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Design and characterization of a submicronized o/w emulsion of diazepam for parenteral use

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Summary

An innovative, injectable, submicronized emulsion delivery system into which diazepam was incorporated has been developed. This emulsion has been formulated to meet all the requirements for injection either i.v. or i.m. The technique utilized a high shear mixer followed by a two stage pressure homogenizer yielding a very fine monodispersed emulsion, the mean droplet size of which ranged from 100 to 150 nm. The combination of purified egg yolk phospholipid and non-ionic emulsifier, as a complex emulgator, together with a highly efficient emulsification technique yielded a fine diazepam emulsion with improved stability properties. An increase in the oil phase volume ratio caused a moderate but significant increase in the mean droplet size of the diazepam emulsion. A sharp and parallel elevation in mean droplet size and viscosity was observed in the emulsions containing 30% or more oily phase. The ζ-potential of the emulsified droplets was affected by the alteration of the pH, whereas no significant effect on mean droplet size was observed. The mean droplet size of diazepam emulsion decreased with increasing concentrations of the non-ionic emulsifier until reaching a minimum constant value. Mean droplet size of diazepam emulsion decreased, while ζ-potential increased with increasing phospholipid concentration. No changes in the various physicochemical properties were observed with increasing diazepam concentration.

Introduction

The i.v. administration of diazepam has been widely used in the control of acute muscle spasms such as tetanus, status epilepticus and convulsions (Kasturilal and Shetti, 1975; Parsonag and Norris, 1967; Tehrani and Cavanaugh, 1977). In addition, diazepam injection has also been administered as a premedication, before induction of general anaesthesia, or as a sole anaesthetic agent before major or minor surgery or dental procedures (Dundee and Haslett, 1970).

The aqueous solubility of diazepam is very low, and the addition of organic solubilizing solvents such as propylene glycol, phenyl carbinol and ethanol (Valium, Stesolid) is needed for total drug dissolution in the aqueous preparation, which is usually intended for parenteral administration. I.v. administration of these preparations is frequently associated with pain and thrombophlebitis (Langdon et al., 1973; Von Dardel et al., 1976; Schou Olesen and Huttel, 1980; Jensen et al., 1981). I.m. injections in most cases induced pain (Assaf et al., 1975; Korttila et al., 1976).

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It has long been known that precipitation occurs when diazepam is diluted either with i.v. fluids or plasma following i.v. injection (Korttila et al., 1976; Jusko et al., 1973). It was postulated that either the drug precipitation or the irritability of the organic solvent vehicles or both may account for the irritation of the vein endothelium which might ultimately result in thrombophlebitis (Von Dardel et al., 1976; Schou Olesen and Huttel, 1980; Jusko et al., 1973; Von Dardel et al., 1983).

Attempts have been made to reduce these side-effects by administering concomitantly either heparin or steroids, or by diluting the drug with saline, but with little success (Langdon et al., 1973). A number of investigations have been conducted to prevent precipitation by means of new diazepam aqueous formulations containing either glycoferol (Apozepam A.L, Norway), cremophor (stesolid MR), or recently, poloxamer surfactants (Schou Olesen and Huttel, 1980; Selander et al., 1981; Prancan et al., 1980; Lin and Kawashima, 1987). It was found that the use of glycoferol–water solution caused local pain and thrombophlebitis as often as reported with the conventional organic solvent systems (Selander et al., 1981). A significant reduction in thrombophlebitis was achieved by dissolving the drug in cremophor. However, the danger of anaphylactic reactions with cremophor markedly minimized its clinical value (Clarke et al., 1975; Dundee, 1976).

Furthermore, another serious technical problem encountered during the i.v. administration of diazepam is the extensive adsorption of the drug by plastic tubing or by plastic containers during continuous infusion (Parker et al., 1979; Cloyd et al., 1986).

A new approach to resolve the side effects associated with diazepam conventional formulations consisted of dissolving the drug in the oleaginous phase of an oil-in-water emulsion (Von Dardel et al., 1976). A significant reduction in thrombophlebitis has been observed using such a fat emulsion as a solvent system upon i.v. administration of diazepam (Schou Olesen and Huttel, 1980; Mattila et al., 1981). Furthermore, no significant differences in the therapeutic effect or in the pharmacokinetic parameters could be demonstrated when compared with Valium injection, but there was a marked reduction in the degree of pain and local venous reactions (Von Dardel et al., 1976, 1983; Thorn-Alquist, 1977).

We have recently developed an innovative injectable submicronized emulsion delivery system into which diazepam was incorporated. The objective of our investigation was to design optimal conditions for large scale manufacturing of an o/w emulsion vehicle for diazepam, appropriately formulated to meet all the requirements for injection either i.v. or i.m. A significant reduction in the degree of thrombophlebitis was observed and reported elsewhere upon i.v. administration of this novel diazepam emulsion to rabbits (Levy et al., 1989).

The present study is an in-vitro evaluation of the physicochemical properties of the diazepam emulsion.

Materials and Methods

Materials

Purified soybean oil was purchased from Bertin Co. (Courbevoie, France). Crude egg yolk phospholipids were supplied by Sigma Chemicals (St. Louis, MO, U.S.A.). Polyoxyethylenepolyoxypropylene emulsifier, poloxamer (Pluronic F-68) was furnished by BASF (Ludwigshafen, F.R.G.). Diazepam was kindly provided by Teva Pharmaceuticals Inc. (Kfar Saba, Israel). All other ingredients used were of pharmaceutical grade. The method used for the extraction of the phospholipids from the crude raw material prior to use, is a modification of an earlier technique (Schuberth and Wrethind, 1961). The composition of the purified and crude lecithin was determined by standard TLC procedures using several eluent systems (Ansel et al., 1973). No change in phospholipid composition was observed following extraction in comparison with the crude mixture. The purified complex emulsifier is composed mainly of phosphatidylcholine and phosphatidylethanolamine, together with small quantities of phosphatidylserine, phosphatidylglycerol and phosphatidic acid.

Methods

Emulsion preparation

The non-ionic emulsifier (poloxamer), osmostic agent (glycerin) and two preservatives methyl and butyl derivatives of *p*-hydroxybenzoic acid were dissolved in the aqueous phase.

The egg yolk phospholipids and diazepam were dissolved in the purified, winterized, stabilized oil phase containing α-tocopherol as antioxidant.

Both phases after appropriate filtration were heated separately to 70 °C and dispersed by a magnetic stirrer. Further heating was applied while mixing until the temperature reached 85 °C. At this temperature, the emulsification was carried out using a high shear mixer, polytron (Kinematica, Luzern, Switzerland). The resulting coarse emulsion was cooled rapidly. A fine monodispersed emulsion was achieved using a two stage homogenizing valve assembly (Gaulin Homogenizer, APV Gaulin, Hilversum, The Netherlands). Finally, the pH of the emulsion was adjusted to the desired value using a sodium hydroxide solution (10%), and the emulsion was filtered to discard the coarse droplets and debris generated during the emulsification and homogenization processes. Samples of the fine clean emulsion were stored in 10 ml brown ampoules. The whole preparation process was conducted under nitrogen atmosphere and aseptic conditions.

A typical formulation (% w/w) consisted of diazepam 0.5, oily phase 20.0, purified fractionated egg yolk phospholipids 1.2, poloxamer 2.0, glycerin 2.25, α-tocopherol 0.02, methyl, butyl *p*-hydroxybenzoic esters 0.2 and 0.075, respectively, and bidistilled water to 100.0 g.

The influence of several types of emulsification equipment on the mean droplet size of diazepam emulsion was determined. The effects of the pH, phase volume ratio, concentration of diazepam, phospholipids and the non-ionic emulsifier on the physicochemical properties of the emulsions formed were also investigated.

Emulsion evaluations

Particle size analysis. Particle size distribution is one of the most important characteristics of an emulsion. For example, the stability of an emulsion can be conveniently monitored by measuring the changes in the droplet size distribution. Moreover, the in vivo fate of the emulsion droplets is also dependent upon their sizes and distribution profiles (Singh and Ravin, 1986).

The mean droplet size of most of the emulsions prepared was found to lie between 150–800 nm. Photon correlation spectroscopy (PCS) is the appropriate method for studying particle sizes below 1 μm (Burnham et al., 1983; Davis and Galloway, 1986). In the present work, the mean droplet size and size distribution were determined by means of a computerized laser light scattering apparatus (Malvern System 4700).

Each emulsion sample was diluted to the appropriate concentration with a filtered isotonic solution (2.5% w/v glycerol in water) before measurement at 25 °C. Each emulsion system was analysed twice, and for each diluted system 10 size determinations were made.

Electrophoretic mobility. The charge on emulsion droplets was measured using the moving boundary electrophoresis technique which has been shown to yield accurate electrophoretic mobility data (Shaw, 1969). The shape of the electrophoresis cell and the way of converting electrophoretic mobility to ζ-potential were discussed in detail in a previous work (Benita et al., 1986). Appropriate experimental conditions were found which yielded initially sharp boundaries without further disturbances. The electrolyte consisted of an aqueous solution containing 1% glycerol and 0.75% poloxamer, which helped to stabilize the boundary without altering the electrophoretic mobility. Each emulsion sample was diluted with distilled water (1 : 10) prior to examination. Further confirmation of the reliability of the method used was demonstrated by obtaining similar ζ-potential values from several emulsion samples which were examined using this method and using the Malvern zetasizer system respectively.

pH. The pH of the emulsion samples was measured and recorded at given time intervals using a pH meter (Radiometer pHM63, Copenhagen, Denmark).

Solubility determination

The solubility of diazepam in both oily and

aqueous phases as a function of temperature was determined. An excess of diazepam was added to samples of each medium tested. The mixtures were shaken at the desired temperature for 24 h until equilibrium was reached. Samples were centrifuged at 2000 rpm for 5 min and supernated liquid was diluted with 0.1 N sulphuric acid in ethanol and assayed spectrophotometrically vs a corresponding blank at 366 nm for diazepam using a calibration curve.

Results and Discussion

The effect of emulsification equipment

The mean droplet size of i.v. emulsions must be smaller than the finest capillaries likely to be encountered in the vascular system, otherwise oil embolism can occur. Emulsions prepared by conventional apparatus, e.g., electric mixer, mechanical stirrer, etc., show not only large droplet sizes but also a wide particle size distribution, and are often unstable.

Submicron emulsions can be prepared by using a two stage pressure homogenizer in which the crude dispersion is forced under high pressure, through the annular space between a spring-loaded valve and the valve seat. The second stage is tandem with the first so that the emulsion is subjected to two very rapid dispersion processes (Hansrani et al., 1983; Takamura et al., 1983). Pre-mixing with ordinary electrical agitators has been found to be very useful before the homogenization step. The influence of various mixers and homogenizers is illustrated in Fig. 1. As can be seen, a coarse dispersion is characteristic of emulsions prepared with magnetic stirrer or using simple homogenizer of the stator-rotor type. The use of high shear homogenizers such as ultra-turrax and polytron decreases the mean droplet size to 1.1 μm and 0.65 μm respectively. A submicron monodispersed emulsion can be prepared efficiently with high pressure homogenizer of the Gaulin type only. In the present work polytron was used as a pre-mixer prior to the homogenization process conducted by repeatedly passing the coarse dispersion through the Gaulin homogenizer.

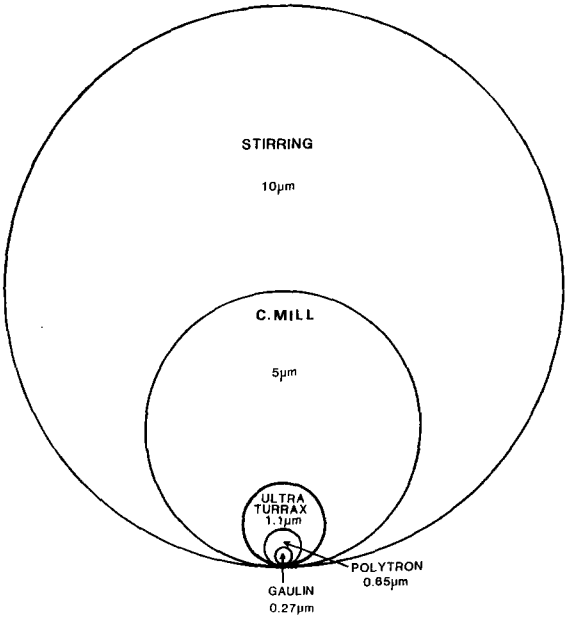

Fig. 1. Effect of emulsification equipment on mean droplet size of diazepam emulsion. Colloid Mill (Model Protol 689, Brogli, Switzerland), Ultra-Turrax (Janke & Kunkel GmBh, F.R.G.) Polytron and Gaulin Homogenizer.

Effect of oil phase nature and temperature on diazepam solubility

It is desirable to incorporate the drug into the innermost phase of the emulsion in order to successfully exploit the advantages of an emulsion dosage form. Incorporation of hydrophobic drugs in the oil phase exhibits special problems related to the solubility of the drug. This is usually overcome by techniques such as temperature elevation and surfactant addition. However, massive heat exposure of the oily phase could catalyze oil degradation and oxidation. The concentration of diazepam in the marketed hydroalcoholic preparation is 5 mg/ml and a total volume of 2 ml is usually injected either i.m. or i.v. as a single dose. Thus a total amount of 25 mg of diazepam should be dissolved in 1 ml of oily phase of the emulsion comprising an oily phase volume ratio of 0.2. Therefore, diazepam solubility as a function of temperature was compared in soya oil and in the final stabilized oily phase. A small increase in diazepam was found in the oily phase as compared with soya oil.

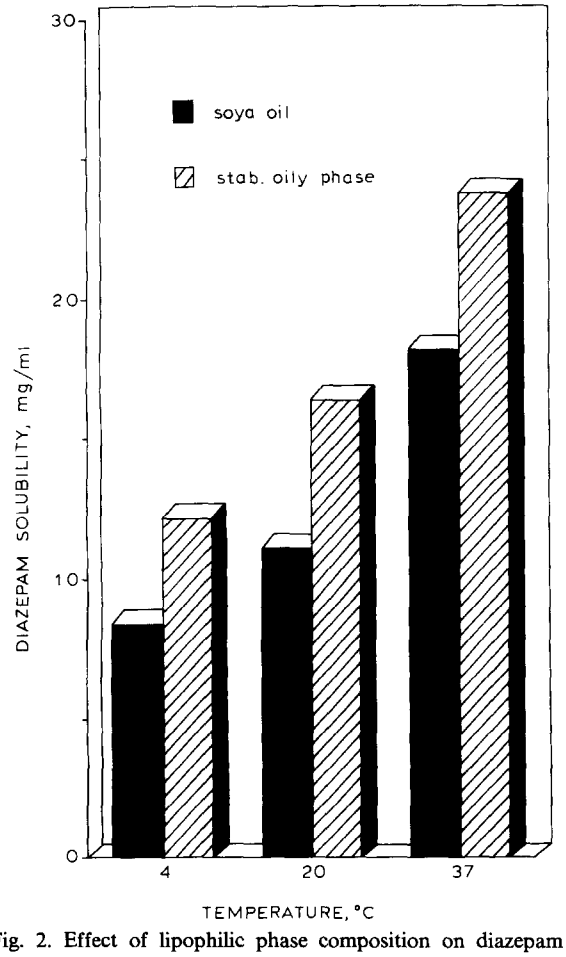

Fig. 2. Effect of lipophilic phase composition on diazepam solubility as a function of temperature.

However, a moderate increase in temperature increased significantly the diazepam solubility in the oily phases (Fig. 2). Thus, the desired concentration of diazepam (5 mg/ml) was achieved by applying further gentle heating over a short period of time. It is clear, as shown in Fig. 3, that the hydrophilic phase also contributed to the total high solubility of the drug in the final emulsion, and this should be attributed mainly to the non-ionic emulsifier present in the aqueous phase.

Effect of the pH

I.v. fat emulsions normally have a pH value close to neutrality. However, the addition of certain additives, e.g., dextrose, amino acids, etc., alter the pH value considerably (Black and Popovich, 1981). Moreover, the free fatty acids formed by the degradation of the oily components during storage can also reduce the pH value of the system with time (Kawilarang et al., 1980). The reduction in pH can significantly affect the physical stability of the emulsion.

Emulsifiers can stabilize the emulsion droplet not just by formation of a mechanical barrier, but also by producing an electrical (electrostatic) barrier or surface charge. The surface potential (ζ-potential) of an emulsion droplet will be dependent upon the extent of ionization of the emulsifying agent. The ionization extent of some phospholipids such as phosphatidylserine, phosphatidylethanolamine and phosphatidic acid is markedly pH-dependent (Bangham, 1968; Rydhag and Wilton, 1981).

The influence of pH on the mean droplet size and the droplet surface charge of diazepam emulsion is shown in Fig. 4. The ζ-potential of the emulsified droplets was affected by the alteration of the pH, whereas no significant effect on mean droplet size was observed. The droplet ζ-potential increased progressively with increasing pH value until a plateau was reached approximately above a pH value of 6.0. A similar phenomenon was also observed by other authors who examined the influence of pH on the ζ-potential of Intralipid as compared to the known effect of pH on the zeta potential of various phospholipids' liquid crystal-

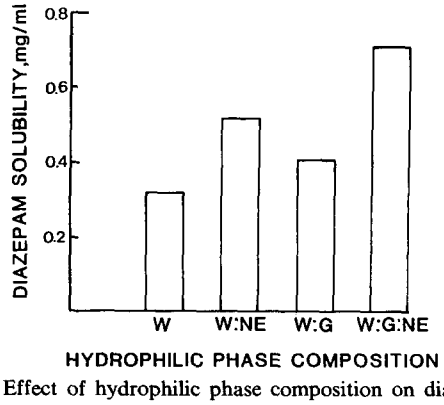

Fig. 3. Effect of hydrophilic phase composition on diazepam solubility at 25 °C using water (W), 2.5% poloxamer solution (W:NE), 2.8% Glycerin solution (W:G), and 2.5%, 2.8% poloxamer and glycerin solution respectively (W:G:NE).

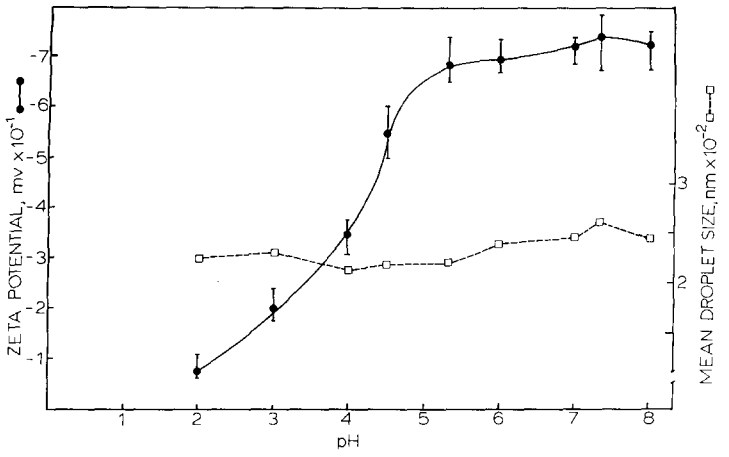

Fig. 4. Influence of pH on the mean droplet size and droplet surface charge of diazepam emulsion. Formulation, diazepam 0.5, α-tocopherol 0.02 stabilized oily phase to 20.0, poloxamer F-68 2.0, glycerin 2.25, methylparaben 0.2, butyl paraben 0.075, bidistilled water 100 g.

line particles (Davis, 1982). It was shown that the ζ-potential elevation was due to the ionization increase with pH of some phospholipids, mainly phosphatidylserine and phosphatidylethanolamine, while phosphatidylcholine, the main constituent of lecithin, maintained its electrical neutrality over a wide range of pH (Davis, 1982; Papahadjopoulos, 1968). The effect of pH on the ζ-potential was exploited to enhance emulsion stability by adjustment of the pH to optimal value, which prevented coalescence of the droplet and ensured emulsion integrity.

Effect of phase volume ratio

The rheological behaviour of the injectable emulsion determined the "syringeability" of the delivery dosage form. I.v. or i.m. administration of viscous preparations is usually painful. Increasing the oily phase volume promoted and augmented the amount of liposoluble drug which could be incorporated in the emulsion dosage form. However, the viscosity of the emulsion also increased and limited the use of these dosage forms (Sherman, 1950).

The mean droplet size of diazepam emulsion increased moderately but significantly with oily volume increase from 15% to 25% (Fig. 5). A sharp and parallel elevation in mean droplet size value and viscosity was observed in the emulsions containing 30% or more oily phase. In fact, these

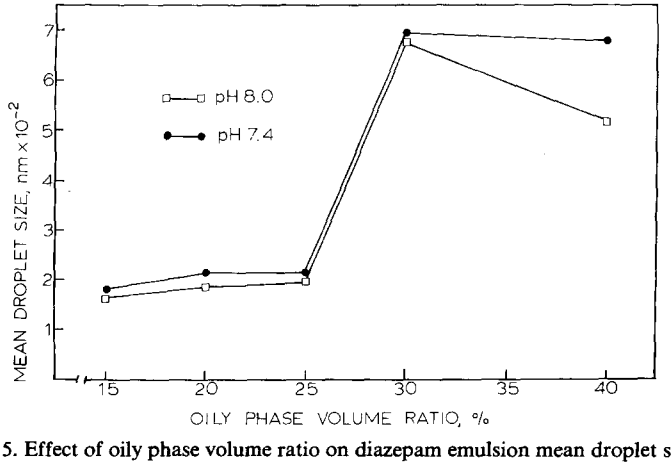

Fig. 5. Effect of oily phase volume ratio on diazepam emulsion mean droplet size.

TABLE 1

Influence of the phase volume ratio on the specific surface and total surface of the emulsified oily droplets

Phase volume ratio (% w/w)	Mean droplet size (nm)	S_w ($cm^2/g \times 10^{-5}$)	Total surface ($cm^2 \times 10^{-5}$)
15	143	4.55	68.25
20	181	3.6	72
25	211	3.08	74
30	670	0.97	29.1
40	630	1.03	41.2

Total surface of the emulsified oily droplets was calculated on the basis of 100 g total emulsion.

emulsions behave as semi-solid products with respect to their flow properties.

A number of investigators who examined the droplet size of various commercial fat emulsions as a function of various oily phase volumes reported similar results (Davis, 1982; Jeppsson et al., 1974, 1976).

It appears therefore that the efficiency of the dispersion process is affected by the rise in viscosity of the dispersed system, promoting ultimately the formation of an emulsion having larger droplets. This was also supported by the results obtained from the calculation of the specific surface and total surface of the dispersed droplets as a function of the oily phase volume (Table 1).

The specific surface can be calculated following the equation: $S_w = 6/pd$ (Martin et al., 1983), in which S_w is the specific surface per unit weight, p is the true density of the particles (assuming the droplets consisted mainly of the oily phase the density value of which was measured and found to be 0.922), and d is the diameter of the particles.

It should be noted that two main combined effects might affect the final droplet size distribution of the emulsion during the manufacturing process, the extent of interfacial coverage of the generated droplets by the various emulsifier molecules, and the viscosity of the medium as previously mentioned. A partial or minimal interfacial coverage might lead to an increase in surface tension which will be compensated by an increase in droplet size. Such a phenomenon could occur if the oily phase volume ratio is increased while the poloxamer concentration is even decreased. Thus, each one of these effects, or both, may account for the increase in droplet size with increasing oily phase volume ratio. It can then be deduced from the results reported in Table 1 that increasing the oily phase volume ratio from 15 to 25% led to a moderate augmentation of the droplet size, which should be attributed to a decrease in the interfacial surface coverage rather than to an increase in viscosity, which was moderately altered by the increase in oil concentration up to 25%. This was also supported by the fact that the regenerated specific surface declined moderately, indicating probably the formation of a stabilizing close-packed mixed film of the emulsifying agents at the interface of the emulsified droplets of progressively increasing size. The growing droplet rate will cease only when a sufficient interfacial high energy barrier will be installed, causing the repulsion of adjacent droplets. This obviously will depend on the extent of surface coverage by the combined emulsifier mixture. The sudden increase in droplet size above 25% oily concentration was considered to be due to a combination of increase in viscosity and decrease in poloxamer surface coverage. The former effect was visually detected and should account for the poor efficiency achieved in the homogenization process. The latter effect should be attributed to an alteration in the molecular composition of the interfacial mixed film due to a diminution of the total poloxamer concentration in the emulsion system. This film is probably less effective in stabilizing the emulsified droplets at an earlier stage of the growing process. It should be added that the mean droplet size as measured above 25% oily concentration should not reflect the actual mean droplet size, which should be larger but could not be effectively measured using the PCS method, as previously mentioned.

Effect of non-ionic emulsifier concentration

The combination of purified egg yolk phospholipid and non-ionic emulsifier (poloxamer) as a complex emulgator in the formation of fat emulsion yielded a fine emulsion with small particles and improved stability properties (Benita et al., 1986; Singleton et al., 1958). The mean droplet size of diazepam emulsion fell sharply at the be-

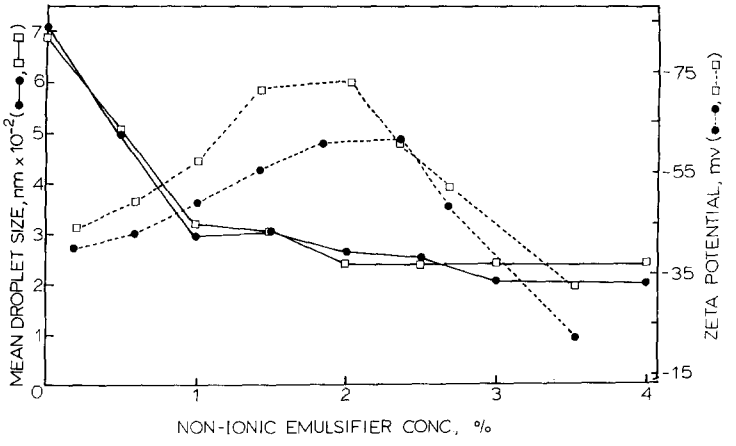

Fig. 6. Effect of non-ionic emulsifier concentration on diazepam emulsion mean droplet size and ζ-potential; (□———□), pH 8 and (●———●), pH 7.4. Same formulation as in Fig. 4.

ginning, then declined moderately and finally reached a minimum constant value at 3.0% with increasing poloxamer concentration (Fig. 6). This gradual decrease behaviour reflects the formation of better close-packed mixed film of both emulsifying agents at the oil–water interface of the emulsified droplets confirming previous results reported earlier. This interfacial film acted as a stabilizer at the earlier stage of the emulsification process by forming a high-energy barrier which caused repulsion of adjacent droplets and led to the formation of stabilized emulsified droplets of progressively decreasing size.

It is interesting to note also from Fig. 6 that the variation of the non-ionic emulsifier concentration affected the ζ-potential of the emulsion. ζ-Potential values increased, reached a plateau and finally decreased with increasing poloxamer concentration.

The initial raising in the surface potential should rather be attributed to the incorporation of polar compounds in the mixed interfacial film in the

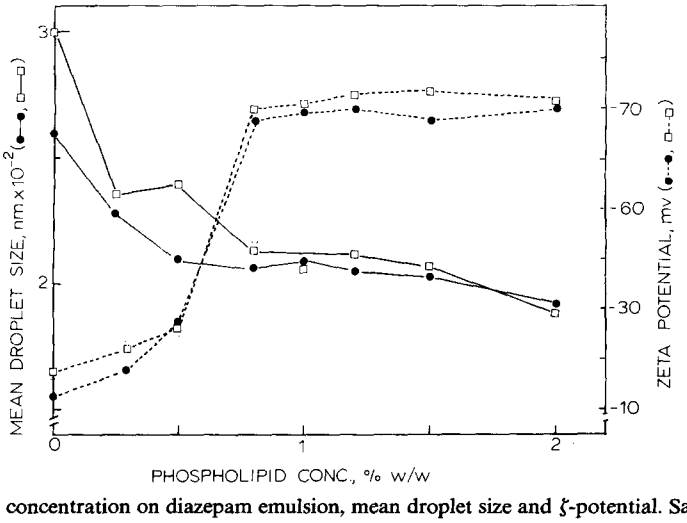

Fig. 7. Effect of phospholipid concentration on diazepam emulsion, mean droplet size and ζ-potential. Same formulation as in Fig. 4; (□———□), pH 8 and (●———●), pH 7.4.

presence of poloxamer. It appeared that interfacial concentration of these polar compounds increased with increasing poloxamer concentration until a saturation was reached at 2.0%.

The final marked reduction in ζ-potential could be explained by further changes in the interfacial layer composition resulting from the removal of the phospholipids, which were replaced by poloxamer molecules, leading to a reduction in ζ-potential values.

Effect of phospholipids concentration

Mean droplet size of diazepam emulsion decreased, while ζ-potential increased with increasing phospholipid concentration (Fig. 7). These findings indicated the formation of a better close-packed ionized interfacial mixed film with improved mechanical and electrostatic properties which led to the formation of stabilized emulsified droplets of progessively diminishing particle size.

It should be noted that at least 0.75% of phospholipids were required to maintain adequate values of ζ-potential and mean droplet size.

A moderate rise in the viscosity of the emulsions containing 2.0% of phospholipids was observed.

Influence of diazepam concentration

It has been shown that usually the incorporation of a lipophilic drug into an emulsion causes a certain degree of instability. However, in our research it was found that the emulsion system containing diazepam had similar physical properties to that of the emulsion system without the drug. Neither change in the mean droplet size, nor in the ζ-potential were observed with increasing diazepam concentration to the required therapeutic concentration (0.5%) in the emulsion. The stabilization of the drug containing emulsion was due to the use of a complex combination of emulsifiers, the phospholipids and poloxamer, together with a highly efficient emulsification process.

This indicates that the developed emulsion system can serve as a safe vehicle for other lipophilic drugs for parenteral administration.

Conclusion

It can be concluded that an optimized large-scale emulsion manufacturing process, involving well known industrial techniques (emulsification, homogenization) was developed. Optimal experimental conditions were found and yielded an optimal, stable diazepam emulsion. The emulsion presents a clear advantage over a marketed diazepam product with respect to venous sequelae incidence as previously shown in animal studies (Levy et al., 1989).

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