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Review

Biodegradable injectable in situ forming drug delivery systems

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Abstract

The ability to inject a drug incorporated into a polymer to a localized site and have the polymer form a semi-solid drug depot has a number of advantages. Among these advantages is ease of application and localized, prolonged drug delivery. For these reasons a large number of in situ setting polymeric delivery systems have been developed and investigated for use in delivering a wide variety of drugs. In this article we introduce the various strategies that have been used to prepare in situ setting systems, and outline their advantages and disadvantages as localized drug delivery systems. © 2002 Published by Elsevier Science B.V.

Keywords: Drug delivery; In situ setting polymers; Thermoresponsive polymers; Thermoplastics; Thermosets; Solvent precipitation; Ionotropic gelation; Photocrosslinking gelation

1. Introduction

The development of new injectable drug delivery systems has received considerable attention over the past few years [1–3]. This interest has been sparked by the advantages these delivery systems possess, which include ease of application, localized delivery for a site-specific action [4–6], prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort. Initial studies examined delivery systems such as emulsions [7–9], liposomes [10–13], biodegradable microspheres [14–16] and micelles [17,18]. Although these formulations have demonstrated some success in certain applications, there still is room for improvement.

Emulsions are used extensively in parenteral products but usually not in long acting formulations because of the stability problems accompanying this dosage form. The possibility of dispersion breakdown or dissolution in the surrounding body fluid has made emulsions a poor choice for long acting formulations [19]. Liposomes are not a promising dosage form for long acting formulations as well. Local retention of liposome-entrapped drugs is likely to be longer than that of free drugs, but it may not always be long enough to maintain local therapeutic drug levels, due in part to rapid clearance by macrophages and other cells [20,21]. Other problems, such as stability issues, sterilization problems and often-low drug entrapment, have played an important role in limiting the utility of liposomes [22]. Microspheres are easy to deliver to the site of

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action but they have several inherent disadvantages. These include the need for reconstitution before injection, a relatively complicated manufacturing procedure to produce a sterile, stable, and reproducible product, and the possibility of microsphere migration from the site of injection [23–27]. Micelles, which are also prone to migration, suffer from the fact that there are a large number of variables, which influence micelle properties. Controlling factors like core block length and corona outer shell length, which significantly influence drug loading and size distribution, at the same time is almost impossible. Furthermore, the stability of micelles is highly dependent on their critical micelle concentration (CMC), which is the minimum polymer concentration required for micelle formation. The lower the value of the CMC, the greater the thermodynamic stability of micelles in dilute solutions. Once diluted below the CMC, micelles begin to spontaneously disassemble into single chains [27,28]. Therefore, dilution upon injection, as well as interaction with lipid components in the blood, may result in dose dumping.

With these shortcomings in mind, injectable, in situ setting semi-solid drug depots are being developed as alternative delivery systems. These implant systems are made of biodegradable products, which can be injected via a syringe into the body and once injected, solidify to form a semi-solid depot [29–31]. Our goal in this paper is to outline the different strategies used to prepare in situ setting drug depots and comment on their advantages and disadvantages. In this review, semi-solid biodegradable injectable implant systems are divided into four categories based on the mechanism of achieving solidification in vivo: (1) thermoplastic pastes, (2) in situ crosslinked systems, (3) in situ precipitation, and (4) in situ solidifying organogels. The coverage of the literature is not encyclopedic; rather, a few select examples have been chosen to highlight certain points. The discussion will emphasize some of the practical issues, problems and unique challenges that are associated with these injectable implant systems.

2. Thermoplastic pastes

Thermoplastic pastes are polymer systems, which are injected into the body as a melt and form a semi-solid upon cooling to body temperature. They are characterized as having a low melting point, ranging from 25 to 65 °C, and an intrinsic viscosity from 0.05 to 0.8 dl/g, measured at 25 °C [32]. It has been reported that an intrinsic viscosity below 0.05 dl/g may fail to significantly impart a delayed release profile to a drug, and a carrier copolymer with an intrinsic viscosity above 0.8 dl/g may be too viscous to be easily administered through a needle [33]. The facile injectability of these systems, when heated slightly above their melting point, is due to their low molecular weight and low T_g (glass transition temperature). These polymeric systems flow easily when pushed or stretched by a load, usually at elevated temperatures. They mostly hold their shape at room temperature and can be formed into different shapes by applying heat [34].

Bioerodible thermoplastic pastes could be prepared from such monomers as D,L-lactide, glycolide, ϵ-caprolactone, trimethylene carbonate, dioxanone and ortho esters [33,35]. Polymers and copolymers of these monomers have been extensively used in a number of biomedical areas, from carriers of pharmaceutical compounds [36] to surgical sutures [37], ocular implants [38], soft tissue repair [39,40] and augmentation materials [41,42]. They therefore have a demonstrated track record of biocompatibility and thus are attractive starting points for new material development. Specific examples of the use of these materials are given in the following paragraphs.

Walter et al., placed a Taxol™-loaded poly [bis(*p*-carboxyphenoxy)propane-sebacic acid] implant beside brain tumors or within tumor resection sites and demonstrated the effectiveness of the method in rats after surgery [43]. In an effort to develop a means of avoiding surgery and circumventing the invasiveness of Walter's method, Zhang et al., developed a thermoplastic triblock polymer system composed of poly(D,L-lactide)–*block*–poly(ethylene glycol)–*block*–poly(D,L-lactide) and blends of low-molecular-weight poly(D,L-lactide) and poly(ϵ-caprolactone) (PCL) for the local delivery of Taxol™ [44]. Both polymeric systems were capable of releasing Taxol™ for a long period of time (greater than 60 days) (Fig. 1), albeit at a very low rate. The advantages of using this system over systemic administration of Taxol™ are reduced side effects due to the local delivery of

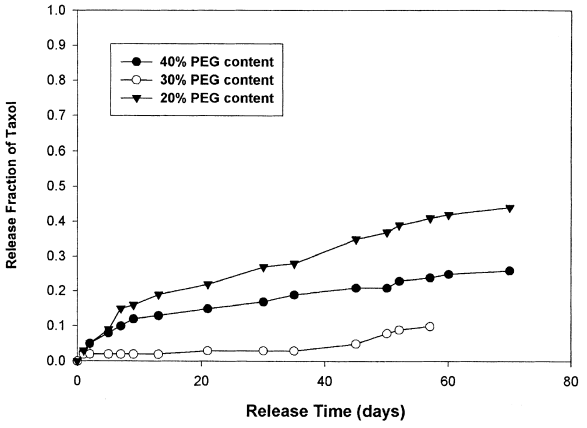

Fig. 1. Cumulative release of taxol from PDLLA–PEG–PDLLA cylinders into phosphate-buffered saline plus albumin at 37 °C. Reproduced from Ref. [44].

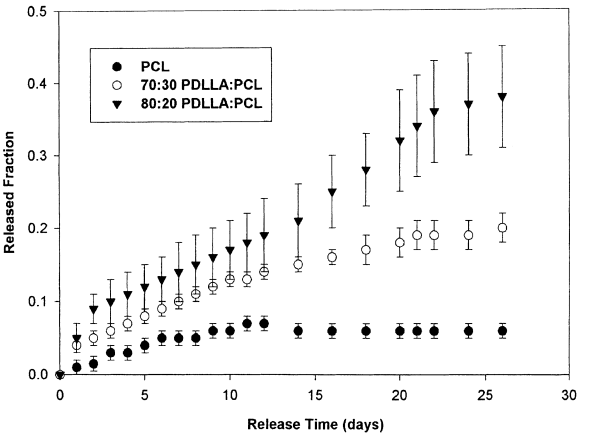

Fig. 2. Cumulative release of taxol from 20% taxol loaded PDLLA:PCL blends and PCL into PBS albumin buffer at 37 °C. Reproduced from Ref. [44].

Taxol™ to the tumor site. There are some noteworthy disadvantages associated with this polymeric system. The melting points of these polymeric pastes were greater than 60 °C, therefore the temperature of the paste at the time of injection was at least 60 °C. This temperature can be very painful for a patient and increases the chance of necrosis and scar tissue formation at the site of injection [45]. The second disadvantage is the very slow rate of drug release (40% drug mass released after 60 days when the block copolymer was used and 35% drug mass released after 30 days when the blend of PDLLA and PCL were used) (Fig. 2). This slow rate of release, which had a significant impact on the efficacy of the polymeric paste formulation to inhibit the tumor growth, may be due to the high molecular weight of PCL, the high degree of crystallinity in the synthesized polymer (PCL) or the affinity of the drug for the polymer versus the aqueous phase.

Dordunoo et al., attempted to overcome the problem of slow Taxol™ release by using poly(ϵ-caprolactone) of molecular weight 10–20 kDa as a polymeric paste [46]. To enhance Taxol release, they examined the effect on the rate of drug release of water-soluble additives such as gelatin, albumin, methylcellulose, dextran and sodium chloride. Cylindrical pellets of Taxol™-loaded paste were prepared by melt extrusion and suspended in phosphate-buffered saline (PBS) for in vitro studies. Addition of the water-soluble additives significantly improved the rate of drug release, especially when gelatin or albumin was used. The authors suggested that the enhanced rate of drug release in the case of gelatin or albumin emanated from the fact that these two additives are water swellable as well as soluble. The swelling of such additives inside the polymeric paste, increased the water imbibitions into the polymer and hence, produced a higher rate of drug dissolution and release. An alternative explanation is that the enhanced release may not have anything to do with swelling, but may be a result of increased Taxol™ solubility in the release medium due to its binding to proteins. Taxol™ has low water solubility (0.2 μg/ml), and the addition of proteins such as albumin can increase the water solubility of Taxol™ up to 3.7 μg/ml [46]. In in vivo studies, pastes containing Taxol™-gelatin particles were prepared and heated to 60 °C and then extruded at the tumor site of eight DBA/2j female mice. Tumor mass was reduced 63±27% with respect to controls. This system has some limiting disadvantages, however. Low-molecular-weight poly(ϵ-caprolactone) has a degree of crystallinity of between 50 and 70% [47]. This high degree of crystallinity would impede the diffusion of the drug within the cylinder. Further, the paste was heated to 60 °C to bring it into a molten state and make it injectable through a needle. This temperature can invoke scar tissue formation, which in turn can encapsulate the polymeric paste and inhibit the diffusion of Taxol™ to the surrounding tumor cells.

In another approach Winternitz et al., added methoxy(polyethylene glycol) (MePEG) in amounts up to 30% to the poly(ϵ-caprolactone) paste, which brought down the melting point from 55 to around 50 °C and increased the crystallinity of the polymer from 42 to 51% [48]. Taxol showed a biphasic in vitro release profile composed of a burst phase during the first couple of days followed by a much slower release rate (Fig. 3). The addition of MePEG increased the amount of water taken up by the polymer blends but decreased the rate of Taxol™ release. This was attributed to an increased degree of crystallinity of PCL resulting in a polymer, which degrades more slowly, and a decreased Taxol™ diffusion coefficient due to the increased tortuosity of the diffusional pathway [48]. Nevertheless, this delivery system, with slight changes with respect to polymer composition, was tested in human prostate LNCaP tumors grown subcutaneous in castrated athymic male mice and promising results were obtained [49].

Thermoplastic injectable implants have even been used for delivery of pharmaceutically active agents into the eye [50]. An injectable implant system was developed by Davis et al., made of copolymers of PCL and poly(ethylene glycol) (PEG) which was capable of being injected through a 25 gauge needle when heated to 50 °C. This invention avoids the hazards of eye surgery to insert the drug delivery device and also avoids possible intraocular chemical reactions. The only problem that this system bears is the temperature of the paste at the time of injection (50 °C), which appears to be too high for the eye environment. It is claimed that in vivo compatibility and degradation life times in the eye can be ascertained by injecting the sterilized paste into both the anterior chamber and vitreous cavity of laboratory rabbits' eyes. Absence of experimental data or examples, regarding the in vivo compatibility of this system, emphasizes the necessity for further studies.

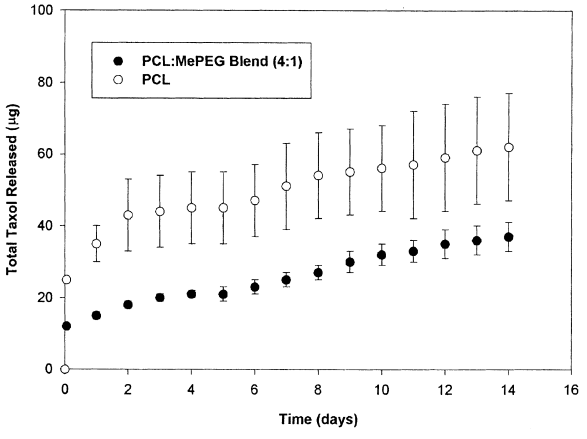

Fig. 3. Taxol release profiles. PCL and PCL:MePEG blend at 30% taxol loading. Reproduced from Ref. [48].

3. In situ crosslinked systems

Crosslinked polymer networks can be formed in situ in a variety of ways, forming solid polymer systems or gels. Means of accomplishing this end include free radical reactions initiated by heat (thermosets) or absorption of photons, or ionic interactions between small cations and polymer anions.

3.1. Thermosets

Thermoset polymers can flow and be molded when initially constituted, but after heating, they set into their final shape. This process is often called ''curing'' and involves the formation of covalent crosslinks between polymer chains to form a macromolecular network. Reheating a cured polymer only degrades the polymer [34]. This curing is usually initiated chemically upon addition of heat. In two US patents, Dunn et al. introduced the application of thermoset systems [39,40]. Unfortunately, there have not been many articles written regarding the application of chemically initiated thermoset systems for the delivery of pharmaceutically active agents into the body. This may be due to the limitations and adverse effects associated with it [51]. In particular, the reaction conditions for in vivo applications are quite stringent, including a narrow range of physiologically acceptable temperatures, requirement for nontoxic monomers and/or solvents, moist and oxygen-rich environments, the need for rapid processing, and clinically suitable rates of polymerization [52]. In this part of the review, the characteristics of thermoset systems and the application of photoinitiated thermoset systems will be investigated.

Dunn et al., used biodegradable copolymers of D,L-lactide or L-lactide with ϵ-caprolactone to prepare

a thermosetting system for prosthetic implants and slow release drug delivery systems. This system is liquid outside the body and is capable of being injected via a syringe and needle and once inside the body, it cures [53]. The multifunctional polymers in their thermosetting system were first synthesized via copolymerization of either D,L-lactide or L-lactide with ϵ-caprolactone using a multifunctional polyol initiator and a catalyst (e.g., peroxides) to form polyol terminated liquid prepolymers. This prepolymer was then converted to an acrylic ester-terminated prepolymer. Curing the liquid acrylic-terminated pre-polymer is initiated by the addition of either benzoyl peroxide or *N,N*-dimethyl-*p*-toluidine, prior to injection into the body. After introduction of the initiator, the polymer system is injected and polymer solidification occurs. The estimated time of reaction is between 5 to 30 min [54].

The advantage of using this system is its facile syringeability. There are a couple of disadvantages accompanied with this system, which have limited its application. When a bioactive agent (e.g., flurbiprofen) was incorporated into this system, a burst in drug release during the first hour was observed (Fig. 4). This burst was due to the lag time for solidification of the polymer. While the cross-linking reaction inside the body is in process and the polymer is in liquid form, the drug can diffuse out of the system more rapidly, thereby causing the burst. This high concentration of drug at the site of reaction may result in the appearance of side effects of the drug. Furthermore, the heat released upon curing (up to 94 °C have been reported for poly(methyl methacrylate) used as a prosthetic bone cement [57]) due to the exothermic nature of the crosslinking reaction, can cause necrosis to the surrounding tissues [51,55–57]. Finally, introduction of free radical producing agents such as benzoyl peroxide into the body may induce tumor promotion [58–60].

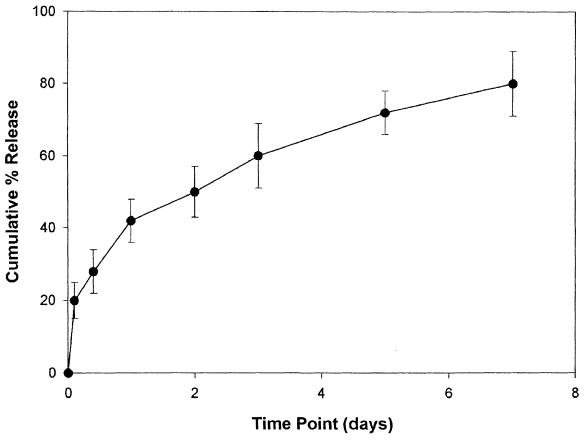

Fig. 4. In vitro drug release from formulations containing 5% flurbiprofen in PBS at 37 °C. Reproduced from Ref. [54].

3.2. Photocrosslinked gels

Photopolymerizable, degradable biomaterials would provide many advantages over chemically initiated thermoset systems. In this approach, prepolymers are introduced to the desired site via injection and photocured in situ with fiber optic cables [52]. This approach has many advantages. Photoinitiated reactions provide rapid polymerization rates at physiological temperatures. Further, because the initial materials are liquid solutions or moldable putties, the systems are easily placed in complex shaped volumes and subsequently reacted to form a polymer of exactly the required dimensions. These characteristics have encouraged the investigation of using this system for tissue engineering [56], orthopedic applications [61], cell transplantation [62], local drug delivery [63,64], dentistry [65,66], and tissue adhesion prevention [67,68].

Hubbell et al., described a photopolymerizable biodegradable hydrogel as a tissue contacting material and controlled release carrier [69]. This system consisted of a macromer with at least two free radical-polymerizable regions (PEG-oligoglycolyl-acrylates), a photosensitive initiator (eosin dye) and a light source (ultraviolet or visible light). By exposing the mixture of macromers and photoinitiator to the light source, the macromer undergoes rapid cross-linking and forms a network. These networks can be used to entrap water-soluble drugs and enzymes and deliver them at a controlled rate. Use of an argon laser as a light source offers a greater depth and degree of polymerization, less time is required and an enhancement of the physical properties of the polymer is realized. These advantages are offset by reports that the increased polymerization caused by the laser results in increased shrinkage and brittleness of the polymer [70].

As an example of the drug delivery capabilities of this approach, the delivery of various proteins from a photopolymerized PEG–PLA hydrogel is illustrated in Fig. 5 [71]. Release of the proteins from these hydrogels was relatively rapid, with completion achieved within 5 days. The release rate was dependent on protein molecular weight, decreasing as molecular weight increased. The release was diffusionally controlled for molecules below a critical molecular weight. For the larger immunoglobulin G, release required the degradation of the hydrogel structure to afford larger openings within the gel to allow for diffusion. Thus, to achieve prolonged release, this delivery system is best suited for large drug molecules.

3.3. Ion-mediated gelation

Alginates are natural polymers, which have been widely investigated for drug delivery [72]. Alginates form a gel upon contact with divalent cations such as calcium ions. They can be used directly as a drug carrier or as a carrier of another delivery system such as liposomes [73]. Liposomes are capable of increasing the local retention of liposome-entrapped drugs over that of free drugs. However, site retention may not always be long enough to maintain local therapeutic levels, due in part to rapid clearance of the liposomal vesicles by macrophages. In order to overcome this problem Cui et al. [73] used thermally sensitive Ca-loaded vesicles, capable of releasing Ca^{2+} when heated to body temperature, along with sodium alginate to form a fluid suspension that gels at 37 °C. 1,2-bis(palmitoyl)-glycero-3-phosphocoline (DPPC) and 1,2-bis(myristoyl)-glycero-3-phophocoline (DMPC) were used to prepare both Ca-loaded and drug loaded phospholipid vesicles. The molar ratio of DPPC:DMPC was adjusted to 9:1 to bring the melting point of the liposomes below body temperature. It is well known that the permeability of the phospholipid bilayers is strongly temperature dependent [74,75]. At temperatures below the lipid chain melting transition, phospholipid bilayers are relatively impermeable to multivalent ions. However, phospholipid permeability has been shown to be several orders of magnitude higher at the melting temperature [74]. The addition of drug-filled liposomes to the formulation resulted in a hydrogel that released entrapped drug (metronidazole) in a controlled manner. Drug release was characterized by a rapid burst-type release followed by a slower controlled release of drug from the hydrogel matrix. Metronidazole was released more rapidly from the 15% DMPC liposome than from the pure DPPC liposome due to the difference in bilayer permeability of the two compositions at the experimental temperature (37 °C) (Fig. 6). This approach clearly improved the half-life of the liposomes and proved to be advantageous for certain local delivery applica-

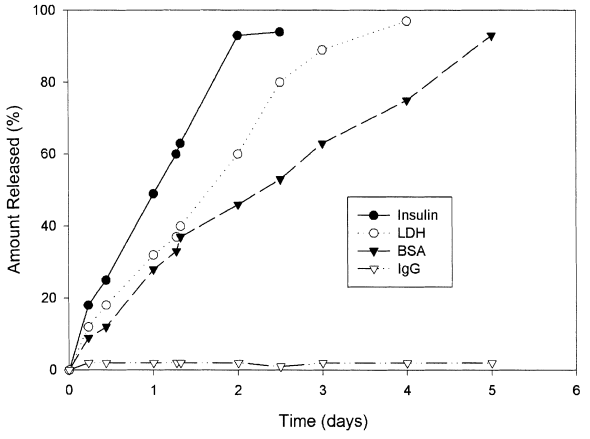

Fig. 5. Protein release from photocrosslinked biodegradable hydrogel. Reproduced from Ref. [71].

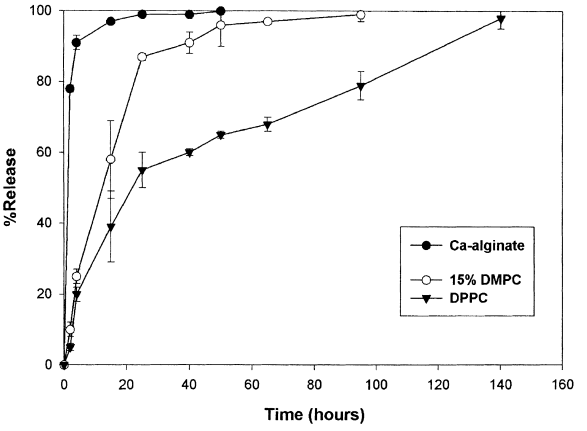

Fig. 6. Release of metronidazole from thermally gelled liposome/alginate hydrogels. Reproduced from Ref. [73].

tions in which in situ gelation is required [76,77]. The disadvantages of using this system are a short shelf life due to the slow leakage of Ca^{2+} from the liposomes and a large amount of drug released in the initial release burst.

Recently, Westhaus and Messersmith [78], introduced thermally triggered Ca^{2+} release from liposomes to form calcium alginate hydrogels, and a protein-based system in which triggered release of calcium activates transglutaminase enzyme-catalyzed cross-linking of proteins. The fundamentals of this system are the same as the Cui et al. hydrogel system mentioned above and has the same problem of calcium leakage out of the liposomes and hence, the same problem with a short shelf life.

Alginate has been used for ophthalmic drug delivery as well [79]. The human eye has enough calcium ions to induce alginate gelation. In a study by Cohen et al. [79], it was demonstrated that an aqueous solution of sodium alginate could gel in the eye without the addition of external calcium ions or other bivalent/polyvalent cations. The concentration of $CaCl_2$ in human eye is 0.008% (w/v). Using this calcium to cause gelation of an alginate–pilocarpine solution; pilocarpine was delivered to the eye in a sustained fashion. Unfortunately, this method of delivery is restricted to the eye due to the lack of calcium concentration in other tissues.

Despite these applications, there are two important factors, which have limited the use of calcium alginate for drug delivery purposes. The first factor is their potential immunogenicity and the second is the long time required for their in vivo degradation [80,81]. For example, cytotoxicity and the non-biodegradable nature of calcium alginate wound dressings induce a chronic foreign-body reaction [80].

4. In situ polymer precipitation

Another strategy that has been utilized to produce an injectable drug delivery depot is the phenomenon of polymer precipitation from solution. This precipitation can be induced by solvent-removal [82,83], a change in temperature [84,85], or a change in pH [86,87].

4.1. Solvent-removal precipitation

Dunn et al., introduced an in vivo setting system made of biodegradable polymers [88], which has been used for human as well as veterinary purposes [89–94]. This injectable implant system is comprised of a water insoluble biodegradable polymer, such as poly(DL-lactide), poly(DL-lactide-co-glycolide) and poly(DL-lactide-co-ϵ-caprolactone), dissolved in a water miscible, physiologically compatible solvent. Upon injection into an aqueous environment, the solvent diffuses into the surrounding aqueous environment while water diffuses into the polymer matrix. Since the polymer is water insoluble, it precipitates upon contact with the water and results in a solid polymeric implant. Solvents which have been used in this approach include *N*-methyl-2-pyrrolidone (NMP), propylene glycol, acetone, dimethyl sulfoxide (DMSO), tetrahydrofuran, 2-pyrrolidone, and triacetin, but the most preferred are NMP and DMSO because of their pharmaceutical precedence [95]. Due to the number of disadvantages inherent in this system, it has not been extensively investigated or endorsed by fellow pharmaceutical scientists.

One of the problems is the possibility of a burst in drug release especially during the first few hours after injection into the body. Since this injectable implant system is administered as a liquid, it is reasonable to assume that there is a lag between the injection and the formation of the solid implant. During this lag time the initial burst of drug may exceed the plasma concentration achieved using conventional implant systems. This initial burst of drug has been linked to tissue irritation and sometimes to systemic toxicity. Due to this unwanted phenomenon, the use of this system has been limited only to drugs with a narrow therapeutic index.

In order to control the burst effect four factors have been examined: the concentration of polymer in the solvent [96], the molecular weight of the polymer [29], the solvent used [29,82,97], and the addition of a surfactant [98]. All of these parameters influence the rate of precipitation of the polymer. For example, Lambert and Peck examined the influence of solvent, polymer molecular weight, and polymer concentration on FITC-bovine serum albumin release from poly(D,L-lactide-co-glycolide) (PLG) precipitated from solution as spheres [29]. They found that, for a

high PLG (75–115 000 Da) molecular weight, the higher the polymer loading in the solvent (10 to 20%), the smaller the burst effect, and the higher the capability of solvent to dissolve the polymeric system the greater the burst effect (Fig. 7A). By using a lower molecular weight PLG (10–15 000 Da), much greater concentrations (33.5 to 40%) of polymer in solution were obtainable, and as a consequence, the initial burst of drug released was eliminated (Fig. 7B). The effect of solvent choice, however, was not as clearly defined. For the high molecular weight polymer case, DMSO provided the greatest burst effect at a given polymer concentration, while the opposite effect was observed for the low molecular weight polymer. Finally, Chandrashekar et al., have reported that the addition of a diblock of PLG–PEG of relatively low molecular weight (5000 Da) was effective in reducing the burst of small drug molecules. For example, the initial burst (% released after 24 h) of leuprolide acetate in PLG dissolved in DMSO (50% PLG, 50% DMSO) was reduced from 50% to 34% of the initially loaded concentration when injected subcutaneously into rats [98] with the incorporation of 10% PLG–PEG in place of 10% of the PLG. Similar results were reported for floxuridine, lidocaine, and lidocaine HCl. The reduction in the burst effect was most notable for the water soluble lidocaine HCl, whose initial burst decreased from 82% to 30%. While the reductions are significant, there still remains a large burst effect with this approach.

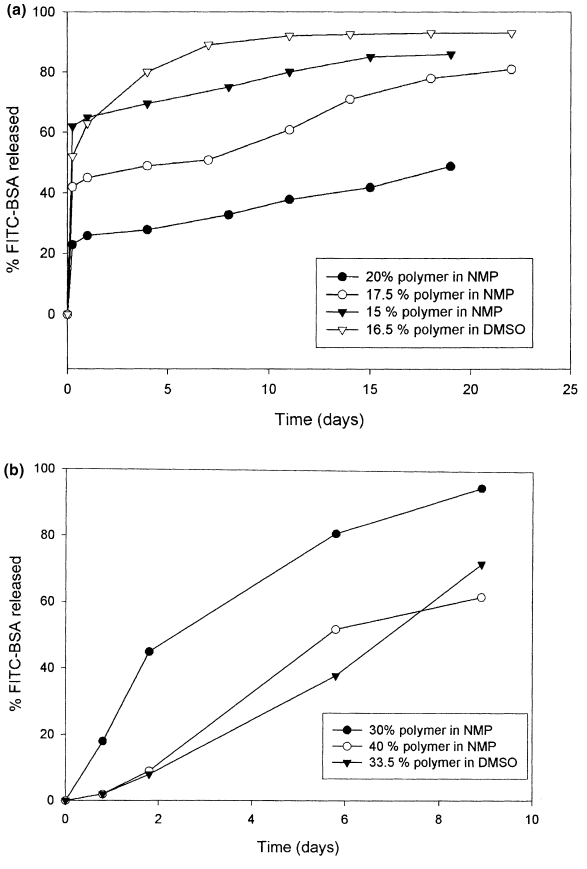

Fig. 7. (A) Release of FTIC-BSA from a high-molecular-weight 50:50 PLGA polymer precipitation system in phosphate-buffered saline. Reproduced from Ref. [29]. (B) Release of FITC-BSA from a low-molecular-weight 50:50 PLGA polymer precipitation system in PBS. Reproduced from Ref. [29].

Another problem with this system is the use of DMSO and NMP, which are highly controversial solvents. There are extensive toxicity data for oral, intraperitoneal and intravenous administration of these solvents, but not for subcutaneous or intramuscular use [99]. In a recent study [100], poly(lactide) or PLG was dissolved in NMP, DMSO or 2-pyrrolidone and injected intramuscularly into Sprague–Dawley rats. It was shown that these solvents are myotoxic and can damage muscles. Chandrashekar and Udupa [101] and Singh et al. [102], tried propylene glycol and triacetin, respectively. However, triacetin suffers from the same problems of NMP and DMSO and propylene glycol is not recommended due to its hemolytic potential [103,104]. Eliaz et al., utilized glycofurol as a solvent to deliver soluble necrosis factor receptor from an in situ forming PLGA implants in vivo [105]. Glycofurol is another solvent, which has been used in parenteral products [106]. Unfortunately, little toxicological data are available in the literature [107]. The only data uncovered were intravenous LD_{50} values of 3.8 g/kg in the mouse [108] and 2.0–4.3 g/kg in the rat [109].

Poly(acrylic acid) and its derivatives have also been examined as precipitating polymers. Haglund et al. [110], investigated the use of poly(ethylene glycol) and poly(methacrylic acid) or poly(acrylic acid) as an injectable drug delivery system. Albumin-FITC and pheniramine were chosen as high- and low-molecular-weight model drugs, respectively.

Since the system is poorly soluble in an aqueous environment, at least 50% ethanol (preferably 60–80%) was added to keep the solution clear. After injection into the body, the ethanol diffuses out and water diffuses into the system, causing the dissolved polymeric network to collapse and precipitate. A similar approach was investigated by Ismail et al. [111], who examined water-soluble polymers such as hydroxypropylmethylcellulose (HPMC)–carbopol system and polymethacrylic acid (PMA)–PEG for plasmid DNA delivery. Carbopol is a pH dependent polymer, which forms a low viscosity gel in alkaline environment (e.g., pH 7.4) and stays in solution in acidic pH. The addition of HPMC, a viscosity inducing agent, to carbopol reduces the carbopol concentration and hence the solution acidity while preserving the viscosity of the in situ gelling system. This system gels upon an increase in pH when injected. The second appraised system consisted of a mixture of PMA and PEG dissolved in NMP–ethanol–buffer (1:1:2). Although the conformational analysis of the released pDNA from these systems showed no sign of degradation in vitro, it was shown that the physical stability of pDNA was compromised (conversion from super coil to open circular form). Both systems were capable of releasing the pDNA but with a 35–70% drug burst in the first couple of hours followed by little to no subsequent release. The lack of significant release beyond the burst phase indicates a strong interaction between the pDNA and the polymers used, which holds a portion of the pDNA within the polymer matrix. The collapse of the polymer network due to the removal of solvent (ethanol) causes a significant change in the volume of the system, which in turn causes the rapid outburst of the drug, especially when the drug is soluble in alcohol. Moreover, the outburst of the alcoholic solvent at the site of injection is likely to cause tissue irritation. Additionally, the in vitro release profiles of Ismail et al. was compared by using a two-way analysis of variance (ANOVA). It is noteworthy that the use of two-way ANOVA to compare dissolution profiles is not recommended by the US Food and Drug Administration (FDA). This is due to the fact that in dissolution profile data all the time points are dependent on each other and this violates the ANOVA assumption of independence between dissolution time points, and the time effect, which is not of interest, consumes too many degrees of freedom in the analysis [112].

In situ forming injectable microspheres is a different approach introduced by Jain et al. [113]. This system is comprised of a stable dispersion of PLGA–solvent solution microglobules (premicrospheres) dispersed in a continuous oil phase. Upon injection into the body, water penetration into the system while the PLGA solvent diffuses out hardens the microglobules into solid microspheres. To prepare this system PLGA was first dissolved in triacetin and a solution of PEG 400 and the drug (e.g., myoglobin M_w=16 950 or cytochrome c M_w=12 327) was added into followed by an addition of Tween 80. This mixture, called oil phase I, was added into a mixture of Span 80 and Miglyol 812 (oil phase II) dropwise with continuous homogenization to form the premicrospheres. Drug entrapment efficiency was reported to be between 60 and 90% by varying the PEG 400 and drug concentration in the formulation. The effects of using different vehicles were investigated and compared (triacetin with triethyl citrate and miglyol 812 with soybean oil). It was shown that there is no effect in myoglobin release profile when the different vehicles were used. Overall, both systems were able to release the drugs in a sustained fashion for 15 days with a burst in the drug release of between 30 and 50% during the first day. This was attributed to the free and unencapsulated drug present in the vehicle. The molecular weight of the drug had an impact on the in vitro release rate, and circular dichroism (CD) spectroscopy showed no sign of physical instability in the proteins' native conformation. Although the system is readily injectable, the same can be said about the injection of drug-loaded PLGA hardened microspheres in a suitable vehicle, which would not have the disadvantage of the presence of an undesirable solvent phase.

These examples illustrate that the burst effect obtained depends on the nature of the drug incorporated [82]. For hydrophobic drugs, the burst effect depends on the affinity of the drug for the solvent–water phase versus the solvent–polymer phase. If the drug has a higher affinity for the solvent–water phase, which initially surrounds the device, then a high burst effect will be observed. For hydrophilic drugs, such as FITC-bovine serum albumin, which

are injected as suspensions in the polymer–solvent solution, the burst effect is determined by the number of drug particles which reside at the implant surface during polymer precipitation. This is affected by the viscosity of the solution, which governs particle settling, and the degree of mixing of the solution. Viscosity increases as polymer molecular weight and concentration increase, for a given solvent. Viscosity is also influenced by the polymer–solvent interaction. The greater the affinity of the polymer for the solvent, the greater the expansion of the polymer chain in solution and thus the greater the number of polymer chain entanglements which produces a more viscous solution. The burst effect is thus a complex situation and difficult to predict a priori.

A non-polymeric approach using the same precipitation strategy has also been developed. Smith and Tipton [114] introduced sucrose acetate isobutyrate (SAIB), a noncrystalline, viscous compound, which dissolves in solvents such as DMSO and ethanol, to form a solution having the same viscosity of vegetable oil (50–200 cP). SAIB is reported to be bioerodible and essentially insoluble in water. Upon injection of SAIB dissolved in, for example, ethanol at 50% concentration, into tissue, the solution increases dramatically as the solvent diffuses away. The SAIB then forms a depot, which sustains the release of the drug. Drugs that have been examined for release by this system include gonadotropin hormone [115], chlorhexidrine, doxycycline, diclofenac, flurbiprofen, naproxen, and theophylline [116]. Although sustained release is achieved, the same problems inherent with the polymeric systems exist with this system, i.e., high initial burst, relatively rapid release rates, and use of controversial solvents. Furthermore, although SAIB has been approved as a food additive, it has not received approval as a parenteral compound.

4.2. Thermally-induced sol–gel transitions

Many polymers undergo abrupt changes in solubility in response to changes in environmental temperature [117–119]. This physical characteristic has been employed to form drug depots by using polymer systems, which undergo a sol–gel transition upon injection into the body.

Poly(*N*-isopropyl acrylamide) [poly(NIPAAM)] is an example of a thermosensitive polymer. It exhibits the phenomenon of lower critical solution temperature (LCST) phase separation. Reviews of poly-NIPAAM and its gel applications are numerous [120–122]. PolyNIPAAM shows a very well defined LCST at about 32 °C, which can be shifted to body temperature by formulating polyNIPAAM based gels with salts and surfactants [123,124]. Although numerous poly(*N*-alkylacrylamides) and polymers possess LCSTs [125,126], polyNIPAAM is unique with respect to the sharpness of its almost discontinuous transition, which is usually observed only with ionizable polymers [127]. These features make poly(NIPAAM) an interesting potential material for use in in situ setting dug delivery. However, acrylamide based polymers with quaternary ammonium in their structure, in general, are not suitable for implantation purposes due to cell toxicity [128]. The observation that acrylamide-based polymers activate platelets on contact with blood [129], along with the poorly understood metabolism of polyNIPAAM and its non-degradability [130], make it difficult to win FDA approval. Therefore, the vast majority of the drug delivery systems which employ LCST, use block copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) simply because of FDA approval [131].

Triblock PEO–PPO–PEO copolymers (Pluronics®, or Poloxamers®) are available in a variety of lengths and are of particular interest, as their gelation phenomena have been extensively studied [132–134]. The properties of some Pluronic copolymers frequently used in drug delivery studies are collected in Table 1 [135]. It is significant to note that, although most of the Pluronics listed in Table 1 have a LCST well above normal body temperature, they do exhibit gelation at body temperature in concentrated solutions [136]. However, application of concentrated polymer solutions (>16 wt.%) in drug delivery may be disadvantageous as it changes the osmolality of the formulation, kinetics of gelation, and causes discomfort in ophthalmic applications due to vision blurring and crusting [137]. Since F127 has been reported to be the least toxic of the commercially available Pluronics® [138], it has been used most extensively in drug delivery studies. One other reason for the popularity of Pluronics® is its inhib-

Table 1
Properties of the Pluronic copolymers

Copolymer[a]	Composition	Average M_w	M_{ppo}	PEO (wt.%)	CP[b] (°C)	HLB[c]
L64	$EO_{13}PO_{30}EO_{13}$	2900	1740	50	58	12–18
F68	$EO_{76}PO_{29}EO_{76}$	8400	1680	80	>100	>24
F88	$EO_{103}PO_{39}EO_{103}$	11 400	2280	80	>100	>24
P103	$EO_{17}PO_{60}EO_{17}$	4950	3465	30	86	7–12
P104	$EO_{27}PO_{61}EO_{27}$	5900	3540	40	81	12–18
P105	$EO_{37}PO_{56}EO_{37}$	6500	3250	50	91	12–18
P108	$EO_{132}PO_{30}EO_{132}$	14 600	2920	80	>100	>24
F127	$EO_{100}PO_{65}EO_{100}$	12 600	3780	70	>100	18–23
L122	$EO_{12}PO_{67}EO_{12}$	5000	3600	20	19	1–7

Reproduced from Ref. [135].
[a] L, F and P indicate liquid, flakes and paste, respectively.
[b] Cloud point in aqueous 1 wt.% solution.
[c] Hydrophilic–lipophilic balance.

itory effect on P-glycoprotein [139,140]. Certain Pluronics®, e.g., P85, strikingly increase the cytotoxicity of drugs such as daunorubicin, against multidrug cell overexpressing P-glycoprotein [141]. It appears that unimers of Pluronics are able to inhibit P-glycoprotein. The mechanism of inhibition is unclear, but it may be related to the changes at a membrane level induced by Pluronics. This may inhibit P-glycoprotein or enhance cellular uptake of drugs [142].

Veyries et al. [143], demonstrated the possibility of controlled release of vancomycin from Pluronnic® F127. They investigated Poloxamer® 407 (Pluronic® F127) 25% formulations aimed at prolonging the residence time of vancomycin, a time dependent antibiotic, in a body site with a high infectious risk. It appeared that neither the rheological properties of the Poloxamer® matrices nor the antibacterial activity of vancomycin was altered by their combination. Two formulations were prepared, one saturated and one unsaturated (solubilized) with vancomycin. In vitro, the dispersed form (saturated) exhibited prolonged release, with a lower diffusion coefficient of vancomycin compared to the solubilized form (4.7×10^{-8} vs. 2.1×10^{-7} cm^2 s^{-1}) (Fig. 8). In rats, a single dose was well tolerated and resulted in a high local concentration for 24 h (>131 mg/l), followed by lower but effective antibacterial levels for at least 8 days. Based on the release profiles, good preservation of vancomycin activity, good tolerability in rats, and ease of administration, it was concluded that Poloxamer® 407 might be useful as a vancomycin delivery vehicle for local prophylaxis of infections, especially in prosthetic surgery. In another study by Miyazaki et al., the antitumor effect of Pluronic® F-127 containing mitomycin C (MMC) on sarcoma-180 ascites tumor mice was evaluated [144]. The Pluronic® F-127 gels were evaluated as a sustained release vehicle for intraperitoneal administration of MMC in order to enhance the therapeutic effects of MMC. Tumor cell injections were made on day 0 and injections of MMC in 25% (w/w) Pluronic® F-127 on day 1, both intraperitoneally. A prolongation of the life span of tumor-bearing mice following injection of therapeutic Pluronic® F-127 was noted, and Pluronic® F-127 containing MMC was therapeutically more active than free drug. The in vitro

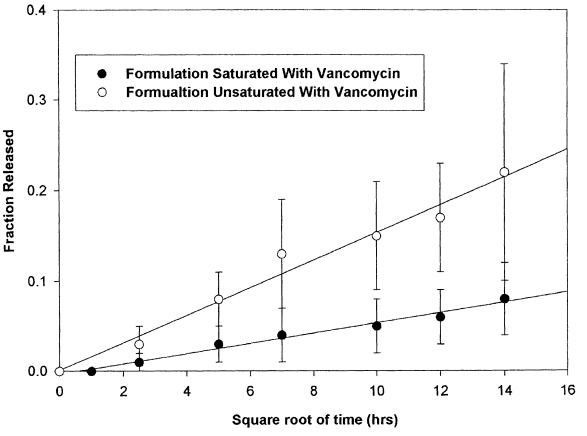

Fig. 8. In vitro vancomycin release from poloxamer 407 gels at 37 °C. Reproduced from Ref. [140].

release experiments indicated that Pluronic® gel might serve as rate-controlling barrier and be useful as a vehicle for sustained release preparations of MMC to be administered intraperitoneally (Fig. 9).

As discussed previously, Pluronic® concentrations above 16 wt.% may show toxicity particularly when intended for intraperitoneal administration. This problem led the Miyazaki's group to study the potential of natural polymers as vehicles for drug delivery. Xyloglucan polysaccharide derived from tamarind seeds is composed of a (1–4)-β-D-glucan backbone chain, which has (1–6)-α-D-xylose branches that are partially substituted by (1–2)-β-D-galactoxylose. When partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation in dilute aqueous solutions [145]. An important difference between the gelation properties of the xyloglucan gels and block copolymers such as Pluronic® F-127 from a toxicity viewpoint is that xyloglucan forms gels at much lower concentrations. In an in vitro study, the cumulative release of MMC from an aqueous solution of concentration 0.025% (w/v) and from xyloglucan gels with gel concentrations of 0.5, 1 and 1.5% (w/w) was compared at 37 °C (Fig. 10). It was determined that the in vitro release of MMC from xyloglucan gels is diffusionally controlled. The figure demonstrates that the system has the potential for sustained drug release. The advantage of xyloglucan gel is its gel formation at low concentrations. However, there is no published information on their biodegradability or tissue biocompatibility, which may limit its suitability for use.

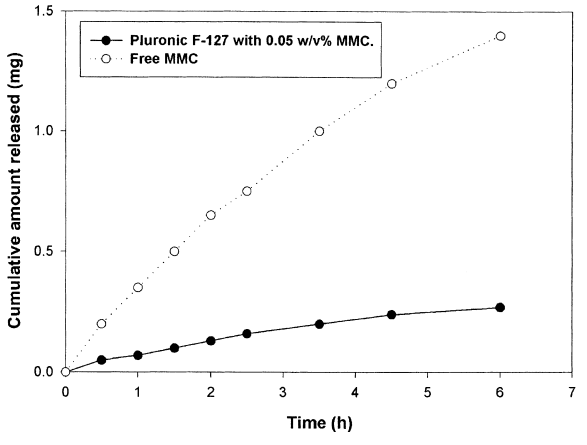

Fig. 9. Release of mitomycin C from pluronic F-127 gel at 37 °C. Reproduced from Ref. [141].

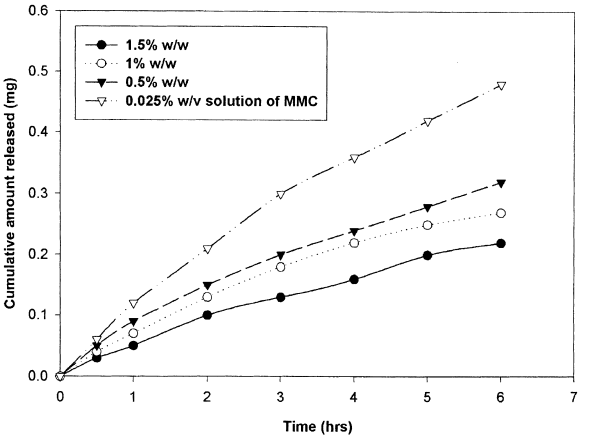

Fig. 10. Cumulative amount of mitomycin C released as a function of time at 37 °C from xyloglucan gels of concentrations 0.5, 1 and 1.5% containing an initial concentration of 0.025% (w/v) and from 0.025% (w/v) solution of mitomycin C. Reproduced from Ref. [142].

Besides the two examples given above, Poloxamer® 407 has been studied in a series of papers for controlled delivery of low- and high-molecular-weight bioactive agents such as melanotan-I [146], lidocaine [147], ibuprofen [148], pilocarpine [149] and interleukin-2 [150]. One important drawback of Poloxamer® 407 is hypertriglyceridemia induction following intraperitoneal injection. Johnston and co-workers in two studies [151,152], demonstrated that Poloxamer® 407 injected into rats by intraperitoneal injection resulted in sustained hypercholesterolemia and hypertriglyceridemia. This phenomenon was due to stimulation of 3-hydroxy-3-methylglutaryl-co-enzyme A (HMG-CoA) reductase activity in the liver by the Poloxamer® vehicle. Thus, elevated levels of plasma cholesterol and triglycerides resulting from the chronic administration of Poloxamer® containing drug formulations to patients may potentially hinder therapeutic outcome.

Jeong et al. in 1997 [130,153], reported a hydrogel consisting blocks of poly(ethylene oxide) and poly(L-lactic acid). Aqueous solutions of these copolymers exhibited temperature-dependent reversible gel–sol transitions. The advantage of this system over sol-

vent-removal gelation is the absence of any organic solvent. Unfortunately, this system can only be loaded with bioactive molecules in an aqueous phase at an elevated temperature (around 45 °C), where it forms a sol. This loading procedure limits the nature of the drugs that can be incorporated in the delivery system to those that are not prone to hydrolysis. Moreover, this temperature can cause protein denaturation.

Jeong et al. [154], also designed another thermoresponsive hydrogel made of PEG–PLGA–PEG triblock copolymers. This type of polymer mixture demonstrates phase separation behavior as the temperature increases above the LCST. The polymer is in sol form at room temperature and once inside the body, it turns into a gel and forms a viscous polymer solution. No organic solvent is required for this system but it bears the problem common to sol–gel systems, which is the high initial burst effect. When a polymer system goes through the sol–gel process, it shrinks and its volume changes dramatically. This phenomenon can exude significant amounts of the encapsulated bioactive agent out of the hydrogel and create an initial burst.

Chenite et al., developed a novel hydrogel system composed of neutral solutions of chitosan [155]. Chitosan is obtained by alkaline deacetylation of chitin, a natural component of shrimp or crab shells. Chitosan is a biocompatible pH-dependent cationic polymer, which remains dissolved in aqueous solution up to a pH of 6.2. Neutralization of chitosan aqueous solutions to a pH exceeding 6.2 leads to the formation of a hydrated gel-like precipitate. In this study, pH-gelling cationic polysaccharide solutions were transformed into thermally sensitive pH-dependent gel-forming aqueous solutions, without any chemical modification or crosslinking. This was done by addition of polyol salts bearing a single anionic head, such as glycerol-, sorbitol-, fructose- or glucose-phosphate salts to chitosan aqueous solutions. This system was examined for delivery of biologically active growth factors in vivo as well as encapsulation of living chondrocytes for tissue engineering. Although this transformation has solved the non-degradability problem of chitosan and can be considered as an advantage for this system, there is a lack of data presented regarding the volume change of the hydrogel and release profile data for the growth factor. Therefore, its suitability as a drug delivery vehicle requires further examination.

Other thermally sensitive polymer systems have also been developed. For example, the concept of stereocomplex formation was exploited recently to form a novel hydrogel, based on self-assembling of entantiomeric lactic acid oligomers grafted to dextran [156]. L- and D-lactic acid oligomers were coupled to dextran, yielding dex-(L)lactate and dex-(D)lactate, respectively. Upon dissolving each product in water separately and mixing the solutions, a hydrogel formed at room temperature. Although no drug delivery applications have been demonstrated to date, this approach can be manipulated for delivering pharmaceutically active agents into the body without the need for crosslinking agents and organic solvents. In two reports, one published in *Science* (1998) [157] and the other in *Nature* (1999) [158], protein domains were used to form hydrogels. Petka et al., used recombinant DNA methods to create artificial proteins that undergo reversible gelation in response to changes in pH or temperature [157]. The proteins consisted of terminal leucine zipper domains flanking a central, flexible, water-soluble polyelectrolyte segment. Formation of coiled-coil aggregates of the terminal domains in near-neutral aqueous solutions triggers formation of a three-dimensional polymer network. Dissociation of the coiled-coil aggregates through elevation of pH or temperature causes dissolution of the gel and showing viscous behavior. Wang et al., reported a hybrid hydrogel system assembled from water-soluble synthetic polymers and a well-defined protein-folding motif, the coiled coil [158]. These hydrogels undergo temperature-induced collapse owing to the cooperative conformational transition of the coiled-coil protein domain. Such new systems are still in the development stage and need more experimental studies.

5. In situ solidifying organogels

Organogels or oleaginous gels are composed of water-insoluble amphiphilic lipids, which swell in water and form various types of lyotropic liquid crystals. The nature of the liquid crystalline phase formed depends on the structural properties of the lipid, temperature, nature of the drug incorporated,

and the amount of water in the system [159]. The amphiphilic lipids examined to date for drug delivery are primarily glycerol esters of fatty acids, for example glycerol monooleate, glycerol monopalmitostearate, and glycerol monolinoleate which are waxes at room temperature. These compounds form a cubic liquid crystal phase upon injection [159] into an aqueous medium. The cubic phase consists of a three-dimensional lipid bilayer separated by water channels. This liquid crystalline structure is gel-like and highly viscous.

This gel forming nature has been used to form drug depot systems for the delivery of both water soluble and water insoluble drugs. For example, Ericsson et al. [160] used a glycerol monooleate system to deliver somatostatin subcutaneously in rabbits while Yim et al. developed a formulation for interferon-α composed primarily of aluminum monostearate and peanut oil [19], and Gao and co-workers [161,162] demonstrated the use of a glycerol palmitostearate (Precirol) system to deliver the lipophilic drugs levonorgestrel and ethinyl estradiol. The equilibrium water content of the organogel formed is typically approximately 35%, which therefore produces relatively short release duration for hydrophilic drugs. For the somatostatin example given above, somatostatin release lasted for only 6 h. Much more sustained release can be achieved using a lipophilic drug. In the work of Gao and co-workers [161,162], in vitro release of levonorgestrel was observed for up to 14 days [162], while in vivo studies of levonorgestrel in the organogel injected subcutaneously into rabbits demonstrated an estrus blockage for up to 40 days [161].

Although they can be formulated with a low concentration of water, the viscosity of the system is reduced by mixing with vegetable oils. Reducing the viscosity in this manner eases injectability and increases the release duration, particularly for lipophilic drugs. For example, Gao et al. found that incorporating glycolyzed apricot kernel oil (Labrafil 1944 CS) reduced the in vitro release rate of levonorgestrel, from 36.2 to 19.9 $\mu g/cm^2$ at day 14 for 0 and 20% oil incorporation, respectively [162]. Lipophilic drug release from these organogels is also dependent upon the solubility of the drug in the cubic phase. If the drug concentration exceeds its solubility in the cubic phase then drug particles will form. The presence of these solid particles has been demonstrated to produce zero-order release kinetics, with a rate which increases as particle size decreases [162].

Another advantage of these systems is that they are biodegradable. Biodegradation occurs through the action of lipases [163] and for the glycerol palmitostearate/Labrafil 1944 CS system, requires between 5 to 6 weeks [161]. An inflammatory reaction was observed for this system, which lasted for 7 days and then dissipated.

Organogels thus are a promising injectable delivery system for lipophilic compounds. There are some disadvantages inherent to this approach. Purity of waxes and stability of oils are the major issues that need to be addressed. There are number of waxes such as carnauba wax, wool wax, spermaceti wax and esparto wax, used for cosmetic purposes but not for parenteral applications. Only beeswax is readily available in various purification grades. Oils usually need a stabilizer, antioxidant and preservative to increase their shelf life and stability. Moreover, the difference between the melting point of waxes and oils makes this system susceptible to phase separation. Labrafil and Precirol are a mixture of many different vegetable oils and glyceryl esters of fatty acids, respectively. Unfortunately, there is still concern over the purity and lack of toxicity data for these waxes and oils. Another drawback is the need to apply heat to mix the oil and wax phase. Temperatures of up to 60 °C for 30 min have been used [161,164]. Temperatures this high can easily reduce the potency of some drugs.

6. Summary

We have reviewed a large cross-section of biodegradable in situ forming drug delivery systems for local delivery of drugs (Table 2). These delivery systems have unique challenges associated with their development that are related to drug stability, drug release kinetics and the conditions under which the system is delivered to the body. The continuous advances in biotechnology and drug development will produce more pharmaceutically active agents that will be difficult to administer by conventional

Table 2
Biodegradable in situ solid forming delivery systems

Delivery system	Common problems	Common components
Thermoplastic pastes	High temperature at the time of injection	PLA, PLGA & PCL. Alcohols as initiator.
In situ crosslinked systems		Stannous Octoate as catalyst.
Thermosets	•Unacceptable level of heat released during reaction •Burst in drug release •Toxicity of un-reacted monomers	Oligomers of PLA, PDLLA & PCL, Polyols as initiator and peroxides as curing agent.
Photocrosslinked gels	•Shrinkage and brittleness of the polymer due to high degree of crosslinking	PGA, PLA, PCL & PEG, initiators such as eosin dye, light source (e.g., UV or laser)
Ion mediated gelation	•Low shelf life •Burst in drug release •Long degradation time	alginate with Ca^{2+} as gelling agent
In situ polymer precipitation	•Burst in drug release	PDLLA, PCL & PLA. Solvents such as DMSO or NMP.
Solvent-removal	•Burst in drug release	
Precipitation	•Application of organic solvents	
Thermally induced sol–gel transition	•Stability of oils and purity of waxes	NIPAAM, PEG, PLA, PLGA & Chitosan, Pluronics
Organogels	•Lack of toxicity data •Phase separation	Oils such as peanut oil & Labrafil, waxes (e.g., Beeswax & Pericerol).

means, and an increased demand for controlled or site-specific delivery system is anticipated.

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