Review Article

Theme Issue: Critical Variables in the In Vitro and In Vivo Performance of Parenteral Sustained Release Products
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Critical Factors Influencing the *In Vivo* Performance of Long-acting Lipophilic Solutions—Impact on *In Vitro* Release Method Design

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Abstract. Parenteral long-acting lipophilic solutions have been used for decades and might in the future be used in the design of depots with tailored delivery characteristics. The present review highlights major factors influencing the *in vivo* performance of lipophilic solutions. Furthermore, an account is given of the characteristics of employed *in vitro* release methods with a focus on the “state” of sink condition, the stirring conditions, and the oil–water interfacial area. Finally, the capability of *in vitro* release data to predict the *in vivo* performance of drug substances administered in the form of lipophilic solutions is discussed. It is suggested that as long as the major rate-limiting *in vivo* release mechanism is governed by the drug partitioning between the oil vehicle and the tissue fluid, the use of *in vitro* release testing in quality control appears to be realistic. With increasing lipophilicity of the drug substances and longer duration of action, the *in vivo* drug release process may become more complex. As discussed, practical analytical problems together with the inability of release methods to mimic two or more concomitant *in vivo* events may constitute severe impediments for establishment of *in vitro* *in vivo* correlations.

KEY WORDS: depot effect; lipophilic solutions; *in vitro* *in vivo* correlations; *in vitro* release methods; parenteral administration.

INTRODUCTION

Parenteral long-acting lipophilic solutions (mainly for intramuscular injection) have been in clinical use for more than 3 decades in the field of schizophrenia and hormone replacement therapy (1–3). Prolonged duration of action is accomplished by employing a suitable vegetable oil-containing vehicle and a lipophilic prodrug derivative of the parent drug (Table I) (4). Compared with other parenteral depot principles, key attributes of such relatively simple oil-based solutions include uncomplicated manufacture (including terminal sterilization) and feasible long-term stability. Also, this formulation principle opens up the possibility of designing depots with tailored delivery characteristics since manipulation of oil–water distribution coefficients might be obtained through prodrug formation or optimization of oil vehicle composition (5–7). Therefore, this type of formulation appears to be attractive not only for intramuscular injection of novel drug candidates which require transport to the target area via the systemic circulation but also to provide sustained local drug action, for example, in the joint cavity upon intra-articular administration (8).

In vitro drug release models for lipophilic solutions could be used for formulation design purposes or in quality control (including establishment of *in-vitro*–*in-vivo* correlations (IVIVC)). Ideally, *in vitro* model development should be based on knowledge of the *in vivo* drug release mechanism(s) (9–11). It is well established that the drug release rate from lipophilic solutions is, at least partly, governed by the drug partitioning between the oil vehicle and the tissue fluid (2,3,12–14). However, several other factors, such as the shape of the depot at the injection site and the fate of the oil vehicle per se, might affect the overall pharmacokinetic fate of the therapeutic agent. The main objectives of the present review are (a) to highlight factors influencing the *in vivo* performance of lipophilic solutions (b) to comment on various *in vitro* release methods used in the characterization of lipophilic drug solutions, and (c) to discuss the capability of *in vitro* release data to predict the *in vivo* performance of drug substances administered in the form of lipophilic solutions.

FACTORS INFLUENCING THE *IN VIVO* PERFORMANCE OF LIPOPHILIC SOLUTIONS

After depot instillation, the fate of the drug is potentially influenced by a number of processes (Fig. 1). The relative importance of these may to some extent vary with the route of administration. The main focus of this contribution is on intramuscular (i.m.) depot injection, which is the preferred route of administration in human therapy. Several of the

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Table I. Examples of Lipophilic Depot Solutions Used for i.m. Administration in Humans

Prodrug	Product (manufacturer)	Oil vehicle
Estradiol valerate	Delestrogen® (Monarch Pharmaceuticals)	Castor oil
Fluphenazine decanoate	Prolixin Decanoate® (Bristol-Myers Squibb)	Sesame oil
Haloperidol decanoate	Haldol Decanoate® (Ortho-McNeil Pharmaceutical)	Sesame oil
Testosterone cypionate	Depo-Testosterone® (Pharmacia and Upjohn)	Cottonseed oil
Zuclopenthixol acetate	Cisordinol-Acutard® (Lundbeck)	Fractionated coconut oil

issues treated may be of equal importance for the understanding of the *in vivo* behavior of lipophilic solutions injected subcutaneously (s.c.). In the veterinary field, the subcutaneous injection is common practice (15).

Disregarding local metabolic activity, the drug substance (or a prodrug derivative of the active pharmaceutical ingredient) can be removed from the site of administration by two different processes. The active agent may be released from the oil vehicle into the tissue fluid followed by transport to the microvessels, where the average length of diffusion to a capillary is influenced by the extent of spreading of the depot at the injection site. Alternatively, small oil drops containing the drug may be cleared from the local injection site by transport into the lymphatics. Where prodrugs are used, the site of prodrug conversion as well as uptake into fatty tissues ("second depot") may also have an impact on the shape of plasma profile of the parent drug as discussed in the sections below (Table II, Fig. 1).

Drug Partition from Lipophilic Solution to Tissue Fluid

Release of lipophilic drugs from oil vehicles is suggested to constitute the rate-limiting step governing the rate of drug appearance in the systemic circulation after intramuscular administration (2,16,17). This transport process has been suggested to involve partitioning of the drug between the oil vehicle and the tissue fluid (2,3,12–14). In support of this suggestion, linear relationships between log absorption rate and log oil–water distribution coefficient have been reported (18–21). Thus, the rate of drug release from the vehicle can be manipulated by choice of transport group for prodrug design and/or vehicle composition.

As to the exploitation of the prodrug approach, significantly different drug pharmacokinetics were observed following i.m. injection of sesame oil solutions containing buprenorphine base and the corresponding decanoate ester in rats (22) (Fig. 2). The effect of variation of the chemical structure of the transport group on *in vivo* depot properties has been reported for a few drugs only. Duration of action of zuclopenthixol acetate and decanoate dissolved in fractionated coconut oil (Viscoleo®) were 2–3 days and 2–4 weeks, respectively (23). Likewise, in rats, i.m. injection of ester derivatives of buprenorphine resulted in longer absorption half-lives with increasing ester lipophilicity (22) (Fig. 2). Also in case of fluphenazine and testosterone, animal studies have revealed enhanced plasma residence times of the decanoate esters compared to those of the corresponding enanthate esters (16,24).

Employment of different oil vehicles may affect absorption rates to a minor extent following i.m. and s.c. injection.

Several *in vivo* studies have been conducted (18–20,25–28; see for instance Fig. 3), though the reported data do not allow for a ranking of the various vehicles with respect to their prolonged release capabilities.

Spreading of the Lipophilic Solution at the Injection Site

At the site of injection, solute transfer from the oil phase into the tissue fluid requires drug transport (diffusion in oil vehicle) to the oil-tissue fluid interface followed by partitioning into the aqueous phase. Thus, vehicle viscosity and the interfacial area between the oil and aqueous phase are determinants of the total amount of drug released per time unit. Upon i.m. injection, significant spreading (and consequently increase of interfacial area) along the muscle fibrils takes place. Moreover, s.c. injection leads to spreading and dispersion of lipophilic solutions (19,20,29,30). Minimal drug release per unit volume of oil vehicle might be observed in body compartments such as the joint where the original oil drop shape is expected to be at least partly restored. Additional factors that potentially might affect the spreading of the depot formulation include the physiology of the injection site (14,31–33), injection volume (19,20,26), the

Fig. 1. Overview of processes influencing the pharmacokinetics fate of drug substances after injection of lipophilic drug solutions. The drug substance (*D*) may represent the parent drug compound or a prodrug derivative

Table II. Potential Factors Influencing the Release Mechanism and Rate

Factors	Comments
Drug partition from oil vehicle to tissue fluid	Suggested to be the major factor controlling the drug release (at least for substances with moderate lipophilicity)
Spreading and dispersion of the lipophilic solution at the injection site	Can affect the release rate by changing the oil/water interfacial area. Variation in spreading/dispersion might be affected by injection volume, oil viscosity, interfacial tension of the oil, injection site, and injection technique
Injection site	Variations of physiological factors such as blood and lymph flow, number of blood vessels, and injection depth may influence the rate and extent of absorption
Injection volume	Affects the absorption rate by changing the surface-to-volume ratio of the oil vehicle at the injection site (further studies are needed)
Viscosity of the oil vehicle	Currently, no correlation between oil viscosity and absorption (and release) rate has been found
Diffusion of drug in oil vehicle	To be clarified
Drug transport in tissue fluid by diffusion/convection	Dictate the "degree" of sink conditions in the vicinity of the injection site
Disappearance of oil vehicle from the injection site	Has to be considered primarily for highly lipophilic drugs
Hydrolysis of ester prodrug at the oil/tissue fluid interface	Possible release mechanism mediated by lipases

injection technique (33), and the interfacial tension of the oil (26).

Lymphatic and Second Depot Uptake

After i.m. injection (rats) of sesame oil solutions of fluphenazine (13,34) and haloperidol (35,36), in the form of their decanoic acid esters, drugs were detected in the lymph system with the highest concentrations found in the lymph

nodes close to the injection site. It was suggested that small oil drops containing the drugs were most likely absorbed by the local lymphatic system followed by drug release from the depot (Fig. 1). In contrast, the presence of drug in peripheral lymph nodes was most likely a result of drug uptake from the blood (13,35,36). Enhanced drug residence time in the blood might also be brought about by drug uptake from the systemic circulation into so-called second depots including fatty tissue (12,21), entero-hepatic circulation (18) and protein binding (37,38).

Additional *In Vivo* Events Potentially Affecting the Rate of Drug Appearance in the Systemic Circulation

The clearance of various oil vehicles from the i.m. and s.c. injection sites have been investigated in various animal species, revealing half-lives of disappearance in the range of 2–30 days (26,29,30,39–41). In most cases, fractionated coconut oil was cleared faster from the injection site than other oil vehicles investigated (29,30,41). In Table III, representative oil dis-

Fig. 2. Plasma concentration-time profiles of buprenorphine in plasma after intramuscular injection of parent buprenorphine and the propionate, enanthate, and decanoate esters in sesame oil in rats. Values are expressed as mean $\pm$ SEM ($n=3$). Reprinted with permission from (22)

Fig. 3. Serum concentrations (mean $\pm$ SEM) of testosterone after single-dose intramuscular injection of 1,000 mg testosterone undecanoate in tea seed oil in seven hypogonadal men (filled square) or castor oil in 14 hypogonadal men (empty circle). Broken lines indicate normal range of testosterone. Reprinted with permission from (25)

Table III. Mean HALF-lives ($t_{1/2}$, $n=6$) for the Clearance of Oil Vehicles after i.m. or s.c. Administration to pigs (29)

Oil vehicle	$t_{1/2}$ in days
Castor oil	20
Fractionated coconut oil	14
Isopropyl myristate	20
Sesame oil	23

appearance half-lives are given. There were obtained by monitoring the amount of ^{125}I -labeled oil remaining at the i.m. injection site (in pigs) as a function of time by non-invasive gamma-scintigraphy (29). As previously mentioned, removal of the drug substance from the tissue site may be a result of two parallel processes. The drug may be released from the oil vehicle into the tissue fluid followed by transport to the microvessels, and small oil drops containing the drug may be cleared from the local injection site by transport into the lymphatics. Hence, the contribution of the latter (slow) process to the overall rate of disappearance from the injection site is

Table IV. Selected *In Vitro* Release Models for Oil-based Injectables

Principle of the model	Schematic illustration/short description	Reference
Lipophilic solution floating on the top of the release medium	<p>The lipophilic formulation is floating on the release medium. Constant stirring in both phases.</p>	(61) ^a
	<p>Inverted cup: The lipophilic formulation is held in the inverted cup which is placed in the release medium. Constant stirring.</p>	(49) ^b
	<p>The lipophilic formulation is placed in the eccentrically open tube on the top of the release medium. Constant stirring.</p>	(47,48) ^c
	<p>Single drop technique: The lipophilic formulation is dispersed as a single drop in the release medium. The drop was held in a continuously rotating down-ward flow of solution inside the tube.</p>	(49,62,71) ^b

Table IV. (continued)

Dialysis techniques		(5-7,50-56,72) ^d
	<p>Rotating dialysis cell. The dialysis cell containing the lipophilic formulations and the donor medium is placed in the large acceptor phase. Constant stirring of the dialysis cell.</p>
	(54,73) ^e
	<p>Float A Lyzer[®]: The lipophilic formulation is installed in the donor phase inside the dialysis tube which is placed in the acceptor phase. Constant stirring.</p>
	(68,69) ^f
	<p>Reverse dialysis bag method: The lipophilic formulation is placed into the large donor phase in which numerous dialysis bags containing the acceptor medium are immersed. At appropriate time intervals, dialysis bags are withdrawn. Constant stirring.</p>	
Continuous flow methods		(57-59)
	<p>The lipophilic formulation floats on the release medium. The medium is circulated through the cell.</p>	

^aReproduced with permission from (61)

^bReprinted from (49) and with permission from Elsevier

^cReprinted from (48) and with permission from Elsevier

^dReprinted from (52) and with permission from Elsevier

^eReprinted with permission from (73)

^fReprinted with permission from (68)

expected to increase with increasing lipophilicity of the drug. Likewise, local metabolic degradation of the oil vehicle might also affect the *in vivo* performance of the depot products.

In the field of oil depot injectables, exploitation of the prodrug approach may be used to tailor the rate of drug release from the vehicle. Also, prodrug derivatization constitutes a means to provide the necessary oil solubility.

Hydrolysis of the ester prodrug bond is not usually considered to be the rate-limiting step in the overall absorption process, except for the (hypothetical) situation involving parent drug regeneration at the oil-tissue fluid interface as a result of enzyme-mediated cleavage of the prodrug ester bond. Especially for highly lipophilic prodrug derivatives such as enzyme-facilitated hydrolysis might lead to unexpectedly fast release of the less lipophilic parent drug. Lipases which are active at oil-water interfaces have been shown to hydrolyze ester derivatives (for example triglyceride-like conjugates (42–45) and fatty acid esters (46)) at oil/water interfaces *in vitro*.

Minor changes in the formulation or the manufacturing process may result in the introduction of new chemical substances in the depot formulation. Such “impurities” may be a result of shifts in supplier of a pharmaceutical excipient, for example, or in the degradation profile of the product due to alteration in the sterilization procedure. Unless embraced by the specifications, even small amounts of impurities may in principle affect several of the above-mentioned factors, as well as the host response, and may therefore cause non-predictable variability in drug pharmacokinetics. Since the effect of changes in host response cannot be simulated by using an *in vitro* release model, the adequacy of a change in formulation/manufacture should preferably be documented by bioequivalence studies performed in a suitable animal model.

IN VITRO RELEASE TESTING

No regulatory approved standard methods exist for testing drug release from sustained release parenteral products, despite the long-recognized need for such *in vitro* release methods (9,10). For oil-based injectables, the release models used (Table IV) so far can be roughly divided into three categories: (a) models with the lipophilic solution floating on the top (or dispersed as a single drop) of the release medium, (b) dialysis techniques, and (c) continuous flow methods. Comparison of release data obtained by using the various methods is far from straightforward, since different experimental setup influence three key parameters (oil–water interfacial area, hydrodynamics (stirring conditions) and “state of sink condition”) differently, as discussed in the paragraphs below.

Oil–Water Interfacial Area

The drug release rate is proportional to the total oil–water interfacial area. Thus, this parameter should ideally be kept constant during the release process. This can be effectuated by using models with the oil phase floating on the release medium. In this type of model, the area may easily be altered by inserting tubes/cubes of different sizes/geometries (47–49). As regards the dialysis techniques, the interfacial area for mass transfer is equal to the total membrane area as long as an amount of oil sufficient to cover the entire inner surface is added to the donor compartment (6,7,50–52). Conversely, if the lipophilic solution is added to a donor cell containing an aqueous buffer, proper agitation may create a very large oil–water interface (53–56) (Fig. 4). In such a case, equilibrium of partitioning between the oil and aqueous phase is maintained at all times in the

Fig. 4. Schematic presentation of the transport processes in the three compartment model obtained after instillation of a drug substance (X) in a lipophilic solution into an aqueous donor compartment separated from a large aqueous acceptor compartment by a dialysis membrane. The transport processes include (i) drug release from oil vehicle ($[X]_o$) into the aqueous donor phase ($[X]_{Dw}$) governed by the partitioning process between the two immiscible phases and (ii) diffusion of drug out of the donor cell into the acceptor phase ($[X]_A$). Reprinted from (53) and with permission from Elsevier

dialysis cell. For this reason, determinations of the drug partition coefficient may eventually replace release experiments. Decisions have to be made on case-by-case basis, preferably based on a dialog between the company and the regulators. In the present context, the applicability of continuous flow methods, in general, seems to be questionable due to difficulties in controlling the interface between oil vehicle and the aqueous phase. One exception might be the flow-through cell initially developed for *in vitro* release testing of suppositories (57–59).

Stirring Conditions

Agitation of the acceptor compartment is a prerequisite for maintaining homogenous drug concentrations in the aqueous release medium. However, stirring of the oil phase might be left optional, depending on the *in vivo* conditions to be mimicked. In release studies characterized by an unstirred oil phase floating either on the release medium (60) or at the ceilings of a flow-through cell (59), linear relationships between percent drug released (up to 30%) and square root of time were observed. These observations led to the (speculative) suggestion that solute diffusion in the unstirred oil phase to the oil–water interface is the rate-determining step in the overall release process. In contrast, drug transfer rates from the oil to the aqueous phase obeyed first-order kinetics using models characterized by stirring of the oil phase (6,7,49–51,53–55,61,62).

Sink Conditions

The impact of maintaining sink conditions during *in vitro* release testing of sustained release products has been subject to debate (see for example the reviews (11,63–65)). According to the USP, sink condition is defined as being one third of the saturation value (66). Alternative definitions include that the bulk drug concentration during the entire release process should not exceed 10% of the drug saturation concentration.

Exploitation of sink conditions during *in vitro* release experiments may ease the mathematical analyses (65,67). On the other hand, the relevance of such model conditions may be questionable since perfect sink conditions are less likely to prevail locally at the site of administration of the depot formulation (53,55,63–65,67). In this context, it might be mentioned that excellent quantitative descriptions of *in vitro* drug release rates from lipophilic solutions under non-sink conditions have been accomplished using the rotating dialysis cell model (6,50,51,53–55). If release testing under approximated sink condition is desirable, flow-through systems might appear attractive (59). Also, the reverse dialysis bag method previously used for measuring drug release rates from emulsions (68,69) might be advantageous.

CAPABILITY OF *IN VITRO* RELEASE DATA TO PREDICT *IN VIVO* DRUG PERFORMANCE

Design of a suitable *in vitro* release model is a key activity in the development of a novel parenteral depot formulation. In the Chemistry, Manufacturing, and Control (CMC) regulatory file, the pharmaceutical company needs to convince regulatory agencies of the adequacy of the discriminatory release model constructed for use in batch release. 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 behavior. At best, the *in vitro* model may mimic the *in vivo* conditions to such an extent that a level A IVIVC can be established. In this case, performance of *in vitro* release tests might be accepted instead of an *in vivo* bioequivalence study (biowaiver) for the evaluation of minor changes in the composition or manufacturing process of an already marketed product. In the field of lipophilic solutions, apparently only one attempt to establish an IVIVC has been reported in the literature. A relation between *in vitro* and *in vivo* drug release was demonstrated for an oil solution containing bupivacaine (a moderate lipophilic drug), which showed an analgesic effect lasting for about 1 day (70). The lack of true IVIVCs can most likely be ascribed to barriers related to intrinsic drug properties or the inability to design *in vitro* methods sufficiently capable of mimicking the *in vivo* conditions at the injection site.

Attributes of highly lipophilic drugs (or prodrug derivatives) include high vegetable oil solubility and slow partitioning from oil vehicles into an aqueous phase. However, such compounds may exhibit extremely low aqueous solubility. The latter physicochemical property may make *in vitro* release testing of highly lipophilic drug substances problematic. This analytical challenge relates to detection of the compounds as well as performance of release experiments endowed with acceptable run times. These problems might to some extent be reduced by introducing a means to enhance drug solubility in the release medium (such as addition of cyclodextrin (7)). Also, faster drug release from the lipophilic formulation might result from enhancing the oil–water interfacial area (this approach might also be suitable for accelerated *in vitro* release testing) (55).

In vivo phenomena which are difficult to simulate using an *in vitro* release model include spreading and dispersion of the oil vehicle at the injection site. Variable spacial expansion of the depot at the injection site (and thus creation of variable

interfacial areas of drug transfer) is dictated mainly by the injection technique and the choice of specific administration site. Hence, variable vehicle spreading, per se, is not considered a drug product quality issue, although this parameter may indeed affect drug release rates. Also, the situation, where two or more events in parallel contribute to the overall rate of disappearance of the drug from the injection site, may be difficult to mimic *in vitro*. Transport of microdroplets containing the drug to the lymphatics and enzyme-mediated prodrug cleavage at the oil surface may represent examples of such contributing processes. Apparently, little is known about the clearance of different oil vehicles from the injection site in humans, and to which extent host responses affect the disappearance rates. Animal studies (in pigs) have revealed only minor differences in disappearance rates of various vegetable oils with half-lives of about 14 to 23 days (29). Often, it has been emphasized that *in vitro* release model development should be based on knowledge of the *in vivo* drug release mechanism (9–11). So far, elucidation of effects of hydrodynamics, convective diffusion mass transfer and “state of sink condition” on drug fate at the injection site is still lacking.

In case of relatively short-acting oil depots comprising moderately lipophilic drug substances the generation of *in vitro* release data for prediction of *in vivo* drug performance appears reasonably realistic given that drug release from the lipophilic vehicle constitutes the rate-limiting step *in vivo*. For long-acting depot formulations, the disappearance of the oil containing the drug from the injection site (and potentially other drug release processes) contributes to the overall rate of drug release from the local administration site. The latter *in vivo* conditions are difficult to simulate by use of an *in vitro* drug release model and may become prohibitive for the establishment of an IVIVC.

CONCLUSION

Although parenteral long-acting lipophilic solutions have been used for intramuscular administration for several decades, the *in vivo* drug release mechanism(s) are far from fully elucidated. To this end, literature data may suggest the involvement of two major contributing *in vivo* processes (a) drug release from the lipophilic solution governed by the partitioning of the drug between the oil vehicle and the tissue fluid and (b) disappearance of the oil vehicle containing the drug from the injection site. Alterations in these processes caused by formulation changes might therefore influence the overall *in vivo* performance of the drug product. In the case of relatively short-acting oil depots comprising moderately lipophilic drug substances, the generation of *in vitro* release data for prediction of *in vivo* drug performance appears to be reasonably realistic given that drug release from the lipophilic vehicle constitutes the rate-limiting step *in vivo*. With increasing duration of therapeutic activity, two or more *in vivo* events (including non-predictable host responses) may contribute to the overall drug release characteristics. Thus, it is suggested that the probability for successful development of a suitable *in vitro* release model (for batch control and IVIVC), so to speak, is inversely proportional to the intended duration of action of the lipophilic solution.

REFERENCES

- Benson HAE, Prankerd RJ. Optimisation of drug delivery 6. Modified-release parenterals. *Aust J Hosp Pharm*. 1998;28(2):99–104.
- Chien YW. Long-acting parenteral drug formulations. *J Parenter Sci Technol*. 1981;35(3):106–39.
- Zuidema J, Kadir F, Titulaer HAC, Oussoren C. Release and absorption rates of intramuscularly and subcutaneously injected pharmaceuticals (II). *Int J Pharm*. 1994;105(3):189–207.
- Murdan S, Florence AT. Non-aqueous solutions and suspensions as sustained-release injectable formulations. In: Senior J, Radomsky M, editors. *Sustained-release injectable products*. Denver: Interpharm Press; 2000. p. 71–107.
- Fredholt K, Larsen DH, Larsen C. Modification of *in vitro* drug release rate from oily parenteral depots using a formulation approach. *Eur J Pharm Sci*. 2000;11(3):231–7.
- Larsen DB, Fredholt K, Larsen C. Addition of hydrogen bond donating excipients to oil solution: effect on *in vitro* drug release rate and viscosity. *Eur J Pharm Sci*. 2001;13(4):403–10.
- Larsen SW, Thomsen AE, Rinvar E, Friis GJ, Larsen C. Effect of drug lipophilicity on *in vitro* release rate from oil vehicles using nicotinic acid esters as model prodrug derivatives. *Int J Pharm*. 2001;216(1–2):83–93.
- Larsen C, Ostergaard J, Larsen SW, Jensen H, Jacobsen S, Lindegaard C, et al. Intra-articular depot formulation principles: role in the management of postoperative pain and arthritic disorders. *J Pharm Sci*. 2008;97(11):4622–54.
- Burgess DJ, Hussain AS, Ingallinera TS, Chen ML. Assuring quality and performance of sustained and controlled release parenterals: workshop report. *AAPS PharmSci*. 2002;4(2):E7.
- Burgess DJ, Crommelin DJ, Hussain AS, Chen ML. Assuring quality and performance of sustained and controlled released parenterals. *Eur J Pharm Sci*. 2004;21(5):679–90.
- Martinez M, Rathbone M, Burgess D, Huynh M. *In vitro* and *in vivo* considerations associated with parenteral sustained release products: a review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting. *J Control Release*. 2008;129(2):79–87.
- Armstrong NA, James KC. Drug release from lipid-based dosage forms. I. *Int J Pharm*. 1980;6(3–4):185–93.
- Luo J, Hubbard JW, Midha KK. The roles of depot injection sites and proximal lymph nodes in the presystemic absorption of fluphenazine decanoate and fluphenazine: *ex vivo* experiments in rats. *Pharm Res*. 1998;15(8):1485–9.
- Minto CF, Howe C, Wishart S, Conway AJ, Handelsman DJ. Pharmacokinetics and pharmacodynamics of nandrolone esters in oil vehicle: effects of ester, injection site and injection volume. *J Pharmacol Exp Ther*. 1997;281(1):93–102.
- Medlicott NJ, Waldron NA, Foster TP. Sustained release veterinary parenteral products. *Adv Drug Deliv Rev*. 2004;56(10):1345–65.
- Dreyfuss J, Ross JJ, Shaw JM, Miller I, Schreiber EC. Release and elimination of ¹⁴C-fluphenazine enanthate and decanoate esters administered in sesame oil to dogs. *J Pharm Sci*. 1976;65(4):502–7.
- Jann MW, Ereshefsky L, Saklad SR. Clinical pharmacokinetics of the depot antipsychotics. *Clin Pharmacokinet*. 1985;10(4):315–33.
- Al-Hindawi MK, James KC, Nicholls PJ. Influence of solvent on the availability of testosterone propionate from oily, intramuscular injections in the rat. *J Pharm Pharmacol*. 1987;39(2):90–5.
- Hirano K, Ichihashi T, Yamada H. Studies on the absorption of practically water-insoluble drugs following injection V: subcutaneous absorption in rats from solutions in water immiscible oils. *J Pharm Sci*. 1982;71(5):495–500.
- Hirano K, Ichihashi T, Yamada H. Studies on the absorption of practically water-insoluble drugs following injection. I. Intramuscular absorption from water-immiscible oil solutions in rats. *Chem Pharm Bull (Tokyo)*. 1981;29(2):519–31.
- James KC, Nicholls PJ, Roberts M. Biological half-lives of [⁴⁻¹⁴C]testosterone and some of its esters after injection into the rat. *J Pharm Pharmacol*. 1969;21(1):24–7.
- Liu KS, Tzeng JI, Chen YW, Huang KL, Kuei CH, Wang JJ. Novel depots of buprenorphine prodrugs have a long-acting antinociceptive effect. *Anesth Analg*. 2006;102(5):1445–51.
- Aaes-Jørgensen T. Pharmacokinetics of three different injectable zuclopenthixol preparations. *Prog Neuro-psychopharmacol Biol Psychiatry*. 1989;13(1–2):77–85.
- Partsch CJ, Weinbauer GF, Fang R, Nieschlag E. Injectable testosterone undecanoate has more favourable pharmacokinetics and pharmacodynamics than testosterone enanthate. *Eur J Endocrinol*. 1995;132(4):514–9.
- Behre HM, Abshagen K, Oettel M, Hubler D, Nieschlag E. Intramuscular injection of testosterone undecanoate for the treatment of male hypogonadism: phase I studies. *Eur J Endocrinol*. 1999;140(5):414–9.
- Tanaka T, Kobayashi H, Okumura K, Muranishi S, Sezaki H. Intramuscular absorption of drugs from oily solutions in the rat. *Chem Pharm Bull*. 1974;22(6):1275–84.
- Wicks SR, Kaye B, Weatherley AJ, Lewis D, Davison E, Gibson SP, et al. Effect of formulation on the pharmacokinetics and efficacy of doramectin. *Vet Parasitol*. 1993;49(1):17–26.
- Wistuba J, Marc LC, Kamischke A, Gu YQ, Schlatt S, Simoni M, et al. Pharmacokinetics and pharmacodynamics of injectable testosterone undecanoate in castrated cynomolgus monkeys (*Macaca fascicularis*) are independent of different oil vehicles. *J Med Primatol*. 2005;34(4):178–87.
- Larsen SW, Rinvar E, Svendsen O, Lykkesfeldt J, Friis GJ, Larsen C. Determination of the disappearance rate of iodine-125 labelled oils from the injection site after intramuscular and subcutaneous administration to pigs. *Int J Pharm*. 2001;230(1–2):67–75.
- Schultz K, Mollgaard B, Fisher AN, Illum L, Larsen C. Intramuscular rate of disappearance of oily vehicles in rabbits investigated by gamma-scintigraphy. *Int J Pharm*. 1998;169(1):121–6.
- Houpt P, Combrisson H, Le NS, Autefage A, Toutain PL. Intra- vs intermuscular injections in swine. *Vet Res*. 1993;24(3):278–85.
- Contreras-Solis I, Gomez-Brunet A, Encinas T, Gonzalez-Bulnes A, Santiago-Moreno J, Lopez-Sebastian A. Influence of vehicle on kinetics of exogenous progesterone administered either by subcutaneous and intramuscular routes to sheep. *Res Vet Sci*. 2008;85(1):162–5.
- Zuidema J, Pieters FAJM, Duchateau GSMJ. Release and absorption rate aspects of intramuscularly injected pharmaceuticals. *Int J Pharm*. 1988;47(1–3):1–12.
- Huang Y, Hubbard JW, Midha KK. The role of the lymphatic system in the presystemic absorption of fluphenazine after intramuscular administration of fluphenazine decanoate in rats. *Eur J Pharm Sci*. 1995;3(1):15–20.
- Matsunaga Y, Nambu K, OH-E Y, Miyazaki H, Hashimoto M. Absorption of intramuscularly administered [¹⁴C]haloperidol decanoate in rats. *Eur J Drug Metab Pharmacokinet*. 1987;12(3):175–81.
- Oh-E Y, Miyazaki H, Matsunaga Y, Hashimoto M. Pharmacokinetics of haloperidol decanoate in rats. *J Pharmacobio-Dyn*. 1991;14(11):615–22.
- Belkien L, Schürmeyer T, Hano R, Gunnarsson PO, Nieschlag E. Pharmacokinetics of 19-nortestosterone esters in normal men. *J Steroid Biochem*. 1985;22(5):623–9.
- Nambu K, Miyazaki H, Nakanishi Y, Oh-E Y, Matsunaga Y, Hashimoto M. Enzymatic hydrolysis of haloperidol decanoate and its inhibition by proteins. *Biochem Pharmacol*. 1987;36(10):1715–22.
- Howard JR, Hadgraft J. The clearance of oily vehicles following intramuscular and subcutaneous injections in rabbits. *Int J Pharm*. 1983;16(1):31–9.
- Larsen C, Schultz K, Fisher AN, Illum L. Intramuscular fate of C-14- and I-131-labelled triglycerides. *Int J Pharm*. 1998;166(2):227–30.

41. Svendsen O, Aaes-Jørgensen T. Studies on the fate of vegetable oil after intramuscular injection into experimental animals. *Acta Pharmacol Toxicol.* 1979;45:352–78.
42. Delie F, Couvreur P, Nisato D, Michel JB, Puisieux F, Letourneux Y. Synthesis and in-vitro study of a diglyceride prodrug of a peptide. *Pharm Res.* 1994;11(8):1082–7.
43. Delie F, Letourneux Y, Nisato D, Puisieux F, Couvreur P. Oral-administration of peptides—study of a glycerolipidic prodrug. *Int J Pharm.* 1995;115(1):45–52.
44. Scriba GKE. Phenytoin lipid conjugates—chemical, plasma esterase-mediated, and pancreatic lipase-mediated hydrolysis in-vitro. *Pharm Res.* 1993;10(8):1181–6.
45. Scriba GKE, Lambert DM, Poupaert JH. Bioavailability of phenytoin following oral administration of phenytoin-lipid conjugates to rats. *J Pharm Pharmacol.* 1995;47(11):945–8.
46. Alvarez FJ, Stella VJ. Pancreatic lipase-catalyzed hydrolysis of esters of hydroxymethyl phenytoin dissolved in various metabolizable vehicles, dispersed in micellar systems, and in aqueous suspensions. *Pharm Res.* 1989;6(7):555–63.
47. Crommelin DJA, Deblaey CJ. *In vitro* release studies on drugs suspended in non-polar media. II. The release of paracetamol and chloramphenicol from suspensions in liquid paraffin. *Int J Pharm.* 1980;6(1):29–42.
48. Crommelin DJA, Deblaey CJ. *In vitro* release studies on drugs suspended in non-polar media. I. Release of sodium chloride from suspensions in liquid paraffin. *Int J Pharm.* 1980;5(4):305–16.
49. Soderberg L, Dyhre H, Roth B, Bjorkman S. The “inverted cup”—a novel *in vitro* release technique for drugs in lipid formulations. *J Control Release.* 2006;113(1):80–8.
50. Larsen DB, Parshad H, Fredholt K, Larsen C. Characteristics of drug substances in oily solutions. Drug release rate, partitioning and solubility. *Int J Pharm.* 2002;232(1–2):107–17.
51. Larsen DH, Fredholt K, Larsen C. Assessment of rate of drug release from oil vehicle using a rotating dialysis cell. *Eur J Pharm Sci.* 2000;11(3):223–9.
52. Schultz K, Mollgaard B, Frøkjær S, Larsen C. Rotating dialysis cell as *in vitro* release method for oily parenteral depot solutions. *Int J Pharm.* 1997;157(2):163–9.
53. Larsen SW, Ostergaard J, Friberg-Johansen H, Jessen MN, Larsen C. *In vitro* assessment of drug release rates from oil depot formulations intended for intra-articular administration. *Eur J Pharm Sci.* 2006;29(5):348–54.
54. Larsen SW, Frost AB, Ostergaard J, Marcher H, Larsen C. On the mechanism of drug release from oil suspensions *in vitro* using local anesthetics as model drug compounds. *Eur J Pharm Sci.* 2008;34(1):37–44.
55. Larsen SW, Jessen MN, Ostergaard J, Larsen C. Assessment of drug release from oil depot formulations using an *in vitro* model—potential applicability in accelerated release testing. *Drug Dev Ind Pharm.* 2008;34(3):297–304.
56. Pedersen BT, Larsen SW, Ostergaard J, Larsen C. *In vitro* assessment of lidocaine release from aqueous and oil solutions and from preformed and *in situ* formed aqueous and oil suspensions. Parenteral depots for intra-articular administration. *Drug Deliv.* 2008;15(1):23–30.
57. Janicki S, Sznitowska M, Zebrowska W, Gabiga H, Kupiec M. Evaluation of paracetamol suppositories by a pharmacopoeial dissolution test—comments on methodology. *Eur J Pharm Biopharm.* 2001;52(2):249–54.
58. Lootvoet G, Beyssac E, Shiu GK, Aiache JM, Ritschel WA. Study on the release of indomethacin from suppositories—*in vitro-in vivo* correlation. *Int J Pharm.* 1992;85(1–3):113–20.
59. Schultz K. Parenteral oily depot formulations. *In vitro* and *in vivo* characterisation. Ph.D. Thesis, The Royal Danish School of Pharmacy, Copenhagen; 1997.
60. Radd BL, Newman AC, Fegely BJ, Chrzanowski FA, Lichten JL, Walkling WD. Development of haloperidol in oil injection formulations. *J Parenteral Sci Tech.* 1985;39(1):48–9.
61. Kakemi K, Sezaki H, Muranishi S, Ogata H, Giga K. Mechanism of intestinal absorption of drugs from oil in water emulsion. II. Absorption from oily solutions. *Chem Pharm Bull.* 1972;20(4):715–20.
62. Soderberg L, Dyhre H, Roth B, Bjorkman S. In-vitro release of bupivacaine from injectable lipid formulations investigated by a single drop technique—relation to duration of action in-vivo. *J Pharm Pharmacol.* 2002;54(6):747–55.
63. D'Souza SS, Deluca PP. Methods to assess *in vitro* drug release from injectable polymeric particulate systems. *Pharm Res.* 2006;23(3):460–74.
64. Iyer SS, Barr WH, Karnes HT. Profiling *in vitro* drug release from subcutaneous implants: a review of current status and potential implications on drug product development. *Biopharm Drug Dispos.* 2006;27(4):157–70.
65. Washington C. Drug release from microdisperse systems—a critical-review. *Int J Pharm.* 1990;58(1):1–12.
66. <1092> The dissolution procedure: development and validation. *Pharmacopoeial Forum.* 2005;31(5):1463.
67. Washington C. Evaluation of non-sink dialysis methods for the measurement of drug release from colloids—effects of drug partition. *Int J Pharm.* 1989;56(1):71–4.
68. Chidambaram N, Burgess DJ. A novel *in vitro* release method for submicron sized dispersed systems. *AAPS PharmSci.* 1999;1(3):E11.
69. Levy MY, Benita S. Drug release from submicronized o/w emulsion—a new *in vitro* kinetic evaluation model. *Int J Pharm.* 1990;66(1–3):29–37.
70. Larsen DB, Joergensen S, Olsen NV, Hansen SH, Larsen C. *In vivo* release of bupivacaine from subcutaneously administered oily solution. Comparison with *in vitro* release. *J Control Release.* 2002;81(1–2):145–54.
71. Soderberg L, Dyhre H, Roth B, Bjorkman S. Ultralong peripheral nerve block by lidocaine:prilocaine 1:1 mixture in a lipid depot formulation: comparison of *in vitro*, *in vivo*, and effect kinetics. *Anesthesiology.* 2006;104(1):110–21.
72. Dibbern HW, Wirbitzki E. Possibilities for determining the active substance release from hydrophobic carriers especially as suppositories. *Pharm Ind.* 1983;45(10):985–90.
73. D'Souza SS, Deluca PP. Development of a dialysis *in vitro* release method for biodegradable microspheres. *AAPS PharmSciTech.* 2005;6(2):E323–8.