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Review

PLGA *in situ* implants formed by phase inversion: Critical physicochemical parameters to modulate drug release

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ABSTRACT

In situ forming implants (ISI) based on phase separation by solvent exchange represent an attractive alternative to conventional preformed implants and microparticles for parenteral applications. They are indeed easier to manufacture and their administration does not require surgery, therefore improving patient compliance. They consist of polymeric solutions precipitating at the site of injection and thus forming a drug eluting depot. Drug release from ISI is typically divided into three phases: burst during precipitation of the depot, diffusion of drug through the polymeric matrix and finally drug release by system degradation. This review gives a comprehensive overview on (i) the theoretical bases of these three phases, (ii) the parameters influencing them and (iii) the remaining drawbacks which have to be addressed to enlarge their commercial opportunities. Indeed, although some of them are already commercialized, ISI still suffer from limitations: mainly lack of reproducibility in depot shape, burst during solidification and potential toxicity. Nevertheless, depending on the targeted therapeutic application, these shortcomings may be transformed into advantages. As a result, keys are given in order to tailor these formulations in view of the desired application so that ISI could gain further clinical importance in the following years.

Contents

Abbreviations: BA, benzyl alcohol; BB, benzyl benzoate; DMSO, dimethyl sulfoxide; EA, ethyl acetate; ISI, *in situ* forming implants; ISM, *in situ* forming microparticles; GA, glycolide; GRAS, generally recognized as safe; IIG, inactive ingredient; LA, lactide; LD50, lethal dose 50; NMP, *N*-methyl-2-pyrrolidone; PEO, poly(ethylene oxide); PDLA, poly(D,L-lactide); PGA, poly(glycolide); PLA, poly(lactide); PLGA, poly(lactide-*co*-glycolide); PLLA, poly(L-lactide); PPO, poly(propylene oxide); PVP, polyvinylpyrrolidone; TA, triacetin; 2P, 2-pyrrolidone.

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1. Introduction

Despite the fact that oral route ought to be considered as highly desirable by the patients it still represents a huge challenge in many applications. Indeed, an increasing number of new active pharmaceutical ingredients belong to the family of peptides or proteins and suffer from low bioavailability after oral administration. Alternative routes of administration (pulmonary, nasal, buccal, transdermal, ocular, and rectal) have also shown drawbacks such as enzymatic degradation or low/variable absorption [1]. As a result, there is a renewed interest in parenteral administration, especially as many improvements have been done in pain reduction. Nevertheless, many drugs are characterized not only by a high activity but also by a short half-live. In this context, sustained release forms are highly desirable to avoid continuous infusions or frequent injections. They display several advantages, such as enhanced patient compliance and the avoidance of peaks and valleys in plasma concentrations, thus allowing a reduction of the total dose and minimizing potential side effects.

The development of new injectable drug delivery systems has received considerable attention over the past few years, and many systems have been developed: *e.g.* microparticles, nanoparticles, liposomes and micelles [2–4]. Besides, significant advances have been made in the field of implants and microparticles. Implants classically formed by melt extrusion must be implanted into the patients, either surgically or through large diameter needles, and surgically retrieved after use unless they are biodegradable. Microparticles can be injected through smaller needles causing less pain to the patient, but the multi-step production processes are costly, the scale-up more difficult to achieve and the encapsulation efficiencies often low [5]. Therefore new alternatives have been studied to allow simple and painless administration as well as easy manufacturing. One of these alternatives is the development of smart systems, especially *in situ* forming depots, which can be injected into the body in liquid form and then solidify *in vivo* at the place of injection.

In situ forming systems have been commonly classified according to their mechanism of formation as: (i) *in situ* solidifying organogels; (ii) *in situ* cross-linking systems and (iii) *in situ* precipitating systems. Previous papers offered comprehensive reviews of all these *in situ* forming systems [5–8]. By contrast, this review will exclusively focus on the *in situ* forming implants (ISI) based on poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA), which precipitate following phase separation triggered by solvent/non-solvent exchange. Among the first been described, these systems have been used for the delivery of various drugs. As they can be easily modulated, cover a wide range of release periods and are based on biodegradable polymers with excellent biocompatibility approved for parenteral administration, they represent a valuable strategy for controlled drug release applications.

The concept of *in situ* forming implants was first introduced by Dunn et al. in the 90's [9,10]. A water-insoluble biodegradable polymer is dissolved in a pharmaceutically acceptable organic solvent, miscible or partially miscible with water. Drug is added to this polymeric solution to form either a solution or a suspension. Following injection into an aqueous medium, a phase separation occurs as the solvent diffuses towards the surrounding aqueous environment while water/body fluids penetrate into the organic phase. This results in polymer precipitation and formation of a depot entrapping the drug at the injection site.

Two products have been commercialized, both using *N*-methyl-2-pyrrolidone (NMP) as solvent and PLA or PLGA as biodegradable biocompatible water-insoluble polymers. On the one hand, Eligard© (Sanofi) contains leuprolide acetate (a luteinizing hormone-releasing hormone agonist) and is injected subcutaneously at 1 to 6-month intervals for the treatment of advanced prostate cancer [11]. On the other hand, Atridox© (Tolmar Inc.) offers a local sustained release of doxycycline over a week after direct injection into the periodontal pocket. It is indicated in the treatment of chronic periodontitis for both human and veterinary purposes [12].

In view of the above, many other PLA- or PLGA-based ISI have been developed for local or systemic delivery of drugs (including peptides, proteins and nucleic acids), covering a wide range of therapeutics, which shows the potential of *in situ* implants in a broad range of indications (Table 1). Furthermore, some tissue engineering applications have been explored [12,13]. Drug release from these systems is generally characterized by an initial burst during the solidification of the matrix, followed by a second period mainly controlled by diffusion processes. Finally, subsequent drug release is driven by the polymeric carrier degradation and erosion [14]. Matrix formation, drug diffusion, matrix degradation and therefore drug release could be modulated varying several parameters. This review contains the theoretical basis of drug release from *in situ* forming PLGA implants, the parameters influencing the drug release characteristics as well as a critical discussion of potential toxicity issues and industrialization challenges. Finally, a decision-scheme is proposed to tailor the formulation depending on the drug and the targeted clinical application.

2. *In situ* implants — fate and drug release kinetics

This chapter reviews the theoretical basis of the solidification process of the *in situ* forming implants, the degradation of the polymeric matrix and the mechanisms of drug release.

2.1. Matrix solidification phase

2.1.1. Phase inversion dynamics

Contact of *in situ* forming PLGA implants into an aqueous medium or body fluids triggers a phase inversion process in the polymer solutions, which finally results in polymer precipitation. Nevertheless, the formation of the final solid/semi-solid depot is not instantaneous but depends on the kinetics of the phase inversion.

The dynamics of non-solvent induced phase inversion have been extensively studied with polymer-membranes designed for purification applications and the underlying thermodynamics and mass-transfer laws as well as corresponding morphologies of the membranes have been elucidated [40]. After immersion of the polymeric solution into an aqueous medium, an exchange between polymer solvent and non-solvent (*i.e.* water or body fluids) occurs. Induced by this diffusion process the polymer solution turns into a thermodynamically metastable or unstable state. Driven by a decrease of the free energy of the system the homogeneous solution can be separated into two phases of different compositions — a polymer-lean and a polymer-rich phase. Ternary phase diagrams with the composition in solvent, non-solvent and polymer represent a useful tool to follow or predict the phase transitions which occur after injection of the polymeric solution into aqueous environments (Fig. 1A). The phase diagrams visualize compositions were the polymer solution consists of a single homogeneous phase as well as an area representing the liquid–liquid demixing gap delimited by the binodal curve. This demixing gap is again divided into a domain delimited by the spinodal curve representing unstable compositions (Fig. 1A: area II) as well as a domain between the spinodal and the binodal curves representing metastable compositions (Fig. 1A: areas I and III). Demixing of the latter compositions occurs according to a binodal decomposition through the generation and growth of stable nuclei of a polymer-lean phase at higher polymer concentrations

Table 1
Results published on PLA/PLGA-based ISI categorized for indications.

Molecule	Polymer	Solvent	Main outcomes	Ref
Cancer therapy				
Cisplatin	PLGA	NMP	*In vivo* sustained delivery (rats) for 7 days Increased maximum tolerated dose and tumor suppression effect (mice) *vs.* free cisplatin	[15]
	PLGA	DMSO	*In vivo* sustained release (dogs) but lack of effect (inactivation of cisplatin bioactivity by interaction with DMSO)	[16]
Fenretinide	PLGA	NMP	*In vitro* 1-month sustained release	[17]
Hormonal therapy				
Human growth hormone	PLGA	BB	Sustained serum levels for 28 days (rats)	[18]
Levonorgestrel	PLA	BB/BA	Zero-order drug release *in vitro* lasting on 90 days	[19]
Testosterone				[20]
Calcitonin				[21]
Immunomodulation				
Betamethasone	PLGA	NMP	*In vitro* releases from sterilized formulations from 24 to 90 days, depending on the PLGA composition	[22]
Thymosin-1-alpha	PLGA	NMP (± TA)	Significant increases of thymic and spleen indexes (immunosuppressive mice)	[23]
Soluble tumor necrosis factor (TNFα) receptor	PLGA	Glycofurol	*In vitro*: burst (<20%) then continuous release (20 days) *In vivo*: long-lasting protection against pathological effects of TNFα (mice)	[24]
Anti-infectious therapy				
Ivermectine	PLA	NMP, 2P, TA, BB	*In vitro* release rates (96 days) with speeds ranked in the order NMP > 2P > TA > BB	[25]
Secnidazole doxycycline	PLA PLGA	NMP	*In vitro* release (3 days) with high bursts (>30%, desired) and suitable antimicrobial activity	[26]
Tinidazole	PLA	NMP	Significant decrease in periodontitis symptoms (dogs, 7-day local delivery)	[27]
HIV-fusion inhibitor	PLGA	DMSO/TA	Drug plasma concentration in the therapeutic range up to 48 h (rats)	[28]
Analgesia/anesthesia				
Aspirin	PLGA	NMP	7-day controlled release *in vitro* Faster polymer degradation with aspirin	[29]
Ketoprofen	PLGA	NMP	Effective plasma levels maintained about 8 weeks (rats)	[30]
Bupivacaine	PLGA	2P	Reduction of plasmatic concentration (systemic side effects) while local analgesic effect was maintained for 6 h (rats)	[31]
Neurological disorders				
Haloperidol	PLGA	NMP	20–30 days of *in vivo* release (rats)	[32]
Risperidone	PLGA	BB/BA	Prolonged mean residence time: 32.6 h *vs.* 5.8 h for risperidone solution (rabbits)	[33]
Risperidone	PLGA	DMSO	3-week sustained release (dogs)	[34]
Paliperidone			Sustained suppressive effect of psychotic behavior during 38 days (mice)	
Naltrexone	PLGA	NMP	30-day sustained release *in vitro* (burst >40%)	[35]
Metabolic disorders				
Rosiglitazone	PLGA	NMP, TA	Sustained *in vitro* release up to 8 days with lower burst for TA (<20%) than NMP (20–60%)	[36]
Insulin	PLGA	BB/BA	Better pharmacological response during 15 days after a single injection *vs.* routine once-a-day administration of insulin (mice)	[37]
Gene delivery				
Model plasmid DNA	PLGA	Glycofurol	2-month *in vitro* release (burst <20%) with transfection activity maintained *In vivo* transfection with 10-fold higher protein expression (up to 67 days) *vs.* plasmid solution (mice)	[38]
Model plasmid DNA-containing PLGA microspheres	PLGA	Glycofurol	*In vitro* controlled release during 70 days with transfection activity maintained	[39]
Tissular reconstruction				
Bone morphogenetic proteins	PLGA	NMP	Trend without statistical increase in bone formation *vs.* blank formulations but less inflammatory response (rats)	[13]

NMP: *N*-methyl-2-pyrrolidone; DMSO: dimethylsulfoxide; BB: benzyl benzoate; BA: benzyl alcohol; TA: triacetin; 2P: 2-pyrrolidone.

(area I) or of a polymer-rich phase at lower polymer concentrations (area III), which affects the morphology of the resulting system significantly (Fig. 1B).

Binodal and spinodal curves meet at only one point, called the "critical composition" (indicated by the cross in Fig. 1A), at which transition from the homogeneous to the unstable domain and hence phase separation occurs spontaneously by spinodal decomposition resulting in a bicontinuous structure of polymer-lean and polymer-rich phase (Fig. 1B, II).

After injection of ISI formulation into aqueous medium, water concentration in the polymeric solution increases until finally the demixing gap is reached. In most cases, the phase inversion process crosses the area I, as polymer concentrations used are commonly above the critical point. Hence, droplets of polymer-lean phase form within the polymer-rich phase during the demixing process. These two liquid phases are in thermodynamic equilibrium and hence evolve in parallel until the polymer concentration of the polymer-rich phase becomes high enough to solidify the structure (Fig. 1A: solidification region) due to the continuous loss of polymer solvent to the aqueous surrounding.

Key parameters of the phase inversion dynamics of *in situ* forming systems are thus the influx of non-solvent (*i.e.* water) as well as the outflow of polymer solvent. Several scenarios are possible depending for example on whether the solvent used has high or low miscibility with the non-solvent (Fig. 2).

In case of water-miscible solvents, injection of the polymeric solution into water leads to a fast diffusion of the solvent towards the aqueous medium (*e.g.* NMP; fast inverting systems). The solution is quenched immediately and the composition path crosses the binodal curve without delay time. This triggers the formation of a solidified polymer layer at the top of the depot (Fig. 2: point D), sublayers still consisting in homogeneous polymer/solvent solution (phase A). Then, the top layer forms a barrier to the entry of water. Following the progressive

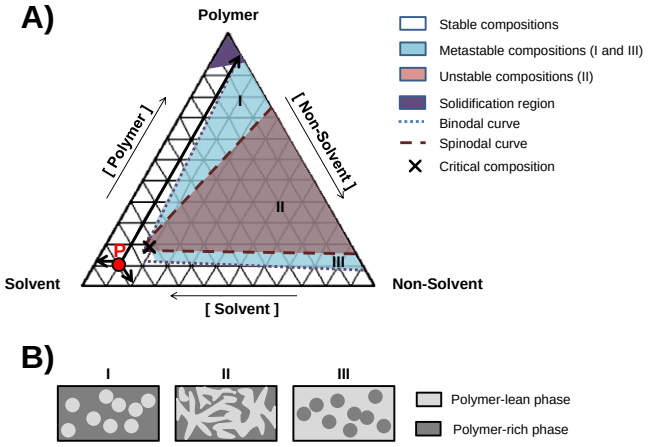

Fig. 1. Model of a ternary phase diagram of a mixture of solvent, non-solvent and polymer (A). As an example, P point corresponds approximately to 8% of polymer, 8% of non-solvent and 84% of solvent. In the areas I and III, demixing occurs through binodal decomposition, with nucleation and growth of a polymer-lean phase (area I) or a polymer-rich phase (area III) (B). Area II, area of absolute instability, direct transition leading to spontaneous formation of bicontinuous structures.

penetration of water, the sublayer composition enters progressively the metastable region leading to phase separation (phases B and C). The core of the nascent pore consists of a water-rich polymer-lean phase (C), while the surrounding is a polymer-rich phase (B). If the solvent has a high affinity for water, demixing requires low quantities of water. The resulting structure is a thin top layer on a pore-rich region. Furthermore, solidification of the pore wall is slow because solvent diffuses rapidly and in large quantities into the nascent pore. As the water penetration front progresses, pores are supplied with both solvent and water. Pore growth is stopped by the solidification of the polymer-rich phase surrounding it (phase D). As penetration of water is fast, the polymer-rich phase part of the pore situated close to the implant surface will solidify while the part far from the surface will stay liquid longer. As a result, typical "finger-like" pore structures (Fig. 3A) are generated [40].

In case of solvents immiscible with water, liquid–liquid demixing is delayed when the formulation is injected into water, as diffusion rates are slower (*e.g.* TA; slow inverting systems). As a result, the top layer does not solidify so fast and therefore affects in a lower manner solvent and water diffusions from/into the sublayers. Consequently, the final structure is more homogeneous. Solvent exhibiting low affinity for water, nucleation starts later as it requires a higher quantity of penetrated water. Consequently, numerous nuclei are initiated at the same time and because each of them consumes solvent, the growth of each nucleus is limited by its neighbors. Moreover, solidification of the pore wall is fast because a lower proportion of solvent is present in the polymer-lean phase. In this case "sponge-like" implant morphologies (Fig. 3B) are observed [41].

The dynamics of the phase inversion can be tailored by modulating the *in situ* implant composition, *i.e.* polymer solvent and/or polymer. These changes, however, impact the initial drug release within the first day (burst) directly but also affect the following diffusion- and erosion-controlled release phases, due to effects on the matrix morphology and the degradation of the solidified matrix.

2.1.2. Drug release during matrix solidification

During the first periods after injection of ISI, drug release is essentially occurring by drug transport either through the polymer-rich or through the polymer-lean phase of the forming implants [14,41]. Although, it was recently hypothesized that convection might contribute to the initial drug release of *in situ* forming implant systems [42], a diffusion-controlled drug and solvent release is generally accepted as the applicable transport mechanisms during the solidification [41].

According to a diffusion-controlled release, the quantity of drug initially released can be attributed to physicochemical constants of the drug such as solubility, diffusivity, partition coefficient, dissociation constant and molecular weight [43,44]. The water-miscibility of the polymer solvent, however, plays a crucial role for drug release, due to its impact on the environment governing drug diffusion.

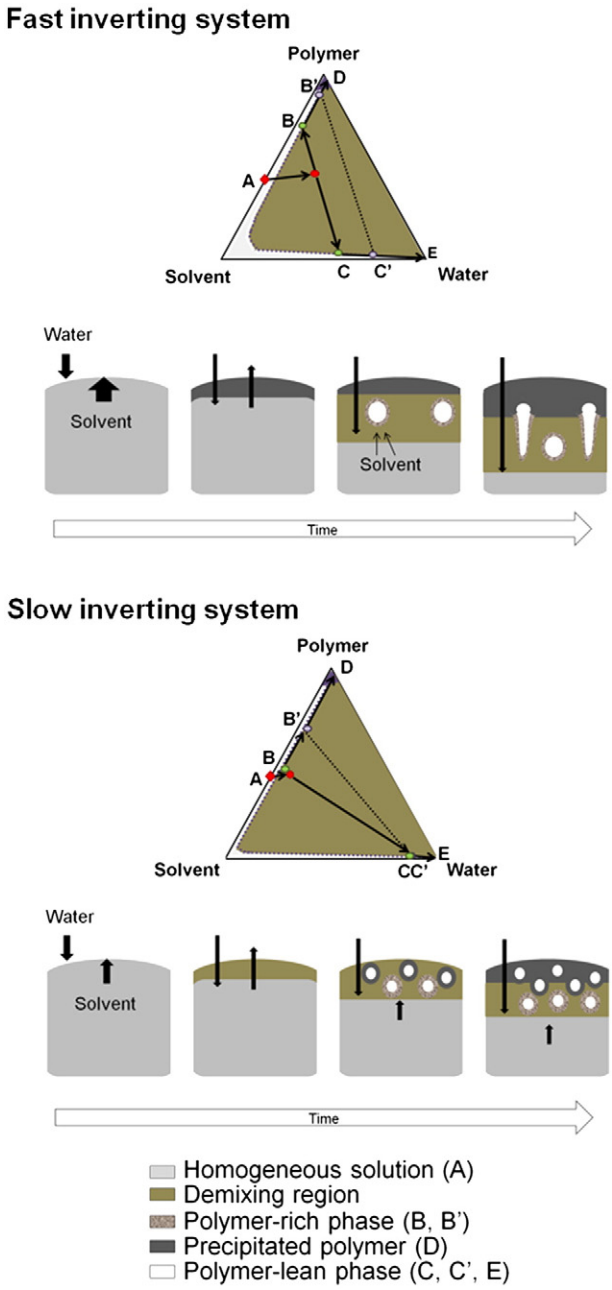

Fig. 2. Typical ternary phase diagrams and matrix structures observed with a fast inverting system (for example when solvent has high water miscibility, *e.g.* NMP) and a slow inverting system (when solvent is partially water miscible, *e.g.* TA). Dotted black lines on the phase diagram represent the tie lines between the two compositions in equilibrium. After injection, the single phase mixture A splits into two phases B and C which then evolve through B' and C' and finally to D (in the solidification region) and E. In the first case, relatively large increases in polymer concentration of polymer-rich phase will occur quickly upon the separation, as indicated by the steep slopes of tie lines. In the second case, the tie lines have lower slopes and cross the horizontal axis of the diagram at fixed and low solvent compositions (related to water solubility). Thus, a less concentrated, more fluid polymer-rich phase is generated.

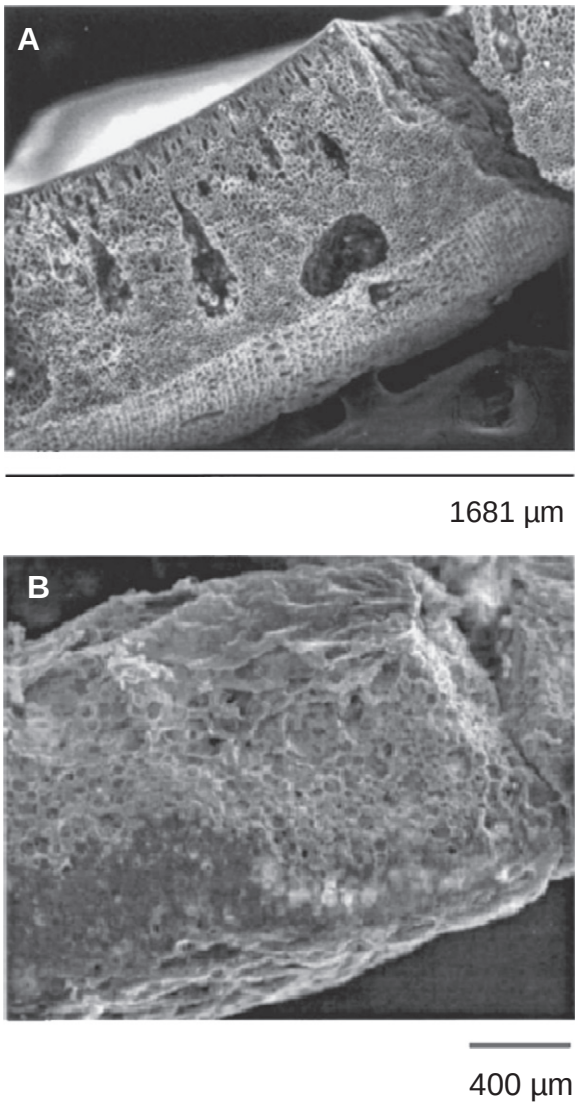

Fig. 3. Examples of "finger-like" structures (A) and "sponge-like" structure (B) in a fast and a slow inverting *in situ* formulation, respectively. Formulation: 40% m/m PLGA (50:50, M_w 10,000) in NMP (A) or TA (B). Reprinted from [41] copyright (1999), with permission from Elsevier.

Fast inverting systems based on water-miscible solvents are often characterized by a high drug burst [25]. This is usually attributed to an increased probability for the formation of an interconnected network of polymer-lean phase, representing an implant domain with high drug diffusivity (Fig. 4) [14,41]. Diffusion coefficients are considered to be around 10^{-5} cm^2/s in the polymer-lean phase of the nascent implant system (Fig. 2: phase C) and about 10^{-7}–10^{-8} cm^2/s in the corresponding polymer-rich phase (Fig. 2: phase B) as well as in the homogeneous polymer solution (Fig. 2: phase A) [45]. An interconnected network increases the chance for the incorporated drug load to have access to the surface of the solidifying matrix and hence to be rapidly released [46]. Once the porous volume is depleted and the remaining drug is entrapped, drug is released much slower by diffusion through the hardened polymeric matrix (Fig. 4).

As an example, large hydrophilic molecules like peptides and proteins are transported preferentially through the interconnected polymer-lean phase of a fast inverting system and hence released in form of a burst. A retarded diffusion is obtained, however, if these drugs are forced through the polymer-rich phase as reported for slowly inverting *in situ* implant formulation (Fig. 4) [41].

Hence, slow inverting systems are characterized by reduced bursts, because the extent of the formation of a polymer-lean phase is limited by the limited water-miscibility of the systems [14], which causes the depot to stay more or less viscous during a prolonged solidification process and produces a lower porosity in the hardening structure. As a consequence the drug diffusivity is decreased as well as the diffusion path length increased, resulting in a more gradual drug release.

Small or hydrophobic drugs, however, can efficiently diffuse through the polymer-rich and even the hardened polymer phase into the aqueous surrounding [47]. If drugs in close contact to the polymer are ionizable, polymer–drug interactions can modify the diffusion rates further [48].

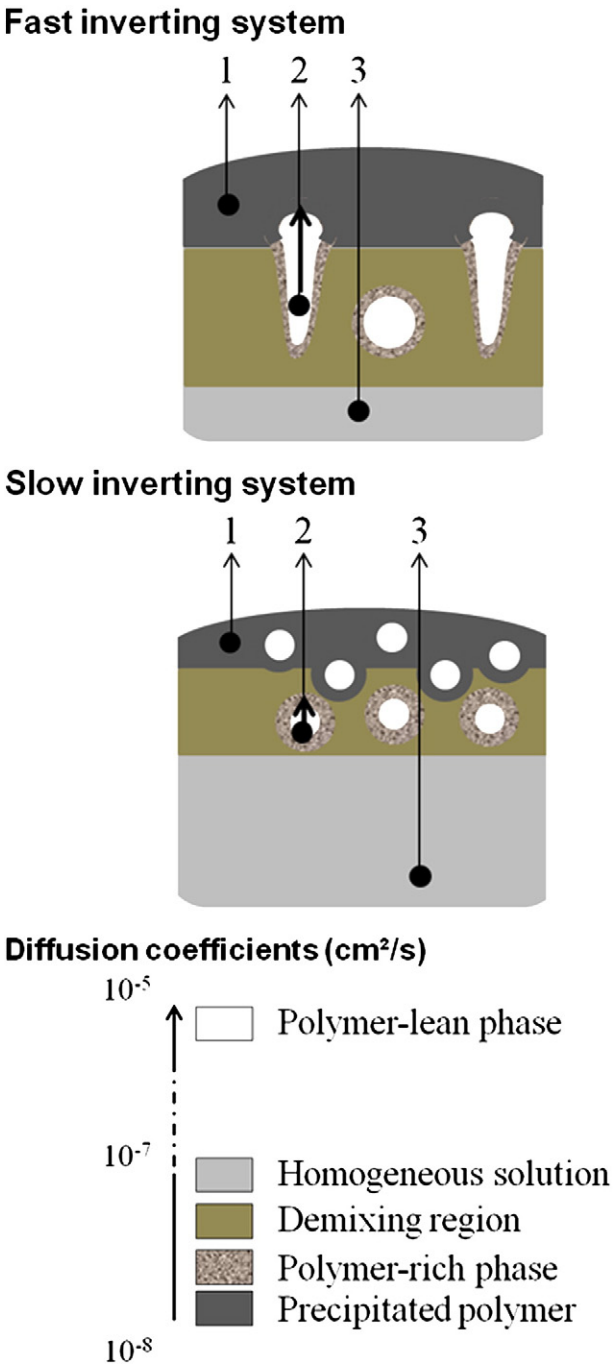

Fig. 4. Release of drugs during implant hardening in relation to their location in fast and slow inverting systems: 1. drug close to the surface = fast release, 2. drug in the polymer-lean phase = fast release, 3. drug in the polymer solution = slow release.

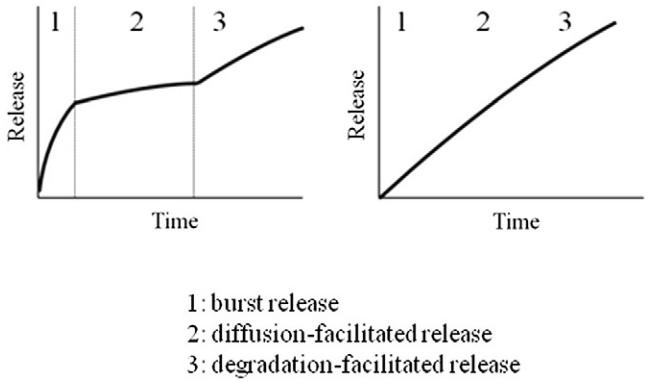

Fig. 5. Typical release profiles of fast (left) and slow (right) inverting systems.

2.2. Drug diffusion phase

Drug diffusion out of the hardened implant needs to proceed according to the same principles as applicable to conventional matrices, like implants and microparticles, consisting of drug and polymer only. According to applicable laws, drug diffusion is a function of the physico-chemical properties of the drug, the polymer as well as the matrix morphology. The main factors affecting the release can thus be derived; being the drug diffusivity within the polymer matrix, the drug solubility, drug loading, but also the matrix dimensions (*i.e.* surface area) as well as its porosity and the tortuosity of the diffusion pathway [43,49].

In a number of cases the fraction of drug released within this phase is small [17,19,20], which can result in a release plateau after the initial phase and before the erosion-controlled release phase commences (Fig. 5 left) [50].

A careful modulation of the *in situ* implant composition and hence the phase inversion dynamics, however, can lead to continuous diffusion-controlled release (Fig. 5 right) of drugs over time-frames exceeding the hardening phase of the formulations [18,21,23].

2.3. Implant erosion phase

Polyester degradation is the hydrolytic chain scission process cutting polymer chains into oligomers and finally monomers [51]. The degradation of the polyesters poly(lactide) (PLA), poly(glycolide) (PGA) and poly(lactide-*co*-glycolide) (PLGA) is the prerequisite for the erosion of the polymer, which is the loss of implant mass due to the release of water-soluble PLGA degradation products (critical molecular weight $<10^3$ g/mol [52]) from the polymer matrix. The monomeric end-products of the hydrolysis process are lactic and/or glycolic acid, which are eliminated from the body through the Krebs cycle.

Water uptake and hence degradation of the polyesters actually starts immediately after injection of the ISI formulation into aqueous medium (water or body-fluids). However, it is not before water-soluble oligomers are formed that the polymer matrix starts to erode due to the release of the mobilized molecules [53]. Additionally, these products have carboxylic chain ends which are able to autocatalyze the ester bond hydrolysis and hence lead to a faster degradation of the entire polymer matrix.

In case of the PLA, PGA and PLGA water penetration into the polymer is faster than the degradation of polymer bonds (Fig. 6). Hence, the polymer is hydrolyzed over the entire matrix leading to a homogeneous formation of degradation products and finally to bulk erosion [54]. On the contrary, if the chain scission occurs faster than diffusion of water, as for poly(ortho esters) for example [51], the hydrolysis is confined to the matrix surface and the polymer matrix undergoes surface erosion. Although these polymers could be potentially used for biodegradable *in situ* implants, they are not approved for parenteral administrations yet.

The release of the formed oligomers is not necessarily immediate as shown for large polymer matrices [55]. In thick structures lactic acid oligomers can form salts differing from the protonated acids in solubility characteristics [51]. Accumulation of degradation products in the core of such a matrix and the entering of buffer ions from the matrix surface favor a crystallization of insoluble salts in the outer shell of the polymer matrix. Besides the formation of insoluble salts the formation of stereocomplexes between poly(D-lactic acid) and poly(L-lactic acid) oligomers appears to be another reason for the insoluble residual of PL(G)A matrices undergoing "heterogeneous" bulk erosion [51].

Moreover, it was hypothesized that the pH gradient developing between the matrix surface, which is in contact with buffered medium, and an oligomers-enriched inner core results in a slower degradation of the shell *vs.* the center [56]. This, however, seems to be inconsistent with recent insights into the hydrolytic degradation of PLA oligomers: stability of oligomers is indeed nonlinearly related to the pH with a stability maximum at acidic conditions and not at neutral pH [52]. In such conditions, faster degradation in the center is probably related to the accumulation of –OH and –COOH functions in the core (by random hydrolysis) resulting in increased hydrophilicity [52].

The erosion of the polymer matrix facilitates the release of efficiently entrapped drug molecules, probably situated in the polymer-rich phase during phase inversion [14]. Erosion starts when the degradation is sufficient to result in soluble oligomers forming a porous network, which enables the release of oligomers as well as the entrapped drugs [57].

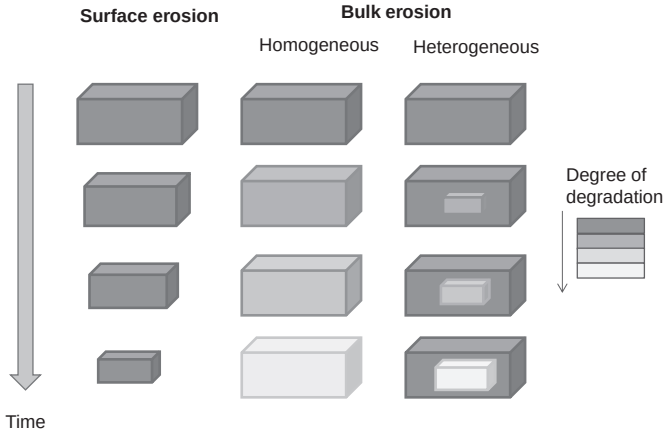

Fig. 6. Different degradation pathways of polymeric matrices.

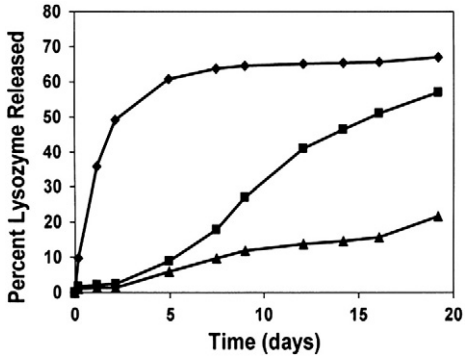

Fig. 7. Typical example of *in vitro* release profiles of a protein illustrating the impact of water-affinity of solvents on drug release, reprinted from [14] Copyright (1999), with permission from Elsevier. (♦) NMP; (■) TA; (▲) ethyl benzoate.

In summary, water uptake is the main trigger of matrix degradation and erosion, resulting in a calculable change of the polymer molecular weight. During degradation, however, physical changes can occur such as softening, pore creation/closure due to the decrease in polymer molecular weight, pH changes due to the formation of acidic degradants and crystallization. All of these events can affect the drug release during this phase making it a complex phenomenon. As for conventional biodegradable matrices, however, modulations are generally feasible by the choice of the polymer grade as well as by addition of additives like pore-formers [58], pH modifiers [59], or plasticizers [57].

It can be concluded, that the drug release from *in situ* forming implants is highly dependent on the matrix structure resulting from the phase inversion process as well as the polymer and drug properties.

3. Key parameters of *in situ* implant composition to obtain a suitable drug release

This part highlights the main aspects currently encountered in the literature. As previously seen, the matrix structure is highly dependent on the solvent used, especially on its affinity for water. Although this has been studied from the very beginning of the ISI concept [9,10], other key parameters such as polymer, drug, additives, or injection site have progressively emerged and will be discussed in this section.

3.1. Solvent

The ideal solvent or solvent blend for *in situ* systems needs to possess suitable properties in terms of water affinity, viscosity, ability to dissolve the polymer and last but not least, safety.

A low water affinity assists controlling the phase inversion/matrix formation and thus the drug burst. Reducing the affinity of solvent for water by replacing part or the totality of the solvent by a water-immiscible one slows the phase inversion rate increasing the chance for a more uniform to zero-order release pattern over an extended time-period [14,41]. This trend has been observed both *in vitro* [19,25,32] and *in vivo* [18,20,23] typically with solvents like benzyl benzoate, ethyl acetate, ethyl benzoate or TA. A good example of the influence of water-immiscible solvents is given in Fig. 7, from the study of Graham et al. [40]. In another *in vitro* study, Ahmed et al. reported haloperidol bursts from a PLGA-based ISI (50:50, M_w 60,000–70,000 g/mol; 20% w/v) of 20% with DMSO, 18% with NMP, 9% with ethyl acetate and only 7% with TA [32]. Durations of release were also affected by the solvents: being 24 and 28 days for DMSO and NMP (water-miscible) *vs.* a 60 day extended release for ethyl acetate and TA (water-immiscible).

The solvent should further have a viscosity facilitating the easy injection of the formulations (also referred to as "syringeability"). Systems based on some water-immiscible solvents, for example, could have viscosities making their own injection difficult, which could necessitate implementing a warm-up step before injection, as previously reported [20].

In addition to the viscosity of the solvent itself, syringeability is also facilitated by solvent affinity to the polymer ("good" solvent). This will indeed not only ease the dissolution step but also decrease the viscosity of the overall concentrated polymeric solutions used for *in situ* systems. As a result, in "good" solvents, polymer–solvent interactions predominate over polymer–polymer ones, therefore lowering the viscosity. By contrast, in "poor" solvents of the polymer, polymer–polymer interactions are favored, leading to the formation of aggregates and an increased viscosity [25]. "Good" solvents for the polymer also present the advantage of a reduced injected volume, due to the possibility to achieve higher polymer and drug loadings in the formulations. This is beneficial in two aspects, (i) volume constraints of subcutaneous or intramuscular injections (<1 mL [60]) and (ii) a lower amount of organic solvent administered. Beyond its impact on the injectability of the formulations, viscosity also affects the diffusion of species in the solidifying matrix. In this respect, a high viscosity will slow down the entry of water and the drug diffusion. Therefore it reduces the burst as well as delays polymer degradation.

Thirdly, good compatibility of the solvent with the polymer and the drug is a prerequisite. For instance, Dong et al. reported an influence of the solvent properties on the storage stability of PLGA [61] and Dernell et al. reported an interaction between DMSO and cisplatin, diminishing its biological activity [16].

Finally, biocompatibility or at least low toxicity is required for pharmaceutical acceptance (see also Section 4.1.). Several solvents have already been used for *in situ* depots formulation (Table 2). Of course, none of these gathered all the previously mentioned qualifications. NMP and DMSO seem to be safe due to pharmaceutical precedence in approved parenteral products, but they are freely miscible with water resulting in a rapid solvent and drug burst, as discussed above. One has to consider, that rapid initial release of large amounts of drug and solvent within a short time frame (minutes to hours) is undesirable, since it may result in local tissue irritation or even systemic side-effects not predicted by an estimated average dose, which considers a constant administration over the entire drug delivery period.

On that basis, water-immiscible solvents are attractive but challenge the injectability of the formulation. Hence, the better alternative can be a blend of water-immiscible and water-miscible solvents to obtain acceptable viscosity, suitable phase inversion rate and low burst.

Table 2
Common solvents in ISI formulations and their main characteristics.

Solvents	Water miscibility (mg/mL)	Viscosity (cP) at 20 °C	Classification	LD50 oral rat (mg/kg)
Glycofurol	miscible in all proportions[a]	8–18[a]	/	980[c]
DMSO	miscible[a]	2.19[d]	ICH class III[e]	14,500[c]
NMP	1000[b]	1.89[d]	ICH class II[e]	3914[c]
2P	1000[b]	14.66[d]	/	328[c]
TA	64[b]	19.7[d]	FDA GRAS[a]	3000[c]
BA	35[c]	5.81[d]	FDA IIG[a]	1 230[c]
BB	Insoluble[b]	8.67[d]	FDA IIG[a]	1 680[c]

NMP: *N*-methyl-2-pyrrolidone; DMSO: dimethyl sulfoxide; 2P: 2-pyrrolidone; TA: triacetin; BB: benzyl benzoate; BA: benzyl alcohol; GRAS: generally recognized as safe; IIG: inactive ingredients. LD50: lethal dose 50.
[a] Data are from [62].
[b] Data are from [25].
[c] Data are from Material Data Safety Sheet.
[d] Data are from [63].
[e] Data are from [64].

3.2. Polymer

The characteristics of the biodegradable polymer strongly impact the degradation and hence not only the erosion of the matrix but also the phase inversion dynamics.

PLGA is a copolymer of D,L-lactic and glycolic acid obtained by ring-opening copolymerization of D,L-lactide (LA) and glycolide (GA) (Fig. 8) [65]. Polymer grades are available with lactide/glycolide molar ratios 100:0 to 0:100 and molecular weights from below 10,000 up to 200,000 g/mol. As a result, PLGA copolymers provide a wide range of physicochemical and degradation characteristics for controlled drug delivery applications.

The composition of the copolymer is a critical point. LA/GA ratio and their distribution inside the chains (*i.e.* copolymer microstructure) are complex parameters, modulating the hydrophobicity and crystallinity of the system. Indeed, lactide exists in three different forms due to its two asymmetric carbons: L-lactide, D-lactide or *meso*-lactide (Fig. 8). L-lactide and D,L-lactide (*i.e.* the racemic mixture of L- and D-lactide) are the most commonly used forms for drug delivery applications. Poly(L-lactide) (PLLA) is a semi-crystalline material while poly(D,L-lactide) (PDLA) is amorphous [66]. PGA is also crystalline but

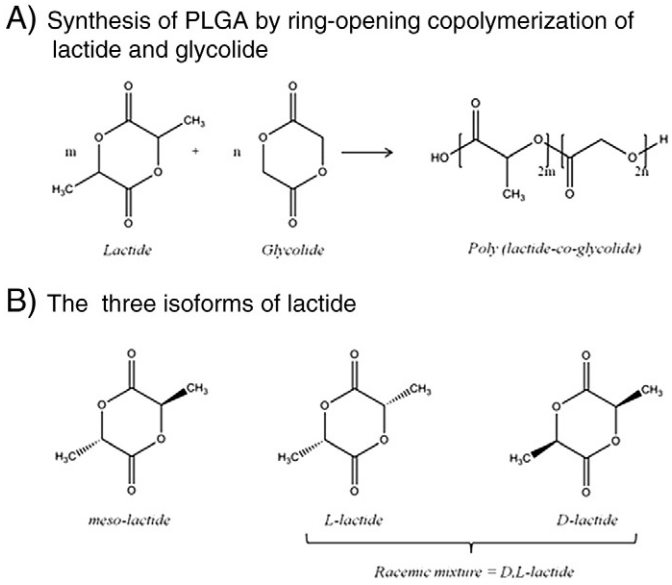

Fig. 8. Principle of PLGA synthesis through ring-opening copolymerization of lactide and glycolide (A) and existence of three isoforms of lactide (B).

PLGA, the copolymer of LA and GA, can be again amorphous depending on the polymer composition and microstructure.

The hydrolysis proceeds faster in the amorphous polymer parts compared to the crystalline ones resulting in a faster onset of polymer erosion and thus erosion-controlled drug release [67]. Degradants of the initially amorphous D,L-PLA, however, can crystallize during the hydrolysis resulting not only in a slower degradation of these domains [68] but also in a compromised biocompatibility of the polymeric device [69]. Crystallization occurs especially if the degree of randomness of the polymer is low, *i.e.* the segment lengths of monomeric repeat units in the polymer backbone are long. During crystallization, drugs can be excluded from the newly formed domains to the amorphous or already porous region, therefore accelerating their releases [70].

Modulating the polymer hydrophobicity could impact both the burst by influences on the solvent–water exchange rate and the degradation. As GA is slightly more hydrophilic than LA and introduces the more labile GA–LA bonds into the polymer backbone [71], the higher the GA content, the faster is the hydrolysis rate. Due to the crystalline nature of PGA, however, the degradation of PLGA containing 40 to 70% of GA is the fastest. Thus, for drug release applications for up to 2 months, PLGA 50:50 is often used [72].

The molecular weight of the polyester is another factor affecting matrix erosion and also the initial drug release. Several studies showed indeed that smaller bursts occur with low molecular weight PLGA compared with medium or high molecular weight PLGA. Patel et al. [73], for example, reported a burst of 28.2% fluorescein from PLGA implants (50:50, 36.5% m/m in NMP) for low molecular weight PLGA (M_w 16,000 g/mol), *vs.* 55.1% for high molecular weight PLGA (M_w 60,000 g/mol). Results from Luan et al. [46] followed the same pattern with a burst of leuprolide acetate of 18.8% and 48.1% from implants based on PLGA 50:50 with low or high molecular weight (M_w 7000–17,000 and 24,000–38,000 g/mol; 30% m/m of polymer in NMP), respectively. In both studies a slower diffusion of NMP from the lower molecular weight-PLGA solution and thus a slower phase transition was hypothesized, due the higher hydrophilicity of this polymer. This affinity for water also explains their higher tendency to swell [42,74], probably driven by an osmotic process.

The degradation process is obviously a function of the initial polymer molecular weight, since it is the starting point for the pseudo-first order decrease of the molecular weight over time [75]. Consequently, the higher the molecular weight initially, the longer is the time needed to produce water-soluble oligomers and hence the induction period until matrix erosion commences [53].

To summarize, low molecular weight-PLGA formulations often exhibit a lower initial release than high molecular weight-PLGA systems, but onset of the erosion-controlled release is accelerated. Intermediate properties can be achieved using a blend of PLGA with various molecular weights [74]. However, instead of a simple average of the release profiles of the pure polymers, the release can be primarily controlled by the polymer with the higher molecular weight during the burst phase, while degradation-facilitated release seemed to be controlled by the lower molecular weight polymer in the first place. Intermediate behavior was maintained until a 10:1 ratio of both polymer types.

The last parameter modulating the hydrophobicity of PLGA is the kind of chemical moieties at the chain ends. PLGA could be provided either with free carboxylic acids at the ends of polymeric backbone chain, or end-capped with alcohols. The end-cappings decrease PLGA hydrolysis rates, because they make the polymer somewhat more hydrophobic [75], leading to a decrease in water uptake and most importantly because capped acids cannot participate to the autocatalysis of the ester bonds. However, reduced burst might also be obtained with uncapped polymers in the case of active drugs having functional groups interacting with polymer acidic ends [46,76]. Covalent modifications of the polymeric backbone itself with a hydrophilic polymers, like poly(ethylene glycol), have been investigated but lack yet of pharmaceutical acceptance [77,78].

The simplest modulation to reduce the burst of *in situ* forming implants is an increase of the polymer concentration, due to the decreased water affinity of the solution, the thicker solidified polymer skin and the generally lower diffusivities in the system, which slows the solvent/water exchange and generates a less porous structure. This approach has been extensively studied [19,23,26,41,79,80]. For example, haloperidol initial release from ISI was decreased, increasing the polymer concentration (20, 30, 40% m/v), whatever solvent was used (NMP, DMSO, TA, ethyl acetate) [32]. Additionally, increasing polymer concentration also extended the duration of release, *e.g.* 28, 35 and 47 days for ISI–NMP [32]. However, the limitation of this method is the concomitant increase in system viscosity, which could quickly hamper injectability [60].

To conclude, polymer characteristics influence on the one hand the hydrophobicity of the system, which impacts the initial as well as the diffusion-controlled drug release *via* its effect on the phase inversion dynamics, and on the other hand the erosion-controlled release out of the matrix according to the initial molecular weight and the degradation rate.

3.3. Drug

Injectable formulations with high drug loadings have the advantage, that the volume of the formulation can be reduced considering a fixed dose to be administered. This is interesting from an economical point of view and from the patients' point of view since pain exposure times are reduced [60].

The effect of the low loading on release of *in situ* implants was investigated in a number of studies. Wang et al. [81] varied ketoprofen from 4 to 10% m/m (PLGA 70:30, 35% m/m in NMP) without significant effect in drug release *in vitro*. Ravivarapu et al. [82] obtained the same results *in vivo* with leuprolide acetate 3 to 6% m/m (PLGA 75:25, 45% m/m in NMP). Interestingly, Chen et al. [20] observed also the same but at much higher drug loadings of 20–60% (PDLA 5% m/v in BB/BA 85/15 v/v). Although the release was increased in absolute terms (0.33 to 0.85 mg/day), a 3-fold increase of the drug loading led to an about 3-fold increase of the absolute amount of testosterone released within a 3-month period, which means that the fraction of drug released (%) remained the same.

According to the solubility of the drug in the polymer solution and its concentration, the drug can be either dissolved or dispersed in the *in situ*

forming implant. Based on this state, different behaviors have been described. Körber and Bodmeier [83] found a faster release of lysozyme (4% based on polymer) when it was dispersed rather than dissolved in the polymer solution (PLGA 50:50, 40% m/m in DMSO). This was attributed to drug particles sedimentation and heterogeneity of the resulting polymer–solvent–drug mixture. Large drug aggregates were created at the surface of the matrix, and released rapidly. In agreement with this, Brodbeck et al. [18] investigated human growth hormone formulations, which showed a large burst, when the bulky lyophilized powder was suspended in the polymer solution, whereas a low burst was obtained for the material obtained after densification of the lyophilized material, which was attributed to a reduced water uptake of the formulations.

Another point of particular importance is their stability. As previously mentioned and as in any galenic formulation, drug interaction with excipients can also occur in ISI. On one side, a drug can accelerate polymer degradation [13,29,38,61], either because it contains water or possesses H-bond donating functional groups which can interact catalytically with polymer chains and therefore increase the exposition of ester bonds to water. On the other side, polymer and/or solvent can degrade or inactivate a drug [23,29,61]. In this sense, PLGA based-ISI are not suitable for drugs degradable by water or highly sensitive to the acidic environment formed within the degrading polymer matrix. However, stabilization strategies such as the formation of insoluble salts or complexes [18] or addition of basic additives [84] have not yet been fully explored.

3.4. Additives

An alternative method to scale the burst of ISI is the incorporation of additives into the polymer solution. The use of hydrophilic additives is thereby expected to accelerate the liquid–liquid demixing and hence generate higher bursts, as shown for mannitol [85] or polyvinylpyrrolidone (PVP) [41] addition. Interestingly, Graham et al. [41] observed an 8-fold increase in the separation rate with only 3% (m/m) PVP. Accelerating the phase separation permits the faster emergence of zones where species have great diffusion capacity (see Fig. 4). Such additives appear to impact the initial period of release only [85], which could be explained by a lack of changes in terms of water penetration or overall morphology due to the rapid leaching of these additives.

Addition of amphiphilic or hydrophobic additives reduces the burst while also modifying the morphology of the system, with a transition towards a sponge structure. Glycerol monostearate, ethyl heptanoate, stearic acid, ethyl heptanoate, methyl heptanoate and ethyl nonoate, for example, were added to ISI formulations, from 1 to 10% m/m [33,35,86,87]. Whatever the active ingredient, formulations with these additives retained more solvent than control ISI, consisted in less porous and even sponge-like matrices, which allow lower bursts and extended releases. In addition, these effects were proportional to the amount of incorporated additive and to its hydrophobicity. Noteworthy, the highest tested quantities of these additives did not result in increased viscosity as it would be expected for a burst reduction by an increase of the polymer concentration. To illustrate, risperidone burst from ISI studied by Dong et al. [33] (PLGA 70:30, 30% m/v in BB/BA 90:10 v/v) was reduced *in vitro* from 32.2% to 4.7% in formulations containing glycerol monostearate. A higher mean-residence time was also obtained *in vivo* (86.8 h for modified ISI *vs.* 32.6 h for control ISI *vs.* 5.8 h for risperidone solution). A very smooth surface was observed for modified ISI, probably because of a specific location of the additive filling the pores on the surface. A similar burst reduction has been reported by DesNoyer and McHugh [58] after addition of Pluronic (triblock copolymer poly(ethylene)oxide/poly(propylene)oxide/poly(ethylene)oxide, PEO-PPO-PEO) in a formulation of PDLA in NMP. Nevertheless in this case, no significant morphological change was observed, yet the protein burst was strongly reduced. This was explained by a preferential segregation of Pluronic to the phase boundary during phase inversion. The hydrophobic PPO chains are anchored into the polymer matrix, while the hydrophilic PEO ends deploy into the surrounding aqueous environment, both at the implant surface or into the pores. The resulting hydrophilic coating of the surface might improve the biocompatibility of the system and hinder protein adsorption as proposed elsewhere [88]. At the same time, PEO segments can fill the pores thereby creating a diffusion barrier. Optimization of Pluronic concentration or structure (ratio PEO/PPO, PEO chain length) is necessary to displace the balance between increased water absorption (hydrophilicity of the PEO chains) and diffusion barrier. Although authors considered that only this physical interaction is involved, they only studied one model protein (lysozyme) and additional interaction might occur with the drug, impacting its release. Depending on the application aimed, additives may also enhance drug activity, such as in anticancer therapy with some Pluronic potentially exerting chemo-sensitizing action [89].

3.5. External phase to create in situ forming microparticles

To address the burst while maintaining a low viscosity of the system, a novel approach has been developed [31,80,90,91]. The drug-containing polymeric solution (inner phase) is thereby emulsified with an oily or aqueous external phase containing a stabilizer. Droplets of the internal phase solidify upon contact with body fluids to form *in situ* forming microparticles (ISM).

One advantage of such a formulation is that it has lower viscosity than ISI formulations, because viscosity is determined by the external phase [63]. Consequently, higher polymer concentrations can be used without rendering injection difficult or painful. Additionally, ISM showed both reduced burst [31,32] and lower myotoxicity [92], as external phase creates a supplementary barrier slowing down solvent/drug leaching and water entry during solidification. Another advantage is the regular shape of formed ISM, which is determined by the size of the previous emulsion droplets and therefore minimizes morphological variations hence providing a more consistent and reproducible drug release than ISI. Nevertheless, as for every emulsified system, despite the use of surfactants, the main drawback could be a lack of emulsion stability. In the case of ISM, as the emulsion is extemporaneously prepared, this does not challenge the injection itself. However the coalescence of emulsion droplets could occur during their solidification [60].

Similarly to ISI, influence of several parameters on ISM systems has been studied [31,32,46,80,90]. In the same manner as ISI, burst decreases depending on the solvent and polymer used, increasing polymer concentration or decreasing drug loading. Additionally, higher viscosity of the external phase, faster emulsification rate or lower internal/external phase ratio can also contribute to burst reduction. In this respect, Li et al. [93] optimized the preparation of vinpocetine-ISM using a spherical symmetric design–response surface methodology considering drug loading, surfactant concentration and internal/external phase ratio. Optimized ISM had a very smooth surface and displayed a very gradual release with low burst *in vitro* (6.64% released the first day).

4. Remaining limitations to be addressed

In situ forming implants are promising galenic tools to administer, protect and release a wide range of compounds in a sustained fashion. They avoid frequent administrations, painful surgical procedures, allow localized or systemic drug delivery and are self-eliminating. And yet only two products have been commercialized until now. This could be potentially explained by the several issues which have to be solved: (i) drug burst release, (ii) safety and tolerability, (iii) reproducibility, and (iv) sterilization and stability. Means of controlling the first point have been extensively developed above; remaining points will be detailed below.

4.1. Safety

Despite the fact that some ISI products have already been commercialized, injection of organic solvent(s) remains a cause for concern. To choose the best solvent is quite difficult, because only little toxicological data concerning their parenteral administration are available (Table 2). However, NMP, DMSO, BA, BB and glycofurol have been used in injectable products for human use [94,95] and 2P and TA in veterinary products [96].

Only few toxicological and histological studies of ISI are reported in the literature, on several animal species and with sometimes contradictory results. For example, Bodmeier et al. used a rat model to evaluate the acute myotoxicity after single intramuscular injection [92,97]. In the first study, they tested *in vitro* NMP, DMSO and 2P as pure solvents: only 2P toxicity was significantly lower than the positive control [92]. Injections of ISI based on these solvents led to toxicities comparable to pure solvents. In the second study, they tested BA, ethyl acetate, propylene carbonate, TA and triethyl citrate and except for ethyl acetate, all solvents caused high muscle toxicity, especially BA [97]. The evaluation of muscle damage during 72 h *in vivo* after intramuscular injection of ISI or ISM based on 2P or ethyl acetate revealed that myotoxicity of ISI was not different from pure solvents, while ISM were found to be much less myotoxic because of the 1 to 10-fold dilution of the solvent with the external phase. Results from a study conducted over a longer time frame on monkeys are not consistent with the foregoing [98]. Authors injected animals both subcutaneously and intramuscularly with ISI formulations based on NMP or DMSO. No safety concern appeared, as animals maintained normal behaviors during the study and histological analysis after 1 month showed tissue reaction similar to those reported for usual preformed biodegradable implants. However, recent concerns about the reproductive toxicity of NMP administered orally and dermally resulted in a tightening of the permitted daily exposure information included in the International Conference on Harmonisation guidelines Q3C (R5) on impurities in 2006.

Finally, in rabbits, ISI based on glycofurol or on a mixture of BB and BA showed no irritation after subcutaneous or intramuscular injections and/or normal inflammatory and foreign body reactions similar to blank and pure drug solutions [33,38,99]. In addition to the modulation of solvent mixture hydrophobicity, the advantage to use BA is its local anesthetic effect, which could avoid or reduce pain during injection, especially for viscous solutions [5].

Overall, ISI formulations appear to be well tolerated. Systems with hydrophobic solvent are thought to be less irritating, as solvent diffuses more slowly into the surrounding tissues. For the same reason, ISM-systems are generally regarded as less irritant than ISI, because the external oily phase forms a barrier between the muscle and the internal phase, thus limiting the amount of solvent in immediate contact with the muscle after injection.

To conclude, toxicity evaluations of ISI seem to differ depending on the route of administration, the method employed, the time of evaluation and eventually the animal model. Extensive toxicological studies, with a more harmonized methodology, are really needed to widen the future of ISI. They will represent an important cost, but also a beneficial step for other drug delivery systems, as many of the newly discovered drugs have low water-solubility. New solvents such as low molecular poly(ethylene glycol) (PEG) have been introduced [100–102]. Nevertheless, authors reported that conventional PEG accelerates the degradation of PLGA in solutions [61] by a trans-esterification mechanism [101]. As a result, significant improvement was obtained by alkyl-capping of the polymer ends. Additionally, transient edema was reported with PEG [103].

An alternative approach to enhance the safety of *in situ* systems is to reduce or suppress the need of organic solvents through the development of new polymers. For example, alkyl substituted polylactides were recently synthesized from the monomer 2-hydroxyoctanoic acid [104]. Resulting biodegradable polymers ("hexyl-substituted polylactides" or "hex-PLA") form viscous solutions, are injectable without or with only small amount of NMP (<5%) and showed good biocompatibility [105].

4.2. Reproducibility

A key feature of drug delivery systems is the reproducibility of the drug release characteristics. This is obviously conditioned by reproducibility in terms of shape, size and structure. Structure of ISI is highly dependent on the components and rate of phase inversion, as discussed above. Concerning shape and size, however, they are influenced by several factors related to the formulation, the administration procedure and the environment [7].

As the solidification of the *in situ* systems takes place at the site of injection, it is easily understandable that it will be particularly sensitive to environmental changes. Accordingly, good correlations between polymer precipitation and drug release have been obtained both *in vitro* and *in vivo* while establishing correlations between *in vitro* and *in vivo* drug releases remains challenging [42,74]. Even between *in vitro* results, comparisons are difficult, as several protocols for drug release assays from ISI are described in the literature. Variations include the composition, volume and pH of the aqueous medium, use of shaking device, dialysis membrane [31] or even home-made structures [106] and agarose phantoms [42].

Only a few studies have been conducted to evaluate how the aqueous environment composition influences the system *in vitro*. Brodbeck et al. found that organic salt or small chain triglyceride in the aqueous bath impact demixing kinetics and lysozyme release (PLGA 50% m/m in NMP, TA or EB) [14]. Formulations based on solvent with high affinity for the non-solvent bath (NMP), however, were less strongly affected. In another study, quenching of a PLGA 50% m/m/DMSO solution with water, a phosphate buffer solution or with horse serum did not result in any change neither in phase inversion dynamic nor in the depot morphology [41]. The use of DMSO, a freely water miscible solvent, in this latter study is a potential explanation for the lack of change.

In addition to potential differences in the external tissue surroundings composition (proteins, salts, acids *etc.*), one of the main factor of influence *in vivo* is the tissue stiffness. ISI were indeed originally intended to be injected subcutaneously or intramuscularly. Nevertheless, emerging applications are moving towards more unusual sites of injection, such as directly into specific area (the eye [107], the brain [108]), physiological pockets [12,26,27], tumors [109–111] or bone defects [13]. This is achieved due to the capacity of ISI to adjust readily to the surrounding tissue, providing a high level of contact. However, the final shape of the injected implant will affect the diffusion conditions and thus drug release. Patel et al. [110] obtained for instance uniform spherical shape after *in vitro* injection, when flat disc like shape was observed after subcutaneous injection and multi-lobular shape after intratumoral injection. Additionally, they tested three different PLGA in ISI injected subcutaneously; fluorescein burst release was always higher *in vivo* than *in vitro*. They attributed this trend to the limitation of implant swelling and expansion *in vivo*, due to interstitial pressure or compressive forces exerted by the surrounding tissue. Other authors proposed the use of ISM systems to improve reproducibility, as regular shape is ensured by the emulsion droplets solidification [90].

Injection sites might also diverge in terms of tolerance. *In situ* forming formulations are generally described as well tolerated, although a fibrous capsule surrounding the implant has been described [103]. Such a structure would constitute a barrier impeding water/solvent exchanges and drug release. Moreover, entrapment of polymeric oligomers produced during degradation within the capsule might result in a lower pH inside the matrix and thus accelerated matrix degradation. In this respect, non-invasive imagery techniques (electron paramagnetic resonance [112], magnetic resonance imaging [103], and ultrasound imaging [42,74,110]) can be useful for the real-time monitoring of ISI. They provide information about the implant itself (solidification, shape, size,

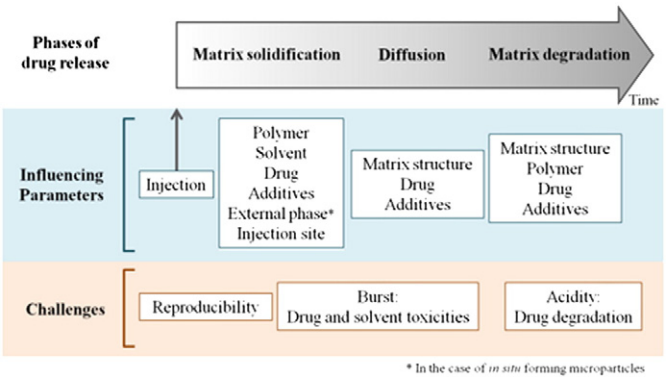

Fig. 9. The three phases of drug release from ISI and critical parameters affecting them.

and disappearance) in addition to the eventual biological response to implantation (edema, inflammation, and encapsulation).

Finally, the conception of injection devices in order to standardize the deepness and the speed of injection might broaden clinical applications of ISI in the future.

4.3. Industrial development

From an industrial point of view, *in situ* forming systems provide several benefits. First, the development of these injectable sustained-release formulations might extend the life cycle of a drug. Second, preparation process is very simple, accomplishes essentially 100% encapsulation efficiency and avoids the use of high temperatures or high shear methods which can be deleterious for fragile compounds. Consequently, a wide range of molecules can be incorporated into ISI, including peptides and nucleic acids.

ISI formulations are intended to be injected into the body and thus have to fulfill parenteral requirements including sterility. Gamma-irradiation is widely accepted for terminal sterilization of biodegradable polymer systems such as ISI since it results only in a slight decrease in molecular weight (*e.g.* [13,82]). If the drug is not stable into the polymeric solution, it can be provided as a lyophilizate in a separate syringe: sterilization is obtained *via* sterile filtration or aseptic manufacturing. Dong et al. [61] have also proposed to freeze–dry a drug-containing PLGA solution in dioxane or acetic acid. The obtained sponges can be dissolved in the ISI solvent just before injection. However residual dioxane or acetic acid can be a problem for this approach.

Interestingly, delivery time of drugs from ISI can be modulated over a large range. Consequently ISI offer an alternative to oral (24 h), transdermal systems (1–7 days) and conventional implants (months–years).

A key element of the *in situ* systems is the polymer: safe use history, FDA approval and wide range of products available have made PLA and PLGA very popular. Nevertheless, these synthetic polymers are costly (2000–6000 $/kg) and change in supplier as well as batch-to-batch variations could lead to different properties. For example, copolymer microstructure (*i.e.* alternation of GA/LA units) is strongly influenced by the polymerization conditions, which determine the importance of the main secondary reactions (redistribution reactions) during the polymerization process [65]. Furthermore, despite PLGA non-toxicity and good *in vivo* degradation properties, polymer–drug interactions or acidic microenvironment created during the degradation may represent additional obstacles. Switch towards other polymers ongoing surface erosion or homogeneous bulk erosion could represent a valid approach.

The use of potentially toxic organic solvents, however, remains a major issue for these formulations. Therefore, toxicological studies and the elucidation of new solvents could help to further develop ISI systems.

Finally, a comprehensive overview of the main points discussed in the different sections of this review is proposed in Fig. 9.

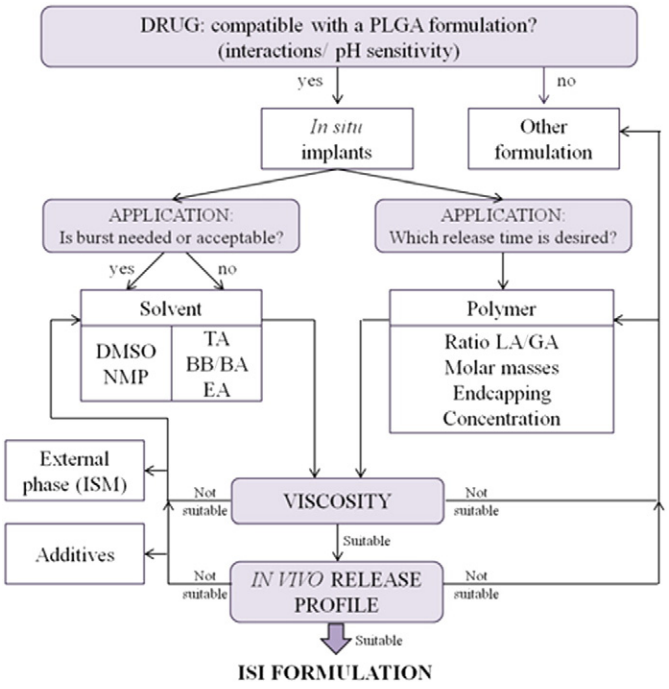

Fig. 10. Schematic representation for a rational design of *in situ* implants. DMSO: dimethylsulfoxide; NMP: *N*-methyl-2-pyrrolidone; TA: triacetin; BB: benzyl benzoate; BA: benzyl alcohol; EA: ethyl acetate; LA: lactide; GA: glycolide; ISM: *in situ* forming microparticles.

5. Conclusion

Polymeric *in situ* implants formed by phase separation are promising minimally invasive parenteral formulations applicable in many therapeutic fields. A loading dose followed by a sustained release at lower concentration may be desirable especially for hormonal castration, anticancer therapy, for antibiotic applications as well as for local anesthesia. Apart from the traditional use as delivery systems for chemical compounds, *in situ* implants also offer potential uses as gene delivery platform or tissue repair scaffold. In the future, one might imagine further applications, for example in vaccination or in allergic desensitization, as already shown with other polymeric forms [113,114]. Finally, composite forms might also be designed, where ISI will allow site-specific delivery of other drug-containing systems. One example is already encountered in the literature: Yehia et al. demonstrated recently the feasibility of ISI-containing liposphere [115].

This review gives an overview of the actual knowledge about design and performance of *in situ* forming implants and it offers the keys to a rational development of products (Fig. 10) with potential benefits in terms of costs and patient compliance.

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