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Lipid based intramuscular long-acting injectables: current state of the art

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Abstract

Long acting injectables (LAI) have received increased research and commercial interest due to their potential for improving treatment effectiveness and adherence for antipsychotic, antiviral and addiction treatments. A range of materials have been used to formulate LAI products, including lipids and polymers. Classic lipid-based LAI, such as oil solutions of antipsychotic drugs, have been widely prescribed to patients. Clinical evidence has shown significantly improved key therapeutic markers such as reduction of relapses in the case of schizophrenia patients. The commercial LAI products can be given either via subcutaneous or intramuscular injection. The main types of lipid-based LAI formulations include oil solutions, lipid-based nanoparticles and lipid based liquid crystal formulations, which are currently clinically available, and oil suspensions and oleogels and which currently have no commercial products available. This review will discuss all relevant aspects related to the development of lipid-based long acting injectables with a special focus on intramuscular (IM) injectables. It aims to provide useful guidance on effective future LAI product design and development. Lipid-based nanoformulations are not discussed in this review as they are thoroughly reviewed in literature elsewhere.

Keywords: lipid-based formulations, long-acting injectables, intramuscular, oil solutions, lipid based liquid crystals, oleogels.

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1. Introduction

Long-acting injectable (LAI) products providing sustained drug release have been developed and used clinically for the treatment of psychotic diseases from as early as 1957, where the first approved product was developed and launched by E.R. Squibb and Son [1, 2]. The LAI discussed in this review are limited to the products that can be directly injected and do not require any level of surgical implantation. In the literature, LAI is often loosely used to cover a wide spread of formulations that can be either defined by the key functional excipient (i.e. being lipid-based [3] or polymer-based [4] [5, 6] or the macro- to micron-scale of the structure of the formulations. For lipid-based formulations, if classifying is based on the structure of the formulation, LAI can range from simple oil-based solutions and suspensions [7] to complex formulations including lipid-based nanocarriers such as liposomes [8, 9], emulsions [10] lipid-based depot systems such as oleogels [11], and *in-situ* forming liquid crystals [12]. In this review of lipid based LAIs, the key focus is the comparison of simple oil solution-based formulations, which dominate the marketed products, and the more complex *in situ* forming depot formulations, including oelogels and lipid-based liquid crystalline systems. Lipid-based nanoformulations are not discussed in this review as they are thoroughly reviewed in literature elsewhere [13].

LAIs ensure a significant reduction of the dosing frequency for the patients and are well suited for managing chronic conditions that require frequent repeated dosing. LAI products have proved to lead to improved patient compliance and, more importantly, improved therapeutic outcomes, with a decreased incidence of side effects, leading to an overall cost reduction of medical care [14, 15]. LAI anti-psychotic drugs for managing and reducing relapse in patients with schizophrenia are an excellent example of the clinical benefit that these formulations provide for the patients, health care systems and society [16, 17]. In a

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recent systematic review and meta-analysis of long-term studies, LAI antipsychotics were reported to reduce the relapse rates of outpatients with schizophrenia from an average of 33% to 21% [18]. A nationwide cohort study from Finland concluded that the use of LAI formulations was associated with a significantly lower risk of re-hospitalization compared to oral formulations of the same antipsychotic [19]. Another potential application for LAIs would be patient populations that have poor and/or limited access to medical contact and care, for treating diseases such as HIV [20-22]. The ease of the procedure and the low associated costs of administration of injectables in comparison to implantable formulations have led to an increased interest in the development of LAI products in general [23-25].

There are a range of LAI products on the market utilizing different technologies, including oil-based systems, aqueous suspensions, polymeric microspheres, *in situ* forming gels, where the choice of the formulation technology is heavily dependent upon the physicochemical properties of the active pharmaceutical ingredient (API) [26]. As described above, lipid-based formulations were the first formulation strategy used to generate an LAI [3]. However, since the first wave of lipid based LAIs, few products have entered the market. Simple oil-based formulations, such as oil solutions in which the drug is dissolved in the oil carrier (most commonly used ones are vegetable oil or medium chain triglyceride oil), with or without a cosolvent, are easy to manufacture. However, with a high number of lipophilic compounds in the pharmaceutical pipelines over the last three decades, the approach may once more be relevant to consider in relation to LAI products. There are many excellent reviews on the current LAI products and different types of formulations of LAI [27-31] [26, 32] [3, 33]. This review will specifically focus on the lipid-based LAI products, excluding lipid-based nanoformulations which are reviewed elsewhere, and their biopharmaceutics aspects when given intramuscularly.

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2. Lipid based long-acting injectable (LAI) formulations

2.1. Formulation considerations

2.1.1 Drug selection for oil-based LAI product development

As the primary goal of LAI is to achieve the continuous, but prolonged release of drug lasting from weeks to months, a high partitioning or solubility of the drug in the LAI matrices is necessary. As shown in **Table 1**, for oil solution-based LAI products given intramuscularly, drugs, prodrugs and conjugates with high log P values are often the preferred, but not exclusively used, candidates [34, 35]. In most cases, the drug release is driven by passive diffusion due to the concentration gradient between the tissue fluid and LAI matrices, combined with local digestion of the oil depot [36]. To achieve a higher solubility to the oil vehicle, several oil depot products include esterified pro-drugs of the active component. Esterification of alcohol groups within the drug molecule with fatty acids can significantly reduce the drug's aqueous solubility and increase its oil solubility. With the large range of endogenous fatty acids [37], the esterification modification of the drug molecule can be performed to adjust the physical properties, including melting point, solubility, and partition coefficient.

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Table 1. Examples of regulatory approved oil-based LAI products delivered by intramuscular injections

Oil	Manufacturer	Drug Product	LogP	Use	Reference
Castor Oil	AstraZeneca	Fulvestrat (Faslodex)	8.9	Breast Carcinoma	[38]
	Monach Pharmaceuticals	Estradial Varerate (Delestrogen)	6	Treatment of menopause symptoms	[39]
	Bayer	Norethisterone enantate (Noristerat)	6	Contraceptive	[40]

	Bayer	Testosterone Undecanoate (Nebido)	8.5	Testosterone deficiency	[41, 42]
	Bayer	Cyproterone acetate (Androcur Depot)	3.6	Prostate Cancer	[43]
Cottonseed Oil	Pfizer	Testosterone cypionate (Depo-Testosterone)	6.4	Testosterone deficiency	[44]
Sesame Oil	Jassen Pharmaceuticals	Haloperidol Decanoate (Haldol)	4.3	Antipsychotic	[45]
	Sanofi	Fluphenazine decanoate (Prolixin)* Discontinued	9	Antipsychotic	[46-48]
	Bristol-Myers Squibb	Fluphenazine decanoate (Modecate)* Discontinued	8.2	Antipsychotic	[48]
	Sanofi	Pipotiazine palmitate (Piportil)* Discontinued	10.5	Antipsychotic	[49]
Fractionated Coconut oil	Lundbeck Ltd	Zuclopenthixol decanaote (Clopixol)	9	Antipsychotic	[50]
	Lundbeck Ltd	Flupentixol decanoate (Depixol)	8.8	Antipsychotic	[51]

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Esterification is one of the common approaches to form more lipophilic prodrugs. As an example, the effect of ester tail length of the prodrug on the drug release were studied with fluphenazine. It was found that the drug release from oils was slower for the prodrugs with longer chain esters [52]. This could simply be that the longer the easter chain, the higher the molecular weight of the prodrug which led to slower diffusion through the oil. After being released, most ester prodrugs can efficiently be cleaved in the body to the active form by esterases. Remenar summarized the patents and literature data and concluded that the esterification using decanoate (C10) appeared to provide the most optimal release rate for many drug molecules [53].

A secondary delay of effective absorption of the parent drug is the hydrolysis process of the prodrug back into the parent drug after release in the case of oil solution and suspension-

based LAI. If the hydrolysis of the esterified prodrug is slow the vehicle needs to be adjusted accordingly otherwise it may risk reducing the compound potency and increasing the risk of failure in toxicity studies [53]. Hence, the overall absorption rate of the injected pro-drug is a result of the combination effect of the release from the vehicle and the speed and place at which (enzymatic) hydrolysis occurs. In this case, the injection site and muscle activities may have a role to play in influencing the drug release from the LAI depot.

For drugs that naturally have a high LogP and are highly lipophilic, the absorption after IM injection is most likely be predominated by local tissue drainage and lymphatic uptake [54]. The local metabolic activity may also affect the rate and level of absorption of the depot injections. In addition to the consideration of the lipophilicity (here defined through LogP) of the drug, the solubility in the aqueous phase and the pK_a value is also important for influencing the speed of drug release and uptake. More hydrophilic compounds can diffuse directly into the central circulation. In these cases, additional biopharmaceutics factors associated with administration need to be considered. For example, during IM injection of LAI of an anti-psychotic drug, olanzapine, if a blood vessel is punctured by the needle causing leakage of blood into the muscle, this may lead the local pH to drop from around 7.4 to 6.5 in response to the trauma caused by injection and a post-injection delirium/sedation syndrome (PDSS) may arise [55]. Although this cannot be generalized to all drugs delivered via IM injection. If the compound has a pH sensitivity release, it may potentially lead to dose dumping, which is not desired both for safety and efficacy reasons. Molecular modification strategies, such as using carbamate linked esters, have been investigated for olanzapine to reduce the differences in solubility between buffer and plasma with the intention of reducing or eliminating the PDSS effect caused rapid release of drug [56].

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2.1.2 Oil-based LAI products

An oil-based depot injection often consists of the active pharmaceutical ingredient in combination with an oil and potentially a cosolvent. For many antipsychotics, the drug compounds in the commercial products are often esterified with decanoate or palmitate. Many vegetable and synthetic oils have been used as the vehicle oil in LAIs products, including castor oil, soybean oil, sesame oil, cottonseed oil, and fractioned coconut oil. A single dose of the depot normally contains 2-8 weeks dose of the active ingredient. When it is administered it cannot be removed in case of poor patient tolerance [57]. For the antipsychotic products, a test dose (an initial low dose of an oil-based depot injection) is therefore recommended, for those where an oral version does not exist, to assess the tolerability to both the active ingredient and the vehicle, before administration of the full dose to the patients. If the patient shows good tolerance, the full does should be give after 5-7 days of the test dose [58].

Viscosity of the injectable oil has been correlated to the *in vivo* clearance rate of the oil when the injection volume is above 300 µL [59]. Animal studies on the comparison of clearance rate of oils with different viscosities revealed that the viscosity of the oil used can have a significant impact on the release from the vehicle and hence the apparent plasma half-life. As an example, peanut oil with a viscosity (39 cps) 10 times higher than ethyl oleate (3.9 cps) was reported to have more than doubled plasma half-life in relative terms [59]. Sesame oil is one of the most widely employed oil vehicles with a relatively high viscosity and good *in-vivo* tolerance profile following IM injection. To adjust/lower the viscosity of the formulation, benzyl alcohol can be added [60]. However, the effects discussed above are injection volume dependent and can be neglected when low injection volumes (50 uL) are used [61]. Similar findings have been reported for organogels with a higher overall viscosity,

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where more retarded drug release was observed from a gel than a classical oil-based depot [62]. These findings aligning well with the classical theory on drug release being dominated by drug diffusion/partition from oil/gel into the body fluid.

It should be highlighted that some research data has shown differences in the rate of *in vivo* clearance of the oil depots between animals and humans. For example, the MRI data of the disappearance rate of sesame oil mixed with 10% (w/v) benzyl alcohol after IM injection in human volunteers was within one week, whereas in rats even after 31 days there was still microscopic oil depots visible [54]. The authors speculated that the differences in body factors such as the activated immune system can influence this with macrophages [42].

Drug suspension in oil is another alternative to oil-based LAI. Most LAI suspensions are aqueous-based suspensions that require reconstitution prior to the injection. There are existing patents on suspension of drug crystals in oil, but currently no product on the market [63]. In comparison to aqueous suspensions, oil suspensions may be more difficult and painful for injection and would be less favored. The release of drug from oil suspension can be divided in three subsequent steps [64]. Initially the drug must diffuse through the oil, then passed through the oil-water interface and eventually dissolves in the water layer. In the case of water-soluble compounds, *in vitro* experiments have shown that the rate limiting step was the diffusion in the oil medium, which was affected by particle dimension and oil viscosity [64]. The influence of the oil viscosity on the drug release of ondansetron hydrochloride from drug loaded methylcellulose microparticles suspended in oils was studied [65]. Three oils with increased viscosities were investigated, medium chain triglycerides, corn oil and castor oil. Corn oil was selected as the appropriate oil for suspension in *in vivo* subcutaneous (SC) injection experiments in rats due to the sustained drug release performance and the

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appropriate viscosity for injection [65]. Although the *in vivo* results of the formulations with three different oils were not compared, the oil suspension using corn oil was compared to an ondansetron hydrochloride aqueous solution. The *in vivo* data showed a significantly more sustained drug release over 72 hours from the oil suspensions than the aqueous drug solution [65].

The addition of gelators has been used as an alternative method to increase the viscosity of the oil and enhanced the sustained drug release performance of oil suspensions [59-61]. In the case of the steroid ZK28, the gelation of medium chain triglycerides with methyl cholate was reported to produce a more constant and sustained release of the compound *in vivo* in Wistar rats [66]. A microcrystal suspension of drospirenone in medium chain triglycerides was compared to the same suspension that was stabilized by the addition of various gelators to increase the viscosity of the external oil phase [67]. The gelators used were aluminium stearate, silica, methyl cholate, cholesteryl stearate and dextrin palmitate derivatives [67]. The micro-suspension in the gelled oil improved the storage and lead to a longer drug release of drospirenone *in vitro*, in comparison to the non-gelled oil formulation. However, the choice of gelators made no significant differences in the drug release rates from the gelled oils. For the delivery of Procaine Penicillin G, beeswax and aluminium stearate were used as gelling agents for peanut oil, but in this case the type of gelator was important in determining the drug release properties [68]. No difference was seen in the drug release from the oil suspensions with and without beeswax, but the oil suspensions containing aluminium stearates showed a prolonged drug release. The *in vivo* results of the study on human subjects confirmed the slower release of the formulation gelled with 2% aluminium stearate compared to the formulations without gelator. In addition, the results also demonstrated that the gelled formulation containing small particle sizes provided more prolonged drug release after IM

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injection than the ones with large particle sizes [68]. As there is no existing marketed oil-based suspension product, this categorise of formulation will not be discussed further in this review.

2.1.3 Oleogels

An oleogel system is a semi-solid material composed of an organic solvent, such as liquid oil, that is immobilized by an oleogelator via physical or chemical interactions, into a continuous network [69-72]. Oleogels have been investigated for a wide range of routes of drug delivery, including injectable, topical and oral drug delivery applications [11, 28, 73-80]. Structurally, oleogels are distinctly different from oil solution and suspensions, as shown in **Figure 1**. This review focuses on the discussion of the potential uses of oleogels as an *in situ* forming LAI delivered by IM administration.

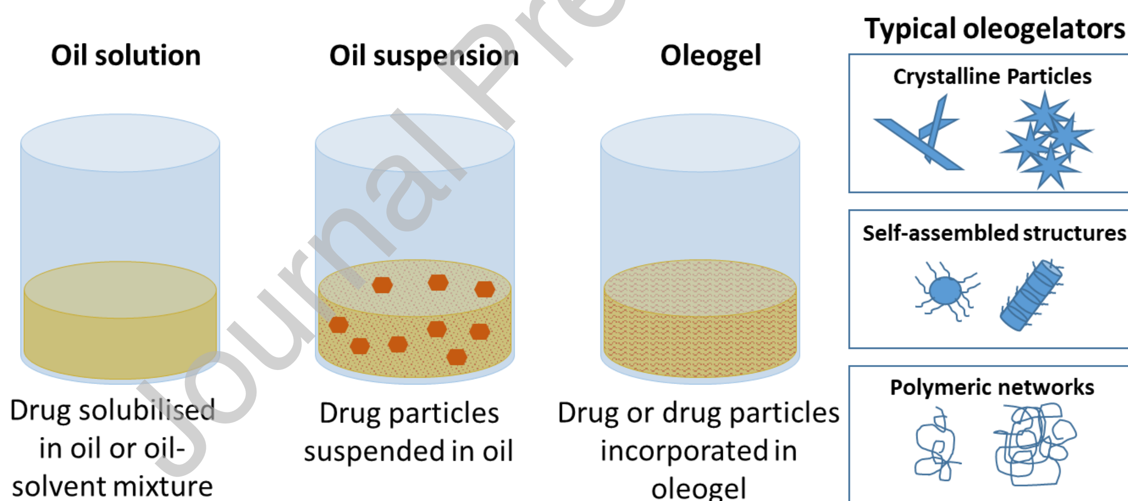

Figure 1. Graphic illustration of oil solution, oil suspension and oleogels.

Oleogels are formed as the oil phase is immobilized within spaces of a three-dimensional network formed due to physical interaction among the self-assembling structure of oleogelators (see **Figure 1**). Such a network is formed through either chemical or physical cross-linking [31]. In the literature, chemically cross-linked oleogels are rarely used for drug

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delivery and not discussed further here [81]. Oleogels formed via physical interactions mainly rely on supra molecular self-assemblies which are usually stabilized by non-covalent interactions, such as *H*-bonding, van der Waals attractive interactions and π–π stacking [27, 82]. The gelation process of the *in situ* forming oleogel is triggered by one or multiple stimuli, such as pH changes, temperature modulation, mechanical stress, metal anions and solvent exchange, which induce structural changes, such as the crystallisation of oleogelators [29, 83].

Commonly reported oleogels for drug delivery, mostly as a LAI depot delivered via subcutaneous injection, contain an oil carrier, an oleogelator, a cosolvent and an API. The solubilities of the oleogelators in the oil phase and in the cosolvent phase can significantly affect the quality of the gelation. Limited solubilities in both phases ensures rapid crystallisation/precipitation of the oleogelator in the oil when the cosolvent diffuses out of the oil matrix. From a structural perspective, weak interactions between oil and oleogelator may result in the formation of a continuous network; on the other hand, a strong gelator–gelator interaction can lead to phase separation due to the precipitation of crystalline or amorphous molecular clusters of the gelator [72]. In some cases , the oleogels are also be prepared using a heat-cool method with or without a cosolvent [84]. The oleogelators molecules are either melted or dissolved by the cosolvent at a low concentration (typically ≤15 wt%) at elevated temperature until a clear solution is formed. After cooling, the solubility of the gelator reduces and the gelator molecules either crystallise or self-assemble into fibrous aggregates [85]. The gelation process produces a homogeneous dispersion of the gelator assemblies in the oil carrier and slow down the drug diffusion from the oil phase into the body fluid [27, 31, 73, 82, 86, 87]. The drug release kinetics of the *in situ* formed oleogels are governed by a range of physicochemical and biochemical mechanisms, such as drug and cosolvent (if used)

diffusion, drug solubilization in the matrix and lipid degradation by endogenous lipases. In comparison to polymeric microparticle-based delivery systems, oleogels form depots with much lower specific surface area after injection and gelation, which reduces the risk of surface-area related initial burst release [67].

In order to use oleogels as an injectable product, i.e. as liquids instead of a viscous gel, there are two approaches described in the literature: 1) directly using the formulation containing oil, gelator and API with no addition of cosolvent, and heating prior to injection to melt and dissolve the gelator into the oil phase in order to the convert the gel into a liquid form [73]; 2) adding an organic cosolvent to the formulation to form a liquid injectable at room temperature and the gelation occurs after the diffusion of cosolvent post-injection [11, 74-78, 87]. When the *in situ* formation of the oleogel relies on the temperature change, the melting point of the gelators must be below 50 °C as to avoid tissue necrosis and a painful injection [88], but above 37 °C as to allow the formation of the gel at body temperature. Some fatty acid based oleogels fall within this range and have been used for SC administration [73]. However hot injection above 37 °C is not favoured in clinical practice for multiple reasons: 1) it may cause an increased level of discomfort at the injection site; 2) it may change the local blood supply for the injected muscle site; 3) it requires additional preparation and facilities to heat and maintain the formulation heated until administration.

To overcome the unfavourable features of oleogels, the use of an organic solvent as a gelation inhibitor is a more explored method to facilitate injection [75, 87]. Addition of a small amount of a solvent, e.g. N-methyl pyrrolidone (NMP), is necessary in order to partially or completely inhibit gelation at room temperature and allow the injection of the formulation. NMP is a biocompatible and water-soluble hydrophilic organic solvent [11, 78].

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Due to its water solubility, NMP diffuses into the surrounding tissues after injection allowing the formation of the gel. NMP was show to effectively disrupt the gelation of a gel containing 12-hydroxystearic acid [78] or amino acid based gelators [11], allowing their subcutaneous injection. *In situ* forming implants made with N-Stearoyl-l-alanine methyl ester (SAM), safflower oil and NMP were found to be well tolerated in rats and effective in delivering rivastigmine in a prolonged manner. Drug release studies performed on these formulations with and without the addition of NMP showed that the addition of NMP had no effect on the drug release rate [11]. On the contrary, the amount of NMP added in an oleogel containing tyrosine-based gelators and safflower oil, significantly reduced the burst release of rivastigmine hydrogen tartrate in *in vitro* studies [11, 89].

2.1.4 Lipid-based liquid crystal-forming systems

Lipid based liquid crystals or lyotropic liquid crystals form an interesting alternative long-acting formulation option in addition to fatty acid based oleogels. Liquid crystalline mesophases can be spontaneously formed from the liquid crystal-forming system (LCFS) in an aqueous fluid and provide a controlled drug delivery system that is able to entrap drug molecules. The sustained drug release is a result of the slow diffusion of drug through the tortuous networks of aqueous nano-channels in the mesophases [12, 90-92]. There are three types of mesophases; lamellar, hexagonal, and cubic with hexagonal and cubic being the most widely studied for controlled release due to their internal structure [93]. As shown in **Figure 2**, lamellar phase ($L\alpha$) is a 1-D network of planar lipid bilayers that slide over each other resulting in their low viscosity, hexagonal (H) is a 2-D lattice arrangement of infinite cylindrical micelles and cubic (Q) is a three-dimensional bicontinuous structure composed of continuous water channels that do not come into contact of each other [94]. The arrangement of these mesophases formed can be predicted by the critical packing parameter (CPP) of the

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lipid excipients used and identified under polarizing light microscopy by “fan -like textures”, “maltase crosses” and birefringence [95].

Both low molecular-weight and macromolecular drugs including proteins, peptides and nucleic acids have been reported to be delivered using the reversed hexagonal phase (H2) and the reversed cubic phase (Q2) of the lyotropic LC system [96]. As an example, the LCFS containing sorbitan monooleate (SMO) showed sustained release of leuprolide for 1 month (**Figure 2**). The treatment efficacy from the animal in vivo data indicated that the 90 μL LCFS s.c. injection was as effective as conventional PLGA solid microparticles depots [94]. For low molecular-weight drug, functionalization of LCs is often needed to enhance their loading and release properties [97]. The use of anionic phospholipids or cationic surfactants as an anchoring material (via electrostatic interaction between the excipient and the drug molecules) have been explored by serval groups [98, 99], [100] (**Figure 2**). Lim and co-workers used the hydrophilic charged drugs, entecavir, as an example and demonstrated the ‘anchoring’ effect and its ability to sustain the drug release [98].

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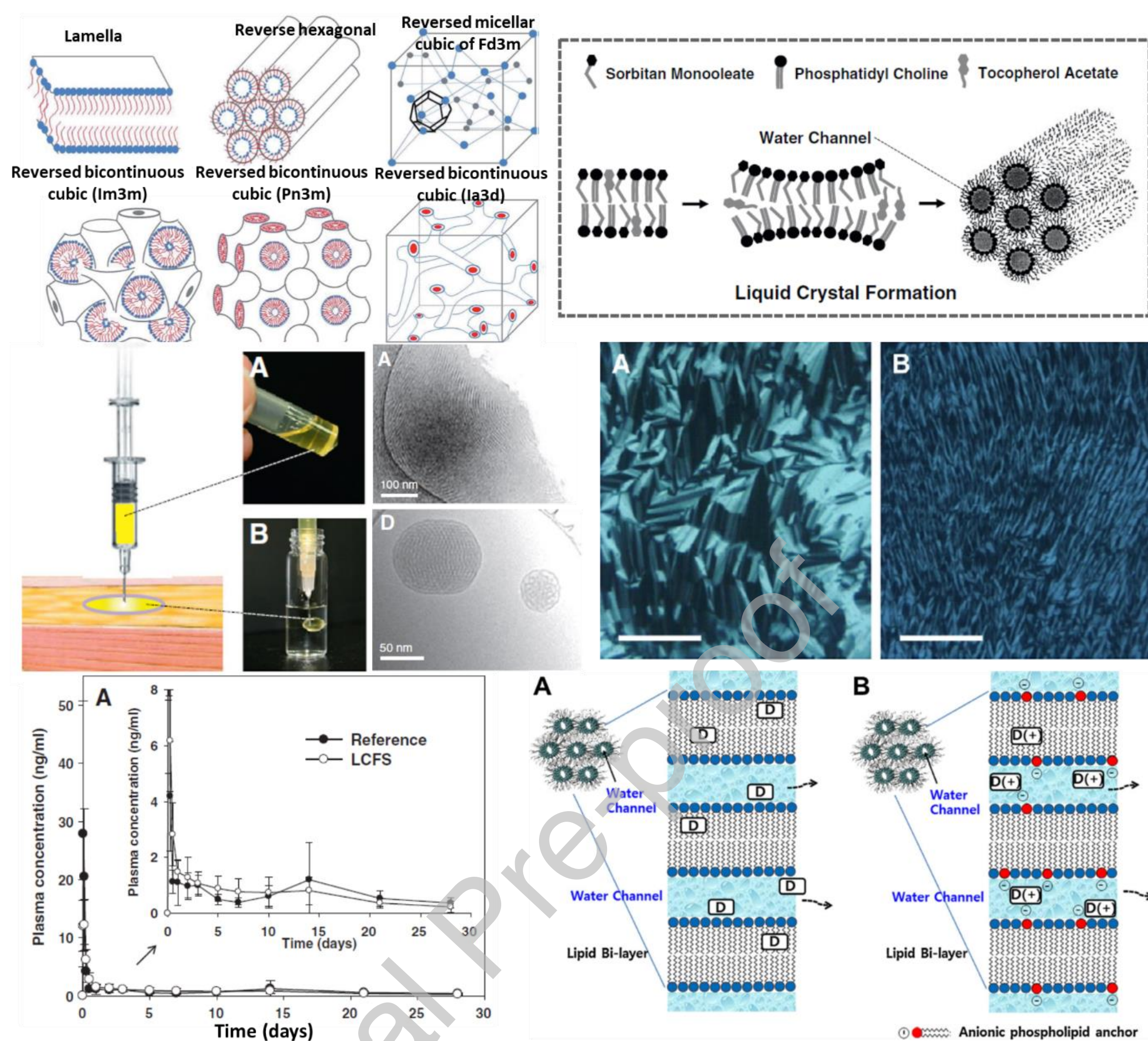

Figure 2 Top left: schematic illustration of the three different lipid based liquid crystalline structures, used with permission of [95]; top right: illustration of the LCFS formation of SMO containing system [94]; middle left: liquid crystal formation of SMO containing LCFS in water after injection [94]; middle: cryo-SEM images of the inner structures of the bulk mesophase and dispersed mesophase particles formed from LCFS [94]; middle-right: polarise optical microscopic images of the angular and anisotropic textures of the hexagonal LC phases [94]; bottom-left: plasma concentrations of leuprolide after SC injections of PLGA solid microparticles (as reference) and LCFS (90 μL injection volume) in dogs used with permission of [94]; bottom-right: illustration of additional 'anchoring' effect in LC mesophase for achieving sustained release of cationic drugs, used with permission of [98].

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2.2. Excipients

2.2.1 Oils

Oily solution based LAI products are easy and simple to formulate, manufacture, sterilise and have good long-term stability [2]. **Table 1** summarises the most commonly used oils in parenteral depot products. Unlike hydrogels, oils are particularly suitable for hydrolysis sensitive API's. Most oils are generally known for their high biocompatibility upon injection [101]. Application of super-refined oils can lead to reduced oxidation and hydrolysis of the solubilised compounds [102]. In general, oil solution based LAI products have a retention time limited to around four to six weeks [2], but it is also drug-dependent. As an example, Nebido solution for injection containing testosterone undecanoate only requires one injection of 750-1000 mg testosterone undecanoate administered every 10-14 weeks [103].

2.2.2 Oleogelators

For oleogels, in addition to oil, gelators are needed to enable the formation of the network and the immobilisation of liquid oil. The gelators generally fall into two categories; low molecular weight oleogelators (LMOGs) (<1 kDa) and high molecular weight polymeric oleogelators (POGs) (>2 kDa) [82]. Low molecular weight gelators can gel an oil simply by direct dispersion, i.e. being dissolved in the oil by heating the mixture and then letting it crystallize upon cooling. Among the high molecular weight gelators, only ethylcellulose has enough solubility for the oil to form a gel by direct dispersion, but it is not biodegradable thus not ideal to be included [72]. Structuring the oil with more hydrophilic biopolymers, such as proteins, is possible, but requires indirect methods as they are generally not dispersible in oil [104]. This class of gelators is not discussed here as they are out of the scope of this review.

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In addition to their stability in water, one of the main problems of administering oleogels is their injectability, both with respect to the force required to inject them and their rheological destruction after passing through the needle. *In situ* forming oleogels are a promising tool to overcome this obstacle. Low molecular weight gelators have favourable properties for formulating *in situ* forming oleogels, including thermoreversibility and, for some of them, their ability to self-assemble after the diffusion of a small amount of co-solvent. Despite the existence of a wide range of materials that can be used as oleogelators, for developing LAI products, the biocompatibility and a good solubility in a biocompatible organic solvent of the material needs also to be taken into consideration.

Fatty acids

Medium to long chain fatty acids, such as stearic acid, palmitic acid, myristic acid, and arachidic acid are widely studied as gelators for oleogels and are good candidates for pharmaceutical and food application due to their good biocompatibility. Preparation of these oleogels normally involve heating and melting of the fatty acid in oil followed by cooling which, causes crystallisation of the fatty acid and the formation of a solid network. Studies by Wang *et al.* [73] argued that in order to prevent tissue necrosis at the injection site, the ideal gelling temperature of these systems after the injection should be between body temperature (37 °C) and 50 °C. Using this temperature limitation, oleogels prepared using palmitic and myristic acid could not meet the standard. The oleogels containing stearic acid and arachidic acid at 7.5% and 5% concentration, respectively, can be prepared and then injected within this temperature range. The release of paliperidone from these oleogels were studied *in vitro* and compared to oil and aqueous suspensions. The oil suspension without any oleogelator extended the release to three days, compared to the 24 hours release of drug from the aqueous suspension. The addition of oleogelators to form oleogels further prolonged the release to

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seven days, with a most sustained release achieved with 5% arachidic acid [73]. The study also found that increasing the drug concentration also led to extension of drug release. The *in vivo* rodent data confirmed that the arachidic containing oleogels were able to form effective drug delivery depots post-injection and sustain the drug release over seven days governed by both diffusion and erosion mechanisms. After nine days the depot had completely disappeared (i.e. had been fully absorbed) [73].

12-Hydroxy stearic Acid (12-HSA)

12-HSA is a LMOG that can form oleogels at very low concentrations (<1% w/w) in oils such as canola, peanut and soybean oils [78, 105, 106] as the continuous phase. As seen in **Figure 3a**, the phase diagram demonstrates that 12-HSA can form gels with canola oil at low temperatures when a critical concentration of 0.5% w/w is reached. The strength and appearance of the gels are 12-HSA concentration and temperature dependent. In addition, external factors such as shear stress and cooling rate during the preparation of the 12-HSA containing oleogels can also affect the microstructures of the gels. Co and Marangoni studied the combined effects of applied laminar oscillatory shear and cooling rate applied during crystallization on the microstructures of 12-HSA/canola oil oleogels [106, 107] . They reported that crystallization at a high cooling rate (30 ºC/min) resulted in a spherulitic microstructure (**Figure 3b**) with a higher oil-binding capacity, lower storage modulus and lower yield stress compared with a material (with a fibrillar microstructure) crystallized at a slow cooling rate (1 ºC/min) [107]. As seen in **Figure 3b**, increasing the frequency of the oscillatory shear applied increased the incidence of nucleation for the rapidly cooled gel, but had no effect on the slow cooled gel [107]. With sufficiently high frequency (i.e. 10.0 Hz, **Figure 3b**, iv) of oscillation, the spherulisation (fibre branching) and growth was inhibited, but the mechanism of this observation is unclear [107].

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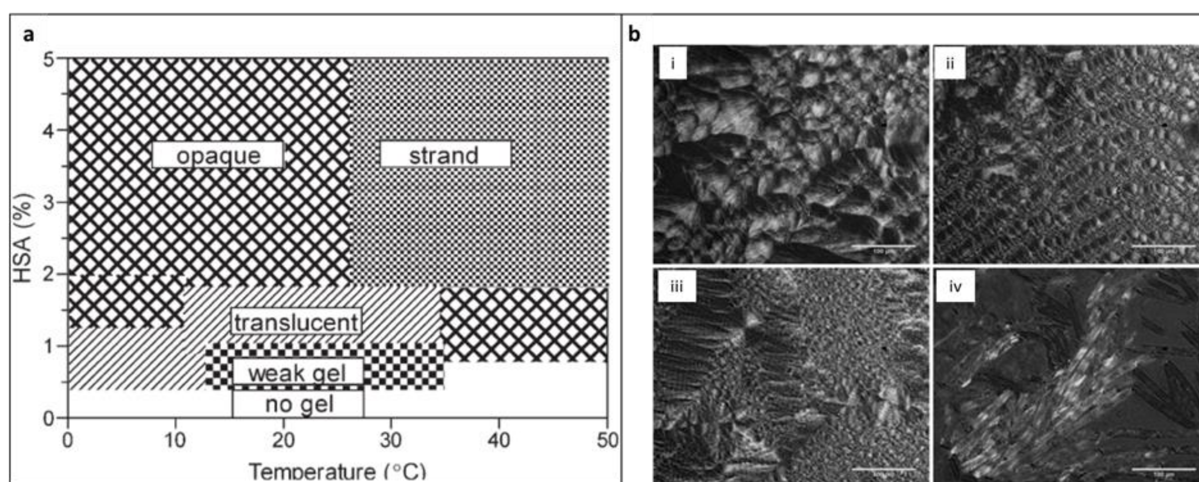

Figure 3. (a) The phase diagram of gelation behaviour when canola oil and 12-HSA are combined. Rogers *et al.*, 2009 [106] with permission; (b) The microstructure of 12-HSA/canola oleogels cooled at 30 ºC/min and shear-crystallized (oscillatory) under various frequencies
(strain = 1,500 %): 2.5 Hz (i), 5.0 Hz (ii), 7.5 Hz (iii), 10.0 Hz (iv). Co and Marangoni, 2013 [107] with permission.

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To formulate *in situ* forming oleogels, 12-HSA was used with solvent/gelation inhibitors including 2-pyrrolidone, ethyl acetate, NMP, DMSO, glycofurol, and PEG 400 and NMP was selected as optimal for its good oil miscibility and high solvating power for 12-HSA [78] . Tantishaiyakul *et al.* reported that 4% ethanol inhibited gelation of 12-HSA in coconut oil and the formulation could be easily injected through a 21-gauge needle. The *in vitro* drug release of the gels containing of the model lipophilic and hydrophilic drugs, piroxicam and diclofenac acid, were slower when compared to oily suspensions [76]. For delivering testosterone enanthate and leuprolide acetate using *in situ* forming oleogels, the addition of no more than 15% of NMP was sufficient to completely inhibit gelation of peanut oil-based formulations containing 5 % and 7 % of 12-HSA and form solutions which were stable for 6 months and injectable through 26-gauge needles [78]. However, the authors highlighted that

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convincing data interpretation and prediction of the *in vivo* drug release of the oleogels was difficult. This is not only due to the small sample sizes of the *in vivo* data, but also due multiple factors relating to the complex phase transformation (*in situ* solidification upon NMP extraction which also was believed to be at least partially responsible for the observed initial burst release) of the formulation after injections. Post injection, the surface erosion, fragmentation and deformation of the implants may also contribute to the unpredictability of the measured *in vivo* behaviour of the formulation [78] .

In its native form, due to its free carboxylic group, 12-HSA is suggested to cause skin irritation, thus work has been carried out to modify 12-HSA by ethoxylation in order to reduce the toxic effects. Berkhardt and co-workers [105] reported that ethoxylation of 12-HSA provided an structure capable of gelling a variety of solvents, including decamethylene cyclopentasiloxane, 2-ethylhexyl palmitate and bis(2-ethylhexylcarbonate), and oils (paraffin and caprinic/caprylic triglyceride). Interestingly, 12-HSA with the highest degree of ethoxlaytion provided a gelator with the most potent gelling capability [105].

Amino acid derivates

Amino acid based gelators are the most studied for biomedical applications for their intrinsic biocompatibility. Fatty acid derivatives of amino acids have also proved to selectively gel oil in an aqueous environment, making them optimal candidates as oleogels for injection in human body. Amphiphilic derivatives of L-alanine have been shown to form oleogels when combined with oils and in the presence of water [77, 89, 102, 108]. N-Stearoyl-l-alanine methyl ester (SAM) and N-stearoyl L-alanine ethyl ester (SAE) were used as gelators to produce gelled water-in-oil emulsions loaded with leuprolide. They were produced with safflower oil as the continuous phase and NMP as a gelation inhibitor (**Figure 4**).

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Characterisation of the depots by differential scanning calorimetry (DSC) and texture analysis showed that the 10% SAM system exhibited a higher gel–sol phase transition temperature than its SAE counterpart derivative and a 30% increase in strength and retained leuprolide for a longer period (24.0 ± 1.4 (SAM) versus 34.4 ± 5.9% (SAE) released after 72 hours), as demonstrated in **Figure 4b [75]**. It was suggested that the difference may be related to fast gelation kinetics which led to the low porosity and high stiffness of the formed gel. The greater stiffness of SAM gels was attributed to a higher level of H-bonding between the amide functions of the oleogelator, which, in case of SAE, was partially hindered by the ethyl group. For this reason, the most promising derivative for forming LAIs in the L-alanine class seems to be SAM. Oleogels prepared with SAM show greater drug retention properties than hydrogels and comparable release profile to polymeric microspheres [75].

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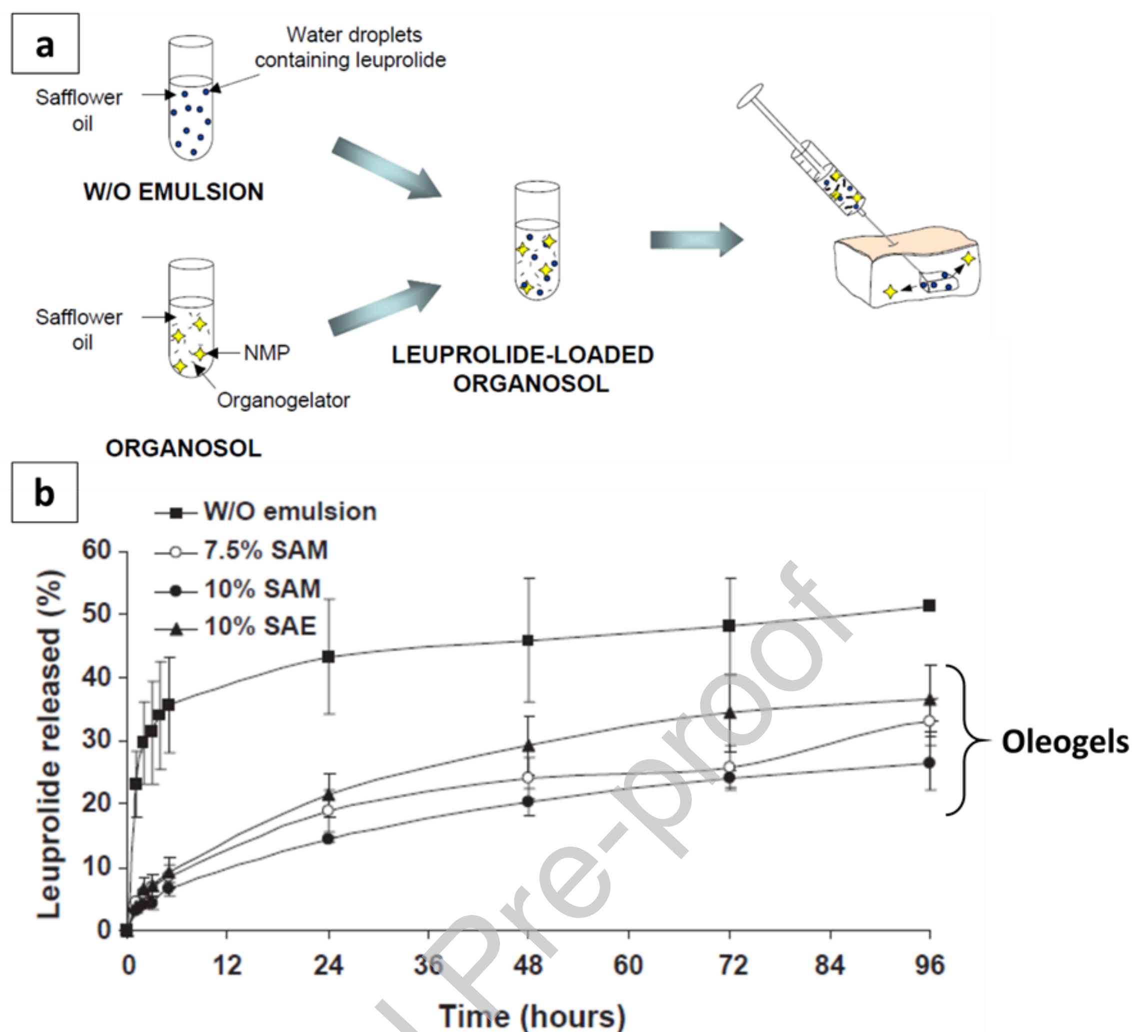

Figure 4 (a) Schematic of the preparation of a safflower and L-alanine oleogel to deliver Leuprolide and (b) the kinetics of release. Plourde *et al.* 2005 [75] with permission.

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SAM was used to gel sunflower oil containing rivastigmine or rivastigmine hydrogen tartrate in a dissolved or dispersed state respectively [11]. The *in vitro* data showed that rivastigmine oil suspension had a considerably smaller burst drug release effect in comparison to the oil solution [11]. The pharmacokinetics results of SC injected suspension to rats showed no statistical difference in values of area under the rivastigmine blood concentration versus time curve (AUC) for 14 days between oil based and oleogel formulations. Nevertheless, the burst release decreased with increasing gelator concentration, contrary to *in vitro* studies. Decreasing the burst release could allow the incorporation of a higher amount of drug, thus

prolonging the release. The authors hypothesised that higher SAM oleogelator concentration led to the higher density of network. This improved cohesiveness of these gels, which may reduce the subcutaneous spreading of the formulation after injection. Minimal spreading gave a potentially small surface area which generated minimized drug contact with the aqueous environment, contributing to a decrease in burst release [11].

Work has also been carried out on the *in vitro* and *in vivo* degradation of SAM oleogels combined with soybean oil and NMP [87]. A correlation between the concentration of SAM in the oleogel and the time it took to degrade in PBS (8%, 10%, 12%, and 15% SAM oleogels degraded by 35.40%, 31.32%, 25.39%, and 17.50% over 40 days) was reported suggesting that a dense gel could block water molecules from entering the 3D network. An *in vivo* study demonstrated that the 12% SAM oleogel was biodegradable with very little remaining after six-weeks, where a gel weight loss of 94.57% was observed, which was significantly faster than the *in vitro* results (25.39%). The distinct difference between the *in vivo* and *in vitro* methods of evaluation may raise questions on the direct correlations between *in vivo* and *in vitro* data for both biodegradability and drug release. In terms of toxicity, MTT cell viability studies of the SAM oleogels using mouse fibroblasts (L929) showed some evidence of toxicity when the gel extracts were diluted 1:5. When diluted further to 1:20, no toxicity was detected [73].

Despite the promising results, L-alanine based gelators assemble through relatively weak molecular interactions, leading to the formation of gels with poor mechanical properties and require a high concentration of gelator. Tyrosine based gelators, in particular the N-behenoyl L-tyrosine methyl ester (BTM), has shown to have better gelation ability, rheological properties and drug release profiles compared to L-alanine based gelators *in vitro* [89].

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Animal studies conducted in rats concluded that both SAM and BTM were able to prolong the release of rivastigmine hydrogen tartrate compared to the oil suspension, from 2 to 10 days [108]. The gelation of the oil greatly decreased the burst release, allowing the administration of a greater amount of drug. This effect was two times greater for BTM compared to SAM.

Methyl (S)-2,5-ditetradecanamidopentanoate (MDP) was synthetized as a new oleogelator to improve the mechanical properties of oleogels formed with L-amino acid derivatives [109]. The amounts of MDP necessary to structure oils, such as injectable soybean oil, olive oil and corn oil, were below 5% (w/v) which was much less compared to SAM in which the gelator concentration was between 7.5 to 10 % (w/v) in safflower oil. This improvement was attributed to the more H-bonding sites, s as a result of the greater number of amide groups. The release of candesartan cilexetil was investigated *in vitro* as to optimize the amount of gelator, drug and gelator inhibitor. The chosen formulation contained 4.9% of MDP and 30% of NMP and was tested *in vivo* and compared to an oil solution. The oleogel formulation significantly prolonged the release and had good biocompatibility.

2.2.3 Solvents

For LAIs, solvents are used in *in situ* forming dosage forms, such as oleogels and LCFSs. Solvents reduce the viscosity of the formulation by inhibiting crystallisation and preventing lipid solidification and ensure the formulation is injectable. Using NMP containing oleogels as examples, NMP will partially disrupt the interactions between the oleogelator molecules, thereby maintaining the formulation in a liquid state and hence ease the injection. Once *in situ*, the solvent diffuses into the surrounding tissue, allowing the oil and gelator to interact and form a solid depot [29, 75, 78]. The diffusion of the solvent may, in some cases, led to

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burst drug release if the drug has a high solubility in the specific solvent, thus the optimisation of the quantity used is required. The commonly used organic solvents in oleogels and LCFSs are NMP, DMSO, ethanol [11, 75-78, 87].

The use of solvents in IM injectable formulations is, however, controversial. Some studies suggested that the solvent may cause potential muscle damage at the injection site. Kranz *et al.* [110] evaluated muscle damage *in vivo* by measuring the cumulative creatine kinase (CK) release from the muscles after injection with either the pure solvent, including NMP, DMSO and 2-pyrrolidone or *in situ* forming depots containing the same solvent. Their results showed a significant increase in CK when the solvent was injected alone in comparison to a control injection of saline. 2-pyrrolidone demonstrated the lowest toxicity in comparison to other solvents investigated [110]. *In situ* forming depot solutions containing 40% PLA, and the same solvents discussed above, showed comparable CK-efflux to the pure solvents. However, when peanut oil was added to the formulation the CK-levels were significantly lower and decreased with increasing amount of external oil phase. The authors hypothesized that the external oil in the *in situ* forming formulations acted as a barrier and prevented the immediate contact of the solvent with the muscle [110]. There are other studies indicating that the irritation caused by the use of solvent in the IM injectables is minimal when using inflammation as a marker. For example, Motlusky and colleagues used NMP in an oleogel composed of L-alanine and safflower oil [102]. The biocompatibility study showed a minimal inflammatory reaction which was limited to the immediate area around the gel and within the range of expected foreign body reaction. In comparison to polymeric microsphere based LAIs, the significantly lower surface area/volume may also play a role in the low tissue inflammation observed [111].

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2.2.4 Lipid-based liquid crystalline forming materials

Liquid crystal forming depots are mostly formulated with lipid excipients that can spontaneously self-assemble and form a range of thermodynamically stable liquid crystal phases either by hydration or solvation and without any external influences. Some examples of these include, glycerol monooleate (GMO) , glycerol dioleate (GDO) , oleyl glycerate (OG) , phytantriol (PT) [112] and sorbitan monooleate (SMO) [95], with GMO being the most studied lipid for liquid crystal forming depots. This can be attributed to GMO's non-toxicity and biocompatibility properties together with its high swelling capacity in aqueous solutions. The amphiphilic nature of these lipids is why they form various lyotropic liquid crystals with their hydrophobic tails as the driving force [95].

Among the materials mentioned above, glycerol monooleate is the amphiphile of choice in food and pharmaceutical applications, mostly due to its emulsifying and biodegradability properties which is a result of glycerol monooleate susceptibility to lipolysis due to the different kinds of esterase activity in different tissues [113]. Furthermore, glycerol monooleate has the unique ability of being able to form the lamellar, hexagonal, bicontinuous cubic, sponge and discrete micellar phase [113].

Both glycerol monooleate and sorbitan monooleate were used as the key excipients for LAI depots of leuprolide acetate with promising results [75, 93, 94]. The depot formulation containing sorbitan monooleate demonstrated a significantly reduced initial burst compared to a commercial depot of leuprolide acetate and no signs of chronic inflammation at the injection site in the animal models [75]. Such liquid crystalline based products have been recently commercialised. The Fluid crystal® injection depot (Camurus, Sweden) is such example [114]. Fluid crystal® is a lipid-based liquid crystalline formulation platform that

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spontaneously forms a nanostructure in an aqueous environment and can provide sustained release of therapeutic compounds over weeks to months [114].

3 Formulation characterisation

3.1 Physicochemical material characterisations

Due to the simplicity of the formulations, characterisation of the oil solution product (containing API with either oil or oil with a cosolvent) is relatively simple in comparison to other lipid-based formulations, such as oleogels and liquid crystal-based products. Chemical stabilities of oil solution products are mainly related to degradation of the oil and API over shelf life. The physical stability of the oil solutions can be monitored by visual inspection of formation of particulates; hence the main focus of this section is related to the characterisation of more complex formulations, including oleogels and liquid crystal-based products.

During the formation of an oleogel, a gelator self-assembles in a three-dimensional structure network interacting via non-covalent bonds [106]. The strength of the gels at the macroscopic scale depends on how the strands interact (the interactions are based on hydrogen bonding, van der Waals interactions or π- π stacking). The particulars of the interaction depend on the type of gelator and concentration, the solvent used [76], the cooling rate during gel preparation (if heating is used), external oscillatory shear applied during preparation [107, 115], and the storage temperature of the gel [106]. Additives such as ethyl cellulose, Span 60 or Tween 20 can also modify the gel strength and/or structure to obtain a crystalline structure amenable to the purpose of the work [116, 117]. For liquid crystal-based formulations, the self-assembly arrangement of the liquid and surface-active molecules determines the mechanical and drug delivery functional behaviour of the resulting formulation [12]. The

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arrangement into a cubic lattice or cylindrical micelles allows the controlled delivery of model drugs. For example the sustained release of glucose was achieved through the liquid crystal phase transition from cubic to hexagonal [118]. Sustained release can be achieved through these phases due to physicochemical properties such as viscosity and mechanical strength. Therefore, determining the molecular, structural, and mechanical properties is an essential part in the development of an organogel formulation. In this review, focus will be on the selection of most effective microscopic, spectroscopic, structural, and mechanical characterisation methods.

Microscopy

A variety of microscopy approaches are readily used to characterise the microstructure of oleogels. Polarised light microscopy (PLM), scanning (SEM) and transmission electron microscopies (TEM) are particularly useful tools for characterising the morphology of oleogels and distinguish the type of liquid crystals present. Hot-stage PLM can provide an interesting platform to study the behaviour and thermo-reversibility of oleogels as a function of temperature.

PLM is a convenient means of visualising the microstructure of oleogels and to identify the type of liquid crystals. Samples require minimal preparation and qualitative data are rapidly obtained at typical scales ranging from 50 to 300 μm. As an example, needle-shaped fibers can be observed for stearic acid based oleogels [119] while 12-HSA based oleogels was reported to show fibrillar crystals of various brightness intensity which reflect the twisted nature of the 12-HSA strands [115, 120]. Oleogels are usually prepared by melting the oleogel (and surfactant if applicable) in the solvent at elevated temperatures, thoroughly mixing and dissolution of drug then letting the mixture to cool down. PLM can also be used

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to investigate the effect of the cooling rate which is an important factor that can affect the integrity of the 3-dimensional crystal network. As an example, 2.5% 12-HSA in canola oil showed spherulitic aggregates seen as Maltese crosses when cooled at 30 ºC/min [107] (**Figure 5a**) as opposed to the fibrillar structures showing segmented birefringence observed at lower cooling rates (**Figure 5b**) [115, 116, 120].

PLM is particularly useful to establish the structure type of the major mesophases of lyotropic liquid crystal systems [121]. Monoglycerides such as GMO or SMO are amphiphilic lipids that can self-assemble upon addition to water to form mesophases. Cubic phases (Q_{II}), often displayed by systems consisting mainly of glycerol monooleate or phytantriol, with or without a small portion of inhibitor, (NMP for example) are characterised by a micrograph with a black background representing a lack of birefringence [121]. With addition of NMP and water and reduction of phytantriol, the formulations formed a lamellar phase (L_{α}) displaying a maltese cross pattern over a black background (**Figure 5c**) [122] Addition of tricaprylin to glycerol monooleate or phytantriol resulted in the formation of a hexagonal phase (H_{II}) characterised by intense fan-like patterns (**Figure 5d**) [121, 123, 124].

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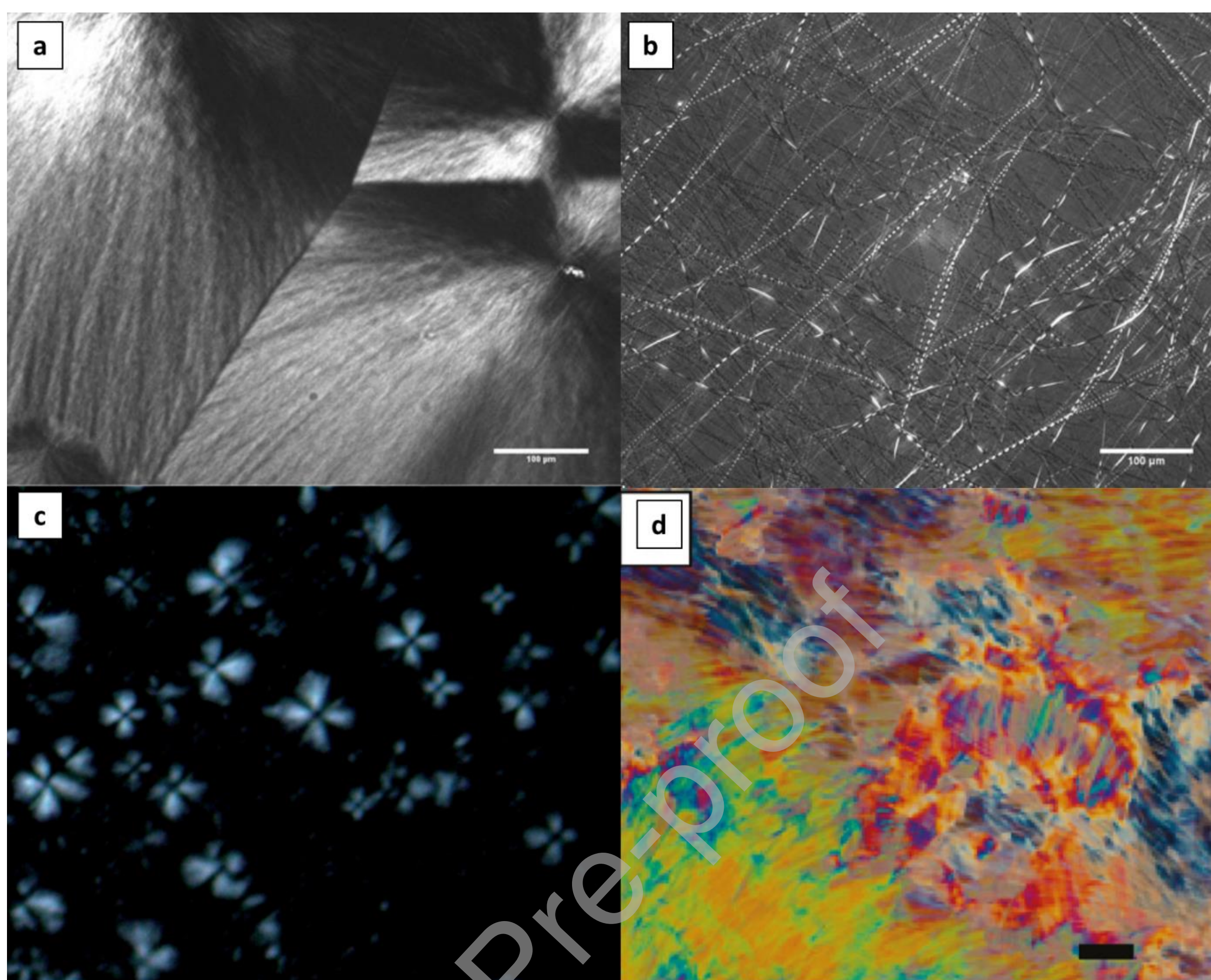

Figure 5 PLM images of oleogles formed with 12-HSA in canola oil showing the fibrillar microstructures of the oleogels being affected by cooling rate [107]. An increased incidence of spherulite nucleation in the oleogels that were rapidly cooled at 30 °C/min (a) and thickening of fibres observed in the oleogels slowly cooled at 1 °C/min (b). PLM of a lamellar phase Lα formed by phytantriol, water and NMP [122] (c), and a hexagonal phase H_{II} from a formulation containing GMO and tricaprylin [124] (d). The images were reproduced with permission.

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SEM and TEM were used to characterise 12-HSA oleogels and nanoparticles formed from liquid crystal systems (cubosomes and hexosomes) [94, 106, 125]. However, in the case of conventional SEM, the samples need to be dried out for testing which can significantly alter the structure of the samples. The cryo/freeze-fracture approach applied to SEM and TEM

can preserve the structural morphology of gel-like samples and allow more direct observation of the microstructures of the samples, such as oleogels and liquid crystals [126]. As an example, **Figure 6** shows some examples of the cryo-SEM and cryo-TEM images of organogel and lipid based liquid crystalline nanoparticles to reveal their microstructure [100, 106, 127].

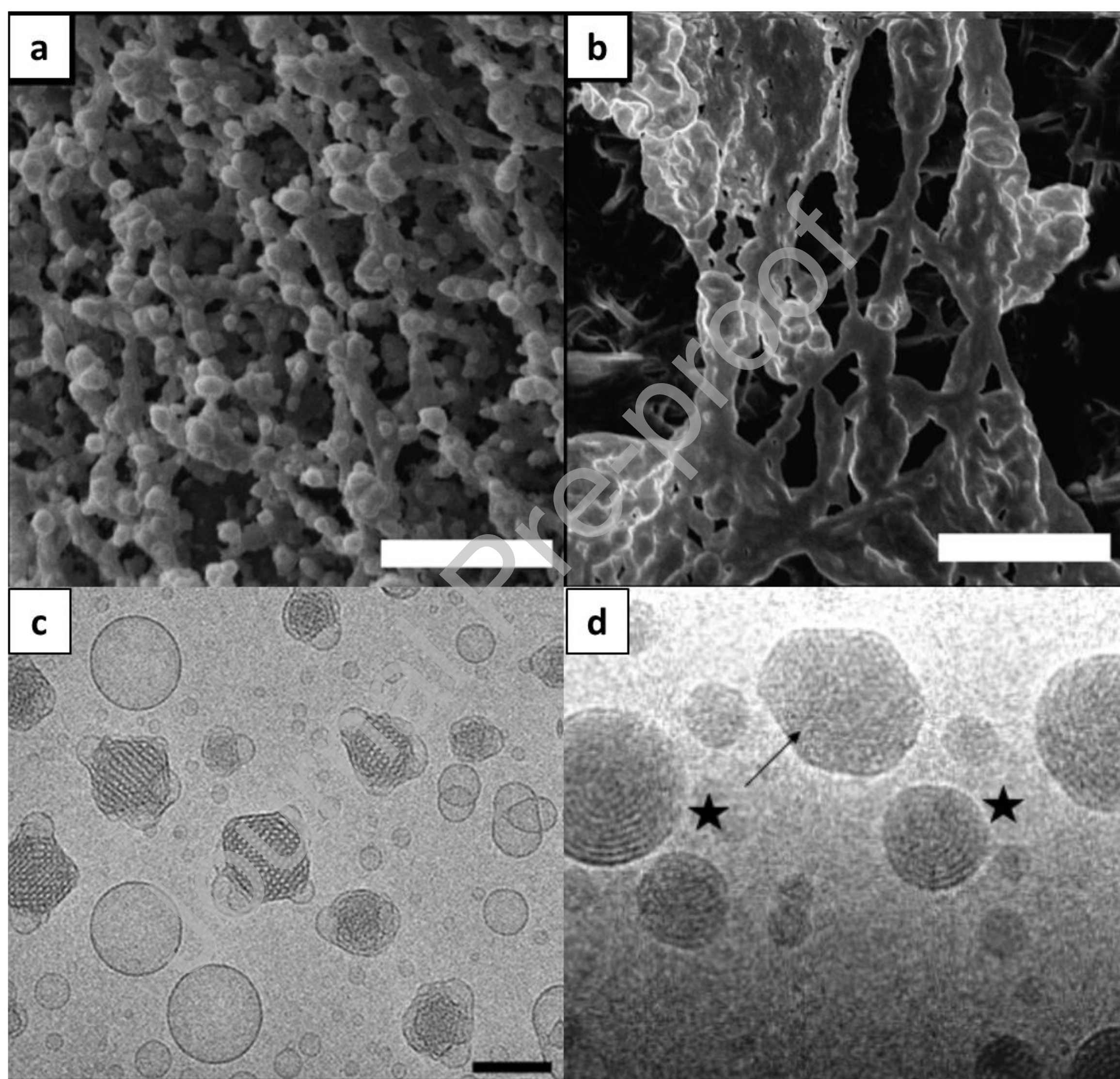

Figure 6. Cryogenic SEM images of 2% 12HSA-canola oil oleogel stored for 24 h. Samples treatment: after storing for 24 h at 5 °C (a) and 30 °C (b) the samples were either treated with 80/20 v:v hexane/acetone, taken with permission from [106]; cryo-TEM images of dispersed cubic nanoparticles made from GMO [100] (c); cryo-TEM image of monolinolein-based

hexosomes with the presence of curved striation (stars) and hexagonal internal symmetry (arrows) [127] (d). All images were taken with permission.

Structural characterisation

Wide angle and small angle X-ray scattering (WAXS and SAXS) can provide critical information on the crystalline structure and molecular arrangements of liquid crystalline and oleogels. For example, WAXS is relevant to studies at the nanometre scale and identifies polymorphisms in oleogels such as those made from stearic acid and stearic acid-stearyl alcohol mixtures [114], while SAXS have the ability to detect inhomogeneities at the mesoscopic level [128]. SAXS is the essential structural method of choice to characterise the mesophase type in liquid crystal systems [122, 123, 125, 129, 130].

Fourier Transfer Infrared Spectroscopy (FTIR)

Attenuated total reflection (ATR)- Fourier Transfer Infrared Spectroscopy (FTIR) is a versatile tool used to elucidate the chemical structure of materials and the molecular interactions in composite materials. For lipid-based formulations, the characteristic carbonyl band, typically in the 1700- 1750 cm^{-1} region, is sensitive to hydrogen bonding as is the hydrogen bonded OH-H bend around 940 cm^{-1} [106]. For liquid crystal-based gels, ATR-FTIR has been used not only to investigate interaction between the liquid crystal forming materials, but also drug-excipient interaction. As an example, the incorporation of gallic acid (as the model drug) and polypropyleneimine in a liquid crystal forming system consisting of GMO and water shifted the H-O-H bending band at ~ 1650 cm^{-1}. This peak was used to characterise the hydrogen bonding with the GMO headgroups within the cubic phase as a result of competitive water interaction [121].

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is a highly effective tool to gain molecular level information as well as larger scale structural information on hydrogels, oleogels and liquid crystals that cannot be obtained by other characterisation methods.

NMR can explore both the range of dynamics within a system and obtain information about molecular interactions and structure. By use of imaging techniques and diffusion measurements it can also give information about the meso- and macro- structure of the system [108, 118,119]

Typically, diffusion measurements have been used to explore structure in mesophases [108] and to characterise the hysteresis of gelation in the low-molecular weight oleogelator 2,3-di-*n*-decyloxyanthracene [131]. Measurement of proton relaxation times readily distinguishes between solid and liquid phases. For example, it was used to evaluate the degree of crystallinity in 12-HSA canola oil oleogels [106]. The wider field of the applications of NMR to hydrogels and oleogels has been reviewed by Shapiro [132] .

Thermal characterisation

Differential scanning calorimetry (DSC) analysis measures temperature induced transitional events that exhibit enthalpic changes. For characterisation of lipid-based formulations, DSC is useful determine how excipient-excipient and drug-excipient interactions may affect the melting, crystallisation and mesophase transformations. A decrease of melting temperature usually indicates a reduction in crystalline structure in the material while an increase confers the possibly of an increase in crystal size and/or a better crystalline structure. This was observed for 12-HSA oleogels when 12-HSA was added at levels of 1, 2, 3 and 5% [106].

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Similarly, addition of ethylcellulose to SA:SO oleogel formulations resulted in a larger melting enthalpy and higher crystallisation temperatures [133]. Applying heat-cool cycles to test the samples was used to evaluate the thermal stability of the oleogel [133]. For identifying phase transitions, DSC was used in combination with PLM to confirm the nature of the birefringence transitions observed in a phytantriol-based liquid crystalline formulation was the transition between cubic and hexagonal phases [134].

Mechanical characterisation

The viscoelastic properties are one of the most important mechanical properties of lipid-based LAI products. This is because they can directly impact on the injectability of the product. Using oscillatory rheology measurements, samples are submitted to known amounts of mechanical strains for a determined frame of time and analysed for their viscous and elastic properties. In the context of gels, the liquid component (loss modulus; G'') should be considerably smaller than the solid component (storage modulus; G') [28, 133]. As an example, injectability of a lyotropic liquid crystalline precursor was evaluated by measuring the viscosity and shear stress as a function of shear rate [123]. A low viscosity (0.6 Pa s) and a decrease in the value of shear stress/shear rate may be indicative of a shear thinning property, a favourable trait for injectable products. In addition to predicting injectability, for oleogel and liquid crystal-based formulations, these properties can also provide some level of indications to the likelihood of *in vivo* fragmentation and shape deformation. For example, the viscosity of stearic acid based oleogels was shown to be a function of shear rate applied during measurement [119] and the application of oscillatory shear resulted in the modification of the microstructures and mechanical properties of 12-HSA oleogels in a cooling rate-dependent fashion [106, 107]. This may potentially to be used to simulate *in vivo* stress and provide implications to their *in vivo* degradation.

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In addition to rheology measurements, texture analysis is another important methodology for obtaining firmness and subsequently injectability of oleogel and lipid-based liquid crystal formulations. Firmness of the oleogels can be determined by back extrusion or probe penetration tests using a texture analyser [128, 133, 135]. The force expressed in Newtons (N) applied to the sample can be measured as a function of distance in millimetres travelled by the probe [133] or simply determined at fixed penetration depth and probe speed [128, 135]. Adhesiveness can also be measured alongside gel strength by measuring the force values while the probe is withdrawn at a set speed [123]. In a step closer to *ex vivo* injectability assessment, Rungseevijitprapa and Bodmeier evaluated the injectability of *in situ* forming systems using fresh chicken meat model where the injection force was measured using a texture analyser (as illustrated in **Figure 7**) [136]. To determine injection force into muscular tissues, the needle was inserted 0.5 inches under the skin using various needle sizes [136].

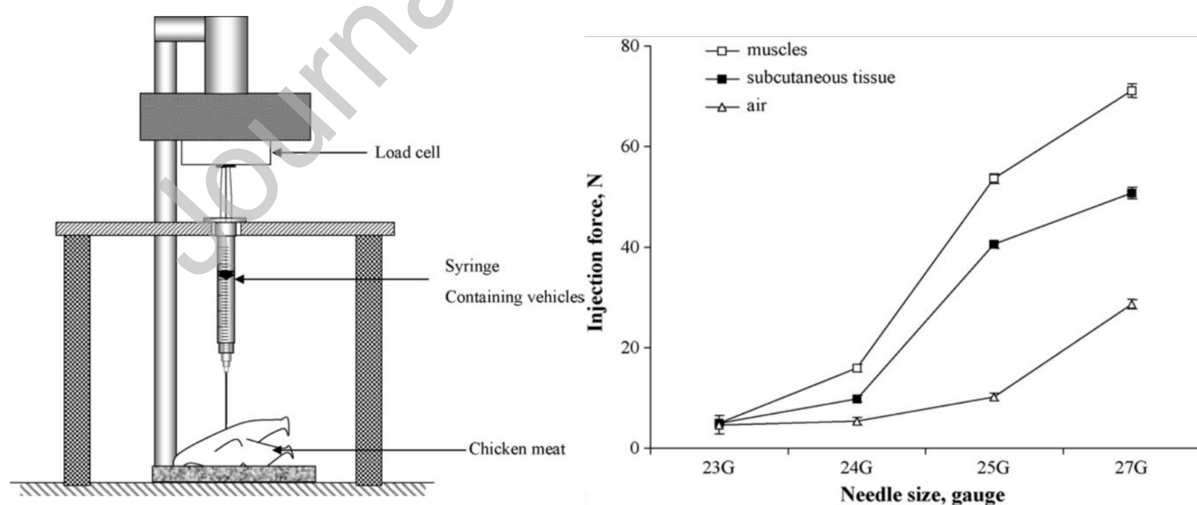

Figure 7. Illustration of the setup for injection force measurement with chicken meat model (left) and injection force of O/W– *in situ* forming microparticle formulations with varying

needle sizes and injection site, injection speed 100 mm/min, taken with permission from [136].

3.2. *In vitro* drug release characterisation

Currently there is no unified pharmacopeia guidance for *in vitro* drug release assessment of IM injectable products. In 2009, Larsen and Larsen reviewed and illustrated a selection of *in vitro* methods to determine drug release from oil-based parental solutions [2]. Oleogels prior injection are often in the form of a liquid, thus these *in vitro* dissolution methods may be applicable to injectable oleogels. However, in order to more closely mimicking the *in vivo* post-injection scenario of the oleogels, methodology modifications are required to take the solidification process post-injection of oleogels into consideration. **Table 2** summarise the most common *in vitro* drug release testing set up described in the literature for lipid based LAIs, including solutions and *in situ* forming systems. For *in situ* forming LAI system, **Figure 8** summarises the graphic illustration of the dissolution set ups that have been used in the literature. From the analysis of the literature data, it is apparent that the choice of the apparatus to investigate drug release from parenteral formulations can significant affect the *in vitro* drug release results measured [11, 67, 89, 93, 137-139]. Often the choice of the *in vitro* testing method is driven by the primary purpose of the *in vitro* testing, whether being discriminating of the performance difference between formulations or establishing IVIVC correlation.

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Table 2. Comparison of *in vitro* drug release methods used to test *in-situ* forming lipid-based LAI formulations, including example lipid-based systems and hydrogels.

Method	**Formulations Tested**	**Description**	**Results and In-Vivo Correlation**	**Reference**
Non-Membrane	1. Fatty acid gelators in soybean oil (Paliperidone and Coumarin-6) 2. Aqueous solution of Pluronic F127 (Oxytocin) 3. Aromatic amino acids, Safflower Oil and Rivastigmine	Sample is allowed to transform into its gel phase inside a container and dissolution medium, usually phosphate buffer, carefully added so as not to disrupt the formulation. Then tube is then incubated in a water bath at 37°C	***In vitro*:** Steady drug release over 14 days with more sustained profile as drug concentration increased. Good discriminatory power between release profiles **Correlation to *in vivo*:** Poor IVIVC correlation due to shorter drug release *in-vivo*, likely due to lack of degradation of oily matrix in-vitro. Drug release rate is largely underestimated using in-vitro studies	[89, 137, 138]
Dialysis tubing	1. Liquid Crystal systems (Leuprolide acetate). 2. Microcrystal suspensions in peanut oil (Drospirenone). 3. Alanine based gelator and Safflower oil (rivastigmine). 4. Aqueous solution of Pluronic F127 (Oxytocin)	Tubing is submerged into a physiological buffer (pH7.4) and held at 37°C in a water bath. The bag can be placed either in USP apparatus 2 or agitated using a stirrer. Dialyzate is withdrawn frequently to gain release of drug at specified time points and replaced with fresh buffer to ensure sink conditions maintained	***In vitro*:** Burst release was observed. Poor discriminatory power as the drug dissolution from the matrix is unaffected by the density of the gel. **Correlation to *in vivo*:** Sink conditions are available *in vitro* although may not be available *in vivo* until the drug reaches the circulation, as such, drug release profiles show greater release *in-vitro*. *In-vivo* studies showed greater differences in drug release from gels containing differing concentrations of gelator.	[11, 67, 138, 140]

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USP Apparatus 1 (basket)	1. Aqueous solution of Pluronic F127 (Oxytocin) 2. Glutamate based gelators and soybean or medium chain triglycerides (MCT)	Basket is used to contain the LAI sample with or without mould	***In vitro*:** Useful method if drug release by degradation (plus lipases) is investigated but not used widely. Degradation drug-release studies by Hu at al (2018) showed good discrimination between gelator types. This discriminatory power was not as robust in work done by Chaibva and Walker, (2007). **Correlation to *in vivo*:** Data from *in vivo* mass loss profiles and *in vitro* drug release both fit the Hixson-Crowell erosion model. Drug release using the basket with lipase could predict degradation *in vivo*.	[138, 139]
USP Apparatus 2 (paddle)	Aqueous solution of Pluronic F127 (Oxytocin)	Hydrogel contained within mould and placed at the bottom of dissolution vessel with paddles above	***In vitro***: No significant differences shown between the release profiles when using this method **Correlation to *in vivo***: No comparison	[138]
USP Apparatus 3 (VanKel Bio-Dis)	Aqueous solution of Pluronic F127 (Oxytocin)	This dissolution set-up comprises of a screen mesh containing pores (177µm) which retains the semi-solid formulation away from the dissolution media.	***In vitro*:** Method gave the greatest discriminatory power between formulations when compared to Non-Membrane, Dialysis tubing or USP apparatus 1 and 2. **Correlation to *in vivo***: No comparison	[138]

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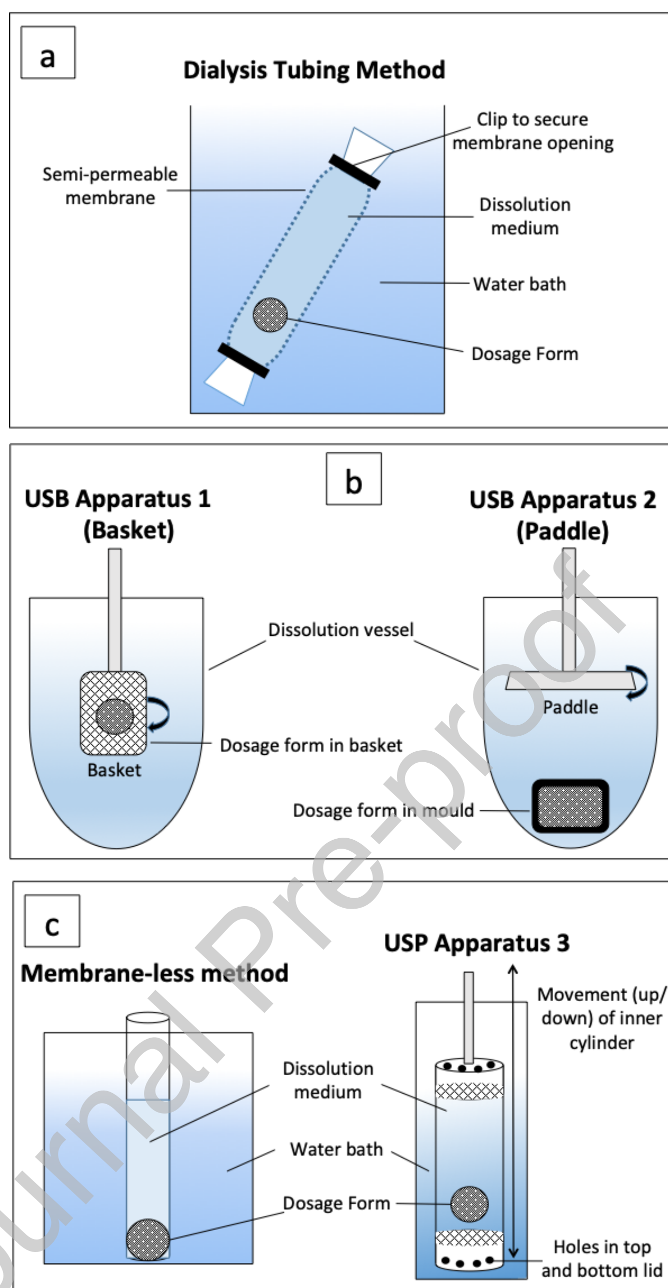

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Figure 8 Graphic illustration of the *in vitro* drug release testing for *in situ* forming LAI products (a) dialysis tube method set up; (b) modified USB apparatus 1 and 2 set up; (c) the membrane-less dissolution method and the USP apparatus 3 dissolution set up.

Dialysis bags and tubes are commonly used to evaluate drug release from oleogel [11, 67] and liquid crystalline formulations [11, 67, 138, 140]. The dialysis bags and tubes are chosen based upon the molecular weight cut off required for the drug of interest. The dialysis tubing

set up (**Figure 8a**) has the advantage of containing the oily solutions thereby preventing floating and breaking up of the gel after its formation. Typically, the tubing is submerged into a physiological buffer (pH 7.4) and held at 37 °C in a water bath. The bag can be placed either in USP apparatus 2 or agitated using a stirrer. However, it appears that the use of dialysis bags provides low discriminatory power between different formulations [11, 67, 138]. Nippe and colleagues discussed this point at length and pointed to the fact that a poor discriminating model was most likely the background for the poor IVIVC obtained [67].

Modified USP 1 (basket) and 2 (paddle) methods (by securing the sample inside a mould) are not commonly used to evaluate drug release from LAI's [141] (**Figure 8b**). Hu and colleagues used the modified baskets as a technique to solve the problems associated with dialysis bags as discussed by Nippe [67] and proposed that it to be a useful technique to evaluate degradation driven drug release when lipase was added into the dissolution media. Rotation speed is a factor to be considered since minimal hydrodynamic agitation of the oleogel might be expected in the muscle. As such, a lower rotation speed (25rpm) was chosen by Chaibva and Walker [138] for their experiments. In terms of comparing USP 1 and 2, Chaibva and Walker [138] reported that both methods were not accurate enough to distinguish differences in drug release between different Pluronic F127 gel concentrations (20%, 25% and 30%). In contrast, the dialysis tubing combined with apparatus 2 provided improved differentiation between the formulations [138].

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Further work by Chaibva and Walker [138] tested the capability of a modified USP 3 method (**Figure 8c**) to discriminate between the release of oxytocin from thermo-setting hydrogels of Pluronic® 127 with three different Pluronic concentrations. This dissolution set-up comprises of a screen mesh containing pores (177 µm) that retains the semi-solid formulation from the

dissolution media. The release profile of oxytocin demonstrated clearly defined differences in the drug release kinetics compared to the other methods (USP 1, USP 2 ± mould/dialysis tubing/ membrane-less method).

A similar set-up to USP 3, but without the membrane, so called membrane-less method was also reported in the literature used for the evaluation of drug release from a range of gel-based formulations [73, 89, 138, 142, 143]. The sample was allowed to transform into its gel phase inside a container and dissolution medium (usually phosphate buffer) carefully added not to disrupt the formulation. The tube was subsequently incubated in a water bath at 37°C (**Figure 8c**). This system was also reported to have good discriminatory power between formulations. Wang *et al.* [73] used a membrane free system and discovered that the fatty acid oleogels provided sustained release over seven days with data that clearly differentiated between different concentrations of arachidic acid.

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3.3 *In vitro-in vivo* (IVIVC) correlation

Biocompatibility, drug release and biodegradation studies with LAIs have been carried out in different animal models. The most commonly used *in vivo* models used for LAI's are rodents [67, 73, 75, 78, 94, 102, 108, 110, 139, 144] and one IM injection study uses primates [145]. In most cases the *in vivo* drug release was evaluated via generation of a plasma concentration curve through venous blood sampling. Some studies employed *in vivo* fluorescent imaging and biocompatibility was studied after sacrifice of the animals by collecting tissues, fixing and staining with hemotoxylin and eosin (H&E) to study tissue damage by inflammation [73]. Importantly, it must be noted that most of the *in vivo* studies have been conducted with SC administration of the formulation rather than IM, which raises questions about the *in vivo* correlation of the *in vitro* drug release due to the differences of injecting IM versus SC.

There is a general consensus in the published literature that most studies show a lack of correlation between *in vitro* and *in vivo* studies when investigating drug release from oleogels [11, 67, 73, 75, 139]. The disparity of the *in vivo* and *in vitro* results may be attributed to many factors including the lack of absorption (frame erosion) of the oleogel in the *in vitro* model, compared to enzymic biological erosion [75] within the body and the inability of *in vitro* methods to distinguish between formulations when a distinct difference was observed in release in *in vivo* studies, illustrated particularly well by Nipps and co-workers when studying the *in vitro* release of drospirenone microcrystal suspensions in either an aqueous or peanut oil based formulation via a dialysis tubing [67]. In the study by Pluore and co-workers [75], the *in vitro* tests indicated that both formulations demonstrated a slow drug release, whereas *in vivo* the pharmacokinetics of the two formulations in rats differed significantly with slower absorption from the aqueous formulations compared to those produced with peanut oil. The authors concluded that some IVIVC correlation could be found, however, the predictability based on the *in vitro* test was restricted.

4. Biopharmaceutical performance of lipid-based LAI administered via intramuscular (IM) injection

Medicines commonly administered IM include sedatives, hormonal therapies, long-acting antipsychotics, immunosuppressants, chemotherapeutics, neuroleptics and vaccinations [146, 147]. Example antipsychotic drugs that are currently used in the clinical treatment and administered IM using oil-based formulations (with vegetable oil as the base) include zuclopenthixol decanoate, flupentixol decanoate, haloperidol decanoate, pipotiazine palmitate. For IM injection, it is widely known that skeletal muscle is highly vascularised receiving 20% of the total cardiac output at rest, which equals one litre of blood passing

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through the organ every minute. The oxygenated blood passes through a network of capillaries, known as a microvascular unit, estimated to be between 200-400 in every cubic millimetre of muscle [148-150]. In comparison to SC injections, larger volumes of formulations may be administered IM due to the highly vascularised regions within the muscle fibres [151]. Depending on the doses required for each patient, the injection volume can range from 1 to 5 mL after IM injections, whereas SC only up to 1.5-2 mL. In this section, the focus will be on the biopharmaceutical events and performance of LAI administered IM.

4.1. General physiological events following IM injection

Post injection the spreading of the injectate and the drug absorption can be affected by various factors. The physiological factors include the fat content of the local injection site and the blood flow. In terms of local environment of the formulations post-injection, there are both the compositional factors and mechanical factors affecting the spreading and the absorption of the injected depot. At the injection site, the muscle fibres contain bundles of actin and myosin fibrils which are the contractile units of muscle fibres [149] (**Figure 9**). The contraction of the muscle fibres provides movement and causes the spreading of the formulation along the muscle fibres. This spreading of the injectate increases the interfacial area. In addition to the interfacial area between the formulation and the aqueous environment of the interstitial fluid, the vehicle viscosity also can affect the spreading and subsequently the rate of drug absorption [2].

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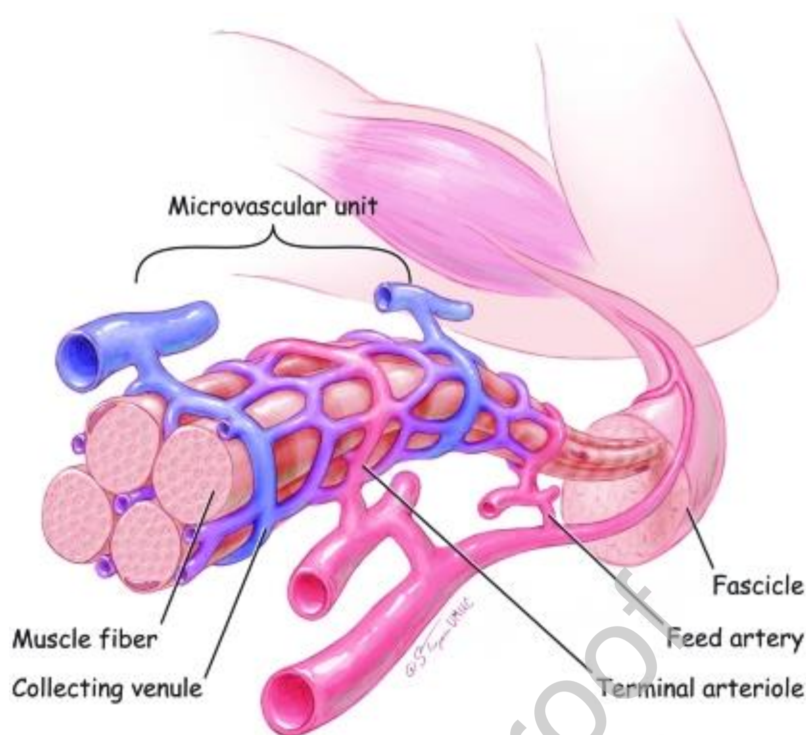

Figure 9. Illustration demonstrating the highly vascularised fibres of skeletal muscles. Korthuis et al, 2011 [150] with permission.

Drug partitioning into the muscle interstitial fluid is the first step towards drug absorption. Interstitial fluid is a filtrate of plasma through the capillary walls, which is highly permeable to water, electrolytes, and solutes with a lower molecular weight [152]. pH is 7.38±0.02 for quadriceps skeletal muscle at rest [153] and 7.17±0.01 for vastus lateralis [154]. Exercise can cause a reduction of the pH of interstitial fluid to be close to 7 which can be recovered with a mean half-time of 5.2 minutes [153]. Such change may affect the drug solubility, absorption and oil excipient degradation rate (as it may catalyse the oxidation reaction of the oil used in the formulation), but no existing study has been performed to study this aspect.

Once the drug has partitioned into the aqueous muscle interstitial fluid, it is ready for uptake by either the systemic blood circulation running through the muscles or the lymphatics depending on the lipophilicity and size of the drug [2]. Highly lipophilic drugs and high

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molecular weight molecules are often absorbed by the lymphatics. The lymph vessels are blind-ended endothelial tubes that run though-out skeletal muscle [150] and exist to drain interstitial fluid and transport leukocytes between the tissues and lymph nodes for immune surveillance [155, 156]. Within the muscle tissue, the vessels rely on the pulsation of the muscular arterioles to compress and propel the lymph centrally as well as the physical contraction of the skeletal muscles themselves. The physicochemical properties of the drug also play an essential role in influencing the drug absorption, which will be discussed below.

4.2 Absorption of lipid-based LAI administered by IM injection

For a drug capable of fulfilling the basic pharmacokinetic (PK) and pharmacodynamic (PD) requirements to be included into a LAI formulation, which includes high potency, low dose and wide therapeutic widow, the absorption of the drug is highly dependent on a range of formulation factors which controls the drug release prior to absorption. The drug release from the depot and the drug absorption are two distinct processes. For an oil-based depot, the drug release is mainly diffusion controlled, i.e. there is a range of relevant parameters that can be manipulated for the purpose of modulating the drug release rate. These theoretically include a) the drug concentration in the oil, b) the diffusion length and diffusion coefficient of the drug from the LAI matrices to tissue, c) the surface area of the LAI depot, d) the partition coefficient (P) between LAI matrices and tissue fluid, e) the thickness of the diffusion layer in the aqueous phase as well as the diffusivity in this compartment [157], and importantly f) the degradation rate of the oil depot.

There are few special considerations associated with IM injection. The APIs in most commercial oil depot products administered IM are prodrugs which require hydrolysis post-injection to become therapeutically active. The hydrolysis has been reported mainly to occur

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in the blood. Although surrounding tissue cells contain the appropriate esterases for hydrolysis, the poor tissue permeation of the lipophilic prodrugs means that these cannot access the local enzymes. Therefore, Kalicharan and colleagues argued that the inactive prodrug has to be drained via the lymphatic system in order to access blood cells in the systemic circulation where it can be hydrolysed into the parent compound [60]. This leads to a lag time between injection and drug release from the depot and therefore also entry of the active substance into the systemic circulation (being bioavailable, as illustrated in **Figure 10**). Kalicharan and the colleagues defined the rate of the drug substance that enters the blood stream as the absorption rate constant (*ka*). Their experimental data indicated that *ka* was mainly determined by the partition coefficient of the prodrug and the site of injection [158] . In comparison to SC injection, their results showed a lower drug (nandrolone) absorption rate constant and a relatively shorter lag time than after IM injections at different muscle sites.

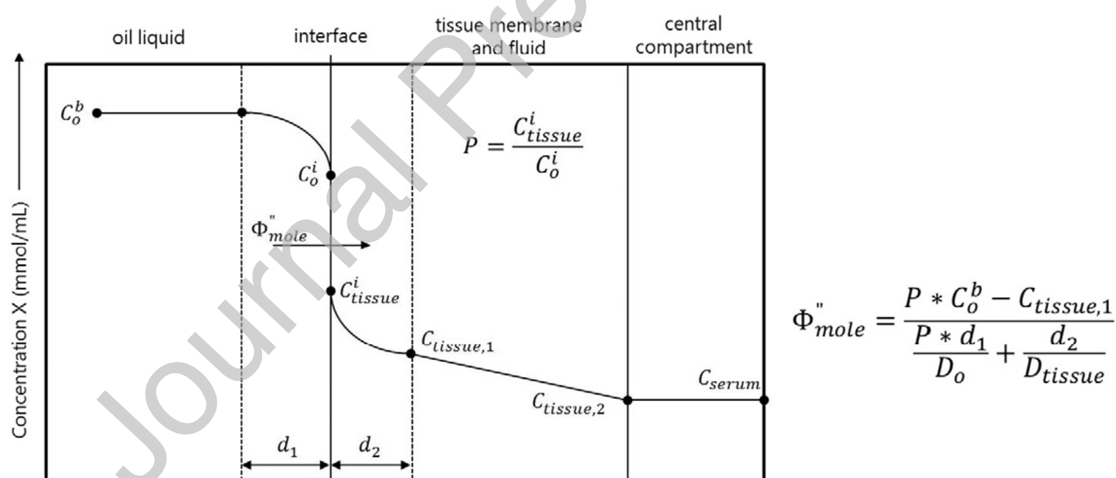

Figure 10. Schematic overview of the *in-vivo* situation (left). Equation on the right presents the parameters which contribute to the mole flux (Φ_{mole}) of drug transport. Abbreviations: bulk concentration of the drug in oil (C_o^b), at oil interface (C_o^i), at tissue interface (C_{tissue}), in tissue beginning ($C_{tissue,\ 1}$), before entering central compartment ($C_{tissue,\ 2}$) and in serum (C_{serum}); d = diffusion layer in oil (d_1) and tissue fluid (d_2); P = partition coefficient; D =

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diffusion coefficient in oil (D_o) and in tissue fluid (D_{tissue}). Kalicharan *et al*. 2016 [157] with permission.

In terms of the intrinsic properties of the drug, the diffusion coefficient of the drug molecule out of the oil depot is, as described above, influenced by the partition coefficient between the two phases, which can be manipulated by optimising the matrix materials, i.e. the formulation, or by adjusting the properties of the compound, i.e. the prodrug concept discussed above. For oil solution-based depots, increasing the viscosity of oil could reduce the diffusion coefficient. However, caution should be taken as increased viscosity could be associated with an increased pain sensation upon administration. In the case of more complex matrices, such as liquid crystalline based depot andoleogels, the presence of a solid gelator and the biocontinuous structures, such as cubic phase, could increase the practical diffusion path length and thus increase the diffusion time needed of the drug to be released. Therefore the overall drug release pattern is the interplay between molecular properties and delivery strategies [53].

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Increased amount of research effort has been given to identify the *in vivo* relevant factors that can affect drug absorption. Using surface area as an example, previously it has been assumed that the injected depot forms a spherical shape in the muscle tissue. With this assumption a 0.5 mL injection would lead to an approximated total surface area of 750 mm^2. However, this has now been questioned through review of histopathology and MRI data reported by Kalicharan *et al.* [54] on oil solution injections. As shown in **Figure 11**, oil depot does not represent a discrete, continuous phase, but have been reported to disperse into separate non-spherical droplets that follows the axis of the surrounding muscle tissue. However, as oil solutions are liquid injection, breaking the oil droplets into smaller droplets is highly

probable. Such droplet size reduction will naturally increase the surface area which, in theory, would increase the drug release rate and consequently the absorption. On the basis of the SC injectable literature, we hypothesise that, for IM administration, this disadvantage could theoretically be overcome by developing *in situ* forming depots with much more solid texture, such as oleogels.

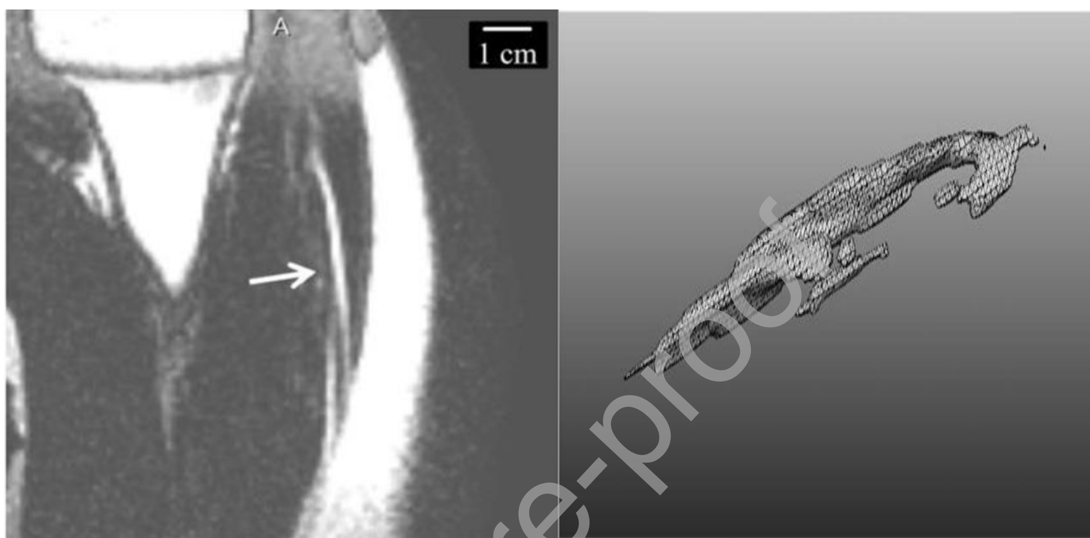

Figure 11. Visualisation of the oil depot in the deltoid muscle of a volunteer. In the image, the white arrow indicates the oil depot in tissue (left) and the 3D-picture of the oil depot is given in the image (right) [54]. The images were taken with permission.

In cases where the drug is suspended in the oil/matrix, the particle size of the drug can play a significant role in controlling the release rate. Firstly, in such products, particle size must be controlled to ensure that the solid particles fit through the injection needle without clogging during injection [159]. The smaller the particle size, the more rapid release is expected. Due to the much higher viscosity of oil and lipid-based excipients in comparison to aqueous preparations, the common agglomeration and "Ostwald ripening' effect may be much less in oil-based formulations than aqueous suspensions. Factors such as compound dissolution rate in a classical aqueous suspension can also influence the release rate as well as the digestion of the oil.

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4.3. Effects of injection site on drug absorption

The injection site can lead to significant variation in the drug absorption. Gluteal (dorsogluteal and ventrogluteal), vastus lateralis, deltoid and rectus femoris muscles are the main injection sites for most licensed products. The oil solution depot product of flupentixol and zuclopebnthixol are intended for deep IM administration into the gluteal or vastus lateralis muscle [160]. The ventrogluteal muscle, which has the greatest thickness among the gluteal muscles, few nerves and only a thin layer of fat [58, 144], has been recommended as the main site for IM injection. Injections into this muscle are associated with low risk for unintentional delivery to the subcutaneous tissue, which may result in poor systemic absorption of the drug [58, 161]. On the contrary, the dorsogluteal region often has a variable amount of subcutaneous tissue, thus it is not a recommend site for IM injection [161]. Although the ventrogluteal site is a frequent location for IM injection, there are other injection sites commonly used including vastus lateralis and rectus femoris and deltoid muscle [144]. With respect to injection site, the highest bioavailability was reported following gluteal injection, for the injection volume the highest bioavailability was reported for the low volume. Minto *et al* [162] demonstrated that the IM injection for delivery 100 mg of nandrolone esters in arachis oil had higher bioavailability and more sustained physiological effects when the gluteal was used as the injection site compared to the deltoid region. The higher fat content and lower blood-flow in the gluteal location allowed slow partitioning of the drug into the muscle tissue and contributed to the slower release kinetics. IM injection of lidocaine provided higher blood concentrations and more rapid development of peak concentration when was administrated as intradeltoid injections than the intraglutial injection [163].

4.4. Injection technique

The absorption of the drug through IM injection is highly dependent on injection depth, [164] site of injection [165, 166], massage before injection and muscle activity [165]. The injection depth can be controlled by the injection technique. As an example, a study showed that plasma diazepam concentrations injected with a 4 cm needle where more than twice that of injections with a 3 cm needle after 90 minutes [167]. This was further highlighted with the finding that gluteal fat has a depth of 3.5 cm and thickness is greater in women than in men suggesting that gender differences in drug delivery after IM injection may be observed [168].

For paediatric patients it should also be considered that the thicknesses of the subcutaneous fat tissue and muscle layers may differ from adults, thus the appropriate use of the length of needles for injection may need adjustment to prevent under- or over-penetration during IM also in the light that in overweight/obese patients, standard 37 mm needle may not be long enough to penetrate sufficiently deep to reach an IM injection [168-170]. Therefore, for gluteal administration of antipsychotics LAIs the recommended needle is a 38 mm (1.5 inch), 22-gauge hypodermic safety needle for patients with a normal BMI (Body Mass Index); whereas for obese patients with a BMI > 28 kg/m2, a 50 mm (2 inch), 21-gauge hypodermic safety needle should be used. For deltoid administration the recommended needle is a 25 mm (1 inch), 23-gauge hypodermic safety needle; for obese patients, a 38 mm (1.5 inch), 22-gauge hypodermic safety needle should be used [58]

5. Feasibility for large-scale batch manufacturing

The number of lipid-based LAI product that have entered the market is still relatively limited. Most of these products are oil-based products with a few products based on sophisticated structures such as liposomes that are out of the scope of discussion of this review. Oil-based

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LAI products have a relatively simple large-scale manufacturing process. From a dosage form perspective, the simplest lipid based LAIs are oil solutions for injection, all defined as small-volume injections, where the drug is dissolved in an oil or an oil-cosolvent system, which, after injection, will ensure a controlled release. The complication of the process is related to the parenteral status of the formulation, as they will be injected. The drug is normally dissolved in the oil, filtered under pressure to reduce the bioburden and distributed into ampoules. After sealing, the ampoules may be sterilized by dry heat sterilisation at temperature typically at or above 160 ºC for several hours. For formulations that contains components that are heat sensitive, the process will be different in order to avoid potential degradation. Here sterile drug and excipients will be used, i.e. the oil will be heat sterilised, mixed with compound, sterilised by aseptic manufacturing techniques and once dissolved, passed through a filter for sterilization into pre-sterilised ampules. From a pharmaceutical manufacturing perspective, this process is relatively simple, hence manufacturing of lipid based LAIs is a relatively simple parenteral process, which in general should not provide any limitation to their application.

6. Conclusion and future outlook

LAI formulations have multiple clinical applications and in this context the lipid-based LAI poses a number of advantages and challenges, which have been discussed in this review. Currently, lipid-based LAIs that have reached the markets are dominated by oil-solution based formulations. Although the conventional oil-solution based LAIs are simple to formulate, the criteria for drug candidates that can successful be loaded and delivered by oil-solution based LAIs are rather restrictive and narrow. From a manufacturing perspective the oil-based formulations are simple and can, in cases where the compound is not heat sensitive, be terminal sterilised, i.e. the drug product will be very safe to use for the patient. In addition,

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with the low volume of injection, the formulations generally have relatively low drug loading which works best for highly potent drug.

Extending the success of oil-solution based LAIs, the development of novel lipid based LAIs such as oleogels and liquid crystal-based systems is urgently needed to fill the gap that oil-solution based products cannot fill. Modified oleogels and liquid crystal-based formulations that are optimised to have rapid sol-gel phase transitions post-injection can form a gel depot *in situ* and have the potential of extending the release rate beyond the 4-6 weeks that oil solutions can support. They pose the possibility of making good LAIs for mild to highly lipophilic compounds, i.e. supplementing the biodegradable polymer based depot products. A significant amount of research is still needed in this field to truly define the formulation parameters, establish relevant *in vitro* dissolution methods that may provide a predictive IVIVC to optimise the formulations. From a manufacturing perspective, the systems would in general be easy to commercialise.

The accumulated literature has built a good understanding on the potential vehicles, the interlink to the release and the *in vitro* methods are overall well established. The areas missing are *in vivo* data to truly define the IVIVC for the applied *in vitro* dissolution methods in the field and an in depth understanding of the effects of route of administration (i.e. SC versus IM and different sites of IM injection) on *in vivo* drug absorption. This knowledge is critical for building meaningful in-silico prediction models for rapid product development.

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Reference:

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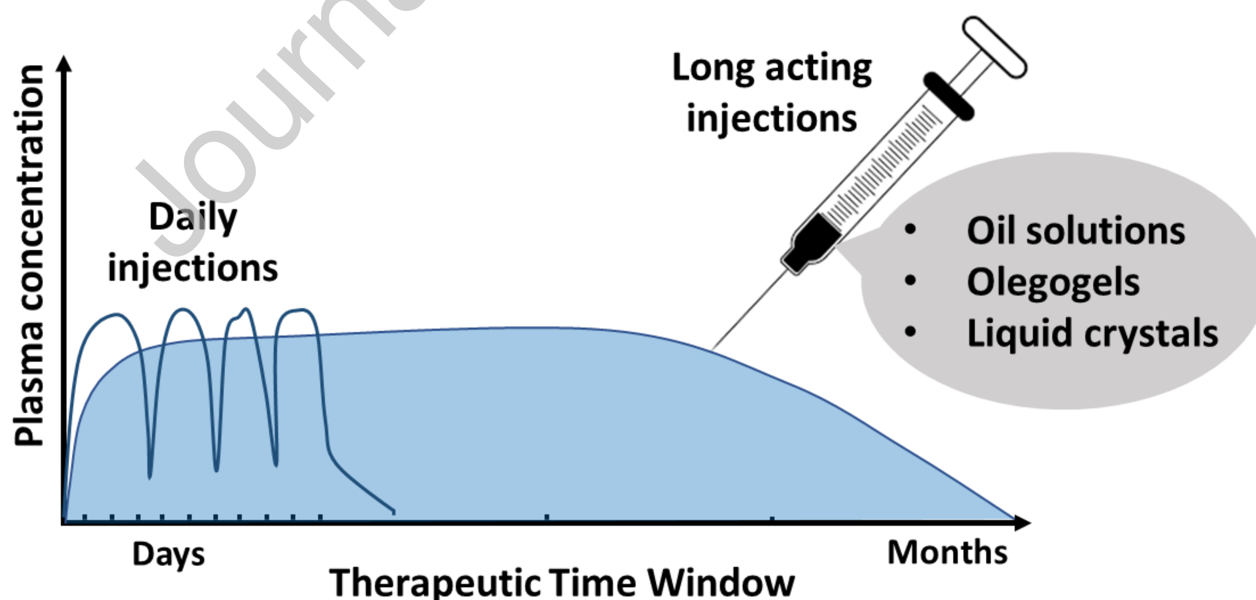
Graphic abstract



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