Experimental Design in Formulation of Diazepam Nanoemulsions: Physicochemical and Pharmacokinetic Performances

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ABSTRACT: With the aid of experimental design, we developed and characterized nanoemulsions for parenteral drug delivery. Formulations containing a mixture of medium-chain triglycerides and soybean oil as oil phase, lecithin (soybean/egg) and polysorbate 80 as emulsifiers, and 0.1 M phosphate buffer solution (pH 8) as aqueous phase were prepared by cold high-pressure homogenization. To study the effects of the oil content, lecithin type, and the presence of diazepam as a model drug and their interactions on physicochemical characteristics of nanoemulsions, a three factor two-level full factorial design was applied. The nanoemulsions were evaluated concerning droplet size and size distribution, surface charge, viscosity, morphology, drug–excipient interactions, and physical stability. The characterization revealed the small spherical droplets in the range 195 –220 nm with polydispersity index below 0.15 and zeta potential between −30 and − 60 mV. Interactions among the investigated factors, rather than factors alone, were shown to more profoundly affect nanoemulsion characteristics. *In vivo* pharmacokinetic study of selected diazepam nanoemulsions with different oil content (20%, 30%, and 40%, w/w) demonstrated fast and intense initial distribution into rat brain of diazepam from nanoemulsions with 20% and 30% (w/w) oil content, suggesting their applicability in urgent situations. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci
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INTRODUCTION

During the last decades, nanoemulsions have been established as useful colloidal drug carriers for different routes of administration, especially for parenteral application. Considering the formulation and physicochemical characteristics, their possible advantages are low surfactant concentration, uniform and very small droplet size, low viscosity, kinetic stability, high solubilization capacity for lipophilic drugs; therefore, an improved penetration through biological barriers, possible enhanced bioavailability, and organ targeting could be expected with these systems.[1–6] Although lipid nanoemulsions have been clinically approved for parenteral nutrition and intravenous delivery of lipophilic drugs (e.g., diazepam, propofol, and etomidat), additional efforts are required to optimize nanoemulsion formulation, stability, drug delivery, and overall pharmacokinetic behavior, giving this topic a renewed and growing interest.[5–8]

In general, parenteral nanoemulsions are prepared with highly safe and biocompatible oils and emulsifiers, most frequently using high-pressure homogenization (HPH) technique.[1,6,9,10] It is well known that both, formulation parameters (type and concentration of oils and emulsifiers) and processing parameters (homogenization temperature, pressure, and number of cycles) can affect the physicochemical properties and stability of the nanoemulsions.[3,4,6,7,9–11] Further, the way of incorporation of the lipophilic drug in the internal oil phase of the nanoemulsion may also affect its physicochemical characteristics and stability.[3,6,12–15] For instance, Levy and Benita[16,17] have been studied injectable diazepam (DZM) submicron emulsions containing the combination of egg yolk phospholipids and poloxamer as emulsifiers, employing the hot HPH technique. They evaluated nanoemulsion physicochemical properties and short- and long-term stability, but without deeper insight into interactions of formulation variables and their influence on the system characteristics. Furthermore, until now, only a few studies have been investigating the impact of formulation or processing variables on nanoemulsion properties and stability using experimental design, which was introduced as a useful tool in formulation development.[18,19]

Based on this, the aim of the present study was to develop lecithin and polysorbate 80-containing oil-in-water (o/w) nanoemulsions for parenteral drug delivery by means of experimental design, and to characterize them in terms of droplet size, surface charge, viscosity, morphology, and drug–vehicle interactions. For this purpose, blank and DZM-loaded nanoemulsions were prepared through cold HPH method. DZM, a lipophilic poorly water-soluble compound, was chosen as the referent benzodiazepine commonly used in preclinical studies of novel psychotropic drugs.[20] During the formulation development, the

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simultaneous influence of oil content, lecithin type, and the drug presence on the nanoemulsion droplet size, polydispersity index (PDI), zeta potential (ZP), and viscosity was evaluated employing the full factorial experimental design. In addition, the stability of the obtained nanoemulsions during 2 months storage was also investigated.

Although DZM has been already formulated as a commercial injectable emulsion (under the trade name Diazemuls), data on its pharmacokinetic profile are generally lacking.[21] As it is known that the nanoemulsion system on its own may affect the pharmacokinetic behavior of the incorporated drug, and consequently its therapeutic effect,[5,22] the final aim of the study was to investigate the plasma and brain pharmacokinetics of DZM after intraperitoneal administration of a series of nanoemulsions in rats. We assessed whether increasing the oil content (from 20% to 40%) affects the DZM pharmacokinetics, including drug brain to plasma ratios and hence the brain targeting efficiency after intraperitoneal administration of DZM nanoemulsions.

MATERIALS AND METHODS

Materials

DZM was kindly donated by Galenika a.d. (Belgrade, Serbia). Medium-chain triglycerides (MCT) were purchased from Fagron GmbH & KG (Barsbüttel, Germany). Soybean oil (Lipoid Purified Soybean Oil 700), soybean lecithin (Lipoid S 75, fat-free soybean phospholipids with 70% phosphatidylcholine), and egg lecithin (Lipoid E 80, egg phospholipids with 80% phosphatidylcholine) were generously gifted by Lipoid GmbH (Ludwigshaften, Germany). Polysorbate 80 (polyoxyethylensorbitan monooleate) and butylhydroxytoluene (BHT) were obtained from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany), whereas glycerol was provided by Merck KGaA (Darmstandt, Germany). Sodium hydroxide and potassium phosphate monobasic were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Water used in the preparation of formulations was double distilled, whereas ultrapure water, used in analyses, was obtained with a GenPure apparatus (TKA Wasseranfbereitungssysteme GmbH, Neiderelbert, Germany). All other chemicals and reagents used were of pharmaceutical or HPLC grade and used as received without further purification.

Solubility Study

The solubility of DZM in MCT, soybean oil, and MCT–soybean oil mixtures at different ratios (1:1, 2:1, 3:1, and 4:1, v/v) was determined by shake flask method. An excess amount of DZM was added to 5 mL of both oils and their mixtures in Erlenmeyer flasks, which were tightly closed and shaken on orbital shaker (IKA® KS 260 basic; IKA® Werke GmbH & Company KG, Staufen, Germany) at 250 rpm at 25°C for 24 h. The samples were then centrifuged (Centrifuge MPW-56; MPW Med. Instruments, Warszawa, Poland) at 3000 rpm for 30 min to separate the undissolved drug. An aliquot of the supernatant was properly diluted with methanol and DZM concentration was assayed spectrophotometrically using a Varian Cary-100 UV–VIS spectrophotometer (Varian BV, Middelburg, The Netherlands) at 254 nm. The calibration curve for DZM in methanol was linear in the range from 1 to 10 μg/mL ($r^2 = 0.99895$).

Preparation of Nanoemulsions

All nanoemulsions were prepared by cold HPH (room temperature) according to the previously described,[9,11] but slightly modified procedure.

The oil and the aqueous phases were first separately prepared. The oil phase, consisting of oil (a mixture of MCT and soybean oil at a mass ratio of 4:1), lipophilic emulsifier (soybean or egg lecithin), and antioxidant (BHT), was heated at 70°C under slight stirring (RH basic 2 IKAMAG® Magnetic Stirrer; IKA®-Werke GmbH & Company KG), until lecithin was completely dissolved. The obtained oil phase was then cooled down to the room temperature (25°C). Afterward, DZM was added and dissolved in the oil phase.

The aqueous phase was prepared by dissolving hydrophilic emulsifier (polysorbate 80) in 0.1 M phosphate buffer solution (PBS) (pH 8), containing sodium hydroxide and potassium phosphate monobasic. To adjust isotonicity, glycerol (2.5%, w/w) was added to the aqueous phase, which was kept at 25°C.

The phases were combined by adding the aqueous phase to the oil phase, both being kept at 25°C, and further prehomogenized with a rotor-stator homogenizer IKA Ultra-Turrax® T25 digital (IKA®-Werke GmbH & Company KG) at 8000 rpm for 3 min. The obtained coarse emulsion was subsequently homogenized with a high-pressure homogenizer (EmulsiFlex-C3; Avestin Inc., Ottawa, Canada) at 500 bar. The HPH process was discontinuous, meaning that the product was manually transferred back into the chamber for nine repeated cycles. The resulting nanoemulsion was aseptically filtered through Millipore membrane filter (MCE 0.22 μm; Merck Millipore, Billerica, Massachusetts) and filled into 20 mL head-space glass vials with crimp cups. Blank nanoemulsions were also prepared as the above procedure without DZM.

All formulations were stored at 25°C and after 24 h, their characterization was performed. All measurements were carried out in triplicate. Code names and compositions of all developed nanoemulsions are given in Table 1.

Full Factorial Experimental Design

A two-level full factorial experimental design was used to estimate the main effects and the interaction effects of three different formulation factors (variables) on the nanoemulsion physicochemical properties. The investigated factors were: the oil content (A), the type of lecithin (B), and the presence of DZM as model drug (C). Each factor was varied at two coded (−1, +1) levels, in which the −1 level corresponds to the lower value and +1 to the upper value of each independent variable (Table 2). According to the applied design, a total of eight (2^3) experimental runs were generated and the run order was randomized to satisfy the statistical requirement of independence of observations. Different formulations were prepared in duplicate. As the response variables, the droplet size, PDI, ZP, and viscosity of the prepared nanoemulsions were determined. The factorial design matrix and the responses of each experiment are shown in Table 3. To fit the experimental data, a first-order polynomial (factorial) model composed of a list of coefficients multiplied by associated factor levels was applied (Eq. (1)):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{123} ABC \quad (1)$$

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