# 3 Controlled Release of Poorly Soluble Active Ingredients from Bioresorbable Polymers

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## 3.1 Introduction

Drug delivery is of significant importance to medical and healthcare sectors. It is a broad term that covers issues such as formulation, technology and the system for transporting active pharmaceutical ingredients (API) in the body [1]. The main objective of any drug-delivery device is to safely achieve the desired therapeutic result with minimum side effects. However, in some cases, such as chemotherapy for cancer, current treatment methods rely primarily on the use of conventional cytotoxic drugs that have an adverse side effect and only limited effectiveness. Many studies have indicated that these problems could be attributed to the lack of target specificity of state-of-the-art anti-tumour drugs. To overcome this hurdle the demand for drug delivery is growing and, in the US alone, the drug-delivery market was valued at >$135 billion in 2015 as a result of the growth of 9% each year since 2010. The largest share is expected to be produced by oral, parenteral or injectable drugs [1].

Controlled drug delivery improves bioavailability (i.e., the proportion of a drug that enters the circulation when introduced into the body and so is able to have an active effect) by preventing premature degradation and enhancing uptake by controlling drug-release rates and reducing side effects by targeting the diseases or defective site. Since the first US Food and Drug Administration approved

drug-delivery system, liposomal amphotericin in 1990, various drug-delivery systems have become available commercially to treat diseases ranging from cancer to fungal infections and muscular degeneration [2]. Polymers show improved pharmacokinetics, longer circulation times and more efficient tissue targeting in comparison with small-molecule drugs. They can be used as the polymeric drug, as a drug carrier, or be combined with small molecules such as proteins. The field is usually characterised by the term 'polymer therapeutics' and can be divided into five subclasses: polymeric drugs, polymer–drug conjugates, polymer–protein conjugates, polymeric micelles, and polyplexes.

If the polymeric material is not acting as a drug, it can be used as a drug carrier to reduce immunogenicity, toxicity or degradation while simultaneously improving circulation times and potentially having a passive targeting function. Polyethylene oxide (PEO) is a widely studied polymer in this field due to its biocompatibility, non-toxicity, water solubility and, most importantly, prolonged residence time in the body. PEO has been investigated to bypass normal physiological defences to protect chemical or biological conjugates by entrapping the PEO chains onto conjugates to induce a stealth effect to decrease immunogenicity and increase the stability of the conjugates against enzyme hydrolysis [3].

The success of an efficient drug-delivery device hinges upon its ability to construct biocompatible carriers that allow high loading of drug molecules without premature release of the drug cargo before reaching the target organ [4]. Other factors to consider are that the carrier material should be compatible, and have high-loading capacity and a controlled release rate of drugs. For example, a novel polyethylene glycol 400 (PEG400)-mediated lipid nanoemulsion as a drug delivery carrier for paclitaxel (PTX) was developed by Jing and co-workers [5]. They demonstrated that PEG400 could be used to carry an anticancer drug that is used for cancers of the breast, prostate gland and lung. Their investigation showed that a two-vial formulation comprising a PEG400 solution of the drug and a commercially available 20% lipid emulsion could be used to form

paclitaxel-loaded lipid nanoemulsion (TPLE). Results demonstrated that TPLE could significantly reduce extraction of reticuloendothelial system organs and increase tumour uptake, which exhibited more potent antitumor efficacy in mice compared to conventional TPLE. TPLE did not cause haemolysis or an intravenous irritation response and showed the same cytotoxicity against HeLa cells as Taxol® [6].

Drug carriers are used to enhance therapeutic efficiency *via* encapsulation of active molecules. This encapsulation enhances the stability of drug molecules, improves targeting properties, and prolongs pharmacological activity *via* continuous local release of active molecules [7]. There is an array of drug-delivery devices, such as plasmids, viral, liposomes, micelles, dendrimers and nanoconjugates.

## 3.2 Poorly Water-soluble Drugs

Poorly water-soluble (hydrophobic) drugs have poor lipophilicity and, therefore, low stability, which are necessary constituents for desired bioavailability [8]. The study of drug-delivery systems for hydrophobic drugs is important because large amounts of active substances are difficult to formulate by normal approaches. This leads to undesirable distribution in the body due to their lack of solubility in the human body [9]. Enhancing the delivery technologies for hydrophobic drugs is very expensive because, according to van Hoogevest [8], various undesirable effects might occur: lack of stability in the solid state (chlorhexidine); extreme hygroscopicity (ibuprofen); extreme light sensitivity (nimodipine); and extremely low solubility (fenofibrate).

Although the lack of solubility in the human body does to not prevent targeting the delivery of hydrophobic drugs [10], strategies have been developed to overcome the problems associated with solubility. Essentially, there are three main formulations for hydrophobic drug technologies: solubilised, amorphous and crystalline.

Solubilised formulations are generally used for oral drugs where the permeability through the intestinal epithelial membrane is not limited. The advantage of solubilised formulations is that dissolution of the drug is not necessary because the drug is already dissolved. However, precipitation of the drug may occur after release in the human body due to the dilution of the solubilising component if it is water-soluble; for an immiscible soluble component such precipitation does not occur unless the component remains intact [11, 12].

If the drug cannot solubilise for the method applied it is carried by an amorphous formulation of the drug, such as a solid dispersion. This process increases oral absorption but lacks stability, which leads to crystallisation [13, 14].

An amorphous formulation is the most robust; the drug needs to dissolve and is dependent on the crystalline phase and particle size. Nonetheless, to increase the dissolution rate, the particle size must be as small as possible. This leads to nano-sized particles, which are widely studied in this field [15].

Amorphous formulations can offer high free energy and lead to improved solubility [16]. However, the use of amorphous formulations is limited due to their thermodynamic instability, which causes higher chemical degradation and recrystallisation. In addition, solid dispersions often lead to poor reproducibility of physicochemical properties, difficulties in development of dosage forms and potential physical instability [17].

It is also necessary to analyse the route where the drug will be administered. Different administration routes might cause different problems due to the low dissolution of hydrophobic drugs.

The oral route is the most favoured because it can be being easily handled and is painless. Drugs administered *via* this route can also be produced in different dosages with low costs [18]. One of the reasons is that the gastrointestinal tract (GIT) has a high

intestinal surface area and rich mucosal vasculature, which offers high absorption following bioavailability. GIT fluids dissolve the drug so it can be absorbed onto the intestinal mucosa and be delivered into the circulation. In this case, the aqueous solubility and intestinal permeability are key factors for this delivery route [19]. In the case of poorly water-soluble drugs, their behaviour results in low bioavailability and is highly sensitive to food intake [20]. Normally, to prevent some complications that may occur with the interaction of the drug and food intake, patients are suggested to follow food restrictions.

The parental route is the injection of drugs *via* subcutaneous, intra-arterial or intravenous routes. The drug is delivered into the blood circulation and allows rapid exertion of its pharmaceutical effect. This route is preferred for drugs for which the oral route might lead to low bioavailability. In addition, this route provides more predictable pharmacokinetic and pharmacodynamics profiles than that for oral administration [21]. However, despite the benefits of the pharmaceutical effects, drugs must pass some regulatory requirements and this is not feasible for poorly water-soluble drugs. To overcome this issue hydrophobic drugs are mixed with organic solvents, which might lead to a decrease in bioavailability and cause haemolysis and drug precipitation [22].

The pulmonary route is aimed towards diseases such as asthma. It leads to enhanced delivery of the pharmaceutical ingredient to tissue with a large surface area and thin epithelial barrier [19]. The particle size of the drug determines where it will be deposited into the lungs. For example, particles of size 1–3 μm are deposited deep within the lungs; those of 5 μm in the upper airways, and particles <1 μm are not deposited and instead exhaled after inspiration [19, 23]. Apart from this limitation of particle size, the pulmonary route is also limited by the small number of safe ingredients for this delivery route.

To overcome the issues associated with bioavailability and controlled release of poorly water-soluble drugs, the introduction of polymers

for drug carriers has been investigated. In earlier studies, drug encapsulation was used merely for improvement of drug-delivery efficiency [24]. Currently, controlled-release mechanisms have been used to: target delivery of hydrophobic drugs *via* amorphous solid dispersions; improve API stabilisation through the inhibition of nucleation and crystallisation in the solid state; enhance effective solubility; minimise drug degradation; reduce drug toxicity; facilitate the control of drug uptake [25].

## 3.3 Mechanisms of Drug Release from Biodegradable Polymers

The mechanism of controlled release from biodegradable polymers can occur as diffusion through a rate-controlling membrane, osmosis, ion exchange or degradation. The advantage of drug delivery from biodegradable polymers is that these materials do not need to be removed from the body after drug delivery has been completed. Normally, bioresorbable polymers are usually considered because they degrade slowly and it is possible to control drug delivery for longer periods of time [26]. The mechanism of drug release from biodegradable polymers as well as their release kinetics is dependent on the choice of polymer and formulation method used [27, 28]. The hydrolytic cleavage of the polymer chains results in erosion of the polymer matrix, with two main mechanisms of degradation, bulk and surface, taking place [29, 30]. Whether bulk degradation or surface degradation is the dominant mechanism is dependent on the rate of water penetration and hydrolysis of the polymer backbone and is, therefore, influenced by the: rate of water diffusion through the polymer; size of the device; polymer porosity; reactivity of the functional groups of the polymer [31]. If the rate of water penetration is significantly higher compared with the rate of polymer hydrolysis, bulk degradation will be the dominant mechanism. This is because degradation of the polymer backbone will spread through the entire device as a result of the fast-penetrating water before surface degradation can take place. The opposite is true for surface

degradation, with it being the dominant mechanism if degradation of the polymer matrix is faster than the rate of water diffusion into the device. This is due to surface erosion taking place before water has penetrated into the device [32].

Surface-degrading polymers can provide improved controlled-release kinetics compared with bulk-degrading polymers and thus have greater potential in drug-delivery applications. Polyanhydrides and polyorthoesters are two types of surface-degrading polymers that have been researched extensively for drug delivery. The chemical bonds (anhydride bonds and orthoesters, respectively) of their polymer backbone are hydrolysed faster than water can diffuse into the polymer, which results in surface degradation [33–35]. In theory, if the surface-degradation process is spread evenly and confined to the surface of the device or formulation, then drug release will be controlled by surface degradation and exhibit zero-order release kinetics [36]. However, actual drug release may deviate from zero-order release kinetics because it is dependent on the type, chemistry, molecular weight (MW), crystallinity, porosity and glass transition temperature of the polymer.

Bulk-degrading polymers such as polycaprolactone (PCL) and poly(lactic-*co*-glycolic acid) (PLGA) are the most commonly used biodegradable polymers in drug-delivery applications. The release rate, duration of release, and release profile (mono- or multi-phasic profiles) can be controlled by the degradation of the polymer. Therefore, a particular release profile can be achieved by selecting or modifying a polymer with an appropriate degradation rate or behaviour [37]. The rate of degradation and degradation behaviour of bulk-degrading polymers is influenced by several parameters:

1. Polymer composition – in PLGA, the glycolide group degrades faster than the lactide group, thus the release rate from PLGA can be adjusted by varying the ratio of these two groups [38, 39].
2. MW and polydispersity – the lower the MW of the polymer, the faster its degradation process will be. Therefore, the choice of a low-MW polymer will lead to increased drug release.

3. Polymer crystallinity – the more amorphous the polymer the faster rate of water penetration into the polymer and thus, the faster the polymer degrades [40].
4. The size of the drug-delivery device or formulation – larger devices can undergo autocatalytic degradation because the degradation products remain within the device and catalyse the degradation process further, thus increasing the degradation rate [41].

However, depending on the degradation rate, it could take longer for water to penetrate larger devices, which may slow down the degradation process. The nature and type or drug, drug loading, and porosity of the polymer can also influence release.

The process of degradation and thus drug release for bulk-degrading polymers involves four key steps:

1. The water or dissolution medium penetrates into the amorphous regions of the polymer and begins to disrupt the tertiary structure, which is stabilised by hydrogen bonds and van der Waal's forces.
2. The ester bonds in the polymer backbone are cleaved to generate carboxylic acids, which autocatalyse the hydrolysis process. The MW of the polymer will begin to decrease and the device or formulation will begin to lose its mechanical strength.
3. Cleavage of the ester bonds on the polymer backbone continues with a significant loss of the MW of the polymer as well as the mechanical strength of the device or formulation.
4. The oligomers are solubilised into the dissolution medium and the polymer breaks down into smaller fragments, which are subsequently hydrolysed into free acids.

The drug-release profile of bulk-degrading polymers is typically triphasic. An initial burst is followed by a period of zero-order drug release, controlled by a combination of drug diffusion through the polymer and degradation. The third phase is a rapid release of drug due to degradation of the polymer device or formulation [42–44].

## 3.4 Polymers for Controlled Release of Poorly Water-soluble Drugs

### *3.4.1 Controlled Release from Water-soluble/Bioresorbable Hydrogels*

Hydrogels are three-dimensional crosslinked polymeric networks that can swell and absorb large quantities of water or biological fluids to form a soft and elastic material that does not dissolve instantly. Swelling is due to the hydrophilic blocks of the group chains. With lower hydrophilicity, the polymer swells in water but, with a further increase in hydrophilicity, the polymer becomes water soluble [6, 45, 46]. Certain hydrogels can show responses to a stimulus in the environment such as volume, viscosity, pH, temperature or mechanical stress by incorporation of co-monomers into the network [45, 47, 48]. These hydrogels are termed 'stimuli-sensitive' or 'smart' hydrogels.

Hydrogels can be obtained naturally or by syntheses and have elicited considerable interest because, due to their properties, it is possible to obtain controlled drug delivery such as: coatings for the oral route and as dissolution and binding agents in capsules of drugs [49, 50]; encapsulation of cells for repairing and regenerating organs and tissues [46, 51]. Hydrogels have been investigated for targeted delivery of poorly water-soluble drugs. The incorporation of poorly water-soluble drugs improves their solubility and achieves longer periods of delivery, which increases the chances of intra-tumour uptake of drugs compared with free drugs [52].

Although the popular term 'hydrogel' refers to gels made by water-soluble stable polymers, they can be modified to become biodegradable. A common technique for the production of biodegradable hydrogels is to utilise low-MW polymers, which have a low degree of crosslinking. However, another approach is to produce hydrogels which are crosslinked with hydrolytically and enzymatically biodegradable chains. An example of this is

the use of hydroxyethyl methacrylate which is crosslinked with PCL-based crosslinking agent rendering the entire hydrogel biodegradable [53].

Early studies on the drug release of biodegradable hydrogels [26] showed interesting behaviour. The drug will be in contact with water, so the drug properties are important and its solubility must be addressed. This strategy is usually employed for macromolecular drugs, which are entrapped in the gel network until the gel is degraded.

The pathways for biodegradability or dissolution may occur *via* enzymatic means, hydrolytic means, or *via* the environment. For drug delivery, degradation is not always desirable due to the timescale and location for the drug-delivery device.

An amorphous solid dispersion is the most used formulation when incorporating poorly water-soluble drugs onto hydrogels. Crosslinked hydrogels, compared with water-soluble polymers for poorly water-soluble drugs, have considerable advantages. Sun and Lee [54] stated that, when using amorphous solid dispersions on water-soluble polymers, a highly supersaturated environment is created. This results in recrystallisation of the dissolved drug, which reduces the drug concentration and supersaturation limit of the solid dispersion. Conversely, crosslinked hydrogels ensure that the drug is 'tangled' and protected by the hydrogel layer. This helps control drug delivery by reducing diffusion to the external core and preventing supersaturation in the environment. This could lead to a critical supersaturation above which spontaneous nucleation and crystallisation would occur (**Figure 3.1**).

However, hydrogels have limited capacity for loading of poorly water-soluble drugs. Two methods have been reported [52, 55]: incorporation of polymeric micelles onto hydrogels that can loco-regionally deliver multiple drugs in a single dose [56]; and electrospinning to produce nanofibres with poorly water-soluble drugs to enhance the solubility, drug release and effectiveness of the active ingredient [55].

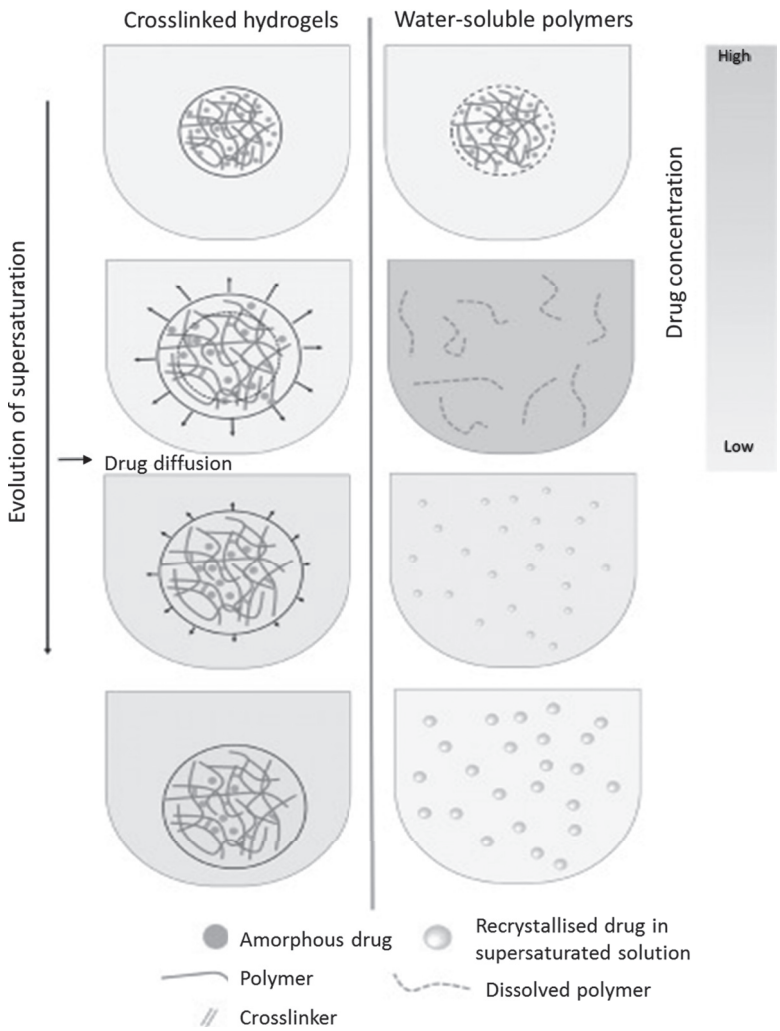


**Figure 3.1** Drug-release mechanism of an amorphous drug in crosslinked hydrogels and water-soluble polymers. Reproduced with permission from D.D. Sun and P.I. Lee, *Acta Pharmaceutica Sinica: B*, 2014, **4**, 26. ©2013, Elsevier [54]

### 3.4.2 Controlled Release from Polymeric Micelles

As discussed before, nanoparticles help to improve poor solubility. In this regard, polymer micelles have been used in multifunctional-based delivery systems for poorly water-soluble drugs. Polymeric micelles consist of a hydrophobic and hydrophilic block domain that are self-assembled and which can form a 'core-shell' structure due to the interaction of the hydrophobic and hydrophilic parts named as 'micelles' [57–59]. The interior part, the hydrophobic block, protects the drug and the hydrophilic part confers solubility [59]. Micelle formation follows after the colloid aggregates, and the concentration range at which these chemical structures are formed is above the critical micelle concentration [60].

The most used polymer to form this structure is polyethylene glycol (PEG) at a MW range of 2–15 kDa. It can stabilise the micelle structure and interactions with cells and proteins. Subsequently, a hydrophobic block consisting usually of PCL [61], poly(b-benzyl-l-aspartate) [62] or polypropylene oxide (PPO) [63] acts as a shell to protect the hydrophobic drug from making contact with the aqueous solution and hindering enzymatic degradation of bioactive molecules [64]. Use of these polymers is based primarily due to their non-toxicity, biodegradability and biocompatibility. Also, PEG can decrease opsonin adhesion in plasma and increase the circulation time of the structures in the human body [65]. The advantage of using polymeric micelles compared with micelles is that they have a very low critical micelle concentration, which results in relatively stable systems which are not easily dissociable *in vivo* [66, 67]. This stability ensures the controlled administration of the pharmaceutical ingredient for longer periods of time.

In general, it is possible to prepare polymeric micelles with many copolymers but the choice becomes limited because of biocompatibility and biodegradability [68]. The most used block copolymers are the ones characterised by their hydrophobic blocks, such as polyethers, polyamides and polyesters.

Drug loading onto polymeric micelles can be influenced by the hydrophobic effect and the interaction between the micelle blocks.

Several studies [65, 69] have shown that the interaction between hydrophobic and hydrophilic parts is significant. For example, PEG-*b*-PCL polymeric micelles that have a longer hydrophobic block improve drug capabilities [70], but the partition coefficient can deteriorate with a longer PEG chain. Miscibility is the main factor that can improve drug loading and the initial amount of drug influences the entrapment efficiency which is also related to the aggregation number of the polymer block [71, 72].

The advantages of using polymeric micelles for poorly water-soluble drugs are: their very small size (which helps to target drug-administration routes such as blood vessels); high-structural stability due to their core-shell structure [73], which helps to improve bioavailability; large amount of drug loading and water solubility due to their inherent layer of hydrophilic/hydrophobic parts [74]; incorporation of various diagnostic agents.

## 3.5 Recent Strategies for Poorly Water-soluble Drugs

### *3.5.1 Hydrogels/Polymeric Micelles*

Recent research shows that incorporation of micelles as a hydrophobic block into the hydrogel structure can resemble the behaviour of semicrystalline polymers which contains a mixture of crystalline and amorphous regions [75]. This balance can provide water absorption, fluid flow, and controlled release based on the hydrophilic block, whereas the hydrophobic segment can provide the strength, tear and shear resistance [76]. This structure is quite advantageous for poorly water-soluble drugs. Ju and co-workers [77] demonstrated that when using PTX (an anticancer agent with poor water solubility), micellar encapsulation onto the hydrogel reduced the toxicity of PTX and the whole system demonstrated prolonged drug release (>15 days).

A block of polymeric micelles in the hydrogel structure helps solubilise poorly water-soluble drugs and acts as a 'shell' for transport and controlled delivery [78]. A system for oral delivery of the anticancer

agent docetaxel was developed to target the drug onto the intestinal tract while improving its solubility. Polymeric micelles were mixed with docetaxel, which showed better solubility and permeability. In addition, encapsulation of the micelle block helped prevent identification by proteins that could cause efflux and change delivery within the body.

Three main methods can be used to incorporate hydrogels containing micelles, as shown below.

1. Hydrogels with distributed micelles – using this method, a mixture of micelles and a hydrogel matrix is formed [69]. Hydrogels with micelles have a complex structure that can host hydrophilic and hydrophobic drugs. Anirudhan and co-workers showed that the release of a hydrophilic drug (tramadol hydrochloride) was controlled mainly by the hydrogel and release of a hydrophobic drug (cefixime trihydrate) was controlled mainly by the polymeric micelle [79].
2. Hydrogels with integrated micelles – using this method, it is possible to obtain two type of structures:
    - Dispersed micelles anchored in crosslinked chains –very insightful work by Yom-Tov and co-workers [79] used pluronic micelles in a hydrogel while anchoring most of their molecules to the surrounding network through their end groups. This led to anchored molecules that facilitated the crosslinking between the hydrogel and stabilised the network.
    - Micelles formed by hydrophobic segments located directly on the gel – Inoue and co-workers [80] grafted oligo(methyl methacrylate) as a hydrophobic block onto hydrophilic polyacrylic acid (PAA) and used model hydrophobic drugs. The hydrophobic drug was released slowly from the hydrogels compared with an ungrafted-PAA hydrogel. The authors hypothesized that this was due to favoured absorption of the hydrophobic drug onto the hydrophobic block and lack of interaction, which led to faster drug release for hydrophilic drugs.
3. Hydrogels with micellar crosslinks – in this method, the polymeric micelles form a chemical or physical crosslinking network with

the hydrogel. Missirlis and co-workers [81] used inverse emulsion photocopolymerisation with PEG diacrylate to produce pluronic-based nanoparticles. The hydrophobic blocks within these biodegradable nanoparticles solubilised a poorly water-soluble drug, doxorubicin, up to almost 10% (w/w).

Loading of a hydrophobic drug onto hydrogel-polymeric micelles involves mixing a solution of micelles with the drug followed by the incorporation onto the hydrogel. These processes include: simple equilibrium (dissolution of the drug into a solution of pre-formed micelles); chemical conjugation; dialysis; oil–in–water emulsions; solution casting; and freeze-drying. For the detailed process, the reader should find [60, 64] relevant.

**Figure 3.2** shows how micelles are embedded in a hydrogel structure. In this example, the polymeric micelles entrap the hydrophobic drugs acting as a core-shell for the drug, following immersion mixing with the hydrogel. The representation shows well-aligned micelles between the hydrogel. However, as explained above, one can obtain three major structures with very good bioavailability and low toxicity without altering the biodegradability of the hydrogel for targeted delivery of the drug.

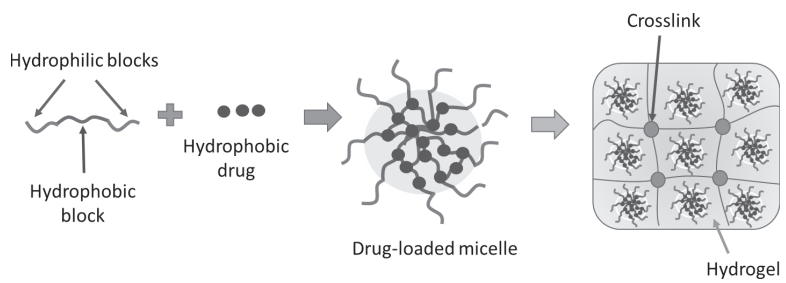


**Figure 3.2** Nanoscale drug-entrapment strategy in hydrogels (schematic)

### *3.5.2 Nanocomposite Hydrogels*

Due to the deficiency of loading for hydrophobic drugs, hydrogels have been produced at the nanoscale [82]. Gwak and co-workers

[83] prepared a hydrogel–inorganic hybrid material with agarose mixed with zinc basic salt, and the results showed sustained release. Apart from micelles, biodegradable cyclodextrin (CD) hydrogels have also been studied for complex poorly water-soluble drugs [84] to increase their solubility. The incorporation of CD into hydrogels does not only maintain the swelling properties of the hydrogel but the hydrophobic interior can facilitate the capture and sustained release of a hydrophobic drug. The process of CD incorporation is similar to that for micelles [82], but the drug can be added onto the hydrogel during or after gel crosslinking.

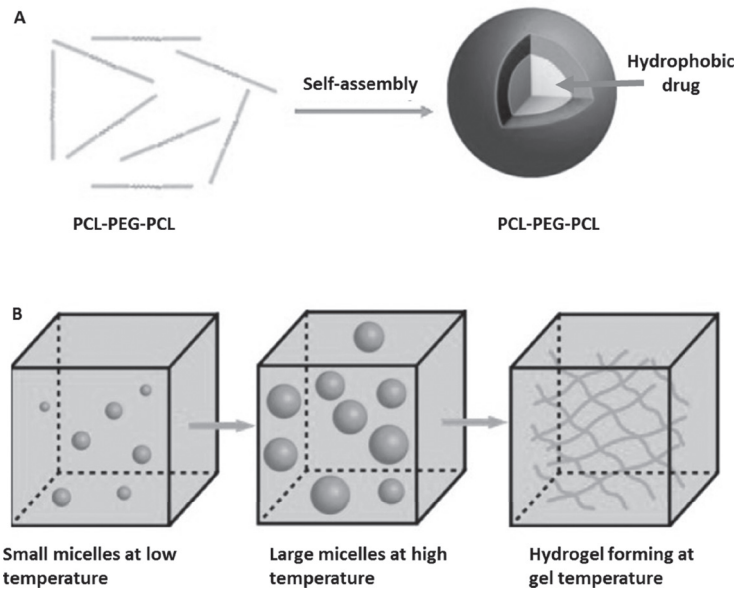


**Figure 3.3** A thermosensitive PCEC hydrogel (schematic). (A) Assembling of PCEC triblock copolymers and (B) due, to the sensitivity of these micelles, they became larger with increasing temperature, and a hydrogel was formed when the temperature reached the gelation temperature. Reproduced with permission from X. Gao, X. Deng, X. Wei, H. Shi, F. Wang, T. Ye, B. Shao, W. Nie, Y. Li, M. Luo, C. Gong and N. Huang, *International Journal of Nanomedicine*, 2013, **8**, 2453. ©2013, Dove Medical Ltd [85]

Gao and co-workers [85] produced a thermosensitive hydrogel-based poly(ε-caprolactone)–polyethylene glycol–poly(ε-caprolactone) (PCEC), which has the potential for preventing formation of postoperative adhesions. They used ring-opening polymerisation of the PCEC copolymer to dissolve it into PCEC micelles, which resulted in spheres of mean size 25 nm (**Figure 3.3A**). This polymer was thermosensitive and at body temperature the PCEC micelles were converted into a hydrogel (**Figure 3.3B**). The results showed no signs of adhesion *in vivo* and of total degradation after 6 weeks. According to Gao and co-workers, this system can also be used for delivery of two drugs.

## 3.6 Stimuli-responsive Hydrogels

During the 1970s, hydrogel research shifted from water-swollen networks to hydrogels that were capable of responding to changes in environmental conditions [86]. In the past two decades, these stimuli-responsive hydrogels have received increasing attention in relation to several biomedical applications, such as drug-delivery systems and tissue engineering [87, 88]. Thus, stimuli-responsive hydrogels have been suggested to be superior to conventional hydrogels as sensors [89]. Stimuli-responsive hydrogels can react to minor changes in physical or chemical environments. One of the most promising applications for a stimuli-responsive hydrogel is drug delivery. This type of delivery allows the drug to increase its concentration in a specific organ or tissue, which can improve the efficiency and safety of some medicines that are already on the market. The development and success of targeted drug-delivery systems has led to novel strategies, such as delivery of anticancer drugs and gene therapy [90]. This helps the efficacy of the drug by reducing side effects. Treatment of most diseases is reliant on effective, safe, targeted drug-delivery systems [90]. Several types of stimuli-responsive hydrogels can react to a number of different environmental conditions. These can be classified into two main categories, which are displayed in [91, 92].

| Table 3.1 Different types of stimuli | |
|---|---|
| **Physical stimuli** | **Chemical stimuli** |
| Temperature | pH |
| Mechanical stress | Ionic |
| Magnetic | – |
| Electric | – |

The most frequently used stimuli are pH and temperature. Among all stimuli-responsive hydrogels, temperature-responsive hydrogel systems have received most interest because temperature is a vital physiological factor in the body. Some diseases manifest themselves by a change in temperature [93–96].

Dual-sensitive hydrogels can also be produced; this is where two stimuli-responsive monomers are bonded together to create a hydrogel that can respond to both stimuli. For example, combining pH- and temperature-responsive monomers together will result in pH–temperature-sensitive hydrogels, where the phase-separation temperature will depend on the pH of the medium. Combining a pH monomer to a temperature-responsive hydrogel benefits an oral drug-delivery system. That is, the pH hydrogel will protect the drug in the presence of an acid pH (e.g., stomach) and deliver it to an area of alkaline pH (e.g., small intestine).

### 3.6.1 Hydrogels that are Responsive to Temperature

Temperature-responsive hydrogels are the most commonly studied due to their physiological importance. Temperature-responsive hydrogels contain hydrophilic and hydrophobic segments. If the temperature is within the range of phase transition, the balance between the hydrophilic and hydrophobic segments is altered and phase separation or sol-gel transition can follow [97]. Temperature-responsive hydrogels that can undergo a sol-gel transition have been shown to have applications in biomedical fields, particularly in drug delivery and injectable-tissue engineering [97–99]. These

are solutions of hydrogels that can be injected into any tissue, organ or body cavity in a minimally invasive manner prior to sol-gel transition [86, 100].

Hydrogels that display a sol-gel transition around room temperature to body temperature are predicted to be the most beneficial [101]. Some hydrogels can comprise drugs or cells and such systems aid in delivery to a desired site. During a sol-gel transition, all of the pharmaceutical agents in the system can be released in a controlled manner [102]. Hydrogels that can undergo sol-gel transition offer several advantages over systems that must be formed into their final shape before implantation [86].

A temperature-sensitive hydrogel shows a thermally induced phase transition in solutions. The phase behaviour of temperature-sensitive hydrogels is governed by the Gibbs' free energy of mixing (**Equation 3.1**):

$$\Delta G_m = \Delta H_m - T\Delta S \qquad (3.1)$$

where $\Delta H_m$ and $T\Delta S$ are the enthalpy and the entropy of mixing, respectively.

Two components will mix only if the Gibbs' free energy of mixing is negative: $\Delta G_m < 0$. Upon heating, the entropy of mixing can dominate and lead to decreasing miscibility with increasing temperature. A typical phase diagram consists of two-phase boundaries. The lower boundary corresponds to thermally induced mixing (known as the upper critical solution temperature) and the upper boundary corresponds to thermally induced demixing [known as the lower critical solution temperature (LCST)]. Temperature-responsive hydrogels, which become insoluble upon heating, have a LCST in which the hydrophilic and hydrophobic balance changes. Hydrogel systems that exhibit LCST behaviour have gained significant attention in biomedical applications in recent years. The change in temperature affects the hydrogen bonding of the polymer molecules, which results in a volume-phase transition [103].

One of the most renowned temperature-responsive hydrogels is poly(N-isopropylacrylamide) (PNIPAAm), which exhibits a phase transition of ≈32 °C in aqueous solution. The aim of researching temperature as a release trigger is due to controlled drug release by precisely controlling the temperature sensitivity of the hydrogel to the environmental temperature of the target site. Temperature-responsive hydrogels have become attractive candidates for designing nano-vehicles to target specific tissues. The temperature range at which these nano-vehicles should release is 37–42 °C [104].

Low-MW poly(N-vinylcaprolactam) (PNVCL) is known for its superior biocompatibility, solubility, thermosensitivity and having non-ionic and non-toxic features. Moreover, PNVCL has a similar LCST range compared with PNIPAAm, which is between the physiological range of 32 and 38 °C [105]. The LCST behaviour of PNVCL is sensitive to alterations in the polymer concentration, the MW of the polymer and also the composition of the solution [106]. PNVCL sensitivity to these alterations allows for 'tuned' LCST behaviour.

### *3.6.2 Hydrogels that are Responsive to pH*

Hydrogels that are responsive to pH are also very popular stimuli. The pH sensitivity of a hydrogel results from weak acid or weak base functionality on the polymer backbone, which accepts or releases protons depending upon external conditions [107]. Thus, at certain pH values, they can dissociate in aqueous solutions to form polyelectrolytes. Commonly used weak acids are acrylic acid and methacrylic acid [49, 108]. A frequently used weak base is dimethylaminoethyl methacrylate. The pH at which hydrogels show volume changes depends on the type of weak acid or base used. Some hydrogels can contain weak acid functionality; they will swell as the pH of the medium increases. Hydrogels that are pH-sensitive are commonly used to develop controlled-release formulations for oral administration [109]. Certain body tissues have different pH values, such as the stomach, which is quite different from the

neutral pH in the intestine, and more changes occur within various body tissues. Chronic wounds can have a pH range between 7.4 and 5.4, and cancer tissue has been reported to be acidic extracellularly [103]. Cancer tissue usually has a pH <7 and the pH of heathy body tissue is 7.2–7.4. Tumours with low pH result primarily from a high glycolysis rate, which can produce lactic acid. This low pH benefits tumour cells and promotes invasive cell growth. The low pH in the tumour provides a tissue-specific stimulus that may be exploited to target applications. pH-responsive hydrogels can be tailored to carry, deliver, and control the release of a therapeutic agent in cancer tissue (**Figure 3.4** [110]).

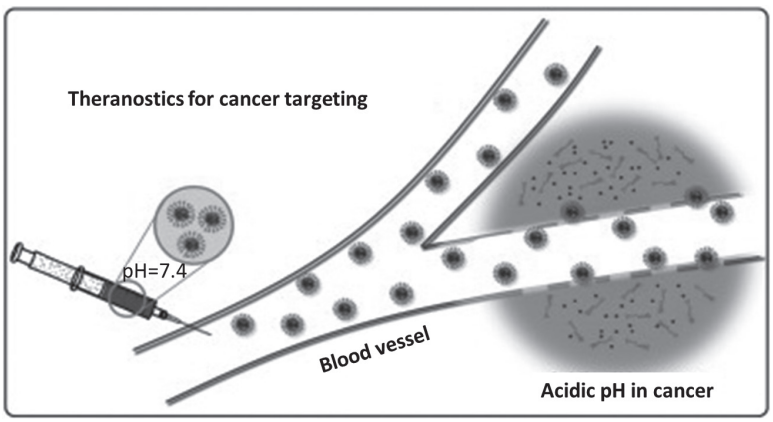


**Figure 3.4** The effect and acidic controlled release system in a cancer area (schematic). Reproduced with permission from G.H. Gao, Y. Li and D.S. Lee, *Journal of Controlled Release*, 2013, **169**, 180. ©2013, Elsevier [110]

### *3.6.3 Combining pH- and Temperature-responsive Hydrogels*

Hydrogels can be sensitive to both pH and temperature. The presence of pH-sensitive co-monomers, which are charged, increases the LCST of the thermosensitive co-monomers due to an increase in

the hydrophilicity of the polymer. pH- and temperature-sensitive hydrogels can undergo volume changes in response to changes in temperature and pH. The magnitude and location of temperature-induced gel collapse can be altered significantly by changing the pH of the solution. This allows tailoring of a thermosensitive hydrogel to meet specific process or system needs. These unique characteristics are of great interest in drug delivery [111] and cell encapsulation [112].

An injectable pH- and temperature-sensitive biodegradable hydrogel poly(β-amino ester urethane) and triblock poly(ε-caprolactone lactide) (PCLA)–PEG–PCLA was used to enhance sustained release by dual ionic interactions. The injectable hydrogel suppressed the initial burst release and extended the release period for 13 days *in vitro* and 5 days *in vivo* [113].

## 3.7 Applications of Stimuli-responsive Hydrogels

Applications that require the delivery system not to be degradable, such as in oral drug delivery, do not offers problems. However, this becomes a serious limitation in other applications. One major disadvantage of temperature-responsive hydrogels such as PNIPAAm and PNVCL is that they are not degradable. Thus, recent research has focused on development of the biodegradable ability of these hydrogels. For a hydrogel to be biodegradable, the presence of hydrolytic or proteolytic labile bonds in their backbone is essential to allow it to degrade. Several research teams have attempted to incorporate a biodegradable backbone to enhance the biodegradable properties of these hydrogels [114].

Rejinold and co-workers [115] developed curcumin-loaded chitosan (CS)-*g*-PNVCL nanoparticles for cancer therapy. This hydrogel system had biodegradable, biocompatible and temperature-responsive properties. It was extremely efficacious against cancer cells with minimal toxicity to normal cells *in vitro*.

Gan and co-workers synthesised novel temperature-sensitive biodegradable hydrogels from N-isopropylacrylamide and two biodegradable crosslinkers: PCL dimethacrylate and *bis*(acryloyl) cystamine. At physiological pH, the hydrogels could be biodegraded slowly in glutathione at 37 °C [116].

Kokuryo and co-workers [117] measured the release of anticancer drugs by dual-sensitive hydrogels (pH-temperature). They noted a decrease in tumour size after treatment.

### *3.7.1 Injectable Hydrogels in Drug Delivery*

Hydrogels that can be formed *in situ* can be clear polymer solutions prior to administration, and undergo a sol-gel transition in response to changes in stimuli. Physically crosslinked injectable hydrogels are reversible networks and can be developed by varying the environmental stimuli. Several appealing biomedical applications have been proposed for hydrogels that can be formed *in situ*, particularly in the areas of drug delivery and cell culture. Due to their unique advantages, such as minimal invasion, lack of organic solvents or photoinitiators, site specificity, and ability to deliver hydrophobic/hydrophilic drugs, stimuli-sensitive hydrogels are injectable. For example, drugs or cells can be encapsulated with *in situ*-forming hydrogels solutions at low temperature, and the mixed solutions rapidly undergo sol-gel transition after injection into the body [118]. This large gain in entropy is caused by the release of bound water molecules from the hydrophobic segments at sol-gel transition. The polymer accumulates and the phase-separated depot then slowly releases the drug by dissolution and diffusion of the drug.

In drug delivery, the release of a drug could be in response to a temperature increase that makes the temperature-responsive hydrogel undergo phase transition. The temperature at the target site is slightly higher (e.g., in solid tumours) than normal body temperature. By adjusting the sol-gel transition of the temperature-responsive hydrogel

between the body temperature and the higher temperature of the tumour, it is possible for the drug-delivery system to accumulate within the tumour [119].

Prabaharan and co-workers entrapped self-assembled stable micelles of PNVCL-*b*-PEG block copolymers coupled with folic acid as an anticancer drug carrier. The diameter of these micelles in a dehydrated state was ≈150 nm. At 37 °C, the release profile of these polymeric micelles showed a slower and more controlled release of entrapped 5-fluorouracil (5-FU) than that at 25 °C. 5-FU-loaded micelles did not induce remarkable cytotoxicity. However, they showed a cytotoxic effect against 4T1 mouse mammary carcinoma cells due to the availability of loaded anticancer drugs delivered to the inside of cancer cells by a folate receptor-mediated endocytosis process [120, 121]

Yerriswamy and co-workers prepared PNVCL-*co*-VAc microspheres by free-radical emulsion polymerisation in the presence of 5-FU [122]. The authors reported higher release rates of 5-FU for the formulations prepared with higher amounts of N-vinylcaprolactam (NVCL) than formulations prepared using lower amounts of NVCL. Slower drug release was observed from formulations prepared with a lower amount of NVCL, which was attributed to the hydrophilic nature of the drug and NVCL in the copolymer [122].

Prabaharan and co-workers used biodegradable CS-*g*-PNVCL for the controlled release of the hydrophobic drug ketoprofen. These were stabilised by crosslinking with a solution of sodium tripolyphosphate. The release behaviour was influenced by the pH and temperature of the medium. At pH 7.4 and 37 °C, CS-g-PNVCL showed a compact structure with reduced pore size and strong hydrophobic interactions with drug molecules, resulting in a slow and steady release of the drug from the system [123].

Thermogelling hydrogels can be incorporated into contact lenses. Thermogelling hydrogels are becoming popular in eye treatment because conventional eye drops move away quickly from the surface of the eye, resulting in poor bioavailability. To improve such poor

bioavailability, Cho and co-workers developed a temperature-sensitive biodegradable hexanoyl glycol chitosan (HGC) as a carrier for topical drug delivery to the eye. The researchers found that controlling the amount of hexanoyl added to the glycol CS could control the thermogelling behaviour for glaucoma therapy. *In vivo* experiments demonstrated HGC to be maintained on the periocular surface for a longer period of time due to its increased viscosity at body temperature [124].

## 3.8 Summary

This chapter discussed the various difficulties associated with poorly water-soluble drugs for targeted delivery and bioavailability. Various strategies have been studied to improve it, but this field needs further work. The ability of drug holding and shell encapsulation offer hope in terms of attachment of cells and biocompatibility. In addition, targeted delivery with poorly water-soluble drugs will be able, in the near future, to localise where the drug needs to be delivered independent of the route chosen. Currently, pH and temperature-hydrogels allow a new future in biomedical applications using al and sol-gel transition. Although there have been achievements in pH and temperature-responsive injectable hydrogels, several challenges for clinical applications remain.

## References

1. E. Skorupska, A. Jeziorna, S. Kazmierski and M.J. Potrzebowski, *Solid State Nuclear Magnetic Resonance*, 2014, **57**, 2.

2. Y. Zhang, H.F. Chan and K.W. Leong, *Advanced Drug Delivery Reviews*, 2013, **65**, 104.

3. S. Stolnik, L. Illum and S.S. Davis, *Advanced Drug Delivery Reviews*, 1995, **16**, 195.

4. I.I. Slowing, J.L. Vivero-Escoto, C-W. Wu and V.S-Y. Lin, *Advanced Drug Delivery Reviews*, 2008, **60**, 1278.

5. X. Jing, L. Deng, B. Gao, L. Xiao, Y. Zhang, X. Ke, J. Lian, Q. Zhao, L. Ma, J. Yao and J. Chen, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2014, **10**, 371.

6. Y. Jiang, J. Chen, C. Deng, E.J. Suuronen and Z. Zhong, *Biomaterials*, 2014, **35**, 4969.

7. K. Miladi, S. Sfar, H. Fessi and A. Elaissari, *International Journal of Pharmaceutics*, 2013, **445**, 181.

8. P. van Hoogevest, X. Liu and A. Fahr, *Expert Opinion on Drug Delivery*, 2011, **8**, 1481.

9. A. Fahr and X. Liu, *Expert Opinion on Drug Delivery*, 2007, **4**, 403.

10. A. Fahr, P. van Hoogevest, S. May, N. Bergstrand and M.L.S. Leigh, *European Journal of Pharmaceutical Sciences*, 2005, **26**, 251.

11. R.G. Strickley, *Pharmaceutical Research*, 2004, **21**, 201.

12. C.W. Pouton, *European Journal of Pharmaceutical Sciences*, 2006, **29**, 278.

13. Y. Kawabata, K. Wada, M. Nakatani, S. Yamada and S. Onoue, *International Journal of Pharmaceutics*, 2011, **420**, 1.

14. C. Brough, and R.O. Williams, *International Journal of Pharmaceutics*, 2013, **453**, 157.

15. C.M. Keck and R.H. Müller, *European Journal of Pharmaceutics and Biopharmaceutics*, 2006, **62**, 3.

16. P. Gupta, G. Chawla and A.K. Bansal, *Molecular Pharmaceutics*, 2004, **1**, 406.

17. P.H.L. Tran, T.T.D. Tran, J.B. Park and B.J. Lee, *Pharmaceutical Research*, 2011, **28**, 2353.

18. F. Gabor, C. Fillafer, L. Neutsch, G. Ratzinger and M. Wirth in *Drug Delivery*, Springer, Amsterdam, The Netherlands, 2010, p.345.

19. S. Bosselmann and R.O. Williams, III., in *Formulating Poorly Water Soluble Drugs*, Springer, New York, NY, USA, 2012, p.1.

20. G.L. Amidon, H. Lennernäs, V.P. Shah and J.R. Crison, *Pharmaceutical Research*, 1995, **12**, 413.

21. C.B. Packhaeuser, J. Schnieders, C.G. Oster and T. Kissel, *European Journal of Pharmaceutics and Biopharmaceutics*, 2004, **58**, 445.

22. S.H. Yalkowsky, J.F. Krzyzaniak and G.H. Ward, *Journal of Pharmaceutical Sciences*, 1998, **87**, 787.

23. J. Heyder, J. Gebhart, G. Rudolf, C.F. Schiller and W. Stahlhofen, *Journal of Aerosol Science*, 1986, **17**, 811.

24. A.P. Simonelli, S.C. Mehta and W.I. Higuchi, *Journal of Pharmaceutical Sciences*, 2016, **58**, 538.

25. J. Liu, and L. Li, *European Journal of Pharmaceutical Sciences*, 2005, **25**, 237.

26. H. Park, K. Park and W.S.W. Shalaby in *Biodegradable Hydrogels for Drug Delivery*, CRC Press, Boca Raton, FL, USA, 2011, p.1.

27. C. McConville, P. Tawari and W. Wang, *International Journal of Pharmaceutics*, 2015, **494**, 73.

28. I. Zembko, I. Ahmed, A. Farooq, J. Dail, P. Tawari, W. Wang and C. Mcconville, *Journal of Pharmaceutical Sciences*, 2015, **104**, 1076.

29. D.Y. Arifin, L.Y. Lee and C-H. Wang, *Advanced Drug Delivery Reviews*, 2006, **58**, 1274.

30. K.L. Poetz, H.S. Mohammed, B.L. Snyder, G. Liddil, D.S.K. Samways and D.A. Shipp, *Biomacromolecules*, 2014, **15**, 2573.

31. F. von Burkersroda, L. Schedl and A. Göpferich, *Biomaterials*, 2002, **23**, 4221.

32. S. You, Z. Yang and C-H. Wang, *Journal of Pharmaceutical Sciences*, 2016, **105**, 1934.

33. A. Göpferich, *Biomaterials*, 1996, **17**, 103.

34. J. Tamada and R. Langer, *Journal of Biomaterials Science, Polymer Edition*, 1992, **3**, 315.

35. B. Wei, Y. Tao, X. Wang, R. Tang, J. Wang, R. Wang and L. Qiu, *ACS Applied Materials & Interfaces*, 2015, **7**, 10436.

36. J. Heller and J. Barr, *Biomacromolecules*, 2004, **5**, 1625.

37. L.L. Lao, N.A. Peppas, F.Y.C. Boey and S.S. Venkatraman, *International Journal of Pharmaceutics*, 2011, **418**, 28.

38. S. Li, *Journal of Biomedical Materials Research*, 1999, **48**, 342.

39. X.S. Wu and N. Wang, *Journal of Biomaterials Science, Polymer Edition*, 2001, **12**, 21.

40. M. Vert, S. Li and H. Garreau, *Journal of Controlled Release*, 1991, **16**, 15.

41. S. Li, H. Garreau and M. Vert, *Journal of Materials Science: Materials in Medicine*, 1990, **1**, 198.

42. B.S. Zolnik, P.E. Leary and D.J. Burgess, *Journal of Controlled Release*, 2006, **112**, 293.

43. N.S. Berchane, K.H. Carson, A.C. Rice-Ficht and M.J. Andrews, *International Journal of Pharmaceutics*, 2007, **337**, 118.

44. Q. Xu and J.T. Czernuszka, *Journal of Controlled Release*, 2008, **127**, 146.

45. G.G. de Lima, D. Kanwar, D. Macken, L. Geever, D.M. Devine and M.J.D. Nugent in *Handbook of Polymers for Pharmaceutical Technologies*, Eds., V.K. Thakur and M.K. Thakur, John Wiley & Sons, Inc., New York, NY, USA, 2015, p.1.

46. A.S. Hoffman, *Advanced Drug Delivery Reviews*, 2012, **64**, 18.

47. S. Chaterji, I.K. Kwon and K. Park, *Progress in Polymer Science*, 2007, **32**, 1083.

48. Y. Samchenko, Z. Ulberg and O. Korotych, *Advances in Colloid and Interface Science*, 2011, **168**, 247.

49. M. Canillas, G.G. de Lima, M.A. Rodríguez, M.J.D. Nugent and D.M. Devine, *Journal of Polymer Science, Part B: Polymer Physics*, 2015, **54**, 761.

50. T.G. Park, *Biomaterials*, 1999, **20**, 517.

51. N.A. Peppas, P. Bures, W. Leobandung and H. Ichikawa, *European Journal of Pharmaceutics and Biopharmaceutics*, 2000, **50**, 27.

52. M. McKenzie, D. Betts, A. Suh, K. Bui, L.D. Kim and H. Cho, *Molecules*, 2015, **20**, 20397.

53. S. Atzet, S. Curtin, P. Trinh, S. Bryant and B. Ratner, *Biomacromolecules*, 2008, **9**, 3370.

54. D.D. Sun, and P.I. Lee, *Acta Pharmaceutica Sinica: B*, 2014, **4**, 26.

55. U. Paaver, J. Heinämäki, I. Laidmäe, A. Lust, J. Kozlova, E. Sillaste, K. Kirsimäe, P. Veski and K.Kogermann, *International Journal of Pharmaceutics*, 2015, **479**, 252.

56. S.A. Abouelmagd, B. Sun, A.C. Chang, Y.J. Ku and Y. Yeo, *Molecular Pharmaceutics*, 2015, **12**, 997.

57. V.P. Torchilin, *Journal of Controlled Release*, 2001, **73**, 137.

58. P. Mukerjee, *Journal of Pharmaceutical Sciences*, 1971, **60**, 1531.

59. A.S. Mikhail and C. Allen, *Journal of Controlled Release*, 2009, **138**, 214.

60. B.P.K. Reddy, H.K.S. Yadav, D.K. Nagesha, A. Raizaday and A. Karim, *Journal of Nanoscience and Nanotechnology*, 2015, **15**, 4009.

61. H. Ai, C. Flask, B. Weinberg, X. Shuai, M.D. Pagel, D. Farrell, J. Duerk and J. Gao, *Advanced Materials*, 2005, **17**, 1949.

62. G. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai and K. Kataoka, *Langmuir*, 1993, **9**, 945.

63. K.N. Prasad, T.T. Luong, A.T. FlorenceJoelle Paris, C. Vaution, M. Seiller and F. Puisieux, *Journal of Colloid and Interface Science*, 1979, **69**, 225.

64. S. Biswas, O.S. Vaze, S. Movassaghian and V.P. Torchilin in *Drug Delivery Strategies for Poorly Water-Soluble Drugs*, John Wiley & Sons Ltd, Hoboken, NJ, USA, 2013, p.411.

65. Y. Lu, and K. Park, *International Journal of Pharmaceutics*, 2013, **453**, 198.

66. S.B. La, T. Okano and K. Kataoka, *Journal of Pharmaceutical Sciences*, 1996, **85**, 85.

67. A.V. Kabanov, E.V. Batrakova and V.Y. Alakhov, *Journal of Controlled Release*, 2002, **82**, 189.

68. A.V. Kabanov and V.Y. Alakhov in *Critical ReviewsTM in Therapeutic Drug Carrier Systems*, Ed., M. Sachdeva, Begell House, Inc., Banbury, CT, USA, 2002, p.19.

69. Y. Wang, L. Chen, L. Tan, Q. Zhao, F. Luo, Y. Wei and Z. Qian, *Biomaterials*, 2014, **35**, 6972.

70. K. Letchford, R. Liggins and H. Burt, *Journal of Pharmaceutical Sciences*, 2008, **97**, 1179.

71. J. Lee, S.C. Lee, G. Acharya, C. Chang and K. Park, *Pharmaceutical Research*, 2003, **20**, 1022.

72. K.M. Huh, S.C. Lee, Y.W. Cho, J. Lee, J.H. Jeong and K. Park, *Journal of Controlled Release*, 2005, **101**, 59.

73. M. Morishita and N.A. Peppas, *Drug Discovery Today*, 2006, **11**, 905.

74. V.P. Torchilin, *Pharmaceutical research*, 2007, **24**, 1.

75. S.K. Shukla, A.W. Shaikh, N. Gunari, A.K. Bajpai and R.A. Kulkarni, *Journal of Applied Polymer Science*, 2009, **111**, 1300.

76. M. Pekař, *Frontiers in Materials*, 2015, **1**, 35.

77. C. Ju, J. Sun, P. Zi, X. Jin and C. Zhang, *Journal of Pharmaceutical Sciences*, 2013, **102**, 2707.

78. L. Bromberg, *Expert Opinion on Drug Delivery*, 2005, **2**, 1003.

79. T.S. Anirudhan, J. Parvathy and A.S. Nair, *Carbohydrate Polymers*, 2016, **136**, 1118.

80. T. Inoue, G. Chen, K. Nakamae and A.S. Hoffman, *Journal of Controlled Release*, 1997, **49**, 167.

81. D. Missirlis, N. Tirelli and J.A. Hubbell, *Langmuir*, 2005, **21**, 2605.

82. S. Merino, C. Martín, K. Kostarelos, M. Prato and E. Vázquez, *ACS Nano*, 2015, **9**, 4686.

83. G.H. Gwak, S.M. Paek and J.M. Oh, *European Journal of Inorganic Chemistry*, 2012, 5269.

84. J. Zhang and P.X. Ma, *Advanced Drug Delivery Reviews*, 2013, **65**, 1215.

85. X. Gao, X. Deng, X. Wei, H. Shi, F. Wang, T. Ye, B. Shao, W. Nie, Y. Li, M. Luo, C. Gong and N. Huang, *International Journal of Nanomedicine*, 2013, **8**, 2453.

86. S.J. Buwalda, K.W.M. Boere, P.J. Dijkstra, J. Feijen, T. Vermonden and W.E. Hennink, *Journal of Controlled Release*, 2014, **190**, 254.

87. P. Matricardi, C. Di Meo, T. Coviello, W.E. Hennink and F. Alhaique, *Advanced Drug Delivery Reviews*, 2013, **65**, 1172.

88. A.S. Hoffman, *Advanced Drug Delivery Reviews*, 2013, **65**, 10.

89. N.A. Peppas and D.S. Van Blarcom, *Journal of Controlled Release*, 2015, **240**, 142.

90. R.K. Verma and S. Garg, *Pharmaceutical Technology*, 2001, **25**, 1.

91. D. Klinger, and K. Landfester, *Polymer*, 2012, **53**, 5209.

92. R.A. Siegel, *Journal of Controlled Release*, 2014, **190**, 337.

93. Q. Wu, L. Wang, X. Fu, X. Song, Q. Yang and G. Zhang, *Journal of Polymer Research*, 2014, 1.

94. E.S. Gil, and S.H. Hudson, *Progress in Polymer Science*, 2004, **29**, 1173.

95. H. Tian, Z. Tang, X. Zhuang, X. Chen and X. Jing, *Progress in Polymer Science*, 2012, **37**, 237.

96. E. Cabane, X. Zhang, K. Langowska, C.G. Palivan and W. Meier, *Biointerphases*, 2012, **7**, 9.

97. M.A. Ward and T.K. Georgiou, *Polymers*, 2011, **3**, 1215

98. I.Y. Galaev and B. Mattiasson, *Trends in Biotechnology*, 1999, **17**, 335

99. Z. Li, S. Cho, I.C. Kwon, M.M. Janát-Amsbury and K.M. Huh, *Carbohydrate Polymers*, 2013, **92**, 2267.

100. D. Chitkara, A. Shikanov, N. Kumar and A.J. Domb, *Macromolecular Bioscience*, 2006, **6**, 977.

101. S. Chen, H. Zhong, B. Gu, Y. Wang, X. Li, Z. Cheng, L. Zhang and C. Yao, *Materials Science and Engineering C*, 2012, **32**, 2199.

102. D. Ko, U. Shinde, B. Yeon and B. Jeong, *Progress in Polymer Science*, 2013, **38**, 672.

103. D. Schmaljohann, *Advanced Drug Delivery Reviews*, 2006, **58**, 1655.

104. E. Fleige, M.A. Quadir and R. Haag, *Advanced Drug Delivery Reviews*, 2012, **64**, 866.

105. A. Imaz, J.I. Miranda, J. Ramos and J. Forcada, *European Polymer Journal*, 2008, **44**, 4002.

106. K. Madhusudana Rao, B. Mallikarjuna, K.S.V. Krishna Rao, S. Siraj, K. Chowdoji Rao and M.C.S. Subha, *Colloids and Surfaces B: Biointerfaces*, 2013, **102**, 891.

107. S. Mendrek, A. Mendrek, H-J. Adler, A. Dworak and D. Kuckling, *Colloid and Polymer Science*, 2010, **288**, 777.

108. D.M. Devine, and C.L. Higginbotham, *European Polymer Journal*, 2005, **41**, 1272.

109. N.V. Gupta and H.G. Shivakumar, *DARU Journal of Pharmaceutical Sciences*, 2010, **18**, 200.

110. G.H. Gao, Y. Li and D.S. Lee, *Journal of Controlled Release*, 2013, **169**, 180.

111. F-J. Xu, E-T. Kang and K-G. Neoh, *Biomaterials*, 2006, **27**, 2787.

112. T. Ishida, Y. Okada, T. Kobayashi and H. Kiwada, *International Journal of Pharmaceutics*, 2006, **309**, 94.

113. N.K. Singh, Q.V. Nguyen, B.S. Kim and D.S. Lee, *Nanoscale*, 2015, **7**, 3043.

114. T.N. Vo, A.K. Ekenseair, F.K. Kasper and A.G. Mikos, *Biomacromolecules*, 2014, **15**, 132.

115. S. Maya, B. Sarmento, A. Nair, N.S. Rejinold, S.V. Nair and R. Jayakumar, *Current Pharmaceutical Design*, 2013, **19**, 7203.

116. J. Gan, X. Guan, J. Zheng, H. Guo, K. Wu, L. Liang and M. Lu, *RSC Advances*, 2016, **6**, 32967.

117. D. Kokuryo, S. Nakashima, F. Ozaki, E. Yuba, K.-H. Chuang, S. Aoshima, Y. Ishizaka, T. Saga, K. Kono and I. Aoki, *Nanomedicine: Nanotechnology, Biology and Medicine*, 2015, **11**, 229.

118. N.K. Singh, and D.S. Lee, *Journal of Controlled Release*, 2014, **193**, 214.

119. K. Kono, T. Ozawa, T. Yoshida, F. Ozaki, Y. Ishizaka, K. Maruyama, C. Kojima, A. Harada and S. Aoshima, *Biomaterials*, 2010, **31**, 7096.

120. N.A. Cortez-Lemus and A. Licea-Claverie, *Progress in Polymer Science*, 2015, **53**, 1.

121. M. Prabaharan, J.J. Grailer, D.A. Steeber and S. Gong, *Macromolecular Bioscience*, 2009, **9**, 744.

122. G. Venkatareddy, M. Subha, C. Rao, B. Yerriswamy, L.N. Reddy and V. Prasad, *Asian Journal of Pharmaceutics*, 2010, **4**, 200.

123. M. Prabaharan, J.J. Grailer, D.A. Steeber and S. Gong, *Macromolecular Bioscience*, 2008, **8**, 843.

124. I.S. Cho, C.G. Park, B.K. Huh, M.O. Cho, Z. Khatun, Z. Li, S-W. Kang, Y. Bin Choy and K.M. Huh, *Acta Biomaterialia*, 2016, **39**, 124.