POLYETHYLENE OXIDE-POLYPROPYLENE OXIDE BASED BLOCK COPOLYMERS AS NANOVEHICLES FOR DRUG FORMULATIONS

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Abstract

Concepts utilizing polymers as therapeutic agents have been widely investigated for a number of decades. Nanosized drug delivery systems formulated from biocompatible and biodegradable polymers constitute an evolving approach to drug delivery and tumor targeting. The increasing interest in Polyethylene oxide (PEO)-Polypropylene oxide (PPO) block copolymers (known as Pluronics®) arises mainly due to their surface-active and self-associative characteristics leading to micellar formation. Pluronic polymers are approved by FDA for pharmaceutical and medical applications and listed in the US and British pharmacopeia as pharmaceutical excipients. The present article will be explained the basics of these smart polymers, its nanomicelles and applications of such nanomicelles in drug formulations specifically for an anticarcinogenic drug, Curcumin for improved solubility, stability, release, and bioavailability.

Keywords: Block copolymers, Pluronic®, Micelles, Nanoparticles, Drug delivery, Curcumin drug

Polymers have been used in a range of pharmaceutical and biotechnology products for more than five decades. Since the 1940s polymers have been explored as therapeutics with a rapidly increase in research publications year on year (Fig. 1a). The number of papers in 2011 interestingly reached to ~9000, still some 10-fold higher than is seen for the descriptor “nanomedicine,” a wider discipline that is growing well.

The term “Polymer therapeutics” was coined to describe polymeric drugs, polymer conjugates of proteins, drugs and aptamers, together with those block copolymer micelles and multicomponent non-viral vectors which contain covalent linkages (Fig. 1b). These often complex, multicomponent constructs are actually “drugs” and “macromolecular prodrugs” in contrast to drug delivery systems that simply entrap (non-covalently) drug molecules (Fig. 1b). They have also been called as nanomedicines.

Nanomedicine can be defined as the design of diagnostics and/or therapeutics on the nanoscale, which provides advantages due to the high degree of coincident transport and delivery of the active species with mediation of their navigation within the biological systems for the treatment, prevention and diagnosis of diseases[1,2].

Fig.1: (a) Literature against “Polymer Therapeutic” in search panel (b) Various Polymer Therapeutics

As drug carriers, the Polymeric micelles have manifested a number of attractive features and advantages over the other types of carriers. Over the years, various synthetic, structural, physicochemical, and biomedical aspects of polymeric micelles have been discussed in a number of excellent reviews[3,4]. In this article, we mainly discussed the Polyethylene oxide(PEO)-Polypropylene oxide(PPO) based block copolymeric micelles as the smart materials for the drug formulations.

Block copolymers are a special type of macromolecules in which each molecule consists of two or more segments of simple polymers (blocks) covalently joined together with and/or radial arrangement. In the
simplest case, a diblock copolymer (A-B) is composed of two different homopolymers linked end to end. An extension of this concept leads to (A-B-A) or (B-A-B) triblocks and to (A-B), linear multiblocks, A and B can be made into a copolymer in many different ways. Where, n block copolymer chains are linked by one of their ends to a multifunctional moiety. Some arrangements are linear, in which the blocks are connected end-to-end, and star, in which all of the blocks are connected via one of their ends at a single junction. The number of monomer types in a block copolymer may be less than or equal to the number of blocks. Even more complex architectures in block copolymers have also been reported, such as cyclic, heteroarm, “palm tree”, H-shaped, dumb-bell structures, etc.

Pluronic® Polymers - The Smart Materials

A number of symmetric tri-block copolymers of propylene oxide (PO) and ethylene oxide (EO) have been commercially available since 1950’s under the generic name Poloxamers and the trade marks Pluronic® (BASF, Mount Olive, NJ, USA) and Synperonic® (ICI, Cleveland, UK). It is a user with fixed number of nonionic surfactants of the type Poloxamer. The polypropylene oxide block, PPO, is sandwiched between two polyethylene oxide (PEO) blocks. PEO blocks are hydrophilic while PPO is a hydrophobic block in the Pluronic molecule. Depending on the number of EO and PO units, various types of Pluronics are available commercially. The critical micelle concentration (CMC) or critical micelle temperature (CMC) in water (Fig.2a). Pluronic micelles can be prepared by several techniques. Even more complex architectures in block copolymers have also been reported, such as cyclic, heteroarm, “palm tree”, H-shaped, dumb-bell structures, etc.

Pluronic micelles can be prepared by several techniques, but most of them are focused on the variations for drug loading. The representative method is a direct dissolution of Pluronic polymer around CMC and followed by the elevation of temperature or the addition of hydrophobic drugs. The other one might be swelling (e.g., DMF, DMSO, acetone, THF) exchange. In this method, drugs are dissolved in a solvent with the block copolymers (rather water insoluble), and then water can be added into this solution or vice versa, viz., the solution can be poured into the water.

Fig.2: (a) Chemical structure of Pluronic® polymers (b) Pluronic grid. Liquids in blue, pastes in green and solids in the pink region. (from Ref.9)

In the Pluronic grid, the alphabetical designation explains the physical form of the product: ‘P’ for pastes, ‘F’ for solid and ‘L’ for liquid form. The first digit (two digits in a three-digit number) in the numerical designation, multiplied by 300, indicates the approximate molecular weight of the hydrophobe part. The last digit, when multiplied by 10, indicates the approximate ethylene oxide content in percentage in the molecules.

Micelles of Pluronic® Polymers

The micellization of PEO–PPO–PEO triblock copolymers in water 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 the critical micelle concentration (CMC) or critical micelle temperature (CMC) in water (Fig.3). In contrast to the conventional nonionic surfactants of the type PEO condensates, Pluronics show some unique behavior in water which mostly arises around room temperature when the PPO no longer remains hydrophobic. The shape or morphology of micelles prepared from amphiphilic Pluronic polymers includes spherical (‘core-corona’ or ‘crew-cut’), network-like, cylindrical, lamellar and so on (Fig.3). The shape varies with the block length ratio and the composition of block copolymers.

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Pluronic micelles as Nanovehicles for Drug Delivery Systems

The Pluronic® polymers are approved by Food and Drug Administration (FDA) also listed in the US and British pharmacopoeia as pharmaceutical excipients and extensively used as emulsification, solubilization, dispersion, thickening, coating, and wetting agents\textsuperscript{[10]}. Pluronic® self-assemble into micelles (5-50 nm) in the aqueous solution with core–shell structure above their CMC and the hydrophobic PPO core of micelles serves as a cargo space for the incorporation of drug molecules, while the hydrophilic PEO shell contributes greatly by maintaining the micelles in a dispersed state as well as by decreasing undesirable drug interactions with cells and proteins through steric-stabilization effects\textsuperscript{[10-13]}. Fig. 4 represented the complete mechanism of an encapsulation of drugs into Pluronic micelles. The incorporation of drugs into the core of the micelle results in increased solubility, metabolic stability and circulation time for the drug. By attaching a peptide or other biospecific molecule that can promote site-specific drug delivery to surface of the EO corona, a micelle can be targeted to a specific site in the body. Not only that, Pluronic® unimers exhibit biological response modifying activities in certain drug formulations. For example, the interactions of the unimers with multidrug-resistant cancer cells result in sensitization of these cells with respect to various anticancer agents. Further, Pluronic® unimers have been shown to inhibit drug efflux transporters in both the blood-brain barrier and in the small intestine. This can provide for enhanced transport of select drugs to the brain and enhanced oral bioavailability\textsuperscript{[12]}. 

There have been many important studies on the solubilization of hydrophobic drugs having different pharmacological activity in Pluronic® micelles were reported. Increased solubility and longer half-life of naproxen and rofecoxib was shown by Suh et al.\textsuperscript{[13]} and Ahuja et al.\textsuperscript{[14]}. Solubility of tropicamide a mydriatic/cycloplegic drug was studied with a series of Pluronic® with different molecular characteristics and found that ocular solubility increased linearly with increasing surfactant concentration and enhanced solubility
for higher EO content\textsuperscript{[15]}. Neuroleptic drug haloperidol showed a 5-fold increase in solubility in P85\textsuperscript{[16]}. A novel doxorubicin formulation (SP1049C) has been developed using a combination of two Pluronics (L61 and F127) by Alakhov et al.\textsuperscript{[17]}. The cytotoxic activity of this product has shown that it is highly effective against MDR cells. Further mechanistic studies have revealed that SP1049C has higher activity than doxorubicin due to increase in the drug uptake, inhibition of the energy-dependent drug efflux and changes in intracellular drug trafficking. The experiments on in vivo tumour models have confirmed high efficacy of SP1049C against drug-resistant tumours, as well as suggested that this product has considerably broader efficacy than doxorubicin. The analysis of pharmacokinetics and biodistribution of SP1049C has shown that it accumulates in tumour tissue more effectively than doxorubicin, while distribution of the formulation in normal tissues is similar to that of doxorubicin. Micelles formed by Pluronic\textsuperscript{®} polymers(PBC) have been studied in multiple applications as drug delivery systems by Kabanov et al.\textsuperscript{[18]}. Hydrophobic PBC forms lamellar aggregates with a higher solubilization capacity than spherical micelles formed by hydrophilic PBC. However, they also have a larger size and low stability. To overcome these limitations, binary mixtures of hydrophobic (L121, L101, L81, and L61) and hydrophilic (F127, P105, F87, P85, and F68) Pluronics were also studied. PBC mixtures were not stable, revealing formation of large aggregates and phase separation within 1–2 day(s). However, stable aqueous dispersions of the particles were obtained upon sonication for 1 or 2 min and/or heating at 70°C for 30 min. In many combinations, L121/F127 mixtures (1:1% weight ratio) formed stable dispersions with a small particle size. The solubilizing capacity of this system was examined using a dye, SudanIII. Mixed L121/F127 aggregates exhibited approximately 10-fold higher solubilization capacity compared to that of F127 micelles. It concluded that the mixed PBC aggregates can efficiently incorporate hydrophobic compounds.

Oh et al.\textsuperscript{[19]} developed a binary mixing system with two Pluronics, L121/P123, as a nano-sized drug delivery carrier. The lamellar-forming Pluronic L121 (0.1 wt%) was incorporated with Pluronic P123 to produce nano-sized dispersions (in case of 0.1 and 0.5 wt% P123) with high stability due to Pluronic P123 and high solubilization capacity due to Pluronic L121. The binary systems were spherical and less than 200-nm diameter, with high thermodynamic stability (at least 2 weeks) in aqueous solution. The CMC of the binary system was located in the middle of the CMC of each polymer. In particular, the solubilization capacity of the binary system (0.1/0.1 wt%) was higher than mono-systems of P123. The main advantage of binary systems is overcoming limitations of mono systems to allow tailored mixing of block copolymers with different physicochemical characteristics. These nano-sized systems may have potential as anticancer drug delivery systems with simple preparation method, high stability, and high loading capacity.

**Nanomicelles of Pluronic\textsuperscript{®} Polymers as nanovehicles for Curcumin drug**

Curcumin is a natural polyphenol molecule derived from the *Curcuma longa* plant which exhibits anticancer, chemo-preventive, chemo- and radio-sensitization properties. Curcumin’s widespread availability, safety, low cost and multiple cancer fighting functions justify its development as a drug for cancer treatment\textsuperscript{[20]}. However, various basic and clinical studies elucidate curcumin’s limited efficacy due to its low solubility, high rate of metabolism, poor bioavailability and pharmacokinetics\textsuperscript{[21]}. To increase its solubility and bioavailability, attempts have been made through encapsulation in liposomes, polymeric and lipo-NPs, biodegradable microspheres, cycloexetrin, and hydrogels. Nano-sized drug delivery systems formulated from Pluronic polymers constitute an evolving approach to drug delivery and tumor targeting. Fig.5 shows the solubilization of curcumin in Pluronic as the nanocarriers.

![Fig.5: Solubilization/encapsulation of Curcumin drug into Pluronic micelles](image-url)
Pluronic® F127 and F68 were used for the encapsulation of curcumin for drug delivery application by Sahu et al.[22]. The encapsulation of drug in pluronic micelle was highly dependent on drug-to-polymer ratio. Copolymer F127 showed better encapsulation efficiency than F68, Tonnesen et al.[23] have been studied that the concentration of solubilized curcumin is related to the number of PO units of Pluronics. All Pluronics showed a maximum solubilizing capacity at a certain drug-to-polymer molar ratio and exceeding this ratio resulted in precipitation of curcumin, when keeping it the samples for 15 days. Addition of PEG-400 could to a certain extent stabilize the supersaturated samples, while ethanol physically destabilized the samples. Ionic strength did not influence the solubilization of curcumin in the Pluronic micelles.

Curcumin-loaded mixed micelles using P123 and F68, was prepared through thin-film hydration method and evaluated in vitro by Zhai and group[24]. The preparation method was optimized with a central composite design (CCD). The average size of the mixed micelles was 68.2 nm, and the encapsulating efficiency for curcumin was 86.93%, and 6.996% drug-loading. Compared with the curcumin in propylene glycol (PG) solutions, the in vitro release of curcumin from loaded mixed P123/F68 micelles showed the sustained-release property. The in vitro cytotoxicity assay showed that the IC_{50} values on MCF-7 cells for Cur-PF and free Cur in DMSO solution were 5.04μg/mL and 8.35 μg/mL, while 2.52 μg/mL and 8.27 μg/mL on MCF-7/ADR cells. Results concluded that mixed P123/F68 micelles might serve as a potential nanovehicles to improve the solubility and biological activity of curcumin.

Bora et al.[25] have also been reported the nanoformulation of curcumin with a tripolymeric composite for delivery to cancer cells. The composite nanoparticles (NPs) were prepared by using three polymers; alginate(Alg), chitosan(CS), and F127 by ionotropic pregelation followed by polycationic cross-linking. F127 was used to enhance the solubility of curcumin in the Alg-CS NPs. The AFM and SEM analysis showed that the particles were spherical in shape with an average size of 100 ± 20 nm. FTIR analysis revealed potential interactions among the constituents in the composite NPs. Encapsulation efficiency(%) of curcumin in composite NPs showed considerable increase over Alg-CS NPs without Pluronics. The in vitro drug release profile alongwith release kinetics and mechanism from the composite NPs were studied under simulated physiological conditions for different incubation periods. A cytotoxicity assay showed that composite NPs at a concentration of 500μg/mL were nontoxic to He-La cells. Cellular internalization of curcumin-loaded composite NPs was confirmed from green fluorescence inside the He-La cells. The half-maximal inhibitory concentrations for free curcumin and encapsulated curcumin were found to be 13.28 and 14.34 μM, respectively.

Pluronic®F127 (EO_{90}-PO_{40}-EO_{90}) has a good solubilizing capacity and is approved by FDA for pharmaceutical and medical applications, including parenteral administration and extensively used as emulsification, solubilization, dispersion and wetting agents. Therefore, it was chosen as parent copolymer for our study too.

Curcumin has negligible solubility (0.11 ng/mL) in water, but a linear increase in solubility with F127 concentrations can simply be attributed to interaction between drug and polymer molecules and increased no. of micelles(shown in Fig.6a). The intensity of yellow colour of drug was clearly increased with the F127 concentrations suggested the high amount of drug in aqueous media. Solubility is almost 1000 folds more in presence of Pluronic micelles encourages to use of such smart materials for encapsulation of curcumin to it.

![Phase solubility curve of curcumin in Pluronic solutions](image1)

![Thin Film Hydration Method](image2)

Fig.6: a) Phase solubility curve of curcumin in Pluronic solutions (b) Synthesis of Curcumin loaded Pluronic nanoparticles by Thin film hydration method.
Curcumin-loaded nanoparticles (CUR-NPs) were prepared by thin-film hydration method and represented in Fig.6b. CURNP1 (using hydrophobic F127 polymer) clearly shows the more intense orange colour in compare to CURNP2 (using hydrophilic F68 polymer). The drug loading and nanoparticles yield of the optimized NPs were 47.72% and 34.51% for CURNP1 and CURNP2, respectively.

![Fig. 6b: Characterization of curcumin loaded Pluronic nanoparticles by various techniques.](image)

The Characterization of the synthesized CURNPs was performed by variety of techniques presented in Fig.7. It can be observed that micelles for both the formulations has the particle sizes were about 99 nm (for CURNP1) and 107 nm (for CURNP2), which are practically useful for drug delivery applications. The SEM pattern of pure curcumin shows its crystalline nature. The Pluronic itself was semi-crystalline, but the SEM of CURNP1 showed absence of major curcumin crystals. It indicates the masking of crystallinity of drug after encapsulation into Pluronic micelles. Results of UV-Visible spectra (1% aqueous solution of NPs) clearly show that drug has weak/negligible absorption (no intense peak) in the wavelength range of 300 to 500 nm as hydrophobic, but CURNPs gave sharp intense peaks of curcumin. Results suggested the encapsulation of drug in Pluronic® micelles. In all the three samples, a characteristic intensity of the O-H stretch is observed around 3500 cm⁻¹. The chemical structure curcumin shows the functional groups of phenolic OH, C=O and aromatic C=C peaks at 1623 cm⁻¹ and 1520 cm⁻¹. The F127 showed two major peaks at 1101 cm⁻¹ of –C–O stretching and 2882 cm⁻¹ of –C–H stretching and 1350 cm⁻¹ of C=C aromatic. The FTIR spectra also confirmed the interaction of curcumin with F127 micelles and identified the formation of NPs between them. The PXRD pattern of pure curcumin shows several characteristic peaks indicative of its crystalline nature. The Pluronic itself was also crystalline in nature. The two sharp peaks at 20 of 20.0°C and 23.9°C were due to the presence of PEO groups present in F127. But the CUR-NPs showed absence, broadening and reduction of major curcumin diffraction peak. And it indicated the masking of crystalline peaks of curcumin after encapsulation into Pluronic micelles. DSC curves obtained for pure curcumin, F127, and CURNP1 are displayed in Fig.7. A broaden melting peak was observed at about 176°C for free curcumin and in the thermogram of F127, a sharp peak (60.05°C) was observed, which was associated with the endothermic melting of F127. In DSC spectra of CURNP1 was showed peak at 59.65 °C for F127, but no peak at around 176°C.

The in vitro release of both the formulations, a typical two-phase release profile was observed(Fig.8). A relatively rapid release was in the first stage followed by a sustained and slow release over a prolonged time up to 11 days. Relase of drug was faster in case of F68 based NPs and almost 80% was released in 10 days while in the CURNP1, 63% drug released. The stronger the interaction between the drug and the core-forming block, the slower the release of drug from micelle.
Conclusions

Overall, Pluronic® block copolymers are among the most potent drug targeting systems with a broad spectrum of biological response modifying activity, and an ability to self-assemble into micelles, incorporate drug molecules and transport them within the body. These properties warrant further investigation regarding the use of Pluronic® formulations as novel promising drug delivery systems. Pluronic micelles as nanovehicles for curcumin drug has not only become a frontier movement but is also future prospects. Nanomicelles of Pluronic resulted in increased solubility, metabolic stability, and improved circulation time for curcumin drug.

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References


Fig.8: In Vitro release of curcumin from CURNPs.

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