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# A Method to Determine the Incorporation Capacity of Camptothecin in Liposomes

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## ABSTRACT

The purpose of this study was to establish a new experimental approach to determine the maximum amount of camptothecin (CPT) that can be incorporated in liposomes, and to use this method to compare the CPT-incorporation capacity of various liposome formulations. Small, CPT-saturated liposomes were prepared by dispersing freeze-dried blends of lipids and drug in phosphate buffer, and subsequent probe-sonication. Excess precipitated CPT could be separated from the liposomes by ultracentrifugation. The small and homogeneous liposome size obtained gave a good and reproducible recovery of liposomes in the supernatant (>80%), whereas the acidic pH (pH 6.0) kept CPT in its hydrophobic lactone form, which is poorly soluble in the buffer. The maximum CPT-incorporation capacity of 12 different liposome formulations was investigated, using the described method, and was found to vary widely. With liposomes made of neutral and anionic phospholipids, the solubility of CPT in the buffer was improved by approximately a factor of 10 (from ~2.7 to 15-50 µg/mL) as compared with buffer. With cationic liposomes containing 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a maximum CPT-solubilization of ~100-fold, the buffer solubility was reached, probably owing to an electrostatic interaction between the cationic lipids and the carboxylate-CPT isomer. Increasing DOTAP fractions within egg-phosphatidylcholine (EPC)/DOTAP liposomes reached a CPT-incorporation plateau at ~20 mol% DOTAP. The presented approach appears suitable to study the incorporation capacity of any drug component within small vesicles as long as the liposome incorporation is high relative to the intrinsic water solubility of the drug.

**KEYWORDS:** liposomes, camptothecin, drug carriers, incorporation, DOTAP.

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## INTRODUCTION

Numerous attempts to overcome poor water solubility of drugs by liposomal formulations are described in the literature.[1-9] Such formulations, unfortunately, are developed mostly on an empirical basis, and systematic quantitative studies on the potential of a given formulation to solubilize a drug are lacking. The incorporation of 20-S-(+)-camptothecin (CPT) into liposomes is described in the literature mostly in a qualitative manner,[10] and the few quantitative data are difficult to compare because of the differences in their experimental approaches.[11-13] Many approaches[9-11,13-15] are aimed at incorporation of CPT or its derivatives into micellar, liposomal, or nanoparticular lipid carriers, separating free from incorporated CPT by various means. From our experience, the results of all these approaches may be hampered by incomplete separation of free and liposomal CPT.

Routine clinical application of CPT has not been achieved to date despite the compound's strong topoisomerase-I inhibitory potential in vitro[16-18] and its broad anticancer activity established in the preclinical athymic mouse model.[19-21] This failure is due to its poor water solubility and hydrolytic conversion of the active lactone form into a less active and more toxic ring-open carboxylate form at physiological pH (Figure 1).[22,23] One approach to overcome the unfavorable physicochemical characteristics of CPT is to synthesize more hydrophilic analogs and prodrugs. Two such drugs, topotecan (Hycamptin) and irinotecan (Camptosar), have been approved by the authorities. The problem of hydrolytic conversion into less active carboxylate isomers, however, persists. Another approach is to solubilize CPT (or its hydrophobic congeners) by lipid-based drug carriers such as micelles, nanoparticles, and liposomes. This method appears to protect CPT from hydrolysis, and may result in a targeted delivery of CPT to solid tumors as observed with other liposome-based cytostatics.[24,25] Despite several attempts over the past 10 years to develop lipid-based drug carriers for CPT, no such formulation has reached the clinic. CPT, although hydrophobic in nature and thus inserted in the hydrophobic core of phospholipid bilayers, appears to have only a limited affinity for liposome carriers as determined by fluorescence anisotropy titration.[12] Thus, the aim of the current study was to establish an alternative experimental approach that allows quantification of the maximum amount of CPT incorporated in the liposomes and to compare the CPT-incorporation capacity of various liposome formulations.

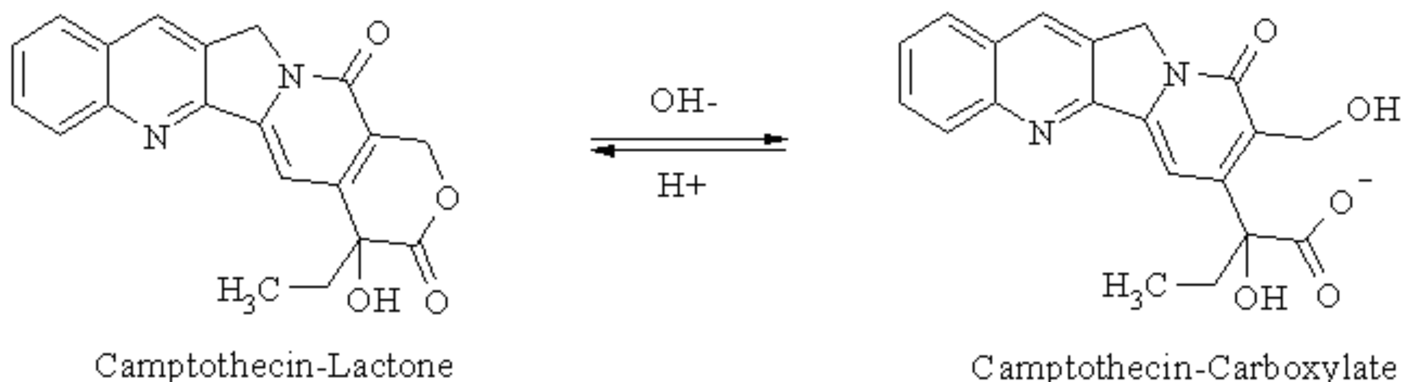


**Figure 1.** The pH-dependent carboxylate-lactone equilibrium of camptothecin.

**Table 1.** Organic Solvents and Lipids Concentrations Used for the Lipid Stock Solutions Prepared Prior to Freeze Drying*

| Lipid | Solvent | Lipid Concentration |
|---|---|---|
| DOPC, DLPC, DMPC, EPC , E-80 | Methanol | 200 mg/mL |
| DOTAP | Chloroform:Methanol (2:1 vol/vol) | 200 mg/mL |
| CHOL | Chloroform:Methanol (2:1 vol/vol) | 100 mg/mL |
| DOPE | Chloroform:Ethanol (2:1 vol/vol) | 200 mg/mL |
| DPPG, DMPG | Chloroform:Ethanol (2:1 vol/vol) | 25 mg/mL |

*DOPC indicates 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; DLPC, 1,2-dilauroyl-sn-glycero-3-phosphatidylcholine; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine; EPC, egg phosphatidylcholine; E-80, Lipoid E-80 (80%-85% EPC); DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine; DPPG, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol; DMPG, 1,2-dimyristoyl-sn-glycero-3-phosphatidylglycerol; CHOL, cholesterol; and PC, phosphatidylcholine.

## MATERIALS AND METHODS

### *Chemicals*

Camptothecin (CPT) was obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Egg phosphatidylcholine (EPC), Lipoid E-80 (80%-85% EPC), 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE), and 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) were obtained from Lipoid GmbH (Ludwigshafen, Germany); and 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), 1,2-dilauroyl-sn-glycero-3-phosphatidylcholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphatidylglycerol (DMPG), and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol (DPPG) were purchased from Genzyme Pharmaceuticals (Liestal, Switzerland). Cholesterol (CHOL) was purchased from Croda Chemicals Ltd (Brighton, UK), and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) was delivered by Avanti Polar (Alabaster, AL). Liquid chromatography gradient grade quality organic solvents were obtained from Merck (Darmstadt, Germany). Water was freshly distilled and buffers filtrated through 0.22-µm pore size filters before use.

### *Liposome Preparation*

CPT was dissolved in dimethylsulfoxide (DMSO) to make up a 4.0 mg/mL stock solution. Lipids were dissolved in organic solvents to make up lipid stock solutions as given in Table 1. The stock solutions were transferred to injection vials and mixed to form 12 different lipid compositions, containing in total 200 or 250 mg lipid and 2.0 or 2.5 mg CPT depending on the volume of the final lipid dispersion, which was either 2.0 or 2.5 mL. The organic mixtures were shock-frozen in liquid nitrogen and placed in a precooled freeze dryer (Beta 2-16 equipped with an LMC-2 controller, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Freeze drying was performed as follows: condenser temperature ≤-80°C, shelf temperature at start -40°C and at the end 45°C, and pressure declining from 800 mbar to 0.008 mbar. The freeze-drying program lasted 65 hours. The residual organic solvent was controlled by weighing the sample vials prior to and after freeze drying and found to be acceptable (<1%). The vials were sealed and stored at -80°C.

Liposome dispersion was formed from a freeze-dried phospholipid (PL)-CPT mixture by hydration with 25 mM phosphate-buffered saline (PBS), pH 6.0, to make up 2.0 mL (screening study) and 2.5 mL (DOTAP-study) of the final liposome formulations, containing 100 mg/mL lipid and 1 mg/mL CPT. For formation of small vesicles (SUVs), the lipid dispersions were probe sonicated (Labsonic U, B. Braun Biotech International, Leverkusen, Germany) at 50 W in an ice bath for 3 cycles of 5 minutes each. After sonication, the liposome dispersion was refrigerated at 4°C to 8°C overnight for equilibration, unless stated otherwise.

### *Ultracentrifugation*

For separation of liposomes and CPT crystals, the liposome dispersions were transferred to 3-mL polycarbonate, thick-wall centrifuge tubes. The 2-mL samples were diluted with 500 µL PBS to fill the tubes to 1/8 inch from the top, as recommended by the manufacturer.[26] An Optima LE-80 ultracentrifuge with an SW60Ti rotor was used for centrifugation (Backman Instruments, Inc. Palo Alto, California). Chosen centrifugation conditions were 100 000*g* for 20 minutes at 10°C for the CPT liposome incorporation study, and 25°C for the CPT DOTAP-liposomes incorporation study.

### *Camptothecin Quantification*

A modification of the high-performance liquid chromatography (HPLC) method previously described by Warner and Burke[27] was used for CPT quantification within the liposomes. Liposome samples were diluted with 9 mM phosphate-buffered solution (PB), pH 3.0, containing 5% (wt/wt) Triton to convert all CPT into the lactone form and to dissolve the liposomes. The HPLC method used mobile-phase gradients from 25% to 35% acetonitrile (AcCN) for 10 minutes in a 1% triethylamine buffer with pH 5.5 and a flow rate of 1 mL/min. The Waters HPLC system was equipped with a 474 scanning fluorescence detector, a 2695 separation module, and a symmetry $C_{18}$-column, 3.9 × 150 mm (wavelengths, excitation $\lambda$ = 360 nm and emission $\lambda$ = 440; injection volume, 10 µL) (Waters Corp, Milford, MA).

### *Phosphatidylcholine Quantification*

The relative content of phosphatidylcholine (PC) in the supernatant as compared with the whole dispersion was determined to quantify the liposome recovery under centrifugation. The PC content was quantified using an enzymatic assay[28] with a commercial test kit (Phospholipids B, Wako Chemicals USA Inc, Richmond, VA). In brief, 50 µL liposome dispersion was dissolved with PBS containing Triton to make up a final concentration of 5% (wt/wt); samples and blank (25 mM PBS, pH 6.0, with 5% Triton) were applied in triplicates; and 250 µL phospholipid B reagent solution was added and the plate incubated at 37°C for 30 to 45 minutes in the reader with shaking for the first 5 minutes. A reddish color appeared due to formation of a phenole-4-aminoantipyridine complex.[29] Absorbance was measured at $\lambda$ = 492 nm using a microplate reader (PolarStar Galaxy, BMG Lab Technologies GmbH, Offenburg, Germany) and Microplate Costar 96 plates (Corning Incorporation, Corning, NY).

### *Photon Correlation Spectroscopy*

The liposome size in the supernatant after centrifugation was measured by photon correlation spectroscopy (PCS) analysis using a Nicomp model 380 particle sizing system (Nicomp Particle Sizing Systems, Santa Barbara, CA) with software version C-370 V-1.51a, and equipped with a fixed 90° external fiber angle and a 632.8-nm, 5-mW He-Ne laser. Sample preparation was done as described in Frantzen et al.[30] All operations were performed in a laminar airflow bench. Test tubes (borosilicate glass) were cleaned by sonication for 15 minutes in an ultrasonic bath containing distilled water and washed with freshly filtrated PBS (syringe filter unit Millex-GS 0.22 µm, Millipore Corp, Billerica, MA). The liposome dispersion was diluted empirically with freshly filtrated PBS until a count range of 250 to 350 kHz was achieved. Instrument parameters chosen for the analysis were as follows: Nicomp distribution and number weighted; every sample was analyzed with an equilibration time of at least 5 minutes and a measurement (data collection) time of 15 minutes.

### *Fluorescence Microscopy*

A fluorescence microscope, Zeiss Axiosert S 100 (Zeiss Corp, Oberkochen, Germany) was used to examine the supernatants of the CamptoLipid formulations for presence of CPT crystals. The supernatant was diluted 1:10 with 25 mM PBS pH 6.0; 1 drop was applied on a clean glass slide and covered with a cover glass. Observations were conducted at ×20 000 magnification.

## RESULTS AND DISCUSSION

### *Experiments for Establishing the Experimental Approach*

For separation of free- and liposome-incorporated CPT, size-exclusion chromatography was first tested, as suggested by Cortesi et al[11] and Sugarman et al.[10] In our hands, size-exclusion chromatographic fractionation (on a Sephadex G-25 column (Pharmacia Biotech AB, Uppsala, Sweden) was hampered by a poor overall recovery (<<10%) of CPT, most likely owing to adhesion of precipitated CPT to the chromatography column. Unfortunately, the recovery of CPT from the columns was not given in the above reports. We cannot therefore judge whether incorporation capacities reported in the literature may have been hampered by this effect. Nevertheless, since no gel material is able to separate drug crystals from liposomes, alternative approaches were sought. Separation of free CPT from liposomes by centrifugation has previously been suggested in literature: Lundberg et al[9] and Sugarman et al[10] pelleted the CPT (crystals) by centrifugation at 10 000*g* for 1 hour or by Ficoll gradient centrifugation 3400 rpm for 30 minutes, respectively. Our results demonstrated that centrifugation at 16 000*g* for 30 minutes yielded insufficient separation as revealed by light microscopic evaluation of drug crystals in the supernatant. These studies do not report whether CPT crystals occurred in the liposome fraction (supernatant). In our hands, ultracentrifu-

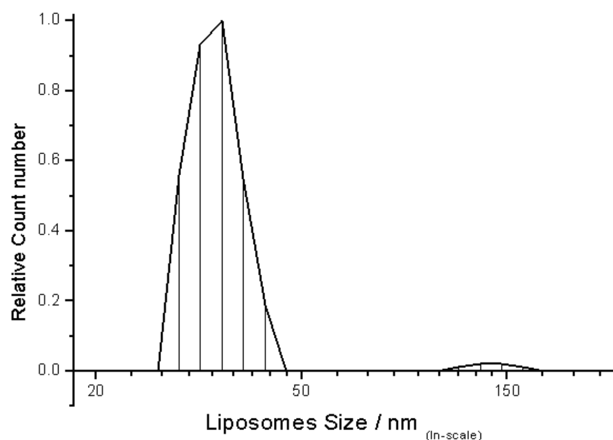


**Figure 2.** A typical size distribution of sonicated and ultra-centrifuged liposomes measured by photon correlation spectroscopy (PCS).

gation at 100 000*g* for 20 minutes gave best separation of free CPT from the liposomes, as no CPT crystals were detectable in the supernatant under these conditions. Daoud et al,[13] in contrast, pelleted multilamellar vesicles (MLVs) (centrifugation at 100 000*g* for 1 hour) and quantified the CPT content within the liposomes in the pellet. The same approach was applied by Zhang et al,[31] who characterized liposomes (~130 nm in diameter) containing SN-38, a hydrophobic CPT derivative. They centrifuged the liposome dispersion for 2 hours at 200 000*g*, and the amount of drug incorporated in the liposomes was calculated as the total drug content minus drug quantified in the supernatant. A major drawback of this approach is that any microcrystals present would end up in the pellet and thus spoil the results.

In contrast, using our experimental approach, the liposomes remain in the supernatant to a high extent due to their very small size. By selecting a pH in which CPT is almost insoluble, ultracentrifugation works properly in terms of spinning down all of the CPT not incorporated into the liposomes. Nevertheless, the PL-recovery (loss of liposomes in the pellet) was quantified, and the incorporation capacity normalized for this loss.

Based on the experiences gained during preliminary experiments, the following final approach was chosen. For liposome preparation, drug and lipids were dissolved in organic solvents suitable for freeze drying. Volumetric mixing of stock solutions of the drug and various lipids, respectively, allowed preparation of a variety of lipid compositions at a minimum risk of contamination. Shock freezing in liquid nitrogen and freeze drying were used to minimize the risk of drug and/or lipid recrystallization in order to prepare homogeneous lipid-drug cakes. After freeze drying, the PL-CPT cakes were hydrated with 25 mM PBS, pH 6.0, where most of the CPT was present in its water-insoluble lactone form. The chosen centrifugation conditions demanded preparation of homogeneously sized SUVs to ensure liposomes would not be trapped along with the CPT crystals in the pellet. Probe sonication was thus performed. Sonication for 3 cycles of 5 minutes at 50 W was found appropriate for reducing the liposome size, as PCS measurements revealed a mean diameter of <50 nm for the vast majority and a minor population of size >100 nm representing <2% of the liposomes (number weighted). A typical size distribution is displayed in Figure 2.

### *Choice of pH for the Formulation*

As CPT is susceptible to spontaneous reversible hydrolysis such that the intact lactone form predominates at acidic pH, whereas the inactive opened-ring carboxylate species is favored at neutral and alkaline pH, CPT formulations should have an acidic pH. In aqueous solutions, the lactone-/carboxylate-equilibrium was found at pH 6.8 (data not shown). On the other hand, buffer pH influences phospholipid hydrolysis.[32] Hydrolysis of phosphatidylcholine in the liposome bilayer is described to be at its minimum around pH 6.5.[33] After considering both these aspects, a pH of 6.0 was chosen for this formulation study. At this pH, ~85% of the CPT was present in its lactone form (data not shown).

### *CPT Liposome Incorporation Study*

Twelve liposome preparations of different lipid compositions were prepared in 3 parallels each, and the content of CPT remaining in the supernatant after centrifugation was quantified; results are given in Table 2. All liposome formulations were found to contain increased amounts of CPT in the supernatant as compared with CPT in buffer. The overall solubility in PBS at the chosen pH was 2.72 µg/mL as measured by HPLC in saturated solutions at 25°C. It is obvious that considerable amounts of CPT were solubilized by the liposomes. As some liposomes may get trapped in the pellet after centrifugation, the amount of PC in the supernatant was quantified in comparison to the PC content before centrifugation (Table 2). PC recovery in the supernatant in general was quite high and varied only modestly, both within parallels and between the various formulations. Nevertheless, in order to compensate for variable PC recovery, each CPT concentration value was adjusted by the corresponding PC-recovery value, such that CPT incorporation was normalized to a 100 mg/mL lipid level after subtracting the water-dissolved fraction of the drug (Figure 3).

Despite the fact that liposome-membrane composition varied considerably in terms of fatty acid chain length and saturation, net charge, and membrane fluidity, the chosen approach seemed to work properly, thus allowing for a direct comparison of the incorporation capacity of different liposome formulations.

Throughout the PC group (DLPC, DMPC, DOPC, EPC, E-80), a maximum solubilization of approximately 30 µg/mL

**Table 2.** Phospholipid Composition of the 12 Formulations in the Screening Study, and Recovery of Camptothecin and Phosphatidylcholine in a 10% (mass/vol) Liposome Dispersion After Ultracentrifugation (n = 3)*

| Phospholipid Composition | Molar Ratios | CPT Concentration in Supernatant µg/mL (mean ± SD) | % PC recovery (mean ± SD) |
|---|---|---|---|
| DLPC | 1 | 27.3 ± 5.6 | 93.9 ± 8.9 |
| DMPC | 1 | 12.3 ± 4.8 | 84.2 ± 22.7 |
| E-80 | 1 | 26.4 ± 3.5 | 96.3 ± 6.7 |
| EPC | 1 | 26.5 ± 3.4 | 86.1 ± 14.3 |
| EPC/DOPC | 0.5/0.5 | 29.5 ± 2.8 | 102.6 ± 10.5 |
| EPC/DOTAP | 0.8/0.2 | 138.1 ± 8.3 | 97.9 ± 4.1 |
| EPC/DOPE/DOTAP | 0.4/0.4/0.2 | 39.6 ± 15.8 | 113.8 ± 6.3 |
| DOPC/DOPE/DOTAP | 0.4/0.4/0.2 | 49.4 ± 8.2 | 92.9 ± 4.6 |
| EPC/DMPG | 0.9/0.1 | 27.3 ± 6.8 | 98.3 ± 7.6 |
| EPC/DPPG | 0.9/0.1 | 50.9 ± 9.5 | 94.5 ± 3.0 |
| E-80/CHOL | 0.5/0.5 | 17.0 ± 3.8 | 85.3 ± 7.8 |
| EPC/CHOL | 0.5/0.5 | 22.2 ± 8.0 | 88.5 ± 7.5 |

*Abbreviations are explained in the footnote to Table 1.

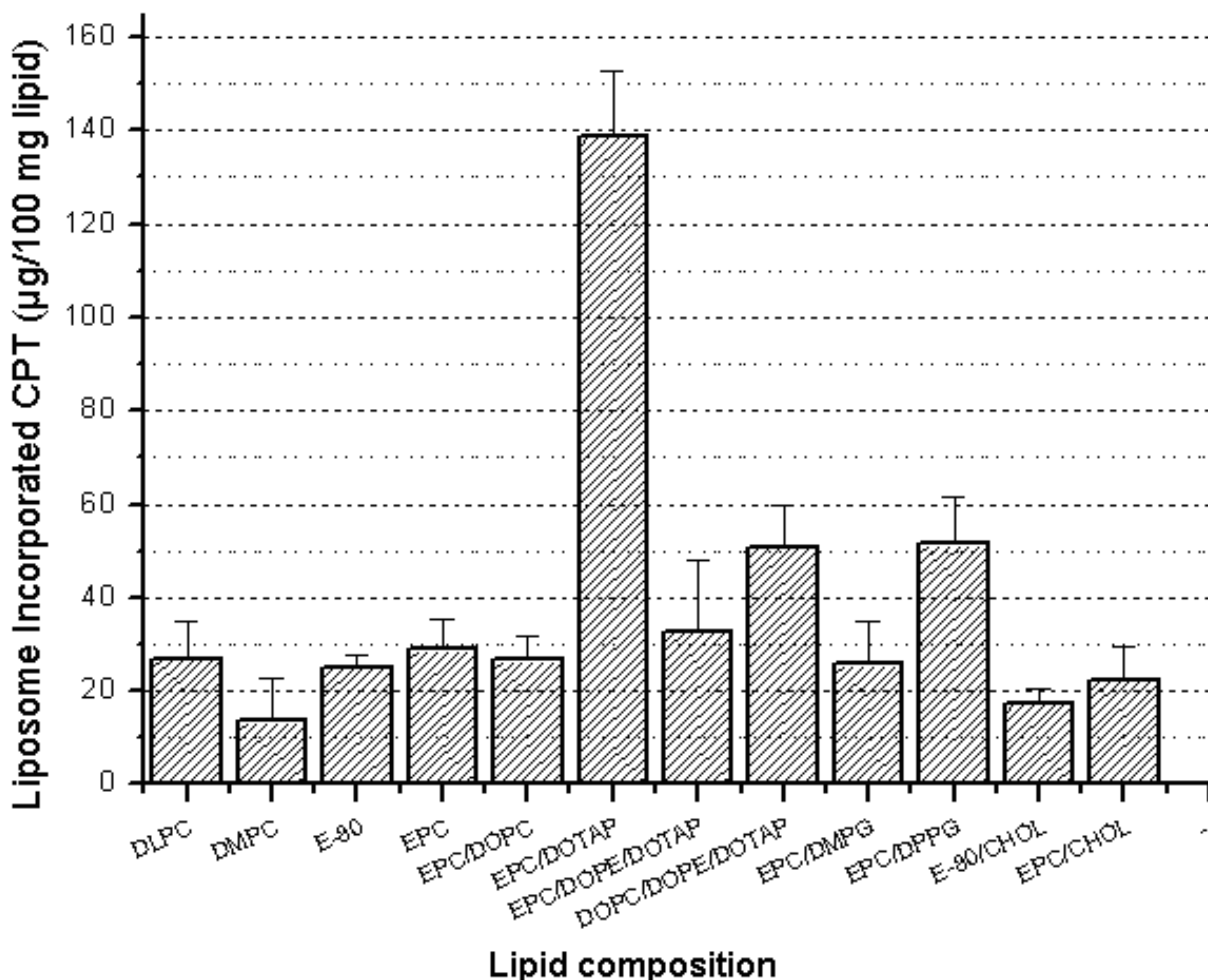


**Figure 3.** Camptothecin-incorporation capacity of 12 liposome formulations, of differing lipid composition (n = 3).

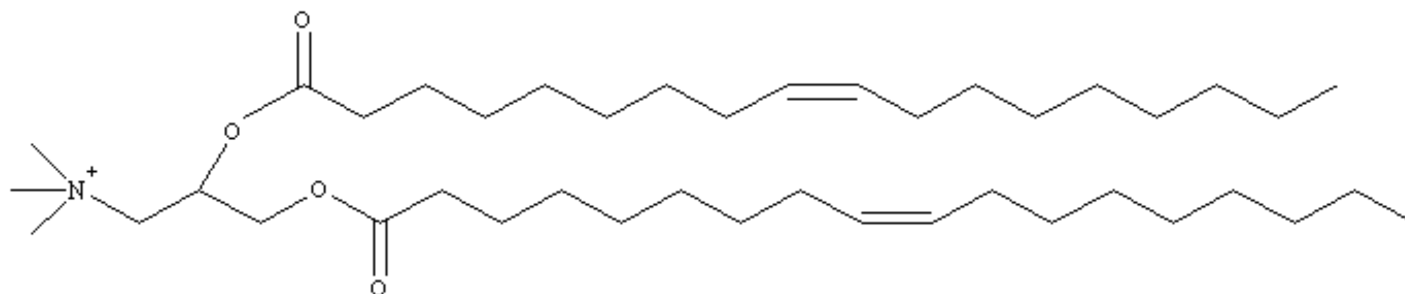


**Figure 4.** The structure formula of 1,2-dioleyl-3-trimethylammonium-propane (DOTAP).

**Table 3.** Recovery of Camptothecin in a 10% (mass/vol) Liposome Dispersion Containing Different Amounts of DOTAP, and the Respective Phosphatidylcholine Recovery in the Samples After Ultracentrifugation (n = 4)*

| Formulation | % (mol) DOTAP | CPT Concentration in supernatant, µg/mL (mean ± SD) | % PC recovery (mean ± SD) |
|---|---|---|---|
| 1 | 0 | 40.5 ± 1.5† | 86.3 ± 2.5† |
| 2 | 2.5 | 46.4 ± 3.8 | 84.5 ± 3.2 |
| 3 | 5 | 58.5 ± 4.6 | 88.1 ± 1.7 |
| 4 | 10 | 163.1 ± 14.0† | 90.6 ± 2.8† |
| 5 | 20 | 206.7 ± 14.1† | 91.6 ± 2.5† |
| 6 | 40 | 213.7 ± 16.1 | 92.3 ± 3.3 |

*DOTAP indicates 1,2-dioleoyl-3-trimethylammonium-propane; CPT, camptothecin; and PC, phosphatidylcholine.
†Owing to experimental problems, n = 3.

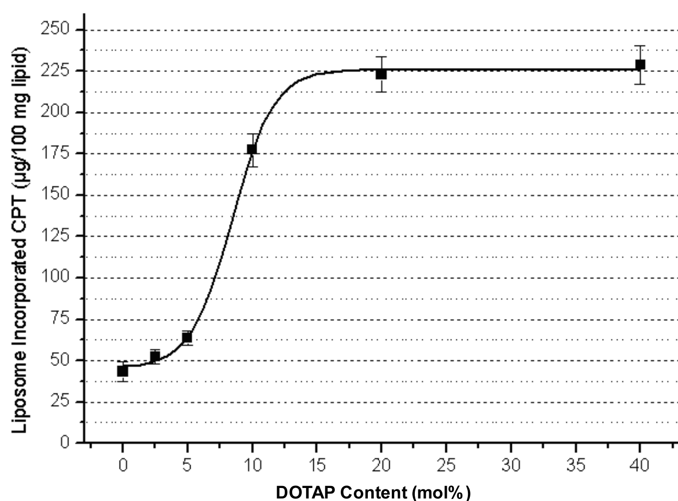


**Figure 5.** Camptothecin-incorporation capacity of 10% (mass/vol) liposome dispersions as function of lipid-mol% of DOTAP in the formulation (n = 4).

was achieved with one minor exception: DMPC liposomes appeared to solubilize slightly less CPT. This may be because DMPC (phase transition temperature Tm = 23°C), unlike the others, is expected to be in the "gel" rather than the "fluid" state at the temperature of incubation (4°C-8°C). For a general conclusion on this aspect, however, more data are needed. A slight tendency toward reduced CPT-incorporation capacity was observed when equimolar amounts of cholesterol were present in the PC membrane (EPC/CHOL and E-80/CHOL as compared with EPC and E-80 alone). Cholesterol does not form bilayers itself but is known to cause condensing and stiffening of PC bilayers above phase transition temperature.

The most prominent effect observed was that CPT appeared to have higher affinity for cationic lipid blends as compared with the electro-neutral ones. This finding agrees well with Perez-Soler's[34] qualitative observation that the least CPT crystals occurred in DOTAP liposomes. Since CPT lactone at the chosen pH is in equilibrium with its negatively charged carboxylate isomere, the latter may, through electrostatic interactions, bind preferentially to cationic liposomes. Separate quantification of the 2 isomers by HPLC in the liposome formulation is not feasible, unfortunately, owing to rapid reequilibration during sample preparation.

In our studies, the amounts of CPT incorporated within the various liposomes deviated, at least in part, considerably from the mass ratios previously given in the literature; we reached a maximum lipid-drug mass ratio of ~700:1 (EPC:DOTAP). In comparison, Daoud et al,[13] Cortesi et al,[11] and Burke et al[12] reported a CPT incorporation capacity as translated to mass ratios corresponding to 200:1, 20:1, and ~330:1, respectively. However, a direct comparison of these values is difficult to make because of the differences in experimental approach, as well as in pH and temperature. During our incorporation experiments, it was revealed that the incorporation capacity of the liposomes is highly temperature sensitive. By increasing the temperature during equilibration overnight from 4°C to 8°C to room temperature (18°C-22°C), and centrifugation temperature from 10°C to 25°C, the incorporation was increased almost 1.6-fold for the

same formulation (EPC:DOTAP, 80:20 mol/mol), from ~140 to 220 μg/100 mg lipid.

### *CPT DOTAP-Liposomes Incorporation Study*

As the EPC/DOTAP formulation showed a superior incorporation of CPT as compared with all other formulations in the screening study, this formulation was chosen as a starting point for comparing the effect of different molar fractions of DOTAP (from 0 to 0.4) within EPC/DOTAP liposomes. The aim of this study was to investigate if CPT incorporation correlated with the amount of DOTAP, and to find the optimal DOTAP content for maximum incorporation of CPT, as DOTAP has a higher toxicity and is more expensive than naturally occurring lipids. The structural formula of this synthetic lipid is shown in Figure 4. Since temperature seemed to have an effect on CPT incorporation, this study was performed at room temperature (18°C-22°C), and temperature at ultracentrifugation was increased from 10°C to 25°C. Measured CPT concentration in the supernatant and recovery of PC after centrifugation are shown in Table 3. In Figure 5, the calculated incorporation capacity of the different liposome composition at 100% PC recovery in the supernatant is plotted as a function of DOTAP concentration in the formulations. In Figure 5, as in Figure 3, the water-soluble fraction (2.7 μg/mL) was subtracted from the results, and only the lipid-associated fraction was included. A sigmoidal relationship between DOTAP concentration and CPT incorporated into the liposomes was obtained, with no further significant increase in CPT-incorporation capacity when increasing DOTAP-mol fraction from 0.2 to 0.4 (incorporation capacity of $223.1 \pm 10.7$ and $228.1 \pm 11.9$ μg/100 mg lipid at 20 and 40 mol% DOTAP, respectively). A more than 5-fold increased incorporation was observed when the DOTAP content was raised from 0 to 0.4 (mol fraction), and the solubility of CPT increased ~80 times as compared with buffer.

## CONCLUSION

A screening method for investigating incorporation capacity of camptothecin (CPT) in probe-sonicated, small unilamellar liposomes was developed and successfully applied in this study. CPT-saturated liposomes were separated from excess CPT crystals by ultracentrifugation. The maximum incorporation capacity of a variety of liposome formulations varied considerably, with a maximum for DOTAP-containing liposomes, an effect that might be explained by electrostatic interaction of the carboxylate CPT isomer with cationic liposomes. The presented approach is expected to be suitable for studying incorporation capacity for any drug component within liposomes as long as their incorporation is high in comparison to the water solubility of the drug.

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