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Formulating and stability of benzodiazepines in a new lipid emulsion formulation

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The objective of the current study was to evaluate the novelty of a new lipid emulsion formulation containing 30% oil phase as a drug delivery system. Therefore different benzodiazepines (BZs), namely diazepam, tetrazepam, clonazepam and lorazepam, were incorporated into this emulsion formulation. This lipid emulsion formulation showed enhanced solubilization capacity as 10 mg/ml, 10 mg/ml, 0.9 mg/ml, and 1.8 mg/ml formulations for diazepam, tetrazepam, clonazepam, and lorazepam were achieved, respectively. Incorporating the drugs into the lipid emulsion did not alter its physicochemical properties. Also the free and the drug emulsion formulations displayed good physical stability after autoclaving and after around one year of storage at shelf, as no changes in the physicochemical properties were observed. Most drugs also showed stable behavior after autoclaving and after ~ 1 year of storage at shelf. The only exception was lorazepam, as only around 50% of the drug was still intact after autoclaving.

1. Introduction

Emergency cases require an immediate action of drugs. In order to achieve this goal, the drugs should be administered intravenously and usually in a soluble form [1, 2]. The first step is to solubilize the drug in a sufficient concentration and in a suitable formulation, which should conform to the requirements for parenteral administration [3, 4]. Therefore many attempts have been made to meet these challenges. For example, co-solvents, micelles, mixed micelles, liposomes, cyclodextrins and lipid emulsion were usually used to enhance the solubility and the stability of the drug [3–9].

Co-solvent toxicity and drug precipitation while diluting with blood result usually in unpleasant side effects. Liposomes, cyclodextrins, micelles and mixed micelles are not always successfully applicable because of either solubility or stability issues, as in the case of benzodiazepines [3, 10–12].

Lipid emulsions have been suggested to solubilize and stabilize drugs with critical stability/solubility properties [13, 14]. Lipid emulsions showed reduced side effects compared to the other systems. For example, diazepam was formulated in a lipid emulsion which showed significantly reduced side effects compared to similar formulations prepared with mixed micelles and co-solvents [15]. However, the conventional lipid emulsion formulation, which usually contains 10 or 20% oil phase consisting of either soybean oil or middle chain triglycerides alone or in mixture (1 : 1), sometimes showed insufficient capacity to solubilize the drugs as well [3]. Consequently, this limits the use of lipid emulsions as a drug delivery system. For example, the conventional lipid emulsion did not sufficiently solubilize some benzodiazepines (e.g. tetrazepam, lorazepam, clonazepam) to achieve the therapeutic dose required [3].

Currently, a new lipid emulsion formulation has been developed with a 30% oil phase consisting of a 1 : 1 mixture of castor oil with middle chain triglycerides [16, 17].

The objective of this study was to evaluate the potentiality of this new lipid emulsion formulation as a drug delivery system. Some benzodiazepines, namely diazepam, tetrazepam, lorazepam and clonazepam, were incorporated into this emulsion formulation, as the conventional lipid emulsion did not show sufficient capacity to solubilize most of these drugs.

2. Investigations, results and discussion

2.1. Production of emulsion formulation with 30% oil phase

The 30% emulsion was produced as follows; a 1 : 1 oil phase mixture of MCT-castor oil was used in this trial. The concentration of the phospholipids (1.5%) was kept as in the 20% commercial emulsions in order to avoid any further increase in the emulsion viscosity, which will limit the parenteral application [19, 20].

The production parameters were first optimized in order to obtain an emulsion formulation with adequate physicochemical properties for parenteral application [16, 17]. As shown in the Fig., increasing the homogenization pressure

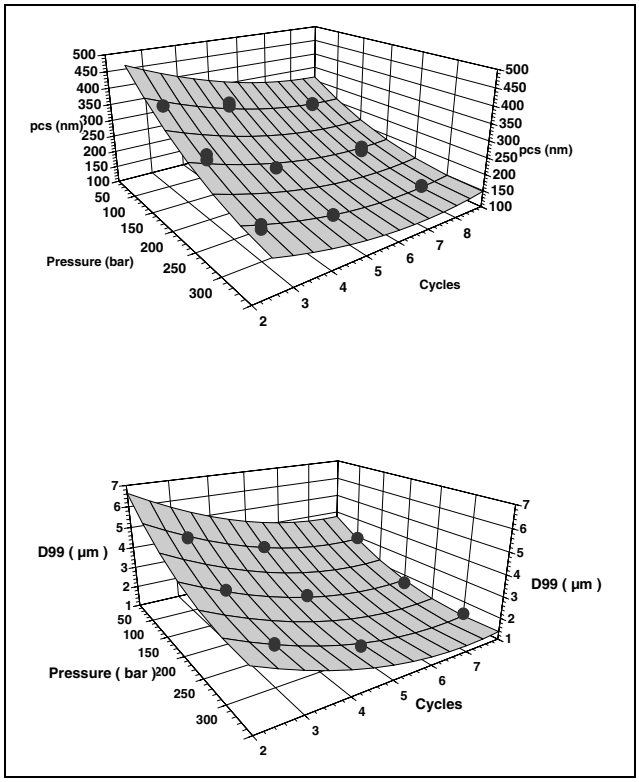


Fig.: Influence of homogenisation conditions on the physicochemical properties of the produced emulsions [mean particle size obtained from PCS (nm) and D99 (µm) obtained from LDA]

Table 1: Long term stability of the 30% emulsions containing an oil phase mixture (MCT-castor oil 1 : 1)

Months	D99 (μm) of castor oil-MCT emulsion	MPS (nm) of castor oil-MCT emulsion
After production	1.43 ± 0.01	144 ± 3.5
After autoclaving	1.44 ± 0.02	148 ± 3.5
6	1.41 ± 0.02	148 ± 2.7
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

and the homogenization cycles lead to significant reduction in the particle sizes (homogenization pressure between 250–300 bar and 8 cycles seem to be the optimum conditions), as all particles are smaller than 1.8 μm. The emulsions produced under these conditions also displayed fine mean particle sizes (around 140 nm) with a low polydispersity (>0.15) indicating a narrow particle size distribution [20]. The emulsions also showed viscosity values lower than 3.9 mPa · s, which is acceptable for parenteral application. More details are explained in our previous papers, in which the development of the 30% emulsion was sufficiently described [16, 17].

The new emulsion formulation displayed excellent stability after autoclaving and on shelf for more than two years (Table 1), as no visible deterioration and also no changes in its physicochemical properties were observed. This excellent stability in spite of increasing the oil ratio (30%) can mostly be attributed to the decrease in the interfacial tension offered by castor oil as a component of the oil phase [21]. This behavior of castor oil might be due to its high amount of the free fatty acid, which can act as co-surfactant [22].

By virtue of the above results, this 30% emulsion formulation showed adequate physico-chemical properties and sufficient stability to be administered parenterally.

2.2. Solubility of BZs in the 30% emulsion formulation

The new lipid emulsion with 30% oil phase significantly enhanced the solubility of the poorly water soluble BZs. As illustrated in Table 2, the solubility of different BZs was enhanced two to three fold compared to the conventional lipid emulsion formulation containing 20% oil phase consisting of soybean oil-MCT (1 : 1), as reported by Bock et al. [3]. Consequently the lack of the solubility of BZs in the conventional lipid emulsion formulation, which has limited the use of lipid emulsions as drug delivery systems for BZs can be overcome. As a result, the required therapeutic dose for parenteral application can be achieved, which allows to deliver the BZs for emergency cases [4]. This enhancement in the solubility can be attributed to the increase in the oil phase ratio and also to the increase in the polarity of the oil phase as a result of using castor oil as a part of the oil phase 23.

Table 2: Solubility of BZs in the developed 30% emulsion compared to some other systems

Drug	Water (mg/ml)	MM (mg/ml)	CE (mg/ml)	NE (mg/Ml)
Tetrazepam	~0.005	1.6*	5	10**
Clonazepam	~0.006	0.45*	0.25	0.9
Lorazepam	~0.048	2.8*	0.5	1.8

* 10% (w/w) mixed micelle concentration
** 10 mg/ml Diazepam was also formulated
MM: mixed micelles, CE: conventional emulsion, NE: new emulsion

Table 3: Physical stability of emulsions containing different BZs after autoclaving

Drug	PCS (nm)		D_{50} (μm)		D_{99} (μm)		D_{max} (μm)	
	A	B	A	B	A	B	A	B
Free	144	148	0.59	0.6	1.43	1.44	1.8	1.8
Diazepam	149	155	0.6	0.62	1.46	1.48	1.8	1.8
Tetrazepam	147	152	0.61	0.60	1.46	1.47	1.8	1.8
Clonazepam	146	150	0.61	0.61	1.47	1.48	1.8	1.8
Lorazepam	148	151	0.61	0.62	1.48	1.46	1.8	1.8

also, no changes were observed after 10 months of storage at shelf temperature

2.3. Physicochemical properties of the emulsions with BZs

The incorporation of drugs into the lipid emulsions may affect their physicochemical properties. The incorporated drugs can affect the pH-value of the emulsion formulation or can directly interact with the emulsifier resulting in a less stable formulation [24, 25]. However, the incorporation of the different BZs into the emulsion did not alter the physicochemical properties of the produced emulsions (Table 3). In addition, all emulsion formulations displayed good physical stability after autoclaving as no difference was observed between the drug and the drug-free emulsions (Table 3). These emulsions also had good shelf stability, as no changes in their particles were observed over more than 10 months of storage. Hence, physically stable emulsions were achievable for the different BZs using the new lipid emulsion formulation.

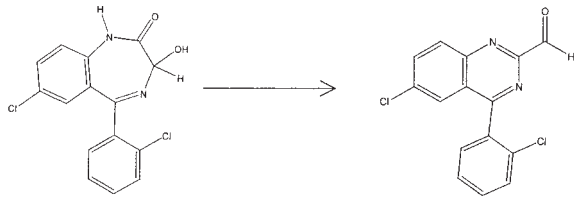
2.4. Chemical stability of BZs in the new lipid emulsion formulation

Not only the physicochemical stability of the lipid emulsions is important but also the chemical stability of the incorporated drugs per se is crucial, as the lipid formulation should be autoclaved and then stored for at least two years. Hence the incorporated drugs should display adequate chemical stability after autoclaving and at shelf. Therefore the chemical stability of BZs in the lipid formulation was followed immediately after autoclaving and at shelf (for more than 10 months). As is shown in Table 4, most BZs displayed good stability after autoclaving, as no obvious change in the amount of the intact drug (also no new degradation peaks were observed in the HPLC chromatogram). The only exception was lorazepam, as only ~50% were still intact after autoclaving. This rapid degradation after autoclaving might mostly be attributed to the loss of water molecules from the parent compound (Scheme). This degradation pathway was studied and reported for lorazepam [26]. The long-term stability of lorazepam was not further followed, as the drug did not show sufficient stability after autoclaving,

Table 4: Stability of BZs after the autoclaving process

Drug	Intact concentration before autoclaving (%)	Intact concentration after autoclaving (%)
Diazepam	102	100
Tetrazepam	104	101
Clonazepam	102	102
Lorazepam	101	47.5

Scheme



Lorazepam

Hydrolytic degradant

(6-Chloro-4-(O-chlorphenyl)-2-quinazolincarboxaaldehyde)

which is required for lipid emulsions to obtain sterile product [27].

Regarding the other BZs with good stability after autoclaving, the long-term stability was further investigated up to 10 months of storage at ambient temperature, which is usually the practical storage temperature. These BZs (diazepam, clonazepam, and tetrazepam) displayed also good stability after 10 months of storage. As shown in Table 5, almost no change was observed in the intact drug after 10 months regardless of the bottle type used (amber or white). Additionally, no new peaks (degradation peaks) were observed in the HPLC chromatogram. Hence this negligible change in the intact drug may be considered as an analytical deviation.

In conclusion the increase in solubility and stability rendered by this novel lipid emulsion (with 30% oil phase) appears very promising for the delivery of BZs.

The BZs emulsions displayed good physicochemical properties, as no changes were observed after autoclaving and after 10 months of storage. Also, most of BZs showed good stability after autoclaving and after 10 months of storage. The only exception was lorazepam, as a significant degradation was observed after autoclaving.

The solubility enhancement could be attributed to the change in the polarity and the ratio of the oil phase, where the stabilizing effect may be attributed to the encapsulation of the drugs in the oil droplets, which can in most cases protect the drugs from hydrolysis and oxidation.

3. Experimental

3.1. Materials

Tetrazepam, diazepam, clonazepam, and lorazepam were 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% lysophophatidylcholine. Purified castor oil was obtained from Henry Lamotte (Bremen, Germany). Medium chain triglycerides (MCT, Miglyol 812) were supplied by Hüls (Witten/Ruhr, Germany). KH_2PO_4 and H_3PO_4 and sorbitol were furnished from Merck (Darmstadt, Germany) and were of reagent grade. All chemicals were used as received. Double distilled water was used for all preparations. All other chemicals were of reagent grade or higher.

Table 5: Stability of BZs after 10 months of storage under ambient conditions

Drug	Intact drug After AC (%)	Intact drug After 10 m (%)*	Intact drug After 10 m (%)**
Diazepam	100	100	99
Tetrazepam	101	98	98
Clonazepam	102	98	99
Lorazepam	42	ND	ND

* amber glass, ** white glass

3.2. Methods

3.2.1. Preparation of the emulsions

Typical oil-in-water (o/w) emulsions were prepared, as previously described [16], using an oil phase mixture of castor oil with medium-chain triglycerides MCT (1 : 1). 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 50–55 °C. The two phases were then mixed and preemulsified with an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). The coarse emulsions were homogenized with a high-pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany). The homogenizer was equipped with a temperature control unit in order 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. Subsequently, the pH of the resulting emulsions was adjusted to about 8 using 0.1 N sodium hydroxide solution (because lipid emulsions are only stable in pH value higher than 7.5) and the emulsions were filled into 15 ml vials. The vials were sealed and the emulsions were sterilized by autoclaving (K15T, Keller, Weinheim, Germany) at 121 °C for 15 min.

3.2.2. 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, U.K, with Helium- Neon laser $\lambda = 632.8$ nm, Siemens, Germany). The width of the size distribution was expressed as polydispersity index (PI). The PI is zero for monodisperse particles, whereas parenteral fat emulsions are typically in the range 0.10 to 0.20 [20]. For size analysis approximately 1 μl fat emulsion was added to 1 ml of 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 Analysis 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 (that means 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.

3.2.3. HPLC analysis

The HPLC analysis of benzodiazepines was performed as described by Hammad et al. [4]. The instrument consisted of a RP-18 (250 × 4.6 mm) 5 μm column (Schambeck SFD, BAD Honnef, Germany), high-precision pump Gynkotek; 300 °C (Gynkotek, München, Germany), autosampler Kontron 360 (Kontron Instruments, München, Germany), UV detector Kontron 742 (Kontron Instrument) and integrator Shimadzu C-R6A Chromatopac (Shimadzu, Kyoto, Japan). The analysis parameters for diazepam were: mobile phase acetonitrile- water (60 : 40 v/v), flow-rate 1 ml/min and wavelength 254 nm, for tetrazepam: mobile phase acetonitrile-0.01 M KH_2PO_4 (adjusted to pH 4.2 with H_3PO_4) (60 : 40 v/v), flow-rate 2 ml/min and wavelength 254 nm, for lorazepam: mobile phase acetonitrile-water-acetic acid (55 : 45 : 1 v/v/v), flow-rate 1 ml/min and wavelength 254 nm, for clonazepam: mobile phase acetonitrile-water (45/55 v/v), flow-rate 1 ml/min and wavelength 310 nm. No interference of the degradation product peaks with the peak of the parent drugs was observed. The calibration curves were linear in a range from 1 to 10 μg/ml (r^2 not less than 0.998).

3.2.4. Solubility determination

For determination of the saturated solubility, excess amounts of the drugs were added to the oil phase (a 1 : 1 mixture of castor oil and middle chain triglycerides MCT) in bottles (in duplicate). The bottles were then tightly closed under nitrogen and shaken in a thermostated shaking water bath at 25 °C until equilibrium was reached (24 to 48 h). The excess amounts of the drugs were separated by filtration (0.2 μm cellulose acetate filter). The first milliliters of the filtrate were rejected to avoid problems arising from adsorption to the filter. Some samples were also subjected to centrifugation. 100 mg of the filtrate was dissolved in dichlomethan-acetonitrile (20 : 80 v/v). This was further diluted with acetonitrile and then subjected to HPLC (in triplicate). No significant difference was observed between the solubility in the filtered and centrifuged samples. The solubility of the different drugs were significantly enhanced using the developed 30% emulsion, as emulsion formulations with 10 mg/ml, 10 mg/ml, 0.9 mg/ml, and 1.8 mg/ml for diazepam, tetrazepam, clonazepam, and lorazepam, respectively, were achievable (Table 2).

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