual drug delivery system might be feasible. However, using peptides to encapsulate drugs is relatively a new field and in vivo work needs to be done further on this delivery system to ensure that this system indeed works as it promises.

Drug was entrapped and not conjugated to the polymer. In vitro drug release was performed in phosphate buffer saline at 37°C. Green circles show the in vitro release profile of quercetin from PEG–PCL micelles. Brown circles show the in vitro release profile of quercetin from PEG–PLLA micelles. Purple circles show the in vitro release profile of doxorubicin (DOX) from N-succinyl,N′-octyl chitosan graft polymer micelles. Release data based on [19]. Red circles demonstrate the in vitro release profile of griseofulvin from PLA-PEG micelles. Orange circles show the in vitro release profile of indomethacin from PLA–PEG micelles. Pink circles show the in vitro release profile of sulindac from PLA–PEO–PLA triblock copolymer micelles. Dark blue circles show the in vitro release profile of tetracaine from PLA–PEO–PLA triblock copolymer micelles. Gray circles show the in vitro release profile of Honokiol from PCL–PEG–PCL triblock copolymer micelles. Release data based on [26]. Yellow circles show the in vitro release profile of paclitaxel from hyper branched polyglycerol-PEG micelles. Light blue circles show the in vitro release profile of prednisone acetate from PLLA–cellulose graft polymer micelles.Light green circles show the in vitro release profile of cisplatin from PEG–polyglutamic acid micelles. The hydrophobic interactions between the PLA polymer and the hydrophobic drug griseofulvin resulted in the most prolonged sustained release. PEG: Polyethylene glycol PEO: Polyethylene oxide; PCL: Polycaprolactone; PLLA: Poly-l-lactic acid.

Reverse micelles

All the above mentioned approaches have been designed for the delivery of largely hydrophobic drugs. However, these approaches are not as useful for the delivery of hydrophilic drugs. Reverse micelles can be used for the delivery of hydrophilic drugs. Figure 1B shows the alignment of the hydrophobic and hydrophilic regions in a reverse micelle. Reverse micelles are especially useful for administration in oily vehicles. Usually the nutrients required for comatose patients are given as oily injections. Moreover, USP injections of steroids can also be made as oily injections. Reverse micelles can prove to be useful for the co administration of hydrophilic drugs in such injections. Some biocompatible oils are also used as vehicles in oral delivery. Thus, reverse micelles may be useful in oral delivery of some drugs by dispersion of micelles in oily vehicles. Reverse micelles may be particularly useful for protein delivery. For instance, ovalbumin was encapsulated in poly caprolactone-poly(2-vinyl pyrrolidone (PCL-b-P2VP; Mn PCL = 35,400 g/mol, Mn P2VP = 20,900 g/mol) reverse micelles and dispersed in an oily medium (oleic acid). The mean hydrodynamic diameter of the ovalbumin-loaded micelles was 157 nm. The protein was entrapped in the aqueous core and the micelles sustained protein release up to 200 h upon contact of the micelle containing oily medium with an aqueous medium. The release of hydrophilic dyes such as fluorescin sodium and trypan blue have been reported from this system up to 60 days. The dyes were dispersed in PLGA polymeric nanoparticles and the nanoparticles were encapsulated in micelles to provide a greater sustained release.

Multilayer micelles with layer by layer assembly

Multi-layer micelle assembly can be used to achieve greater sustained release of drug from micelles than any other techniques. Micelles can be formed from an H-bond acceptor and an H-bond donor can be added to the micellar shell. Then, the micelles can be arranged layer by layer on a support to form a microsized film containing several layers of drug-loaded micelles. The H-bonding can be tailored to be broken under desired conditions to release the micelles. Figure 5 shows a schematic of this strategy. Kim et al. showed sustained release of triclosan (an antibacterial) up to 15 days from these 3 μm thick films of

PEO-b-PCL (PEO-5000, PCL-6500). The mean diameter of the micelles was 71 nm and the CMC of the polymer was 1.2 μg/ml. Upon release, the drug maintained its antibacterial activity. A H-bond donor, polyacrylic acid (PAA; Mw = 90,000 Da), was added to the polymer to introduce H-bonding capability. The carboxylic groups on PAA were crosslinked to retard the release of the drug. The uncrosslinked film released all the drug in 120 min, while the release from the crosslinked films was maintained up to 15 days. This way, more drug can be loaded and a better release profile can be achieved. The release of the drug and the total amount of the drug in these cases depends on the number of layers and the thickness of the film synthesized.

Reverse thermo responsive polymers

These polymers have special properties. They exist as a solid at room temperature but at higher temperatures such as the body temperature, these polymers form gel-like structures. This property can be used to form micelles, which will form a gel-like structure at body temperature. These structures can lock the drug in the core, resulting in a sustained release of the drug. These polymers, however, are complicated to synthesize and very long release times have not been reported. For instance, polyether carbonate copolymers PEG–polypropylene glycol (PPG) have been developed by Yang et al. to prepare such micelles. The polymer had a CMC of 38.5 μg/ml and the mean particle size of the micelles obtained was 65.5 nm. A varying ratio of PEG–PPG was used in the synthesis of these polymers to obtain polymers with molecular weights ranging from 10,416 to 15,238 Da.

Aqueous solutions of these polymers have low viscosity at room temperature but show an increase in viscosity at body temperature. This system showed sustained release of hydroxycamptothecin (HCPT) for 80 h compared with less than 20 h for plain drug. The in vivo pharmacokinetics of HCPT-loaded micelles were studied in rabbits. Plain HCPT, HCPT in pluronic micelles and HCPT in polyether carbonate micelles were compared for in vivo half-life. The HCPT in polyether carbonate micelles increased the in vivo half-life from 1.3 h for plain HCPT and 10.4 h for HCPT in pluronic micelles to 12.4 h in HCPT in polyether carbonate micelles. Similarly, pentablock polymers such as poly(2-diethylaminoethyl-methylmethacrylate) (PDEAEM25)–PEO100–PPO65–PEO100 PDEAEM25 (Mn = 21,900 Da) form a gel-like structure at body temperature. Micelles made out of such pentablock amphiphilic polymer sustained release of lysozyme for up to 80 h. The release was pH dependent and a 49% increase in the lysozyme release was seen at pH 7 when compared with pH 8. PDEAEM has a pKa of 7.8 and the tertiary amines in this polymer are protonated below 7.8, resulting in an elevated polymer hydrophilicity and drug release.

Polymer films converted to micelles at body temperature

This is a unique strategy that involves formation of a drug-containing copolymer film at room temperature. At body temperature, this film collapses into micelle-like particles, which entrap the drug. A higher amount of drug can be entrapped in the micelles with such a technique, and sustained drug release can be achieved. However, the synthesis of polymer films requires great technical expertise and can easily go wrong, resulting in burst release of the drug. Moreover, this approach tends to form micelles of a higher diameter than several of the above approaches. Such a technique using the polymer monomethoxy PEG-block-poly(trimethylene carbonate) (Mn of mPEG = 3100 Da; Mn of polytrimethylene carbonate = 10,800 Da) was investigated for dexamethasone by Zhang et al.. This study reported sustained release of dexamethasone up to 20 days. The CMC of the polymer was 1.35 μg/ml. However, the mean diameter of the micelles was 210 nm, which was higher compared with some of the methods discussed above.

Micelles coated on metal stunts:

Metallic stents are in use for patients suffering from cardiovascular problems. If such patients need to be given medication, the stents can be a good source for drug release. Drug-eluting stents have been long investigated for treatment of lesions and other cardiovascular problems. Drug-loaded micelles can potentially be coated on stents to achieve sustained drug release in patients. In this approach, the stent will perform the function it is supposed to perform, that is, widening the coronary arteries. Second, such a coated stent will release the drug of choice into circulation or artierial walls in a sustained manner. The stents can be appropriately heparinized and chemically treated so that they last for prolonged periods. However, it is a complicated system to manufacture and coating the micelles on the stents can be difficult. One such system was reported by Kim et al. They heparinized the metallic stents to create a nonthrombogenic environment and then PLL (poly-l-lactide) (Mw = 70 kDa) was adsorbed. On this preparation, hyaluronic acid-(Mw = 17 kDa) g-PLGA micelles were coated. The micelles (mean diameter 202.8 nm) were loaded with paclitaxel and the release of the drug was confirmed by release studies as well as the ability of the stent to arrest the growth of human coronary artery muscle cells. The drug release was sustained for at least 25 days. Such a strategy can potentially be used with some of the micelle stabilizing strategies (discussed later) to further sustain drug release up to 6–12 months.

Diamonds show the multi-layer assembly of polyethylene oxide–polycaprolactone micelles as a film using PAA as a hygrogen bond donor. The drug employed was triclosan. Squares show micelles from reverse thermoresponsive polymer poly(ether carbonates). The polymer increases in viscosity at body temperature and hence, sustains the release of hydroxycamptothecin.

Triangles show the release from layer by layer assembly of hyaluronic acid-g-Poly(lactide-co-glycolide) micelles on a heparinized stent. The micelles separate from the stents at body temperature in a sustained manner, thus giving a sustained release of paclitaxel. Circles show the release of polyethene –polycaprolactone polymer and encapsulating prodrug of paclitaxel from micelles, which breaks down into paclitaxel to give a sustained release. Drug release was assessed in phosphate buffered saline at 37°C.

Figure 6 compares in vitro drug release for formulations prepared using various micelle-forming strategies including prodrugs, layer by layer assembly of micelles as a film, use of reverse thermoresponsive polymers to form micelles and layer by layer assembly of micelles on a stent. Figure 7 compares plasma clearance profiles of paclitaxel from PEG–PCL micelles, indomethacin from polyphosphazene micelles, indomethacin from PCL–PEG micelles, cisplatin from PGLA micelles and SN38 from NK012 micelles.

The graph shows the comparison of plasma concentration profiles with the use of different polymeric micelles. PEG–PCL micelles (purple circles) for the administration of prodrug of paclitaxel. Polyphosphazene micelles (green circles) for the administration of indomethacin. PCL–PEG micelles (yellow circles) for the administration of indomethacin. PEG–polyglutamic acid micelles (blue circles) for the administration of cisplatin. NK105 micelles (red circles) for the administration of SN38. PCL: Polycaprolactone; PEG: Polyethylene glycol.

Crosslinking the shell

This strategy involves introduction of cross-linkable groups within the hydrophilic portion of the copolymer and then using polymer chemistry to cross-link the hydrophilic shell portion after the micellization of the polymer. Such cross-linking leads to a stabilization of the micelle system and delays the degradation of the micelle. This chemistry can be used in a biodegradable system and a shell cross-linked micelle can be prepared for drug delivery. The use of shell cross-linking with some other approaches, such as conjugation of the core with the drug, can be useful in preparing sustained-release micellar systems. For instance, multifunctional, multi-armed PEG can be used with some commonly used degradable hydrophobic polymers to form an amphiphilic block copolymer. PEG branching in this polymer can be used to create cross-linkable groups in the system to prepare a shell cross-linked micelle system. Thurmond et al. and Li et al. reported interesting approaches for cross-linking of the micellar shell in order to stabilize the micelles. Thurmond et al. prepared micelles from polyvinyl pyridine-b-poly styrene (Mn = 52,500 Da) block copolymer, which on self-assembly, forms shell cross-linked knedel-like micelles, which appear to be a hybrid between dendrimers, hollow spheres, latex particles and block copolymer micelles. Li et al. reported similar stabilization of the micelles by cross-linking the shell of the micelles made from a poly(styrene-b-butadiene-b-styrene) polymer. Their approach involved formation of the micelles in aqueous medium and then cross-linking the hydrophobic portion of the micelles using chloromethylation and amination. However, the usefulness of the above polystyrene systems for pharmaceutical purposes is unclear at this stage. In these approaches, since these cross-linking groups are on the shell, the individual micelles have to be kept sufficiently away from each other so that these cross-linking groups do not interact with the groups of other micelles but only interact with the cross-linking groups of the same micelle to cross-link the shell. This is required to prevent aggregation of the micelles. Hence, they have to be prepared under highly dilute conditions. Surface functionalities attached to the shell can add a new dimension of targetability to the sustained release already obtained. However, the chemistry used here is not simple to perform. Moreover, the chemical groups added to the polymer may contribute to its toxicity, change its properties and may render the micelle useless for drug delivery. Hence, such an approach should only be followed with some amount of caution.

Cross-linking the core

Crosslinking the shell has been tried by many groups with only moderate success. However, such a shell cross-linking needs preparation at a high dilution. This decreases the efficiency of the process. Hence, the stabilization of micelles needs something other than shell cross-linking. The strategy of core cross-linking involves cross-linking the core to form a matrix that traps the drug inside it, thereby controlling the diffusion of the drug from the core. Such an approach is easy to use and different polymeric core portions can be used to suit the drug that is to be encapsulated. Many approaches have been tried to stabilize the core by cross-linking it with different functional groups. Addition of thiol group to the core of the micelle can be used to cross-link the core with a disulfide group. This has been done with polyion complex micelles by Kakizawa et al. In a polyion complex, the electrostatic interaction between two polymer segments drives association.

Kazikawa et al. synthesized micelles using PEG-5000-b-poly(lysine) diblock copolymer. The polymerization degree of poly(lysine) was 22. The cross-linking of the poly(lysine) core was achieved using thiolation chemistry. The lysines in the core were thiolated and hence, they cross-linked with a disulfide bond. This stabilizes the core of the micelle and increases the micellar stability. A completely biodegradable system was prepared by Hu et al. using the polymer PEG-b-PLA with a 5-methyl-5-allyloxycarbonyl-1,3-dioxane-2-1 (Mn = 4500 Da) group as the polymerizable group for crosslinking the core. The cross-linking was achieved postmicellization by reaction with 2,2-azoisobutyronitrile. The mean diameter of the resultant micelles was reported to be 130 nm. The

micelles were shown to survive water dilution and temperature better than non-crosslinked micelles. This property of the core crosslinked micelles can be utilized to prepare drug-loaded micelles that offer a longer sustained release than non-modified regular micelles. Moreover, this modification can be used along with other techniques to further enhance sustained release by the system. The chemistry involved in such crosslinking is comparatively simpler than the one used to crosslink the shell. Moreover, the core is the part that encapsulates the drug. A stabilized core will hold the drug for a longer period of time. In addition, drug-loaded nanoparticles can also be crosslinked to the core to achieve a higher control over the release of the drug. Strategies like this may make the system highly complicated while allowing formulation of a drug in a controlled delivery system.

Use of a low critical solution temperature hydrogel to stabilize the micelles

We have already discussed the use of polymers that change their viscosity with temperature to form micelles. Similarly, a low critical solution temperature (LCST) hydrogel can be used to stabilize the micelles. An LCST gel can be polymerized along with the core of the micelle to stabilize the core. LCST gels remain in a swollen state at room temperature, allowing drug loading. But at physiological temperatures, these gels collapse and lock the hydrophobic portion of the micelle forming a locked core that contains the drug. Such a locked interpenetrating network in the core prevents the breakdown of the core upon dilution. This means that a drug loaded in the core would remain in the micelles for prolonged release. Such a system with pluronic micelles and an LCST gel was reported by Rapoport. Rapoport suggested three ways to stabilize pluronic micelles, namely, core crosslinking, introducing vegetable oil in the hydrophobic portion to stabilize the micelles and polymerizing an LCST gel with the hydrophobic portion of the micelle to stabilize the core. The core crosslinking strategy decreased the drug loading capacity of the micelle. Addition of vegetable oil to the core increases the hydrophobicity of the core. However, the release is not as sustained as seen with an LCST gel core LCST gel in the core allows incorporation of hydrophilic as well as lipophilic drugs. One major disadvantage of using an LCST gel in the core of the micelle is that it increases the micellar size by several fold. Rapoport reported a size increase from 12–15 nm to 30–400 nm.

COMPARISION WITH OTHER DELIVERY SYSTEMS[5]

Thus far, several strategies that are useful in achieving sustained release micelles have been discussed. Below, some competing alternative delivery systems for sustained drug delivery are briefly discussed. The discussion has been restricted to particulate delivery systems since these are more similar to micelles. However, it should be noted that implantable drug delivery systems are also clinically relevant for prolonged drug delivery.

Polymeric microparticles

Polymeric microparticles have been most successfully employed in the sustained delivery of drugs. Release profiles of drugs up to 6 months have been reported with polymeric microparticles (e.g., Lupron Depot®). Furthermore, sustained release up to 287 days has been shown in dogs with ivermectin in PLGA microparticles. Such formulations are polymeric matrix formulations that are injected as suspensions. These particles are micron size in diameter and hence, they exhibit low burst effects/release. However, such particles are large in size and hence, they are not suitable for applications where the particles need to pass through leaky blood vessels. Also, they are not as effective as micelles in solubilising a poorly soluble drug. Hence, there is a need for better formulations that have both the solubilising capacity of micelles and sustained release capacity of microparticles.

Polymeric nanoparticles

Polymeric nanoparticles have also been used for targeted and sustained release of drugs. For instance, Singh et al. reported targeted gene delivery to the retina following intravenous administration of functionalized PLGA nanoparticles Furthermore, PLGA polymeric nanoparticles have been shown to sustain the release of tetanus toxoid in vivo for 4 months. Nanoparticles increase the surface area of the formulation and hence, result in significant burst effects/release. Moreover, drug loading can be limited in these nano particles. Further, they tend to aggregate more readily, resulting in larger particles compared with micelles less than 100 nm. Hence, polymeric nanoparticles do not completely alleviate the need for a better delivery system at the nanoscale.

Liposomes

Liposomes have also been used for sustained release of drugs. Liposomes are comprised of lipids that are largely endogenous in the human body. Hence, they avoid the toxicity issues. Although, release profiles up to a month have been reported with liposomes for anti-tubercular drugs, they are not the first choice delivery systems for prolonged drug delivery. Doxil, a pegylated liposomal formulation of DOX, demonstrates an in vitro release of 100% over 24 h. Atyabi et al. showed that 100% release of SN-38 occurs from pegylated liposomes in 25 days as compared with 60% from non-pegylated liposomes.

CHARACTERISATION OF POLYMERIC MICELLE [2]

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.

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 quasielastic 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. 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.

***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.

POLYMERIC MICELLAR NANOCARRIERS IN DRUG DELIVERY[3]

The studies on the application of polymer micelles in drug delivery have mostly focused on the following areas that are considered below:

(1) Delivery of anticancer agents to treat tumors
(2) Drug delivery to the brain to treat neurodegenerative diseases
(3) Delivery of antifungal agents
(4) Stimuli-responsive nanocarriers for drug and gene delivery
(5) Ocular drug delivery

Delivery of anticancer agents to treat tumours:

Chemotherapy is an essential component in the multidisciplinary management of most cancers. Cancer is a leading cause of death world-wide and is responsible for approximately 13% of all deaths, according to the World Health Organization. A very promising approach to overcome systemic toxicity is the application of drug-loaded nanosized drug carriers, such as liposome’s, polymeric nanoparticles, dendrimers and micelles. Currently, many drug-loaded polymeric micelles for anticancer therapy are under investigation in preclinical Studies to improve drug efficacy. Five micellar formulations have been tested in clinical trials as follows in table no:3.

Drug delivery to brain to treat neurogenerative diseases

By restricting drug transport to the brain, the blood brain barrier (BBB) represents a formidable impediment for the treatment of brain tumors and neurodegenerative diseases such as HIV-associated dementia, stroke, Parkinson's and Alzheimer's diseases. Two strategies using polymer micelles have been evaluated to enhance delivery of biologically active agents to the brain. The first strategy is based on the modification of polymer micelles with antibodies or ligand molecules capable of transcytosis across brain microvessel endothelial cells, comprising the BBB. The second strategy uses Pluronic block copolymers to inhibit drug efflux systems, particularly, Pgp, and selectively increase the permeability of BBB to Pgp substrates An earlier study used micelles of Pluronic block copolymers for the delivery of the CNS drugs to the brain. These micelles were surface-modified by attaching to the free PEO ends, either polyclonal antibodies against brain-specific antigen, a2-glycoprotein, or insulin to target the receptor at the luminal side of BBB. The modified micelles were used to solubilize fluorescent dye or neuroleptic drug, haloperidol, and these formulations were administered intravenously in mice. Both the antibody and insulin modification of the micelles resulted in enhanced delivery of the fluorescent dye to the brain and drastic increases in neuroleptic effect of haloperidol in the animals. Subsequent studies using *in vitro* BBB models demonstrated that the micelles,

vectorized by insulin, undergo receptor rmediated transport across brain microvessel. endothelial cells**.** Based on one of these observations, one should expect development of novel polymer micelles that target specific receptors at the surface of the BBB to enhance transport of the incorporated drugs to the brain

Delivery of antifungal agents

The need for safe and effective modalities for the delivery of chemotherapeutic agents to treat systemic fungal infections in immune compromised AIDS, surgery, transplant and cancer patients is very high . The challenges to the delivery of antifungal agents include low solubility and sometimes high toxicity of these agents. These agents, such as amphotericin B, have low compatibility with hydrophobic cores of polymer micelles formed by many conventional block copolymers. Thus, to increase solubilization of amphotericin B, the core-forming blocks of methoxy-PEOb- poly(Laspartate) were derivatized with stearate side chains. The resulting block copolymers formed micelles. Amphotericin B interacted strongly with the stearate side chains in the core of the micelles, resulting in an efficient entrapment of the drug in the micelles, as well as subsequent sustained release in the external environment. As a result of solubilization of amphotericin B in the micelles, the onset of hemolytic activity of this drug toward bovine erythrocytes was delayed, relative to that of the free drug. Using a neutropenic murine model of disseminated Candidas, it was shown that micelle incorporated amphotericin B retained potent *in vivo* activity. Pluronic block copolymers were used by the same group for encapsulation of another poorly soluble antifungal agent, nystatin. This is a commercially available drug that has shown potential for systemic administration, but has never been approved for that purpose, due to toxicity issues. The possibility to use Pluronic block copolymers to overcome resistance to certain antifungal agents has also been demonstrated. Overall, one should expect further scientific developments using polymer micelle delivery systems for the treatment of fungal infection.

Stimuli responsive nanocarriers for drug and gene delivery

With parallel recent breakthroughs in molecular understanding of diseases and controlled manipulations of material at the nanometric length scale, nanotechnology offers tremendous promise in disease prevention, diagnosis, and therapy. Among the various approaches for exploiting developments in nanotechnology for biomedical applications, nanoparticulate carriers offer some unique advantages as delivery, sensing and image enhancement agents**.** Many bioactive used for pharmacotherapy, while have a beneficial action, can also exhibit side-effects that may limit their clinical application. There has long been the desire to achieve selective delivery of bioactives to target areas in the body in order to maximize therapeutic potential and minimize side-effects. For example, cytotoxic compounds used in cancer therapy can kill target cells, but also normal cells in the body resulting in undesired side-effects. For achieving better therapeutic application, nanocarriers are considered for target-specific delivery of drugs and gene to various sites in the body in order to improve the therapeutic efficacy, while minimizing undesirable side effects. Improvements in target-to-non-target concentration ratios, increased drug residence at the target site, and improved cellular uptake and intracellular stability are some of the major reasons for greater emphasis on the use of nanoparticulate delivery systems. With nucleic acid-based therapeutic modalities, there is substantial need for the therapeutic molecules to be delivered to desired sub-cellular compartments in an efficient and reproducible manner**.** The use of stimuli-responsive nanocarriers offers an interesting opportunity for drug and gene delivery where the delivery system becomes an active participant, rather than passive vehicle, in the optimization of therapy. Several families of molecular assemblies are employed as stimuli-responsive nanocarriers for either passive or active targeting.

Ocular drug delivery

Various efforts in ocular drug delivery have been made to improve the bioavailability and to prolong the residence time of drugs applied topically onto the eye. Eye is characterized by its complex structure and high resistance to foreign substances including drugs. The anterior and posterior segments of the eye, although in juxtaposition to each other, and very different in their anatomical and physiological aspects, function both independently and in tandem upon application of an ocular preparation. While it has been known since long that conventional topical formulations are amenable to application to the anterior portion, most of the applied dose is lost due to the defensive mechanism of the eye. Consequently, much concerted effort has been directed towards increased retention of the applied dose on the eye surface, with the premise that such increased retention will result in better therapeutic effect and lowered local and/or systemic effects. Since most drugs poorly penetrate the cornea, fulminating diseases of the posterior segment viz. vitreous, retina and choroid are required to be treated with either systemic administration or through intravitreal injections and vitreal implants. While therapy with systemic administration requires large doses due to strong blood-ocular tissue barrier, the other two routes are very invasive requiring skilled administration, and are associated with a high degree of risk, such as development of retinal detachment and endophthalmitis. Clearly there is a strong case in favor of formulating ocular delivery systems by focusing on improved ocular bioavailability and extended drug effect in targeted tissues. Prolonging pre-corneal residence time through viscosity enhancers and

gels has only a limited value, because such liquid formulations are eliminated by the usual routes in the ocular domain. The highly sensitive corneal/conjuctival tissues towards penetration enhancers to maximize drug transport requires great caution in the selection of the enhancer. An alternative approach is to develop a drug delivery system that would circumvent the problems associated with the conventional systems, and provide the advantages of targeted delivery of drugs for extend d periods of time and be patient-friendly. The latter requisite becomes more crucial in cases where the patient has to use the drug preparation throughout his life, e.g. in glaucoma.

These advantages have been reported in the literature through the use of nanoparticles. Micro and nanoparticles for topical ophthalmic application are presently being researched based grossly on nanotechnology in which drugs can be administered as an eye drop. Also poorly water soluble or insoluble drugs can be successfully fabricated as effective systems to provide easy administration toocular tissues and convenience to the patient as well as ophthalmologist to adjustment of dose and dosing frequency according to disease therapy. It has been found that biodegradable polymers can be combined with drugs in such a way that the drug is released into the eye in a very precise and controlled manner. The formulation of biodegradable polymers as colloidal systems holds significant promise for ophthalmic drug delivery, since it is suitable for poorly water-soluble drugs and would allow drop- By interaction with the glycoproteins of the cornea and conjunctiva they can form a precorneal depot resulting in a prolonged release of the bound drug. Nanoparticle formulations provide protection for agents susceptible to degradation or denaturation in region of harsh pH, and also prolong the duration of exposure of a new drug by increasing retention of the formulation through bioadhesion. In this context, more clinical studies are necessary to provide further information and insight into this new ophthalmic drug delivery system.

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. 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. 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. 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. The influence on solubilisation 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 solubilisation capacity was dependent on the hydrophobic block length upto a certain extent (15 units of hydrophobic block), after which the solubilisation capacity became independent of the same. Dong and coworkers also studied the effect of hydrophobic block length on solubilisation of toluene in diblock and triblock polyurethane surfactants. It was concluded that solubilisation capacity of polyurethane surfactants increased with an increase in the hydrophobic segment for the same block chain structure.

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.

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. 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. 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. 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. 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. 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, particular enzyme, etc. or by an external trigger including temperature, light, ultrasound or magnetic field. 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. 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. 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. Two main approaches that have been used for developing pHsensitive 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.

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. 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*. demons t rated the use of poly(Nisopropylacrylamide-co-acrylamide)-b-poly(D,L-lactide) copolymer in tumor targeting of docetaxel. 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. 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. 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'. 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.

Table 1. Structures of micelle forming co polymers

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

*A - hydrophilic unit; B - hydrophobic unit

Table 2. Micellar formulations under clinical trails

Polymeric micelle	Block Copolymer	Drug	Indication	Micelle Size (diameter)
Genexol PM	PEG-P(D,L-lactide)	Paclitaxel	Breast cancer,Pancreatic cancer,Small cell	20-50 nm
SP1049C	Pluronic L61and F127	Doxorubicin	lung cancer, Adenocarcinoma of	22-27 nm
		Cisplatin	oesophagus	30 nm
NC-6004	PEG-Cisplatin	Paclitaxel	Solid tumors	85 nm
NK105	PEG-PGL(PGL)		Advanced stomach	
NK012	PEG-(aspartate) PEG-PGL(SN-38)	SN-38	cancer Breast cancer	20 nm

Fig 1. Design of polymeric micelle carrier system

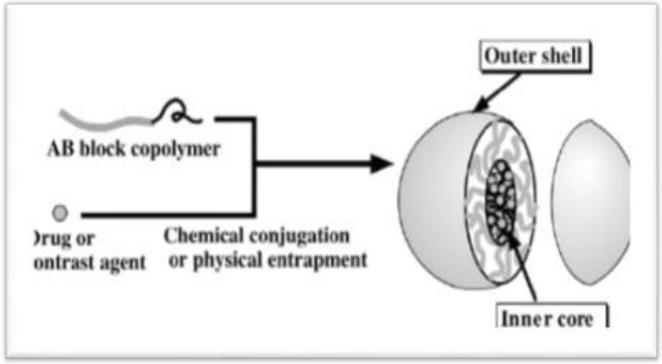


Fig 2. In vitro drug release from micelles prepared by the polymer–drug conjugate strategy.

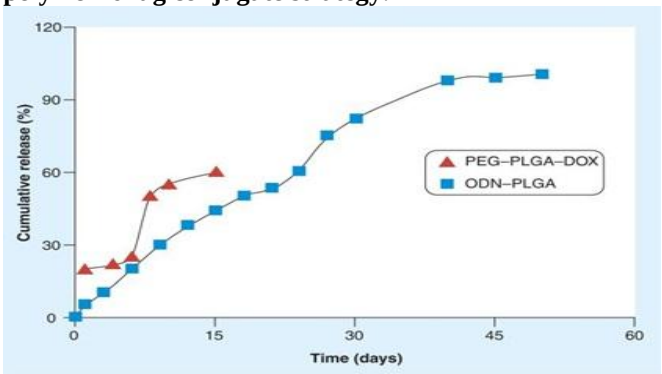


Fig 3. Represents different types of polymers used to prepare micelles

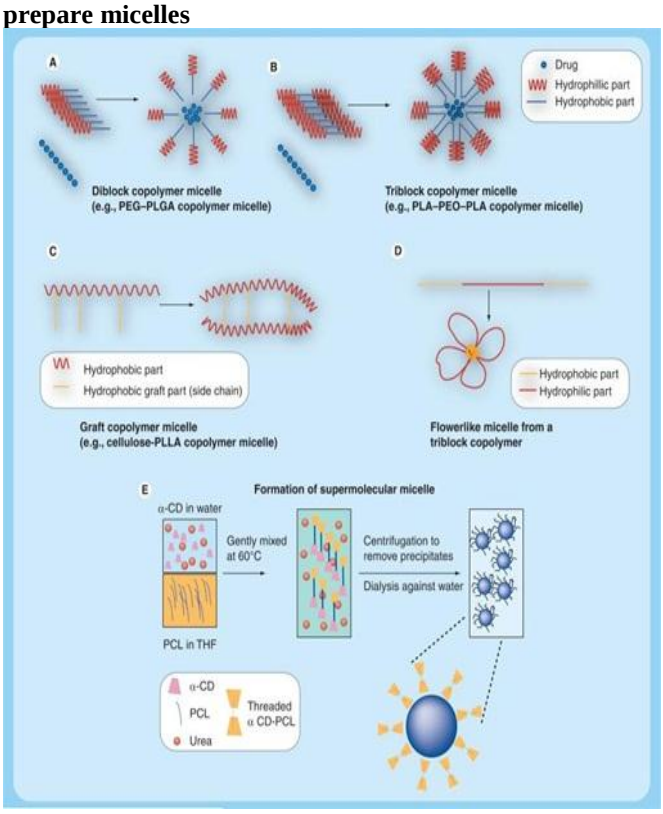


Fig 4. In vitro drug release from polymeric micelles

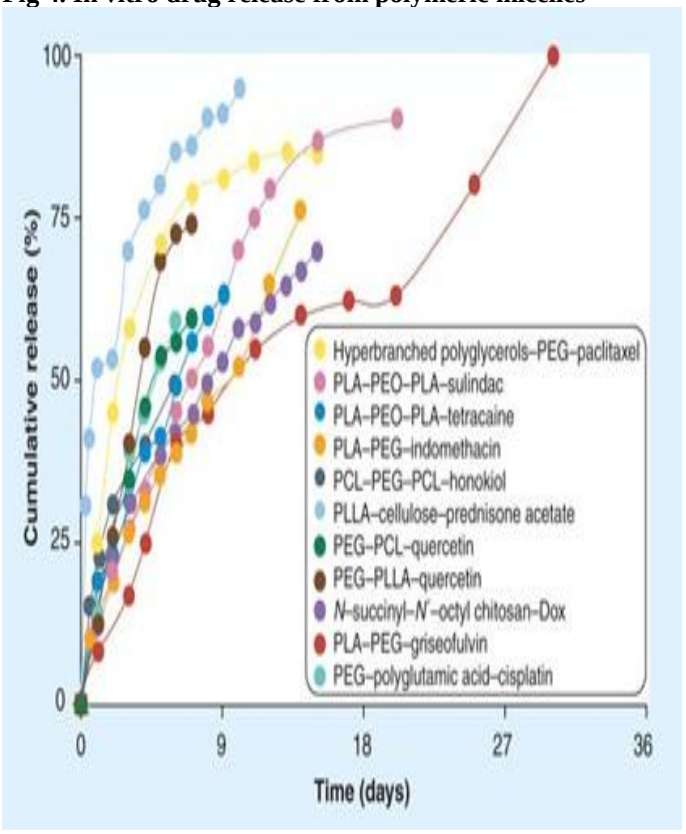


Fig 5. Formation of a film through H-bonding of multiple layers of polymeric micelles PEO–PCL diblock copolymer is used along with PAA as an H-bond donor. The micelles form H-bonds with each other when assembled layer by layer and form a film like structure. These films disintegrate in phosphate buffer saline to release micelles. PEO: Polyethylene oxide; PCL: Polycaprolactone.

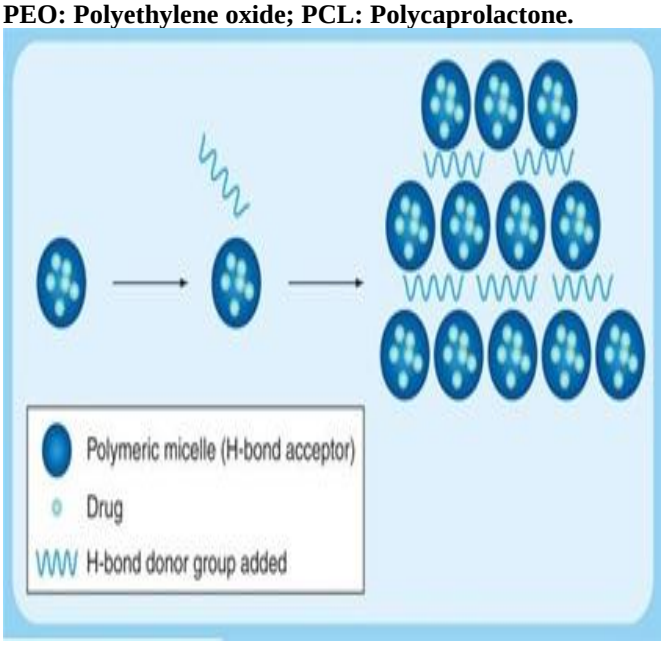


Fig 6. In vitro drug release from micelles formed using various polymers

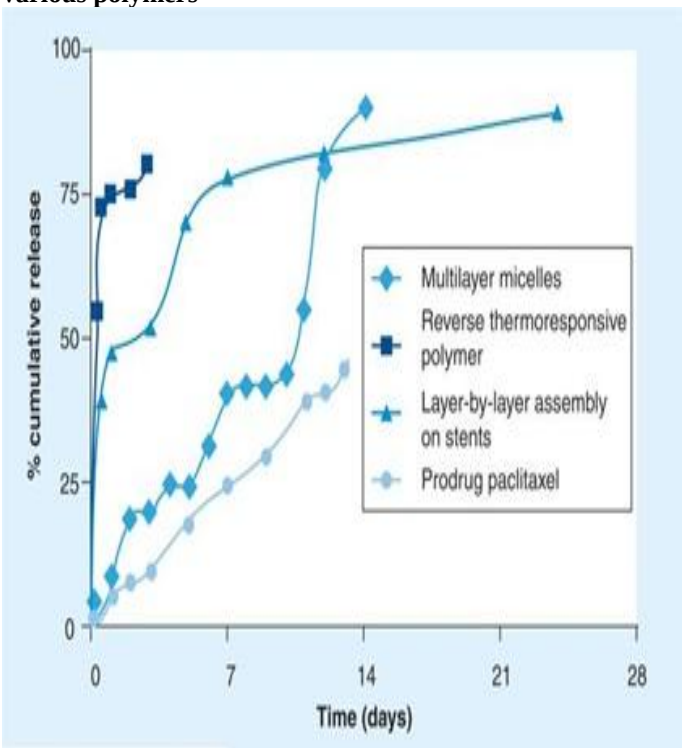


Fig 7. Comparison of in vivo plasma clearance profiles from micelles.

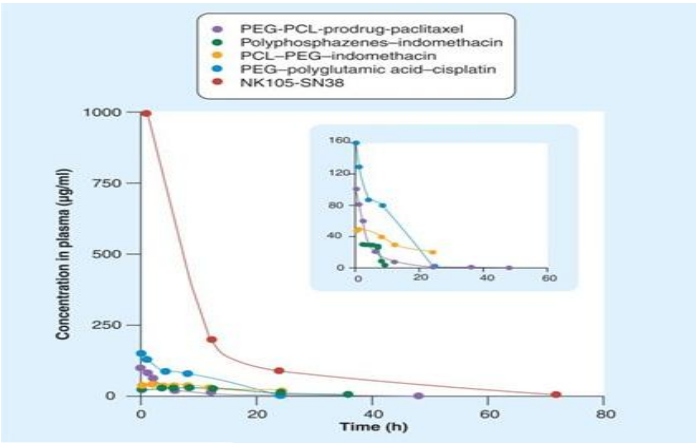
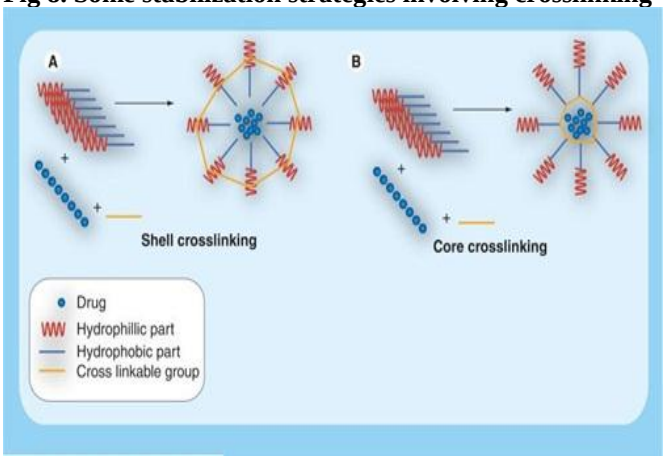


Fig 8. Some stabilization strategies involving crosslinking



CONCLUSION

Nanotechnology with its bottom up techniques welcomed a great era in human history and also its impact in curing life threatening diseases is so exclusive. When nano sized micellar structures enhanced bioavailability of poorly soluble anti-cancer drugs in different compositions attracted researchers' eye. In addition to this their small size demonstrated spontaneous accumulation with enhanced permeability and retention effect in pathological areas with compromised vasculature. When focused on several approaches to sustain the release of drug from polymeric micelles, targeting different sites is also discovered. Compared with other particulate systems like polymeric microparticles, polymeric nanoparticles, liposomes in terms of enhancing solubility, drug loading capacity, sustain release of drugs, nanomicelles has their own architecture of incorporating all these characteristics in a single cage. Thus nanomicellar structures can bring a greater change in increasing bioavailability, sustaining release of drug from dosage forms favoring a year or decade formulations.

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