Expert Opinion

Lipid implants as drug delivery systems

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The parenteral controlled delivery of acid-labile drugs (e.g., proteins) is difficult, because the standard polymer poly(lactic-co-glycolic acid) used to control drug release upon parenteral administration degrades into shorter chain acids, creating acidic microclimates. Lipid implants do not show this disadvantage. The objective of this article is to give an overview on the present state of the art and to highlight the advantages and drawbacks of the different types of systems reported in the literature. The major preparation techniques for lipid implants, underlying mass transport mechanisms, biocompatibility and *in vivo* performance of the most interesting systems are described. Lipid implants offer a great potential as parenteral controlled drug delivery systems, especially for protein-based drugs. A broad spectra of release patterns can be provided and acidic microclimates avoided.

Keywords: compression, controlled drug delivery, extrusion, implant, lipid

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1. Introduction

The appropriate delivery of drugs to their sites of action within the human body can present a major obstacle in the development of novel pharmaco therapies. Even if the drug is known to be effective and (ideally) able to cure the patient, it must reach its target site to become active. In the case of protein-based drugs, for example, oral administration is not (yet) feasible because this type of molecule is degraded within the gastro-intestinal tract and the gastric and intestinal mucosa are poorly permeable for large molecules. In addition, the half-life of many protein-based drugs is short within the human body. Thus, frequent parenteral administrations are required for this type of drug, such as in the case of diabetes treatment with insulin. As frequent injections are inconvenient, patient compliance is often not optimal. To overcome these restrictions, either parenteral drug delivery systems can be used, which release the incorporated drugs over prolonged periods of time (e.g., several weeks or months), or alternative administration routes (e.g., pulmonary or nasal) can be envisaged. Recently, Exubera has been marketed, allowing for the pulmonary administration of insulin [1]. However, the development of this type of advanced drug delivery system is not straightforward as major challenges need to be addressed, including the assurance of reliable and reproducible drug doses that reach the deep lungs; inter- and intra-subject variability; the impact of respiratory diseases/smoking habits; as well as considerable development costs.

Parenteral controlled drug delivery systems are intended to reduce the administration frequency, to protect the drug against degradation upon administration within the human body and to accurately control the resulting release rate, providing optimized therapeutic effects. Nowadays, poly(lactic-co-glycolic acid) (PLGA)-based microparticles are the most frequently used injectable controlled drug delivery systems [2-6]. However, PLGA degrades into smaller chain acids (and alcohols) upon contact with water, which can lead to significant drops in the microenvironmental pH [7-8]. Often, protein-based drugs lose their activity under these

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conditions [9-12]. The use of lipid implants as parenteral controlled drug delivery systems offers an interesting alternative for this type of drugs [13-19]. Obviously, acid-stable drugs can also be incorporated within lipid implants and effectively be protected against physical and/or chemical aggressions (e.g., enzymatic degradation) within the human body. Furthermore, protein-based drugs might lose their activity during the preparation procedure of PLGA-based microparticles [20-24]. For example, changes in the protein structure are likely to occur at liquid–liquid interfaces when using solvent evaporation/extraction techniques [25-28]. During the preparation of lipid implants, such harsh conditions for proteins can often be avoided.

In addition to drug protection and an accurate control of the resulting release rate, lipid implants can also be used to allow for locally restricted drug distribution. This can for example be very helpful to avoid or minimize toxic side effects in the rest of the human body. The idea is to incorporate the drug within the lipid implant, which is administered at (or close to) the site of action. The drug is then released during prolonged periods of time at a pre-determined rate into/nearby the target tissue, providing high local drug concentrations in this/these area(s). At the same time, drug levels in the rest of the human body are reduced, resulting in minimized toxic side effects. An example for advantageous *local* controlled drug delivery is the prophylaxis of prosthetic device-related infections using vancomycin [29]. In the case of Central Nervous System (CNS) diseases, such as brain tumors or neurodegenerative diseases (e.g., Parkinson's and Alzheimer's Disease), lipid implants can be directly administered into the target tissue, thus effectively overcoming the blood brain barrier (BBB). The latter represents a major obstacle for many types of drugs that would be highly efficient for various CNS diseases if they were able to reach their site of action [30-33]. If the implants are cylindrical in shape and exhibit a small diameter, they can be easily injected into or close to the target tissue using standard needles.

Various types of lipids have been proposed in the past for the preparation of implants as advanced drug delivery systems [13,34-39]. Many of them are natural substances. Some are physiological (normal compounds of the human body), for example certain triglycerides and cholesterol. Lipids are also widely used in the cosmetics and food industry and considered to be safe for various types of applications. However, this does not necessarily mean that their use as parenteral drug carrier materials is safe. The biocompatibility of some of the systems described in the literature has been studied *in vivo* and, in certain cases, the pharmacodynamic effects upon administration of drug-loaded devices have been monitored.

This article gives an overview of the different types of implants that have been proposed so far, distinguishing compressed, molten and extruded implants. Special sections have been devoted to: i) the underlying drug release mechanisms of this type of advanced drug delivery systems, allowing for a better understanding of how the systems work; and ii) results reported so far from *in vivo* studies.

2. Compressed implants

If feasible, direct compression of a drug:lipid powder blend is a straightforward and often convenient production procedure for lipid implants. However, caution needs to be exercised and reproducible drug loading and drug distribution within the implants must be guaranteed. In practice, this might be difficult to achieve due to poor flow properties of the powder blends and potential de-mixing tendencies, resulting in inhomogeneous drug loadings and distributions. In order to avoid de-mixing of the drug:lipid powders, different strategies can be followed. In an interesting study, Koennings *et al.* [40] investigated the following four manufacturing procedures for brain-derived neurotrophic factor (BDNF)-loaded, triglyceride-based implants (**Figure 1**):

- Direct compression of drug:lipid powder blends.
- Compression of a lyophilized dispersion of the drug powder in an organic solution of the lipid.
- Compression of a lyophilized dispersion of an aqueous drug solution in an organic solution of the lipid.
- Compression of a lyophilized solid-in-oil dispersion, which is obtained by freeze-drying an aqueous drug:poly(ethylene glycol) (PEG) solution and the subsequent addition of an organic solvent and the lipid powder.

It is important to note that the type of preparation technique strongly affects the resulting drug distribution within the implants, as illustrated in **Figure 2** on the right-hand side: confocal microscopy was used to image the distribution of fluorescence-labelled lysozyme within the systems. As can be seen at the top, a continuous and highly interconnected protein network exists in lipid implants prepared by direct compression of drug:lipid powder blends. In contrast, implants prepared by other preparation techniques showed significantly different protein distribution patterns: the drug-containing regions are not fully interconnected and the channels that can be formed upon water penetration and protein leaching are much narrower and constitute a network with a significantly higher degree of branching. Thus, the probability that a lysozyme molecule may not be released because no continuous water-filled channels can be created to the surface of the device is increased, leading to decreased total amounts of drug released (**Figure 2**, left-hand side, filled diamonds). In addition, the mobility of the drug molecules is reduced in the narrower channels and the drug molecules can more easily "get lost" in the highly branched networks, resulting in decreased apparent protein diffusivity within the matrices and, hence, decreased release rates (**Figure 2**, left-hand side, filled diamonds). Furthermore, there are no major deviations between active and total lysozyme release (dotted and full curves), indicating that these manufacturing procedures are non-destructive for this protein. Only in the case

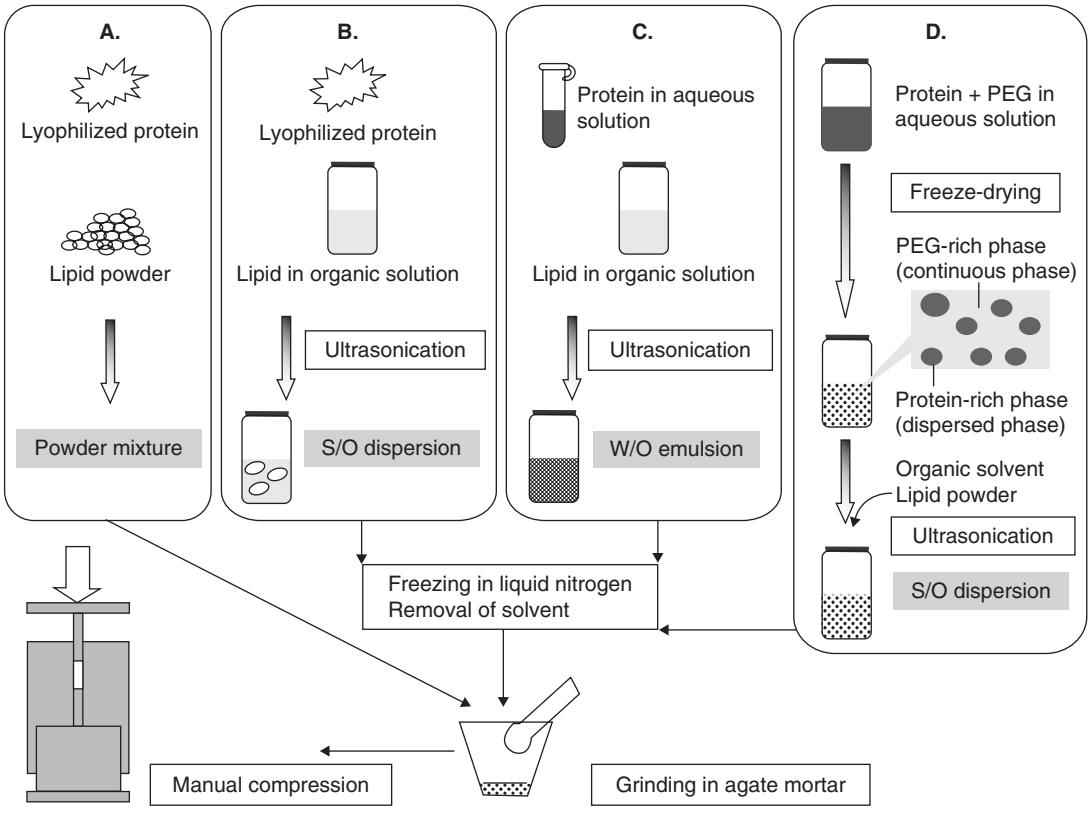

Figure 1. Schemes of the different manufacturing procedures studied by Koennings *et al.* [40] for compressed, protein-loaded lipid implants. Procedure using: (**A**) drug:lipid powder blends; (**B**) lyophilized solid-in-oil dispersions; (**C**) lyophilized water-in-oil emulsions; and (**D**) lyophilized solid-in-oil dispersions, which are obtained from freeze-dried aqueous drug:PEG solutions and the subsequent addition of an organic solvent and the lipid powder.
Reproduced from [40], with permission.

of the lyophilized dispersions of an aqueous drug solution in an organic solution of the lipid, was part of the released protein no longer active (**Figure 2C**). This might be attributable to protein unfolding at liquid–liquid interfaces. Importantly, this phenomenon could successfully be overcome by co-lyophilizing lysozyme with PEG (resulting in a fine dispersion of the protein in a PEG-rich phase [41], as shown in **Figure 1D**) and preparing a solid-in-oil dispersion of the obtained powder within the organic solution of the lipid, which was subsequently lyophilized (**Figure 2D**). Drug release from implants containing 2% protein and 2% PEG are shown on the left-hand side of **Figure 2** (open diamonds). As can be seen, the presence of PEG can be used to alter the resulting protein release kinetics.

In addition to drug distribution, the morphology of lipid implants is also of great interest. **Figure 3** shows, for example, optical and scanning electron microscopy pictures of a glyceryl tripalmitate-based, lysozyme-loaded implant prepared by the emulsion–compression method described above. Clearly, individual lipid plates (consisting of crystalline glyceryl tripalmitate) are visible in **Figure 3B**. In between are numerous cavities allowing for protein release (crystalline lipid plates are not permeable for proteins). Importantly, macroscopically the surface appears to be smooth, allowing for a facilitated administration into the human body.

In order to provide specific, optimized drug release profiles, a variety of formulation and processing variables can be altered when using compressed lipid implants. For example, the type of lipid can be varied, as illustrated in **Figure 4**. The release of lysozyme is shown from compressed implants based on triglycerides differing in the fatty acid chain length. Clearly, the type of matrix former strongly affects the resulting drug release rate. Interestingly, there is no clear relationship between the fatty acid chain length and the resulting drug release rate. In the literature, both decreasing and increasing release rates have been reported when increasing the chain length of the fatty acids in the triglyceride [42-44]. The addition of different amounts of poly(ethylene glycol) PEG or gelatin to lipid-based implants is also a powerful tool to adjust desired drug release rates [16,45-48]. For example, Mohl and Winter 2004 [16] effectively altered the release patterns of rh-interferon α-2a (IFN-α) from glyceryl tristearate-based implants prepared by compression by adding 0 – 20% PEG (**Figure 5**). The variation of the initial drug content within the implant is a further parameter that can significantly affect the resulting drug release rates [43,49-51]. As the porosity

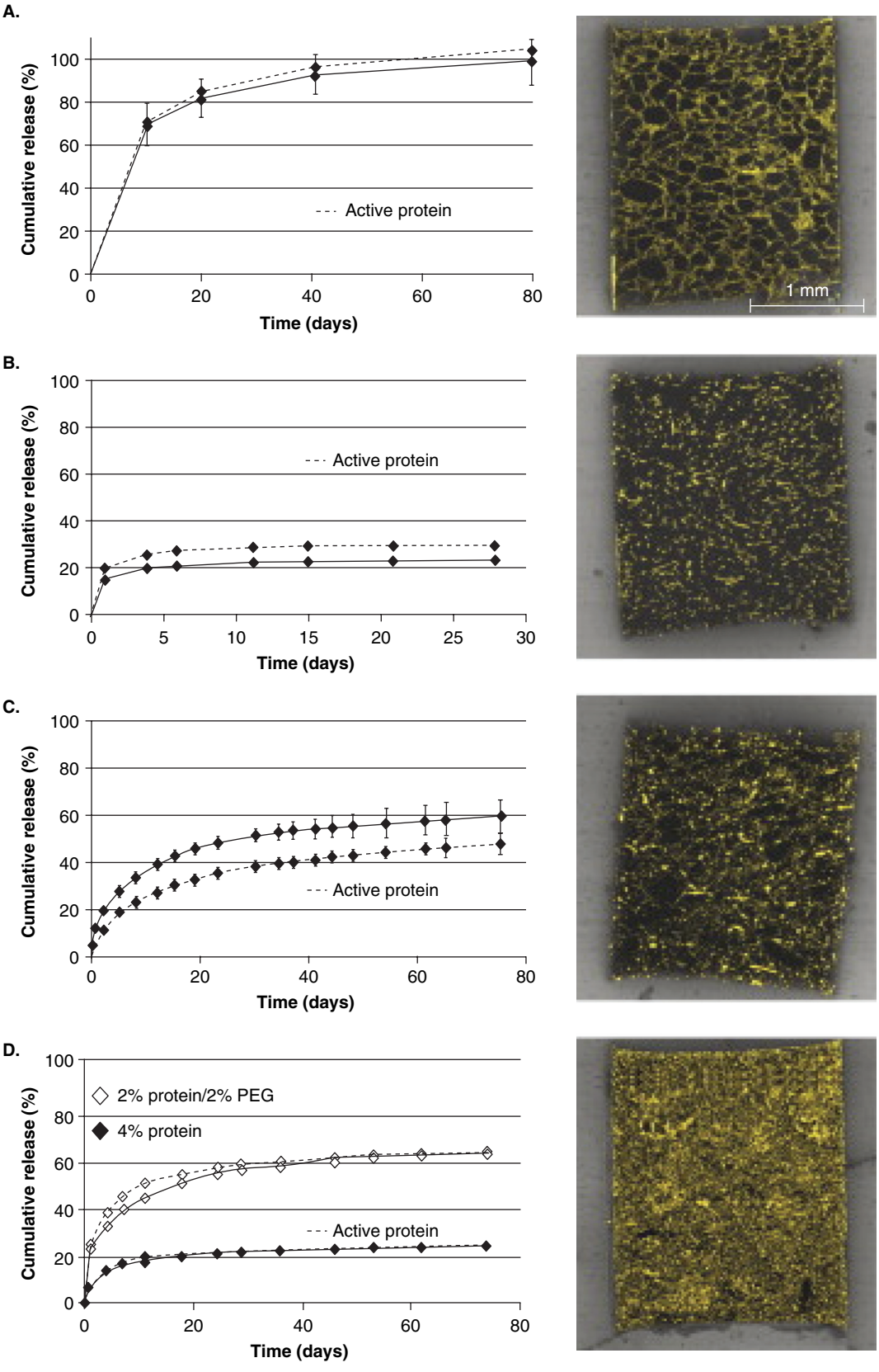

Figure 2. ***In vitro*** **release of lysozyme (♦) from lipid implants loaded with 4% protein (continued).**

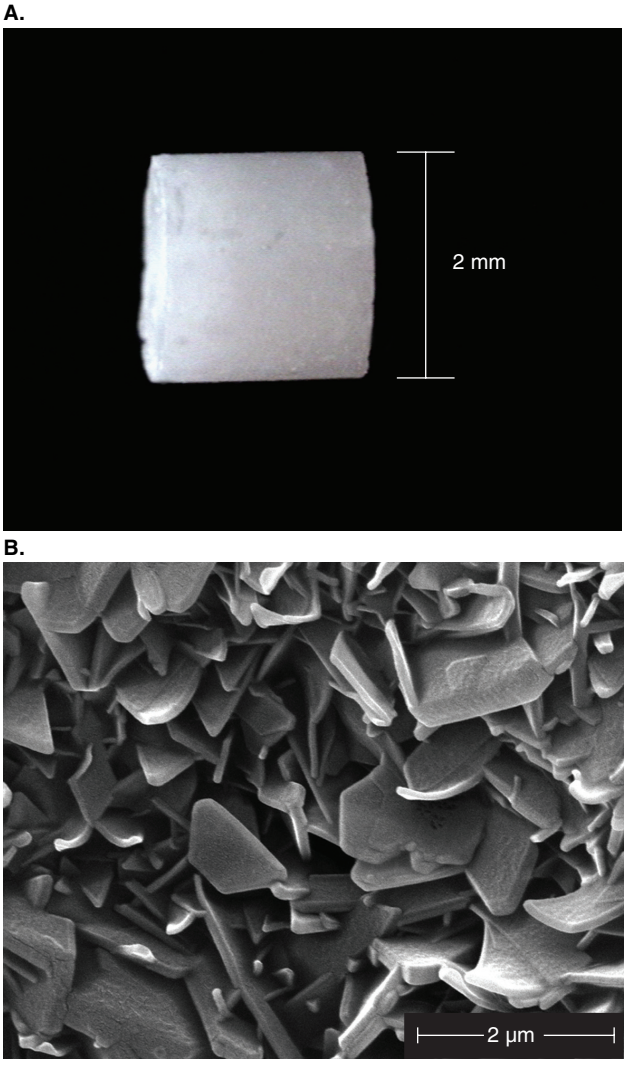

Figure 3. Morphology of glyceryl-tripalmitate-based, lysozyme-loaded implants prepared by the emulsion–compression method. A. Optical microscopy picture of the entire implant. **B.** Scanning electron microscopy picture of the surface of the implant.
Adapted from [43], with permission.

of the implants increases with increasing initial loading upon drug depletion, the mobility of the remaining drug to be released increases, resulting in (generally) increasing drug release rates. However, if the drug solubility within the system is limited, the effects of the initial drug content on the resulting relative release rates can be more complex. In this case not all of the drug is dissolved upon water penetration into the implant: dissolved and non-dissolved drug co-exist within the matrix. Importantly, only the dissolved drug is available for diffusion and able to leach out into the release medium. Thus, when increasing the initial drug content, the absolute amount of drug available for diffusion remains constant (saturated solutions) and so the absolute drug release rate remains constant. However, the 100% reference value increases, resulting in decreasing relative drug release rates. The relative importance of this *limited drug solubility effect* and the above-described *increasing porosity effect* depends on the specific type of system and explains why different effects on the resulting relative drug release rates can be observed when increasing the initial drug loading of the lipid implants.

Obviously, great care has to be taken when defining the compression pressure during implant preparation [43,44,47]: this parameter can crucially affect the resulting implant structure and, thus, resulting drug release kinetics. If the pressure is too low, the mechanical stability of the obtained implant is poor; if the pressure is too high, the porosity of the implant might be insufficient to allow for appropriate drug release rates and production on a large scale might be difficult. Furthermore, the size and size distributions of the compressed particles (e.g., drug and lipid powder, lyophilized solutions/emulsions/dispersions) is of major importance, because these parameters affect the pore size and pore structure of the obtained implants and, thus, the resulting drug release kinetics [40,43,49,50]. It has to be pointed out that the following relationships: "drug particle size – drug release patterns", "lipid particle size – drug release patterns" and "drug:lipid particle size ratio – drug release patterns" are not straightforward. Often, the resulting drug release rate increases simultaneously with an increase of drug and lipid particle size, due to the resulting larger inter-particle voids and, thus, increased implant porosity [43,50]. However, when increasing the drug particle size only (keeping the lipid particle size constant), both increasing and decreasing drug release rates have been reported [49,50].

Interestingly, different lag times prior to the onset of drug release can be provided with lipid implants, if the latter are surrounded by a drug-free, biodegradable polymer layer [52]. The manufacturing procedure for such an implant is illustrated in **Figure 6**. First, the bottom of the implant, consisting in this example of PLGA, is compressed at room temperature. Then the pre-prepared, drug-loaded, lipid implant is inserted as well as the PLGA powder, constituting the top part of the implant. The entire system is then compressed at room temperature, followed by a compression at 48°C in order to

Figure 2. *In vitro* release of lysozyme (♦) from lipid implants loaded with 4% protein (continued). Implants prepared by compression of: (**A**) drug:lipid powder blends; (**B**) lyophilized solid-in-oil dispersions; (**C**) lyophilized water-in-oil emulsions; and (**D**) lyophilized solid-in-oil dispersions, which are obtained from freeze-dried aqueous drug:PEG solutions and the subsequent addition of an organic solvent and the lipid powder. The dotted curves indicate active protein, the open diamonds drug release from implants containing 2% lysozyme and 2% PEG 6000. The pictures on the right-hand side illustrate the distribution of fluorescence-labelled lysozyme within matrices loaded with 4% protein, determined by confocal microscopy.
Adapted from [40], with permission.

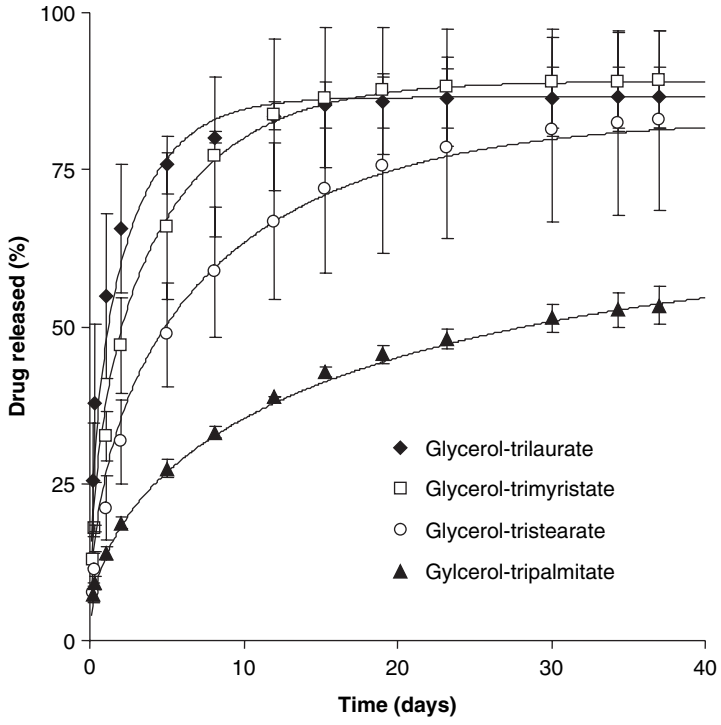

Figure 4. Effects of the type of lipid (indicated in the diagram) on the resulting drug release rate from lysozyme-loaded, triglyceride-based implants prepared by compression (emulsion method described in the text).
Reproduced from [43], with permission.

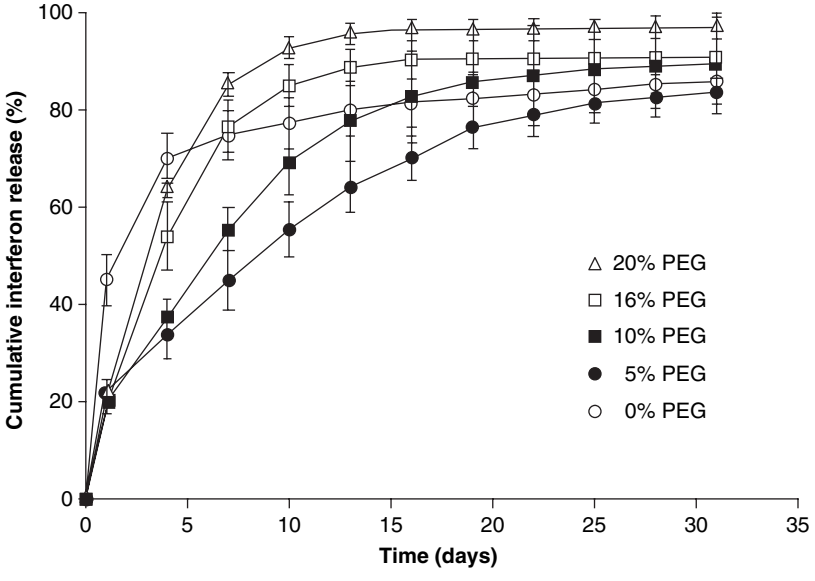

Figure 5. Effects of the addition of different amounts of poly(ethylene glycol) on IFN-α release from glyceryl tristearate-based implants prepared by compression.
Adapted from [16], with permission.

induce pore-closing within the outer PLGA layer (to avoid premature drug release). As can be seen in **Figure 7**, varying the type of biodegradable, drug-free mantle material allows for an efficient adjustment of the lag time period [$PLGA_{10}$ = PLGA 50:50, molecular weight 10 kDa; $PLGA_{17}$ = PLGA 50:50, molecular weight 17 kDa; PLA_{30} = poly(lactic acid), molecular weight 30 kDa]. Furthermore, once drug release starts, the release rate can be effectively adjusted by varying the type of triglyceride the lipid inner core is based on (**Figure 7**: the number of C-atoms of the fatty acid chains is

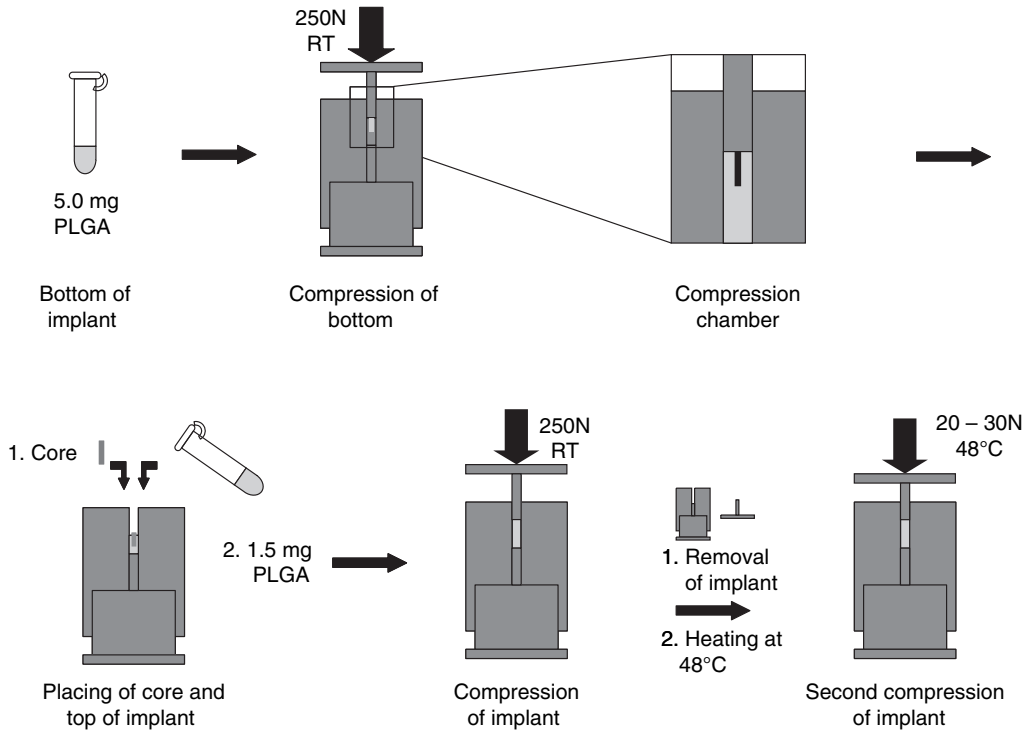

Figure 6. The preparation procedure for lipid implants, which are surrounded by a drug-free, biodegradable polymer layer.
Reproduced from [52], with permission.

indicated in the diagrams). This type of controlled drug release pattern with variable lag times prior to the onset of release can, for example, be very helpful for the design of innovative vaccine delivery systems: administrating several types of implants at the same time containing different types of mantle materials and multiple antigen pulses afterpre-determined lag times can be provided with one single administration.

A potential disadvantage of *compressed* lipid implants might be the challenges during up-scale: if the flowability of the powder blend is poor, either significant amounts of lubricants need to be added (which might significantly alter the resulting drug release patterns), or the production rate might be low. Also the pre-treatment steps required to avoid drug:lipid powder de-mixing might be cumbersome.

3. Molten implants

An interesting alternative to the compression of drug:lipid blends is the suspension and/or dissolution of the drug within the molten lipid carrier material, subsequent casting into moulds and cooling under well controlled conditions. However, care needs to be taken in the case of thermo-sensitive drugs, such as proteins that might (at least partially) lose their biological activity upon heating. Fortunately, the stability of many proteins can be significant at elevated temperatures if water is absent [53,54]. For example, Yamagata [38] measured the activity of interferon-α 2a upon heating to 60°C together with polyglycerol esters of fatty acids. Interestingly, the protein activity decreased only by 5% after 6 min and then remained constant for up to 5 h of heating. Insulin was also shown to be stable when dispersed within glyceryl tripalmitate during spray congealing (2 min exposure to 70 – 80°C): only a minor fraction (≤ 1.6%) of desamidoinsulin was detected [55].

Significantly different implant morphologies can be obtained when preparing the devices by compression as opposed to by melting. As shown by Pongjanyakul *et al.* [46], much smoother implant surfaces can be obtained with a melting method. Also, upon drug release *in vitro*, the surface of molten lipid implants remained relatively non-porous, whereas the surface of compressed implants became highly porous. These significantly different implant structures can at least partially explain the very different drug release kinetics from the systems, which were of identical composition. **Figure 8** shows an example of lysozyme release from cylindrical implants based on glyceryl palmitostearate loaded with different amounts of drug [2% (triangles), 5% (squares) or 10% (circles)]. It is clear that molten implants released the drug much more slowly and to a lesser extent than the respective compressed implants. This can be attributed to the denser matrix structures within molten implants providing narrower pathways for the dissolved drug molecules and a reduced probability of having direct access to the release medium via

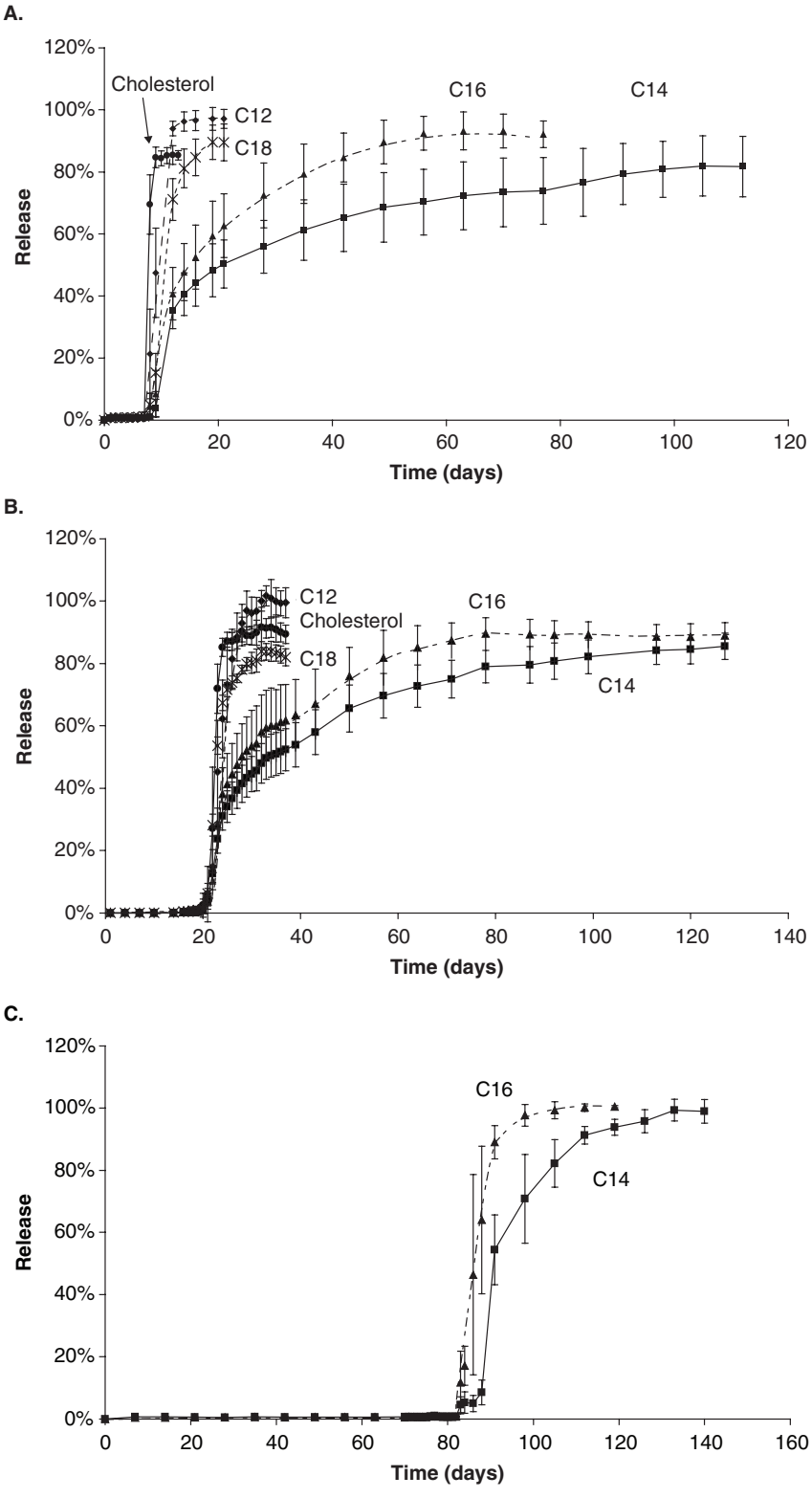

Figure 7. Drug release from lipid implants surrounded by a drug-free, biodegradable polymer mantle, based on: (**A**) $PLGA_{10}$, (**B**) $PLGA_{17}$ and (**C**) PLA_{30} (for preparation procedure see **Figure 6**). The inner drug-loaded, lipid cores are based on triglycerides. The number of C-atoms of the fatty acids is indicated in the figure.

Reproduced from [52], with permission.

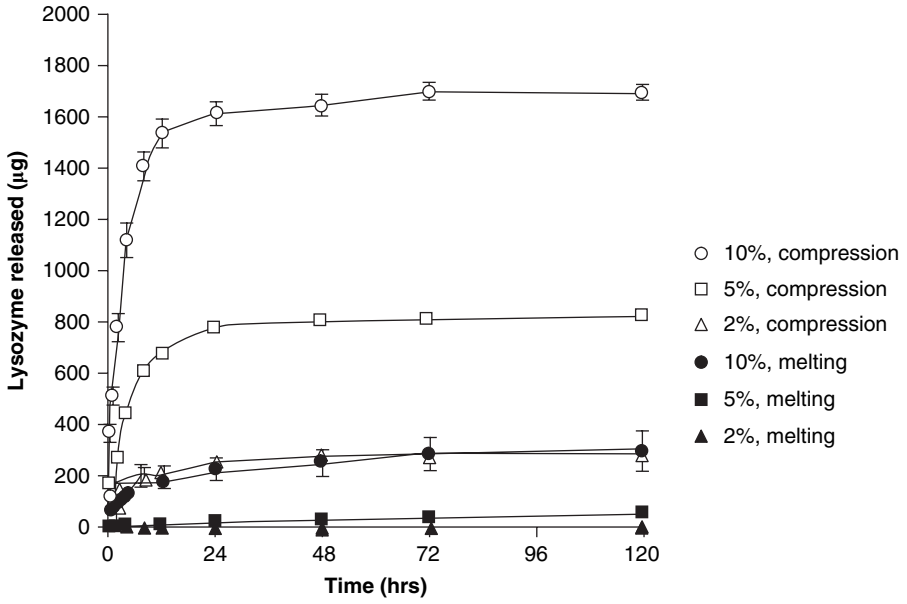

Figure 8. Lysozyme release from glyceryl palmitostearate-based implants prepared by melting or compression. Please note that the ***absolute*** amount of lysozyme released is plotted on the y-axis, not the relative amount.
Adapted from [46], with permission.

interconnected channels for the incorporated drug particles. As can be seen, increasing the initial drug loading from 2 to 10% resulted in increasing absolute drug release rates, irrespective of the type of preparation method.

However, great care must be taken with respect to the physical state of the lipid matrix former. Many lipids are known to be able to form crystals with different inner structures (polymorphism) and thus different physicochemical properties (which can affect the resulting drug release kinetics). If, for example, a meta-stable crystalline form of the lipid is obtained during implant preparation, it can be transformed into a more stable crystalline form during storage [56-61]. This can lead to altered implant properties before as opposed to after storage, including altered drug release patterns. Thus it is of utmost importance to control precisely the physical state of the lipid during this type of preparation method. For example, triglycerides can generally form α, β′ and/or β polymorphs [62-70]. The type(s) of crystal(s) that is/are obtained upon cooling depend(s) on various factors, including the cooling rate, start and end point temperatures, pressure, presence of impurities and/or starter crystals. Even minor changes in these parameters can significantly affect the resulting crystal structure.

4. Extruded implants

The preparation of implants via extrusion might offer interesting advantages compared to the above-described compression and melting methods, including a potentially faster and more easily up-scalable production procedure. For example, implants based on polyglycerol esters (of fatty acids) have been prepared by extrusion of molten drug:lipid blends using a stainless needle. Interferon-α release from these systems was sustained over more than 10 day [38]. Lipid extrudates can also be used for oral controlled drug delivery [71-75]. In this case much shorter release periods (several hours) are targeted, and often high drug loadings are used and hydrophilic fillers added.

Great care needs to be taken when defining the extrusion conditions. An interesting recent report from Reitz and Kleinebudde [76] details the effects of several formulation and processing parameters of theophylline-loaded, glyceryl palmitostearate and glyceryl trimyristate-based extrudates on the resulting systems' properties. As can be seen in **Figure 9**, the extrusion temperature significantly affects the porosity of the obtained systems as well as the inner and outer morphology of the devices. The melting range of glyceryl trimyristate is 55 – 58°C. When extruding above 55°C, cylinders with a relatively high porosity (12.6%) are obtained, providing rather fast drug release (80% drug release within < 8 h). Decreasing the extrusion temperature down to 50.5 and 49.5% leads to significantly decreased device porosities (6.8 and < 2%, respectively) and thus much slower drug release rates (80% drug release within > 15 and > 30 h, respectively). Importantly, the processing of the lipids by *softening* instead of *complete melting* can reduce the risk of the formation of meta-stable polymorphs. It has to be pointed out that the use of elevated temperature during extrusion might not only alter the physical state of the matrix former, but might also lead to drug degradation. In addition, drug–lipid interactions can result in decreased drug

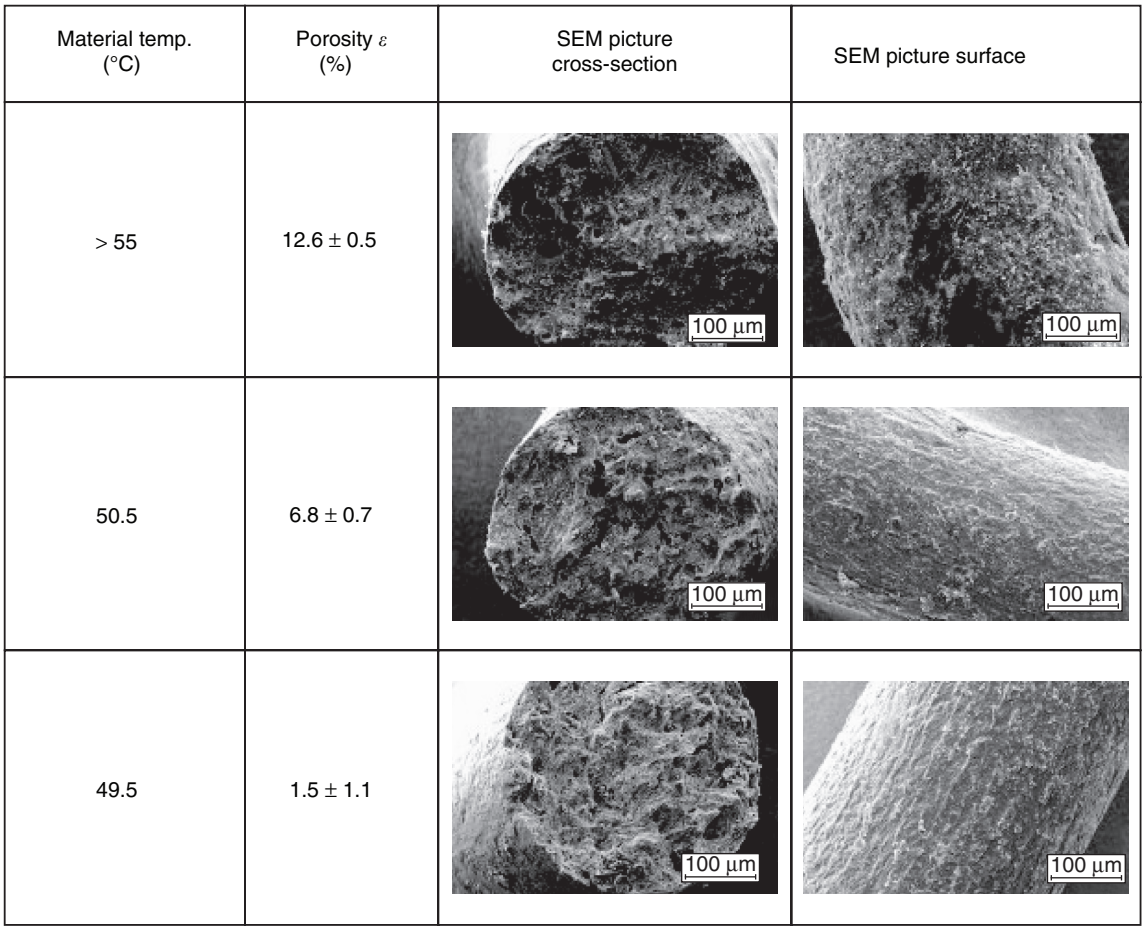

Figure 9. Effects of the extrusion temperature on the resulting porosity as well as the inner and outer structure of lipid cylinders consisting of theophylline:glyceryl trimyristate (50:50) prepared by extrusion.
Reproduced from [76], with permission.

activity, for example in the case of proteins. Lee *et al.* [39] proposed to protect protein drugs with a hydrophilic polymer coating from potentially harmful conditions. For example, interferon-α was spray-dried together with polyethylene glycol 10,000, and human serum albumin was added as a further stabilizer. Importantly, the interferon showed no denaturation in trilaurin-based implants after extrusion using a ram-type extruder at 37°C.

5. Drug release mechanisms

The mechanisms controlling drug release from lipid implants can be complex and may depend on various factors, including the type and amount of lipid used, the type and amount of incorporated drug, the drug distribution within the implant, the type of preparation technique, the type and amount of potential additional excipients present in the formulation and the mechanical stability of the systems. In most cases, diffusion plays a major role. In several types of systems, diffusion is the *dominant* mass transport mechanism. Swelling can generally be neglected because significantly, swelling devices would not be suitable in practice: a considerable increase in implant volume upon administration is likely to cause irritation of the respective human tissue.

In the case of purely diffusion-controlled lipid implants, drug release can be described using Fick's second law considering the respective initial and boundary conditions (e.g., initial drug distribution, geometry of the device, drug concentration within the bulk fluid). **Figure 10** shows the experimentally determined (symbols) and theoretically calculated (curves) release kinetics of pyranine from glyceryl tripalmitate-based implants containing different amounts of saccharose (indicated in the **Figure**). The mathematical model takes into account radial as well as axial mass transport in cylinders and considers the homogeneous initial drug distribution within the devices before exposure to the release medium, as well as perfect sink conditions (which are provided throughout the experiments). Under these conditions, the following analytical solution of Fick's second law can be derived [77]:

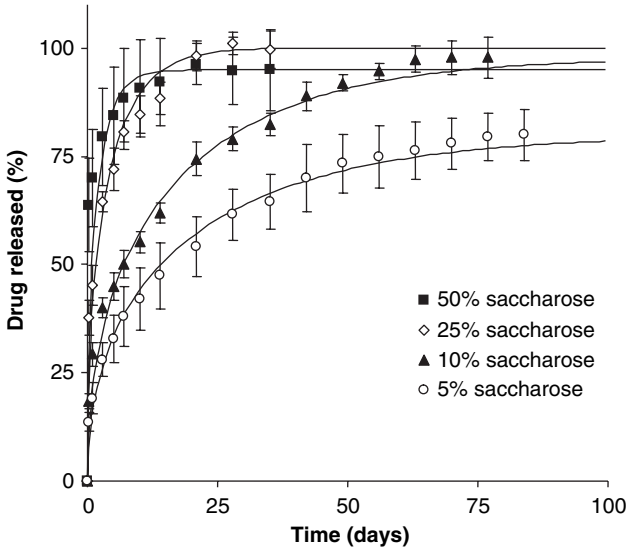

Figure 10. Experiment and theory. Pyranine release from glyceryl tripalmitate-based implants containing different amounts of saccharose (indicated in the **Figure**). The symbols represent the experimental results, the curves the fittings of an appropriate solution of Fick's second law of diffusion considering the given initial and boundary conditions (Equation 1).
Reproduced from [43], with permission.

$$\frac{M_t}{M_\infty} = 1 - \frac{32}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{q_n^2} \cdot \exp\left(-\frac{q_n^2}{R_c^2} \cdot D \cdot t\right) \cdot \sum_{p=0}^{\infty} \frac{1}{(2 \cdot p + 1)^2} \cdot \exp\left(-\frac{(2 \cdot p + 1)^2 \cdot \pi^2}{H^2} \cdot D \cdot t\right) \quad (1)$$

where M_t and M_∞ represent the absolute cumulative amounts of drug released at time t and infinite time, respectively; q_n are the roots of the Bessel function of the first kind of zero order [$J_0(q_n) = 0$], and R and H denote the radius and height of the cylinder; D is the apparent diffusion coefficient of the drug within the lipid matrix. Good agreement between theory and experiment was obtained in all cases (**Figure 10**), indicating that drug release is primarily controlled by pure diffusion, irrespective of the saccharose content in these implants.

When further excipients are added to the implants, the governing mass transport mechanisms can become more complex. For example, Herrmann *et al.* [47] studied IFN-α release from glyceryl tristearate-based implants prepared by compression. In order to modify the resulting protein release kinetics, different amounts of PEG were added. Interestingly, IFN-α release was primarily diffusion controlled in PEG-free devices, but in the presence of this release modifier significant deviations between the respective solution of Fick's second law and the experimental results were observed. This was rather surprising, because the release of PEG itself into the release medium was predominantly diffusion controlled, irrespective of the initial PEG content, as can be seen in **Figure 11**. Good agreement between experiment and theory (Equation 1, with M_t and M_∞ representing the absolute cumulative amounts of PEG released at time t and infinite time, respectively; and with D denoting the apparent diffusion coefficient of PEG within the lipid matrix) was obtained in all cases. Importantly, the significant deviations between the diffusion theory and the experimentally determined protein release kinetics from PEG-containing implants could be attributed to the tremendous decrease in IFN-α solubility in the presence of PEG. **Figure 12** shows the concentration of this protein in phosphate buffer pH 7.4 at 37°C in the presence of different amounts of PEG (filled diamonds). Please note that values indicated for PEG concentrations < 4% do not correspond to the solubility of the protein (the latter being higher), whereas those > 4% do. Clearly, above 4% PEG the solubility of the protein drastically decreases. Thus it can be expected that the amount of protein that is soluble in the water-filled channels within the lipid implants is determined by the PEG concentration in this aqueous phase. Therefore PEG does not only act as a pore former in this case, but mainly as a precipitation agent for the drug. As can be seen in **Figure 12**, the presence of hydroxypropyl-β-cyclodextrin (HP-β-CD, being used as a stabilizing agent for IFN-α) did not affect this phenomenon (open triangles). Importantly, the precipitation effect of PEG was not observed at pH 4.0 (asterisks in **Figure 12**: please note that the indicated protein concentrations do not correspond to the IFN-α solubility, the latter being higher), and drug release was purely diffusion controlled under these conditions [48].

It must be pointed out that not only *drug* diffusion is of importance within lipid implants, but also the diffusion of *water* into the system can play a major role [44,51]. As recently shown by Koennings *et al.* [44], the penetration of the aqueous release medium into glyceryl trimyristate-based implants is not instantaneous and is considerably affected by the presence of different amounts of surfactants in the bulk fluid. **Figure 13** shows confocal microscopy pictures illustrating the penetration of the bulk fluid into the implants as a function of time and the concentration of Tween 20 in the release medium: 0.1% in the upper row, 0.01% in the lower row.

The mechanisms controlling drug release from lipid implants are not yet fully understood. The examples discussed above are intended to give an idea of which type of mass transport processes can be involved and how straightforward or complex the systems can be. A thorough understanding of the underlying drug release mechanisms in a specific device can be highly beneficial, because device optimization and troubleshooting during production is much easier if there is a clear understanding of how the system works. In addition, appropriate mathematical theories can allow for the quantitative prediction of the resulting drug release kinetics as a function of formulation and processing parameters. In this way time- and cost-intensive series of trial and error studies can be avoided.

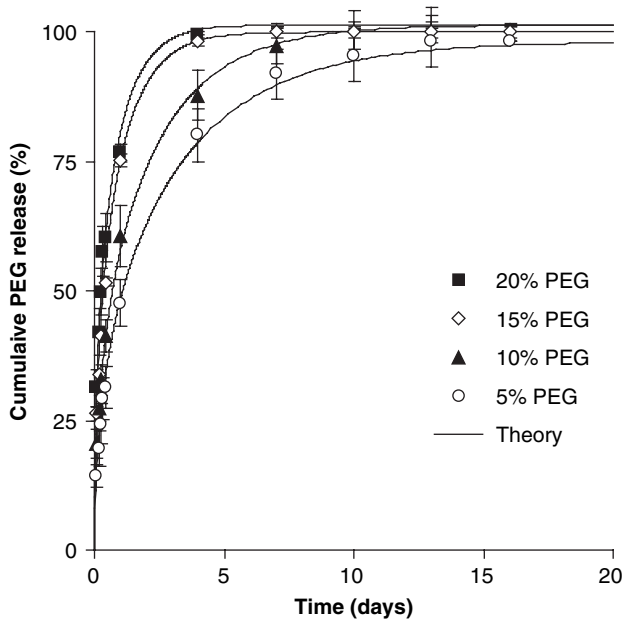

Figure 11. PEG release from glyceryl tristearate-based, IFN-α-loaded implants containing different amounts of this release modifier (indicated in the Figure). Experimental results (symbols) and theory (Equation 1, assuming purely diffusion controlled PEG release).
Reproduced from [47], with permission.

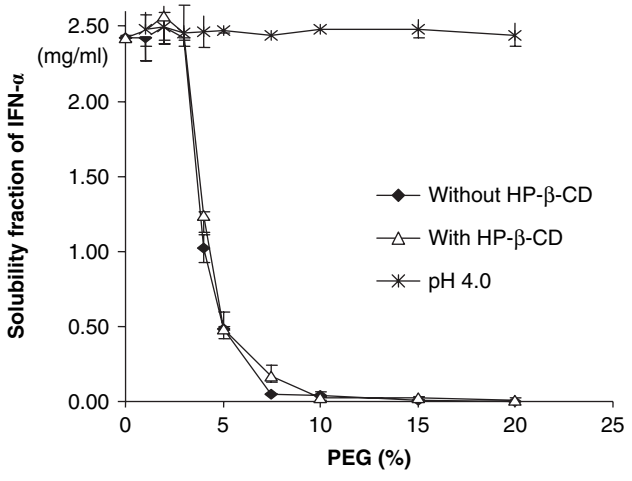

Figure 12. Effects of the presence of different amounts of PEG on the IFN-α concentration in phosphate buffer pH 7.4 at 37°C. For reasons of comparison, the results obtained in the presence of HP-β-CD and at pH 4.0 are also indicated. Please note that the values at PEG concentrations < 4% at pH 7.4 and all PEG concentrations at pH 4.0 do not correspond to protein solubility, in these cases all of the protein used in this experiment was dissolved.
Reproduced from [48], with permission.

6. *In vivo* testing

Most of the lipids used as matrix formers in controlled release implants are known to be safe upon oral administration, because they are common excipients in the food industry. Many of them are physiological substances. However, their safety and fate upon *parenteral* administration in high amounts and in the form of implants is generally unknown. In various cases it is unlikely that serious immune reactions will be caused, but the exact tissue reaction needs to be investigated for each particular type of lipid. Recently, Guse *et al.* [78] reported on the biocompatibility of glyceryl tripalmitate-based cylinders (2 mm in diameter) upon subcutaneous administration into immunocompetent mice. As can be seen in **Figure 14**, the implants were encapsulated by fibroblasts. The picture on the left-hand side shows Masson and Goldner stained tissue after 4 days of implantation, the one on the right-hand side was obtained after 30 days of implantation. Importantly, the intensity of the encapsulation was low and comparable to that of PLGA-based implants. Furthermore, no significant inflammatory reactions were observed and no noteworthy implant swelling was monitored. The implants remained intact during 60 days, demonstrating that they are suitable to provide long-term controlled drug delivery. However, if surgical resection is to be avoided after drug exhaustion, complete biodegradability should be guaranteed. However, the fate of many lipid implants for longer time periods is unknown, although different strategies could be followed if biodegradation is to be accelerated, for example small amounts of gelatin might be included [78]. Walduck [79] administered cholesterol/lecithin-based implants subcutaneously into sheep for the delivery of a recombinant antigen. Interestingly, these implants caused even less local reactions than injected, conventional vaccines. Other biocompatibility studies have been reported for lipids, including different types of devices [80,81]. For example, Reithmeier *et al.* [80] tested glyceryl tripalmitate-based microparticles in mice. The observed reactions were comparable to those obtained with the equivalent PLGA-based systems.

Various *in vivo* studies have clearly provided the proof of concept for the pharmacodynamic efficiency of lipid implants as controlled drug delivery systems [13,36-39,78,79,81-87]. For example, Wang [13] administered insulin-loaded, stearic acid-based implants (which were prepared by compression) subcutaneously into Wistar rats with streptozotocin-induced diabetes. As can be seen in **Figure 15**, the resulting blood glucose levels could be effectively normalized in several weeks by this treatment. Khan *et al.* [87] prepared bovine serum albumin (BSA)-loaded lipid implants (also via compression), BSA serving as model antigen in this case. Cholesterol as well as blends of cholesterol and hydrogenated egg lecithin were used as lipid matrix formers. The devices were implanted subcutaneously into mice and the BSA antibody response was monitored over 10 months. The sustained release of BSA from the implants provided an antibody production in the

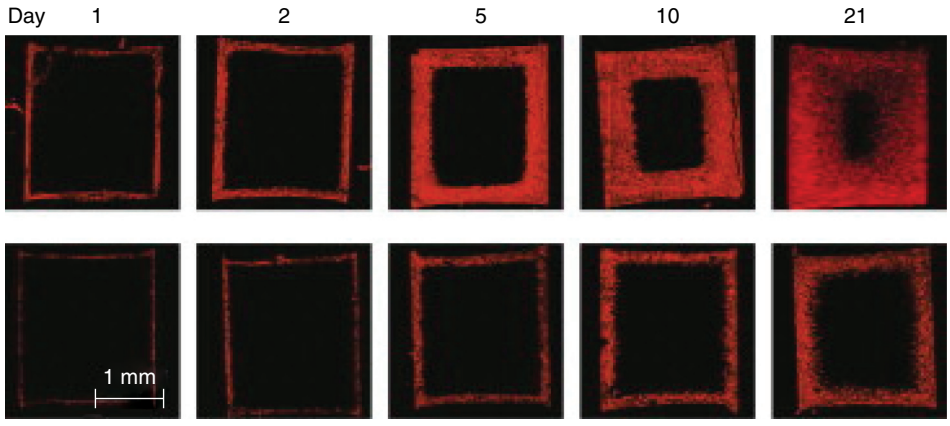

Figure 13. Confocal microscopy pictures illustrating the penetration of the aqueous release medium into trimyristate-based implants as a function of time and the surfactant concentration in the bulk fluid. Upper row, 0.1% Tween 20; lower row, 0.01% Tween 20.
Reproduced from [44], with permission.

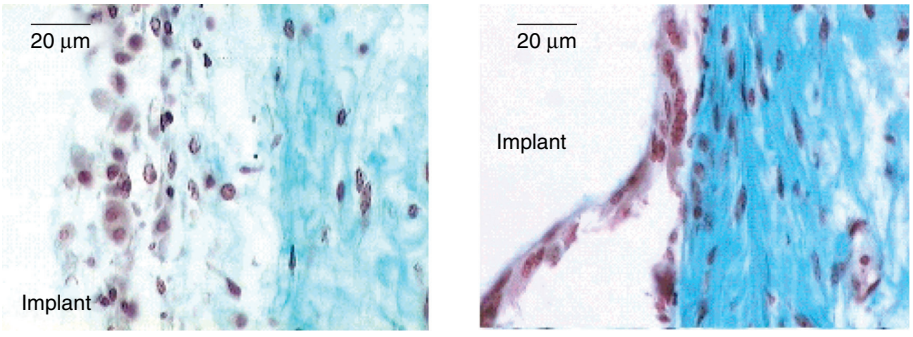

Figure 14. Tissue reaction in immunocompetent NMRI mice upon subcutaneous administration of cylindrical glyceryltripalmitate implants (diameter = 2 mm). Light microscopy pictures obtained upon tissue staining with Masson & Goldner after 4 days (left-hand side) and after 30 days (right-hand side) implantation.
Reproduced from [78], with permission.

animals during the entire observation period, and this response was more pronounced than that induced by three injections of the same BSA dose (studied for reasons of comparison).

7. Conclusion

Lipid implants offer great potential as parenteral controlled drug delivery systems. In particular in the case of acid-labile drugs, which lose their biological activity within PLGA-based devices (due to the creation of acidic micro-environments upon polyester degradation), this type of advanced pharmaceutical dosage forms could become of major practical importance. Interestingly, large spectra of drug release patterns can be provided by varying different formulation and processing parameters. The biocompatibility of the systems can be expected to be good in most cases. Major challenges to be addressed in future developments include a more precise control of the degradation behavior *in vivo* and the establishment of production techniques that are robust and easily up-scalable. A thorough understanding of the underlying drug release mechanisms can be of great help for facilitated device optimization during product development and troubleshooting during production.

8. Expert opinion

Due to significant advances in biotechnology in the last decade, more and more protein-based drugs have become available in significant quantities and at reasonable prices. Many of them are highly promising and might allow for the development of novel therapeutic strategies. This might particularly be true for the treatment of various major diseases in industrial countries, including cancer, cardiovascular and neurodegenerative diseases (e.g., Alzheimer's and Parkinson's Diseases).

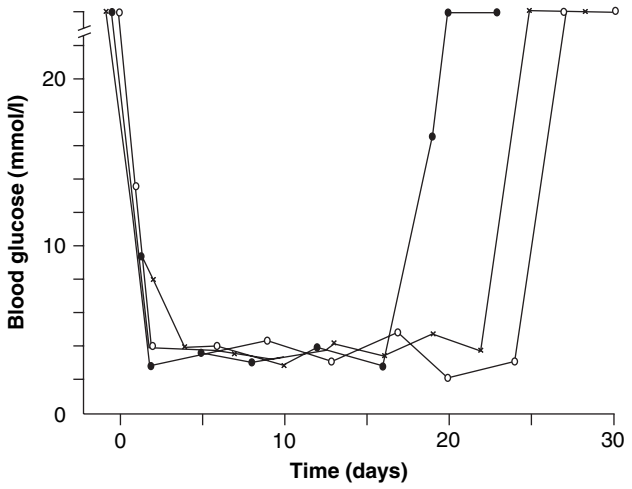

Figure 15. *In vivo* efficiency of lipid-based controlled release implants. Blood glucose levels in three Wistar rats with streptozotocin-induced diabetes upon subcutaneous administration of insulin-loaded, stearic acid-based implants.
Reproduced from [13], with permission.

However, to become active these drugs need to be adequately administered to the patient, and this presents a major challenge during product development for protein-based drugs. At present, oral administration is not feasible because proteins are rapidly degraded by enzymes within the gastrointestinal-tract (GIT), the respective mucosal membranes are poorly permeable for macromolecules and the residence times within the GIT are limited. Various promising research strategies are presently being pursued in order to overcome these restrictions. Some of them are based on bioadhesive, controlled drug delivery systems containing enzyme inhibitors and absorption enhancers, thus allowing for increased residence times, reduced protein degradation and enhanced uptake into the systemic circulation. But the implementation of these strategies and the introduction of real products into the market will take considerable time (if this approach does indeed show to be feasible).

In addition, protein-based drugs generally exhibit very short half-lives *in vivo*. Thus, frequent parenteral administration is required. Time-controlled drug delivery systems, releasing the drug at a pre-determined rate over prolonged periods of time (e.g., several weeks or months) might be used to overcome this restriction. At present, PLGA-based microparticles are the standard parenteral drug delivery system allowing for time-controlled release rates. However, these devices are not suitable for protein-based drugs: upon degradation of the polyester PLGA, shorter chain acids are formed, resulting in potentially significant drops in the micro-environmental pH. Many protein-based drugs lose their activity under these conditions. If no efficient and easy solution allowing for an effective stabilization of acid-labile drugs within degrading PLGA-based microparticles is found, lipid implants have the potential to become one of the standard future parenteral drug delivery devices. They allow for an effective avoidance of acidic micro-climates and can provide large spectra of drug release patterns. Various formulation and processing parameters can be varied in order to achieve the desired drug release profiles and several preparation techniques can be used to incorporate drugs within the lipid matrices. Importantly, major toxic reactions due to the delivery system itself are unlikely, because lipids that are well known from the food industry can be used as matrix formers. Of course, lipid implants can also be highly suitable for the time-controlled, parenteral delivery of acid-stable drugs. The mass transport mechanisms controlling drug release from this type of advanced pharmaceutical dosage forms can be very complex and yet only limited knowledge is available in this field. An important practical benefit of a thorough understanding of how the systems work can be very helpful to more easily develop and optimize this type of advanced drug delivery systems.

Declaration of interest

The authors state no conflict of interest and have received no payment for the preparation of this manuscript.

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