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International Journal of Pharmaceutics 312 (2006) 174–178
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Partitioning of parabens between phases of submicron emulsions stabilized with egg lecithin

Dorota Watrobska-Swietlikowska, Malgorzata Sznitowska *

Department of Pharmaceutical Technology, Medical University of Gdansk, Hallera 107, Gdansk 80-416, Poland

Received 19 July 2005; received in revised form 4 January 2006; accepted 9 January 2006
Available online 10 February 2006

Abstract

Partitioning of methyl and propyl parabens (methyl and propyl hydroxybenzoate, paraben M and P) between the major phases in the parenteral submicron emulsions was studied. The investigated emulsions contained 10% or 20% soya-bean oil, 1.2% or 2.4% egg lecithin, 0.18% or 0.36% paraben M and 0.02% or 0.04% paraben P. The aqueous phase was obtained by ultracentrifugation, and subsequently, it was subjected to ultrafiltration, which procedure allowed to distinguish between the fractions of free preservatives (F_w) and incorporated in the liposomal or micellar region (F_{lm}). The fractions present in the oily phase and in the interface were calculated. Depending on the formulation, F_w was 17–31% and 2.3–6.0% for paraben M and P, respectively. The F_{lm} values were in a very narrow range, i.e. 3.0–6.0% for both preservatives. Substantial accumulation, i.e. 38–58% was found in the interface and the partitioning into this region was related to the oil/lecithin ratio rather than to lipophilicity of the preservative.

Keywords: Emulsion; Preservatives; Parabens; Lecithin

1. Introduction

Pharmaceutical products when distributed into multidose containers, especially for parenteral and ocular administration, should be properly preserved against microbial contamination and proliferation during storage in normal conditions and proper use. Incorporation of preservatives in single-dose vials is also a common procedure, if filtration is used as a sterilization method.

Preservation of emulsions is an important problem because in these systems, due to solubility of the antimicrobial agents in the oily phase and interaction with emulsifiers, they may not attain an effective concentration in the aqueous phase (Shimamoto and Mima, 1979; Kurup et al., 1992; Anger et al., 1996). In recent years, new multiphase and nanoparticular systems were introduced as drug carriers and their preservation is even a more challenging task. Among these formulations are submicron emulsions, generally used as parenteral preparations. They differ from traditional emulsions as they contain lecithin as an emulsifying agent and the size of the oily phase droplets is below 1 μm (mean size 200–500 nm), what results in a large interface region. Submicron emulsions are complex systems where besides oil, water and the interface, other structures like liposomes and various bilayer structures (vesicles) (Westesen and Wehler, 1992; Groves and Herman, 1993; Férézou et al., 2001), as well as, depending on the composition, specific bicompartmental structures (Teixeira et al., 2000) were identified. Although micelles or mixed micelles were not observed by the above mentioned authors, but their existence in submicron emulsions cannot be definitely denied. The interface region, saturated with surfactant can also present complex structures. For example, Groves and Herman (1993) suggested that cubic liquid crystalline phase is formed at the oil/water interface during heat sterilization of phospholipid stabilized emulsions and the bulk of this cubic material is then converted to a lamellar phase on cooling. Considering the complexity of the system, it is clear that preservatives may be more or less available for the contact with microorganisms, depending on the site of their location in the above mentioned structures.

In our previous publication (Sznitowska et al., 2002), preservatives for submicron emulsions were proposed on the basis of their physicochemical compatibility. Among several preserva-

* Corresponding author. Tel.: +48 58 3493180; fax: +48 58 3493190.
E-mail address: msznito@amg.gda.pl (M. Sznitowska).

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doi:10.1016/j.ijpharm.2006.01.005

tives parabens were chosen as the most promising candidates for this purpose.

Up to presence there is no commercial parenteral emulsion which contains preservatives and the research concerning the problem of preservation of submicron emulsions is very limited (Jumaa et al., 2002; Pongcharoenkiat et al., 2002; Han and Washington, 2005). Han and Washington (2005) reported distribution of paraben M added to a commercial intravenous emulsion Diprivan containing 10% (w/w) soya-bean oil and 1.2% (w/w) egg lecithin, and 1% (w/w) propofol as an active agent.

The aim of our study was to investigate distribution of paraben M and paraben P (methyl and propyl hydroxybenzoates) in a drug-free submicron parenteral emulsions in relation to the oily phase and lecithin content, as well as to the paraben concentration.

2. Methods and materials

2.1. Preparation of emulsions

The emulsions (Em 0, I–IV) were prepared according to a standard method employing a hot-stage high-pressure homogenization (Benita and Levy, 1993). Their composition is presented in Table 1: soya-bean oil and egg lecithin Lipoid E 80 (Lipoid, Ludvigshafen, Germany) were used for their preparation, and isotonicity was achieved with glycerol, 2.3% (w/w) (Pollena-Strem, Dabrowa, Poland). The Lipoid E 80 composition according to the manufacturer's specification was as follows (%, w/w): phosphatidylcholine 84, phosphatidylethanolamine 8.0, lysophatidylcholine 2.2, sphingomyeline 2.2, triglycerides 2.2 and cholesterol 0.7. The antimicrobial agents used in the study were methyl and propyl paraben (purity >99%, Fluka, Schnelldorf, Germany). They were added to the emulsions de novo by dissolving in the soya-bean oil before emulsification with water. The emulsions were thermally sterilized by autoclaving (121 °C, 15 min). All formulations were stored in glass vials with Teflon-lined stoppers, at 4–6 °C.

2.2. Analysis of paraben partitioning between emulsion phases

The aqueous phase of the emulsions was separated by ultracentrifugation (ultracentrifuge UP 65, VEB MLV, Engelsdorf, Germany) at 38,000 rpm (147,000 × *g*) for 4 h (25 °C). The collected aqueous phase (w-lm) was opalescent due to liposomal/micellar dispersion, and subsequently, it was subjected to a centrifugal ultrafiltration using Microcon units (Millipore, Bedford, USA) with filters of NMWL 100 kDa, what resulted in a liposomes/micelles-free aqueous phase (w). The ultrafiltration procedure was validated for the recovery of parabens.

Quantitative analysis of the total content of parabens in the emulsions and in the separated aqueous phases was performed using HPLC apparatus (Merck-Hitachi, Darmstadt, Germany) equipped with C18 column (Lichrosphere 5 μm, Merck) and UV–Vis detector at 254 nm. A mixture of methanol and water (65:35, v/v) was used as a mobile phase. Prior the injection, the samples were diluted with methanol.

2.3. Solubility of parabens in emulsion components

The solubility studies were performed at room temperature (20 °C). An excess of paraben M and P were placed in flasks and 20 ml of either water or soya-bean oil or aqueous dispersion of lecithin (1.2% or 2.4%) containing 2.3% (w/w) glycerol were added. The suspensions were stirred with a magnetic stirrer (600 rpm) for 24 h, centrifuged (20 min, 3000 rpm) and the supernatant was analyzed for paraben concentration.

The partition coefficient of parabens between soya-bean oil and water was determined at 20 °C (±1 °C): 10 ml of a saturated aqueous solution of paraben M and P was shaken in a separator with 10 ml of soya-bean oil and the concentration of parabens in the aqueous phase was analyzed. The partition coefficient was calculated from the concentrations of parabens in the saturated solution and the concentration determined after the procedure.

2.4. Physicochemical characterization of the emulsions

Size distribution of the internal phase droplets was measured using laser diffractometer (Mastersizer E, Malvern Instr., Malvern, UK). Calculations were done using the Mie theory model of light scattering. The pH was analyzed with a pH-meter (350 type, Orion, Beverly, USA) by immersion of a Ag/AgCl electrode in the emulsion. Zeta potential was measured using Malvern Zeta Sizer (model 2C, Malvern Instr., Malvern, UK).

Table 1
The composition and physical characteristics of the emulsions

Emulsion	Composition (%, w/w)			Size of oily particles (nm)[a]		pH[b]	Zeta potential (mV)
	Soya-bean oil	Egg lecithin	Paraben M/paraben P	*d*(0.5)	*d*(0.9)		
Em 0	10	1.2	–	346 ± 20	670 ± 55	6.4	−66.24
Em I	10	1.2	0.18/0.02	350 ± 11	690 ± 39	7.8	−69.24
Em II	20	1.2	0.18/0.02	410 ± 7	803 ± 36	7.8	−67.22
Em III	20	2.4	0.18/0.02	349 ± 6	687 ± 40	7.6	−58.26
Em IV	10	1.2	0.36/0.04	308 ± 13	590 ± 28	8.0	−49.98

[a] *d*(0.5), *d*(0.9)—upper limits of diameter for 50% and 90% particles, respectively ($n = 6$).
[b] Deviations of less than ±0.1 were observed ($n = 6$).

3. Results and discussion

Table 1 presents the characteristics of the prepared emulsions. Em I and III did not differ from the preservative-free emulsion in respect of the oily droplet size distribution, as measured by a laser diffractometer (Mastersizer E, Malvern Instr., Malvern, UK). Larger droplets were only observed in Em II, what was not related, however, to the paraben content. On the other hand, the highest concentration of parabens (Em IV) may be a reason for the significantly smaller droplet size ($p < 0.05$), what indicates that parabens can modify the interfacial region (Pongcharoenkiat et al., 2002).

The initially alkaline pH (8.3) of the emulsion decreases during autoclaving due to partial hydrolysis of the lipids (Herman and Groves, 1993), but this phenomenon has been largely inhibited in the emulsions containing parabens and the smallest drop of pH (to pH 8.0) was observed in Em IV, containing the highest concentration of parabens. In this emulsion, the smallest negative charge was measured on the surface of the droplets, while in Em I and Em II containing lower concentration of parabens the zeta potential did not differ significantly ($p < 0.05$) from the value measured in the preservative-free emulsion. No correlation between zeta potential and pH can be demonstrated. Reduced pH drop observed in all emulsions containing parabens may support hypothesis that there is an interaction of parabens with phospholipids and lipids in the interface region. For example, the lipids may be protected from the attack of water molecules due to increased viscosity of the interface layer observed by Zhang and Kirsch (2003). Alternatively, hydrolysis of parabens and the following dissociation of the product accumulated in the interface region may provide hydrogen ions which make the microenvironment more acidic, inhibiting hydrolysis of phospholipids and pH drop (Pongcharoenkiat et al., 2002).

The fractions of paraben M and paraben P determined in the aqueous phases of emulsions obtained by ultrafiltration (F_w) are presented in Table 2. This fraction represents the free preservative active against microorganisms. Depending on a composition of the emulsions, F_w fraction of paraben M is in the range 17–34% and for more lipophilic (Table 3) paraben P is much smaller—2.2–6.0%. As can be expected, considering the log P values, the amounts of paraben M and P in the "w" phase significantly decreased (Student's t-test; $p < 0.05$), when the oil content in the emulsion increased from 10% to 20% (Em I versus Em II). Increased concentration of lecithin (Em III) resulted in further decrease ($p < 0.05$) of the free parabens.

Table 3
Partition coefficients of paraben M and P and their solubility (20 °C) in emulsion components (mean values of two to three determinations)

	Paraben M	Paraben P
Solubility (mg/ml)		
Water	2.06	0.38
Soya-bean oil	17.9	32.9
1.2% Lecithin[a]	2.94	1.26
2.4% Lecithin[a]		
Total	7.38	5.99
In separated lower phase[b]	11.8	13.1
Partition coefficient		
Soya-bean oil/water	7.10	73.8
Octanol/water[c]	2.00	2.98

[a] Dispersion of egg lecithin in water with glycerol corresponding to the aqueous phase of the emulsions.
[b] See description in the text.
[c] KowWin calculation (http://esc.syrres.com).

The main phases of the submicron emulsions are identified as oil (o), water (w), lecithin-rich interface bound to the oily droplets (i) and liposomes and micelles (ml) built in water by an excess of lecithin. In order to elucidate distribution of parabens between these phases, solubility of the compounds in water, oil and lecithin dispersions was studied.

The results of the solubility studies of parabens in water, soya-bean oil and aqueous dispersions of lecithin as well as partition coefficients oil/water and octanol/water are presented in Table 3. During centrifugation of the suspension in 2.4% lecithin dispersion a separation of an opalescent upper micellar phase from the more condensed lecithin dispersion was noted. The volume of the concentrated lecithin phase was approximately 30% of the total volume, what means that the concentration of lecithin in this phase was approximately 6–7%. The concentration of parabens in the concentrated lower phase was additionally analyzed.

The solubility of parabens in water as well as partition coefficient are in agreement with values reported by other authors (McCarthy, 1984; Wan et al., 1986). In the presence of lecithin solubility is increased, particularly for paraben P. Glycerol does

Table 2
Distribution (% of total) of parabens between phases of the investigated emulsions (mean ± S.D.; $n = 5$–10); values F_w and F_{lm}—experimentally determined and F_o and F_i—calculated

Emulsion	Water (F_w)	Oil (F_o)	Liposomes–micelles (F_{lm})	Interface (F_i)
Paraben M				
Em I	31.17 ± 3.01	21.13 ± 2.04	3.41 ± 1.87	44.52 ± 4.11
Em II	22.15 ± 2.23	33.77 ± 3.39	4.45 ± 2.23	39.63 ± 3.39
Em III	17.23 ± 1.03	26.27 ± 1.57	5.95 ± 3.74	50.85 ± 1.65
Em IV	33.99 ± 2.91	23.03 ± 1.97	4.99 ± 2.91	37.99 ± 1.97
Paraben P				
Em I	4.62 ± 0.62	33.86 ± 4.53	3.30 ± 1.04	58.20 ± 5.25
Em II	3.15 ± 0.41	51.82 ± 6.80	3.13 ± 0.41	41.90 ± 6.80
Em III	2.26 ± 0.42	37.30 ± 6.93	4.33 ± 4.84	56.11 ± 7.54
Em IV	6.03 ± 0.49	44.15 ± 3.59	4.28 ± 0.49	45.53 ± 3.59

not exhibit a major effect (data not shown). The solubilization effect is related to the lecithin concentration, although it is not proportional, since the solubility of parabens in 2.4% (w/w) dispersion was more than twice larger than in 1.2% dispersion.

Using the concentrations of parabens determined in the "w" and "w-lm" phases as well as partition coefficients, the amounts of parabens present in the liposomal–micellar phase and in the lecithin-rich interface (mesophase) were calculated. Equations proposed by Han and Washington (2005) were employed:

$$F_w = \frac{C_w V_w}{m}$$

$$F_{lm} = \frac{(C_{uc} - C_w)V_w}{m}$$

$$F_{o+i} = 1 - (F_w + F_i)$$

$$F_o = \frac{C_w V_o P}{m}$$

$$F_i = F_{o+i} - F_o$$

where F_w, F_{lm}, F_o and F_i are fractions of paraben in the aqueous phase (obtained by ultrafiltration), liposomal/micellar and oily phases and in interface, respectively; C_w, C_{uc} the concentration of paraben in the aqueous phase obtained by ultrafiltration and ultracentrifugation, respectively; V_w, V_o the volume fraction of aqueous phase and oily phase, respectively; m the total mass of paraben in emulsion; and P is the partition coefficient of paraben between oil and water.

Table 2 presents the distribution of parabens in all the above phases of the investigated emulsions.

The amount of paraben P in the oily phase is significantly ($p < 0.05$) larger than calculated for paraben M, but the difference is not so big as could be expected from the large difference in the lipophilicity of both compounds. The smallest difference is observed for the Em III with increased lecithin content. On the other hand, similar fractions of paraben M and P were found in the liposomal–micellar structures (F_{lm}). The most interesting is a high content of parabens in the interface region, where depending on the formulation, 38–58% of the total content of added preservatives is present. Partitioning of paraben M and paraben P into this lecithin-rich region was similar, indicating that the process does not depend on the lipophilicity of preservative. This is confirmed by the results of the solubility studies: solubility of both parabens in the concentrated lecithin dispersion was similar (Table 3). The accumulation of parabens in the interface is related to the oil/lecithin ratio, being smaller ($p < 0.05$) for Em II (1:0.6, oil/lecithin ratio) than for Em I and Em III (1:1.2, ratio).

Han and Washington (2005) referring to previous studies (Férézou et al., 2001) estimated that half of the lecithin may be present in the interface while half is located in the liposomal or micellar structures in the continuous aqueous phase. Considering this assumption we have calculated that in the interface region amount of paraben M corresponding to 1 mg of lecithin is: 133, 119, 77 and 227 μg for Em I, II, III and IV, respectively, while for paraben P this order is as follows: 19, 14, 10 and 30 μg. The solubility of parabens in the concentrated lower phase of lecithin dispersion subjected to centrifugation (Table 3) expressed in μg/mg of lecithin is estimated as 213, what allows for conclusion that the above amounts of parabens located in the interface are very likely, although the results should be compared with a great care since the structures present in the aqueous dispersion of lecithin may differ from those formed by lecithin in submicron emulsions.

Our results found for paraben M in the 10% emulsion are similar to those reported by Han and Washington (2005) regarding the free paraben fraction in Diprivan emulsion. However, the authors used centrifugation for separation of the liposome containing phase while in our experiments such procedure did not lead to phase separation and finally ultracentrifugation was used what certainly resulted in the more clear aqueous phase collected, and finally the fractions of paraben M in oily and liposomal phases are smaller than 42% and 10.7%, respectively, as reported by Han and Washington (2005). Consequently, larger fraction of paraben M has been classified by us as located in the interface (44.5% versus 24.0%). In spite of these differences, unavoidable since there is no standard procedure to separate liposomal/micellar fraction, it may be concluded that the results of both studies indicate that the accumulation of parabens in the interface of submicron emulsion is very high, while liposomal/micellar solubilization is not so important. A large capacity of the interface (mesophase) region may be explained by its structure being a mixture of cubic liquid crystalline and lamellar phases (Groves and Herman, 1993). The accumulation of parabens in the interface region may be a reason for reduced zeta potential and decreased oily droplet size (measured for Em IV containing increased amount of parabens) and particularly for the reduction of pH drop during autoclaving (Table 1).

On the other hand, the low concentration of free parabens in water does not allow to achieve a required antimicrobial protection as demonstrated by a pharmacopoeial test (Sznitowska et al., 2002).

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