Department of Pharmaceutical Technology, Medical University of Gdansk, Gdansk, Poland

Diazepam submicron emulsions containing soya-bean oil and intended for oral or rectal delivery

M. Gajewska, M. Sznitowska and S. Janicki

Physically stable diazepam submicron emulsions were prepared using soya-bean oil. Diazepam concentration 4 mg/ml, suitable for rectal or oral delivery, was achieved in 20% emulsions. Mixture of egg lecithin (1.2%) and poloxamer (2.0%) has been chosen as the most suitable emulsifying agent. Composition of the emulsion may be supplemented with α-tocopherol and parabens. However, the system was not stable when either phenylethanol or chlorhexidine gluconate was added. Taste masking agents commonly used as food additives decreased stability of the preparation and were not efficient in elimination of a bitter taste of the drug-loaded emulsions.

1. Introduction

Submicron emulsions are O/W emulsions characterized by a very fine dispersion of the internal oily phase – the sizes of oily droplets are smaller than 1 μm, with a mean diameter as small as 200–400 nm. These systems can be used as a biocompatible drug vehicle, since natural emulsifier, lecithin, and natural oils are main components.

Submicron fat emulsions have been successfully used for more than 30 years for parenteral nutrition. Recently these systems are also used as drug vehicles for intravenous drug delivery and further studies are performed in order to develop these types of formulations for oral, transdermal or ocular applications [1–5]. Although enhanced bioavailability and prolonged activity may be sometimes achieved when a drug is incorporated into a submicron emulsion, these systems are most frequently proposed as vehicles for water insoluble drugs since increased solubility is achieved due to the presence of an oily phase.

Diazepam is an example of a drug only slightly soluble in water, which have been already introduced to the market as an intravenous submicron emulsion (Diazepam®-Lipuro, Diazemuls®, Stesolid®). These preparations are a preferred alternative to solutions, which contain propylene glycol, ethanol, and benzyl alcohol as co-solvents, together with benzoic acid and sodium benzoate as solubilizers [6–10]. Diazepam concentration in both types of formulations is the same i.e. 5 mg/ml. However, emulsion is an organic solvent-free system. The required solubility of diazepam is achieved in emulsions containing 20% oily phase. The composition of diazepam intravenous emulsions is very much alike nutritional emulsions, but the oily phase components contain also MCT oil (Miglyol) or acetylated monoglycerides besides soya-bean oil.

The goal of the present research was to develop diazepam submicron emulsions for rectal or oral drug delivery. Diazepam rectal solutions (2 or 4 mg/ml) or oral solutions (0.4 mg/ml) are potent sedative and antiepileptical drugs which are also frequently used in children [11, 12]. However, ethanol present in these preparations is not a suitable solvent for pediatric dosage forms. Submicron emulsions offer chance to avoid organic solvents present in the commercial preparations. Requirements for oral or rectal formulations differ from those for intravenous use. The main difference is the necessity to incorporate antimicrobials while oral formulations need flavouring agents. The actual experiments were performed to develop a diazepam emulsion containing soya-bean oil as the only oil and lecithin as the main emulsifier to incorporate antimicrobial agents as well as flavouring agents to such system. The composition and technology had to be optimized in order to achieve satisfactory chemical stability of the drug and physical stability of the system.

2. Investigations, results and discussion

2.1. Solubility of diazepam in emulsion

Diazepam solubility in water, lecithin aqueous dispersion, oil and submicron emulsion was studied and the results are given in the Table. Miglyol is a better solvent for diazepam than soya-bean oil, providing at least 2-times higher solubility. From the solubility in soya-bean oil it can be estimated that the target concentration (i.e. 4 mg/ml) cannot be obtained in 20% emulsion. However it was found that solubility in such an emulsion was 1.5 times higher than estimated and the required concentration was achieved. It may be assumed that diazepam is not only dissolved in the oily phase but also incorporated into the interphase or into micelles present in the emulsion. This is confirmed by the finding that diazepam solubility in water is increased 7 times in the presence of lecithin (1.2% w/w). The above preliminary studies allow the conclusion that 20% soya-bean emulsion can be serve as a vehicle for diazepam in a concentration of 4 mg/ml.

It is interesting that poloxamer, when used as a co-emulsifier, had no further effect on diazepam solubility in submicron emulsion. Further increase of concentration could be possible by changing soya-bean oil to Miglyol or adding other oil components, however such modifications were not performed in our studies since higher concentrations of diazepam were not taken into consideration for the intended routes of drug delivery.

Table 1: Diazepam solubility in submicron emulsions and oily and aqueous components (20 °C)

Solvent	Solubility (mg/ml)	Increase of solubility in comparison to water
Water	0.055	–
1.2% Lecithin in water	0.39	7
Soya-bean oil	13.5	245
Miglyol	27.0	490
20% Soya-bean oil emulsion (1.2% lecithin)	4.10	74
20% Soya-bean oil emulsion containing 2% poloxamer (1.2% lecithin)	4.07	72

The determined solubility of diazepam in 20% soya-bean oil emulsion indicates that an emulsion containing 4 mg/ml of diazepam is a saturated system and the drug may precipitate during storage. However during long term stability studies (13 month at 4 °C and 20 °C) precipitation did not occur. It is also probable that solubility of diazepam in the emulsion is elevated when the drug is incorporated to the oily phase in the course of emulsion preparation. Such a conclusion may be also drawn from the observation that in *de novo* prepared emulsions as much as 5 mg/ml of diazepam could be incorporated without initial precipitation. In such emulsions, however, the precipitation is observed after several days, which is also accompanied by coalescence of the oily phase.

2.2. Characteristics and stability of diazepam emulsion

The emulsions containing diazepam (4 mg/ml), 20% soya-bean oil and 1.2% egg lecithin were prepared using a standard method [4]. Glycerol was used in an osmotic agent and the final osmotic pressure was approximately 300 mOsm/l.

It was demonstrated that no significant change in droplet size distribution occurs when diazepam is introduced into the emulsion (Fig.). It can be concluded that diazepam does not influence the sizes of the internal oily phase droplets. The Fig. shows that poloxamer used as a co-emulsifying agent (2.0% w/w) allows to reduce the oily droplet sizes in the emulsion containing diazepam. For further investigations emulsions containing poloxamer were chosen.

Submicron emulsions are usually prepared at 85 °C, but due to sensitivity of diazepam to temperature it could be advantageous to reduce the process temperature. Emulsions prepared at 60, 70 and 85 °C were compared and no difference in appearance or oily droplet sizes was noticed. However during 6-month storage at 20 °C it was observed that much faster creaming process proceeded in emulsions prepared at 60 °C, although no changes in droplet sizes distribution were noted. Emulsions prepared at 70 °C were as stable as those prepared at 85 °C.

Long term stability studies were performed for an emulsion prepared at 70 °C – the emulsion was stored for 13 month at 4 °C and 20 °C. Only slight creaming was observed in the formulations, the intensity was not different in respect to storage temperature. Although an increasing number of larger droplets was observed during storage, the mean droplet size was only slightly changed after 13 month (Table 2). The storage temperature influenced pH – a significant pH decrease to 6.0 was found in the emulsion stored at room temperature while at 4 °C the value dropped to 7.3. Additionally the colour changed to a more creamy appearance when the preparation was stored at 20 °C. HPLC analysis was performed in order to study chemical stability of diazepam in the emulsion and no degradation products were found in emulsions stored at 4 °C. In contrast, in emulsions stored at room temperature one of the degradation products, namely 3-amino-6-chloro-1-methyl-1,4-phenylcarbostyril, was found in concentration 0.15% in respect to the total diazepam content. As USP 23 gives 0.10% as the limit of this impurity in diazepam substance, the content found in the emulsion may be considered as acceptable.

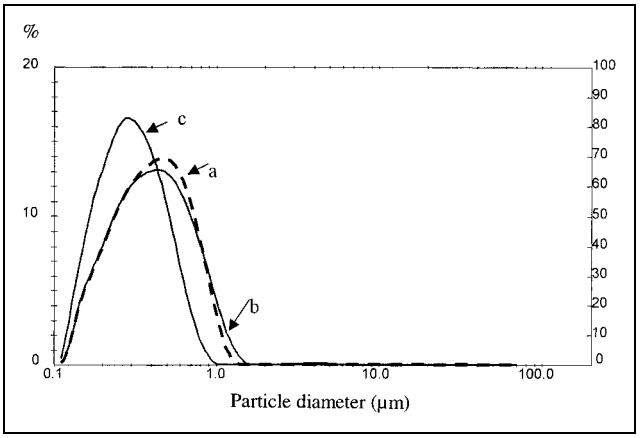

Fig.: Distribution of oily droplet sizes in 20% soya-bean emulsions: a) without diazepam, b) with diazepam, c) with diazepam and poloxamer

Table 2: Effect of additional excipients on oily droplet sizes in diazepam emulsions

Excipient	Storage conditions		Droplet sizes		
	Time (months)	Temperature	d_{50} (µm)	d_{99} (µm)	> 1µm (%)
		Without poloxamer			
–	0	–	0.40	1.23	1.90
		With poloxamer			
Parabens	0	–	0.35	1.00	1.32
α-Tocopherol	6	20 °C	0.36	1.23	2.03
	13		0.37	5.29	4.57
Phenylethanol	0	–	0.53	4.30	41.93
	9	4 °C	1.46	5.29	59.92
		20 °C	0.39	18.54	23.79
Chlorhexidine gluconate	0	–	0.53	3.49	14.22
	8	4 °C	5.84	22.84	100
		20 °C	6.02	52.68	100
Flavouring essence	0	–	0.41	2.30	20.35
	8	4 °C	2.55	8.04	88.62

Limit sizes of 50% (d_{50}) and 99% (d_{99}) of the droplets as well as the fraction of droplets larger than 1 µm are given

2.3. The effect of α-tocopherol, preservatives and flavouring agents on emulsion stability

Preparations intended for oral or rectal drug delivery are subjected to the environmental exposure more than those for parenteral delivery because they are not hermetically packed and in most cases used in multidose containers. For increased stability of oral or rectal emulsions antioxidants and antibacterial agents should be added.

α-Tocopherol was incorporated to the diazepam emulsion in a concentration of 0.02% w/w and droplet size analysis as well as visual inspection showed compatibility of this agent with the preparation (Table 2).

Due to rather high pH only limited choise of antimicrobial agents may be used in the system. Parabens and chlorhexidine gluconate were selected as agents active at pH 8.0 [13]. The third preservative under investigation, phenylethanol, is not active in alkaline pH, and it was incorporated in a diazepam emulsion with a pH adjusted to 7.0. Only emulsions containing paraben M (0.18%) and paraben P (0.02%) were stable during storage (Table 2). In the diazepam emulsion containing chlorhexidine gluconate changes in droplet diameter were observed in comparison to the emulsion without preservative – mean droplet diameter increased from 0.35 to 0.53 µm. The emulsion was not physically stable and as soon as after 1 month of storage intense creaming and coalescence were visually observed, while droplet size measurement showed the pre-

sence of oily particles up to 65 μm in diameter. Destabilization occured also in the presence of β-phenylethanol, although coalescence was significant only at room temperature (Table 2).

Bitter taste of diazepam limits oral delivery of this drug in a fluid form due to patient's compliance, especially when used in pediatrics. Taste masking agents used for pharmaceutical and food products were used to eliminate bitterness of diazepam emulsion. The essences were ethanolic solutions of synthetic flavouring agents. Although no physical changes were observed in emulsions containing these excipients after preparation, very intensive creaming and oily droplet coalescence occurred during storage (Table 2). Moreover, only a very poor taste masking effect was observed and it is concluded that the studied essences must be considered as inadequate components of diazepam emulsions.

3. Experimental

3.1. Chemicals

Diazepam was obtained as a gift from Polfa (Warsaw, Poland), Aseptin M and P (parabens) from Pharmaceutical Works Unia (Warsaw, Poland), chlorhexidine gluconate from Polfa (Lodz, Poland) and flavoring essences: vanilla, cream and strawbery, from Herbapol (Poznan, Poland). Soya-bean oil and egg lecithin, Lipoid E80 was purchased from Lipoid (Ludvigshafen, Germany). Other chemicals were purchased from the following manufacturers: Miglyol 812 (Caelo Caesar & Loretz , Hilden, Germany) poloxamer Syperonic F-68 (Boehringer Ingelheim, Heidelberg, Germany), phennylethanol (Fluka, Buchs, Switzerland), d,l-α-tocopherol (Hoffmann-La Roche, Basel, Switzerland), glycerol (Pollena-Strem, Dabrowa Gornicza, Poland), methanol (Chemical Works Oswiecim, Gliwice, Poland).

3.2. Preparation of emulsions

Egg lecithin (1.2 g) was added to soya-bean oil (10.0 or 20.0 g) and stirred at 70 °C for 30 min. Diazepam (200 mg or 400 mg) was dissolved in the oily phase, which was subsequently filtered using 0.45 μm PTFE filter (Millipore, Bedford, USA). A mixture of 2.25 g glycerol and water (to make total weight of emulsion 100.0 g) was heated to 70 °C. When poloxamer was a component of the emulsion it was also dissolved in the aqueous phase (2.0 g). The oily phase was added to the aqueous phase upon continous stirring. The primary emulsion was stirred using high-shear mixer Ultra-Turrax (Janke & Kunkel, Staufen, Germany) at 20500 rpm. After cooling to room temperature the emulsion was homogenized at 500 bar employing a high pressure homogenizer (AVP Gaulin, Hilversum, Holland). Adjustment of pH to 8.0 was done with 0.1 mol/l NaOH solution. The emulsion was filtered aseptically through a Durapore filter (Millipore, Bedford, USA) and packed in sterile glass vials under nitrogen. The preparations were stored at 4 °C and at room temperature in the dark.

α-Tocopherol (20 mg) or parabens (Aseptin M 180 mg and Aseptin P 20 mg) were dissolved in the oily phase of emulsion, while chlorhexidine gluconate (100 mg) or ethanolic solutions of flavoring agents (100 mg) were added to the ready emulsion.

3.3. Analysis

The emulsions were visually observed: creaming and presence of oily droplets on the surface was examined. Distribution of the oily droplet sizes was measured using laser diffractometer Mastersizer E (Malvern Instr., Malvern, UK).

Concentration of diazepam and the presence of degradation products was analysed using a HPLC technique: the emulsion was dissolved in methanol and injected onto a LiChrosphere RP-18 column (5 μm, 250 × 4 mm, Merck, Darmstadt, Germany), 65% methanol was used as a mobile phase and detection was done at 254 nm.

Solubility of diazepam in water, aqueous phase, lecithin aqueous dispersion, soya-bean oil, Miglyol and emulsion was studied at room temperature. The suspensions containing an excess of diazepam were stirred for 24 h, centrifuged and filtered through 0.45 μm filters. Concentration of diazepam in the filtrate was determined using HPLC.

References

1 Collins-Gold, L. C.; Lyons, R. T.; Bartholow, L. C.: Adv. Drug Deliv. Rev. **5**, 189 (1990)
2 Muchtar, S.; Benita, S.: Colloids and Surfaces **91**, 181 (1994)
3 Elbaz, E.; Zeevi, A.; Klang, S.; Benita, S.: Int. J. Pharm. **96**, R1 (1993)
4 Benita, S.; Levy, M. Y.: J. Pharm. Sci. **82**, 1062 (1993)
5 Schwarz, J. S.; Weisspapir, M. R.; Friedman, D. J.: Pharm. Res. **12**, 687 (1995)
6 von Dardel O.; Mebius C.; Mossberg, T.; Svensson B.: Br. J. Anaesth. **55**, 41 (1983)
7 Trotta, M.; Gasco, M. R.; Carlotti, M. E.: Acta Technol. Legis Medicamenti **1**, 137 (1990)
8 Levy, M. Y.; Benita, S.: Int. J. Pharm. **54**, 103 (1989)
9 Levy, M. Y.; Benita, S.: J. Parenter. Sci. Technol. **45**, 101 (1991)
10 Levy, M. Y.; Schutze, W.; Fuhrer, C.; Benita, S.: J. Microencapsul. **11**, 79 (1994)
11 Korttila, K.; Sothman, A.; Andersson, P.: Acta Pharmacol. Toxicol. **39**, 104 (1976)
12 Martindale. The Extra Pharmacopoeia. 32. Ed., The Pharmaceutical Press, London 1999
13 Kabara, J. J. (Ed.): Cosmetic and Drug Preservation Principles and Practice, Marcel Dekker, New York 1984

Received Febuary 21, 2000
Accepted July 25, 2000

Dr. hab. Malgorzata Sznitowska
Department of Pharmaceutical Technology
Medical University of Gdansk
ul. Hallera 107
80-416 Gdansk
Poland
msznito@farmacja.amg.gda.pl