Entrapping Efficiency and Drug Release Profile of an Oil-in-Water (o/w) Emulsion Formulation Using a Polydimethylsiloxane-Coated Glass Bead Assay

Toshiya Minagawa,[1,2] Yoshiro Kohno,[1] Toshio Suwa,[1] and Akira Tsuji[3]

Received June 11, 1993; accepted October 15, 1993

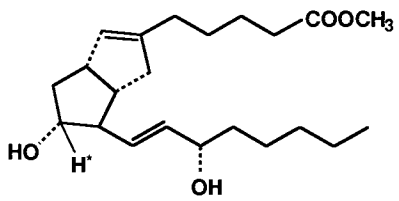
Evaluation of entrapping efficiency is difficult for an o/w emulsion formulation containing a lipophilic oily drug, isocarbacyclin methyl ester (TEI-9090), by commonly employed techniques (dialysis, ultrafiltration, or gel filtration), because of its adsorption to the system materials. Employing this characteristic of TEI-9090, we developed an adsorption technique with polydimethylsiloxane-coated glass beads (PDMS-GB). The assay is based on the quantitative adsorption of unentrapped TEI-9090 to the PDMS-GB. The entrapping efficiency of a 10% soybean oil emulsion containing [^{3}H]TEI-9090 (1 μg/mL) assayed by this method approached 100%. The PDMS-GB assay was performed for the emulsion diluted 100 times with physiological saline at different time intervals after dilution over a period of 24 hr. A plot of [^{3}H]TEI-9090 in the emulsion particles versus time showed rapid release within 1 hr, followed by very slow release, reaching equilibrium. Applying first-order kinetics, the data were found to fit to a biexponential equation over the first hour of release. The terminal release resembled the first-order release of the drug from the phospholipid-rich infranatant, which was separated from the creamy layer by ultracentrifugation of the emulsion and contained 35% [^{3}H]TEI-9090. These results suggest that the drug is released from two components in the emulsion.

KEY WORDS: drug delivery; emulsion; entrapping efficiency; polydimethylsiloxane; glass beads; kinetics.

INTRODUCTION

Parenteral oil-in-water (o/w) emulsions can improve the therapeutic efficacy of drugs (1–5), and successful clinical use of this dosage form has recently been achieved (6–8). The lipophilic modification of drugs, to yield prodrugs, should ensure high entrapment in the oil droplets of emulsions (1,9). Recently it was shown that an o/w emulsion formulation of isocarbacyclin methyl ester, TEI-9090 (10) (Scheme I), dramatically increased its *in vivo* antithrombotic activity compared with the corresponding aqueous solution (11).

The entrapping efficiency of an o/w emulsion formulation of a drug might become an important issue in determining its biological activity, in addition to pharmaceutical characteristics. Although dialysis (9,12), ultrafiltration (13), and gel filtration (14) techniques have commonly been employed, it is difficult to apply these techniques to some lipophilic drugs, such as TEI-9090, because of their adsorption to the dialysis tubes, filters, or resins. This study was designed to establish an alternative method to evaluate rapidly and quantitatively the entrapping efficiency of an emulsion containing TEI-9090, using as an adsorbent, glass beads coated with polydimethylsiloxane (PDMS-GB). Furthermore, using this assay, the drug release profile of the emulsion formulation was characterized.



Scheme I. Chemical structure of [^{3}H]TEI-9090. (*) Labeled position of ^{3}H.

MATERIALS AND METHODS

Chemicals. 11-β-[^{3}H]TEI-9090 (sp act, 0.67 TBq/mmol; >99% pure) and the unlabeled compound were kindly donated by Teijin Co., Ltd., Tokyo. Egg yolk lecithin (Asahi Chemical Industry Co., Tokyo), soybean oil (Ajinomoto Co., Inc., Tokyo), and glycerin (Kozakai Pharmaceutical Co., Ltd., Tokyo) were intravenous grade. Polydimethylsiloxane (PDMS) was purchased from Fuji Systems Corp. (Tokyo). All other chemicals and solvents were analytical grade and were obtained commercially.

Preparation of PDMS-Coated Glass Beads (PDMS-GB). Ten milliliters of PDMS solution was diluted to 500 mL with MeOH. Four hundred grams of glass beads (Toshin-riko Co., Tokyo; 1.0- to 1.4-mm diameter) was washed, dried well, and soaked in the PDMS solution for 24 hr at room temperature. After decantation, the glass beads were washed with 500 mL of MeOH and dried at 40°C. Then the glass beads were heated at 100°C for 1 hr to complete bonding of PDMS to the glass surface.

Preparation and Fractionation of an o/w Emulsion Containing [^{3}H]TEI-9090. An o/w emulsion containing [^{3}H]TEI-9090 was prepared with a French press (Aminco Instrument Co., MD) (15,16). In brief, a mixture of 1000 mg of soybean oil containing 10 μg of [^{3}H]TEI-9090 and unlabeled TEI-9090, 120 mg of egg yolk lecithin, 221 mg of glycerin, and a sufficient volume of distilled water to make a final volume of 10 mL was dispersed in a French pressure cell (2.5-cm inner diameter) at 28,000 psi. The particle size distribution was determined by photon correlation spectroscopy (Nicomp Model 370, Nicomp Instruments, Division Pacific Scientific, CA). Fractionation of the emulsion into the triacylglycerol-rich creamy layer and the infranatant was carried out by the ultracentrifugation method of Hajri *et al.* (17). Total phospholipid and triacylglycerol concentrations

[1] Research Center, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Ohmiya-shi, Saitama 330, Japan.
[2] To whom correspondence should be addressed.
[3] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.

0724-8741/94/0400-0503$07.00/0

were determined using the enzymatic colorimetric kits, Phospholipid C-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Anasolv TG-2 (Daiichi Pure Chemicals Co., Ltd., Tokyo), respectively.

PDMS-GB Assay. Standard solutions of [^{3}H]TEI-9090 were prepared by placing 10 μL of EtOH stock solutions in physiological saline to make a final volume of 1 mL in 3-mL glass assay tubes with polypropylene caps. Dilution of the emulsions with saline was carried out in the same manner in which the standard solutions were prepared. Assay was initiated by adding 3 g of PDMS-GB to the assay tube containing the sample at room temperature (Fig. 1). After shaking the tube vigorously for 15 sec, the assay was terminated by withdrawing a 0.1-mL aliquot. Eppendorf Comfortips used for sampling the aliquot were washed routinely with 0.2 mL of EtOH, which was placed in a counting vial containing the aliquot and 10 mL of Instagel (Packard Instruments Co., IL). The radioactivity was counted in a liquid scintillation counter (LS 6000 TA, Beckman Instruments Inc., CA). To determine the amount of [^{3}H]TEI-9090 adsorbed to the assay tubes, after discarding the contents, the radioactivity in 1 mL of EtOH wash from the inner wall was counted.

The stability of [^{3}H]TEI-9090 was checked by high-performance liquid chromatography (HPLC). The HPLC system consisted of an Auto-Analytical HPLC system (Gilson Medical Electronics Inc., France) and a Ramona LS-90 radiodetector (Raytest Isotopenemeßgeräte GmbH, Germany) operated at 2 mL/min of the liquid scintillator, Instagel. A Lichrosorb RP-18 column (4 × 250 mm, 7 μm; E. Merck, Darmstadt, Germany) was used, and gradient elutions of acetonitrile/water/acetic acid from 50:50:0.1 to 100:0:0.1 (v/v) in 20 min at a flow rate of 1 mL/min were performed. Ten microliters of [^{3}H]TEI-9090 emulsions was diluted with tetrahydrofuran to 0.1 mL, and a 20-μl aliquot was injected.

Recovery Test to Determine Drug Adsorption to the Glass Assay Tubes. The simple recovery tests were performed, without PDMS-GB assay, as follows. The radioactivity in the diluted emulsion containing [^{3}H]TEI-9090, prepared as described above, was determined by counting a 0.1-mL aliquot at different time intervals after dilution. The same evaluation of a 1 μg/mL EtOH solution of [^{3}H]TEI-9090 was also performed in a control study.

Partition Coefficient Determination. A sufficient volume of physiological saline was added to 1.0 g of soybean oil solution containing 10 μg/g of [^{3}H]TEI-9090 to make a final volume of 10 mL. The oil/water mixture was shaken for 24 hr at room temperature and centrifuged at 3500 rpm for 20 min. The radioactivity concentrations in the oil and aqueous layers were determined to calculate the partition coefficient (PC).

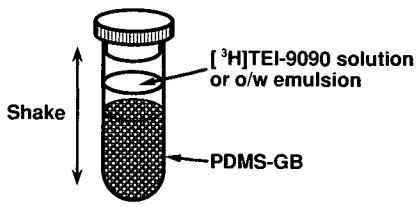


Fig. 1. Setup for PDMS-GB assay for [^{3}H]TEI-9090 solution or an o/w emulsion. See the text for details.

RESULTS AND DISCUSSION

TEI-9090 is a stable prostaglandin I_2 analogue, first synthesized by Shibazaki *et al.* (10), which is practically insoluble in water and is liable to be adsorbed to polymer materials as well as to glass. When 10 ng of [^{3}H]TEI-9090 was added to 1 mL of physiological saline in the glass tubes, 42% of the total radioactivity was adsorbed to the tubes (Table I). Then, employing this characteristic of TEI-9090, we conceived an adsorption technique to evaluate the entrapping efficiency of its o/w emulsion formulation.

Of all the polymers examined, silicone materials adsorbed TEI-9090 to the highest degree, even higher than glass. This prompted us to utilize silicone as the adsorbent, since it could suppress adsorption of the drug to the glass wall. Thereby, glass beads coated with the silicone polymer PDMS, which had enough surface area to adsorb the drug and sufficient weight to sink quickly, were examined in the following experiments.

Standard Assay

The optimum amount of PDMS-GB and assay time were first determined with a saline solution of [^{3}H]TEI-9090. Adsorption of [^{3}H]TEI-9090 increased as the amount of PDMS-GB added was increased (Table I). When more than 2.0 g of the glass beads was added, the residual radioactivity adsorbed to the assay tubes was less than 1.0% of the drug added. However, 3.0 g of the beads was the upper limit in the 3-mL assay tube to remove 0.1 mL of the upper solution accurately without any disturbance by the glass beads. Adsorption of [^{3}H]TEI-9090 to PDMS-GB almost reached saturation in 15 sec, and adsorption to the assay tubes was negligible (data not shown).

The standard curve for [^{3}H]TEI-9090, assayed under the optimum conditions described above, was linear for the addition of up to 1000 ng. The linear regression equation was

$$Y = 0.749X + 1.585 \quad (1)$$

with a correlation coefficient of 0.999. Therefore, for up to 1000 ng of the [^{3}H]TEI-9090 assayed, about 75% of the drug was constantly adsorbed to PDMS-GB. The residual quantity might exist to the aqueous phase in equilibrium with the adsorbed drug. Using the standard curve, the apparent

Table I. Optimum Amount of PDMS-GB for the Assay[a]

Amount of PDMS-GB added (g)	[^{3}H]TEI-9090 (%)[b]	
	Adsorbed to PDMS-GB[c]	Residue in the assay tubes[d]
0	—	42 ± 3
1.0	67 ± 4	2.5 ± 0.4
2.0	71 ± 3	0.9 ± 0.2
3.0	83 ± 1	0.3 ± 0.1

[a] Ten nanograms of [^{3}H]TEI-9090 was added to the tubes and experiments were carried out using an assay time of 15 sec.
[b] Each value represents the mean ± SD of three samples.
[c] Percentage calculated from the difference in radioactivity between the total and the assayed solutions.
[d] Percentage of radioactivity in the EtOH wash from the used tubes.

amount of [^{3}H]TEI-9090 in the aqueous phase ($S_{tot} - S_{sup}$) can be revised to the true amount (S_f), by the following equation:

$$S_f = \frac{(S_{tot} - S_{sup}) - a}{b} \quad [ng] \qquad (2)$$

where S_{tot} is the total radioactivity, S_{sup} is the amount of the drug in the assayed solution in the assay tube, and a and b are the intercept (1.585) and slope (0.749) of the standard curve, respectively. To determine the entrapping efficiency of the o/w emulsion (E), the difference between S_{tot} and S_f can be established [Eq. (3)].

$$E = \left(1 - \frac{S_f}{S_{tot}}\right) \times 100 \quad [\%] \qquad (3)$$

Entrapping Efficiency of the 10% Soybean Oil Emulsion Containing [^{3}H]TEI-9090

PDMS-GB assay for three batches of the 10% soybean oil emulsions containing [^{3}H]TEI-9090 was carried out. In the course of the assay procedure, the mean particle diameters of the emulsions were not changed (Table II), and destruction of [^{3}H]TEI-9090 was not observed on RI-HPLC (Fig. 2). The entrapping efficiency was almost 100%, and the amount of drug in the aqueous phase of the emulsion was negligible (Table II). These results indicate that PDMS-GB did not change the characteristics of the emulsion, or remove TEI-9090 from the emulsion particles, or degrade the drug.

PDMS-GB Assay for the Emulsion Diluted with Saline

A drug-containing o/w emulsion is often diluted in parenteral nutrition for intravenous injection in clinical applications. For an emulsion containing [^{3}H]TEI-9090 diluted 100 times with saline, PDMS-GB assay was performed at different time intervals after dilution. Figure 3 shows a plot of the drug entrapment of the emulsion versus time after dilution. [^{3}H]TEI-9090 in the particles was decreased rapidly within 1 hr, and equilibrium was established over a period greater than 1 hr. In general, release patterns under nonsink conditions were analyzed applying first-order kinetics (18,19). The line in Fig. 3B was generated from the parameters determined by a computer fit of the data to the following biexponential equation using PC-NONLIN (Statistical Consultants Inc., Lexington, KY):

$$A - A_{ss} = 3.61e^{-0.42t} + 0.75e^{-0.0057t} \qquad (4)$$

Table II. Entrapping Efficiency of the 10% Soybean Oil Emulsion Containing [^{3}H]TEI-9090 and Influence of PDMS-GB on the Particle Size Distribution

No.	Entrapment (%)	Particle size (nm)[a]	
		Control	Assayed
1	100.3	218 ± 73	237 ± 85
2	99.9	220 ± 75	224 ± 70
3	100.2	235 ± 64	224 ± 76

[a] Each value represents the mean diameter ± SD.

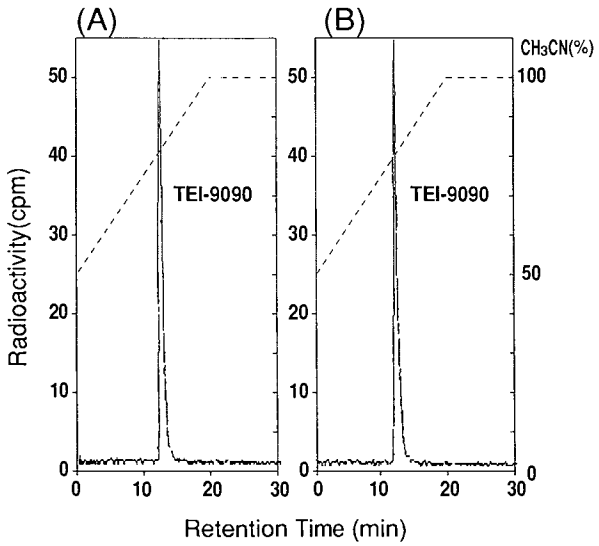


Fig. 2. HPLC of [^{3}H]TEI-9090 in 10% oil emulsion before (A) and after (B) the PDMS-GB assay. The PDMS-GB assay and HPLC procedure were performed under standard conditions as described in the text.

where A is the amount of [^{3}H]TEI-9090 in the emulsion particles and A_{ss} is the amount at steady state (6.30 ng).

The partition coefficient (PC) of [^{3}H]TEI-9090 into the soybean oil and saline was found to be 1300. The volume ratio of the soybean oil (d = 0.926) to the aqueous phase in the 1/100 emulsion was 1/926. The drug in the oil droplets can be calculated to be 5.80 ng in the case of the 1/100 emulsion. However, A_{ss} was obviously higher than the amount in the oil droplets calculated from the PC. This fact revealed that the entrapment and release of the drug cannot be explained with a simple two-phase model of the oil and aqueous phase (20). Therefore, other mechanisms by which the release kinetics in the present study can be explained must be proposed.

Hajri *et al.* (17), Westesen and Wehler (21), and Rotenberg *et al.* (22) have reported that the parenteral o/w emulsion contains phospholipid vesicles as well as oil droplets. When we carried out fractionation of the emulsion containing [^{3}H]TEI-9090 by the ultracentrifugation method (17), phospholipid-rich infranatant, separated from floating

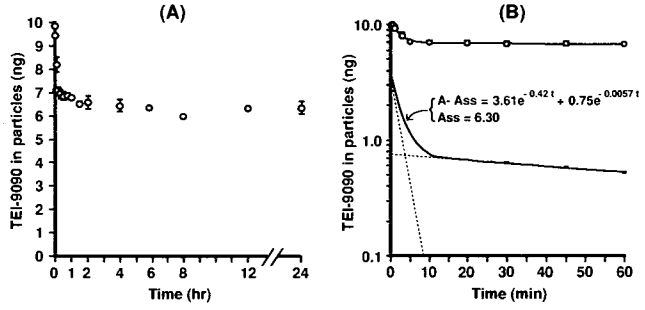


Fig. 3. Plot of the amount of drug in the emulsion particles versus time (A) and semilogarithmic plot of data within 1 hr (B). The emulsion containing [^{3}H]TEI-9090 was diluted 100 times with saline. The line in B was generated by a computer fit of the data according to Eq. (4). Each point represents the mean ± SD of three samples.

Table III. Chemical Composition of Fractions Prepared from the 10% Soybean Oil Emulsion Containing [^{3}H]TEI-9090 by Ultracentrifugation

	Percentage[a]	
	Cream	Infranatant
Radioactivity	67 ± 0	35 ± 1
Triacylglycerols	84 ± 4	10 ± 1
Phospholipids	72 ± 5	30 ± 1

[a] Percentage of total amount of radioactivity, triacylglycerols, and phospholipids in the o/w emulsion containing 1 μg/mL [^{3}H]TEI-9090, respectively. Each value represents the mean ± SE of three samples.

creamy emulsion particles, contained 35% [^{3}H]TEI-9090, as shown in Table III. For the infranatant thus obtained, PDMS-GB assay was carried out after 100 times dilution with saline in the same manner as for the emulsion. As shown in Fig. 4, [^{3}H]TEI-9090 in the infranatant was released slowly, and equilibrium was established over a period of 4 hr. Analysis applying first-order kinetics gave the following equation:

$$A_i - A_{i_{ss}} = 0.23e^{-0.0067t} \quad (5)$$

where A_i is the amount of unreleased drug in the infranatant and $A_{i_{ss}}$ is the amount at steady state (2.75 ng).

The release rate constant, 0.0067 min^{-1} in Fig. 4, resembled the terminal release rate constant (0.0057 min^{-1}) of the drug from the diluted o/w emulsion. These findings suggest that the second linear segment in Fig. 3B would show drug release from the phospholipid vesicles, although further physicochemical characterization is needed to identify the components other than the oil droplets. Then it is likely that the first phase in Fig. 3B would show drug release from the oil droplets. However, other possibilities, such as release of the drug existing at the surface of the oil droplets (23–25), cannot be ruled out at the present time.

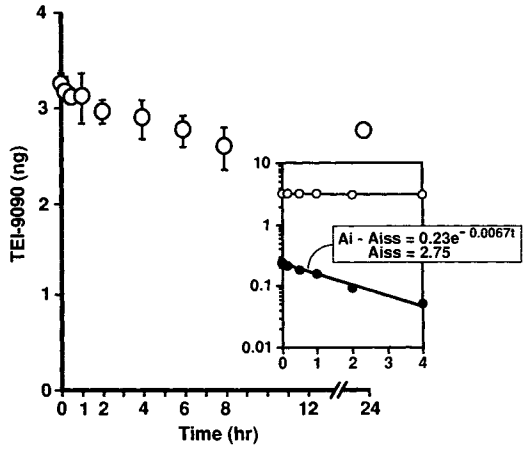


Fig. 4. Plot of the amount of drug in the infranatant prepared from the o/w emulsion containing [^{3}H]TEI-9090 versus time. The inset is a semilogarithmic plot of the data within 4 hr. The infranatant containing [^{3}H]TEI-9090 was diluted 100 times with saline. The line was generated by a computer fit of the data according to Eq. (5). Each point represents the mean ± SD of three samples.

Table IV. Recovery Test for the o/w Emulsion Containing [^{3}H]TEI-9090 Diluted with Saline in Glass Tubes

Formulation	Dilution	[^{3}H]TEI-9090 (ng)	Recovery of [^{3}H]TEI-9090 in solution (%)[a]	
			1 min	1 hr
Saline solution	—	5	48 ± 3	37 ± 6
(1 mL)	—	10	58 ± 3	47 ± 3
o/w emulsion[b]	1/100	10	97 ± 0	98 ± 1
	1/200	5	97 ± 1	92 ± 2

[a] Each value represents the mean ± SD of three samples.
[b] [^{3}H]TEI-9090 emulsion was diluted with saline to make a final volume of 1 mL.

Recovery Test

If [^{3}H]TEI-9090 were released into the aqueous phase from the emulsion particles, adsorption of the drug to the glass tube could be observed. This can be easily examined by a simple recovery test for the drug-containing emulsion diluted with saline in the glass tube, without PDMS-GB assay. While only 37 or 47% of the radioactivity was recovered in the saline containing 5 or 10 ng of [^{3}H]TEI-9090 at 1 hr, the drug was recovered almost-completely in the 1/100 and 1/200 emulsions (Table IV). These data indicate that the drug was not released into the aqueous phase but to the surface area of the particles, where the drug was easily adsorbed to PDMS-GB but not to the glass tubes. Accordingly, the curves in Fig. 3 and Fig. 4 would be regarded as the time-dependent alteration of the distribution of the drug in the particles rather than the drug release.

CONCLUSIONS

A new method for evaluating the entrapping efficiency and drug release profiles of an o/w emulsion containing TEI-9090 has been described. Using our newly developed PDMS-GB assay, it was confirmed that the lipophilic modification of drugs offers a clear advantage in preparing suitable drug-containing o/w emulsion formulations.

REFERENCES

1. Y. Mizushima, T. Hamano, and K. Yokoyama. Tissue distribution and anti-inflammatory activity of corticosteroids incorporated in lipid emulsion. *Ann. Rheum. Dis.* **41**:263–267 (1982).
2. T. Kronevi and S. Ljungberg. Sequelae following intra-arterially injected diazepam formulations. *Acta Pharm. Suec.* **20**:389–396 (1983).
3. H. Yamaguchi, K. Watanabe, M. Hayashi, and S. Awazu. Effect of egg yolk phospholipids plasma elimination and tissue distribution of coenzyme Q_{10} administered to an emulsion to rats. *J. Pharm. Pharmacol.* **36**:768–769 (1984).
4. S. Venkataram, W. M. Awni, K. Jordan, and Y. E. Rahman. Pharmacokinetics of two alternative dosage forms for cyclosporine: Liposomes and Intralipid. *J. Pharm. Sci.* 79:216–219 (1990).
5. K. Iwamoto, T. Kato, M. Kawahara, N. Koyama, S. Watanabe, Y. Miyake, and J. Sunamoto. Polysaccharide-coated oil droplets in oil-in-water emulsions as targetable carriers for lipophilic drugs. *J. Pharm. Sci.* **80**:219–224 (1991).
6. O. Von Dardel, C. Mebius, T. Mossberg, and B. Svensson. Fat

emulsion as a vehicle for diazepam. A study of 9492 patients. *Br. J. Anaesth.* 55:41–47 (1983).
7. Y. Mizushima, A. Yanagawa, and K. Hoshi. Prostaglandin E_1 is more effective when incorporated in lipid microsphere, for treatment of peripheral vascular diseases in man. *J. Pharm. Pharmacol.* 35:666–667 (1983).
8. P. J. Simons, I. D. Cockshott, E. J. Douglas, E. A. Gordon, K. Hopkins, and M. Rowland. Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of ^{14}C-propofol. *Xenobiotica* 18:429–440 (1988).
9. H. Sasaki, Y. Takakura, M. Hashida, T. Kimura, and H. Sezaki. Antitumor activity of lipophilic prodrugs of mitomycin C entrapped in liposome or o/w emulsion. *J. Pharmacobio-Dyn.* 7:120–130 (1984).
10. M. Shibasaki, Y. Torisawa, and S. Ikegami. Synthesis of 9(O)-methano-$\Delta^{6(9a)}$-PGI_1: The highly potent carbon analog of prostacyclin. *Tetrahedron Lett.* 24:3493–3496 (1983).
11. Y. Mizushima, R. Igarashi, K. Hoshi, A. K. Sim, M. E. Cleland, H. Hayashi, and J. Goto. Marked enhancement in antithrombotic activity of isocarbacyclin following its incorporation into lipid microspheres. *Prostaglandins* 33:161–168 (1987).
12. D. Friedman and S. Benita. A mathematical model for drug release from o/w emulsions: Application to controlled release morphine emulsions. *Drug Dev. Ind. Pharm.* 13:2067–2085 (1987).
13. D. L. Teagarden, B. D. Anderson, and W. J. Petre. Determination of the pH-dependent phase distribution of prostaglandin E_1 in a lipid emulsion by ultrafiltration. *Pharm. Res.* 5:482–487 (1988).
14. F. Szoka Jr. and D. Papahadjopoulos. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. *Proc. Natl. Acad. Sci. USA* 75:4194–4198 (1978).
15. Y. Barenholzt, S. Amselem, and D. Lichtenberg. A new method for preparation of phospholipid vesicles (liposomes)—French press. *FEBS Lett.* 99:210–214 (1979).
16. R. L. Hamilton Jr., J. Goerke, L. S. S. Guo, M. C. Williams, and R. J. Havel. Unilamellar liposomes made with the French pressure cell: A simple preparative and semiquantitative technique. *J. Lipid Res.* 21:981–992 (1980).
17. T. Hajrl, J. Ferezou, and C. Lutton. Effects of intravenous infusions of commercial fat emulsions (Intralipid 10 or 20%) on rat plasma lipoproteins: Phospholipids in excess are the main precursors of lipoprotein-X-like particles. *Biochim. Biophys. Acta* 1047:121–130 (1990).
18. J. G. Wagner. Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. *J. Pharm. Sci.* 58:1253–1257 (1969).
19. M. El-Samaligy and P. Rohdewald. Triamcinolone diacetate nanoparticles, a sustained release drug delivery system suitable for parenteral administration. *Pharm. Acta Helv.* 57:201–204 (1982).
20. A. A. El-Sayed and A. J. Repta. Solubilization and stabilization of an investigational antineoplastic drug (NSC no. 278214) in an intravenous formulation using an emulsion vehicle. *Int. J. Pharm.* 13:303–312 (1983).
21. K. Westesen and T. Wehler. Physicochemical characterization of a model intravenous oil-in-water emulsion. *J. Pharm. Sci.* 81:777–786 (1992).
22. M. Rotenberg, M. Rubin, A. Bor, D. Meyuhas, Y. Talmon, and D. Lichtenberg. Physico-chemical characterization of Intralipid™ emulsions. *Biochim. Biophys. Acta* 1086:265–272 (1991).
23. C. Washington, S. J. Taylor, and S. S. Davis. The structure of colloidal formulation of amphotericin B. *Int. J. Pharm.* 46:25–30 (1988).
24. D. L. Teagarden, B. D. Anderson, and W. J. Petre. Dehydration kinetics of prostaglandin E_1 in a lipid emulsion. *Pharm. Res.* 6:210–215 (1989).
25. S. Silvestri, L. L. Wu, and B. Bowser. Release of polyionizable compounds from submicrometer oil-in-water emulsions. *J. Pharm. Sci.* 81:413–418 (1992).