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Formulation and characterization of bupivacaine lipospheres

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Abstract

Bupivacaine lipospheres were prepared as a parenteral sustained-release system for post-operative pain management. Bupivacaine free base was incorporated into micron-sized triglyceride solid particles coated with phospholipids, which were formed via a hot emulsification and cold resolidification process. The bupivacaine liposphere dispersions were characterized with respect to drug loading, particle-size distribution, and morphology. Gelation of the fluid liposphere dispersions was observed at different time intervals upon storage. The type of phospholipids used in the formulation was found to have a major impact on the gelation of the dispersion. The use of synthetic phospholipids instead of the natural phospholipids in the formulation yielded bupivacaine liposphere dispersions exhibiting prolonged gelation time. The addition of a hydrophilic cellulosic polymer can further improve the physical stability of the dispersion.

Keywords: Bupivacaine; Liposphere; Sustained-release; Injectable; Phospholipids

1. Introduction

Bupivacaine has been widely used for local or regional anesthesia after surgery because of its rapid onset and relatively long-lasting anesthetic effect as compared to other commonly used local anesthetics (Physician Desk's Reference, 2003). However, the frequent local administration of a low dose of bupivacaine hydrochloride solution is often required due to its fast local clearance and high systemic absorption which may lead to cardiovascular and central nervous system toxicity. Several injectable sustained-release bupivacaine formulations have been developed. Polymeric-based formulations including polymeric microspheres (Malinovsky et al., 1995), polymeric implants (Masters et al., 1993), microcapsules (Kopacz et al., 2003); and lipid-based formulations such as liposomes (Malinovsky et al., 1997, 1999) and lipospheres (Domb, 1993a) were reported to exhibit long-lasting anesthetic efficacy in vivo.

Lipospheres are a lipid-based drug delivery system comprising micron-sized solid particles with a monolayer of a phospholipid embedded on the surface (Domb, 1993b). The core of a liposphere particle is composed of a hydrophobic active ingredient, such as bupivacaine, dissolved or dispersed in an inert solid hydrophobic material such as triglyceride. Lipospheres can release drug over a period of several hours

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to several days. Liposphere formulations of bupivacaine and lidocaine were previously evaluated as sustained-release delivery systems for local anesthesia (Malinovsky et al., 1999). Natural phospholipids, such as egg phosphatidylcholine (EPC) and soy phosphatidylcholine (SPC), have been used in the preparation of lipospheres. However, these liposphere formulations have exhibited physical stability problems resulting in a short shelf life. Liposphere dispersions formulated with natural phospholipids were found to solidify or gel at room or refrigeration temperature, a few hours to a few days after manufacture. Such a physical instability problem has precluded the development of a ready-to-use product. The present study reports on the use of synthetic phospholipids and a cellulosic polymer to improve the physical stability of bupivacaine liposphere formulations. The effects of other formulation variables such as type of triglycerides and drug concentration on the physical properties and stability of the liposphere dispersions are also discussed.

2. Materials and methods

2.1. Materials

Bupivacaine free base was purchased from Orgamol (Switzerland). EPC and SPC, both 99% pure, were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Synthetic phospholipids: dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), and distearoyl phosphatidylcholine (DSPC), were from Genzyme (Cambridge, MA). Tristearin (TS) was purchased from Condea (Houston, TX). Tricaprin (TC), trilaurin (TL), triarachidin (TA), and carboxymethylcellulose (CMC, low viscosity) were from Sigma Chemical Co. (St. Louis, MO). Phosphate buffer (0.05 M, pH 7.4) was prepared with monopotassium phosphate and dipotassium phosphate, both from J.T. Baker (Phillipsburg, NJ).

2.2. Preparation of lipospheres

Pre-weighed amounts of a triglyceride (tristearin, tricaprin, or trilaurin), a phospholipid (EPC, SPC, DMPC, DPPC, or DSPC), and bupivacaine free base were placed into a glass media jar containing a phosphate buffer. The mixture was heated to 90 °C in a water bath until all of the solid components completely melted. The mixture was subsequently transferred into a pre-heated jacketed beaker and homogenized using a Silverson L4RT high-shear mixer (Silverson, East Longmeadow, MA) at 8000 rpm for 5 min. The hot emulsion was poured into a pre-chilled jacketed beaker maintained at 0 °C. The dispersion was stirred until the temperature dropped to 10 °C, resulting in the formation of solid lipospheres. When preparing formulations containing CMC, a solution of CMC was added into the mixture of bupivacaine free base, triglyceride, phospholipid, and phosphate buffer prior to the heating step. The liposphere formulation was subsequently prepared according to the previously described method.

2.3. Determination of liposphere particle-size distribution

The particle-size distribution of a bupivacaine liposphere formulation was determined using a Horiba LA-920 Laser-Scattering Particle Size Distribution Analyzer (Horiba Instrument, Irvine, CA). Liposphere dispersion was examined under an optical microscope (Optiphot-2-POL, Nikon, Japan). A lyophilized bupivacaine liposphere sample was also prepared and the morphology of the lipospheres was examined using a Leo 1450VP scanning electron microscope (SEM; Leo Electron Microscopy, Germany).

2.4. Determination of total bupivacaine content (potency) and free bupivacaine

The total amount of bupivacaine in a liposphere formulation was determined by high performance liquid chromatography (HPLC). One-milliliter aliquot of the formulation was transferred into a 10-ml volumetric flask, and tetrahydrofuran (THF) was added to volume. The solution was sonicated until lipospheres completely dissolved and was subsequently diluted 25-fold with methanol. The final solution was analyzed for bupivacaine content by using a HPLC (Agilent, Palo Alto, CA) equipped with a C18 reverse phase column (Alltech Alltima, Part no. 88383) and a UV detector set at a wavelength of 263 nm. The mobile phase consisted of a mixture of phosphate buffer (0.2 M, pH 6.8):acetonitrile:water (100:1300:600).

The amount of free (unincorporated) bupivacaine in the formulation was determined by first mixing a 1-ml aliquot of the formulation with 0.01N HCl solution in a 10-ml volumetric flask. The mixture was shaken manually until no bupivacaine free base crystals was seen in the sample under an optical microscope. The time to obtain the complete dissolution of the free bupivacaine in the sample was determined to be 2 min. The sample was subsequently filtered through a 0.2 μm membrane after 2 min of shaking and the amount of bupivacaine in the filtrate was assayed by the previously described HPLC method.

2.5. Determination of injectability

Injectability of a formulation is defined as the smallest needle gauge that a liposphere sample can pass through. The injectability was determined by pushing 4 ml of sample from a 5-ml plastic disposable syringe through hypodermic needles ranging from 18 to 27 gauge. The formulation was first tested using the smallest needle (27 gauge). If the entire content of the sample passed through a 27 gauge needle, its injectability was recorded as 27. If the sample did not pass through a 27 gauge needle, the study was repeated using a 25 gauge needle, followed by the next smaller gauge needle.

2.6. Determination of gelation time

To determine the time for a liposphere formulation to solidify (gelation time), approximately 50 ml of the formulation was transferred in a 60-ml glass jar, sealed, and stored upright at 5 °C or room temperature. At each sampling time point, the jar was tilted 90°. The formulation was considered non-gelling if it flowed freely without agitation. The formulation was considered gelled if it exhibited resistance to free flowing when tilted or showed signs of physical breakdown (i.e. coalesce, coagulate, or phase separate). During the first 8 h of the gelation study, the formulation was evaluated hourly. After the first day of monitoring the formulation was tested daily. Upon a week of monitoring, the sample was examined approximately every 3 days and after 2 weeks, it was tested once a week.

3. Results and discussion

Freshly prepared bupivacaine liposphere formulations were homogeneous dispersions with solid liposphere particles uniformly dispersed in an aqueous buffer medium. The SEM photomicrograph shows that liposphere particles were spherical in shape with a smooth surface (Fig. 1). Some needle-shaped

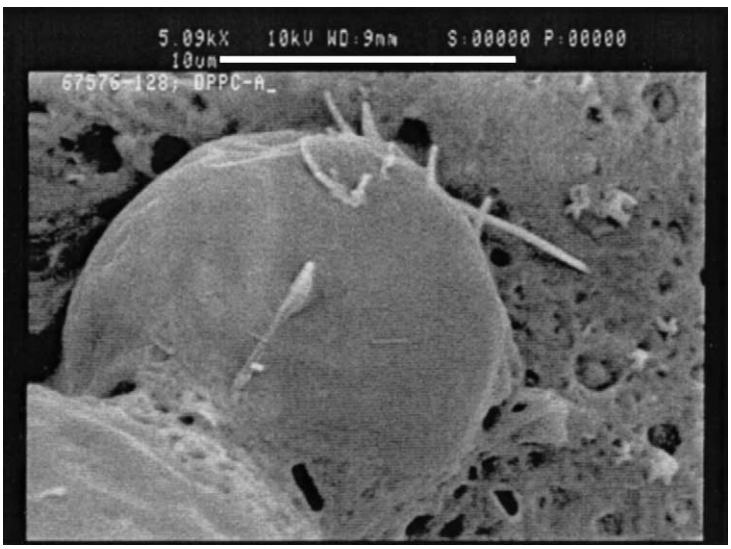

Fig. 1. SEM picture of a liposphere formulation containing 3% bupivacaine, 6% tristearin, and 3% DPPC.

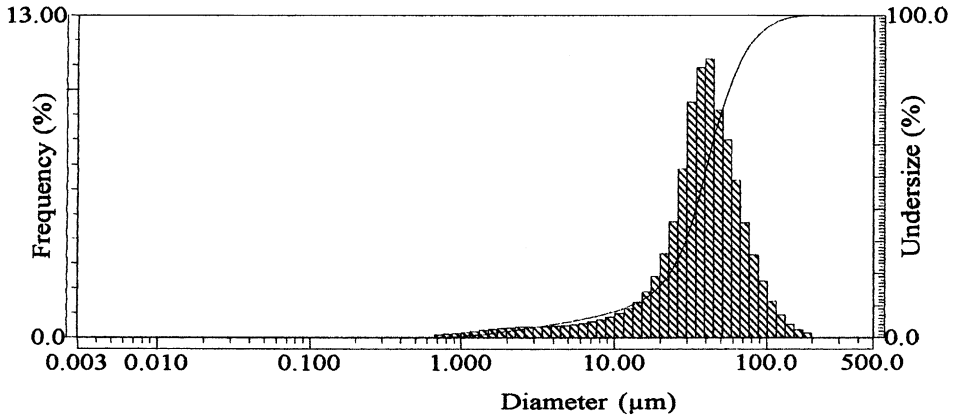

Fig. 2. A typical particle-size distribution plot of a bupivacaine liposphere formulation containing 3% bupivacaine, 6% tristearin, and 0.75% DMPC.

drug crystals were also seen on the surface of lipospheres. These crystals were probably the unincorporated bupivacaine that existed in a solubilized form or as insoluble crystals in the liposphere dispersion. Fig. 2 shows a typical particle-size distribution plot of a bupivacaine liposphere formulation containing 3% bupivacaine, 6% tristearin, and 0.75% DMPC. The diameter of lipospheres formulation ranged from 1 to 300 μm with a mean particle size of 50 μm.

Table 1 lists the compositions and physical properties of liposphere formulations prepared with various types of triglycerides and phospholipids. For all the formulations evaluated, the free drug contents of the formulations containing short chain triglycerides (tricaprin and trilaurin) were significantly higher than those of the formulations prepared with long chain triglycerides (tristearin and triarachidin). This may be attributed to the lower bupivacaine solubility in the lipid as a result of a decrease in hydrophobicity of the short chain triglycerides.

The free drug contents of the formulations prepared with natural phospholipids (EPC or SPC) were significantly higher than those of the formulations containing synthetic phospholipids. The formulation containing 3% bupivacaine, 6% tristearin, and 3% EPC showed 50% in free drug contents, indicating that only half of the drug in this formulation was incorporated in the lipid matrix. The SEM photomicrograph of the lipospheres from this formulation (Fig. 3) shows that there were much more needle-shaped crystals associated with the lipospheres as compared to the lipospheres of the DPPC-containing formulation (Fig. 1).

The combined effect of synthetic phospholipid and tristearin concentration on the free drug contents in 3% bupivacaine liposphere formulations was also investigated and the results are shown in Fig. 4. The response surface plot shows that at a constant tristearin level, an increase in DPPC concentration resulted in higher free drug contents. On the other hand, the free drug contents decreased with increasing tristearin concen-

Table 1
Bupivacaine liposphere formulations containing 3% bupivacaine and various type and concentration of triglycerides and phospholipids

Phospholipid	Triglyceride	Free Drug Content (%)	Injectability[a] (gauge)
3% EPC	6% TC	87.2[b]	ND
3% EPC	6% TL	76.5[b]	ND
3% EPC	6% TS	42.3[b]	ND
3% EPC	6% TA	50.0[b]	ND
3% DPPC	6% TC	43.0[b]	20
3% DPPC	6% TL	55.0 ± 3.8[c]	18
3% DPPC	6% TS	19.3 ± 2.0[c]	18
3% DPPC	6% TA	23.7 ± 1.0[c]	18
3% SPC	6% TS	77.6[b]	ND
3% DSPC	6% TS	16.7 ± 1.7[c]	18
0.75% DPPC	6% TS	10.0 ± 2.1[c]	18
0.75% DMPC	6% TS	19.7 ± 1.0[c]	23

[a] ND denotes that the injectability was not determined due to the gelation of the sample.
[b] $n = 1$.
[c] $n = 2$.

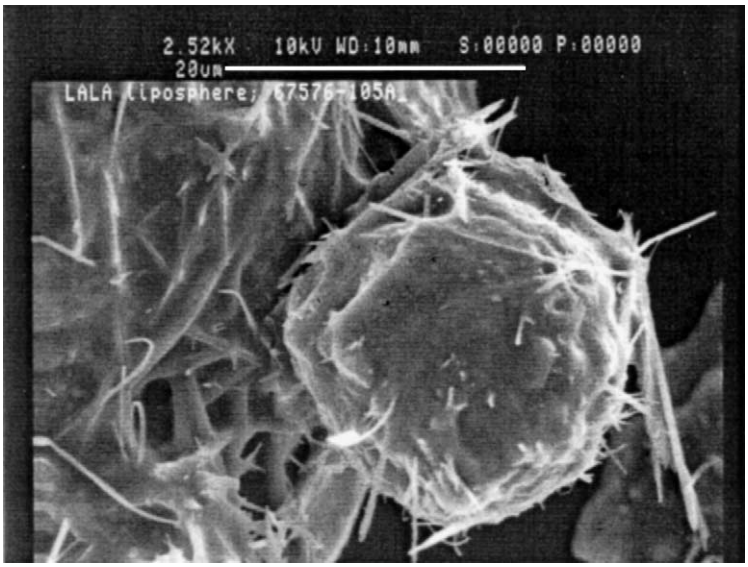

Fig. 3. SEM picture of a liposphere formulation containing 3% bupivacaine, 6% tristearin, and 3% EPC.

tration at a fixed DPPC level. The results also show that a 3% bupivacaine liposphere formulations with low free drug contents (i.e. high drug loading) can be obtained with a low synthetic phospholipid concentration and high triglyceride concentration.

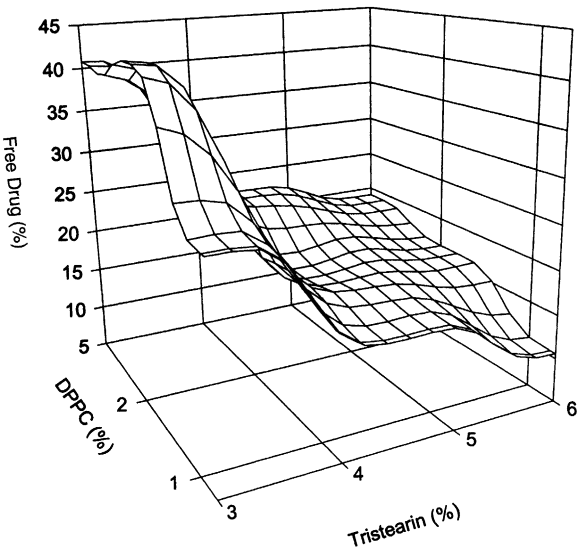

Fig. 4. A response surface plot showing the effect of tristearin and DPPC concentrations on the free drug content of liposphere formulations.

In addition to free drug contents, all formulations listed in Table 1 were evaluated for injectability. The formulations prepared with a natural phospholipid (EPC or SPC) solidified at room temperature within 2 h after preparation. All the formulations containing a synthetic phospholipid remained fluid at room temperature 48 h after preparation. These formulations could also be pushed through a syringe with an 18-gauge needle.

Upon storage at room temperature or 5 °C, some of the liposphere formulations changed from a fluid dispersion to a semisolid (Fig. 5). When a fluid liposphere sample and a gelled sample were examined and compared under an optical microscope, the amounts of needle-shaped drug crystals increased dramatically in the gelled sample (Figs. 6 and 7). The free drug contents in the gelled sample were also found to be two times higher than those in the fluid sample. These results have led us to conclude that the gelling of the liposphere dispersion was the result of formation of bupivacaine crystals in the aqueous medium of the dispersion. Therefore, a bupivacaine liposphere dispersion can remain fluid only if the crystal growth of bupivacaine can be prohibited or significantly retarded.

Table 2 presents the effect of drug concentration, type and concentration of phospholipids (synthetic versus natural) on the gelation time of various bupi-

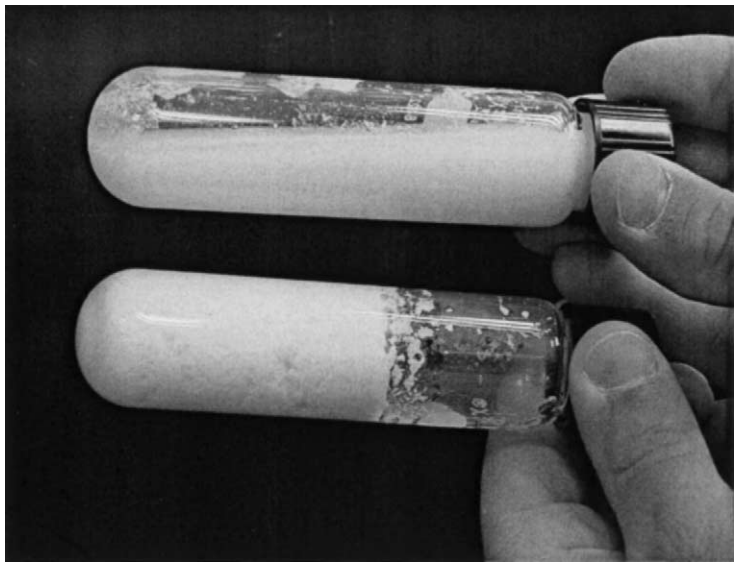

Fig. 5. A picture of a free flowing sample (upper tube) and a gelled sample (lower tube) of liposphere formulations.

vacaine liposphere formulations stored at room temperature and 5 °C. For all the formulations evaluated, the samples stored at room temperature gelled much faster than those stored at 5 °C. The rate of bupivacaine crystal growth was found to be faster at an elevated temperature. This phenomenon can be attributed to the increase in fluidity and permeability of the phospholipid membrane surrounding the liposphere particles at a high temperature. At 2.0 and 3.0% bupivacaine levels, formulations containing DMPC were found to be more stable than those containing EPC. Phospholipid from natural sources is a mixture of

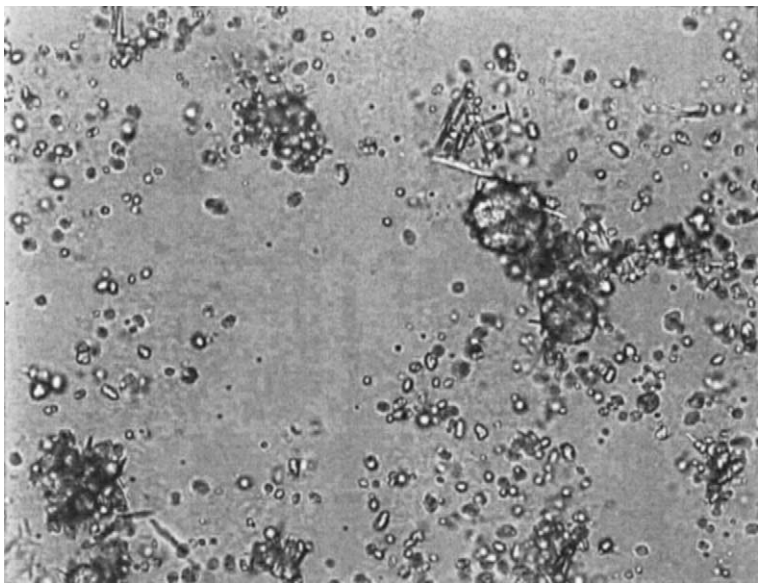

Fig. 6. Optical micrograph of a liposphere formulation containing 3% bupivacaine, 6% tristearin, and 3% EPC before gelling.

phosphatidylcholines with different fatty acid chain lengths and various degrees of saturation. About half of the fatty acids in EPC are unsaturated (Lasic, 1993). Membranes composed of unsaturated lipids are fluid and highly permeable while the membranes composed of synthetic phospholipids with fully saturated fatty acids are more condensed and rigid. The formation of such a condensed membrane of DMPC covering the surface of a bupivacaine liposphere may slow down the transfer of bupivacaine from the liposperes to the bulk solution leading to a slower rate of crystal growth. In addition, phase transition temperatures of natural phospholipid are significantly lower than those of synthetic phospholipid. The phase transition temperature is defined as the temperature required for the lipid physical state to change from the ordered gel phase, where the hydrocarbon chains are fully extended and closely packed, to the disordered liquid crystalline phase, where the hydrocarbon chains are randomly oriented and fluid. The phase behavior of a phospholipid membrane determines its fluidity and permeability. Phase transition temperatures of EPC and DMPC are below 0 and 25 °C, respectively. At storage temperatures of 5 and 25 °C, the natural phospholipid membranes were in leaky fluid-like state which allowed bupivacaine to leach out of the particles more readily resulting in a faster crystal growth rate and a shorter gelation time. The ordered gel phase of synthetic phospholipid membranes at the storage temperatures in this study provided a more stable, non-leaky enclosing system. The results in Table 2 also show that an increase in the concentration of DMPC prolonged the gelling time particularly at a higher drug concentration. A stable 4% bupivacaine liposphere formulation can only be prepared with 3% DMPC.

The rate of crystal growth in a suspension can be significantly slowed down by the addition of a water-soluble polymer (Moustafa et al., 1975; Ziller and Rupprecht, 1988). Cellulosic polymers also exhibit inhibitory effect on precipitation of water-insoluble drug in a supersaturated solution (Usui et al., 1997). The stabilization effect of the polymer is attributed to the increase in viscosity of the aqueous medium and the formation of a protective polymer layer on the surface of the solid particles in the suspension. An increase in viscosity of the medium can result in a slower rate of solute diffusion and the surface polymer layer can function as a barrier to the deposition of solute onto the solid particles. Both mechanisms play an important role in suppressing crystal growth in a suspension. In this study, the effect of CMC on the gelation of liposphere formulations was investigated. A concentrated CMC solution was added into the formulation either before or after the formation of liposperes. Table 3 lists the gelation time results of the formulations containing 3% bupivacaine, 6% tristearin, 0.5% CMC, and

Table 2
Gelation times of liposphere formulations containing 6% tristearin, various concentration of bupivacaine and different type and concentration of phospholipid

Bupivacaine (%)	Phospholipid	Gelation time	
		Room temperature	5 °C
2.0	0.75% EPC	21 h	306 days
	0.75% DMPC	308 days	>602 days
	3.0% EPC	2 h	174 days
	3% DMPC	60 days	>613 days
3.0	0.75% EPC	Coagulate	Coagulate
	0.75% DMPC	58 days	>613 days
	3.0% EPC	2 h	5 h
	3% DMPC	91 days	>597 days
4.0	0.75% EPC	2 h	2 h
	0.75% DMPC	Coagulate	Coagulate
	3.0% EPC	1 h	1 h
	3% DMPC	85 days	>570 days

Table 3
Gelation times of liposphere formulations containing 3% bupivacaine, 6% tristearin, and different type and concentration phospholipid with and without 0.5% CMC

CMC	Phospholipid	Gelation time	
		Room temperature	5 °C
No CMC	0.75% EPC	Coagulate	Coagulate
	0.75% DMPC	58 days	>613 days
	3.0% EPC	2 h	5 h
	3% DMPC	91 days	>597 days
0.5% CMC	0.75% EPC	1 h	12 days
	0.75% DMPC	>468 days[a]	>468 days
	3.0% EPC	1 h	12 days
	3% DMPC	>468 days[a]	>468 days

[a] Sample exhibited sedimentation but was easily redispersed upon gentle agitation.

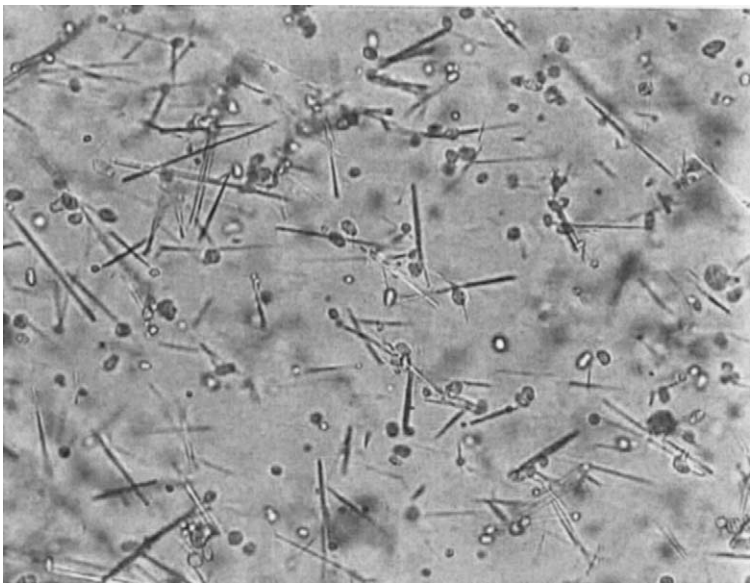

Fig. 7. Optical micrograph of a liposphere formulation containing 3% bupivacaine, 6% tristearin, and 3% EPC after gelling.

phospholipid (0.75 or 3.0% of DMPC or EPC). The presence of CMC has improved the physical stability of liposphere formulations. The gelation time of all DMPC-containing formulations stored at room temperature was significantly prolonged by the addition of CMC. The formulation containing 3% bupivacaine, 6% tristearin, and 0.75% DMPC solidified after 58 days while the same formulation with added 0.5% CMC has remained fluid for at least about 16 months. The addition of CMC in liposphere formulation was also shown to prolong the gelation time of some EPC-containing formulations at both room temperature and 5 °C. Sedimentation has been observed with some room temperature samples, but the samples were easily redispersed with gentle shaking by hand.

4. Conclusions

The crystal growth of bupivacaine in an aqueous dispersion medium was shown to result in the gelation of bupivacaine liposphere formulations during storage. The gelation time was found to be a function of storage temperature, drug concentration, and the type of phospholipids used. Formulations prepared with a synthetic phospholipid exhibited significantly improved physical stability. The addition of CMC in the formulation can result in 3% bupivacaine lipospheres physically stable for more than 1 year upon storage at room temperature.

References

Domb, A.J., 1993a. Liposphere Delivery Systems for Local Anesthetics. US Patent 5,227,165, 13 Jul.

Domb, A.J., 1993b. Lipospheres for Controlled Delivery of Substances. US Patent 5,188,837, 23 Feb.

Kopacz, D.J., Bernards, C.M., Allen, H.W., Landau, C., Nandy, P., Wu, D., Lacouture, P.G., 2003. A method to evaluate the pharmacokinetic and pharmacodynamic variables of extended-release products using in vivo tissue microdialysis in humans: bupivacaine-loaded microcapsules. Anesth. Analg. 97, 124–131.

Lasic, D.D., 1993. Liposomes: a Practical Approach. Elsevier Science Publishers, Amsterdam.

Malinovsky, J.M., Bernard, J.M., Le Corre, P., Dumand, J.B., Lepage, J.Y., Le Verge, R., Souron, R., 1995. Motor and blood pressure effects of epidural sustained-release bupivacaine from polymer microspheres: a dose-response study in rabbits. Anesth. Analg. 81, 519–524.

Malinovsky, J.M., Benhamou, D., Alafandy, M., Mussini, J.M., Coussaert, C., Couarraze, G., Pinaud, M., Legros, F.J., 1997. Neurotoxicological assessment after intracistern al injection of

liposomal bupivacaine in rabbits. Anesth. Analg. 85, 1331–1336.

Malinovsky, J.M., Le Corre, P., Meunier, J.F., Chevanne, F., Pinaud, M., Leverge, R., Legros, F., 1999. A dose-response study of epidural liposomal bupivacaine in rabbits. J. Control. Release 60, 111–119.

Masters, D.B., Berde, C.B., Dutta, S.K., Griggs, C.T., Hu, D., Kupsky, W., Langer, R., 1993. Prolonged regional nerve blockade by controlled release of local anesthetic from a biodegradable polymer matrix. Anesthesiology 79, 340–346.

Moustafa, M.A., Ebian, A.R., Khalil, S.A., Motawi, M.M., 1975. Succinylsulfathiazole crystal forms. III. Crystal growth studies. J. Pharm. Sci. 64, 1485–1489.

Physician Desk's Reference, 2003. Medical Economics. Montvale, NJ, pp. 642–644.

Usui, F., Maeda, K., Kusai, A., Nashimura, K., Yamamoto, K., 1997. Inhibitory effects of water-soluble polymers on precipitation of RS-8359. Int. J. Pharm. 154, 59–66.

Ziller, K.H., Rupprecht, H., 1988. Control of crystal growth in drug suspensions. Drug Dev. Ind. Pharm. 14, 2341–2370.