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Formulation and Comparative Characterization of Chitosan, Gelatin, and Chitosan–Gelatin-Coated Liposomes of CPT-11–HCl

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Liposomes containing phosphatidylcholine and cholesterol (uncoated) and coated by chitosan, gelatin, and combination of chitosan and gelatin were prepared by the modified ethanol injection method. The aim of this work was to formulate and characterize liposomes of camptothecin (CPT)-11–HCl (Irinotecan HCl) containing chitosan, gelatin, and both polymers as coating materials; and also to increase its circulation longevity when compared with the free drug while maintaining the agent in its active lactone form. Size, shape, zeta potential, encapsulation efficiency, stability study, in vitro, and in vivo release study were used for characterization of liposomes. The size of liposomes was in the order of uncoated < chitosan coated < gelatin coated < combination of chitosan and gelatin coated. The zeta potential of liposomes was in the order of combination of chitosan and gelatin coated > chitosan coated > gelatin coated > uncoated. The formulations showed the long-term stability. The encapsulation efficiency of liposomes was in order of combination of chitosan and gelatin coated > gelatin coated > chitosan coated > uncoated. The in vitro and in vivo release of drug was observed in the order of combination chitosan and gelatin coated > gelatin coated > chitosan coated > uncoated.

Keywords liposomes; chitosan; gelatin; CPT-11–HCl

INTRODUCTION

Liposomes are biocompatible and capable of incorporating both hydrophilic and lipophilic drugs and it has been investigated as effective drug carrier (Amamath & Umas, 1997; Gregoriadis, 1995; Lasic, 1998) to improve the therapeutic activity by encapsulation of the active agent, increase drug stability, reduce toxicity, improve drug distribution measures, and most importantly, improve therapeutic effects (Bakker-Woudenberg, Lokerse, & Roerdink, 1989; Madden, Janoff, & Cullis, 1990; Nacucchio, Gatto Bellora, Sordelli, & D'Aquino, 1988). However, the stability problem of liposomes during storage and administration restricts their application and development. Many attempts have been made to enhance the stability of liposomes. The modification of bilayer composition alone is one of the aspects to get stable liposomes (Cocera, Lopez, Coderch, Parra, & de la Maza, 2003). Successful results were obtained by the modification of liposome with several substances, such as poly (ethylene glycol) (Allen, Hansen, & Martin, 1991), poloxamer (Jamshaid, Fair, & Kearney, 1988), polysorbate 80 (Jorg, 2001), carboxymethyl chitin (Dong & Rogers, 1991), chitosan (Guo, Ping, & Jiang, 2003; Rengel & Barisic, 2002), and dextran derivatives (Elferink, Wit, & Veld, 1992), and have been used for the preparation of polymer-coated liposomes.

Irinotecan (camptothecin [CPT]-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]—carbonyloxycamptothecine) is a water-soluble derivative of camptothecine (Sawada et al., 1991) that is currently used in the treatment of advanced colorectal cancer. Irinotecan is a prodrug that needs to be converted to SN-38 (7-ethyl-10-hydroxycamptothecine) (Kawato, Aonuma, Hirota, Kuga, & Sato, 1991) through the action of carboxylesterases (Haaz, Rivory, Riche, Vernillet, & Robert, 1998; Rivory, Bowles, Robert, & Pond, 1996). SN-38 has cytotoxic activity 100–1,000 times greater than that of the parent drug. SN-38 is further conjugated to an inactive glucuronide (SN-38G) by uridine diphosphate glucuronosyltransferases. CPT-11 and its metabolites contain an alpha-hydroxydelta-lactone ring, which is chemically unstable and undergoes pH-dependent reversible hydrolysis to a hydroxylcarboxylate form. An intact lactone group is essential for interaction with the DNA–enzyme complex. Therefore, the pharmacokinetics of CPT-11 lactone and SN-38 lactone are of particular interest (Xie, Mathijssen, Sparreboom, Verweij, & Karlsson, 2002).

The aim of this work was to formulate and characterize liposomes of CPT-11–HCl containing chitosan, gelatin, and both polymers as coating materials; and also to increase its circulation longevity when compared with the free drug while maintaining the agent in its active lactone form.

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MATERIALS AND METHODS

Materials

CPT-11–HCl was supplied by Dr. Reddy's Laboratories (Hyderabad, India). Soybean phosphatidylcholine and cholesterol were purchased from Himedia Laboratories (Mumbai, India). Chitosan and gelatin were obtained from Alkem Laboratories (Mumbai, India). All the other reagents were of analytical grade.

Methods

The composition of different liposomes formulations is shown in Table 1. Liposomes were prepared by the modified ethanol injection method (Skalko, Brandl, Becirevic-Lacan, Filipovic-Grcic, & Jalsenjak, 1996) as follows: lecithin and cholesterol were dissolved in warm absolute ethanol and such a solution is rapidly injected into 10 mL of a magnetically stirred phosphate buffer saline (PBS) (pH 7.4). Immediately after injection, the characteristic opalescence of colloidal dispersions appeared in all preparations. Stirring was continued for 1 h and the liposomal suspension was left overnight at 4°C to stabilize prior to characterization.

Coating of Liposomes

For the preparation of chitosan, gelatin, or chitosan–gelatin-coated liposomes, an appropriate amount of chitosan sample was dissolved in water (in the presence of dil. HCl) or gelatin in hot water or chitosan–gelatin in water. An aliquot of liposome suspension (5 mL) was added dropwise to 20 mL of the above solution under stirring (600 rpm) at room temperature (20°C) for 60 min. The suspension was left overnight at 4°C to stabilize prior to characterization. The coated liposomes were harvested from the reaction mixture by centrifugation at 60,000 × *g* (Optima LE-80 K Ultracentrifuge, Beckman) and resuspended in PBS at pH 7.4.

Characterization of Liposomes

Freeze-Fracture Electron Microscopy

Liposome morphologic analysis was carried out by the freeze-fracture electron microscopy. Samples were quenched using sandwich technique and liquid nitrogen-cooled propane.

Zeta Potential

Zeta potential of liposome was determined by Malvern Zetasizer (Aimili Ltd., London, UK). Measurements were performed at 25°C.

High-Performance Liquid Chromatography Analysis of CPT-11

The concentration of CPT-11 was determined using high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (PerkinElmer series 200; PerkinElmer, Mumbai, India), a Kromasil C18 analytical column (4.6 × 250 mm, 5 μm) maintained at 25°C, UV-detector series 200 (PerkinElmer) at 254 nm, and a data station Total Chrom software (PerkinElmer). The composition of the mobile phase was potassium dihydrogen phosphate buffer (pH adjusted to 3.5 with *ortho*-phosphoric acid) : acetonitrile : methanol (55:25:20, vol/vol). The mobile phase was delivered at a flow rate of 1.0 mL/min and the injection volume was 20 μL.

TABLE 1
Composition of Different Formulations

	Formulations										
Ingredients	*F*0	*F*1	*F*2	*F*3	*F*4	*F*5	*F*6	*F*7	*F*8	*F*9	*F*10
Soya phosphate dylcholine (mg)	—	300	150	100	300	150	100	300	150	100	100
Cholesterol (mg)	—	—	150	200	—	150	200	—	150	200	200
α-Tocopherol acetate	—	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Ethanol (mL)	—	10	10	10	10	10	10	10	10	10	10
Drug	20 mg/ 2 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ 5 mL	50 mg/ mL
Water (mL)	—	25	25	25	—	—	—	—	—	—	—
Chitosan solution (1%) (mL)	—	—	—	—	25	25	25	—	—	—	—
Gelatin solution (1%) (mL)	—	—	—	—	—	—	—	25	25	25	—
Chitosan + gelatin (mL)	—	—	—	—	—	—	—	—	—	—	25

Measurement of Drug Encapsulated in Liposomes

The encapsulation efficiency was calculated according to the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{C_{\text{total}} - C_{\text{out}}}{C_{\text{total}}} \times 100$$

where C_{out} is the liposome suspension diluted with water and ultrafiltered through a Millipore filter (Nihon Millipore Kogyo Co. Ltd., Tokyo, Japan) to remove the liposomes and C_{total} is the liposome suspension diluted with heated ethanol (70°C) in order to disrupt the liposomes completely and release the encapsulated CPT-11–HCl to the solvent. The ethanol solution was cooled and ultrafiltered through a Millipore filter. Concentrations of the drug in the filtrate of C_{out} and C_{total} were quantitatively analyzed using HPLC method (PerkinElmer series 200).

Stability Study—Ageing Conditions

The CPT-11–HCl liposomes and control liposomes were prepared in PBS (pH 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, 7.4, or 7.8) and the hydrolysis was studied upon incubation. For stability testing, samples were stored at room temperature (20–22°C) or in fridge (4–8°C), protected from light and in well-sealed containers. Accelerated ageing was performed in a shaking water bath (Lab India, Mumbai, India) at 60 or 70°C for up to 40 h at 70 rpm.

In Vitro Drug Release

In vitro release of CPT-11–HCl from liposomal formulations were analyzed by membrane dialysis method against phosphate-buffered saline (PBS, pH 7.4) at 37°C. Briefly, a 2 mL aliquot of sample was placed in the dialysis tube (Himedia Lab, Mumbai, India) and then suspended in a temperature-controlled, jacketed flask containing 100 mL of PBS. At various time intervals, aliquot samples were withdrawn and analyzed by UV spectrophotometer (PerkinElmer) at 255 nm.

High-Performance Liquid Chromatography Analysis of CPT-11 in Plasma

The same HPLC conditions were used as described earlier for HPLC analysis of drug with fluorescence detector (1046 A model; PerkinElmer). The mobile phase was 0.1 M potassium dihydrogenphosphate (adjusted to pH 4.2 with 1 M HCl)—acetonitrile (67:33); the flow rate used is 1 mL/min, and the column temperature is set at 30°C. The fluorescence detector excitation wavelength was set at 228 nm and the emission wavelength of CPT-11 was set at 450 nm (Escoriaza, Aldaz, Castellanos, Calvo, & Giráldez, 2000).

In Vivo Study

Liposomal and free CPT-11–HCl was administered intravenously at a dose of 25 mg/kg in female Sprague-Dawley rats (190–220 g). Blood samples (0.2–0.3 mL) were drawn at various times post injection using a heparin-treated syringe. The withdrawn blood volume was replaced using PBS. Blood samples were diluted with 0.3 mL of ice-cold PBS containing 0.04% EDTA, weighed, and centrifuged. Plasma was assayed for CPT-11 by HPLC. The percentage of drug remaining in the liposomes was calculated by dividing the drug in the blood sample to that of the drug in the injected liposomes (taken as 100%) because free CPT-11 is cleared at a much faster rate than liposomes. (A change in the CPT-11-to-liposomes.)

RESULTS AND DISCUSSION

Freeze-Fracture Electron Microscopy

The particle sizes of liposomes of CPT-11–HCl have diameters ranging from 0.17 ± 0.03 to 0.21 ± 0.59 μm (Table 2). In comparison, the mean diameters of different types of liposomes the lowest size of uncoated than those of chitosan and gelatin and combination of chitosan and gelatin. The size of liposomes was found to be uncoated < chitosan coated < gelatin coated < combination of chitosan and gelatin coated (Figures 1–4). Figure 1 shows the drug which is trapped into aqueous compartment of unilamellar uncoated liposomes because CPT-11 is well known to be a water-soluble drug and it is distributed primarily into aqueous compartment of liposomes. Figure 2 shows that the particle size of chitosan-coated liposomes in each formulation is increased with the chitosan solution, probably because of a combination of adsorption coagulation and bridging between liposomes. Figure 3 shows the bigger size of gelatin-coated liposomes because of adhesion and peak detachment force of liposomes. Figure 4 shows the formation of dense layer on liposomes because of the blend of chitosan and gelatin. In addition, gelatin-coated liposomes have bigger particle size than chitosan-coated liposomes because the added gelatin molecules into

TABLE 2
Particle Size of Different Liposomal Formulations

	Particle Size (μm)	
Formulations	Empty	Loaded
*F*1	0.17 ± 0.03	0.19 ± 0.04
*F*2	0.18 ± 0.07	0.20 ± 0.09
*F*3	0.19 ± 0.05	0.21 ± 0.02
*F*4	0.21 ± 0.01	0.23 ± 0.03
*F*5	0.21 ± 0.05	0.23 ± 0.08
*F*6	0.21 ± 0.03	0.24 ± 0.09
*F*7	0.22 ± 0.09	0.25 ± 0.06
*F*8	0.22 ± 0.01	0.25 ± 0.07
*F*9	0.22 ± 0.09	0.26 ± 0.06
*F*10	0.24 ± 0.02	0.27 ± 0.09

Mean ± *SD*; $n = 3$.

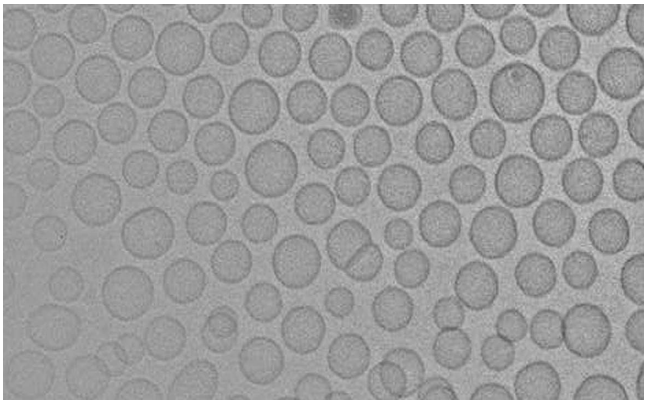

FIGURE 1. Freeze-fracture micrograph of *F*3.

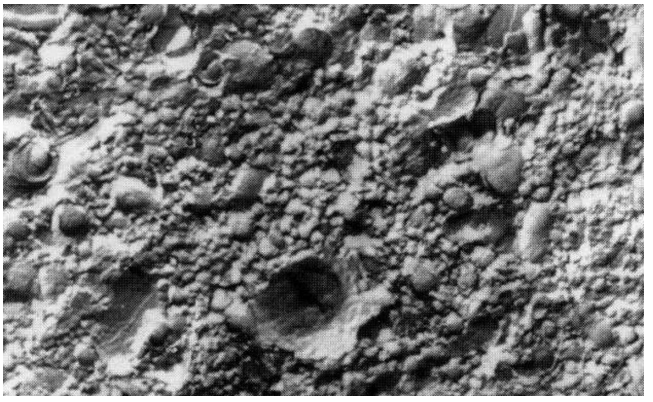

FIGURE 2. Freeze-fracture micrograph of *F*6.

FIGURE 3. Freeze-fracture micrograph of *F*9.

lipid bilayer would increase the glass transition temperature, and resulted in increased rigidity of lipid membrane. The increased size of liposomes may be attributed to the enlarged emulsion globules dispersed in the aqueous phase. On the other hand, it was seen that the increase in cholesterol concentration resulted in an increase in the mean particle size. Furthermore, the increase in the viscosity because of polymer in the aqueous phase interferes with the interfacial hydrodynamic phenomena and is responsible for the spontaneous

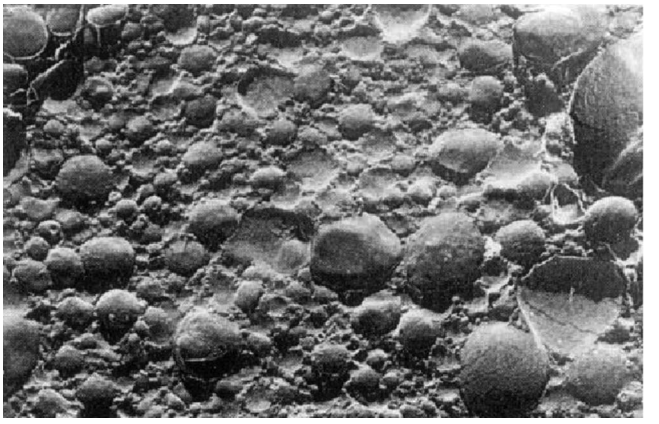

FIGURE 4. Freeze-fracture micrograph of *F*10.

TABLE 3
Zeta Potential of Different Liposomal Formulations

Formulations	Zeta Potential (mV)
*F*1	19.34 ± 3.4
*F*2	19.87 ± 2.1
*F*3	20.3 ± 1.3
*F*4	28.2 ± 2.5
*F*5	30.1 ± 1.7

emulsification of the organic phase when mixed with the aqueous phase.

Zeta Potential

The zeta potential of liposomes was in the order of combination of chitosan and gelatin coated > chitosan coated > gelatin coated > uncoated. The mechanism of coating neutral phosphatidylcholine liposomes by chitosan probably involved hydrogen bonding between the polysaccharide and the phospholipid head groups. All the zeta potentials of chitosan-coated liposomes were positive. As chitosan–gelatin carried high positive charge, their adsorption increased the density of positive charge and made the zeta potential positive than that of chitosan-coated liposomes. Gelatin-coated liposomes and phospholipids involved weak hydrogen bonding and therefore the zeta potential was comparatively less (Table 3).

Sedimentation Volume

The sedimentation volume of liposomes was in the order of uncoated > chitosan coated > gelatin coated > combination chitosan and gelatin coated. As all liposomes possessed positive zeta potential, the zeta potential of particles is thought to play an important role in the resistance to flocculation and coagulation. As to the liposomes of aqueous outer medium, the zeta

potentials were small. Thus, it resulted in flocculation. At relatively high polymer concentrations (0.1%), the surface coverage by the adsorbed polymer is sufficiently high to prevent polymer bridge flocculation. The structured adsorbed polymer film now serves to stabilize the particles against particle–particle interaction presumably by the mechanism of steric stabilization. However, the thick adsorptive layer on the liposomes kept the particles apart and led to stabilization.

Encapsulation Efficiency

The encapsulation efficiency of liposomes was in order of combination of chitosan and gelatin coated > gelatin coated > chitosan coated > uncoated as shown in Figure 5. The encapsulation efficiency of liposomes was more in chitosan–gelatin coated ($F10 = 91.65 \pm 5.43$) than that of gelatin ($F9 = 82.97 \pm 2.54$), chitosan ($F6 = 79.23 \pm 4.12$) and uncoated ($F3 = 70.75 \pm 4.02$) because it provides a firm structure to prevent the gelatin from contracting. The ionized drug fraction is localized in the membrane bilayer partially penetrating into the hydrophobic core of the membrane because of its amphiphilic nature. The encapsulation efficiency of liposomes increased with polymer coating; the highest encapsulation occurred in chitosan–gelatin-coated liposomes. Chitosan and gelatin ionized and significantly competed with CPT-11 in their binding to phosphatidylcholine, thereby making the retention of drug into the vesicles difficult. The encapsulation efficiency of liposomes showed the same order as that of particle size, which concludes that increase in size increases the encapsulation efficiency of CPT-11. Chitosan has a strong affinity for the phospholipids of the vesicle bilayer. Because of these conditions, both CPT-11 and chitosan have the same charge and compete with each other to interact with the phospholipids of the bilayer. The mutual repulsion between the drug molecules and the positively charged chitosan prevents any kind of interaction between them. The presence of a large number of protons resulting from the dissociation of CPT-11–HCl also contributes to this repulsive force, thereby reducing the drug encapsulation efficiency. It was stated that by coating liposomes with collagen (gelatin) it is possible to increase the fraction of liposome-associated drug, and not only the bioadhesive liposomes as a whole had become more favorable for the drugs, but the intraliposomal drug pool had also become more favorable for drug entrapment (Yerushalmi & Margalit, 1994), which was found to be the same as our findings for encapsulation efficiency. Therefore, the encapsulation efficiency is related to the quantity of aqueous phase that is immobilized between the phospholipid bilayers and the concentration of the drug in the aqueous phase.

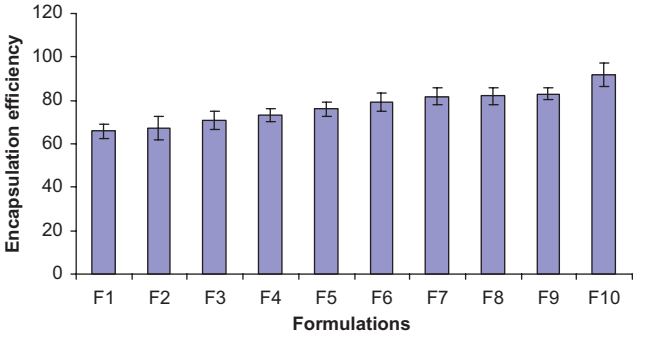

FIGURE 5. Encapsulation efficiency of different liposome formulations.

Stability Studies

In the lipid bilayer, hydrophilic head groups of the phospholipid molecules are positioned outward to the water phase and the hydrophobic chains inward tail to tail. The formulations *F*3, *F*6, *F*9, and *F*10 were stable because of increased concentration of cholesterol in lipid bilayer and surface coating by polymers. Liposome formulations with and without CPT-11–HCl were prepared, autoclaved and used both for an accelerated stability study and storage stability study in fridge (4–8°C) and at room temperature over a period of 2 months. In the accelerated stability study (70°C for 40 h), an initially moderate and later on accelerating increase in phosphatidylcholine content was observed for formulations, and to a higher extent in CPT-11-containing liposomes as shown in Table 4. The phosphatidylcholine content was also observed to increase faster in the CPT-11-containing liposomes than the control during storage in fridge and at room temperature. Hydrolysis stability was poorer at room temperature than in fridge. The observed difference in hydrolysis stability between the two formulations in both the accelerated stability study and the storage stability study was confirmed by calculation; p-values < .05 were attained by the Student's t-test showing a significantly higher increase in phosphatidylcholine concentrations for the CPT-11–HCl liposome formulations when compared with the control liposomes. The process of autoclaving is of first choice to obtain a sterile product for parenteral application (Zuidam, Hirsch-Lerner, Margulies, & Barenholz, 1999). Both the accelerated ageing study and the storage stability study indicate, however, that hydrolytic phosphatidylcholine degradation is not enhanced in the presence of CPT-11, and this catalytic effect of CPT appears to increase with the progress of phospholipid hydrolysis. Further optimalization of the formulation, together with careful processing and handling, is therefore mandatory to obtain a CPT-11 liposome formulation with acceptable storage stability.

In Vitro Drug Release

In the in vitro release study, an aqueous solution of CPT-11 was released rapidly and was almost completed within 5 h. However, the conventional, chitosan, gelatin and chitosan–gelatin-coated liposomes released 73.07 ± 1.24–91.21 ± 2.57% of CPT-11 within 40 h of dialysis as shown in Figure 6. The release of CPT-11 showed an initial burst release phase, releasing

TABLE 4
Stability Study of *F*1–*F*10

	Formulations									
Hours (h)	*F*1	*F*2	*F*3	*F*4	*F*5	*F*6	*F*7	*F*8	*F*9	*F*10
10	98.11	99.13	99.22	99.29	99.34	99.36	99.54	99.54	99.29	99.57
20	95.03	98.14	98.32	98.05	98.31	98.42	98.48	98.04	98.09	98.55
40	95.09	96.22	97.89	96.34	97.76	98.02	97.04	98.05	98.21	98.52

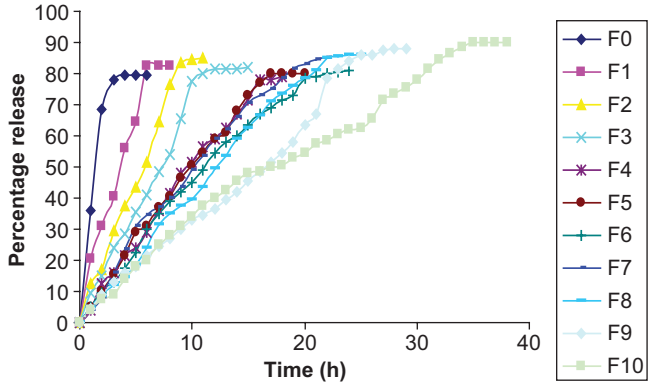

FIGURE 6. In vitro drug release profile of CPT-11–HCl from different formulations.

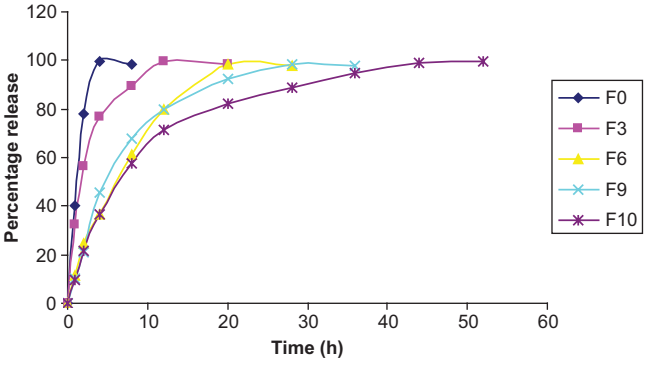

FIGURE 7. In vivo drug release profile of CPT-11–HCl from different formulations (*F*0, *F*3, *F*6, *F*9, and *F*10).

approximately 20% during the first 3 h, and the release rate was reduced thereafter, indicating that the release of CPT-11 reached a slow release status. This result suggests that it takes time for CPT-11 to be released once encapsulated in the liposomes because lipid bilayers are stabilized by cholesterol. Thus a depot effect could be achieved using liposomes, especially in the coated liposomal formulations.

The controlled release of drug was observed in the order of combination of chitosan and gelatin coated > gelatin coated > chitosan coated > uncoated. When the chitosan solution was added to the colloidal dispersion, the chitosan adhered to the liposome surface. The coated liposomes were produced due to high electrostatic interaction between highly positively charged chitosan gelatin and the oppositely charged liposome surface compared to chitosan and gelatin coated liposomes (Filipovic-Grcic, Skalko-Basnet, & Jalsenjak, 2001). The chitosan covers the surface of the liposomes by forming an ion complex with phosphatidylcholine in the liposome formulation. The polymer-coated liposomes, which were confirmed by the increase in particle size and change in zeta potential, may increase the viscosity of formulations and thus it produced the controlled release of CPT-11.

In Vivo Drug Release

Formulations (*F*3, *F*6, *F*9, and *F*10) were selected on the basis of result of long-term stability and controlled release of CPT-11. Free CPT-11 was rapidly cleared from the circulation with $t_{1/2}$ = 0.27 h. Liposome encapsulation was associated with significantly longer circulation times than free drug (Figure 7). This was especially true for uncoated liposomes (*F*3) and coated liposomes of CPT-11 (*F*6, *F*9, and *F*10), with blood half-lives in the order of combination of chitosan and gelatin coated > gelatin coated > chitosan coated > uncoated.

This likely reflects that the $t_{1/2}$ of CPT-11 release from liposomes (*F*3) was 14 h, significantly shorter than that for chitosan–gelatin-coated liposomes with a $t_{1/2}$ of CPT-11 release of 56.8 h.

CONCLUSION

In conclusion, we have investigated the use of gelatin to improve the mechanical and biological properties of a chitosan membrane and also increased its circulation longevity when compared with the free drug while maintaining the agent in its active lactone form. CPT-11–HCl is shown to be an ideal candidate for liposome encapsulation. This study helped to identify the role that different components play in drug encapsulation.

REFERENCES

Allen, T. M., Hansen, C., & Martin, F. (1991). Liposomes containing synthetic lipid derivatives of poly (ethylene glycol) show prolonged circulation half lives in vivo. *Biochim. Biophys. Acta, 1066*, 29–36.

Amamath, S., & Umas, S. (1997). Liposomes in drug delivery: Progress and limitations. *Int. J. Pharm., 154*, 123–140.

Bakker-Woudenberg, I. A., Lokerse, A. F., & Roerdink, F. H. (1989). Antibacterial activity of liposome-entrapped ampicillin in vitro and in vivo in relation to the lipid composition. *J. Pharmacol. Exp. Ther., 251*(1), 321–327.

Cocera, M., Lopez, O., Coderch, L., Parra, J. L., & de la Maza, A. (2003). Permeability investigations of phospholipid liposomes by adding cholesterol. *Coll. Surf. A: Physicochem. Eng. Aspects, 221*, 9–17.

Dong, C., & Rogers, J. A. (1991). Polymer-coated liposomes: Stability coated and release liposomes of ASA from carboxymethyl chitin-coated liposomes. *J. Control. Release, 17*, 217–224.

Elferink, M. L., Wit, J. G., & Veld, G. (1992). The stability and functional properties of proteoliposomes mixed with dextran derivatives bearing hydrophobic anchor groups. *Biochim. Biophys. Acta, 1106*, 23–30.

Escoriaza, J., Aldaz, A., Castellanos, C., Calvo, E., & Giráldez, J. (2000). Simple and rapid determination of irinotecan and its metabolite SN-38 in plasma by high-performance liquid-chromatography: Application to clinical pharmacokinetic studies. *J. Chromatogr. B, 740*, 159–168.

Filipovic-Grcic, J., Skalko-Basnet, N., & Jalsenjak, I. (2001). Mucoadhesive chitosan-coated liposomes: Characteristics and stability. *J. Microencapsul., 18*, 3–12.

Gregoriadis, G. (1995). Engineering liposomes for drug delivery: Progress and problems. *Trend Biotechnol., 13*, 527–537.

Guo, J., Ping, Q., & Jiang, G. (2003). Chitosan-coated liposomes: Characterization and interaction with leuprolide. *Int. J. Pharm., 260*, 167–173.

Haaz, M. C., Rivory, L. P., Riche, C., Vernillet, L., & Robert, J. (1998). Metabolism of CPT-11 by human hepatic microsomes: Participation of cytochrome P-450 3A and drug interactions. *Cancer Res., 58*, 468–472.

Jamshaid, M., Fair, S. J., & Kearney, P. (1988). Poloxamer adsorption on liposomes: Comparison with polystyrene latex and influence on solute efflux. *Int. J. Pharm., 48*, 125–131.

Jorg, K. (2001). Nanoparticulate systems for brain delivery of drugs. *Drug Deliv. Res., 47*, 65–81.

Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H., & Sato, K. (1991). Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res., 51*, 4187–4191.

Lasic, D. D. (1998). Novel applications of liposomes. *TTBETECH, 16*, 307–321.

Madden, T. D., Janoff, A. S., & Cullis, P. R. (1990). Incorporation of amphotericin B into large unilamellar vesicles composed of phosphatidylcholine and phosphatidylglycerol. *Chem. Phys. Lipids, 52*(3–4), 189–198.

Nacucchio, M. C., Gatto Bellora, M. J., Sordelli, D. O., & D'Aquino, M. (1988). Enhanced liposome-mediated antibacterial activity of piperacillin and gentamicin against gram-negative bacilli in vitro. *J Microencapsul., 5*(4), 303–309.

Rengel, R. G., & Barisic, K. P. (2002). High efficiency entrapment of superoxide dismutase into mucoadhesive chitosan-coated liposome. *Eur. J. Pharm. Sci., 15*, 123–129.

Rivory, L. P., Bowles, M. R., Robert, J., & Pond, S. M. (1996). Conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem. Pharmacol., 52*, 1103–1111.

Sawada, S., Okajima, S., Aiyama, R., Nokata, K., Furuta, T., Yokokura, T., Sugino, E., Yamaguchi, K., & Miyasaka, T. (1991). Synthesis and antitumor activity of 20(*S*)-camptothecin derivatives: Carbamate-linked, water-soluble derivatives of 7-ethyl-10-hydroxy-camptothecin. *Chem. Pharm. Bull., 39*, 1446–1454.

Skalko, N., Brandl, M., Becirevic-Lacan, M., Filipovic-Grcic, J., & Jalsenjak, I. (1996). Liposomes with nifedipine and nifedipine-cyclodextrin complex: Calorimetrical and plasma stability comparison. *Eur. J. Pharm. Sci., 4*, 359–366.

Xie, R., Mathijssen, R. H. J., Sparreboom, A., Verweij, J., & Karlsson, M. O. (2002). Clinical pharmacokinetics of irinotecan and its metabolites: A population analysis. *J. Clin. Oncol., 20*, 3293–3301.

Yerushalmi, N., & Margalit, R. (1994). Bioadhesive, collagen-modified liposomes: Molecular and cellular level studies on the kinetic of drug release and on binding to cell monolayers. *BBA-Biomembranes, 1189*, 13–20.

Zuidam, N. J., Hirsch-Lerner, D., Margulies, S., & Barenholz, Y. (1999). Lamellarity of cationic liposomes and mode of preparation of lipoplexes affect transfection efficiency. *Biochim. Biophys. Acta, 1419*, 207–220.