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RESEARCH PAPER

Development of a Novel Parenteral Formulation for Tetrazepam Using a Lipid Emulsion

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ABSTRACT

A novel parenteral formulation for tetrazepam (10 mg/ml) was developed using lipid emulsions. This formulation utilized a new lipid emulsion formulation, which was developed by changing the polarity of the oil phase. It was found that increasing the polarity of the oil phase resulted in enhanced solubility of tetrazepam. Tetrazepam showed higher solubility in a mixture of castor oil and middle-chain triglycerides (MCTs) (1:1) than in any other oil investigated. This mixture resulted in low interfacial tension and moderate viscosity, which seemed to be the optimum oil phase. In addition, to increase the concentration of tetrazepam, an emulsion formulation containing 30% oil phase was produced and optimized. The drug-free emulsion formulation showed fine particle sizes with an imperceptible change in physicochemical properties after more than 2 years on the shelf. As a result, it was possible to produce a parenteral emulsion formulation containing 10 mg/ml tetrazepam. No change in the physicochemical properties of the emulsion was observed after the addition of tetrazepam. The tetrazepam emulsion showed stable behavior during the autoclaving process and good shelf stability for at least 10 months as well. Tetrazepam itself also displayed good stability during the autoclaving process and also showed good shelf stability in this emulsion formulation.

Key Words: *Autoclaving and long-term stability; Lipid emulsion; Parenteral application; Relative oil polarity; Tetrazepam*

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INTRODUCTION

Tetrazepam is a benzodiazepine with skeletal muscle, sedative, and anticonvulsant properties (1). It is practically insoluble in water (~0.005 mg/ml). Besides the hydrolytic degradation, which is well known for benzodiazepines (2), it is also susceptible to autoxidation (3). In addition to its very low solubility and critical stability, it has a high therapeutic dose of more than 20 mg (4). This explains why there is no parenteral formulation for tetrazepam available on the market currently. Overcoming the solubility and stability problems to achieve a suitable formulation for parenteral application is a real challenge (2,4).

This parenteral (intravenous) application is required for emergency administration. Therefore, many attempts have been made to formulate tetrazepam in a suitable parenteral formulation. As an example of this, as mixed micelles (MM) are suitable as a drug carrier for parenteral administration, they have already been used to enhance the solubility and the stability of tetrazepam (5). It was found that tetrazepam solubility could be increased in the best MM formulation up to about 1.7 mg/ml (5% MM). This formulation, however, showed nonacceptable degradation (7%) after 1 year (6). Furthermore, the solubility was still too low relative to the acceptable therapeutic dose; therefore, a large volume had to be administered, which is not acceptable for parenteral application.

The effectiveness of lipid emulsions as drug delivery systems has also been reported in the literature (7–9). Lipid emulsions seem to be suitable carriers for lipophilic drugs if the solubility in the lipid phase is sufficient and the stability of the drugs in the emulsion form is adequate (10). For this reason, lipid emulsions have been investigated as carriers in tetrazepam formulations.

A conventional emulsion formulation using soybean oil or middle-chain triglycerides (MCT), alone or in mixture, has been produced. Using the best formulation, which contained only MCT as the oil phase, a 5 mg/ml concentration of the drug was achieved. This concentration does not suit the therapeutic dose of tetrazepam, and a large amount of this emulsion formulation must be administered. Despite the low solubility, it was observed that the degradation of the drug after 1 year at room temperature was insignificant. This indicates that the drug was quite stable in the emulsion formulation.

The objective of this study was first to investigate the polarity of different oils to determine the solubility of tetrazepam in these different components. Following this, the oil phase and/or oil phase mixture with the best solubilizing efficacy was used to produce a new emulsion formulation. In addition, increasing the oil phase from 20% to 30% could also promote the amount of the liposoluble drug that could be incorporated in the emulsion dosage form, and the injected volume could subsequently be decreased.

EXPERIMENTAL

Materials

Tetrazepam was purchased from Profarmco (Milan, Italy). Lipoid S75 (S75) was obtained from Lipoid GmbH (Ludwigshafen, Germany) and contained a minimum of 70% phosphatidylcholine, 10% phosphatidylethanolamine, and 1.7% lysophosphatidylcholine. Purified castor oil, sesame oil, almond oil, linseed oil, and soybean oil were purchased from Henry Lamotte (Bremen, Germany). Oleic acid was obtained from Merck (Darmstadt, Germany). MCT (Miglyol 812) was supplied by Hüls (Witten/Ruhr, Germany). KH_2PO_4 and H_3PO_4 and sorbitol were provided by Merck and were reagent grade. The cosurfactants used in the polarity estimation (C_4OH, C_4E_2OH, and C_4E_3OH [*n*-butanol, diethylenglycol-monobutylether, and triethylenglycol-monobutylether, respectively]) were obtained from Fulka Chemicals (Switzerland). Double-distilled water was used for all preparations. All other chemicals were reagent grade or higher.

Methods

Estimation of the Relative Polarity

The relative polarity of different oils has been estimated using the method of Kahlweit et al. (11). This method is based on the measurement of the partition of different surfactants between the aqueous phase and the oil phase at 25°C. *n*-Butanol (**A**), diethylenglycol-monobutylether (**B**), and triethylenglycol-monobutylether (**C**) are the surfactants of choice. The surfactant amount (25% w/w) was added to a 1:1 oil:water mixture. After 24 h equilibrium at 25°C, the volumes of the resulting phases were estimated. All investigated oils had a lower density than water. The results were described

as first, the system with two phases (2) and second, the system with three phases (3) if the surfactants were not miscible with both phases. If the volume of the upper phase is larger than that of the lower phase, then a dash is put above the number. When the phases have the same volume, the dash is put in the middle ($\bar{2}$ or $\bar{3}$). If the dash is under the number, this indicates that the lower phase has a larger volume ($\underline{2}$ or $\underline{3}$). For more details, the basic method is described in the paper by Kahlweit and coworkers (11).

Preparation of the Emulsions

Typical oil-in-water (o/w) emulsions were prepared as previously described (12) using an oil phase mixture of castor oil with MCTs (1:1). In this oil mixture, the dug was completely dissolved. Briefly, the phospholipids (1.5%) were dissolved in the oil phase. The oil mixture and the aqueous phase (5% aqueous solution of sorbitol to enable adjustment to isotonicity) were heated separately to about 50°C–55°C. The two phases were then mixed together and preemulsified using an Ultra-Turrax T25 (Janke and Kunkel, Staufen, Germany). The coarse emulsions were homogenized using a high-pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany). The homogenizer was equipped with a temperature control unit to apply high temperatures during the homogenization process and to control the viscosity of the system. The homogenization was performed at a temperature of 40°C. The temperature of the unit was checked at appropriate time intervals. Next, the pH of the resulting emulsions was adjusted to about 8 using 0.1 N sodium hydroxide solution (because lipid emulsions are only stable at pH values higher than 7.5), and the emulsions filled 15-ml vials. The vials were sealed, and the emulsions were sterilized by autoclaving (K15T, Keller, Weinheim, Germany) at 121°C for 15 min.

Emulsion Characterization

The mean diameter of the bulk population was determined by photon correlation spectroscopy (PCS) covering the size range 5 nm to approximately 3 μm (Malvern spectrometer RR 102, Malvern, UK, with helium-neon laser [$\lambda =$ 632.8 nm], Siemens, Germany). The width of the size distribution was expressed as the polydispersity index (PI). The PI is zero for monodisperse particles, whereas parenteral fat emulsions are typically in the range 0.10 to 0.20 (13). For size analysis, approximately 1 μl fat emulsion was added to 1 ml distilled water. The dilution depended on the optimum scattering intensity. Each emulsion sample was analyzed twice, and for each diluted system, 10 size determinations were made.

Larger particles were detected by laser diffraction analyzer (LDA) (Helos, Sympatec, Clausthal-Zellerfeld, Germany) at a focal length of 20 mm, corresponding to a measurement range of 0.18–35 μm. The emulsions were characterized by D_{max} and the D_{50} quantiles of the volumetric distribution (which means that 50% or all of the particles were below the given size).

An Ubbelohde capillary viscosimeter (Schott, Hofheim, Germany) measured the viscosity of the oil phases.

High-Performance Liquid Chromatographic Analysis

The high-performance liquid chromatographic (HPLC) analysis of tetrazepam was performed as described by Hammad et al. (5). The instrument consisted of an RP-18 (250×4.6 mm) 5-μm column (Schambeck SFD, Bad Honnef, Germany); Gynkotek 300C high-precision pump (Gynkotek, Munich, Germany); Kontron 360 autosampler (Kontron Instruments, Munich, Germany); Kontron 742 ultraviolet (UV) detector (Kontron Instruments); and Shimadzu C-R6A chromatopac integrator (Shimadzu, Kyoto, Japan). The mobile phase consisted of a mixture of 0.01 M aqueous solution of KH_2PO_4 (adjusted to pH 4.2 with H_3PO_4) and acetonitrile (40:60 v/v); the flow rate was 2 ml/min, and the detection was at a wavelength of 254 nm. No interference of the degradation product peaks with the peak of tetrazepam was observed.

Solubility Determination

For determination of the saturation solubility of tetrazepam in different oils, excess amounts of tetrazepam were added to the different oils in bottles (in duplicate). The bottles were then tightly closed under N_2 and shaken in a thermostated shaking water bath at 25°C until equilibrium was reached (24 to 48 h). The excess amounts of tetrazepam were separated by filtration (0.2-μm cellulose acetate filter). The first milliliters of the filtrate were

rejected to avoid problems arising from adsorption on the filter. Some samples were also subjected to centrifugation. We dissolved 100 milligram of the filtrate in dichloromethane-acetonitrile (20:80 v/v). This was further diluted with acetonitrile and then subjected to HPLC (in triplicate). The calibration curve was linear in a range from 1 to 10 μg/ml (r^2 not less than 0.998). No significant difference was observed between the solubility in the filtered and centrifuged samples.

RESULTS AND DISCUSSION

Relative Polarity of Different Oils and Solubility of Tetrazepam

As shown in Table 1, oleic acid displayed the highest polarity among the investigated oils. Oleic acid was followed by castor oil, which has a relatively high polarity. Next was MCT, which was followed by soybean oil, which had the lowest value among the oils.

The saturation solubility of tetrazepam was investigated in these oils, as single oils or in mixtures. Two problems were faced during this trial. In oleic acid, the drug showed high solubility, but a rapid change in the drug concentration and in the oil color was observed after a short time. This indicates that oleic oil interacted with tetrazepam, resulting in drug degradation. Such interaction between the oleic oil and the dissolved drug has been reported previously (14).

Because of the high viscosity of castor oil, it was very difficult to determine the saturated solubility of tetrazepam. Increasing the temperature to reduce the viscosity will affect the real saturated solubility. This will affect the long-term stability since recrystallization will occur. However, in a previous paper, it was reported that emulsions prepared with castor oil (as a single oil phase) did not show stable behavior in the long term (12,15). Therefore, it was not investigated extensively. In addition, it was also reported that mixing castor oil with MCT led to a novel oil phase with superior properties (16). As shown in Fig. 1, mixing castor oil with MCT led to a decrease in the viscosity of castor oil and, simultaneously, to a decrease in the interfacial tension. A 1:1 mixture of MCT:castor oil seemed to be the optimal mixture. This could be further utilized to enhance the stability of the emulsions.

Table 1

Relative Polarities of Various Investigated Oils

	Cosurfactants		
Oil	A	B	C
Oleic acid	2	2	2
Castor oil	2	2	~~2~~
MCT	2	2	2
Linseed oil	~~2~~	~~2~~	2
Sesame oil	2	2	~~2~~
Almond oil	2	2	2
Soybean oil	3	2	2

MCT, middle-chain triglycerides.

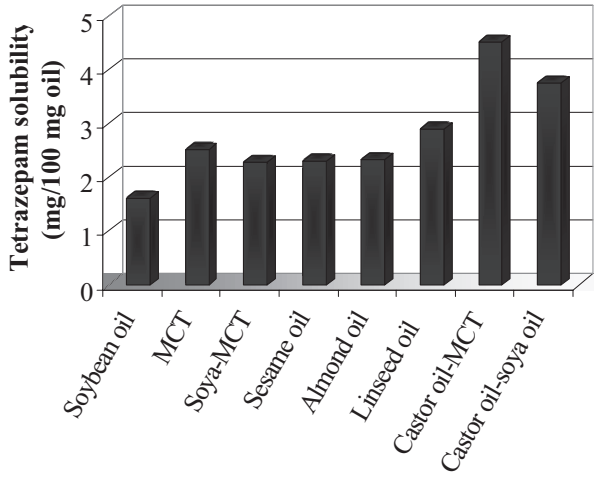

Figure 1. Solubility of tetrazepam in various oils (mg tetrazepam/100 mg oil).

As illustrated in Fig. 1, the highest solubility of tetrazepam was recorded in the oil phase mixtures containing castor oil. However, MCT:castor oil mixture (1:1) displayed higher solubility than the soybean oil:castor oil mixture (1:1). This was because MCT showed a moderate solubility value, whereas soybean oil displayed the lowest value among all the investigated oils. Linseed oil also showed good solubility behavior, but it was avoided because its viscosity was much higher than the viscosity of MCT. Consequently, mixing linseed oil with castor oil did not effectively reduce castor oil viscosity. Producing emulsions using oil phases with high viscosities led to problems during production and of long-term stability (16).

Figure 2 shows the influence of increasing amounts of MCT on surface tension and viscosity of castor oil. Blending castor oil with MCT lowers the viscosity of the first and reduces the interfacial tension of the second. Based on these results, the oil phase of choice was a mixture of MCT:castor oil (1:1) since it showed good solubility, low viscosity, and low interfacial tension.

Production of Emulsion Formulation with 30% Oil Phase

To increase the incorporated amount of tetrazepam, an attempt was made to increase the oil phase from 20% to 30%. The 1:1 oil phase mixture of MCT:castor oil was included in this trial. The concentration of the phospholipids (1.5%) was the same as used in the 20% commercial emulsions (17). The aim was to avoid any further increase in the emulsion viscosity, which will limit the use of the emulsion in parenteral applications (18,19).

The production parameters were first optimized to obtain an emulsion formulation with adequate physicochemical properties for parenteral application (20). Increasing the homogenization pressure and the homogenization cycles led to a significant reduction in the particle sizes. Homogenization pressure between 250 and 300 bar and 8 cycles seem to be the optimum conditions (Fig. 3) because PCS, as well as LDA, showed a minimum in droplet size. Emulsions produced with these conditions displayed mean particle sizes around 140 nm, and all particles were smaller than 1.8 μm. Moreover, these emulsions showed a polydispersity index lower than 0.15, which indicates a narrow particle size distribution (13). The emulsions also showed viscosity values lower than 3.9 mPa · s, which is acceptable for parenteral application.

The stability of this new formulation was investigated after the autoclaving process and after being on the shelf. No changes in the particle size were observed and detected after the application of the autoclaving process (Table 2). In addition, the formulation displayed excellent stability after being on the shelf for more than 2 years. This emulsion showed no visible deterioration and also no changes in its physicochemical properties after 2 years (10,12). This excellent stability could be correlated with the decrease in the interfacial tension offered by castor oil as a component of the oil phase even though the phase ratio was increased to 30% without increasing the emulsifier concentration (21). This behavior of castor oil might be due to its high amount of free fatty acids, which can act as a cosurfactant (22). Thus, the 30% emulsion formulation showed adequate physicochemical properties

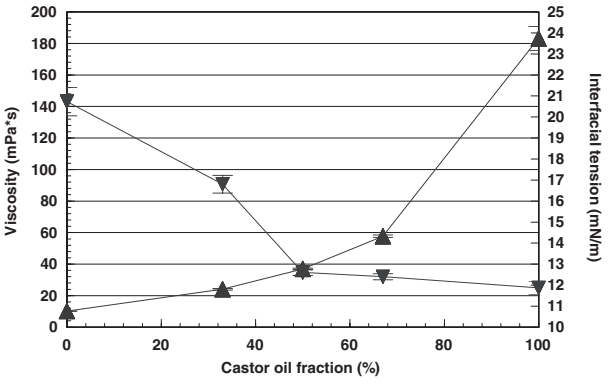

Figure 2. Viscosity *s* and interfacial tension *t* of different castor oil:MCT ratios as a function of castor oil fraction.

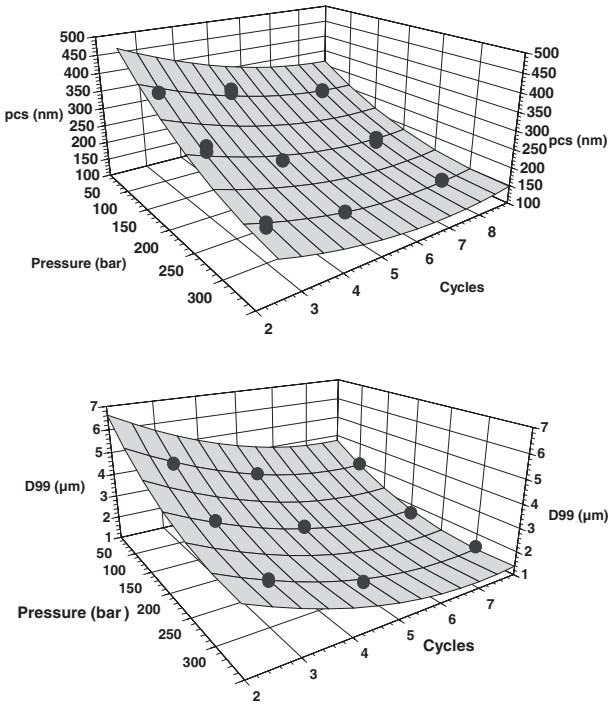

Figure 3. Influence of homogenization conditions on the physicochemical properties of the produced emulsions (mean particle size obtained from PCS [nm] and D99 [μm] obtained from LDA).

and sufficient stability to be used for parenteral administration.

Production of Emulsion Containing 10 mg/ml Tetrazepam

An important aim of the current work was to develop a suitable emulsion formulation for tetrazepam for parenteral application. This formulation had to show sufficient solubilizing efficacy for tetrazepam and also possess satisfactory physicochemical properties. Using the 30% emulsion, a 10-mg/ml formulation was achieved. The drug was dissolved in the oil phase, and the emulsion was produced as explained above. Regarding the physicochemical properties, no obvious changes in the emulsion particle sizes were recorded after incorporation of tetrazepam into the emulsions (Table 3). Moreover, the tetrazepam emulsion in turn showed excellent stability after the autoclaving process (like the drug-free formulation) as no changes in its particle sizes were observed. Furthermore, this emulsion also displayed adequate long-term stability on the shelf. As shown in Table 3, the particle size distribution of this emulsion was constant for more than 9 months at room temperature.

However, not only is the stability of the emulsion important, but also the stability of the drug itself (23). For this reason, the stability of tetrazepam in the emulsion formulation was investigated. Based on the data obtained from HPLC, no loss of tetrazepam and no appearance of degradation products were detected after the application of the stress autoclaving process (Table 4). In addition, negligible degradation was observed in tetrazepam emulsion. More than 97% of the drug was still detected after 10 months of storage. The addition of antioxidant to the emulsion formulation did not have a significant effect on the drug stability. This showed that tetrazepam solubility and stability were clearly enhanced using this new lipid emulsion formulation. The enhancement in the stability could be related to

Table 2

Long-Term Stability of the 30% Emulsions Containing an Oil Phase Mixture of MCT–Castor Oil 1:1

Months	PCS D_{99} Diameter (μm) of Castor Oil–MCT Emulsion	LDA Mean Diameter (nm) of Castor Oil–MCT Emulsion
After production	1.43 ± 0.01	144 ± 3.5
After autoclaving	1.44 ± 0.02	148 ± 3.5
1	1.43 ± 0.02	143 ± 2.8
2	1.42 ± 0.02	144 ± 4.5
4	1.45 ± 0.01	152 ± 3.9
6	1.41 ± 0.02	148 ± 2.7
10	1.42 ± 0.01	144 ± 4.7
14	1.46 ± 0.02	149 ± 3.3
18	1.44 ± 0.02	145 ± 4.1
24	1.45 ± 0.01	150 ± 2.4
30	1.46 ± 0.02	155 ± 3.2

D99 diameter means 99% of the detected droplets are below the stated value.
LDA, laser diffraction analyzer; MCT, middle-chain triglycerides; PCS, photon correlation spectroscopy.

Table 3

Physicochemical Properties of Lipid Emulsions After the Incorporation of 10 mg/ml Tetrazepam and After Autoclaving and Storage at 25°C

Emulsion	PCS Mean Size (nm)	LDA D_{50} (μm)	LDA D_{99} (μm)	LDA D_{max} (μm)
1	147	0.62	1.45	1.8
2	146	0.62	1.45	1.8
3	144	0.61	1.43	1.8
Maximum deviation	6	0.01	0.03	0
After autoclaving	149	0.63	1.45	1.8
After 10 months	152	0.62	1.46	1.8
Maximum deviation	8	0.01	0.01	0

LDA diameters of D50, D99, and D_{max} mean 50%, 99%, and 100%, respectively of the detected droplets are below the stated value.
LDA, laser diffraction analyzer; PCS, photon correlation spectroscopy.

Table 4

Stability of Tetrazepam During the Autoclaving (AC) Process and After 10 Months at Room Temperature (With and Without Ascorbic Acid as Antioxidant)

Emulsion	Before AC (%)	After AC (%)	10 Months (t_{90}) (%)
Clear bottle[a]	102	100	>97
Amber bottle[a]	102	101	>96
Clear bottle [b]	101	102	>96
Amber bottle[b]	101	101	>98

[a]Without antioxidant.
[b]With antioxidant.

the presence of tetrazepam in the oily phase; consequently, the hydrolysis of tetrazepam was minimized (24,25). Moreover, the oxidative reaction was also minimized since the bottles were sealed under nitrogen and because the oxygen amount in the oil phase was very limited (2,5).

CONCLUSION

A new emulsion formulation (30% oil phase) using a mixed oil phase (castor oil:MCT 1:1) was achieved. This formulation showed excellent shelf stability for more than 2 years.

Using this new formulation, a novel stable parenteral emulsion with 10 mg/ml tetrazepam was developed. The emulsion and the tetrazepam showed stable behavior on the shelf after 9 months. The protection against degradation offered by lipid emulsion is caused by the fact that tetrazepam is mostly encapsulated in the droplets of the oil phase.

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