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Lipiodol-based emulsions used for transarterial chemoembolization and drug delivery: Effects of composition on stability and product quality

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ABSTRACT

Transarterial chemoembolization with emulsion-based formulations using doxorubicin hydrochloride (DOX) and Lipiodol® is the golden standard for the loco-regional treatment of unresectable hepatocellular carcinoma (HCC). However, from a pharmaceutical quality perspective these emulsions are poorly characterized. In this study, clinically relevant Lipiodol®-based emulsions were characterized in terms of emulsion stability, continuous phase classification and droplet-size distribution. Also, the solubility of DOX in the different emulsion components and the distribution of DOX to the lipid phase were investigated. These are key features to investigate due to the claimed tumor–seeking properties of Lipiodol®. The *in vitro* release of DOX was studied in a miniaturized dialysis method and an empirical release model was applied to adjust for the passage of DOX across the dialysis membrane. The most stable emulsion (> 72 h) was classified as water-in-oil (w/o), had the highest distribution of DOX to the lipid phase (20%) and an aqueous-to-lipid phase ratio of 1:4. The composition of the aqueous phase was a mixture (v/v) of iohexol (85%) and water (15%). Emulsions containing iohexol and a high aqueous-to-lipid phase ratio (1:2–1:4) displayed prolonged *in vitro* release profiles of DOX. This study further emphasizes the medical need to standardize these emulsion-based drug delivery systems.

1. Introduction

Hepatocellular carcinoma (HCC) is the second most deadly cancer form globally with an incidence of 750,000 per year [1]. The future prevalence of hepatitis C virus (HCV) and nonalcoholic steatohepatitis (NASH) are expected to be reduced and increased, respectively, with NASH emerging as the major contributor of cirrhosis and HCC in the United States [2]. The primary recommended palliative treatment for intermediate stage HCC (Barcelona Clinic Liver Cancer stage B, asymptomatic, non-invasive and multinodular) is local intra-arterial delivery of cytostatic drugs concomitant with a drug delivery system [3–5]. One often explored option is emulsion-based formulations with doxorubicin hydrochloride (DOX). Interventional radiological techniques enable loco-regional administration of the emulsion through a catheter positioned in the hepatic artery feeding the tumor, in a procedure called transarterial chemoembolization (TACE) [3,6,7]. Image-guided oncological treatments often make use of contrast agents based on iodine [8]. In the liver, the emulsion-based formulations releases the drug and causes a partial and temporary embolization of the treated hepatic artery branches feeding the tumor [4].

At physiological conditions the amphiphilic DOX (molecular mass = 543.52 g/mol) exists both as a protonated monovalent cation and a deprotonated neutral entity (Fig. 1A) [9,10]. The two pK_as of DOX depend on the experimental conditions applied. For instance, at 37 °C and an ionic strength of 0.15, DOX has pK_as of 7.5 and 9.5 (the pK_a of 7.5 usually varies between 7 and 8 depending on the experimental conditions) [9,10]. DOX starts to aggregate and form dimers at ~10 μM and at 1 mM aggregates of 40 DOX-molecules have been reported [11]. With increasing DOX concentration, the molecular aggregation number increases, generating both stacking and rod-like micelles of DOX [11–15]. DOX octanol-to-buffer (pH 7.3) partitioning is dependent on the concentration of DOX—below 20 μM the log partitioning coefficient is ~2, whereas at 500 μM it is ~0.4, indicating that a large fraction of DOX is aggregated in the aqueous phase [10]. Clinically, DOX is widely used as a cytostatic agent in Lipiodol®-based emulsions in the treatment of HCC [16]. Although other cytostatic drugs have a reported lower *in vitro* IC50 value against HCC, it is obvious that other factors affect the *in vivo* potency of drug products and other issues affecting its clinical use [17–19].

The lipid phase of these emulsions consists of Lipiodol® which has been proved to accumulate in rabbit tumor-tissue and this is the rational for its use in TACE treatment of HCC [20]. In preclinical models

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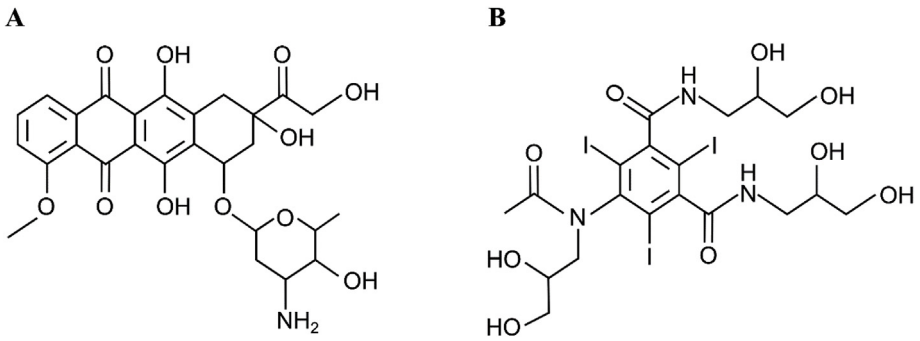

Fig. 1. Chemical structures of molecules in the emulsion-based formulations. A): The active pharmaceutical ingredient doxorubicin hydrochloride (DOX). B): The contrast agent iohexol (main component of Omnipaque™).

the intracellular accumulation in tumor cells has been proposed to be due to cell membrane pumps, pinocytosis and/or reduced degradation by lysosomes [4,21,22]. Lipiodol® has a diverse clinical use and it can be used 1) alone, 2) as emulsion, 3) as emulsion with additional embolization added after emulsion administration [4].

Lipiodol® is an iodinated (480 mg iodine/ml) and ethylated ester derived from poppy seed oil (lineolic (73%), oleic (14%), palmitic (9%), and stearic (3%) acids). However, the exact molecular composition of Lipiodol®, with regard to the iodine molecular position, remains unknown [18,23]. It has a density of 1.28 g/cm^3 and a high viscosity of 25 mPas at 37 °C [4,18]. The final emulsion preparation, i.e. the mixing of the components, is often performed *ex tempore* in the operation theater at ambient temperatures [4,5]. The composition and preparation method of these emulsions affect their *in vitro* evaluation and performance [4,5,24]. For example, it has been suggested that the addition of a contrast agent to the aqueous phase increases emulsion stability and decreases drug release rates [25–28]. One contrast agent used in intra-arterial HCC treatment formulations is iohexol (Fig. 1B). In addition to increasing emulsion stability, this contrast agent also allows for x-ray visibility of the formulation [4].

Furthermore, adjustment of the aqueous-to-lipid phase ratio to a higher ratio of Lipiodol® (1:2–1:4) increases the emulsion stability and reduces the drug release rate from the formulation both *in vitro* and *in vivo* [5,26,29–32]. However, in the clinical setting the composition of the used Lipiodol®-based emulsions are not standardized leading to large variations in its composition, preparation and injectability properties [4].

The overall objective of this study was to evaluate the physicochemical and pharmaceutical factors for the *in vitro* performance of these clinically relevant emulsion-based formulations of DOX. The specific aims were to investigate how the aqueous-to-lipid phase ratio and the aqueous phase composition affected the emulsion stability, the continuous phase of the emulsion and *in vitro* release profile of DOX. Also, DOX distribution and solubility, which can affect the performance of the formulations in the different emulsion components, were investigated.

2. Material and methods

2.1. Drugs and chemicals

Doxorubicin hydrochloride (DOX) was purchased from Toronto Research Chemicals, Canada, and Lipiodol® Ultra Fluid was purchased from Guerbet, France. Warfarin (Sigma-Aldrich, Germany) was used as internal standard in the UPLC-MS analysis. Four aqueous phases with clinical relevance were used in the investigated emulsions (Table 1), purified water, saline 0.9%, Omnipaque™ (iohexol) 300 mg I/mL (GE Healthcare AB, Sweden), and a mixture (v/v) of purified water (15%) and iohexol (85%) [6]. The *in vitro* release experiments used a Pur-A-Lyzer mini dialysis tube, with molecular mass cutoff of 12–14 kDa, (Sigma Aldrich, Sweden) and phosphate buffer saline (PBS) containing 0.01 M Phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride with a pH of 7.4 (Medicago, Uppsala, Sweden) as release medium. All chemicals were of analytical grade or higher. All samples containing DOX were protected from light throughout all experiments in this study.

2.2. Solubility of DOX

The shake-flask method was used to determine DOX solubility (n = 3) at room temperature (during an exceptional Swedish summer 22 °C–30 °C) in Lipiodol®, water, 0.9% saline solution, PBS buffer (pH 7.4), iohexol, and a mixture (v/v) of iohexol (85%) and water (15%) [33]. Room temperature is the most relevant temperature when investigating formulation properties as the emulsions are generally prepared *ex tempore* at room temperature in the operating theatres [34].

In brief, DOX was weighed into Eppendorf vials and an appropriate volume (0.3–1.4 mL) of each solvent was added to reach relevant target concentrations of DOX. To allow for thorough mixing the vials were shaken at 1000 rpm for 24 h. The pH was then measured before the vials were centrifuged at 14,100 *g* for 10 min. After centrifugation a sample was collected from the supernatant, if one was present. If no supernatant was apparent but the contents were highly viscous, a sample was collected from the top of the vial. The samples were transferred to brown HPLC vials (for light protection) and stored at 4 °C until analysis. If no pellet was identifiable after centrifugation (i.e the whole sample was still a clear red solution), the solubility studies were repeated with higher target concentrations of DOX. Prior to quantification with UPLC-MS the solubility samples were appropriately diluted, and the internal standard was added.

2.3. UPLC-MS method

An ACQUITY UPLC I-Class system coupled to a mass single-quadrupole detector (Waters Corporation, Milford, MA) was used to determine DOX concentrations in both aqueous and lipid phases following the distribution and solubility experiments [35]. Stock solutions of DOX at selected concentrations (0.1–2.0 mg/mL) were prepared in DMSO. Linear calibration curves were prepared for the aqueous phase by spiking 5.0 mM ammonium formate (Millipore Corporation, Germany), pH 3.0, with DOX stock solutions to a concentration range of 1.0–20.0 μg/mL DOX. The linear calibration curves for the lipid phase were prepared by spiking Lipiodol® (3.7% v/v) in methanol with the DOX stock solutions to a concentration range of 0.1–10.0 μg/mL of DOX. Warfarin was added as an internal standard to all samples at a fixed concentration of 0.308 μg/mL (1 μM).

The mobile phase consisted of (A) 5.0 mM ammonium formate in water at pH 3.0 and (B) methanol. The gradient used was: initially 25% (B), then 25–75% (B) for 2.50 min, followed by 75% (B) for 0.50 min, 75 – 25% (B) for 0.10 min, and 25% (B) for 1.00 min. The total run time

Table 1
Summary of investigation methods, aqueous phase compositions and aqueous-to-lipid phase ratios of the studied Lipiodol®-based emulsions.

Emulsions	Investigation Methods	Aqueous-phase	Lipid-phase	Aqueous-to-lipid ratios
W/L				
	Distribution to lipid phase	**W**ater	**L**ipiodol®	1:4
	Emulsion stability	**W**ater	**L**ipiodol®	1:1 & 1:4
	Drug release	**W**ater	**L**ipiodol®	1:0, 1:1, 1:2, 1:3 & 1:4
S/L				
	Distribution to lipid phase	**S**aline	**L**ipiodol®	1:4
	Emulsion stability	**S**aline	**L**ipiodol®	1:1 & 1:4
	Drug release	**S**aline	**L**ipiodol®	1:0, 1:1, 1:2, 1:3 & 1:4
I/L				
	Distribution to lipid phase	**I**ohexol[a]	**L**ipiodol®	1:4
	Emulsion stability	**I**ohexol[a]	**L**ipiodol®	1:1 & 1:4
	Drug release	**I**ohexol[a]	**L**ipiodol®	1:0, 1:1, 1:2, 1:3 & 1:4
IW/L				
	Distribution to lipid phase	**I**ohexol:**W**ater[b]	**L**ipiodol®	1:4
	Emulsion stability	**I**ohexol:**W**ater[b]	**L**ipiodol®	1:1 & 1:4
	Drug release	**I**ohexol:**W**ater[b]	**L**ipiodol®	1:0, 1:1, 1:2, 1:3 & 1:4

[a] Iohexol in the form of commercial product Omnipaque™.
[b] Iohexol (85%) and water (15%) mixture (v/v).

was 4.10 min, the flow rate was 500 µL/min, the sample injection volume was 10 µL, and the sample manager temperature was 10 °C. The column was a C_{18} column (ACQUITY UPLC BEH C_{18}, 2.1 × 50 mm, particle size 1.7 µm, Waters Corporation) kept at 60 °C. The positive capillary voltage was set at 0.80 kV and probe and source temperatures were 600 °C and 120 °C, respectively. The quantification was performed in the single-ion recording mode with an electrospray interface operating in the positive mode. The mass detection channels were set to *m/z* 544 (doxorubicin) and 309 (warfarin), each with a cone voltage of 10 V. The sampling frequency was 10 Hz. The collected data were processed using MassLynx 4.1 MS Software (Waters Corporation, Milford, MA).

2.4. Emulsion preparation

All emulsions were prepared with Lipiodol® as the lipid component and with clinically relevant aqueous-to-lipid phase ratios of 1:1–1:4 (Table 1). As stated before, different clinically relevant aqueous phases were used: water (emulsions W/L), 0.9% saline (emulsions S/L), iohexol (emulsions I/L), and a mixture (v/v) of 85% iohexol and 15% water (emulsions IW/L). The preparation of the emulsions, i.e. the *ex tempore* mixing of the components, was made in accordance to the clinical setting where the emulsion preparation takes place in the operating theaters, at room temperature, just prior to the intra-hepatic administration [4,6,34]. The emulsions were prepared by connecting two 5-mL Luer lock syringes (Codan Triplus AB, Sweden) containing appropriate volumes of Lipiodol® and aqueous phase using a fluid dispensing connector (B. Braun Medical, USA). Mixing was done by hand and was initiated by pushing the entire aqueous phase (bright red) into the lipid phase (faintly yellow) as illustrated in step 1 of Fig. 2A [6]. This was followed by 19 pumps back and forth (step 2 and 3, Fig. 2A) resulting in a total of 20 pumps for each emulsion (cloudy orange). The emulsions are referred to by their aqueous-to-lipid phase ratios and aqueous phase compositions (Table 1).

2.5. Stability, classification and droplet sizes

Stability of emulsions with aqueous-to-lipid phase ratios of 1:1 and 1:4 was studied by dissolving DOX in each aqueous phase (2.5 mg/mL). The aqueous phase was then mixed with either one or four parts of Lipiodol®, for example 1 mL of DOX aqueous phase with 1 mL (1:1) or 4 mL (1:4) of Lipiodol®. Directly after mixing (20 pumps) roughly 1 mL of the emulsion was ejected into a vial and used for microscopy and DLS measurements. The emulsion-containing syringe was then left horizontally to equilibrate for 15 min until the drop test. The drop test was used to identify the continuous phase and classify the emulsions as either oil-in-water (o/w) or water-in-oil (w/o) [36]. One to three drops of the prepared emulsions were dripped or "injected" into vials containing either water or Lipiodol® (2 mL). Visual inspection is an established approach to distinguish between separated or maintained emulsions [36]. The appearance of the bright red DOX (aqueous) and a faint yellow Lipiodol® (lipid) phase indicated emulsion separation. Visual inspection of the emulsions was undertaken at 15 min, 1 h and 72 h to determine whether the emulsions were maintained or had separated. A schematic illustration of the timeline for the experiment is presented in Fig. 2B. Samples for Dynamic Light Scattering (DLS) measurements (ca 1 mL) were taken directly after emulsion mixing and analyzed 30 min later. Droplet size determinations were done with a Nicomp 380 ZLS Particle sizer (Particle Sizing Systems, Santa Barbara, CA, USA) equipped with a 5 mW HeNe laser at 632.8 nm and using a 90° scattering angle. Samples for microscopy analysis were taken directly after emulsion mixing. A 20 µL drop of emulsion was placed between two Menzel-Gläser cover slips (Schott AG, Germany). Images were taken over time (5 min–1 h) and specifically at 15 min and 1 h using 20× magnification on an inverted transmitted light microscope (Axio Vert.A1, Carl Zeiss Microscopy GmbH, Germany).

2.6. Distribution of DOX between aqueous and lipid phases

Emulsions with aqueous-to-lipid phase ratio of 1:4 were prepared by dissolving DOX in each aqueous phase (2.5 mg/mL), which was mixed with four parts of Lipiodol®, as previously described. The emulsions were transferred to brown vials and the distribution of DOX between the aqueous and lipid phases was determined at 15 min and 1 h, respectively. The 15 min samples were not processed further whereas the 1 h samples were placed in a plate shaker at 1000 rpm during 1 h to increase the contact between the two phases. All samples were centrifuged at 14,100 *g* for 10 min to separate the two phases. The concentration of DOX in each phase was quantified using UPLC-MS and the percentage DOX distributed to the lipid phase was calculated.

2.7. In vitro release method

The *in vitro* release profiles of DOX (n = 3) from the emulsions were investigated using a modified µDiss profiler method (pION, USA)

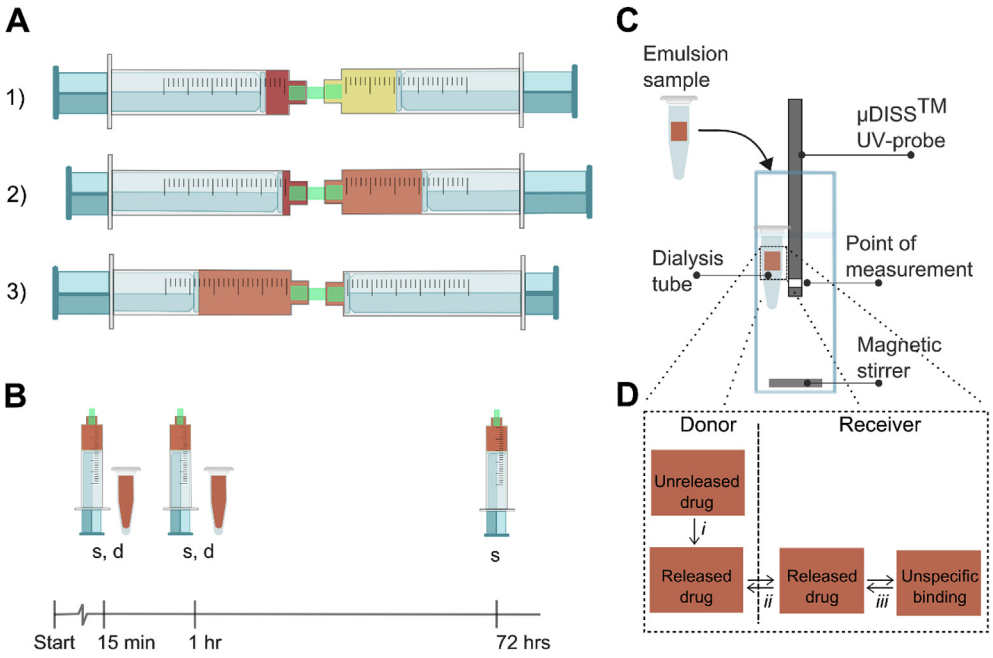

Fig. 2. Schematic illustrations of the methods used in this study. A) Emulsion preparation; the aqueous phase is illustrated by the red solution and Lipiodol® is illustrated by the yellow solution. The emulsions were prepared by pumping Lipiodol® and the aqueous phase back and forth (steps 1–3) between two connected syringes. B) The distribution of DOX between Lipiodol® and the aqueous phase was investigated at an aqueous-to-lipid phase ratio of 1:4 at 0.25 and 1 h. Emulsion stability were investigated at an aqueous-to-lipid phase ratio of 1:1 and 1:4 and was inspected visually at 0.25, 1 and 72 h. C) The release profiles of DOX from the emulsions were investigated in a μDISS profiler. The sample was placed in a dialysis tube which was immersed into a glass vial containing 20 ml of release medium. A magnetic stirrer enabled the stirring (100 rpm) of the release medium. D) A schematic illustration of the empiric release model used to estimate the rate of drug release from the emulsions. The model included drug release (*i*) from the formulation in the donor compartment, bidirectional diffusion across the dialysis membrane (*ii*) separating the donor and receiver chambers and unspecific association (*iii*) in the receiver compartment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

[28,37,38]. Each channel of the μDiss profiler was calibrated individually with stock solutions of DOX in methanol (1–30 μg/ml). The DOX concentration was determined at 553–572 nm as the area under the concentration–wavelength curve of the second-derivative spectrum [39,40]. The samples were separated from the release medium in a dialysis tube (Fig. 2C) [28]. The dialysis tubes were soaked in purified water for at least 30 min before the addition of 0.2 mL of the formulation sample, either as an aqueous phase or an emulsion. The dialysis tube was placed in a glass vial containing 20 mL of PBS buffer pH 7.4. To ensure efficient mixing of released DOX the release medium (37 °C) was stirred with a Teflon coated magnet (100 rpm) throughout the experiment.

The effect of aqueous-to-lipid phase ratios of 1:1, 1:2, 1:3 and 1:4 on the release profiles were investigated by preparing emulsions with a DOX concentration of 2.5 mg/mL (Table 1) [28]. Aqueous and the lipid phases were each prepared with a concentration of 2.5 mg/mL DOX and mixed to form emulsions as described in "Emulsion preparation" above. The passage of DOX across the dialysis membranes was investigated with each aqueous solution (2.5 mg/mL DOX), referred to as emulsions with aqueous-to-lipid phase ratio of 1:0.

2.8. Data analysis and equations

The data were presented as mean values with standard deviations (SD). Statistical comparisons for DOX distribution between the aqueous and lipid phases were performed with multiple analysis of variance and Sidak's correction for multiple comparisons (GraphPad Prism 6.04, GraphPad Software, Inc.).

For the *in vitro* release study, the mean (n = 3) of each experimental release data set was analyzed applying an empiric compartmental *in vitro* release model (Fig. 2D), to enable a comparison of the overall rate of release from the investigated formulations. The drug release rate (v_{rel}) from the emulsion was described by a unidirectional first order process (Eq. (1))

$$v_{rel} = A_f \times k_{rel} \quad (1)$$

where A_f is the amount of drug in formulation and k_{rel} is the release rate constant. The drug release from the formulation was modelled to occur within the donor compartment, where all drug was assumed to be unreleased at time zero and only released drug was allowed to diffuse across the membrane to the receiver compartment. The amount of released drug in the donor compartment (A_{don}) was described by Equation (2)

$$\frac{dA_{don}}{dt} = v_{rel} + k_{mem}\left(\frac{A_{rec}}{V_{rec}} - \frac{A_{don}}{V_{don}}\right) \quad (2)$$

where k_{mem} is the diffusion rate constant over the membrane, A_{rec} is the amount of drug in the receiver compartment, and V_{don} and V_{rec} are the volumes of the donor and receiver compartment, respectively. The bidirectional mass transport across the dialysis membrane assured that the release experiments were conducted up to steady state conditions. V_{don} and V_{rec} were set to 0.2 ml and 20 ml, respectively, in accordance with the experimental setup. A_{rec} was described by Equation (3)

$$\frac{dA_{rec}}{dt} = k_{mem}\left(\frac{A_{don}}{V_{don}} - \frac{A_{rec}}{V_{rec}}\right) + k_a\left(A_{bound} \times K_A - \frac{A_{rec}}{V_{rec}}\right) \quad (3)$$

where the unspecific binding, described by a binding constant (k_a) and an association constant (K_A) in the receiver compartment was included to accommodate for observations of less than the theoretical amount (99%) of DOX in the receiver compartment at steady state. Experiments of aqueous solutions (without Lipiodol® i.e. aqueous-to-lipid phase ratio of 1:0), i.e., all drug released at time zero, were used to estimate k_{mem} for each aqueous-phase (W/L, S/L, I/L and IW/L). k_{rel} was then estimated for each formulation, aqueous-to-lipid phase ratios (1:1–1:4), by adopting the estimate of k_{mem} from the corresponding aqueous solutions.

$$t_{½rel} = \frac{ln2}{k_{rel}} \quad (4)$$

The half-life of release ($t_{½\ rel}$) was calculated according to

Parameter estimation was performed by non-linear least square regression of the model to observations in the receiver compartment using Phoenix 64 WinNonlin software version 6.3 (Certara, L.P.,St. Louis, MO).

Table 2
Solubility (mg/mL) of doxorubicin hydrochloride (DOX) at room temperature after 24 h of mixing in relevant solvents, presented as the mean ± SD (n = 3).

Solvent	Measured Solubility (mg/mL)	pH	Observation
Lipiodol®	0.02 ± 0.01	NM	solid precipitate
Water	> 50	4.2 ± 0.2	free-standing gel
Saline	10.2 ± 1.7	4.6 ± 0.1	gel aggregates
Iohexol[a]	> 75	5.2 ± 0.2	free-standing gel
Iohexol:**W**ater[b]	> 69	5.3 ± 0.04	free-standing gel
PBS	7.1 ± 2.9	6.1 ± 0.1	gel aggregates

[a] Iohexol in the form of commercial product Omnipaque™.
[b] Iohexol (85%) and water (15%) mixture (v/v), PBS; phosphate buffer saline pH 7.4, NM; not measurable.

3. Results

3.1. Solubility of DOX

The results of DOX solubility in PBS and the various emulsion components are summarized in Table 2. The values represent DOX concentrations measured in the sample supernatants, or from the top of the vials, when the whole sample was a self-standing gel and no supernatant was present. Several target concentrations were tested for all solvents (except Lipiodol®) with the aim of achieving saturated solutions. The solubility of DOX in the various solvents ranged from 0.02 to 75 mg/mL with a rank order from lowest to highest solubility for the investigated phases: Lipiodol® < PBS < saline < water < iohexol and water < iohexol. Undissolved DOX formed a solid precipitate only in Lipiodol® (Fig. 3A). In the aqueous solvents viscous gel-like aggregates were observed with increasing concentrations of DOX (Tables 2 and S2). DOX separated into a dense lump at the bottom of the vial, with supernatant on top in both PBS and saline (Fig. 3B). In water, the mixture of iohexol and water, and iohexol, the whole sample was a self-standing gel at concentrations > 50 mg/mL (Table 2, Figs. 3C and S2).

3.2. Stability, classification and droplet sizes

The emulsion stability was studied by visual inspection (example of 1 h time point in Fig. 4) and the drop test (video example in S1) allowed for the classification of the emulsions as either w/o or o/w (Table 3). Samples were taken from each emulsion for microscopy and DLS measurements to characterize the droplet size distribution as displayed in Figs. 5 and 6. The visual inspection showed that two emulsions (emulsion W/L and S/L aqueous-to-lipid phase ratio of 1:1) separated in less than 15 min, and therefore no drop-test was performed for any of these formulations. With the increased lipid ratio (emulsions W/L and S/L aqueous-to-lipid phase ratio of 1:4) stability was increased (> 15 min). This was enough to perform the drop-test, resulting in both emulsions being classified as w/o. However, after 30 min also these emulsions had separated making DLS measurements futile. In the 15 min microscopy images of emulsions W/L and S/L, aqueous-to-lipid phase ratio of 1:1 and 1:4, droplet size determinations proved challenging (Fig. 5). These are very dynamic systems with a wide range of droplet sizes ranging from nm to hundreds of μm. In addition, the emulsions change very rapidly, resulting in blurry images at the earlier time points (2–15 min), this dynamic nature is further illustrated in the Supplementary Content (S3 A-C). However, in the aqueous-to-lipid phase ratio of 1:4 emulsions (Fig. 5C and D) the droplets (aqueous) were more dispersed than in the aqueous-to-lipid phase ratio of 1:1 emulsions, leading to less extensive aggregation and formation of larger droplets or continuous phases (Fig. 5A and B).

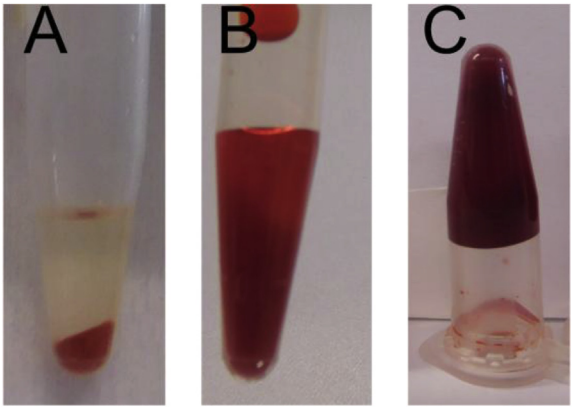

Fig. 3. Pictures from solubility investigaton. A) Solubility of doxorubicin hydrochloride (DOX) in Lipiodol®. B) Saline 0.9%. C) Water, inverted Eppendorf tube. Image of all solubility samples can be found in the Supplementary Content (S2).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.jddst.2019.101143.

The use of iohexol as an aqueous emulsion component substantially increased emulsion stability, with visual inspection qualifying all four emulsions (I/L and IW/L, aqueous-to-lipid phase ratio 1:1 and 1:4) as maintained after 1 h (Table 3). The drop test distinguished the 1:1 emulsions (o/w) from the 1:4 (w/o) emulsions. The results from microscopy and DLS measurements are summarized in Fig. 6. Similarly to aqueous-to-lipid phase ratio of 1:1 emulsions W/L and S/L (Fig. 5A and B), aqueous-to-lipid phase ratio of 1:1 emulsions I/L and IW/L also displayed extensive regions of continuous phases (Fig. 6A and B). Additionally, more complex multiple emulsions were present in the aqueous-to-lipid phase ratio of 1:4 emulsions of both I/L and IW/L (Fig. 6C and D). DLS data are reported as three measurements (á 3 min) of each emulsion sample using both intensity and volume weighing, raw data were fitted using Gaussian analysis. The investigated emulsions were both polydisperse and very concentrated, 20% in aqueous-to-lipid ratio of 1:4 emulsions and 50% in aqueous-to-lipid ratio of 1:1 emulsions (Fig. 6E and F).

3.3. Distribution of DOX between aqueous and lipid phases

The time-dependent distribution of DOX into the lipid phase is shown in Fig. 7. After 15 min no DOX could be detected in the lipid phase of the emulsions W/L and S/L. At 1 h DOX could be detected in the lipid phases of all tested emulsion, where it ranged from 0.5 to 20% distribution to the lipid phase, depending on the composition of the aqueous phase of the emulsion. From 15 min to 1 h there was a 2-fold increase of DOX in the lipid phase of emulsions I/L and IW/L. After 1 h the percentage of DOX distributed to the lipid phase of the emulsions increased from 0.50 ± 0.30% for emulsion W/L to 20 ± 2.3% for emulsion IW/L.

3.4. In vitro release study

The diffusion of DOX from the aqueous solutions (2.5 mg/mL DOX) across the dialysis membrane is shown in Fig. 8A. The aqueous phases containing iohexol generated higher release plateaus of DOX compared to aqueous phases without iohexol. The *in vitro* release profiles of emulsions with DOX concentration of 2.5 mg/mL are presented in Fig. 8B–E. The empirical release model was used to distinguish the release of DOX from the emulsions from the diffusion across the dialysis membrane. The results are presented in Fig. 8F and Table 4. For emulsions I/L and IW/L, the release half-life (h) increased with increasing aqueous-to-lipid phase ratio. The release rate of DOX from emulsions S/L and W/L was unaffected by the increase in lipid ratio.

4. Discussion

A wide range of Lipiodol®-based emulsions are currently in clinical

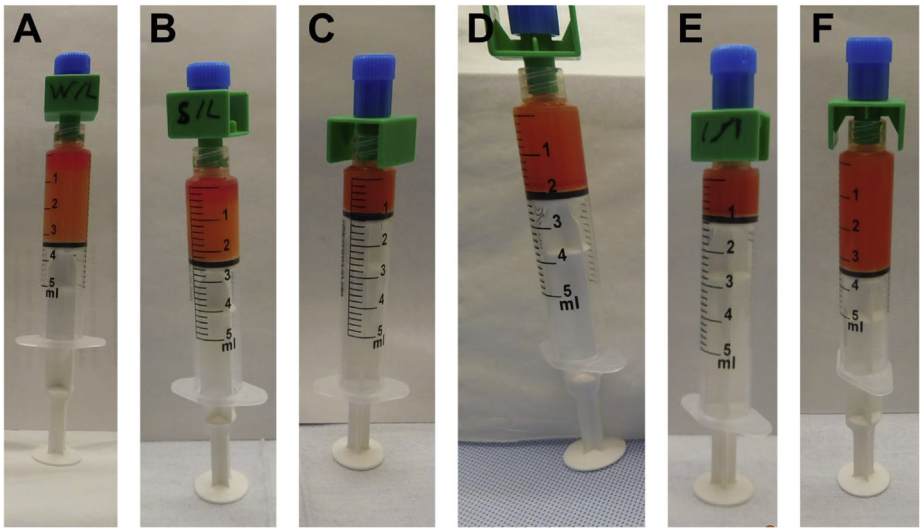

Fig. 4. Visual inspection of the stability of the emulsions at the 1 hr time point. Separated emulsions: A) water and Lipiodol® (W/L) aqueous-to-lipid phase ratio of 1:4, B) saline 0.9% and Lipiodol® (S/L) aqueous-to-lipid phase ratio of 1:4. Maintained emulsions: C) and D) iohexol and Lipiodol® (I/L) aqueous-to-lipid phase ratio of 1:1 and 1:4 respectively, E) and F) mixture (v/v) of iohexol (85%)/water (15%) and Lipiodol® (IW/L) aqueous-to-lipid phase ratio of 1:1 and 1:4 respectively.

Table 3
Stability and classification of the emulsions with aqueous-to-lipid ratio of 1:1 and 1:4 determined with visual inspection and drop test.

Emulsion type	W/L		S/L		I/L		IW/L	
Aqueous-to-lipid ratio	1:1	1:4	1:1	1:4	1:1	1:4	1:1	1:4
Time								
15 min	–	+	–	+	+	+	+	+
Drop test (o/w or w/o)	–	w/o	–	w/o	o/w	w/o	o/w	w/o
30 min *DLS*	–	–	–	–	+	+	+	+
1 h	–	–	–	–	+	+	+	+
72 h	–	–	–	–	–	–	–	+

+ maintained emulsion, - separated emulsion, w/o water-in-oil, o/w oil-in-water. For information on the composition of the emulsions, see Table 1.

use, but the pharmaceutical composition and final *ex tempore* preparation of the emulsions are rarely reported in detail, making comparison of tumor response between clinical studies difficult [4,5,24,41]. Standardization of the composition and final preparation of these formulations is of importance to optimize the clinical utility of these useful but complex pharmaceutical products. In this report, various pharmaceutical quality aspects of clinically relevant Lipiodol® emulsions containing DOX have been evaluated to improve the understanding of these emulsions. The effects of aqueous-phase composition and the aqueous-to-lipid phase ratio on pharmaceutical properties (distribution, stability, continuous phase, droplet-size distribution and drug release profiles) were analyzed and discussed in relation to its clinical use in the transarterial treatment of HCC.

DOX solubility was solvent-dependent and was lowest in Lipiodol®, the only solvent where the precipitate was in a solid state. The most plausible explanation is the ionization of DOX reducing its lipid solubility. In the aqueous solvents gel-like aggregates were observed at increasing DOX concentrations. This is in line with an increase in viscosity reported with increasing DOX concentrations (0–0.4 M) in NaCl solution [13]. Recently, it was reported that the formation of elongated DOX bundles (supramolecular polymer-like aggregates)

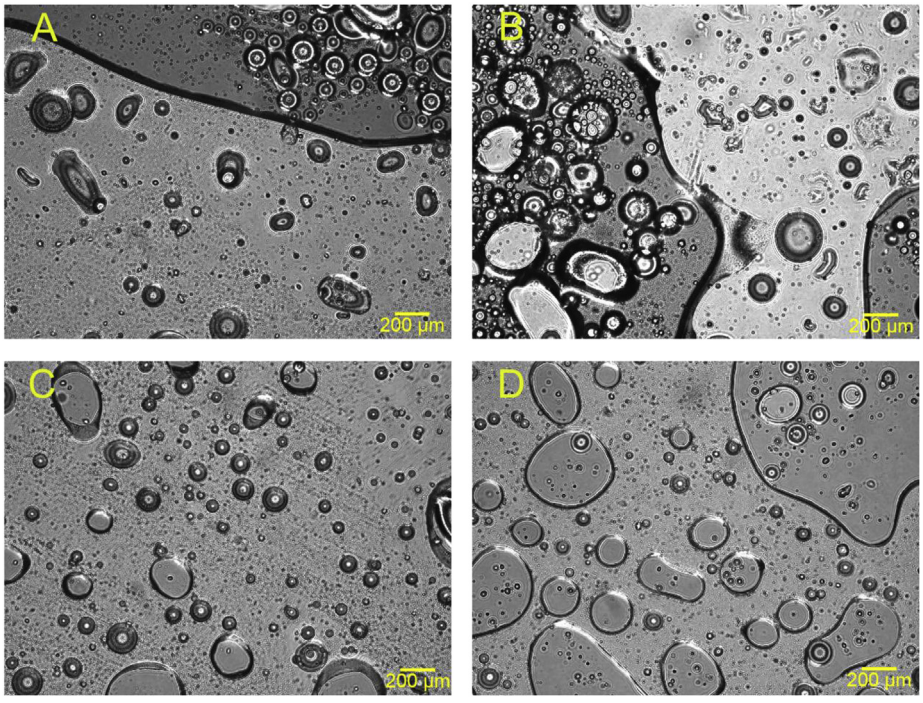

Fig. 5. Microscope images of emulsions with aqueous phase composition of water (W/L) and saline 0.9% (S/L) at 15 min time point. A) Emulsion W/L aqueous-to-lipid phase ratio of 1:1, B) Emulsion S/L aqueous-to-lipid phase ratio of 1:1, C) Emulsion W/L aqueous-to-lipid phase ratio of 1:4 and D) Emulsion S/L aqueous-to-lipid phase ratio of 1:4. All images are at 20× magnification.

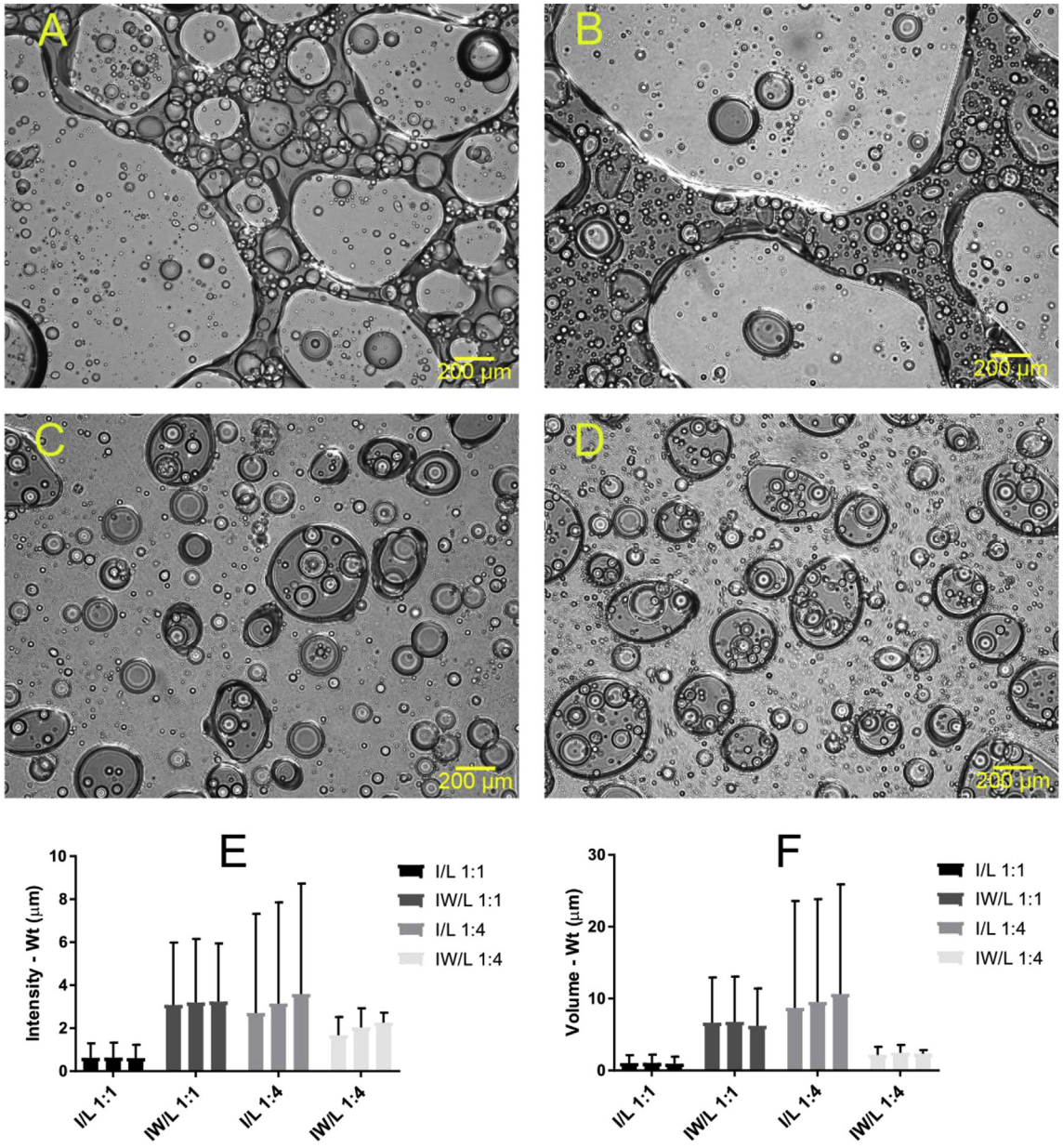

Fig. 6. Microscope images and DLS measurements of emulsions with aqueous phase composition of iohexol (I/L) and mixture (v/v) of iohexol (85%)/water (15%) (IW/L) at 15 min time point. A) Emulsion I/L aqueous-to-lipid phase ratio of 1:1, B) Emulsion IW/L aqueous-to-lipid phase ratio of 1:1, C) Emulsion I/L aqueous-to-lipid phase ratio of 1:4 and D) Emulsion IW/L aqueous-to-lipid phase ratio of 1:4. All images are at 20× magnification. Three DLS measurements (à 3 min) for each emulsion displayed as average diameter ± SD in µm. The raw data was fitted using Gaussian analysis and is reported using intensity E) or volume F) weighing (Wt).

caused the high viscosity [42]. The difference in solubility between water, saline and PBS could be explained as 'salting out', i.e., the entropic penalty of separating out an electrolyte decreases with increasing ionic strength [43]. It may also be described as the de-swelling of a hydrogel-like phase (syneresis). Note that this explanation is not in conflict with the fact that DOX formed gel-like aggregates rather than solid precipitates in the aqueous solvents [13].

More remarkable is the high solubility (> 69 mg/mL) of DOX in iohexol and the iohexol and water mixture found in this study. Iohexol is a basic and polar compound. It is very soluble in water and has a pK_a of 11.4, a log $P_{butanol/water}$ of −1.15, and a log $P_{octanol/water}$ of −3.00 [44–47]. The commercial contrast agent Omnipaque™ contains iohexol at concentrations corresponding to an iodine content of 140–350 mg iodine/mL. Iohexol is considered a low-osmolality compound. However, the concentration used at Uppsala University Hospital is 300 mg iodine/mL, resulting in a 2-fold higher osmolality than blood [45]. Iohexol is a very hydrophilic molecule and has not been shown to self-aggregate in water [48]. Thus, despite DOX's aggregate-forming ability in water it is not likely that iohexol increases the solubility by forming mixed micelles with DOX, however this remains to be further investigated [10–13].

Increased emulsion stability has been shown to increase the drug released into the tumor [18]. Our results suggest that the addition of iohexol generates emulsions that were stable up to 1 h, which is longer than the time between preparation and administration in the clinic [6]. The emulsions with the highest stability were achieved at aqueous-to-lipid phase ratios of 1:4 and classified as w/o emulsions. The aqueous phase of emulsion IW/L has been reported to have the same specific density as Lipiodol® (1.28 g/cm^3) and it was the only emulsion that was stable after 72 h at room temperature at an aqueous-to-lipid phase ratio of 1:4 [4,34]. The increased emulsion stability of the emulsions containing iohexol is due to the densifying properties of iohexol [25–28]. It

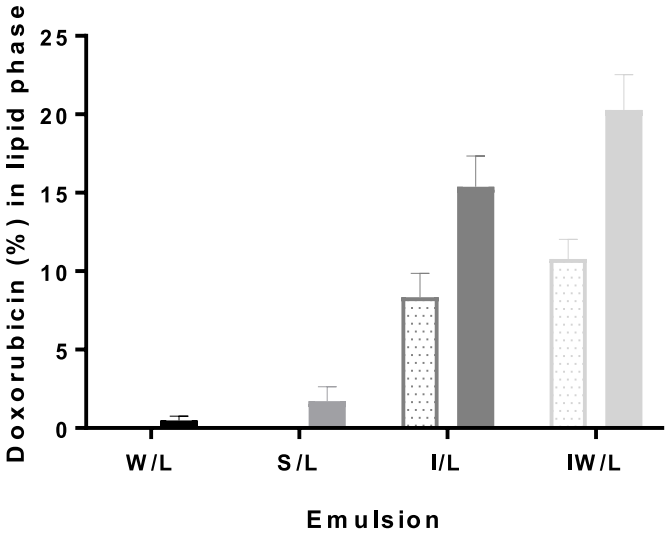

Fig. 7. The distribution of doxorubicin hydrochloride (DOX) to the lipid phase investigated in emulsions with an aqueous-to-lipid phase ratio of 1:4 as a mean (± SD). Dotted bars, 15 min; solid bars, 60 min. Emulsions composition; water and Lipiodol® (W/L), saline 0.9% and Lipiodol® (S/L), iohexol and Lipiodol® (I/L), and a mixture (v/v) of iohexol (85%)/water (15%) and Lipiodol® (IW/L).

is, however, still unclear if iohexol has other stabilizing mechanisms than its densifying properties, such as forming complexes with DOX or reducing the van der Waals attraction between droplets. Other emulsion stabilizing-methods include the addition of polymers or emulsifiers [25,49–51]. The beneficial effect of emulsifiers and/or other stabilizing methods should be systematically investigated and implemented in the clinical use of these emulsions [4,5,52].

The emulsion droplet-sizes were investigated using both microscopy and DLS. DLS offers proper size estimation in dilute and monodisperse samples. However, the investigated emulsions were both polydisperse and very concentrated with extensive physical interaction(s) between droplets. These emulsion properties lead to substantial multiple scattering and explains the unreliable average droplet diameters reported. It has been suggested that accuracy could be improved in larger droplet systems by using multi-angle DLS or complementing with static light scattering measurements [53].

The emulsion droplet-size can be affected by other parameters than aqueous-to-lipid phase ratio and aqueous phase compositions. For example, the drop-size and viscosity of the emulsion can be altered by the number of times and by the speed that the phases are pumped back and forth during the final preparation just prior to administration to the patient [4,32]. Recently a novel robust “pumping emulsification device” was reported that form stable water-in-oil emulsions with homogenous droplet sizes [54]. There are, however, inconsistencies in the reported effect of emulsion droplet-size range on the tumor uptake, emphasizing the need to improve the understanding of the pharmaceutical factors affecting emulsion droplet-size and stability, and to incorporate them into a clinical delivery perspective [55,56].

The distribution of DOX to the lipid phase of the investigated aqueous-to-lipid phase ratios of 1:4 emulsions was in the range of 0.5–20%. For emulsions without iohexol (W/L and S/L), our results are in line with a report of a 1:1 emulsion with the following composition: Lipiodol®, DOX, glucose, water, and polysorbate-80, where 0.5% of DOX distributed to the lipid phase after 1 h [57]. There was a time-dependent increase in distribution indicating that increased equilibration times could increase the distribution of DOX into the lipid phase. This is of importance as *in vivo* studies suggest that Lipiodol® (i.e. the lipid phase) is the only component from the emulsion-based formulations that accumulates in the tumor environment [20]. Accordingly, an increased distribution of DOX to the lipid phase might increase DOX concentrations inside the tumor cells [20,55,58,59]. Also, cytostatic agents with more lipophilic physicochemical properties than DOX (logD for DOX is between ~ 2 and ~0.4 depending on DOX concentration at pH 7.3) might have a greater propensity to accumulate in Lipiodol® and consequently claim some higher degree of tissue targeting property [10].

There exist no standardizations in the *in vitro* release methods of parenteral formulations. This leads to a large variation in the literature guidelines for *in vitro* release of DOX from these emulsions [5,60]. In this study, the addition of a dialysis membrane (which is common when testing drug release from emulsions) to a previously developed μDiss *in vitro* release method, was used to investigate the release of DOX from the emulsions [25,28,37,38,51,61]. For stable emulsions, the release rate depends on factors such as the composition of the aqueous phase, lipid-to-aqueous ratio, viscosity of the continuous phase, droplet-size range and the complexity of the system (i.e., simple or multiple emulsions) [62]. The drug release mechanism from water-in-oil emulsions is suggested to follow first-order kinetics, which is governed by diffusion through both phases [62].

The diffusion of aqueous-phase DOX (emulsion 1:0) across the dialysis membrane was slow and the plateau was reached between 3 and 15 h. Therefore, an empirical model was developed to discriminate between the diffusion of DOX across the dialysis membrane and the release of DOX from the investigated emulsions. The release half-life (h) from the unstable emulsions W/L and S/L was unaffected by the increase in lipid ratio (1:1–1:4) as they still separated quickly. This generated similar DOX release rates for emulsions and aqueous phases. For emulsions I/L and IW/L, the increase in lipid ratio prolonged the estimated release half-life (h). This effect can be attributed to the increased emulsion stability, which forces DOX to diffuse through the increased lipid phase. The estimated release half-life of emulsion IW/L with an aqueous-to-lipid phase ratio of 1:3 was ~1 (0.75–1.5) hr generating an approximate DOX release of 75% after 2 h. In HCC patients Lipiodol®-based emulsions similar to emulsion IW/L with an aqueous-to-lipid phase ratio of 1:3 were reported to release 90% of DOX within 2 h [63]. The difference between the estimated release rate *in vitro* and *in vivo* is most likely attributed to the dynamic exposure of the blood flow *in vivo* causing rapid emulsion separation after intra-hepatic administration to the patients [63].

5. Conclusions

Despite the widespread clinical use of Lipiodol®-based emulsions, the properties of these injectable formulations are in need of extended pharmaceutical characterization and optimization [4,5]. In this study it was demonstrated that the emulsion stability and DOX *in vitro* release profiles depend on the composition of the aqueous phase and the aqueous-to-lipid phase ratio. In addition, the relatively low distribution and solubility of DOX to and in the lipid phase (Lipiodol®) suggest that there is a need for further improvements of this formulation. The methods presented herein can be used for emulsion optimization and pharmaceutical quality assurance. A harmonization of these clinically used drug delivery systems including standardized composition and preparation methods is warranted.

Conflicts of interest

Declare no conflict of interest.

Acknowledgments

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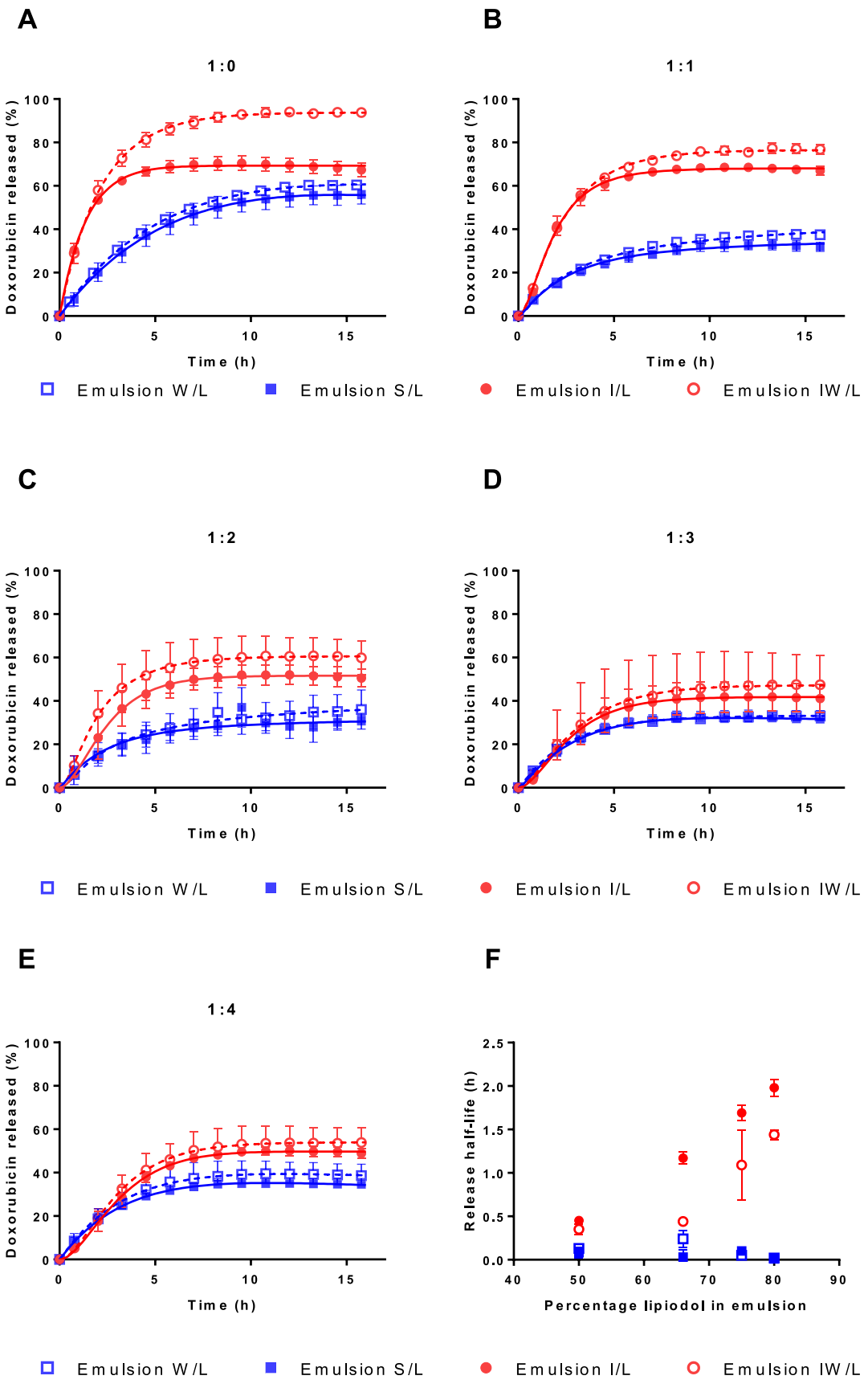

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Fig. 8. *In vitro* doxorubicin hydrochloride (DOX) release from emulsions with DOX concentration of 2.5 mg/ml. Emulsions composition; water and Lipiodol® (W/L), saline 0.9% and Lipiodol® (S/L), iohexol and Lipiodol® (I/L), and a mixture (v/v) of iohexol (85%)/water (15%) and Lipiodol® (IW/L). The aqueous-to-lipid phase ratio was 0:1, 1:1, 1:2, 1:3, and 1:4. All experiments were performed with the μDiss profiler in 20 ml of phosphate buffer saline at a pH of 7.4, 37 °C, with a stirring rate of 100 rpm throughout the experiment. Lines represents the curve-fitted released DOX (%) A) Emulsion 1:0, i.e., only the aqueous phase. DOX has transported across the dialysis membrane, B) 1:1 emulsion, C) 1:2 emulsion, D) 1:3 emulsion, E) 1:4 emulsion, F) release-half life (h) from the empirical model applied to the mean (± SD) release data of each investigated emulsions.

Table 4
Estimated release rate constant (k_{rel}, (h^{-1})) presented as mean and coefficient of variance (CV%) from empiric compartmental model fitted to the observed means of the release data.

	Aqueous phase of emulsion			
	W/L	S/L	I/L	IW/L
Aqueous-to-lipid phase ratio	k_{rel} (CV%)	k_{rel} (CV%)	k_{rel} (CV%)	k_{rel} (CV%)
1:1	5.2 (39)	8.2 (77)	1.5 (9.0)	2.0 (17)
1:2	2.9 (40)	20 (280)	0.59 (5.9)	1.6 (8.7)
1:3	13 (36)	6.8 (11)	0.41 (5.3)	0.63 (37)
1:4	44 (210)	99 (260)	0.35 (4.9)	0.48 (3.9)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2019.101143.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (2015) E359–E386.

[2] O. Ahmed, L. Liu, A. Gayed, A. Baadh, M. Patel, J. Tasse, U. Turba, B. Arslan, The changing face of hepatocellular carcinoma: forecasting prevalence of nonalcoholic steatohepatitis and hepatitis C cirrhosis, J. Clin. Exp.Hepatol. 9 (2019) 50–55.

[3] EASL-EORTC, EASL–EORTC clinical practice guidelines: management of hepatocellular carcinoma, J. Hepatol. 56 (2012) 908–943.

[4] J.-M. Idée, B. Guiu, Use of Lipiodol as a drug-delivery system for transcatheter arterial chemoembolization of hepatocellular carcinoma: a review, Crit. Rev. Oncol. Hematol. 88 (2013) 530–549.

[5] I.R. Dubbelboer, E. Lilienberg, E. Ahnfelt, E. Sjögren, N. Axén, Hans Lennernäs, Treatment of intermediate stage hepatocellular carcinoma: a review of intrahepatic doxorubicin drug-delivery systems, Ther. Deliv. 5 (2014) 447–466.

[6] E. Lilienberg, I.R. Dubbelboer, A. Karalli, R. Axelsson, T.B. Brismar, C. Ebeling Barbier, A. Norén, F. Duraj, M. Hedeland, U. Bondesson, E. Sjögren, P. Stål, R. Nyman, H. Lennernäs, In vivo drug delivery performance of lipiodol-based emulsion or drug-eluting beads in patients with hepatocellular carcinoma, Mol. Pharm. 14 (2017) 448–458.

[7] E. Lilienberg, Biopharmaceutical evaluation of intra-arterial drug-delivery systems for liver cancer: investigations in healthy pigs and liver cancer patients, Acta Universitatis Upsaliensis, 2015.

[8] H. Lusic, M.W. Grinstaff, X-ray-Computed tomography contrast agents, Chem. Rev. 113 (2013) 1641–1666.

[9] M. Dalmark, Characteristics of doxorubicin transport in human red blood cells, Scand. J. Clin. Lab. Investig. 41 (1981) 633–639.

[10] M. Dalmark, H. Storm, A Fickian diffusion transport process with features of transport catalysis. Doxorubicin transport in human red blood cells, J. Gen. Physiol. 78 (1981) 349–364.

[11] Z. Fülöp, R. Gref, T. Loftsson, A permeation method for detection of self-aggregation of doxorubicin in aqueous environment, Int. J. Pharm. 454 (2013) 559–561.

[12] E. Csuhai, S. Kangarlou, T.X. Xiang, A. Ponta, P. Bummer, D. Choi, B.D. Anderson, Determination of key parameters for a mechanism-based model to predict doxorubicin release from actively loaded liposomes, J. Pharm. Sci. 104 (3) (March 2015) 1087–1098.

[13] E. Hayakawa, K. Furuya, H. Ueno, M. Moriyama, A. Kondo, Visible absorption and proton nuclear magnetic resonance studies on the self-association of doxorubicin in aqueous solution, Chem. Pharm. Bull. 39 (1991) 1009–1012.

[14] P. Agrawal, S.K. Barthwal, R. Barthwal, Studies on self-aggregation of anthracycline drugs by restrained molecular dynamics approach using nuclear magnetic resonance spectroscopy supported by absorption, fluorescence, diffusion ordered spectroscopy and mass spectrometry, Eur. J. Med. Chem. 44 (2009) 1437–1451.

[15] X. Li, D.J. Hirsh, D. Cabral-Lilly, A. Zirkel, S.M. Gruner, A.S. Janoff, W.R. Perkins, Doxorubicin physical state in solution and inside liposomes loaded via a pH gradient, Biochim. Biophys. Acta Biomembr. 1415 (1998) 23–40.

[16] R. Lencioni, T. de Baere, M.C. Soulen, W.S. Rilling, J.F.H. Geschwind, Lipiodol transarterial chemoembolization for hepatocellular carcinoma: a systematic review of efficacy and safety data, Hepatology 64 (2016) 106–116.

[17] M. Boulin, S. Guiu, B. Chauffert, S. Aho, J.-P. Cercueil, F. Ghiringhelli, D. Krause, P. Fagnoni, P. Hillon, L. Bedenne, Screening of anticancer drugs for chemoembolization of hepatocellular carcinoma, Anti Canccer Drugs 22 (2011) 741–748.

[18] M. Boulin, A. Schmitt, E. Delhom, J.-P. Cercueil, M. Wendremaire, D.-C. Imbs, A. Fohlen, F. Panaro, A. Herrero, A. Denys, Improved stability of lipiodol–drug emulsion for transarterial chemoembolisation of hepatocellular carcinoma results in improved pharmacokinetic profile: proof of concept using idarubicin, Eur. Radiol. 26 (2016) 601–609.

[19] J. Gabrielsson, L.A. Peletier, S. Hjorth, In vivo potency revisited–keep the target in sight, Pharmacol. Ther. 184 (2018) 177–188.

[20] K. Iwai, H. Maeda, T. Konno, Use of oily contrast medium for selective drug targeting to tumor: enhanced therapeutic effect and X-ray image, Cancer Res. 44 (1984) 2115–2121.

[21] S. Bhattacharya, A. Dhillon, M. Winslet, B. Davidson, N. Shukla, S.D. Gupta, R. Al-Mufti, K. Hobbs, Human liver cancer cells and endothelial cells incorporate iodised oil, Br. J. Canc. 73 (1996) 877.

[22] E. Liapi, J.-F.H. Geschwind, Transcatheter arterial chemoembolization for liver cancer: is it time to distinguish conventional from drug-eluting chemoembolization? Cardiovasc. Interv. Radiol. 34 (2011) 37–49.

[23] J. Wolff, Physiology and pharmacology of iodized oil in goiter prophylaxis, Medicine 80 (2001) 20–36.

[24] T. de Baere, Y. Arai, R. Lencioni, J.-F. Geschwind, W. Rilling, R. Salem, O. Matsui, M.C. Soulen, Treatment of liver tumors with lipiodol TACE: technical recommendations from experts opinion, Cardiovasc. Interv. Radiol. 39 (2016) 334–343.

[25] S.W. Yi, Y.-H. Kim, I.C. Kwon, J.W. Chung, J.H. Park, Y.W. Choi, S.Y. Jeong, Stable lipiodolized emulsions for hepatoma targeting and treatment by transcatheter arterial chemoembolization, J. Control. Release 50 (1998) 135–143.

[26] H. Nakamura, T. Hashimoto, H. Oi, S. Sawada, Transcatheter oily chemoembolization of hepatocellular carcinoma, Radiology 170 (1989) 783–786.

[27] H. Sakaguchi, H. Uchida, Y. Nishimura, Q. Guo, H. Yoshimura, H. Ohishi, [Pharmacokinetic study of adriamycin in the emulsion mixed with lipiodol-difference resulting from composition and methods of preparation, and behavior after mesenteric arterial injection in rat], Gan to kagaku ryoho, Cancer & chemotherapy 18 (1991) 1349–1355.

[28] J.W. Choi, H.-J. Cho, J.-H. Park, S.Y. Baek, J.W. Chung, D.-D. Kim, H.-C. Kim, Comparison of drug release and pharmacokinetics after transarterial chemoembolization using diverse Lipiodol emulsions and drug-eluting beads, PLoS One 9 (2014) e115898.

[29] S.W. Shin, The current practice of transarterial chemoembolization for the treatment of hepatocellular carcinoma, Korean J. Radiol. 10 (2009) 425–434.

[30] T. de Baère, A. Denys, R. Briquet, P. Chevallier, J. Dufaux, A. Roche, Modification of arterial and portal hemodynamics after injection of iodized oils and different emulsions of iodized oils in the hepatic artery: an experimental study, J. Vasc. Interv. Radiol. 9 (1998) 305–310.

[31] B. Abismaïl, J.P. Canselier, A.M. Wilhelm, H. Delmas, C. Gourdon, Emulsification by ultrasound: drop size distribution and stability, Ultrason. Sonochem. 6 (1999) 75–83.

[32] T. Masada, T. Tanaka, H. Nishiofuku, Y. Fukuoka, T. Sato, S. Tatsumoto, N. Marugami, K. Kichikawa, Techniques to form a suitable lipiodol-epirubicin emulsion by using 3-way stopcock methods in transarterial chemoembolization for liver tumor, J. Vasc. Interv. Radiol. 28 (2017) 1461–1466.

[33] C.A.S. Bergström, U. Norinder, K. Luthman, P. Artursson, Experimental and computational screening models for prediction of aqueous drug solubility, Pharm. Res. 19 (2002) 182–188.

[34] A. Norén, J. Urdzik, F. Duraj, C.E. Barbier, B.M. Karlson, U. Haglund, Longterm follow-up after transarterial chemotherapy for hepatocellular carcinoma in a Scandinavian centre, HPB 12 (2010) 637–643.

[35] E. Lilienberg, I.R. Dubbelboer, E. Sjögren, H. Lennernäs, Lipiodol does not affect the tissue distribution of intravenous doxorubicin infusion in pigs, J. Pharm. Pharmacol. 69 (2017) 135–142.

[36] F. Deschamps, L. Moine, T. Isoardo, L. Tselikas, A. Paci, L. Mir, N. Huang, E. Fattal, T. de Baere, Parameters for stable water-in-oil lipiodol emulsion for liver transarterial chemo-eembolization, Cardiovasc. Interv. Radiol. 40 (2017) 1927–1932.

[37] E. Ahnfelt, E. Sjögren, N. Axén, H. Lennernäs, A miniaturized in vitro release method for investigating drug-release mechanisms, Int. J. Pharm. 486 (2015) 339–349.

[38] E. Ahnfelt, E. Sjögren, P. Hansson, H. Lennernäs, In vitro release mechanisms of doxorubicin from a clinical bead drug-delivery system, J. Pharm. Sci. 105 (2016) 3387–3398.

[39] V. Bijlani, D. Yuonayel, S. Katpally, B.N. Chukwumezie, M.C. Adeyeye, Monitoring ibuprofen release from multiparticulates: in situ fiber-optic technique versus the HPLC method: a technical note, AAPS PharmSciTech 8 (2007) E9–E12.

[40] A. Avdeef, K. Tsinman, O. Tsinman, N. Sun, D. Voloboy, Miniaturization of powder dissolution measurement and estimation of particle size, Chem. Biodivers. 6 (2009) 1796–1811.

[41] I.R. Dubbelboer, Biopharmaceutical investigations of doxorubicin formulations

used in liver cancer treatment: studies in healthy pigs and liver cancer patients, combined with pharmacokinetic and biopharmaceutical modelling, Acta Universitatis Upsaliensis, 2017.
[42] E. Tasca, M. D'Abramo, L. Galantini, A.M. Giuliani, N.V. Pavel, G. Palazzo, M. Giustini, A stereochemically driven supramolecular polymerisation, Chem. Eur J. 24 (32) (June 7, 2018) 8195–8204.
[43] K.E. Gutowski, G.A. Broker, H.D. Willauer, J.G. Huddleston, R.P. Swatloski, J.D. Holbrey, R.D. Rogers, Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations, J. Am. Chem. Soc. 125 (2003) 6632–6633.
[44] C.M. Riley, R. McLemore, M.C. Preul, B.L. Vernon, Gelling process differences in reverse emulsion, in situ gelling polymeric materials for intracranial aneurysm embolization, formulated with injectable contrast agents, J. Biomed. Mater. Res. B Appl. Biomater. 96 (2011) 47–56.
[45] K. Eivindvik, C. Sjøgren, Physicochemical properties of iodixanol, Acta Radiol. 36 (1995) 32–38.
[46] Martindale: the complete drug reference, 10 Oct 2016, 2016. https://www.medicinescomplete.com/about/.
[47] A. Hellqvist, R. Heiene, S. De Baere, S. Croubels, Y. Hedeland, Development of a capillary electrophoretic method for determination of plasma clearance of iohexol in dogs and cats, Biomed. Chromatogr. 29 (2015) 504–513.
[48] P.C. Sontum, C. Christiansen, V. Kasparkova, T. Skotland, Evidence against molecular aggregates in concentrated solutions of X-ray contrast media, Int. J. Pharm. 169 (1998) 203–212.
[49] F. Deschamps, K.R. Harris, L. Moine, W. Li, L. Tselikas, T. Isoardo, R.J. Lewandowski, A. Paci, N. Huang, T. de Baere, R. Salem, A.C. Larson, Pickering-emulsion for liver trans-arterial chemo-embolization with oxaliplatin, Cardiovasc. Interv. Radiol. 41 (2018) 781–788.
[50] S.-Y. Lin, W. Wu, W. Lui, In vitro release, pharmacokinetic and tissue distribution studies of doxorubicin hydrochloride (Adriamycin HCl) encapsulated in lipiodolized w/o emulsions and w/o/w multiple emulsions, Die Pharmazie 47 (1992) 439–443.
[51] T.H. Lin, S.Y. Lin, Encapsulation and prolonged release behaviour of w/o/w type multiple emulsions, J. Chin. Chem. Soc. 35 (1988) 463–470.
[52] A. Kabalnov, H. Wennerström, Macroemulsion stability: the oriented wedge theory revisited, Langmuir 12 (1996) 276–292.
[53] C. Goddeeris, F. Cuppo, H. Reynaers, W. Bouwman, G. Van den Mooter, Light scattering measurements on microemulsions: estimation of droplet sizes, Int. J. Pharm. 312 (2006) 187–195.
[54] T. Tanaka, T. Masada, H. Nishiofuku, Y. Fukuoka, T. Sato, S. Tatsumoto, N. Marugami, S. Higashi, K. Kichikawa, Development of Pumping Emulsification Device with Glass Membrane to Form Ideal Lipiodol Emulsion in Transarterial Chemoembolization, European radiology, 2018, pp. 1–5.
[55] O. Cay, J. Kruskal, P. Thomas, M.E. Clouse, Targeting of different ethiodized oil—doxorubicin mixtures to hypovascular hepatic metastases with intraarterial and intraportal injections, J. Vasc. Interv. Radiol. 7 (1996) 409–417.
[56] S. Higashi, N. Tabata, K.-H. Kondo, Y. Maeda, M. Shimizu, T. Nakashima, T. Setoguchi, Size of lipid microdroplets effects results of hepatic arterial chemotherapy with an anticancer agent in water-in-oil-in-water emulsion to hepatocellular carcinoma, J. Pharmacol. Exp. Ther. 289 (1999) 816–819.
[57] P. Favoulet, J.P. Cercueil, P. Faure, L. Osmak, N. Isambert, J.L. Beltramo, F. Cognet, D. Krause, L. Bedenne, B. Chauffert, Increased cytotoxicity and stability of Lipiodol-pirarubicin emulsion compared to classical doxorubicin-Lipiodol: potential advantage for chemoembolization of unresectable hepatocellular carcinoma, Anti Canccer Drugs 12 (2001) 801–806.
[58] S. Baumgarten, R.C. Gaba, R.B. Breemen, Confirmation of drug delivery after liver chemoembolization: direct tissue doxorubicin measurement by UHPLC-MS-MS, Biomed. Chromatogr. 26 (2012) 1529–1533.
[59] R.C. Gaba, S. Baumgarten, B.O. Omene, R.B. Van Breemen, K.D. Garcia, A.C. Larson, R.A. Omary, Ethiodized oil uptake does not predict doxorubicin drug delivery after chemoembolization in VX2 liver tumors, J. Vasc. Interv. Radiol. 23 (2012) 265–273.
[60] M. Martinez, M. Rathbone, D. Burgess, M. Huynh, In vitro and in vivo considerations associated with parenteral sustained release products: a review based upon information presented and points expressed at the 2007 Controlled Release Society Annual Meeting, J. Control. Release 129 (2008) 79–87.
[61] G. Frenning, E. Ahnfelt, E. Sjögren, H. Lennernäs, Computational fluid dynamics (CFD) studies of a miniaturized dissolution system, Int. J. Pharm. 521 (2017) 274–281.
[62] A.T. Florence, D. Whitehill, The formulation and stability of multiple emulsions, Int. J. Pharm. 11 (1982) 277–308.
[63] I.R. Dubbelboer, E. Sjögren, H. Lennernäs, Porcine and human in vivo simulations for doxorubicin-containing formulations used in locoregional hepatocellular carcinoma treatment, AAPS J. 20 (2018) 96.