Chapter 1: Introduction
The research work presented in this thesis deals with the formulation, characterization and release kinetics of some hydrophobic drugs from polymeric micelles. The polymeric micelles used in these studies fall under the broad category of block copolymers. A block copolymer is a polymer consisting of multiple sequences, or blocks, of the same monomer alternating in series with different monomer blocks covalently bound to each other. Block copolymers are classified based on the number of blocks they contain and how the blocks are arranged. For example, block copolymers with two blocks are called diblocks AB; those with three blocks are triblocks ABA, BAB and ABC, ACB, BAC; and those with more than three are called segmented or multiblocks. Nonlinear block copolymers are often called starblock copolymers. The various types of block copolymers are schematically shown in Fig 1.

**Fig. 1. The various block copolymers**

This unique structural architecture makes them behave like amphiphiles or surface active agents (surfactants). A surfactant above certain concentration forms micelles which can be spherical, cylindrical or some other geometry. At much higher concentrations liquid crystalline phase form showing variable rheological properties.
Block copolymers self-assemble in solution and adsorb onto interfaces. In a selective solvent, i.e., good for one block and poor for the other block, these polymers form stable micelles and a variety of structures. Block copolymers with hydrophilic and hydrophobic moieties form micelles in water analogous to conventional surfactants with some unique characteristics of their own. The hydrophilic (A) and hydrophobic (B) blocks can be from different monomers as AB diblock, ABA and BAB triblock and even star and radial block copolymers. For ABC triblock copolymers, any two blocks can be hydrophilic/hydrophobic. Thus, depending on molecular characteristics/structure/type of block copolymer and solution conditions, nanosized aggregates with different morphologies and applications can be produced.
Fig. 3. Block copolymer aggregates in dilute solution

Micellization of amphiphilic block copolymers in aqueous solution has also been examined in details and the most extensively studied copolymers are the ethylene oxide - propylene oxide (EO-PO) based symmetrical triblock copolymers. Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide), (EO)ₙ(PO)ₘEOₙ and poly(propylene oxide)–poly(ethylene oxide)–poly(propylene oxide), (PO)ₙ–(EO)ₘ(PO)ₙ, tri-block copolymers are the products of BASF marketed as Pluronics® and Pluronics® R. Starblock EO-PO copolymers are known as Tetronics®. These copolymers have been known to exhibit unique surface activity, micellization and reversible thermo-rheological phase behaviour in water. The aqueous solution chemistry of these block copolymers has been extensively examined using several techniques for 2 decades. Significant advances have been made over the past few years on the colloid-chemical behaviour of these block copolymers viz. their adsorption onto solid surfaces, colloid stabilization, micellization, micellar transitions and liquid crystalline phases in aqueous and non-aqueous solvents as well as emerging areas of applications.
The micellization of PEO-PPO-PEO in aqueous solution has been a subject of much debate in the past. These are highly surface active compounds and form core-shell micelles with core consisting of hydrophobic middle PPO block surrounded by an outer shell of the hydrated hydrophilic PEO end blocks above critical micelle concentration (CMC) or critical micelle temperature (CMT) in water. In contrast to the conventional nonionic surfactants of the type poly (ethylene oxide) condensates, PEO-PPO-PEO block copolymers show some unique behavior in water, which mostly arises around room temperature when the poly(propylene oxide) remains no longer hydrophobic. Strong concentration/temperature dependent micellization and about 2 magnitude decrease in CMC with a rise in temperature by 20°C is often observed. Anomalous turbidity at certain temperature/concentration below and above which the solutions were clear, solubilization at concentrations below CMC and two break points in surface tension concentration plots as well as the reversible thermo-rheological behavior have also been seen for copolymer solutions (Figs. 4 and 5).

![Fig. 4. Micelles of PEO-PPO surfactants](image)
Nonionic surfactants based on PEO show inverse temperature-solubility relationship in water and the solutions phase separate above a certain temperature called cloud point. Additives influence the cloud point of a nonionic surfactant altering its aqueous solubility and colloid-chemical behavior that determine usefulness for practical purposes. Thus the effect of different types of additives like electrolytes, nonelectrolytes, hydrotropes, and low molecular weight surfactants on micellar and phase behaviour becomes important and has been examined. Aqueous Pluronic® micelles have the ability to solubilize hydrophobic drugs and increase the stability of solubilize. This solubilization behavior has been employed in industrial formulations, particularly in pharmaceutical preparation. Several papers have been published on solubilization of insoluble substances in aqueous solutions of ethylene oxide-propylene oxide block copolymers. EO–PO copolymers represent a bridge between classical oligoethylene glycol condensates type non-ionic surfactants and polymeric surfactants. Their micellization behavior has been extensively studied. The self-assembly generates different aggregated structures (Figs. 6 and 7). The self-assembly, surface activity and gelation behavior depends on the type (linear or nonlinear), (normal and reverse) total mol wt and % block composition, external conditions like temperature, pH, presence of additives.
Fig. 6. Pluronic in aqueous solution.

Fig. 7. Ternary phase diagram of Pluronic P84 /water/p-xylene at room temperature. Nine different phases can be identified: normal (oil-in-water) micellar solution $L_1$, cubic
$I_1$, hexagonal $H_1$, bicontinuous cubic $V_1$, reversed (water-in-oil) micellar solution $L_2$, cubic $I_2$, hexagonal $H_2$, bicontinuous cubic $V_2$ phases, and a lamellar phase $L_\alpha$ (Langmuir 1998).

This thesis mainly concerns with the self-assembly of core-shell nanoaggregates produced by Pluronic® and Tetronic® block copolymeric surfactants in aqueous media. Spectral, chromatographic and thermal methods were used to characterize micellar systems of different linear tri-block (Pluronic®) and star-block (Tetronic®) copolymeric surfactants.

Pluronics® are the most extensively studied amphiphilic block copolymers as drug delivery vehicle due to their surfactant abilities, low toxicity and minimal immune response. Some of them have also been approved by FDA for use in pharmaceutical, biomedical and food applications [1]. Another attractive feature of the Pluronics® is that they are commercially available in a wide range of molecular weights and block ratios, which can be used for specific drug use (Fig. 8).

Fig. 8 The Pluronic grid.
The chemical structure of the copolymer, particularly the size of the PPO (hydrophobic) block, impacts both the CMC and partitioning of hydrophobic molecules into the Pluronic® micelles. Consequently, specific Pluronics can be chosen so as to enhance drug delivery properties. There are several excellent review articles written about the use of Pluronics® in drug delivery [2-4].

Ideal polymeric drug delivery systems should fulfill several requirements such as significant increase in therapeutic effect with respect to the free drug, good biocompatibility and the possibility to scale up its production. In addition, for an ideal micellar drug delivery system:

i) should have long circulating properties and adequate stability in the blood

ii) have a high drug loading capacity

iii) should be able to selectively accumulate at the target site

iv) should offer the possibility to control the release of the drug at the target site by external stimuli and the ability to be degraded and excreted from body after the drug is released.

Pluronics® are used in drug delivery in two distinct forms:

i) Polymeric micelles and

ii) Hydrogels.

Pluronics® have also been evaluated in gene delivery and drug resistant tumors.

**Polymeric micelles:**

As described earlier, in aqueous solutions above CMC, Pluronics® self assemble into micelles. These micelles can be spherical, rod-like or lamellar depending upon the length of EO and PO chains, concentration of the block copolymer, temperature etc. All these micelles have hydrophobic core formed by PO chains and hydrophilic shell formed by EO chains. The core formed by PO chains is water incompatible which is separated from the aqueous environment by hydrophilic chains of EO corona, thereby forming a reservoir for the incorporation of various hydrophobic therapeutic agents. Therefore, Pluronics® can be used as efficient drug carriers for compounds which exhibit poor solubility, undesired pharmacokinetics and low stability in physiological environment.
The ideal polymeric drug delivery system should remain in the systemic circulation for a long period of time. However, human immune system rapidly recognizes and eliminates foreign objects via adsorption of opsonic proteins onto their surfaces. Therefore, for a prolonged circulation of nano drug carriers, the aim is to reduce the rate and extent of this opsonization, and recognition by cells of reticuloendothelial system (RES). It has been shown that coating of the particle surface with hydrophilic polymers, called the steric stabilization, effectively reduces the interactions with opsonic proteins, and thereby the uptake by RES cells of the liver, spleen, and bone marrow.

PEO is FDA approved due to its low toxicity and has long been shown [5-7] to minimize protein adsorption to the surfaces. Due to the hydrophilicity, minimal interfacial energy with water, high aqueous solubility, high mobility and larger exclusion volume [6], the attachment of the PEO group to the hydrophobic surfaces reduces the protein adsorption and thereby improving the biocompatibility of foreign materials. Therefore, encapsulation of therapeutic agents in Pluronic® micelles may prolong the circulation time and helps in maintaining the micelles in dispersed state and decreases the undesirable drug interactions with cells and proteins through steric-stabilization effect [8-9].

**Size and CMC of the micelles:**

The CMCs and size of the micelles are the most important parameters from the drug delivery standpoint. The average hydrodynamic diameter range of spherical micelle formed by Pluronics® is ca. 20 to about 80 nm and aggregation number ranges from 10 to several dozen [10]. This size variation in nanoscale range strongly affects the blood circulation times and bioavailability of the particles within the body [11-15]. The preferred size for many pharmaceuticals applications using nanoparticles is ca. 10 to 100 nm. The micelles of Pluronics® are within this range. For example, particles ranging from ca. 10 to 100 nm offer effective distribution in certain tissues [11-12]. Also, particles which are smaller than 100 nm can also be accommodated in endocytic vesicles allowing entry into target cells via endocytosis [16]. Following systemic administration in the body, particles ranging from 70 to 200 nm have been shown to have the most prolonged circulation times [11, 14]. However, deviation from the preferred size range is usually accompanied by the decrease in the blood circulation times. The particles which are larger
than 200 nm are frequently sequestered by the spleen due to mechanical filtration, followed by eventual removal by the cells of the phagocyte system [11, 13]. On the other hand, particles smaller than 5-10 nm are rapidly removed through extravasation and renal clearance [13]. The CMCs of the Pluronics copolymers used in the drug delivery typically are in the range of 1µM to 1mM [17]. The CMC determines the stability of micelles against the dilution of the drug delivery systems in the body fluids and maximum achievable concentration of Pluronics unimers to cells will be exposed.

**Stability of micelles:**

The stability of the micelles can be either thermodynamic or kinetic. Polymeric micelles are thermodynamically stable when the concentration of the copolymer in water is above CMC. Below the CMC, the block copolymers are present as single chains in bulk and at the air-water interface. At concentrations above CMC, they are present as aggregates due to the hydrophobic interactions between the hydrophobic blocks. The dilution of the micelles upon intravenous injection results in a decrease in the portion of micelle containing solubilized drug. If the system is diluted below CMC, the micelles are completely disintegrated and the drug is released in the media.

The standard change in free energy for the micellization process, \( \Delta G \) is represented as;

\[ \Delta G = RT \ln \text{CMC} \]

Where R is gas constant and T is the temperature of the system. The CMCs of Pluronics® are in the order of 1µM -1mM [18-20] whereas that of low molecular weight surfactants is on the order of 10-10M. Therefore, the micelles formed by block copolymers are more thermodynamically stable as compared to the micelles formed by surfactants. Increased thermodynamic stability indicates that they are less prone to disassembly at low concentration than low molecular weight surfactants.

The kinetic stability is related to the exchange rate of single polymer chain between the micelles and the bulk. Micelles formed by block copolymers are kinetically stable even when the system is subjected to extreme dilution. The rate of disassembly is related to the strength of the interaction in micellar core which depends on many factors such as physical state of the core forming polymer, the ratio between the hydrophilic-hydrophobic block of the copolymer and encapsulation of the hydrophobic compound. Stability toward
dilution is of particular concern for any polymeric micellar based drug delivery system which is intended for parenteral administration and has implications for drug release. For example, a less thermodynamically and kinetically stable carrier might release drug prematurely because of dissociation of the micelle structure, whereas a more stable system might be preferred for long-circulating, sustained-release delivery systems.

**Thermo reversible gelation:**

Another property of Pluronics® which makes them attractive to use in drug delivery systems is that they exhibit a sol-gel transition below or close to the physiological temperature and gel-sol transition around 50°C, called a thermo reversible gelation. At a given temperature, micellization occurs in dilute solution of Pluronics® in selected solvents above the CMC, but at higher concentration and / or elevated temperatures, these micelles associate and form various Lyotropie liquid crystalline phases. [21-24].

At low temperature in aqueous solutions, a hydration layer surrounds Pluronics® molecules. As the temperature is increased, the hydrophilic chains of the copolymers are desolvated due to the breakage of the hydrogen bond between the solvent and the chains. This favors the hydrophobic interactions among the polyoxypropylene domains and leads to gel formation. A liquid micellar phase is stable at low temperatures and transforms into cubic structure by increasing the temperature. As the temperature is further increased, a phase of hexagonal packed structure is formed.

The regions of stability of the ordered nano structures of Pluronics®, in particular cubic three-dimensional lattices which is a gel, shift towards lower concentration at a higher temperature. Thus, a Pluronics® solution may be designed that gels at body temperature by forming a liquid crystalline phase due to increasing intermicellar interactions. As the temperature is further increased, the gel melts again. It has been shown that PEO chains in the micellar structure strongly interpenetrate. [25, 26]. Also, the gelation onset and temperature and thermal stability of the gel increase with increasing length of the PEO block.

The liquid crystalline nanostructures form a thermo reversible gel which is capable of solubilizing poorly soluble drugs in hydrophobic core of the micelle. Due to the low toxicity, and optical clarity, Pluronics® have been extensively studies in various pharmaceutical formulations, in particular, ophthalmic area. Since F127 is the least toxic
of all the commercially available Pluronics®, it is the most extensively studied Pluronics® in drug delivery studies.

**Solubilization:**

Several studies have shown that the most important factor related to the drug solubilization capacity is the compatibility between the solubilizate and the core forming block. Nagarajan [27] demonstrated that the amount of the incorporated solubilizate increases as the molecular volume of the solubilizate decreases. Further, the solubilization capacity is higher when core block-solubilizate interactions are favorable and solubilizate water surface tension is lower. As a result, Aromatic hydrocarbons are incorporated in Pluronics micelles to great extent than aliphatic hydrocarbons.

As compared to conventional low molecular weight surfactants, Pluronics block copolymers have much higher solubilization capacities and are more selective towards aromatic and heterocyclic compounds than towards aliphatic compounds. Since many drugs molecules contain aromatic and heterocyclic groups, Pluronics micelles appear to be particularly suited for the preparation of pharmaceutical formulation of such drugs.

**Biodistribution:**

Kabanov and co-workers [1-3, 17] have extensively studied the use of Pluronics® in drug delivery. They studied the biodistribution and pharmacokinetics of 3H-labeled P85 after a single i.v.-bolus administration in mice. It was found that Pluronics® remained in the circulation for a substantial period of time following a single injection. They also compared the biodistribution of various Pluronics® copolymers having various ratios of lengths of the EO and PO segments. This study determined the area under the curve (AUC) for blood and tissue distribution coefficients for liver and spleen. Results from this study showed that the tissue distribution coefficients increase in the following Pluronics® order: F68 < F108 < P85 < L61 suggesting that the retention of the block copolymer increases as the length of the hydrophobic PO block increases. This study also showed that Pluronics® concentration in the plasma remains quite high for several hours following administration. These concentrations are in the same range as the established CMC for the studied Pluronics which suggests that micelles might be present in the circulation.
**Drug loading into micelles:**

The incorporation of drugs in polymeric micelles may be achieved through chemical and physical routes. Chemical routes involve covalent coupling of the drug to the hydrophilic block of the various copolymers leading to micelles of block copolymer-drug conjugates [28-31]. For example, the pharmacokinetics and distribution of Dox in polymeric micelles formed by a drug-polymer conjugate was studied by Kataoka et al. [9]. They found that the conjugate circulated in the form of micelles much longer in blood than when introduced in free drug form. The uptake of drug conjugate by various non-targeted organs proceeded much slower than that of free drug. Other studies by the same group showed that compared to free drug, lower levels of conjugate were found in the heart, lung, and liver, whereas a much higher conjugate level was found in the tumor [32].

**Release kinetics of drugs:**

The rate of release of drugs from the micelles will depend on the rate of biodegradation of the micelles and the rate of diffusion of drugs from the micelles. Other factors which affect the release of the drugs are micelle stability, localization of the drug within the micelle, the strength of the interaction between the drug and core forming block. Depending on the hydrophobicity, the incorporated drug may reside within the micelle core which is hydrophobic, at the interface between the micelle core and the corona or within the corona itself. Researchers [28-32] have shown that hydrophilic drugs are localized in the inner corona or at the interface between the micelle core-corona while the hydrophobic drugs tend to reside in the micelle core. The rate of release of the drug localized in the outer corona or at the interface has been shown to be much faster than for the drug which is localized in the micelle core as outer corona is quite mobile. The release of the drug which resides in the micelle core will depend on length of the core forming block and the interaction of the drug and micelles core. The longer core forming block will result in larger core, and slower the release of the drugs from the micelle. Also, the drug which resides in the outer corona or at the corona-core interface does not have to diffuse through the core and is not affected by the core radius. The stronger the interaction between the drug and the core, the slower is diffusion. Also, the size or the molecular volume of the drug will also affect its rate of diffusion. The drugs with larger molecular volume will have smaller diffusion coefficient which results in a decreased release rate.
**Drug resistant tumors:**

Chemotherapy, the widely used treatment for the cancer, is severely limited by drug resistance [33-34]. As a result, the response rate following treatment remain very low for many types of cancer and long term survival rates becomes very low for patients with prior treatment with antineoplastic agents [33-35]. There are several mechanisms through which tumor can become resistant to various chemotherapies and tumors with multidrug resistant (MDR) are the most difficult types to treat. It has been shown [33] that tumors with MDR over express efflux transporters belonging to ATP binding proteins such P-glycoprotein (P-gP) and multi drug resistance associated proteins (MPR). Several other proteins (e.g. glutathione-s-transferase, topoisomerase I, II etc.) are also believed to contribute to the resistant phenotype as well. Development of new anticancer drugs has not been very successful because the administration of drugs alone does not result in their high tumor concentration and reduction in systemic toxicity. To improve the chemotherapy, an efficient drug delivery system is needed to minimize the systemic drug exposure and brings the drugs to site of interest.

Alakhov and coworkers [36-38] demonstrated that Pluronics® sensitize resistant cells, which results in an increase in the cytotoxic activity of the drug by two to three orders of magnitude. By addition of P85 or L61, the cytotoxic effects of doxorubicin in the resistant cell line significantly surpassed those observed in the sensitive lines. They also conducted an in-vivo study in mice bearing drug-sensitive and drug-resistant tumors. The mice were treated with doxorubicin alone and doxorubicin in Pluronics compositions. A significant tumor inhibition was observed in all of the tumor models studied whereas doxorubicin alone was effective only in the selected tumors. It has been shown that the effect of Pluronics® on P-GP-mediated efflux is mediated primarily by the unimers. Numerous cytotoxic agents, e.g. doxorubicin, Daunorubicin, Vinblastine, Mitomycin, Taxol and Valproic acid etc. have been loaded into Pluronic® micelles [36-38].The efflux proteins are also expressed in blood brain barrier (BBB). The BBB is a barrier between central nervous system (CNS) and systemic circulation. These characteristics make the BBB a substantial obstacle to CNS delivery. Inhibition of P-gp efflux transport system by Pluronics may facilitate the delivery of therapeutic agents to the CNS. The co-administration of digoxin and Pluronics 85 increases the delivery of digoxin across the
BBB in in-vitro by inhibiting P-gp mediated drug transport and enhanced delivery to the brain. Pluronics 85 have been used to increase the transport of a number of therapeutic agents across a BBB and Caco-2 cells.

It has been shown that blood vessels in tumors greatly differ from blood vessels in normal tissues [39-40]. These blood vessels show an abnormal vascular architecture and deficient lymphatic drainage system. These abnormalities are responsible for enhanced vascular permeability for macromolecules, which remained for extended periods of times. This phenomenon is called enhanced permeation and retention (EPR) effect. Pluronics® micelles have appropriate size to extravasate through leaky blood vessels resulting leaky blood vessels resulting in micelle-encapsulated drug in the interstitial space between the tumor cells enabling passive targeting to tumor via EPR. Though encapsulation of drug into micelles decreases systemic concentration of free drug, the encapsulation of drug also decreases its uptake by cancerous cells. It has been demonstrated that drug uptake can be enhanced by the application of ultrasound. Ultrasound induces formation of cavitations in cellular membrane which results in increased membrane permeability. The most detailed study of the ultrasonic enhancement of the doxorubicin uptake by the cancerous cells from Pluronics was done by Marin et al [35]. They have shown that ultrasound enhances the drug uptake in vitro by increasing both micelle uptake by HL-60 cells and concentration of free drug in incubation medium. Bromberg [41] have explored the use of polymeric micelles, Pluronics-poly (acrylic acid) (PAA), for oral administration of anticancer drugs traditionally administered intravenously. In Pluronics-PAA copolymers, PAA and polyether segments are bonded via pharmaceutically acceptable C-C bond without any foreign chemical entities, combine ionizable and hydrolytic groups that change copolymer conformation in aqueous solution in response to pH and temperature [42].

Animal studies with Pluronics-PAA have demonstrated that after oral administration these molecules do not absorb into systemic circulation and are fully excreted [43]. The Pluronics-PAA self assemble into inter and intramolecular micelles with hydrophobic group consisting of dehydrated PPO and multilayered corona of hydrophilic PEO and partially ionized PAA. The ionizable carboxyl groups in corona provide a strong carboxyl-mucin interaction and polyether fragments interpenetrate into and anchor the
copolymer in mucosa resulting in strong muco-adhesive property of these copolymers [44]. In a rheological study of Pluronics-PAA copolymer [45] it was shown that upon oral administration of aqueous polymer formulation, a dramatic increase in viscosity occurs in-situ at body temperature which results in a soft, shear thinning gels that spread on and simply adhere to mucous surface of oesophagus and GIT.

In an in-vivo study with rats, a significant increase in megestrol acetate bioavailability was observed when drug was formulated with Pluronics (L92)-PAA microgel and administered orally at high dose 10mg/kg. [43]. In contrast, an aqueous micellar formulation comprising a mixture of hydrophobic L61 and hydrophilic F127 copolymer significantly increased the bioavailability of drug at relatively low doses (1 mg/kg – 5mg/kg), but did not provide any significant increase at higher doses. The enhanced bioavailability using microgels was correlated to the enhanced retention of the microgel in the GIT. In an in-vitro experiment, to study the effect of Pluronics-PAA copolymers (L92 and F127) lightly cross-linked by ethylene glycol dimethacrylate, free Pluronics L61, Pluronics L92 and Verapamil, a known inhibitor of P-gP on doxorubicin transport and absorption through Caco-2 cells, it was shown that P-gp mediated doxorubicin efflux inhibited and passive influx was increased, thereby, enhancing the overall absorption of drug. The enhancement was more pronounced with L61 and was comparable to L92 [46].

**Gene delivery:**

Delivery of nucleic acids using a viral vectors is highly efficient [47, 48] but due to the crucial safety concerns such as immunogenicity and insertional mutagenesis [49, 50], the therapeutic use has been limited. Non viral gene therapy has been shown to overcome these problems. Non-viral vectors which contain cationic polymers are better alternative due to viral gene therapy due to low toxicity. Polycations bind to the nucleic acids via formation of salt bonds between the cationic groups of the polycation and phosphate groups of the nucleotides. Polycations such as linear and cross linked poly(ethylene imine) or cationic methacrylates have been shown to be efficient in-vivo transfection agents [51,52].

Although, Polycations vectors have been shown to possess sufficient colloidal stability and significant circulation time of their complexes with DNA in the blood stream, the Polycation-DNA complexes tend to aggregate in aqueous solutions [53]. This
is due to charge neutralization and the lack of electrostatic repulsion between the particle aggregates. The colloidal stability of polycation-DNA can be further enhanced by binding hydrophilic polymers onto polycations and poly(ethylene oxide). PEO is the most prominent hydrophilic polymer used for steric stabilization [54,55]. Modification with PEO improved the colloidal properties of the complexes. However, attachment of PEO to polycations have shown to interfere with cellular processing which results in reduced transfection efficiency in vivo at lower doses. [56]. Bromeberg [57] used the polycations which were modified by conjugation with self-assembling Pluronic® to deliver nucleic acids into human cells to elicit a therapeutic response.

Several studies have shown that Pluronics® are promising agents for utilization in gene therapy applications. Certain Pluronics® block copolymers significantly increase the expression of Plasmid DNA in skeletal muscle in mice [58]. These authors identified a formulation based on the mixture of Pluronics L61 and F127 that increase the gene expression by 5-20 folds compared to the naked DNA. Intramuscular administration of SP1017 was shown to promote the expression of both the reporter and therapeutic genes [59]. According to the dose dependency study, maximal stimulation of gene expression with SP1017 was obtained at relatively low concentration of block (0.01%) and providing at least 500-fold safety margin in animals [58].

**Protein delivery:**

Park et al. [60, 61] studied the controlled release of proteins using Pluronics® formulations. A biodegradable polymer, poly-lactic acid was blended with Pluronics® and fluorescein-labeled bovine-serum albumin, and the blend films were tested for protein release. It was shown that protein dumping at the initial stage of the release was lowered in the presence of Pluronics® and the ability of the Pluronics® to delay protein release and prolong the release period increased in the range of Pluronics® F108 < L101. According to the authors, the diffusion coefficient of the larger proteins in the gel structure of Pluronics® is reduced compared to that of the aqueous phase. Johnson and co-workers used F127 [62-65] gels for sustained delivery of interleukin-2 following intraperitoneal injection in mice and rats, and to achieve sustained release of urease from gel matrix. Pluronics® environment was shown to enhance the stability of both the proteins in aqueous solutions. Banga et al. [66] studied sustained release and stability of human
growth hormone using Pluronic gels and observed zero-order release profile at 37°C and controlled release in vivo profile, following intramuscular and subcutaneous injections [80]. Again, Pluronics® were shown to enhance the protein stability. Bromberg et al. [67] studied the release period of proteins, Zn²⁺-insulin, hemoglobin and lysozyme from Smart hydrogels and observed that the ability of Smart hydrogels to lower the burst effect and prolonged the release period of proteins increased as solution gelled at body temperature. The enzymatic activity of lysozyme loaded into smart hydrogels was shown to be preserved indicating the absence of any deleterious processes in enzyme upon contact with polymer. It was also suggested that protein remain in their native state when released from Smart Hydrogels into the aqueous medium. Coeshott et al. [68] developed a vaccine delivery system based on F127 to deliver a variety of clinically useful antigens in vaccination scheme. Authors formulated protein antigens tetanus toxoid (TT), diphtheria toxoid (DT) and anthrax recombinant protective antigens (rPA) with F127 in combination with CpG motifs or chitosan, as immunomodulators and compared to more traditional adjuvants in mice. It was founds F127 and selected immunomodulators for systemic immunizations that IgG antibody responses were significantly enhanced by F127/CpG and F127/Chitosan combination compared to antigen mixed with CpG or Chitosan alone.

Other formulation applications:

The thermo reversible gelation behavior and promising drug release characteristics of F127 makes it an attractive vehicle drug delivery through various routes of administration such topical/dermal [69,70], rectal and vaginal [71-73], parenteral [74] and for the covering of wounds. Investigations into ophthalmic use have investigated using pilocarpine as the model drug [75]. It has been shown that Pluronics are resistant to protein adsorption. They have lower or suppressed interactions with proteins like immunoglobulins which identify drug vehicles as foreign, prevent the adhesion of drug vehicles onto the surfaces of phagocytes, thereby preventing the clearance of vehicle and allowing them to circulate for a long time. This allows the use of Pluronics® in the creation of biocompatible surfaces in devices for blood contact, membranes for blood purification and contact lenses as well as conditioning of micro and nanospheres.

Miyazaki et al. evaluated the gels of F127 for percutaneous [76] and rectal [77] administration of indomethacin using a rat and rabbit model, respectively. They found
20% aqueous gel to be of practical use as a base for topical administration of the drug. Indomethacin gels, when administered in rabbits rectally, did not show a sharp peak in plasma concentration and produced a sustained plateau level from 10 to 15 hours. Thus, indomethacin preparations based on PF-127 aqueous gels appeared to be practically useful as a rectal preparation with prolonged action. Zhang et al. [78] developed a sustained release formulation which contained F127 alone or with polyvinyl pyrrolidone (PVP), carboxymethyl cellulose (CMC) or hydroxypropyl methylcellulose (HPMC) as an additive of ceftiofur for treating foot infections in cattle. They studied the in vitro release profiles of ceftiofur from F127 formulation and gel dissolution profiles and found that ceftiofur release followed zero order kinetics and correlated well with weight percentage of F127 dissolved. This indicated that overall rate release of ceftiofur is controlled by dissolution of F127. Desai and Blanchard [79] prepared a biodegradable polyisobutylcyanoacrylate nanocapsules (PIBCA-NC) colloidal particulate system of pilocarpine and incorporated it into a F127 based delivery system to study the release profile of pilocarpine. Pilocarpine ophthalmic is used to treat glaucoma by lowering the pressure inside the eye. PIBA-NC of pilocarpine was prepared by interfacial polymerization. The PIBA-NC dispersion of 1% pilocarpine alone (I) and after its incorporation into F127 gel delivery system (II) were compared against 1% pilocarpine incorporated into F127 gel containing 5% methylcellulose (III). Measurement of mitotic response in rabbit eye were performed and statistical analysis indicated a rank order of II>III>I. Thus, the formulation (II) appears to increase the contact time of pilocarpine with absorbing tissue in the eye and thereby, improving the ocular bioavailability. Lin and Sung [80] developed and characterized a series of carbopol and Pluronics® (F127) based solutions as in situ gelling vehicles for ophthalmic delivery of pilocarpine. The rheological properties, in-vitro release and in vivo pharmacological response of various polymer solutions, including carbopol, Pluronics® and carbopol/ Pluronics® solutions were evaluated. It was found that optimum concentration of carbopol solution for in situ gel forming delivery system was 0.3% (w/w), and that of Pluronic® solution was 14% (w/w). When two individual solutions (0.3% carbopol and 14% Pluronics®) were combined, the gel strength under physiological conditions was significantly increased. Also, this solution was free flowing at under non-physiological conditions and its
rheological properties were not affected by the incorporation of drug. In-vitro and in vivo results indicated the combined polymer system performed better in retaining drugs than the individual systems. F127 gels sufficiently mimic normal functions of the skin epidermis by acting as artificial skin and also act as a vehicle to carry small mitogenic protein such epidermal growth factor (EGF) to accelerate wound healing in thermal burns. Di Biase et al. [81] evaluated F127 gel as a potential vehicle for EGF and concluded that it was feasible to develop a topical formulation with EGF in F127 gels with a shelf life of 3 months.

In the foregoing chapters a study on micellar behaviour of some nonionic surfactants, Pluronics in particular, has been made using a variety of experimental techniques. Special attention is given to solubilization of some hydrophobic drugs in Pluronic® micelles.

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