Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 323 (2006) 161–167
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

A less irritant norcantharidin lipid microspheres: Formulation and drug distribution

Wang Lixin [b], He Haibing [a], Tang Xing [a,*], Shao Ruiying [a], Chen Dawei [a]

[a] *Department of Pharmaceutics, Shen Yang Pharmaceutical University,Wen Hua Road No. 103, Shen yang 110016, People's Republic of China*
[b] *Liaoning Medical Apparatus and Instrument Institute, Shen Yang 110000, People's Republic of China*

Received 29 November 2005; received in revised form 7 April 2006; accepted 28 May 2006
Available online 3 June 2006

Abstract

Lipid microspheres (LM) have recently been used as intravenous (i.v.) carriers for drugs, which are sufficiently soluble in oil. However, in the case of norcantharidin (NCTD), which is poorly soluble in both the water and oil phases, this approach is not feasible. In this study, NCTD-loaded LM was prepared by transferring the drug to the interfacial surface of the oil and aqueous phases to produce a less irritating i.v. formulation of NCTD. A probe type sonicator was used to disperse NCTD into the oil phase together with lecithin and Tween 80. A high-pressure homogenization process was used to prepare the lipid microspheres and localize the drug at the surfactant layer. The LM loaded with NCTD consisted of 0.02% drug. Characterization of LMs and short-term stability was performed by photon correlation spectroscopy (PCS) and a centrifugation test was also carried out. The results showed that NCTD-loaded LM (2 mg/ml) with over 80% NCTD loaded in the interfacial surface were stable for a period of 2 months, and were suitable for i.v. injection in terms of size and stability, whether be diluted or not. Such formulations produced less pain and irritation in animal studies.

Keywords: Norcantharidin (NCTD); Lipid microspheres (LM); Poor solubility; Surfactant layer; Short-term stablility

1. Introduction

Lipid microspheres (LM), also referred to as lipid emulsions, with a mean diameter of 0.2 μm produced from soybean oil and egg yolk lecithin, are used to supply calories to patients unable to obtain adequate nourishment from a normal diet. The clinically well-accepted usage of fat emulsions, such as Intralipid® and Lipofundin®, offers the possibility of using the internal oil core of o/w LM as a carrier of lipophlilic drugs to enhance their therapeutic action (Nasirideen, 1998; Igarashi et al., 1996). Because the incorporated drug produces less irritation and fewer toxic effects and because of it exhibits sustained release and targeted delivery to various organs, LM are a promising alternative vehicle for parenteral drug administration. Furthermore, reduced drug hydrolysis or increased bioavailability by incorporation of drugs into i.v LM has been reported, as well as a much more potent pharmacological activity compared with administration as a solution (Nasirideen, 1998). However, the number of drug-loaded emulsions for i.v. injection on the market is very limited, i.e. diazepam, etomidate and propofol. The main reason for this is that poorly water-soluble drug do not have a sufficiently high solubility in the registered oils (e.g. soya oil) (Müller et al., 2004). The issue of dissolving poorly soluble drugs simultaneously in the water and oil phases and their formulation has attracted increasing attention in the last 5 years and is one of the "hot topics" in pharmaceutical formulation development (Müller et al., 2004). The small particle size (approximately 0.1–0.2 μm) of the emulsified oil droplets in the LM result in a sizeable interfacial surface area. Teagarden et al. estimated that the interfacial area for LM containing 0.1–0.2 μm droplets was 10–20-fold higher than would be needed for PGE_1 present in emulsions containing up to 200 μg/ml of drug (Teagarden et al., 1988). Aslihan and Müller showed that a solvent-free approach, involving SolEmuls®, is able to locate poorly soluble drugs in the interfacial area of emulsions (Aslihan and Müller, 2003).

Much attention has been focused on drug delivery systems (DDS) for cancer chemotherapy which aim at the specific targeting of tumor cells or tumor tissues, thus enhancing the effi-

* Corresponding author. Tel.: +86 24 23986343; fax: +86 24 23911736.
E-mail address: tangpharm@sina.com.cn (T. Xing).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.05.060

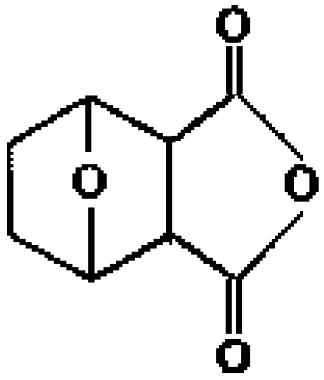

Fig. 1. Structure of norcantharidin (NCTD).

cacy and reducing the toxicity of the antitumor agents involved (Mitsuko, 1996). LM are a suitable carrier for such agents, most of which are not readily soluble in the oil phase. NCTD (Fig. 1) is one of the new chemotherapy agents that have been shown to be very effective against cancer, especially against primary hepatic carcinoma. At the same time, it has a marked effect on leukocyte accretion. Its main clinically adverse effect is that it produces urinary system toxicity. Following aqueous injection, it produces intense irritation at the injection site. The maximum solubility of NCTD in water is 2.5 mg/ml at pH 6.0 and this increases to 9.5 mg/ml at pH 9.5. Clinically, NCTD is mostly given by injection of its sodium salt (5 mg/ml, 2 ml) at a pH of about 9.0. The high pH is an important cause of this irritation following intravenous injection.

In the present study, a new formulation has been developed to locate the poorly soluble drug in the interfacial region of LM. Different injectable excipients were compared to improve the incorporation capacity of the LM system and increasing concentrations of drug were incorporated to study the effect of drug concentration on the mean size, size distribution of emulsion droplets and the distribution of drug in the three phases of LM. This study had four objectives: (1) to incorporate NCTD into the oil phase or the interfacial surface of the LM without any crystals or, if there were any, to see if it was possible to administer such hybrid dispersions intravenously; (2) to determine the loading capacity of the LM system and the effect on the lecithin-layer of additional drug; (3) to study the drug distribution in the water phase, oil phase and the interfacial surface and identify the factors affecting such distribution; (4) to see if the DDS (drug delivery system) of LM was able to reduce in situ irritation.

2. Material and methods

2.1. Materials

Lipoid E80® and MCT (Lipoid KG, Ludwigshafen, Germany), Lutrol (Pluronic) F-68® (BASF AG, Ludwigshafen, Germany), Tween 80 for parenteral use (Shenyu Medicine and Chemical Industry Limited Co., Shanghai, China), soybean oil for parenteral use (Tieling BeiYa Pharmaceutical Co., Tieling, China) and NCTD (Surui Medicine and Chemical Industry Limited Co., Suzhou, China) were used. All other chemicals and reagents were of analytical or chromatographic grade.

2.2. Formulation and preparation of LM

The following is the basic formula of the LM used in this study: a mixture of soybean oil and MCT, 10 g; lecithin, 1.2 g; Tween 80, 20 mg; glycerol, 2.5 g; DL-α-tocopherol, 300 mg; sodium oleate, 30 mg; EDTA, 20 mg; NCTD, 200 mg; doubly distilled water, qs 100.0 g.

The aqueous phase was prepared by dispersing the glycerol, sodium oleate, and EDTA in water using constant speed stirrer at 1500 rpm. The oil phase was prepared by combining a mixture of soybean oil and MCT, lecithin, Tween 80 and DL-α-tocopherol at 80 °C and then the drug powder was added when the lecithin dissolved. A probe type sonicator, SONICS vibra cell™ (Sonic & Materials Inc., USA) was used to disperse the obtained dispersion further. A primary emulsion was achieved by mixing the oil and aqueous phases for 20 min using a high-speed stirrer. High-pressure homogenization was performed using a Niro Soavi NS10012k homogenization apparatus (Via M. da Erba, 29/A-43100 PARMA, Italy). Homogenization conditions were typically 60–120 MP, and 4–20 cycles at 40 °C. After adjusting the pH to 4–9 with 0.1N NaOH, the emulsion was autoclaved for 15 min at 121 °C, 0.095 MP (15 bar).

2.3. Particle size measurement

The particle size measurement was carried out by photon correlation spectroscopy (PCS, dynamic light scattering, DLS) using a Nicomp™ 380 particle sizing system (Santa Barbara, USA). The PSS covers the size range of about 5 nm–3 μm and has, therefore, been extensively used for particle size analysis of LM (Komatsu et al., 1995). The Nicomp and Guassian distributions of particle size were obtained at the same time with intensity-weighting (z-average), volume-weighting and number-weighting while the value of S.D., instead of PI, gave the width of distribution. The characterization parameters of PCS diameters 50, 90, 95 and 99% were also calculated. The LM were diluted 1:5000 immediately before measurement with doubly distilled water that had been passed through a 0.22 μm membrane filter and it was verified beforehand that dilution of the samples did not alter the size distributions obtained (David, 2002; Müller et al., 2004).

2.4. Zeta potential measurement

The Nicomp™ 380 was also used to measure the zeta potential by electrophoretic light scattering (ELS). The ELS technique is based on the scattering of light from particles that move in liquid under the influence of an applied electric field. The value of the mean zeta potential ζ is obtained from the electrophoretic mobility μ which is computed from the measured Doppler shift $\Delta\nu$, for a given applied electric field strength E of 15 V/cm. Samples were diluted as described for particle size measurement, except that the water was adjusted to the desired pH with 0.1N HCl or NaOH beforehand.

2.5. Centrifugation

Centrifugation was carried out on a HITACHI ultracentrifugation apparatus at 46,000 rpm (=approximately 107,000 × *g*) for 4 h. The sample temperature was preset to 10 °C. Polyallomer tubes were used and their bottoms were pricked after centrifugation with a syringe needle to collect the aqueous phase. Creamed oil and pelleted material could then be collected without further washing steps (Groves et al., 1985; Férézou et al., 1994).

2.6. High-performance liquid chromatography (HPLC)

An anion-exchange HPLC method was established for the determination of norcantharidin in LM. The separation was performed on an Hypersil SAX column (250 mm × 4.6 mm, 5 μm). The mobile phase was 3% phosphate monobasic-methyl alcohol (75:25) at a flow rate of 0.6 ml/min. Detection was carried out with a UV detector at 210 nm. The column was operated at room temperature.

2.7. Safety test

Pain on injection of NCTD formulations was evaluated by the rat paw-lick test (Celozzi et al., 1980). Ten weanling rats (Wistar, 70–120 g each) were used to test each formulation. Each rat was given a single injection of 0.1 ml of one of the test formulations into the footpad of the right hind paw. The number of times the paw was licked was counted over a 15 min time period (five separate 3 min intervals for each rat) and the total amount of time each rat licked its paw was also recorded.

The rabbit ear vein test was performed at the same time. Rabbits were divided into test groups of three rabbits (New Zealand white, 2–3 kg). Each animal was given a single infusion of 3 mg/kg at a rate of 1 ml/min into the marginal ear vein. Visual observations of the appearance of the veins were made at intervals until 24 h after the injection by an experienced unbiased observer.

3. Results and discussion

3.1. Production of LM

The most commonly used method of preparation of drug loaded LM is to dissolve the drug in the oil phase. A method has been reported in which the drug particles were added to a preformed emulsion, e.g. parenteral emulsions such as Lipofundin® or Intralipid®. Then the mixture is homogenized until the drug crystals are dissolved. The result of this process is that an ultrafine emulsion is obtained with the drug located in the interfacial lecithin layer without any drug crystals being present (Aslihan and Müller, 2003). However, for NCTD, this is not a feasible option because the acidity of NCTD will lead to a significant drop in the pH of the LM system from 7.6 to 4.2. As is well known, the phospholipid is liable to hydrolysis at acidic pH and results in the breakdown of the LM. In this study, LM was prepared in the following way: lecithin, Tween 80 and DL-α-tocopherol were dissolved in oil phase. Heating the oil to 70–80 °C was essential for the lecithin to dissolve, but the high temperature was no longer necessary when the lecithin had dissolved completely. The drug powder was then added and the suspension was homogenized with a probe type sonicator, at 50 W, 4 °C for 3 min × 5 times (on: 2 s, pause: 1 s). Then the oil phase with the dispersed drug crystals was slowly incorporated into the aqueous phase. An ultra-turax was used to produce a pre-emulsion with a rather wide particle size distribution and then the rest of the process was performed. After the pH was adjusted to 7.5–8.0, autoclaving was performed.

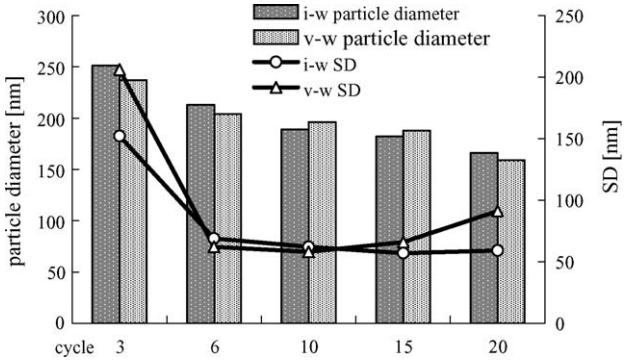

Fig. 2. Photon correlation spectroscopy (PCS) diameter Nicomp distribution (intensity-weight (i-v) and volume weight (v-w)) and standard deviation (S.D.) of the homogenized LM as a function of cycle numbers at 60 Mpa.

A concentration of 2 mg/ml NCTD in the LM was chosen for the preparation of the drug-loaded LM. No drug crystals were detected in the NCTD-loaded LM by microscope with an enlargement factor of 1600. The homogenization was considered to be the most crucial step that affected the particle size of the LM. Higher-pressure emulsification produces a more rapid reduction in particle size. The coarse emulsions were homogenized for 20 homogenization cycles applying individually 60 and 100 MPa at 40 °C, samples were collected after 3, 6, 10, 15, 20 cycles and the mean droplet size, droplet size distribution and the presence drug crystal were determined. There was a slight decrease in mean droplet size (intensity-weight) on increasing the homogenized pressure and cycle times. This means that the final particle sizes were lowest (166 nm) with no over processing on applying the highest pressure (100 MPa, 20 cycles). However, for volume-weight distribution, as typically reported, the few larger particles can be detected more easily, and the model emulsions produced at 100 MPa have a slightly broader distribution than others. Fig. 2 shows the PCS mean diameters of the drug-loaded emulsions. The result showed that just 60 MPa for 6–10 cycles appears to be sufficient to produce sufficiently small mean diameters with a small S.D.

3.2. Analysis of the loading capacity of the LM

PCS covers the size range of about 5 nm–3 μm and is limited to the detection of particles undergoing Brownian motion (Chambrier et al., 1999), so that particles larger than approximately 3 μm are undetectable. In addition, it is difficult for laser diffraction to identify solid drug particles in an ivory-white emulsion and it cannot differentiate between similarly sized

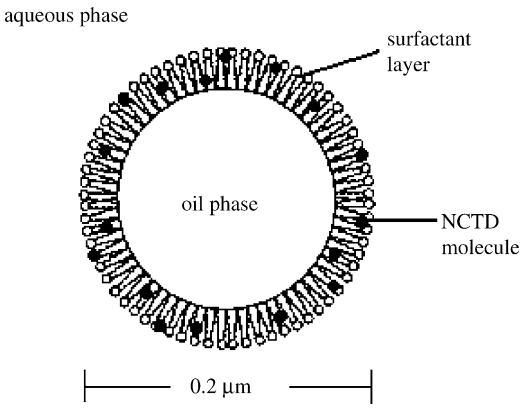

Fig. 3. Drug incorporation into the surfactant layer of the LM.

droplets and crystals. Accordingly, light microscopy associated with centrifugation was applied to examine LM with concentrations ranging from 0.5 to 10 mg/ml to investigate the loading capacity of the emulsions. The LM was not diluted to increase the probability of detecting the presence of even only a few large particles.

The small particle size (approximately 100–200 nm) of the emulsified oil droplets in the LM resulted in a sizeable interfacial surface area. So, for poorly soluble drugs, if there is insufficient loading capability in the aqueous and oil phases simultaneously, a third phase, the interfacial surface, should be taken into account. The ultrasonic and subsequent homogenization processes led to a further size reduction of the drug powder, increasing the surface area and, thus, accelerating the dissolution velocity. The dissolution pressure (and consequent saturation solubility) increases with decreasing particle size, which leads to an increasing concentration gradient $(C_s\text{-}C_x)/h$ in the Noyes–Whitney equation and a higher dissolution velocity (C_s, saturation solubility; C_x, bulk concentration; h, diffusion distance). Furthermore, depending on its emulsifying properties, there is no doubt that lecithin improves the solubility of NCTD to a certain extent. Therefore, an "inlay-model" was used to describe the state of NCTD in the LM where the main principle was that the molecules of surfactant were arranged in order between the water and oil interface with NCTD molecules located between them (Fig. 3). Such a patch-wise arrangement is less likely to lead to reduce interfacial tension and improved dispersion properties of such a mixture (Aslihan and Müller, 2003). The permeation of NCTD into the surfactant layer is thought to be complete during the homogenization and autoclaving processes and the added drug must affect the structure and then the character of the LM system.

3.3. Effect of added drug on LM

There is no doubt that the addition of NCTD and its permeation through the interfacial surface changed the emulsifying properties of the surfactant. A series of LM with different drug concentrations was studied to determine the influence.

It has been reported that all emulsions consist of oil droplets in the submicron range together with small unilamellar liposomes (SUVs) expressing an excess of lecithin (Groves et al., 1985). Dispersions consisting of single, free mobile SUVs of about 50–100 nm were still detected in the blank emulsion in this study. Size analysis by PCS showed that increasing the drug load had a clear effect on the mean diameter of the bulk droplet population as well as the SUVs (Müller et al., 2004). A reduction in particle size was observed in this study below a concentration of 6 mg/ml. The mean PCS diameters as well as the D_{90} decreased with increasing drug load, e.g. 213 nm at 0.5 mg/ml–186 nm at 6 mg/ml (60 MPa, 10 cycles). Also, with the drug concentration increasing from 0.5 to 6 mg/ml, the percentage of the second peak of about 50–100 nm (intensity-weight) dropped from 24.6% to 3.2%. Fig. 4 shows the Nicomp distribution of three kinds of LM. The only explanation for these changes was that when there was a higher energy input, more lecithin was needed to solubilize and provide enough sites for the insoluble drug present in the pre-formed emulsion. So, the superfluous lecithin in the LM system, attributed to SUVs was reduced and another result of the more effective use of lecithin was the reduction in the mean size of the oil droplets. However, it appeared impossible to eliminate the SUVs below an NCTD concentration of 6 mg/ml. Alternatively, 0.6% poloxamer F68 was added as co-emulsifier with the same amount of lecithin. This showed that the SUVs could be avoided with no significant change in droplet size at an NCTD concentration of 2 mg/ml.

However, when the concentration of NCTD was increased to 10 mg/ml, some other peaks at about 500–600 nm with a broad distribution were detected irrespective of whether only lecithin was used or F68 was added. This result suggests that when the drug concentration exceeded 10 mg/ml, the LM system became potentially unstable. An increase in drug concentration also leads to a slight change in zeta potential from −39 to −28 mV. Addition of sodium oleate or adjusting the pH to alkaline values prior

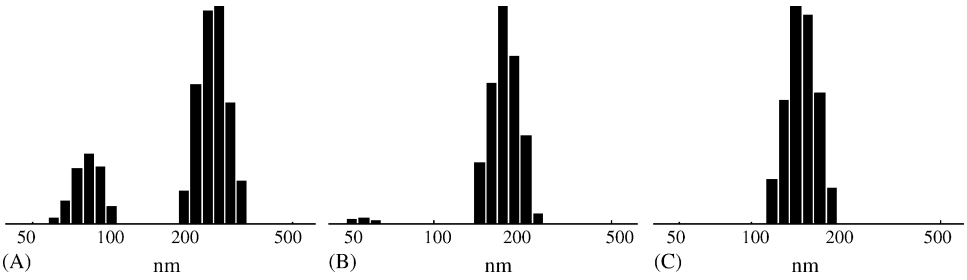

Fig. 4. Particle size distribution (Nicomp, intensity-weight) of three kinds of LM. (A) 1.2% lecithin, 0.5 mg/ml; NCTD; (B) 1.2% lecithin, 6 mg/ml; NCTD; (C) 0.6% lecithin–0.6% poloxamer F68, 2 mg/ml NCTD.

Table 1
Drug distribution with increasing NCTD concentrations at pH 8.0

	NCTD concentration in LM (mg/ml)					
	0.2	0.5	1	2	4	8
$NCTD_A$ (%)	76.5	34.2	22.5	17.1	17.1	14.85
$NCTD_O$ (%)	0.52	0.59	0.47	0.61	0.57	0.6
$NCTD_I$ (%)	22.98	65.21	77.03	82.29	82.33	84.55

$NCTD_I$: amount of NCTD at the interface; $NCTD_A$: NCTD in the aqueous phase; $NCTD_O$: NCTD in the oil phase.

to autoclaving can help to increase the zeta potential by some 5–15 mV. The pH study also showed that the addition of drug aggravate the drop to a different degree during autoclaving. The pH of drug-loaded LM was adjusted to 8.5 before autoclaving and, when the drug concentration was low, there was only a minor change to 8.3 for NCTD 0.5 mg/ml and to 8.0 for 2 mg/ml after sterilization. However the change was more marked when the NCTD concentration increased to 6 mg/ml, namely, a fall to 6.2. In this study, the drug concentration in aqueous phase was detected before sterilization and after sterilization. The entrapment efficiency (EE) of NCTD was degraded after sterilization, and the degree of decreasing was increased with the increasing of drug load. Since the aqueous solution of NCTD was acidity, the increasing of drug concentration in aqueous phase would lead to the pH of drug-load LM drop to a lower degree. No drug degradation observed by HPLC during sterilization.

3.4. Distribution of NCTD in LM

A three-phase model, which assumes that the drug might be present in the aqueous phase, the oil phase, or at the oil–water interface was used in this study. Ultracentrifugation (46,000 rpm) was carried out for 4 h then the uppermost oil layer and the lowest aqueous layer were removed. The relationship described below is mostly common used to determine the drug at the interface:

$$NCTD_I = NCTD_T - NCTD_A - NCTD_O$$

where $NCTD_I$ is the amount of NCTD at the interface, $NCTD_T$ the total NCTD, $NCTD_A$ the NCTD in the aqueous phase and $NCTD_O$ the NCTD in the oil phase.

A comparison of the drug distribution with increasing drug concentration revealed that the percentage of NCTD present at the interface tended to be constant (about 83%) when the concentration of total LM was between 2 and 8 mg/ml. Although the drug concentration of the aqueous phase increased from 0.17 to 1.32 mg/ml when the total NCTD concentration of the LM increased from 0.2 to 2 mg/ml, its distribution percentage dropped more rapidly. As expected, there was little change in the concentration in the oil phase due to the limited solubility of the drug in oil (0.05 mg/ml). This result suggests that, for a poorly soluble drug-loaded LM, it is the surfactant layer that provides the drug a residence, which makes the drug incorporation possible (Table 1).

The pH affected the distribution to a certain extent. This means that when the pH > 9.0, the distribution of drug in the aqueous phase increased dramatically as a result of its increasing solubility in water. The particle size seemed to have no effect on the drug distribution because the surface area was sufficiently high.

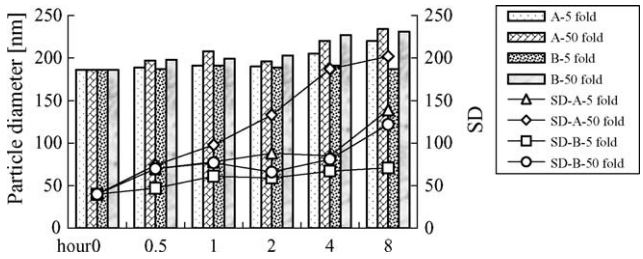

Fig. 5. Mean particle diameter and S.D. of emulsion diluted with different solvents as a function of the time after dilution. (A) Diluted with saline solution (0.9%); (B) diluted with glucose solution (5.4%).

3.5. Study of dilution solvent

Sometimes, dilution is needed before intravenous injection to avoid in situ or blood vessel irritation. Saline (0.9%) and glucose (5.4%) solutions are the mostly common solvents. In this study, drug-loaded LM (2 mg/ml) with added F68 was selected as a model sample to observe the changes in NCTD LM after dilution with the above two solvents. The mean particle size, zeta potential and the drug distribution in the three phases of the diluted emulsion were determined at 30 min, 1, 2, 4 and 8 h after dilution. The particle size results showed that there was no apparent changes in mean particle size with all samples within 8 h after dilution and the particle size distribution showed a notable difference. Fig. 5 shows the change for all samples. The S.D. of the samples diluted with saline solution seemed to increase continuously from 40 to 139 nm at a five-fold dilution and to 202 nm at a 50-fold dilution while particles above 2 μm were detected. The series diluted with glucose solution showed comparatively smaller changes involving an increase to 71 nm at a five-fold dilution and to 122 nm at a 50-fold dilution during the monitoring period and no particles above 1 μm were detected.

The zeta potential dropped markedly at 2 h following dilution of the LM with saline solution, at both a five-fold and 50-fold dilution. It should be pointed out that some commercial LMs (e.g. Diprivan®) can be diluted with saline solution (Bock, 1994). So the effect of sodium chloride on the NCTD loaded LM was thought to be mainly the destruction of the NCTD-interface configuration thereby stabilizing the LM. The destruction mechanism is still being investigated.

The drug distribution in LM after dilution was also investigated and this is shown in Fig. 6. In the case of the oil phase, separation was difficult and only the percentage of NCTD in the aqueous phase was calculated to estimate the change in drug distribution after dilution.

Only about 20% of the total drug was transferred to the aqueous phase after 2 h following five-fold dilution with glucose solution, and this increased to 41% after 8 h, which meant that the drug mostly present at the interface even within 8 h of dilution. This incorporated drug was sequestered by direct contact

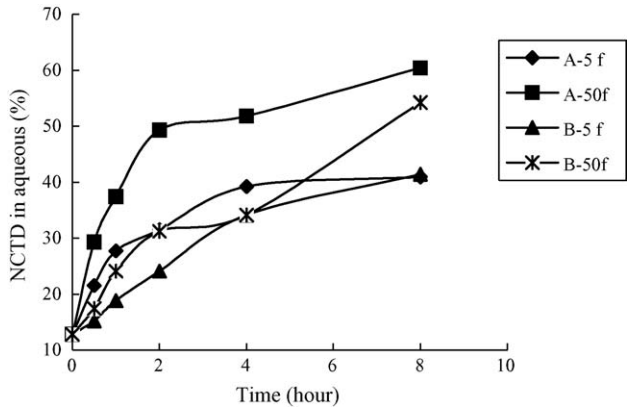

Fig. 6. Drug in aqueous phase at different times after dilution. (A) Diluted with saline solution (0.9%); (B) diluted with glucose solution (5.4%).

with body fluids and tissues and largely avoided irritation and toxic effects. Increasing the degree of dilution markedly had an adverse effect as far as allowing the drug to settle at the interface was concerned. The drug distribution results were consistent with the results of the particle size measurement, suggesting that the incorporation of the drug at the interface was due to the stability of the LM.

3.6. Short-term stability of NCTD LM

It is well known that incorporation of a drug into the interfacial layer of LM can impair their physical stability. Therefore, a short-term stability study was carried out over 6 months. The variations observed in the PCS data were within the range of normal fluctuations. A slight increase was observed for LM with 2 mg/ml NCTD, the zeta potential for all samples was in a similar range about −38 mv and about 85% of the drug was located at the interface. From this, it can only be concluded that NCTD incorporated LM (2 mg/ml) did not destroy the interfacial properties of the stabilizing surfactant layer leading to LM breakdown, at least in terms of the data collected until now.

3.7. Safety test

In order to obtain a better appreciation of the level of pain reduction due to drug delivery as lipid microspheres, an alternative animal model called the rat paw lick was investigated. The basis of this test is that the more painful the formulations, the greater the number of paw licks per animal. In addition, the number of times an animal licks its paw also increases with pain and/or irritation. Table 2 summarizes the data from the rat paw lick study. Both groups of rats were found to lick their paws. The total licking times of two groups were similar. However when the LM was injected, the animals licked their paws only 8.7 times, while the corresponding number of times in the solution group was 18.2. This test suggests that injection of LM may be only 50% as painful as injection of the solution.

Table 2
Results of the rat paw lick test

Formulation tested	Number of animals licking	Average number of times each rat licked	Average total licking time (s)
LM	100% (10/10)	8.7	31.1
Solution	100% (10/10)	18.2	35

The rabbit ear vein irritation test was used to evaluate the irritation of drug formulations following i.v. injection and the observations were compared for LM and solution. While having the advantage of being an i.v. injection, these models involved erythema, discoloration and other visible damage to the vein used for injection, rather than pain upon injection. The area around the rabbit ear vein in the group receiving LM remained normal over the study period. With the solution, the ears of all animals were flushed with blood during the administrations. Based on these observations, the LM formulation was found to be less irritating than the solution.

4. Conclusion

The NCTD-loaded LM could be prepared up to a concentration of 8 mg/ml. Over 80% of the drug was located at the surfactant interface. The composition of the surfactant and inner oil phase and the pH of the LM system affected the distribution of drug to a great extent. Short-term stability investigations showed that the LM of 2 mg/ml was stable for a period of 2 months. The technology used in this study to locate the drug at the interface was in expectation of application to drugs, which are simultaneously poorly soluble in water and oil. The results showed that the LM produced less pain and irritation. Furthermore, it is a good alternative dosage form for drugs given by the injection of solutions that cause irritation.

References

Aslihan, A., Müller, R.H., 2003. Intravenous intraconazole emulsions produced by SolEmuls technology. Eur. J. Pharm. Biopharm. 56, 29–36.

Bock,T., 1994. Emulsionen als parenterale Arzneistofftraegersysteme-Herstellung, Charakterisierung und Optimierung, Ph.D. Thesis, Christian-Albrechts-University, Kiel.

Celozzi, E., Lotti, V.J., Stapley, E.O., Miller, A.K., 1980. An animal model for assessing pain-on-injection of antibiotics. J. Pharmacol. Met. 4, 285–289.

Chambrier, C., Guriraud, M., Gibault, J.P., 1999. Medium and long-chain triacglycerols in postoperatice patients: structured lipid versus a physical mixture. Nutrition 15, 274–277.

David, D.F., 2002. The significance of particle/globule-sizing measurements in the safe use of intravenous lipid emulsions. J. Dispersion Sci. Technol. 23, 679–687.

Férézou, J., Lai, N.T., Leray, C., Hajri, T., Frey, A., Cabaret, Y., Courtieu, J., Lutton, C., Bach, A., 1994. Lipid composition and structure of commercial parenteral emulsions. Biochim. Biophys. Acta 1213, 149–158.

Groves, M.J., Wineberg, M., Brain, A.P.R., 1985. The presence of liposomal material inphosphatide stabilized emulsions. J. Dispersion Sci. Technol. 2, 237–243.

Igarashi, R., Takenaga, M., Matsuda, T., 1996. Distribution of lipid microsphere preparations. Adv. Drug. Deliv. Rev. 20, 147–154.

Komatsu, H., Kitajima, A., Okada, S., 1995. Pharmaceutical characterization of commercially available intravenous fat emulsions: estimation of average particle size, size distribution and surface potential using photon correlation spectroscopy. Chem. Pharm. Bull. 43, 1412–1415.

Mitsuko, T., 1996. Application of lipid microspheres for the treatment of cancer. Adv. Drug. Deliv. Rev. 20, 209–219.

Müller, R.H., Schmidt, S., Buttle, I., Akkar, A., Schmitt, J., Brömer, S., 2004. Solemuls-novel technology for the formulation of i.v. emulsions with poorly soluble drugs. Int. J. Pharm. 269, 293–302.

Nasirideen, S., 1998. Naproxen incorporated lipid emulsion. I. Fomulation and stability study. J. Clin. Pharm. Ther. 23, 57–65.

Teagarden, D.L., Anderson, B.D., Petre, W.J., 1988. Determination of the pH-dependent phase distribution of prostaglandin E_1 in a lipid emulsion by ultrafiltration. Pharm. Res. 5, 482–487.