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Use of ultrasound to prepare lipid emulsions of lorazepam for intravenous injection

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Abstract

Lipid emulsions can be used as a vehicle for the production of low-volume injectable preparations with minimally water-soluble active ingredients. First, we focus on the galenic and technological conditions established by ultrasound techniques. A 2^5 factorial design was used to optimize the carrier emulsion. The study then deals with the development of a parenteral emulsion formulation for lorazepam (1 mg/ml), which is compared with the highest concentration (0.05 mg/ml) achieved in the optimal aqueous diluent for lorazepam (dextrose 5% in water). The physical and chemical stability of lorazepam in the emulsion was examined for 7 months.

Keywords: Lorazepam; Parenteral emulsions; Ultrasound

1. Introduction

Lipid emulsions for intravenous injections are of the lipophilic/hydrophilic type (O/W). This allows transport in the lipid phase of active ingredients that are barely soluble in water (Coudert, 1991; Alison and Sunil, 1996).

The particle size of the droplets, which is a relevant property of a drug-carrier emulsion, must be < 1 μm and generally ranges from 100 to 500 nm. With larger particle sizes, embolism may occur (Davis et al., 1985).

Among the techniques for preparing injectable lipid emulsions, sonication enables not only complete dispersion of the active ingredient in the emulsion, homogenisation and stabilisation, but also its direct emulsification (Jill and Kirsten, 1984; Saleh and Rippie, 1994)

The study first establishes the galenic (formulation) and technological conditions (the manufacturing process variables) for optimising the production of injectable lipid emulsions intended to contain the active ingredient (lorazepam), or to be its vehicle. These emulsions are unavailable in injectable pharmaceutical form because of physical and chemical stability problems (Boullata et al., 1996), high manufacturing costs, lack of profits for industrial-scale production, and so on.

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We then focus on the development of lipid emulsions of lorazepam for intravenous injections for premedication and sedation before an operation (Yalin et al., 1997). These emulsions are easy to prepare and available for direct administration.

Lorazepam is usually administered as a solution in organic solvents such as propylene glycol. It is well-known that such formulations can cause serious side-effects, such as pain and tissue damage at the site of injection (AHFS Drug Information, 1998). However, a concentration of 1 mg/ml of lorazepam can be achieved when it is prepared in a fat emulsion, while the highest concentration (0.05 mg/ml) (Product Information, Ativan (Lorazepam), 1995; Boullata and Gelone, 1996) is achieved in the best aqueous diluent for lorazepam (dextrose 5% in water) (Micromedex Inc., 1974–1998; The Extra Pharmacopoeia Martindale, 1996). Here, we study a parenteral emulsion of lorazepam prepared by ultrasound as an alternative carrier to organic solvents or aqueous diluent (Pohlman et al., 1994; Trissel, 1994).

2. Materials and methods

2.1. Samples

The formulation consisted of purified soya oil (Karlshmann), 10 (-1) or 20 ($+1$)% (Product Information, Intralipid, 1995), as emulsifiers, purified soybean lecithin (Phospholipon 90, Nattermann Phospholipid GmbH. Rhône-Poulenc Rorer) ($+1$) (Coudert, 1991; Faulí, 1993) or Poloxamer 188 (*n*-polyethylene-*n*-polypropylene glycol) (Empilan P7068, Allbright and Wilson Group, Tenneco, Spain) (-1), 1.2 (-1) or 2.0 ($+1$)% (Ishii et al., 1990; Wade and Weller, 1994). The isotonicity of these emulsions was adjusted by 2.5 ml of glycerin (Acofarma) (Arkd et al., 1986; Benita and Levi, 1993), 0.100 g of lorazepam (Impex Química) and water for injection, q.s. 100 ml.

The numbers in parentheses represent the symbols used for each level; upper level ($+1$), lower level (-1).

2.2. Methods

For Phospholipon 90 emulsion, the oily phase and emulsifier were heated while stirring (heating plate, Agimatic E, 100°C, and magnetic stirrer at 1600 rpm). The aqueous phase (water and glycerine) was transferred into a 250 ml precipitating vessel, 12 cm tall with a base diameter of 6 cm, and heated in a water bath to 70 ± 2°C.

The oily phase was then added to the aqueous phase, and after stabilising at 70 ± 2°C, the mixture was sonicated (Branson 250 Sonifier, standard probe 12.5 mm EDP 101-147-056), with the probe placed vertically in the centre of the precipitating vessel 2 mm from the bottom (Higgins and Skauen, 1972; Skauen, 1974).

Sonication heats the samples, and this was avoided by placing the precipitating vessel in an ice bath. Sonication was carried out for 20 min at a constant 90 W, after which, in order to standardise the cooling kinetics, the sonication power was gradually reduced to 55 W at a rate of 10% per second until the sample reached 20 ± 2°C. At this point, the emulsions were filtered (level $+1$) using a cellulose acetate membrane filter 0.8 μm in diameter (Schleicher and Schuell) or left intact (-1) (Alison and Sunil, 1996), and sterilised (level $+1$) by autoclaving for 20 min at 1.2 kg/cm^2 (Microclave P Selecta) or left unsterilised (-1). The samples were conditioned in type I transparent glass vials and stored under nitrogen, and the vials were then closed with an elastomeric stopper sealed by an aluminium cap.

The Empilan P7068 samples were manufactured and conditioned similarly to the Phospholipon 90 emulsions, except that the emulsifying agent was dissolved in the aqueous phase.

An experimental design 2^5 was used to investigate the most stable carrier emulsion in a climatised chamber at 25°C for 90 days, the duration of the study, at t_0 (1 h after preparing the samples), and t_{90} (90 days after preparing the samples).

2.2.1. Particle size

The dispersed-phase droplet size was determined by means of photonic correlation spectrometry (Malvern Autosizer 2 C). The instrument also calculated the polydispersion between 0 and 1 (Haskell, 1998).

This parameter determined whether the droplet population followed a monomodal distribution (0–0.2). Otherwise, the average value did not correctly characterise the whole set of observations. The response parameter used was the dispersed-phase droplet size. As a complementary technique, each sample was examined under an optical microscope (Olympus B $\times 40$) using differential interference contrasts (filter Olympus U-POT). We thus identified large droplets in the sample and crystalline structures, and we estimated that about 97% of the oil was in emulsion particles ≤ 1 μm. Particles ≥ 5 μm were rarely noted. A Coulter or Accusizer measurement could also be a good complementary particle size analysis method.

A statistical analysis was performed by means of the Statgraphics Plus 2.1 software pack (Pérez, 1996). The analysis of variance (ANOVA) — of a droplet size 90 days after preparation — also considered (Table 1) the type of emulsifier (A), the percentage of oily phase (B), the percentage of emulsifier (C), filtration (D) and sterilisation (E). Table 1 depicts three factors with their respective level of statistical significance at $P < 0.005$: A, C and the interaction AC.

The interaction factor (Fig. 1) indicates that better results are achieved with 2% Phospholipon 90 than with the synthetic emulsifier.

The final formulation was as follows:

Purified soya oil: 20% (v/v)
Phospholipon 90: 1.2% (w/v)
Glycerine: 2.5% (v/v)
Lorazepam: 1.0% (w/v)
Water for injection q.s.: 100 ml.

In this final formulation, lorazepam (Impex Química) was dissolved in the oily phase under a laminar air flow (Singh and Ravin, 1986; Colling et al., 1990). This solution was added to the aqueous phase (filtered through 0.22 μm in diameter-Schleicher and Shuell ref: FP 030/30-) under aseptic conditions.

Autoclave sterilization causes some hydrolysis (Fig. 2a and b), resulting in the liberation of free

Table 1
ANOVA (analysis of variance) on the dispersed-phase droplet size

Factors	Sum of squares	Degrees of freedom	F	P
A. Type of emulsifier	13932.0	1	35.43	$<1\times10^{-5}$
B. Percentage oily phase	1294.13	1	3.29	0.0833
C. Emulsifying agent's concentration	1214.5	1	30.90	$<1\times10^{-5}$
D. Filtration	1400.5	1	3.56	0.0724
E. Sterilisation	1771.6	1	4.51	0.0453
BC	934.2	1	2.38	0.1375
AC	13 370.2	1	34.00	$<1\times10^{-5}$
AE	650.7	1	1.65	0.2117
DE	361.1	22	0.92	0.3483
Residual	8650.3	31		
Total	54 513.3	31		

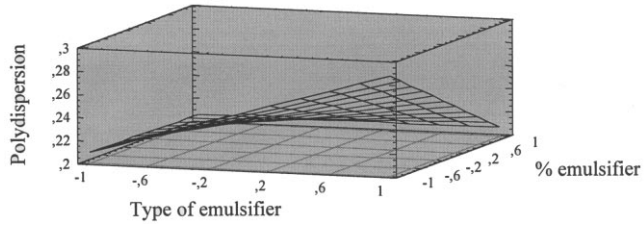

Fig. 1. Estimated response surface.

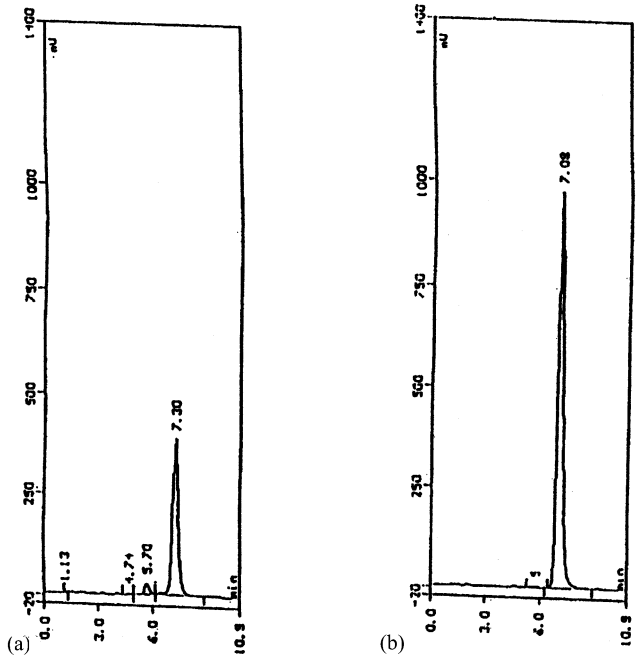

Fig. 2. (a) HPLC analysis: lorazepam emulsion after 48 h of storage in a light-resistant glass vial, temperature 8 ± 1°C and autoclaving. (b) HPLC analysis: lorazepam emulsion after 30 days of storage in a light-resistant glass vial, temperature 8 ± 1°C and unsterilised.

fatty acids and a decrease in pH (Arakane et al., 1995; Alison and Sunil, 1996).

2.2.2. HPLC analysis

The emulsion of lorazepam was analysed using a Kontron Instruments 325 System pump, a Kontron Instruments HPLC 335 detector set at 240 nm, a Kontron Instruments Autosampler HPLC 360 injector fitted with a 20 μl loop and a 250 mm × 4 mm column packed with 10 μm C_{18} Nucleosil. For the mobile phase a mixture of methanol (Promochem quality HPLC) and 0.05 M monobasic ammonium phosphate (50:50) (Panreac Química) was prepared. This was then adjusted with ammonium hydroxide to pH 6.5, filtered and degassed.

The flow rate was 2 ml/min, the retention time was 7–8 min for lorazepam and 5 min for the main degradation product, and calibration curves, 80–240 μg/ml, were constructed from linear plots of peak area versus concentration.

2.2.2.1. Standard preparation. An accurately weighed quantity of lorazepam was dissolved in methanol to obtain a solution with concentration of about 1.0 mg/ml (50 mg of lorazepam/50 ml of methanol). Four millilitres of this solution were transferred to a 25 ml volumetric flask, diluted with mobile phase to volume, and mixed to obtain a solution with a concentration of about 0.16 mg/ml.

2.2.2.2. Assay preparation. An accurately measured volume of parenteral emulsion was transferred to about 4 mg of lorazepam (4.0 ml of lorazepam emulsion), to a 25 ml volumetric flask, diluted with mobile phase to volume and mixed (Castro, 1989; US Pharmacopeia, 1995)

2.2.3. Zeta potential

The zeta potential of the lorazepam emulsions was measured in emulsion samples. A drop of sample was diluted in 20.0 ml of buffer solution Hepes pH 7.4 (1/100 M) ref: H9897. The zeta

potential of each emulsion was measured using the electrophoretic mobility, by a Zeta-meter (Malvern Instruments, UK) and calculated using the Smoluchowski equation. The droplets were charge-stabilized colloids, since they had a surface charge of -30 to -50 mV, although this can increase with time due to the production of fatty acids by phospholipid hydrolysis (Daves and Groves, 1978; Davis et al., 1985; Washington et al., 1989; Washington, 1992).

2.2.4. pH, viscosity and osmolarity

The pH of the emulsions was measured using a Crison mod. 2001 pH meter.

2.2.5. Kinematic viscosity

The rheological measurements were carried out with a capillar viscosimetre Hagenbach (Schott Geräte) equipped with a capillary tube I_c (viscosimetre constant, $k = 0.03$).

Twenty millilitres of emulsion (adjusted to 20.0 ± 0.1°C) were poured into the filling tube and transferred to the capillary tube by gentle suction. The time was recorded, in seconds, for the liquid to flow from the upper to the lower mark in the capillary tube.

2.2.6. Osmolarity

A Roebling 1313 DR Autocal osmometer was used to measure the freezing point of samples in terms of milliosmolality, or its equivalent for dilute solutions, milliosmolarity. The instrument was calibrated by using two standard solutions, water for injection and sodium chloride 300 mOsM. The ideal osmolarity was about 286 milliosmol/l (sodium chloride injection of 0.9%) (US Pharmacopeia, 1995).

2.2.7. Microscopic studies

A JSM-840 Jeol/Criotrans-ZT 1500 (Oxford Instruments) scanning electron microscope (SEM) was used. The three-dimensional appearance of the fat droplets obtained using the SEM facilitated interpretation of the results. However, lipids were extremely difficult to fix.

2.2.8. Freeze-fracturing techniques

This method requires the use of a special vacuum coater, and the preparation involves the rapid freezing of emulsions and fracturing of the frozen emulsion. The fat droplets require a layer of silver or gold to provide a conductive path between the cement surface and the substrate (Du Plessi et al., 1988; Dykstra, 1992; Hyatt and Eaton, 1993).

Fig. 3b revealed the most narrow particle size distribution versus Fig. 3a. Emulsions with an oil content of 20% showed almost spherical, individual droplets and some spherical aggregates of at least two drops. After 8 months of storage, the macroscopic appearance and consistency had not changed. Accordingly, the particle size of stored samples was similar to that of freshly prepared samples (Fig. 3a).

3. Results

Six storage conditions of lorazepam emulsions were studied: room temperature (20 ± 5°C) in a transparent glass vial and unsterilised, room temperature (20 ± 5°C) in a light-resistant glass vial and unsterilised, temperature 8 ± 1°C in a light-resistant glass vial and unsterilised, room temperature (20 ± 5°C) in a transparent glass vial and autoclaving, room temperature (20 ± 5°C) in a light-resistant glass vial and autoclaving and temperature 8 ± 1°C in a light-resistant glass vial and autoclaving (Hoey et al., 1996; Seay et al., 1997; Share et al., 1998). High-performance liquid chromatograghy (HPLC) was used to measure the lorazepam concentration after: 24, 48 and 72 h and 10 and 30 days.

The limits of lorazepam emulsions required according to USP XXIII were 90.0–110.0% (US Pharmacopeia, 1995).

Lorazepam loss was lowest when stored in a light-resistant glass container at 8 ± 1°C and unsterilised (Table 2).

3.1. Storage stability

The stability of lorazepam emulsions after manufacturing under aseptic conditions and storage in

a light-resistant glass container at 8 ± 1°C was followed by particle size analysis, pH, viscosity, osmolarity, zeta potential, assay of lorazepam and microscopic studies for 24, 48 and 72 h, 10 days and 1, 2, and 7 months (Table 3).

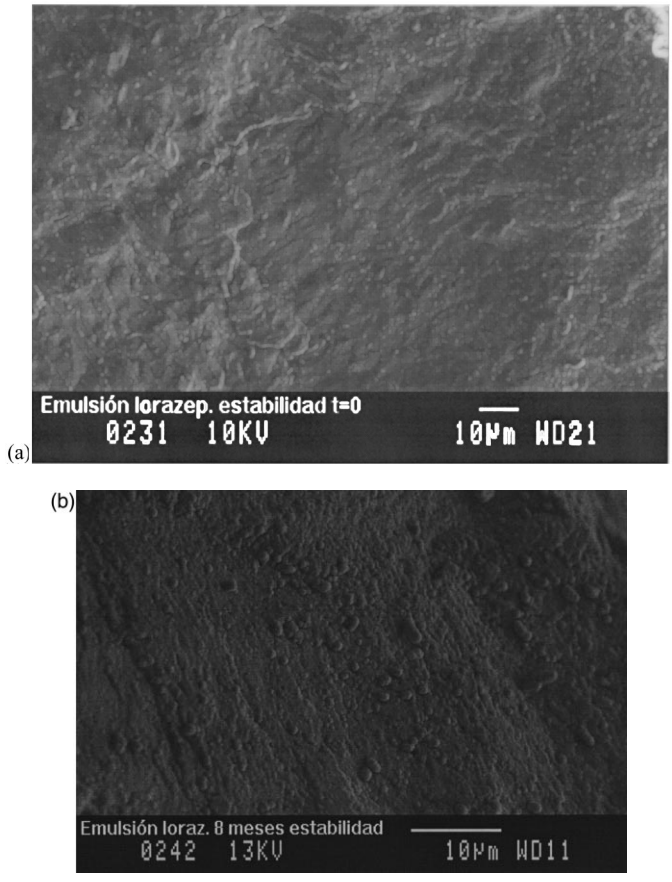

Fig. 3. (a) Scanning reflection electron microscope of freeze-fractured and replicated samples of freshly prepared lorazepam emulsions. Bar: 10 μm. (b) Scanning reflection electron microscope of freeze-fractured and replicated samples of lorazepam emulsions 8 months after preparation (long-term test). Bar: 10 μm.

Table 2
Assay of lorazepam (%)[a]

	Room temperature (20 ± 5°C)				Temperature (8 ± 1°C)	
	(a)	(b)	(c)	(d)	(e)	(f)
24 h	108.2	105.4	37.6	105.4	103.5	36.9
48 h	102.5	102.3	34.3	102.3	102.9	33.9
72 h	99.2	99.3		99.3	101.9	
10 days	97.2	95.1		95.1	96.4	
30 days	63.1	59.3		59.3	95.8	

[a] (a) Transparent glass vial, unsterilised by autoclaving. (b) Light-resistant glass vial, unsterilised. (c) Transparent glass vial and autoclaving. (d) and (e) Light-resistant glass vial, unsterilised. (f) Light- resistant glass vial and autoclaving.

Table 3
Storage stability

	Particle size (nm)	pH	Viscosity (cSt)	Osmolarity (mOsm/kg)	Zeta potential (mV)	Assay of lorazepam (%)	Aspect[a]
24 h	221.8	4.50	1.887	461	−35.9	99.8	Correct
48 h	219.8	4.49	1.887	460	−35.8	99.3	Correct
72 h	215.8	4.49	1.887	463	−36.6	99.3	Correct
10 days	218.5	4.38	1.887	445	−37.6	97.1	Correct
1 month	232.6	4.36	1.887	446	−38.5	94.9	Correct
2 months	232.4	4.34	1.887	454	−39.3	90.3	Correct
7 months	261.6	4.32	1.887	465	−43.6	72.5	Correct

[a] Microscopic studies.

4. Discussion

The use of an experimental design allows a rational approach to both pre-formulation and galenic formulation. The most favourable conditions for obtaining injectable lipid emulsions as vehicles for active ingredients administered parenterally can be established (better results are achieved with 2.0% natural emulsifier than with the synthetic emulsifier). Such products must be both sterile and physically stable, and this can be achieved by means of an emulsification–homogenisation ultrasound technique. Terminal sterilization is achieved by the maintenance of aseptic conditions due to the sensitivity of the lorazepam to high temperatures. Intravenous fat emulsions of lorazepam were stable for 7 months' storage in a light-resistant glass container at 8 ± 1°C. Significant chemical changes in the concentration of lorazepam took place 2 months after the preparation. An emulsion formulation of lorazepam (1 mg/ml) for premedication and sedation, may be preferable to the solutions currently used (0.05 mg/ml), since it reduces the volume when administered by continuous infusion; however, ultrasound would not be acceptable for commercial formulation of an intravenous emulsion, since it does not produce emulsions of a sufficiently high quality.

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