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Role of *in vitro* release models in formulation development and quality control of parenteral depots

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This review article provides an assessment of advantages/limitations of the use of current *in vitro* release models to predict *in vivo* performance of parenteral sustained release products (injectable depots). As highlighted, key characteristics influencing the *in vivo* drug fate may vary with the route of administration and the type of sustained release formulation. To this end, an account is given on three representative injection sites (intramuscular, subcutaneous and intra-articular) as well as on *in vitro* release mechanism(s) of drugs from five commonly investigated depot principles (suspensions, microspheres, hydrogels, lipophilic solutions, and liposomes/other nano-size formulations). Current *in vitro* release models are, to a different extent, able to mimic the rate, transport and equilibrium processes that the drug substance may experience in the environment of the administration site. Their utility for the purpose of quality control including *in vitro–in vivo* correlations and formulation design is discussed.

Keywords: convection and diffusion-controlled mass transfer, drug release mechanism, injection site, *in vitro–in vivo* correlation, *in vitro* release model, parenteral depot

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1. Introduction

Injection constitutes often the only realistic route of administration for active agents with low oral bioavailability resulting from poor biomembrane transport properties or lack of stability in the environment of the gastrointestinal (GI) tract. In case chronic use is required, maintenance of therapeutic drug concentrations over extended periods of time can be achieved by frequently repeated injections or, more ideally, by immobilisation of the active agent in the form of an injectable sustained release formulation (depot) from which the drug is released in a controlled manner. Injectable depots enabling drug release in the vicinity of the target area (localised drug delivery) reduce drug exposure to inappropriate sites and hold promise, for example, in the field of osteoarthritis [1].

For new therapeutic macromolecules the drug delivery methodology may impact efficacy as much as the nature of the drug itself [2]. Also, the selection of proper parenteral depot technologies for small-molecule drug candidates can affect the therapeutic value significantly. Demands for an advanced drug delivery system (DDS) may markedly influence overall project time as well as costs. In fact, the development of a new parenteral depot formulation of an active pharmaceutical ingredient (API) may constitute a significant challenge. From a regulatory point of view the drug product specifications is a key document through which reproducible product quality, or in other words batch-to-batch consistency of product performance,

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has to be documented, including drug release rate from the formulation. Recent workshops on *in vitro* release methods have emphasised the need for *in vitro* release methods [3-5]. In contrast to oral as well as certain special dosage forms [6], no regulatory approved standard methods exist at present for testing drug release from sustained release parenteral products.

2. *In vitro* release models – basic principles

The development of suitable *in vitro* release models (for quality control as well as formulation development purposes) is a critical activity, which, preferably, should be initiated in the early depot design phase. These efforts should ideally lead to the establishment of an *in vitro–in vivo* correlation (IVIVC). This usually requires that drug release from the depot be the rate-limiting step in the absorption process and that the drug release mechanism is the same *in vitro* and *in vivo*. Most often such point-to-point relationships are linear; however, nonlinear correlations are also acceptable [7]. The ideal approach to IVIVC modelling is to develop one IVIVC model for the total plasma profile, but other approaches might also be pursued [8]. Importantly, the development of a true IVIVC requires that a mathematical model describes the *in vitro–in vivo* relationship for two or more formulations showing different release characteristics [9]. When a meaningful IVIVC has been established, it can be used as a surrogate for bioequivalence and for minimising the number of bioequivalence studies to be performed during drug product development [7]. As regards the evaluation of the effect of minor changes in the composition or manufacturing process of an already marketed product on the *in vivo* performance of the parent drug, *in vitro* release profiling might be of value and eventually form the basis for granting a biowaiver.

Independent of formulation principle and injection site, a common feature of depots comprising poorly water-soluble drugs (such as aqueous suspensions and lipophilic solutions) is that analytical sensitivity may become critical, necessitating: i) recycling of the release medium [10]; ii) the involvement of solubilisation principles such as addition of β-cyclodextrins [11]; or iii) the use of radiolabelled drug substances [12]. Prohibitive for release model development might also be problems with drug stability and drug adhesion to surfaces.

In the field of sustained release parenterals, *in vitro* release methods used might be divided into three broad categories: i) sample and separate (S-S) methods; ii) continuous flow (CF) methods; and iii) dialysis membrane-based (DMB) techniques [13]. Within each category a variety of experimental set ups have been used, as is apparent from recent reviews dealing with the feasibility of various methods used for the study of drug release from polymeric particulate systems [14] and lipophilic solutions [1]. A brief account is given below of the three different types of *in vitro* release method, the basic principles of which are sketched in **Figure 1**. The common characteristic of the S-S methodologies is that the formulation (microsphere-based or a gel) is introduced into a vessel/vial containing the release medium (e.g., phosphate buffer pH 7.4). The closed system is left at constant temperature, usually subjected to agitation. At predetermined time intervals aliquots are taken from the supernatant and analysed for released drug. To maintain a constant volume of the release medium an identical volume of fresh buffer is added to the release system after each sampling. In the CF technologies, which encompass the USP (United States Pharmacopeia) apparatus 4, the microparticulate formulation is placed in a (cylindrical) release cell. At constant flow rate, release buffer is pumped into the cell where the concentration of drug released from the depot might be monitored directly from the effluent (open system) or the release buffer is recirculated (closed system). In the latter case samples for analysis are taken from a well-stirred reservoir. Among the DMB methods, which most often can be referred to as two-compartment release models, the rotating dialysis cell model has been characterised extensively [15]. This model consists of a small donor compartment (5 – 8 ml) separated from a large acceptor compartment (1000 ml) by a dialysis membrane. Under different experimental set ups it has been used to investigate drug release from lipophilic (oily) solutions, aqueous suspensions, microspheres and liposomes [1]. Key model parameters of DMB techniques that can be varied include type/mode of agitation, ratio between donor and acceptor cell volumes, and molecular mass cutoff value of the dialysis membrane.

Obviously, the ability of the above-mentioned methods to simulate the conditions prevailing in the vicinity of the injection site is expected to vary considerably. Furthermore, for a given depot formulation significantly different drug release profiles might be generated using *in vitro* release methods differing with respect to sink conditions as well as hydrodynamics (geometries/flow conditions).

According to the dissolution procedures described in the USP, sink conditions exist when the volume of the medium is at least three times that required to form a saturated solution of the drug substance (USP [16]). With reference to the Noyes-Whitney equation, sink conditions are achieved as long as bulk drug concentration, C_b (in the concentration gradient term ($C_s - C_b$)) remains less than one-third of the drug saturation solubility (C_s). Previously, it has been recommended that in general the drug concentration in the sink phase in dissolution experiments should be kept below 10% of saturation [13]. Maintenance of sink conditions has long been aimed at in the *in vitro* release testing of oral formulations based on the rationale that the diffusional pathway to the absorption site in the GI tract is very short, and the drug molecules are almost instantly absorbed into what is, for all practical purposes, a perfect sink [17]. Although the degree of deviation from a perfect sink seems to affect the shape of *in vitro* drug release profiles [18], quantitative descriptions of the relationship between sink condition and drug release rates obtained using S-S, DMB and closed CF methodologies are apparently lacking.

Less well-defined hydrodynamics have been a contributing factor to the unsatisfactory reproducibility of dissolution data

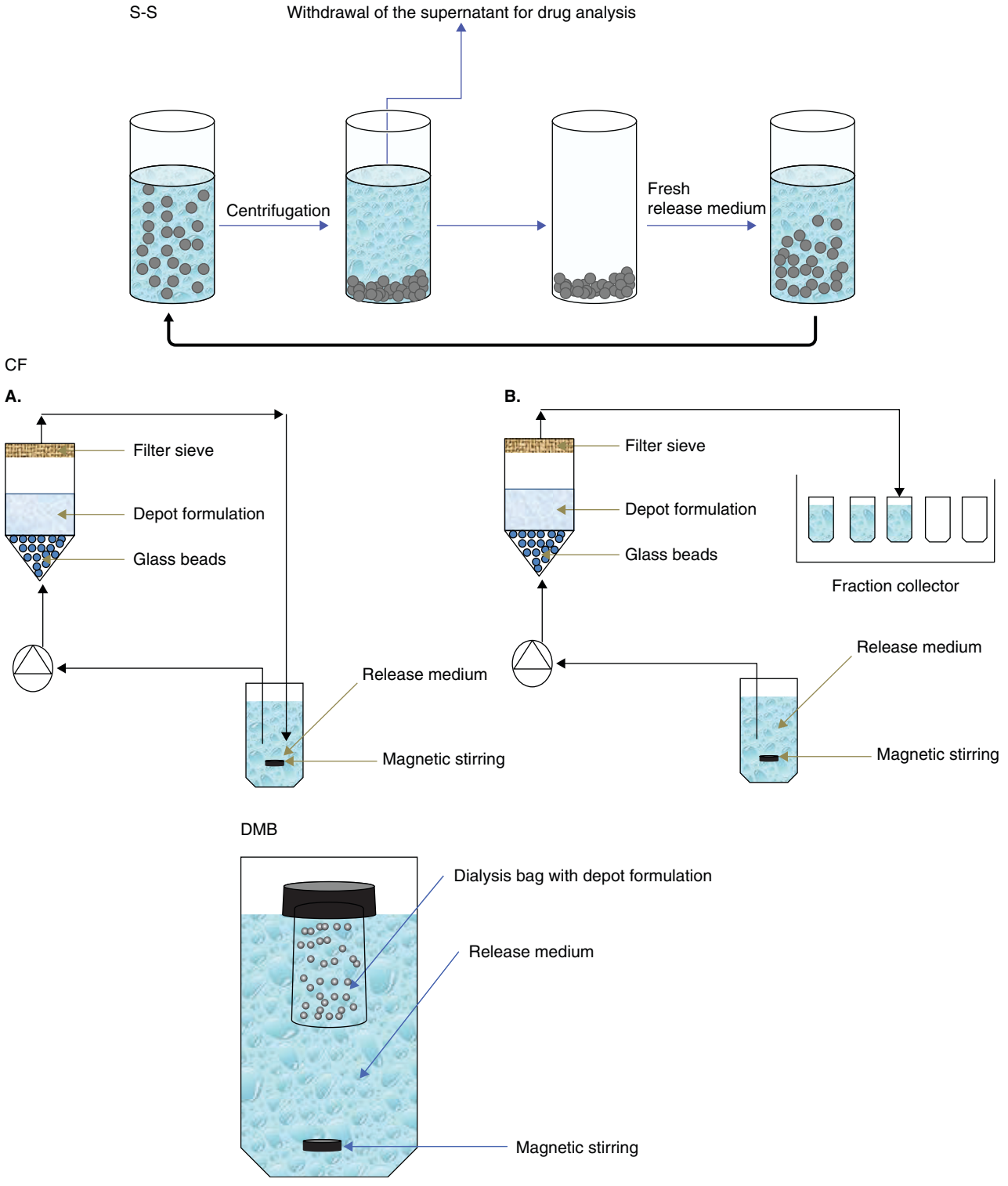

Figure 1. Basic principles of three different types of *in vitro* release sketched as examples of a sample and separation method (S-S), a closed (A) and an open (B) continuous flow method (CF) and a dialysis membrane-based technique (DMB).

sometimes observed by using early compendial dissolution systems, including the USP type 1 and type 2 methods (rotating basket and paddle) [10]. The inherent complexity of this process might be reflected in the fact that only under rather idealised conditions has a convective diffusion model been found to enable a quantitative description of initial drug dissolution in terms of solubility, diffusivity, rate of shear (agitation), and geometry and orientation of the surface relative to flow [19,20]. The mathematical expression for mass transport by diffusion and convection is given by the equation:

$$\frac{\partial c}{\partial t} = D \cdot \nabla^2 c - V \cdot \nabla c \quad (1)$$

where c and D are the local solute concentration and diffusivity, respectively. In the present context ∇ is the three-dimensional partial derivative operator. V is the local velocity of the solvent. The latter parameter might be determined from an independent solution of the Navier-Stokes equation. The first term on the right-side is the diffusion contribution, whereas the second arises from convection. In the field of release testing of sustained release formulations a modified version of Equation 1 might be useful:

$$\frac{\partial c}{\partial t} = D \cdot \nabla^2 c - V \cdot \nabla c + q \quad (2)$$

where the parameter q is a drug release term, the form of which depends on the *in vivo* drug release mechanism from the depot formulation in question [21]. Numerical solutions to Equation 2 might be obtained by, for example, the finite element method [21] (possibly in combination with the Navier-Stokes equation).

3. Common routes of administration for sustained release parenterals

The therapeutic agent, released from the depot, might exert local drug action in the vicinity of the administration site (e.g., after intra-articular [i.a.] injection) or the active agent has to be transported to the site of action through the general circulation. In case of intramuscular (i.m.) or subcutaneous (s.c.) instillation, the drug is released into the tissue fluid and conveyed to the capillaries by diffusion and/or convective processes. A brief treatise of the biological environments of these three main routes of depot administration is given below. As to the i.a. route of administration, long-lasting corticosteroid suspensions have been used for more than three decades, providing symptomatic relief of pain in rheumatoid arthritis (RA) and osteoarthritis (OA) [22]. Also, parenteral long-acting lipophilic (vegetable oil) solutions, mainly for i.m. injection, have been used clinically for many years in the field of schizophrenia and hormone replacement therapy [23,24]. Various insulin products are given s.c. [25]. Subcutaneous administration presumably continues to represent the primary route of delivery for protein-based drugs [26].

The bones of a synovial joint are separated by an articular cavity containing the synovial fluid (SF). The adjoining surfaces of the bones are covered with a layer of articular cartilage and a two-layer joint capsule encloses the cavity. The joint capsule consists of an outer fibrous capsule and an inner synovial membrane (synovium). The various cell types populating the articular cartilage and the synovium as well as components of the synovial fluid are major targets for RA and OA-related drug therapies [1]. In the joint cavity, the solute drug molecule, once released from the immobilised depot, may take part in several reactions and distribution (equilibrium) processes before it is eventually cleared from the synovial space (**Figure 2**). Owing to its architecture, the synovium constitutes the main barrier for drug transport out of the joint cavity. The healthy synovial lining is thin and discontinuous without intercellular junctions. Together with the extracellular matrix (ECM), the synoviocytes function as a permeable, inhomogeneous matrix [27]. The concentration gradient required for effective clearance (diffusion through ECM) of small molecules from the joint is maintained by the synovial blood flow [24,28]. By contrast, escape of proteins from the joint occurs through the lymphatic system, a process that is not size-selective for molecules with the size of plasma proteins [29-31]. To this end, lymphatic clearance of albumin has been suggested to contribute to the efflux of albumin-bound drugs [32]. It appears, however, that synovial disappearance rates may not depend just on drug affinity for proteins, but on binding avidity as well [33].

Drug absorption from i.m. and s.c. sites of depot injection may share some gross common features [34]. Absorptive processes that may occur on injection into muscle or subcutaneous sites are presented in **Figure 3**. The active agent is released into the tissue fluid and has to traverse the interstitium to reach a blood capillary or a lymphatic vessel. The basic structure of the interstitium is similar in all tissues consisting of a collagen fibre framework that contains a gel phase made up of glycosaminoglycans, a salt solution, and proteins derived from plasma. Although the components are principally the same in all tissues, their relative amounts vary greatly [35]. The size exclusion-like properties of the interstitium significantly reduce diffusion of plasma proteins and other macromolecules in the ECM. Compared with the interstitial fluid drainage by the lymph, the rate of filtration and reabsorption of fluid across the vascular capillaries is ~ 10-fold higher. Thus, drug molecules (with molecular masses below ~ 2 kDa), which are capable of entering blood and lymph capillaries at comparable rates, will be cleared predominantly by the blood vessels [26]. On the other hand, macromolecules above ~ 16 kDa as well as particulates are preferentially removed from the tissue by the lymphatics [36]. The contribution of lymphatic uptake of macromolecules to the overall absorption process seems to be of most importance in relation to the s.c. injection site because only a few lymph vessels are located in muscle tissues [37]. After i.m. injection (rats) of sesame oil containing fluphenazine decanoate, however, drug was detected in lymph nodes located in the vicinity of the

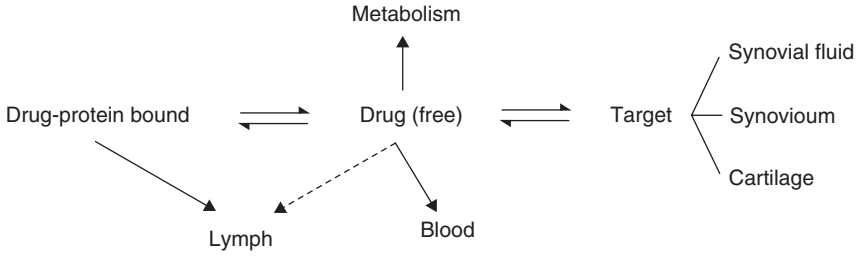

Figure 2. Schematic representation of drug transport and distribution processes of the joint.
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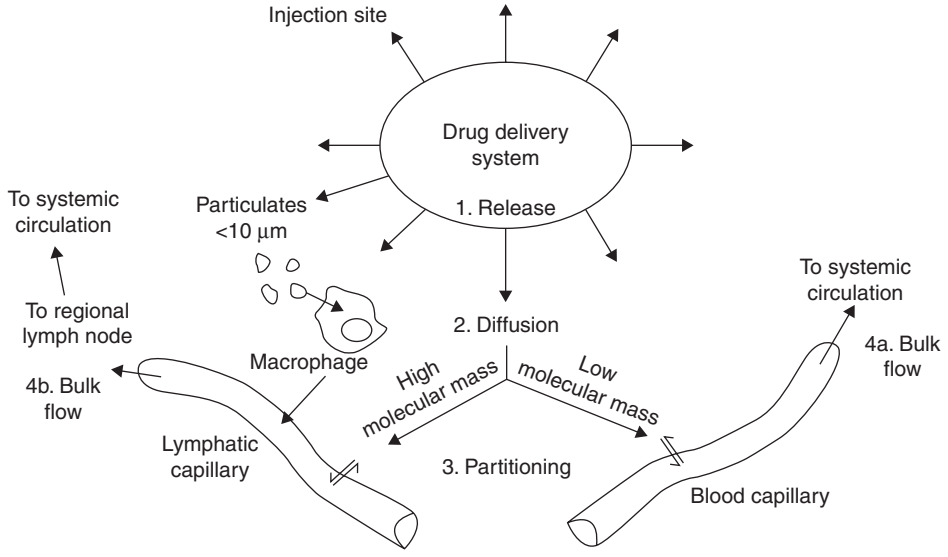

Figure 3. Possible pathways for absorption of drugs from controlled release parenteral dosage forms at intramuscular or subcutaneous sites.
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injection site. It was suggested that small (drug-containing) oil drops were absorbed by the local lymphatic system [38,39].

Abundance and flow rate of blood and lymphatic supplies are contributing factors to differences in drug absorption rates for immediate release products administered at the various injection sites. In sustained release parenterals, however, the rate-limiting step in the absorption is controlled by the delivery system, thereby reducing potential inter-injection and inter-patient variability resulting from differences in injection site perfusion [34]. The impact of the interfacial area between depot surface and the aqueous tissue fluid and tissue responses to depot instillation is dealt with in Section 5.

4. Mechanism(s) of *in vitro* drug release from commonly investigated depot principles

As outlined below, drug release patterns from various depot types may vary depending on the *in vitro* release model used. In most cases investigations were performed under aimed sink conditions, providing a feasible 'reference release condition' that may not necessarily mimic the release processes taking place at the injection site *in vivo*.

4.1 Lipophilic solutions

Parenteral long-acting lipophilic solutions comprise a lipophilic drug (or more often a lipophilic prodrug derivative) dissolved in a vegetable oil and, occasionally, a few extra excipients such as benzyl benzoate (solubilising agent) or tocopherols (antioxidants). Frequently used oil vehicles include sesame oil, miglyols (medium chain triglycerides) and castor oil. The oils differ with respect to the fatty acid chain length and the ratio between the content of saturated and unsaturated fatty acids as well as the viscosity. Compared with other parenteral depot principles, key attributes of such relatively simple oil-based solutions encompass uncomplicated manufacture (including terminal sterilisation) and feasible long-term stability. Also, this formulation principle opens up the possibility of the design of depots with tailored delivery

characteristics because manipulation of oil–water distribution coefficients might be obtained through prodrug formation or optimisation of oil vehicle composition [40-42].

It is generally accepted that the drug release rate from lipophilic solutions is governed at least partly by drug partitioning between the oil vehicle and the aqueous tissue fluid [24,39,43]. Injection volume as well as interfacial tension of the oil appear to be among the factors affecting spreading [44]. The latter parameter, together with the fate of the oil vehicle *per se*, might affect the overall pharmacokinetic fate of the therapeutic agent. The rotating dialysis cell model has been used to study drug release from oil vehicles under two different donor cell conditions: i) the oil depot was added to an empty donor compartment; and (ii) the oil depot was added to the donor cell containing an aqueous buffer solution. In the former set up a linear relationship was established between the logarithmic forms of the partitioning pseudo-first-order rate constant and the drug distribution coefficient (oil-buffer) [40,41]. By contrast, with aqueous buffer present in the donor cell the rate constant could be expressed quantitatively by several parameters, including the drug permeability coefficient (transport across the dialysis membrane) and the oil-buffer distribution coefficient (oil-aqueous buffer) [45]. Similar release experiments were performed using the commercially available Float A Lyzer® system as an alternative dialysis membrane-based model, revealing slower drug release rates not obeying first-order kinetics [46]. These observations most probably reflect that the Float A Lyzer model, in contrast to the rotating dialysis cell, works with only gentle agitation of the donor compartment, resulting in lower drug transport rates. As demonstrated for a s.c. injected oil solution of bupivacaine (analgesic effect lasted for ~ 1 day) [47], linear *in vitro–in vivo* relationships might be established. First-order *in vivo* drug absorption was observed, indicating that solute diffusion in the oil phase was not the rate-limiting step in the overall release process.

4.2 Liposomes and other nano-size lipidic drug delivery systems

In addition to liposomes, nano-size lipidic DDS embody solid lipid nanoparticles [48] and different nanostructured aqueous dispersions [49]. Numerous reports, particularly in the field of liposomes, indicate that such advanced DDS might be of potential utility to overcome various barriers to drug delivery, including biocompatibility, fast elimination and target access [48,50,51] after intravenous, i.a. or s.c. injection. Sustained release activities after i.a. injection of such DDS have been reported [50]. Their sustained release properties are, however, less easy to assess because poorly water-soluble drugs, for example cortisol 21-palmitate [52], most often were incorporated into these systems. In this context it should be mentioned that the DepoFoam® technology, which shows some resemblance to multivesicular liposomes, seems feasible for sustained drug delivery [53].

For nanostructural lyotropic liquid crystalline phases most release experiments have been done using a rotating basket method where a micro-beaker containing the drug formulation (ensuring a well-defined surface area) was placed inside the basket [54]. In the *in vitro* release testing of liposomes, DMB techniques have frequently been used. This also seems to be the case for solid lipid nanoparticles [55,56]. Recently, CF methods have also been recommended [57]. The application of S-S methods for liposomes is not appropriate because the separation process can result in sedimentation of the lipids. In the DMB methods, the nanoparticulate system is separated from the acceptor phase by a semipermeable membrane. This might lead to violation of sink conditions in the donor compartment during the release experiments. Alternatively, a reverse dialysis bag technique developed for colloidal carriers [58] might be applicable. In spite of the large number of studies related to liposomal formulations, *in vitro* drug release mechanisms are not fully elucidated. Drug release may involve drug diffusion across the lipidic bilayer or degradation of the membrane [57,59]. In a few cases first-order drug release kinetics has been reported [60]. It is worth noting that drug release from such vesicles is strongly related to the physical and chemical stability of the liposomes. Loss of the entrapped drug is influenced by the liposome composition, its size and the physical state of the bilayer [60,61]. *In vitro* liposome stability data are of minor if any use in the prediction of the stability of this formulation type *in vivo* [60,62].

4.3 Microspheres

Sustained release properties of biodegradable microsphere preparations, with the drug substance dispersed in the polymer-based matrix, are well recognised. Synthetic polymers, including polyanhydrides [63], poly(ortho esters) [64] and poly-ε caprolactone [65], as well as polymers of natural origin, such as albumin [66] and chitosan [67], have been used in the design of microspheres. Owing to their excellent biocompatibility, the biodegradable polyesters poly(lactide-*co*-glycolide) (PLGA) are the most frequently used materials for microsphere design. Although several PLGA-based products have been launched with durations of action ranging from 2 weeks to 3 months [68], their manufacture might be far from straightforward [69,70]. Often, *in vitro* drug release from microspheres have a characteristic triphasic profile: an initial fast burst release, followed by a slower sustained release and finally a faster release resulting from (bulk) degradation of the microspheres [71], creating an acidic environment within the microsphere [72]. It should be emphasised that the underlying processes involved in the control of drug release are not fully understood [73]. This can be ascribed to the fact that release characteristics are affected by several parameters, including composition/geometry [73,74], size distribution [71,75] and release conditions [76]. Interestingly, microsphere degradation by bulk erosion decreases with decreasing particle size [75], whereas low polydispersity may lead to significantly reduced burst release [2]. Typically, the *in vitro* release from microspheres has been investigated using the S-S

methodology [14]. This technique has been criticised because of the risk of aggregation of the particles [77]. This problem might be circumvented by using a modified USP apparatus 4 method [77]. On the other hand, the latter phenomenon might also be expected to occur *in vivo*. In most of the *in vitro* models used, convective mass transport plays a significant role. Recently, release data were generated by using a purely diffusion-based model as well as a traditional S-S method [78]. Perhaps rather counter-intuitively, the former model gave rise to the fastest release profile [78]. *In vitro* characterisation and comparison with *in vivo* data (most often after s.c. injection) has been done in several studies [77,79-81]. However, often the agreement between *in vitro* and *in vivo* data for PLGA microspheres is rather poor and apparently true IVIVCs are lacking at present.

4.4 Suspensions

Parenteral suspensions are dispersed, heterogeneous systems with the solid particles (usually 1 – 10 μm) suspended in a continuous phase comprising an aqueous or vegetable oil phase [82]. Long-acting suspensions for injection may be advantageous from the perspective that high drug load can be achieved and only a minimum of pharmaceutical excipients is needed. Suspension-based depot injectables are available for i.a. (methylprednisolone acetate, Depo-Medrol®, Pfizer, Denmark), i.m. (medroxyprogesterone acetate, Depo-Provera®, Pfizer, Denmark) and s.c. (protamine insulin, Insulatard®, NovoNordisk, Denmark) administration. Despite the apparent simplicity of this formulation type, parenteral suspensions pose certain challenges to the manufacturing process and physical stability [83]. Hence, *in situ* suspension-forming DDSs might constitute potential alternatives [84,85].

In vivo performance of several oil suspension-based injectables has been investigated [86]. Little is known, however, about the mechanism(s) of drug release from such particulate systems [46]. Two mechanisms describing drug liberation from oil suspensions have been proposed. This includes dissolution of the active agent in the lipophilic vehicle before release into the aqueous phase by partitioning [87]. Alternatively, the solid drug particles in the oil phase may be transported by sedimentation to the oil–water interface or directly into the aqueous medium where the drug undergoes dissolution [88,89]. Recent *in vitro* findings indicate that the oil film surrounding the particles is unstable. Thus, aqueous and oil suspensions are expected to have quite similar drug release profiles [46]. Dissolution testing of suspension dosage forms has in general been very limited, partly because of the lack of official suspension-dissolution requirements [90]. Stout *et al.* [90] have provided a brief overview of different methods used for dissolution testing of pharmaceutical suspensions. Recently, *in vitro* drug release profiles from suspensions intended for i.a. administration have been established by using the rotating dialysis cell model as well as the Float A Lyzer model [46,91]. As regards the s.c. route, an agarose-based (S-S type) model has been utilised [92].

4.5 *In situ* formed gels and implants

Considerable interest has been directed at utilising *in situ* gel/implant-forming DDSs for attaining sustained release of, in particular, peptides and proteins on injection. A depot injectable is now commercially available for s.c. injection (leuprolide acetate, Eligard®) [93]. Drug transport in gel structures such as hydrogels is well characterised [94]. Although new insights have been achieved, the complex processes governing the *in situ* formation of gel depots and related implants on injection are less well described. *In situ* gel formation or polymer precipitation for sustained drug release may be achieved through various means, as reviewed elsewhere [84,95,96]; for example, through the use of thermosensitive polymer systems or the exploitation of solvent-mediated phase transitions.

In situ gelling may be achieved through changes in temperature. For some block copolymers dehydration of polymer chains takes place with increasing temperature and a transition from aqueous liquid to gel occurs at the sol-gel transition temperature [95,97,98]. Polymer solutions capable of forming gels on injection may also be composed of water-insoluble polymers, such as PLGA, and one or a few organic solvents in addition to the drug substance. For the polymer solutions containing fully or partially water-miscible biocompatible solvents, the *in situ* gel is formed as a result of a phase inversion mechanism [99,100]. On injection, the organic solvent will escape into the tissue fluids simultaneously with the onset of water influx, leaving the water-insoluble polymer behind, which causes polymer precipitation or gelling. The solvent-dependent kinetics of the phase inversion process has a profound effect on the drug release characteristics of the *in situ* formed depot and can lead to burst/shutdown as well as zero-order release profiles [99].

For the *in situ* forming gel formulations the vast majority of published *in vitro* release experiments have been performed using variants of the S-S methodology. The pre-gelled formulation may be brought in contact with the buffer solution used as release medium (thus minimising possible initial burst effects) [97,101,102], an aliquot of buffer is added to the preformulation, which sometimes is held in a special retainer to achieve a defined geometry or formulation-buffer interfacial area [100,103,104], or the preformulation is injected into the buffer release medium using a syringe [98,105-107]. Owing to the architecture of the formulation, agitation is often accomplished using a shaking device. Sample volumes withdrawn for analysis are replaced with fresh buffer. Apparently the potential variability introduced by manually injecting the preformulation into the buffer has received little attention. Except for a few cases, a buffer containing surfactants and/or preservatives has been used as the release medium. However, it has been found that the presence of additives or serum in the acceptor medium may affect the drug release profiles [100,108]. Wang *et al.* used cerebrospinal fluid as the release medium for the *in vitro* evaluation of an injectable hyaluronan – methylcellulose hydrogel prepared

for intrathecal drug delivery [105]. Similar approaches may be of utility in the *in vitro* evaluation of other parenteral routes of administration. In addition to the S-S *in vitro* release methodology, release models utilising the DMB principle [103,109-111] have found use in the evaluation of *in situ* forming gel-type depots. The effect of placing only the formulation in the donor compartment of the dialysis system on the *in vitro* release profiles is unclear (cf. lipophilic solutions in Section 4).

In vitro as well as *in vivo* characterisation of *in situ* gel systems has been performed in several studies, for example [97-99,101,102,106,110-112]. Apparently, IVIVCs have not been established. However, recently Kempe *et al.* found quantitative agreement between the *in vitro* and *in vivo* polymer precipitation kinetics and *N*-methyl pyrrolidone-water exchange kinetics for a PLGA/*N*-methyl pyrrolidone *in situ* forming implant administered subcutaneously to mice by the use of electron paramagnetic resonance (EPR) [107].

5. Expert opinion

As long-acting depot injectables often contain substantial amounts of a potent drug substance, dose dumping or too fast release following injection may lead to severe toxicity. On *in vivo* instillation, depot removal might be difficult if not impossible. Hence, *in vitro* release testing has to be performed before batch release. For this purpose a discriminatory release model has to be constructed. Optimally, batch variations, which would lead to unacceptable changes in, for example, the pharmacokinetic profile of the drug, should be reflected in the *in vitro* release behaviour. Ideally, the *in vitro* model may simulate the *in vivo* conditions to such an extent that a level A IVIVC can be established.

The utility of *in vitro* release data as a surrogate for *in vivo* drug performance (Section 2) has given impetus to the search for more 'biorelevant' *in vitro* release methodologies [78,113,114]. To the authors' best knowledge such improvements are expected to emerge from future efforts to gain a more in-depth understanding of the processes taking place in the immediate surroundings of the administration site. On the other hand, there seem to be *in vivo* conditions that cannot easily be simulated *in vitro*. Some of these events influence the *in vivo* fate of the drug, imparting variability to the pharmacological response. In such cases release specifications may, at best, ensure that the injected formulation does not add more variability to the *in vivo* effect. This might be the case when a so-called 'second depot' (into which the drug compound is distributed) is controlling the appearance of the drug in the blood. This behaviour is related mainly to highly lipophilic drugs (or prodrugs). For testosterone esters, residence times within the body (rats) far exceeded those associated the disappearance of the derivatives (dissolved in a synthetic oil) from the i.m. injection site. These observations indicate that the lipophilic agents were distributed into fatty tissues [115]. Also, differences in the interfacial area between the surface of the depot and the aqueous tissue fluid, which cause variability in the overall apparent release rate, may arise from less predictable spreading (oils and gels) or aggregation (microspheres) phenomena taking place at both s.c. and i.m injection sites [116-118]. Owing to insertion of the needle and the injection of a certain volume of the formulation, minor pain and injury at the injection site may be anticipated even in the case where the injected material is totally inert (isotonic saline). Following depot instillation, the tissue reaction (intensity as well as duration of the inflammatory and wound healing processes) is dependent on the size, shape, and chemical and physical properties of the biomaterial [119]. The host responds to the tissue injury with a well-defined sequence of events, including fibrosis [120]. The formation of a fibrous tissue surrounding the depot system may take place over days to weeks and for more long-acting depots, this event may contribute to the overall variability of *in vivo* drug performance. Also, the fact that microsphere particles < 10 μm may be phagocytosed or engulfed by macrophages and foreign body giant cells in the inflammatory and healing responses [120] may affect variability.

After depot injection into subcutaneous and intramuscular tissues the drug is released into the soft tissue fluid. Drug solute transport within the space between blood capillaries (several hundred micrometres in soft tissues) can be described by Equation 2 [121]. Although much of the interstitial fluid is in constant movement owing to the Starling's forces, the flow is slow. Consequently, the convective term in Equation 2 typically will be relatively small in the case of small-molecule drugs [121]. Whereas physiological Péclet numbers (the ratio between convective and diffusion transport contributions) are, in general, in the range 0.1 – 10 [122], mass transfer in the interstitium can be characterised by Péclet numbers below unity. Diffusion is inversely related to molecular size, which means that for larger molecules convection becomes more important in governing their transport compared with diffusion [121]. The above observations suggest that *in vitro* release models intended to simulate s.c. and i.m. conditions should feasibly operate under minor agitation/flow. On a case-by-case basis the extent of agitation/flow has to be decided because this parameter may influence significantly the shape of the *in vitro* drug release profile [123,124]. From a practical point of view, column-type CF-based *in vitro* models seem to be useful, especially when convective transport is minimised by introducing a gel matrix, such as an agarose gel, into the column [78]. At low flow rates, drug release most probably proceeds under non-sink conditions, a situation that also may prevail at the subcutaneous site [125]. Differences in deviations from perfect sink conditions may result in different drug release profiles *in vitro* and *in vivo*, possibly due to: i) a shift in drug release mechanism (a change in the q term in Equation 2); or ii) a shift in the relative contribution of q compared with convection and diffusion (see Equation 2). Injection of the formulation directly into the gel matrix through an injection port attached to the column might provide important information

about the effect of spreading or aggregation on drug release rates. Such *in vitro* release models should also facilitate, at least in the formulation design phase, the study of *in situ* DDS formation as well as drug release from such *in situ* DDSs formed [84,96] (activities within this field are continuing at present in the authors' laboratory).

Particularly for long-acting depots, accelerated *in vitro* drug release testing is desirable [126]. In case the ultimate goal remains establishment of IVIVCs, the successful outcome of the above-mentioned *in vitro* activities presumably is inversely related to the complexity of the release and transport processes occurring at the administration site. Perhaps implementation of accelerated conditions might be easier for less complex *in vitro* release methods feasible for batch control when attempts to obtain an IVIVC have failed. In the latter situation proper specifications have to be worked out to minimise unnecessary *in vivo* variability. This could include the use of a level B IVIVC involving statistical moment analysis [127] in combination with narrow specifications for the API as well as the pharmaceutical excipients. Science-based specification settings may appropriately arise from a dialogue between the company and the regulatory authorities.

Although some *in vitro* release methods are expected to be in use for quality control of existing depot formulations, the need for new release methods for use in the area of parenteral depots has been stressed frequently in recent years. Stimulation of research within academia might constitute a means to accomplish enhanced activities in this particular field. Under such conditions, progress gained in the understanding of the transport processes and solute drug interactions taking place in the vicinity of the site of injection together with the use of different simulation tools may give optimism concerning the emergence of improved *in vitro* release methods in years to come.

Declaration of interest

The authors state no conflicts of interest in the preparation of this manuscript.

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