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Chitosan-Based Thermosensitive Hydrogel Containing Liposomes for Sustained Delivery of Cytarabine

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Sustained release thermosensitive solution containing cytarabine-loaded liposome delivery system offers the possibility of reduced dosing frequency and sustained drug action. Biodegradable and biocompatible chitosan-beta-glycerophosphate (C-GP) thermosensitive solution having the property to gel at body temperature and to maintain its physical integrity for longer period of time was used. The C-GP solution containing cytarabine-loaded liposomes (CGPCLL) was studied, and the results showed that the cytarabine liposomes were capable of high encapsulation efficiency (85.2 ± 2.58%) with the mean diameter of 220 ± 6.9 nm of extruded cytarabine-loaded liposome. Furthermore, transmission electron microscopy showed spherical-shaped liposomes after extrusion with smooth surface. In vitro studies of CGPCLL in PBS buffer showed that this system can sustain release of encapsulated drug for more than 60 h compared with drug-loaded liposomal suspension (upto 48 h). Pharmacokinetic studies of CGPCLL resulted in higher $t_{1/2}$ (28.86 h) and AUC 2526.88 μg/mL h compared with cytarabine-loaded liposomal suspension (CLLS) and C-GP containing free cytarabine (CGPFC) in rats. CGPCLL was capable of sustaining the cytarabine release for more than 60 h in vivo compared with CLLS and CGPFC which showed maximum amount of drug release within 42 and 10 h, respectively. Thus, these results showed that the CGPCLL gels at body temperature and can sustain the delivery of cytarabine effectively.

Keywords thermosensitive; biocompatible; chitosan; sustained release; liposome

INTRODUCTION

In situ gelling has been investigated extensively for its biomedical application since last decade, particularly in drug delivery (Haglund, Joshi, & Himmelstein, 1996). Various materials have been used till date for in situ gel-forming implant systems, of which some have certain advantages and disadvantages. Among these different materials, such as poly(lactide) and poly(lactide-co-glycolide), copolymers in various solvents and complex of poly(methacrylic acid) and poly(ethyleneglycol) dissolved in a hydroalcoholic solvent containing ethanol have been studied by different investigators showing some drawbacks of these systems, which are very crucial considering biomedical applications (Eliaz & Kost, 2000; Peppas, Bures, Leobandung, & Ichikawa, 2000; Shively, Coonts, Renner, Southard, & Bennet, 1995). Some of these polymers require photopolymerization, and it requires the presence of photoinitiator at the site of gelation. Also the organic solvents used to solubilize some polymers can physically denature labile compounds such as proteins. With precipitation method used for gelation, incomplete gelation may occur leading to high burst release and may cause local or systemic toxicity. Thermosensitive polymers such as poloxamers and copolymers of *N*-isopropylacrylamide are not biodegradable, thereby their use in parenteral formulations is limited (Johnston, Punjabi, & Froelich, 1992; Okano, Bae, Jacobs, & Kim, 1990). Recently, polyethylene glycol (PEG) and poly(lactic acid) block copolymer systems are used as a thermosensitive combination. These block copolymers remain in sol state at around 45°C and get converted to gel upon cooling near to body temperature; thereby, they are associated with major limitation as the system needs heating to incorporate the drug as well as to inject it in the body (Affrasiabi & Dong, 1986; Jeong, Bae, Lee, & Kim, 1997; Lin & Cheng, 2001).

In conclusion, as these systems are not fully convincing there is a need to develop a parenteral drug delivery system capable of forming stable gel at body temperature within short period after injection and maintaining its physical integrity for prolonged duration of time. Ruel Gariepy et al. have reported that thermosensitive chitosan-beta-glycerophosphate (C-GP) solution containing model drug (carboxyfluorescein)-loaded liposomes remains in sol state at 8–15°C and turns into a gel at body temperature after s.c./i.m. injection. This system is reported to be able to sustain the drug release for more than 2 weeks. The major advantage of combining liposomes with C-GP hydrogel system is more sustained release of drug along with overall stability compared with liposomal suspension. Chitosan is a biodegradable, biocompatible, and mucoadhesive

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biopolymer, which is emerging to play a significant role in biomedical applications, because of its abundance and wide scope of use (Berger et al., 2004, 2005; Molinaro, Leroux, Damas, & Adam, 2002). The combination of chitosan with GP, which is also a biocompatible agent, to form a thermosensitive hydrogel for the delivery of therapeutic agents is a promising achievement in the drug delivery arena. The advantages like absence of any surfactant, cross-linking agent, or any organic solvent renders this system compatible with most sensitive biologics (Chenite et al., 2000).

The formulation of sustained release parenterals of low-molecular weight hydrophilic drugs is a challenging task. The cytarabine was selected as a drug of choice because of its low plasma half-life ($t_{1/2}$), high aqueous solubility, and low-molecular weight. All these characters make cytarabine difficult to formulate into sustained release formulation. So, the main aim of this study was to develop novel parenteral formulation of cytarabine for sustained release drug delivery.

MATERIALS AND METHODS

Materials

Egg phosphatidylcholine (EPC), cholesterol (Chol), DL-α-tocopherol, and beta glycerophosphate (β-GP) were purchased from Sigma-Aldrich (Munich, Germany). Chitosan (90% deacetylated) was obtained from DKSH (Mumbai, India). Cytarabine was a kind gift from Neon Labs (Mumbai, India). Nuclepore Trach-Etch Membrane, 0.2 μm pore diameter, was purchased from Whatman. All other chemicals were of analytical grade and were used without further purification.

Methods

Preparation of Liposomes

Cytarabine-loaded liposomes composed of EPC and Chol were prepared by thin-film hydration method (1:20 drug : lipid molar ratio and 60:40 EPC : Chol % ratio) (Betagiri, Jenkins, & Parsons, 1993). For optimization, drug : lipid (molar ratio) and EPC : Chol (% ratio) were taken as independent variables, whereas particle size and percent drug entrapment (PDE) were taken as dependent variables. By applying 3^2 factorial design, optimization was carried out. Briefly, EPC : Chol (246.70: 164.47 μmol) and DL-α-tocopherol (0.5 mL of 1%, wt/vol in chloroform : methanol mixture) were dissolved in 10 mL of chloroform : methanol mixture (2:1) in 250 mL round-bottomed flask and dried to thin lipid film with rotary evaporator at 40°C under controlled vacuum. The thin film was flashed with nitrogen gas to eliminate traces of chloroform and methanol. Lipid film was then hydrated with distilled water containing cytarabine (0.1%, wt/vol). The formed multilamellar vesicles (MLVs) were extruded eight times through extruder (Avestin Emulsiflex® C-5, Canada), using polycarbonate membrane of 200-nm pore diameter to get small unilamellar vesicles (SUVs). The liposomes were collected by ultracentrifugation (244 × *g*, 1 h, 4°C, 3 times; Backman TL-100). The liposomes were dried under vacuum and resuspended in water. Finally, liposomes were lyophilized and stored at 2–8°C.

Characterization of Drug-Loaded Liposome

- *Particle size analysis*: The particle size of the prepared liposomes was determined by dynamic light scattering using Malvern particle analyzer (Mastersizer Malvern SM 2000). Average diameter was measured by scattered intensity at 25°C.
- *Transmission electron microscopy (TEM)*: The shape and surface morphology of liposomes was examined by TEM (CM12; Philips, Bothell, Washington, USA) by negative staining with sodium phosphotungstate solution (0.2%, wt/vol).
- *Determination of drug loading efficiency*: The liposomes obtained after ultracentrifugation (as described above) were digested in methanol containing 0.1% (wt/vol) Triton X-100. The digested homogenates were centrifuged at 244 × *g* for 40 min, and the supernatant was analyzed for drug content by UV spectroscopy (Shimadzu, Kyoto, Japan) at 254 nm.

Preparation of the Chitosan-Beta-Glycerophosphate Solution Containing Cytarabine-Loaded Liposomes

C-GP solution was prepared by slight modifications in the method described previously by Ruel-Gariepy et al. (2002). For optimization of C-GP solution, the concentration of chitosan and GP were taken as independent variables, and gelation temperature and gelation time were taken as dependent variables. By using 3^2 factorial design, nine batches were prepared for the optimization. Briefly, to the prechilled solutions of chitosan (1.8%, wt/vol in 0.1 M HCl), prechilled β-GP solution (5.7%, wt/vol in distilled water) was added under continuous stirring and the solution was further stirred for 15 min. Drug-loaded liposomes were added to the C-GP solution (15 μmol/mL) and stirred gently for 10 min for uniform distribution of liposomes in the C-GP solution. The drug-loaded liposome dispersion was added such that it would remain iso-osmotic with C-GP solution (Bochot, Fattal, Gulik, Couarraze, & Couvreur, 1998; Ruel-Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002). The resulting solution containing liposome was stored at 2–8°C until further use.

Characterization of C-GP Solution

Determination of Gelling Temperature by Rheological Method. Rheological study of plain C-GP solution and C-GP solution containing cytarabine-loaded liposomes (CGPCLL) was performed with a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LVDV III, Brookfield Engineering Laboratories, Middleboro, MA, USA), fitted with CP-52 spindle. The cone/plate geometry was used. The cone had a 1.2 cm radius and an angle of 3°. The shear stress was controlled to maintain a shear rate of 10 s^{-1}. This value was

chosen to allow precise determination of the gelling temperature. The temperature was increased in steps of 1°C/min, from 25 to 40°C to locate the solution/gel transition point. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (mPas) as a function of the temperature (°C). Each sample was tested in triplicate to control the repeatability of the measurement (Chenite, Buschmann, Wang, Chaput, & Kandani, 2001; Ruel-Gariepy, Chenite, Chaput, Guirguis, & Leroux, 2000).

In Vitro Release Study

In vitro release study of C-GP solution containing free cytarabine (CGPFC) (chitosan [1.8%, wt/vol], β-GP [5.7%, wt/vol], and cytarabine [0.1%, wt/vol]), cytarabine-loaded liposomal suspension (CLLS) (cytarabine [0.1%, wt/vol], drug : lipid [1:20 mol ratio], EPC : Chol [60:40% ratio]), and C-GP solution containing cytarabine-loaded liposomes (CGPCLL) (15 μmol of CLLS/mL of C-GP solution) was investigated in closed double-jacketed thermostatic chamber at 37 ± 2°C using in vitro dialyzing method (dialysis tubing 12,000 MWCO) and dialyzed against phosphate buffer saline pH 7.4 dialyzing medium under continuous stirring. After 60 h, Triton X-100 was added to the donor compartment to solubilize the liposomes still entrapped in the gel to release the remaining drug. Aliquots from the receptor compartment were withdrawn at predetermined intervals and analyzed for drug content by UV spectrophotometer. All experiments were carried out in triplicate and average values are presented (Chenite et al., 2001; Ruel-Gariepy et al., 2000).

In Vivo Pharmacokinetic Study

Albino rats (Wistar strain) of either sex weighing 200–250 g were used for the study. The studies were carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The animals were fasted overnight and divided into four groups of 15 rats each. Dose equivalent to 5.4 mg cytarabine/kg body weight of CGPFC (chitosan [1.8%, wt/vol], β-GP [5.7%, wt/vol], and cytarabine [0.1%, wt/vol]), CLLS (cytarabine [0.1%, wt/vol], drug : lipid [1:20 mol ratio], EPC : Chol [60:40% ratio]), and CGPCLL (15 μmol of CLLS/mL of C-GP solution), respectively, were administered i.m. to the animals of the first, second, and third group. The blood samples were collected from retro-orbital vein at predetermined time intervals. The collected samples were analyzed for drug content by using high-performance liquid chromatograph (HPLC) method as described below. The plasma concentration was analyzed based on one compartmental pharmacokinetic model using QuickCalc Software (Plexus, Ahmedabad, India), which utilizes curve-stripping procedure to calculate the pharmacokinetic parameters.

HPLC Analysis of Cytarabine in Plasma

Calibration standards were prepared by spiking cytarabine solutions (in phosphate buffer pH 7.4) into drug-free rat plasma to yield concentrations of cytarabine in the range of 0.5–20 ppm, and then plasma samples were estimated by precipitating plasma proteins from the plasma by addition of equal volume of acetonitrile. The supernatant was used for the estimation of cytarabine by HPLC system that consisted of a UV variable wavelength detector (S 3210 variable-wavelength UV-Vis detector (Sykam GmbH, Gewerbering, Eresing, Germany) set at 254 nm as detection wavelength, a EZChrom™ Chromatography Data System, version 6.8 (Scientific Software Inc., Pleasanton, CA, USA, on an IBM-compatible PC connected to a printer). The chromatographic analysis was carried out on Thermo-Hypersil C18 (250 mm × 4.6 mm) column. Cytarabine was eluted using a mobile phase composition of phosphate buffer (pH 7.4) : methanol (70:30, vol/vol) at a flow rate of 1.0 mL/min and retention times of 3 min. Injection volume of the samples was 100 μL (Fahmy, Korani, & Maher, 2004). The peaks were determined using a UV detector set at wavelength 254 nm.

Statistics

Data were expressed as $M \pm SD$. Statistical analysis of data was performed using analysis of variance (ANOVA) followed by the Dunnett multiple comparison test. A p-value <.01 was considered significant.

RESULTS AND DISCUSSION

Characterization of Liposomes

Liposome suspension obtained by thin-film hydration usually consists of MLVs of heterogenous size in the range of about 0.1–10 μm (Szoka, 1980). The SUVs formed by passing liposomal suspension through polycarbonate membrane (0.2-μm pore diameter size) for eight cycles resulted in average particle size 220 ± 3.9 nm and polydispersity index (PdI) 0.213 ± 0.011 (Figure 1). Figure 1 shows a typical profile of particle size distribution in the prepared liposomes. The particle size distributions were unimodal, extended from 80 to 450 nm. The mean particle size of the different batches of factorial design ranged between 220 and 290 nm, with the PdI being in the range of 0.198–0.310. The PdI value measures the range of the particle size distribution, and thus, a small PdI value indicates a narrow particle size distribution (Couvreur, Gref, & Costantini, 2003). All the prepared batches of liposomes exhibited a relatively narrow range of size distribution with the PdI values smaller than 0.31 (0 being the smallest and 1 the largest possible values). The effect of drug : lipid (mol ratio) and EPC : Chol (% ratio) on mean particle size and PdI of all the nine batches of factorial design is shown in Figures 2 and 3. TEM photograph of the liposomes after extrusion showed spherical-shaped liposomes with smooth surface (Figure 4). From TEM, particle size was observed to be slightly larger than 200 nm in diameter, which supported the results of particle size measurement by dynamic light scattering method with Malvern mastersizer. From the factorial design study, the maximum PDE was

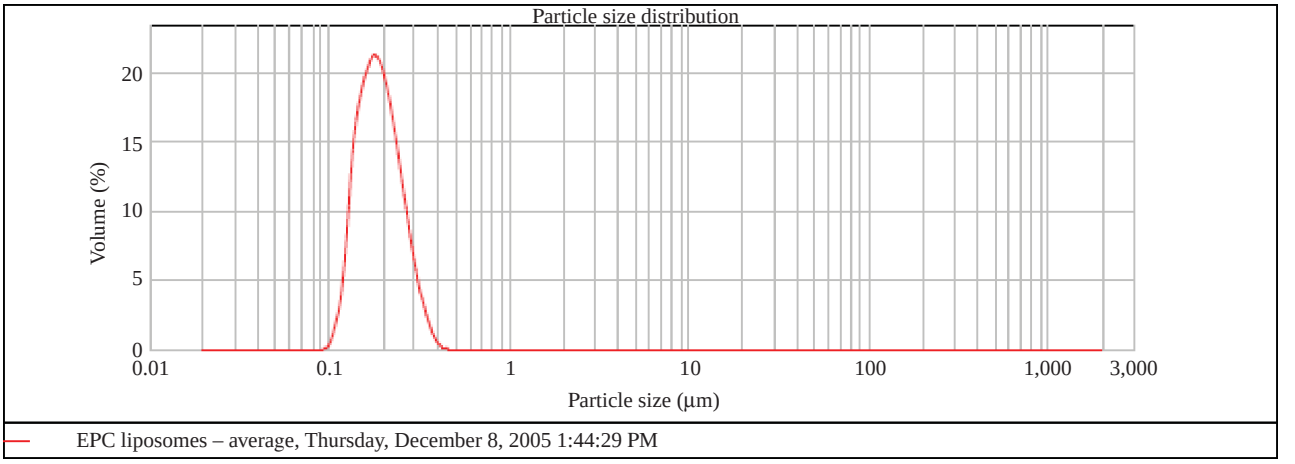

FIGURE 1. Particle size analysis of cytarabine-loaded liposomes by dynamic light scattering method. Results are expressed as $M \pm SD$ ($n = 3$).

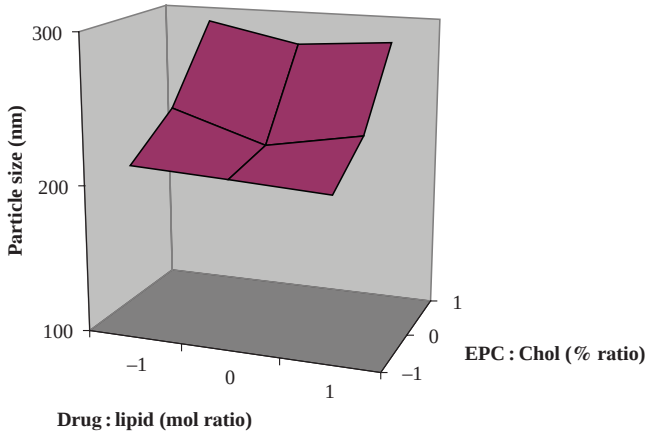

FIGURE 2. The effect of drug : lipid (mol ratio) and EPC : Chol (% ratio) on particle size of all the nine batches of factorial design. On *x*-axis: drug : lipid mol ratio, –1 = 1:17, 0 = 1:20, and 1 = 1:23; and on *y*-axis: EPC : Chol % ratio, –1 = 50:50, 0 = 60:40, and 1 = 70:30. The values are expressed as $M \pm SD$ ($n = 3$).

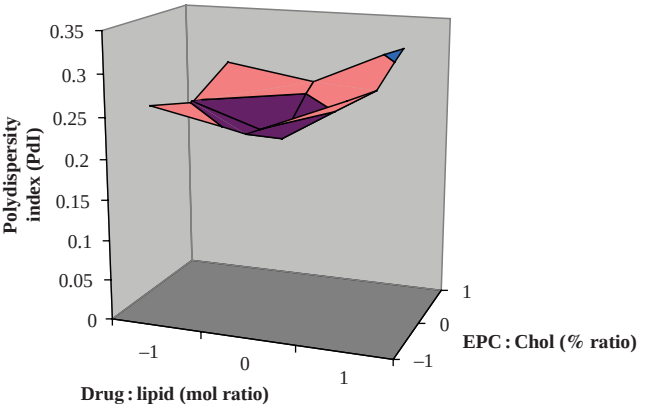

FIGURE 3. The effect of drug : lipid (mol ratio) and EPC : Chol (% ratio) on polydispersity index (PdI) of all the nine batches of factorial design. On *x*-axis: drug : lipid mol ratio, –1 = 1:17, 0 = 1:20, and 1 = 1:23; and on *y*-axis: EPC : Chol % ratio, –1 = 50:50, 0 = 60:40, and 1 = 70:30. The values are expressed as $M \pm SD$ ($n = 3$).

found to be 85.20 ± 2.58%. The effect of drug : lipid (mol ratio) and EPC : Chol (% ratio) on PDE is shown in Figure 5. The comparative results of particle size analysis, PdI, and PDE of all nine batches of factorial design are shown in Table 1. For factorial design, three levels each of drug : lipid (molar ratio) and EPC : Chol (% ratio) were used. Among the three levels of drug : lipid (molar ratio), 1:20 was found to be good as we get desirable mean particle size and excellent PDE compared with 1:17 and 1:23 (Figures 2, 3, and 5). The batches with 1:17 molar ratio leads to significantly less PDE than 1:20 and 1:23, because of less lipid concentration, while those with 1:23 molar ratio resulted in significantly larger particle size, because of increased lipid and cholesterol concentration compared with 1:17 and 1:20 (Figures 2 and 5). In case of EPC : Chol (% ratio), all batches with 50:50 (% ratio) resulted in slightly larger particle size due to increased cholesterol concentration in comparison with 60:40 and 70:30, whereas 70:30 (% ratio) resulted in slightly less PDE due to reduced cholesterol concentration (Figures 2 and 5). So, in conclusion, batches with 1:20 (drug : lipid molar ratio) and 60:40 (EPC : Chol % ratio) seems to be effective as it leads to better PDE, mean particle size, and also less PdI.

Determination of Gelling Temperature by Rheological Method

Gelation temperature lower than 25°C of a thermoreversible formulation would lead to difficulty in manufacturing and administration due to gel formation at room temperature and

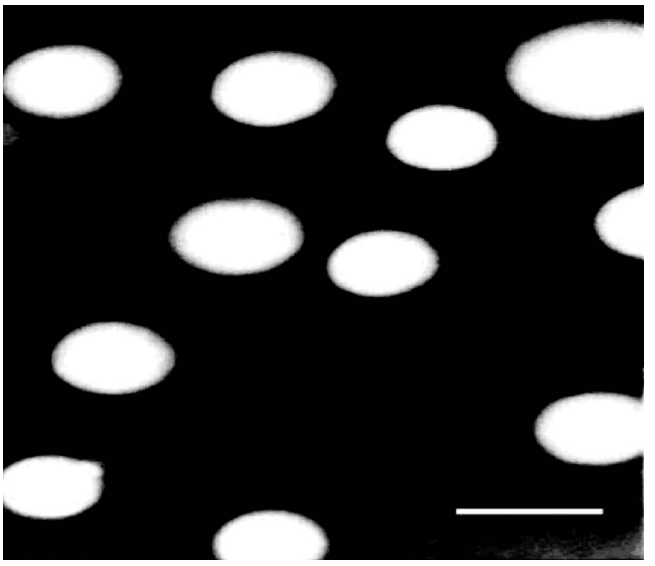

FIGURE 4. Transmission electron micrograph (TEM) of cytarabine-loaded liposomes produced by thin-film hydration followed by extrusion through 200-nm pore diameter membrane filter. The liposome composition was drug : lipid (1:20 mol ratio) and EPC : Chol (60:40% ratio). The magnification bar is 300 nm.

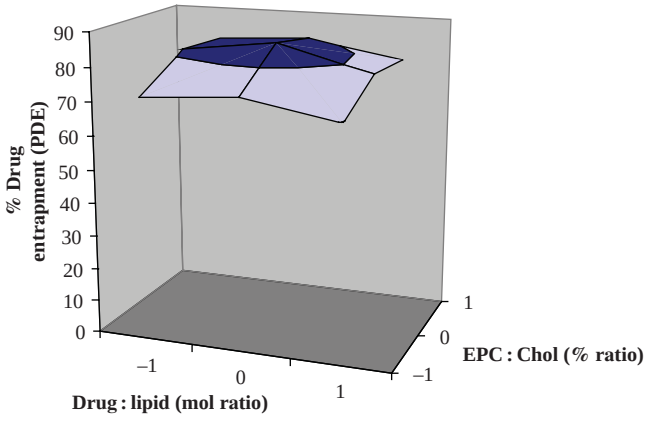

FIGURE 5. The effect of drug : lipid (mol ratio) and EPC : Chol (% ratio) on percent drug entrapment (PDE) of all the nine batches of factorial design. On *x*-axis: drug : lipid mol ratio, −1 = 1:17, 0 = 1:20, and 1 = 1:23; and on *y*-axis: EPC : Chol % ratio, −1 = 50:50, 0 = 60:40, and 1 = 70:30. The values are expressed as $M \pm SD$ ($n = 3$).

for the formulation having gelation temperature higher than 37°C, a liquid dosage form still exists at the body temperature, resulting in the rapid clearance of the administered drugs at an early stage. So, we have tried to prepare a liquid formulation that will gel at or just below body temperature. From the optimization study by factorial design, it was determined that optimum concentration of both chitosan and GP were important for gelation. Three levels of chitosan (1.5, 1.8, and 2.1%, wt/vol) and GP (4.7, 5.7, and 6.7%, wt/vol) were used for the optimization study. The study showed that 1.5% (wt/vol) chitosan with every level of GP either resulted in no gelation or unstable gel with gelation time of 15–20 min, whereas 2.1% (wt/vol) chitosan with every level of GP resulted in stable gel but with gelation temperature between 24 and 28°C. On the contrary, 4.7% (wt/vol) GP with every level of chitosan resulted in no gelation, and 6.7% (wt/vol) GP with every level of chitosan resulted in either unstable gel with gelation time between 15 and 20 min or stable gel with gelation temperature between 22–24°C. The desired gelation time and gelation temperature were achieved with 1.8% (wt/vol) chitosan and 5.7% (wt/vol) GP, respectively. The gelation time and gelation temperature with these concentrations were found to be less than 5 min and 36.6°C, respectively. The gelling temperature of plain C-GP solution and CGPCLL was determined graphically as the inflection point on the curve of the apparent viscosity (mPas) as a function of the temperature. Sudden change in the viscosity of plain C-GP solution and CGPCLL was observed at 36.6 and 37.3°C, respectively, indicating gel formation (Figure 6). At this temperature, gel formation is due to the neutralization of chitosan amine groups by the phosphate group of glycerophosphate and the consequent removal of repulsive interchain electrostatic forces which subsequently allows for extensive hydrogen bonding and hydrophobic interactions between chains (Chenite et al., 2000).

In Vitro Release Study

The in vitro release study conducted for the CGPFC, CLLS, and CGPCLL is presented in Figure 7. It revealed that about 90% of the drug was released within 12 h for CGPFC, whereas in the case of CLLS and CGPCLL, release of cytarabine follows a biphasic pattern. An initial rapid release of the drug occurs, followed by a subsequent, slow, and sustained release. While the unencapsulated fraction of the drug is responsible for the initial burst release (or the drug leached during mixing of cytarabine-loaded liposome with C-GP solution in case of CGPCLL), the later part of the release profile can be attributed to the release of the encapsulated fraction of the drug from liposomes for CLLS and encapsulated fraction of drug from liposomes as well as diffusion of released drug from the gel in case of CGPCLL. From the study, it was determined that CLLS sustains the drug release for 48 h, whereas CGPCLL sustains the drug release for more than 60 h. The burst release pattern obtained after the addition of Triton X-100 to the donor compartment after 60 h in case of CGPCLL indicated that the intact liposomes were still present in the C-GP gel at the end of study (Gabrijelcic & Sentjurc, 1995; Henriksen, Smistad, & Karlsen, 1994). Moreover, release of cytarabine from CGPCLL was significantly slower (86.5% released after >60 h) than the release from CPGFC (89.2% released within 12 h) and CLLS (85.4 released after 48 h), which confirms that addition of cytarabine-loaded liposomes in C-GP gel (CGPCLL) resulted in a slower drug release rate due to two factors: one, rigidity of the phospholipid bilayer and second, gel matrix

TABLE 1
Mean Particle Size and Polydispersity Index Determined by Dynamic Light Scattering and Percent Drug Entrapment Determined by Ultracentrifugation of Nine Different Batches (by 3^2 Factorial Design) of Cytarabine-Loaded Liposomes ($n = 3$)

Cytarabine-Loaded Liposomes (CLL)	Composition	Mean Size ($M \pm SD$) (nm)	Polydispersity Index (PdI) $M \pm SD$	Percent Drug Entrapment (PDE $\pm$ *SD*)
CLL-1	1:17 drug : lipid (mol ratio), 50:50 EPC : Chol (% ratio)	217 ± 2.58	0.268 ± 0.021	72.3 ± 2.74
CLL-2	1:17 drug : lipid (mol ratio), 60:40 EPC : Chol (% ratio)	214 ± 3.45	0.249 ± 0.034	74.6 ± 1.23
CLL-3	1:17 drug : lipid (mol ratio), 70:30 EPC : Chol (% ratio)	210 ± 2.87	0.279 ± 0.026	69.77 ± 2.99
CLL-4	1:20 drug : lipid (mol ratio), 50:50 EPC : Chol (% ratio)	241 ± 3.2	0.244 ± 0.021	81.4 ± 3.41
CLL-5[a]	1:20 drug : lipid (mol ratio), 60:40 EPC : Chol (% ratio)	220 ± 3.9	0.213 ± 0.011	85.20 ± 2.58
CLL-6	1:20 drug : lipid (mol ratio), 70:30 EPC : Chol (% ratio)	232 ± 3.45	0.261 ± 0.031	77.56 ± 2.79
CLL-7	1:23 drug : lipid (mol ratio), 50:50 EPC : Chol (% ratio)	290 ± 4.12	0.290 ± 0.039	80.28 ± 3.12
CLL-8	1:23 drug : lipid (mol ratio), 60:40 EPC : Chol (% ratio)	278 ± 3.98	0.279 ± 0.033	81.78 ± 2.17
CLL-9	1:23 drug : lipid (mol ratio), 70:30 EPC : Chol (% ratio)	282 ± 4.54	0.310 ± 0.044	76.58 ± 3.28

The values are expressed as $M \pm SD$.
[a]Optimized batch.

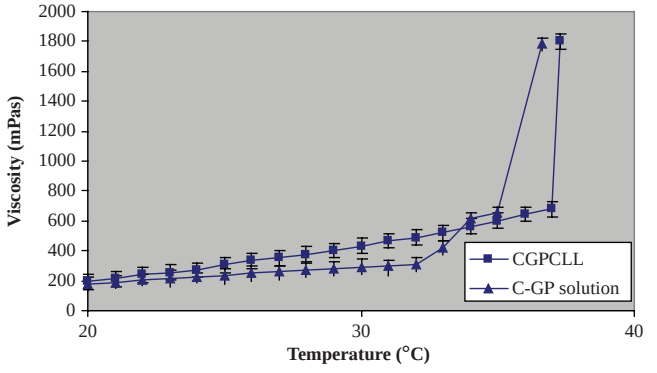

FIGURE 6. Effect of temperature on the viscosity of C-GP-containing cytarabine-loaded liposomes (■, CGPCLL) and C-GP solution (▲) measured at 10 s^{-1} shear rate. Values are expressed as $M \pm SD$ ($n = 3$).

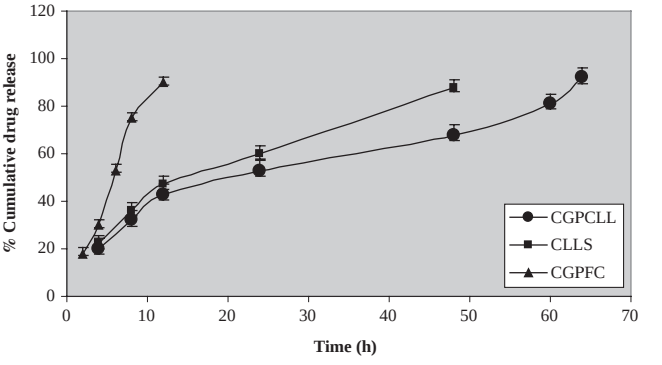

FIGURE 7. In vitro drug release profile of C-GP-containing cytarabine-loaded liposomes (●, CGPCLL), cytarabine-loaded liposomal suspension (■, CLLS), and C-GP-containing free cytarabine (▲, CGPFC) in PBS at 37°C. Each point represents the $M \pm SD$ ($n = 3$).

which drug has to diffuse after it gets released from liposomes. From this study, it was confirmed that release of cytarabine can be sustained for more than 60 h by incorporating cytarabine-loaded liposomes in C-GP system.

In Vivo Pharmacokinetic Study

The in vivo pharmacokinetic study of CGPFC, CLLS, and CGPCLL was carried out in albino rats. All three formulations were given to rats intramuscularly at a dose equivalent to 5.4 mg cytarabine/kg body weight, and drug was recovered from plasma at different time intervals. It was clear that CGPCLL sustained the release of drug for more than 60 h, whereas CLLS and CGPFC released maximum amount of drug (near about 90%) within 48 and 10 h, respectively (Figure 8). The rate of drug release obtained from in vitro study was almost the same as that of in vivo study as seen from Figures 7 and 8;

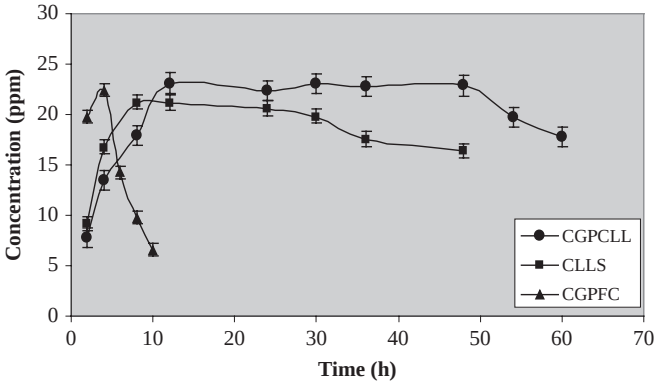

FIGURE 8. Plasma profile of cytarabine following i.m. administration of C-GP-containing cytarabine-loaded liposomes (●, CGPCLL), cytarabine-loaded liposomal suspension (■, CLLS), and C-GP-containing free cytarabine (▲, CGPFC) into rats at 5.4 mg cytarabine/kg body weight. Each point represents the $M \pm SD$ ($n = 3$).

TABLE 2
Various Pharmacokinetic Parameters of Cytarabine as Determined on the Intramuscular Administration of C-GP Containing Free Cytarabine (CGPFC), Cytarabine-Loaded Liposomal Suspension (CLLS), and C-GP Containing Cytarabine-Loaded Liposomes (CGPCLL) Derived Using the Mean ($n = 3$) Cytarabine Levels in Plasma Versus Time Data Using QuickCalc Software by Noncompartmental Analysis ($n = 3$)

Pharmacokinetic Parameter	CGPFC	CLLS	CGPCLL
C_{max} (ppm)	22.34 ± 0.782	21.42 ± 0.587	23.12 ± 0.664
$t_{1/2}$ (h)	3.077 ± 0.0021	19.931 ± 0.019	28.86 ± 0.025
K_{el} (mL/min/kg)	0.225 ± 0.01	0.077 ± 0.009	0.0513 ± 0.0083
Vd (mL)	8.023 ± 0.534	24.28 ± 0.778	27.317 ± 0.892
$AUC_{0-\infty}$ (h mg/mol)	189.16 ± 1.13	2318.82 ± 3.38	2526.88 ± 5.52
$MRT_{0-\infty}$ (h)	9.8952 ± 0.226	46.5288 ± 0.975	64.2892 ± 1.075

The values are expressed as $M \pm SD$.

wherein, the time taken by CGPFC, CLLS, and CGPCLL, respectively, to release about 90% of the drug both in in vitro and in vivo study was almost same. The existence of significant differences ($p < .01$) in various pharmacokinetic parameters between different formulations derived using Quick Calc software is clearly evident from Table 2. The area under the curve (AUC), mean residence time (MRT), volume of distribution (Vd), and $t_{1/2}$ were observed for the determination of most effective formulation among CGPFC, CLLS, and CGPCLL for sustained drug release. The higher plasma AUC, MRT, Vd, and $t_{1/2}$ for CGPCLL and CLLS were observed compared with CGPFC, showing that both CGPCLL and CLLS sustained the drug release for longer duration compared with CGPFC. Between CGPCLL and CLLS, CGPCLL sustained the drug release for longer duration compared with CLLS (Table 2). Another important parameter for the evaluation of sustained release by CGPCLL is elimination rate constant (K_{el}). From the values shown in Table 2, it was observed that CGPCLL showed lower K_{el} compared with CLLS and CGPFC indicating that drug release rate is slow and drug remains in the body for longer period in the case of CGPCLL (Table 2).

CONCLUSION

The developed injectable thermogelling C-GP system has opened up a new strategy in the field of minimally invasive and site-specific in situ forming implants as it can sustain the release of low-molecular weight hydrophilic drug-like cytarabine for more than 60 h, thereby having an advantage over the conventional liposome formulation. Moreover, the system is biodegradable, biocompatible, devoid of surfactant, and minimal mechanical irritation upon in vivo implantation because of its soft and elastic nature. Further, detailed study of this system with respect to its anticancer activity and organ toxicity can lead to a development of novel delivery system for the treatment of cancer.

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