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Preparation and *In Vitro* Evaluation of a Pegylated Nano-Liposomal Formulation Containing Docetaxel

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Abstract

An improved pegylated liposomal formulation of docetaxel has been developed with the purpose of improving the docetaxel solubility without any need to use tween80 that is responsible for hypersensitivities following administration. Liposomes all had spherical shape with size of 130–160 nm. The most important finding of this study is that pegylated liposomes were prepared with significant increase in docetaxel encapsulation efficiency and stability of the formulation in comparison with last reports on docetaxel liposomes. In vitro release studies revealed that such a formulation could be stable in the blood circulation and meet the requirements for an effective drug delivery system.

Keywords

Docetaxel • Liposome • Stability • Pegylated • Nanoparticle

Introduction

Over the past few decades, increasing attention has been given to drug targeting in order to reduce side-effects and improve therapeutic efficacy by preventing undesired drug localization in healthy tissue sites and decreasing rapid degradation or elimination of drugs [1–3]. Among a variety of targeted drug carrier systems, liposomes have been studied extensively because of their capability to accommodate a large variety of drugs, alongside

their good biocompatibility, low toxicity and lack of immune system activation or suppression [4]. In general, an optimized system is consisted of nanoliposomes, which possess a long circulation lifetime. Such liposomes will circulate sufficiently long to accumulate at sites of disease, such as tumors, as a result of the leaky vasculature and reduced blood flow exhibited by the diseased tissue [5].

A number of drugs have already been successfully encapsulated in liposomes, from anti-bacterials [6] and interferons [3] to antitumor drugs such as doxorubicin [1], mitoxantrone [7], paclitaxel [8–12], and docetaxel [13, 14].

Docetaxel (N-debenzoyl-N-tert-butoxycarbonyl-10-deacetyl paclitaxel) is a semisynthetic derivative of the taxoid family of antineoplastic agents. It is an analog of paclitaxel which is extracted from the needles of the European yew tree (Taxus baccata L.) [15]. Docetaxel has been effective against breast, ovarian, lung, and head and neck cancers. Being a microtubule stabilizing agent, it inhibits microtubule disassembly and consequently inhibits cell proliferation [16]. Due to the poor solubility of docetaxel in water, tween80 (polysorbate 80) and ethanol (50:50, v/v) are used for the formulations currently available in the market. Both tween80 and ethanol are responsible for hypersensitivities that occur after docetaxel administration and make premedication of the patients with corticosteroids and antihistamines a necessity. To overcome this problem and to improve efficacy, novel formulations of docetaxel have been introduced. Immordino et al achieved a better antitumor activity and a long circulation time in body by encapsulating docetaxel in liposomes [13]. As the work of Einhaus et al has shown, therapeutic efficacy is increased by entrapping docetaxel in micronized droplets of olive oil coated with functional fibrinogen. However, effectiveness may be limited in the long-term because of provoking an immune response [17]. Musumeci et al reported an increase in the solubility of docetaxel when incorporated in PLA/PLGA nanospheres although their formulation still contained tween80 [14]. Docetaxel-encapsulated nanoparticles formulated with poly(D,L-lactic-*co*-glycolic acid)-block-poly(ethylene glycol) (PLGA-b-PEG) copolymer and surface functionalized with the A 10 2'-fluoropyrimidine RNA aptamers, were found to exhibit remarkable efficacy and reduced toxicity [18]. Stability of the liposomal formulation is one of the important factors in evaluating the success of such a formulation especially for its industrial manufacturing.

In this study, different parameters were investigated for formulation of docetaxel liposomes in order to reach to the best liposome size, drug loading, in vitro release and stability.

Results and Discussion

Physicochemical properties of the liposomes

Formation of the DSPE-PEG conjugate was confirmed by IR spectra (Fig. 1).

The IR-spectrum of DSPE-PEG conjugate (A) showed additionally a small peak at 1679 cm^{-1} (corresponding to a NH–C=O group) missing in the spectrum of DSPE (B). The peak was also present in m-PEG spectrum (C). This peak illustrated that coupling reaction with probable formation of amide bonds occurred when conjugate was made.

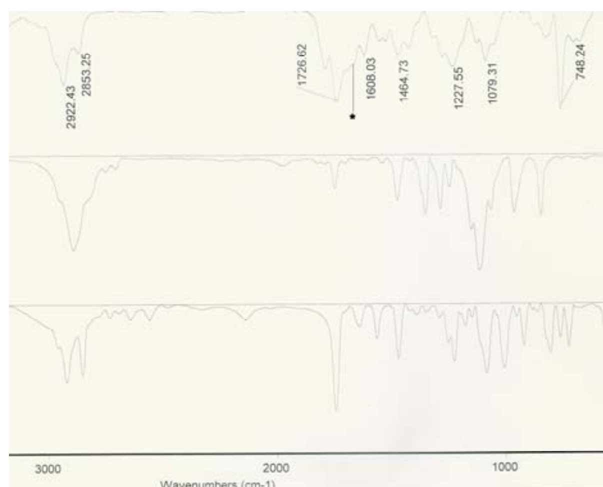

Fig. 1. IR spectra of A) mPEG-DSPE, B) DSPE, C) mPEG, (*) shows the mPEG-DSPE conjugate peak.

Several docetaxel liposome formulations were investigated by using different number of extrusion cycles, type of lipids and lipid combinations to see the effects on drug entrapment efficiency and particle size. Upon further optimization, a lead formulation of liposome-based docetaxel was developed (S3). Basic characteristics of some formulation are summarized in Tab. 1 and 2.

Tab. 1. Various formulations of docetaxel-loaded liposomes

Sample	Phospholipid Composition				
	CHOL (%)	DSPC (%)	DPPC (%)	ePG (%)	DSPE-mPEG (%)
S1	30	53	13	0	0
S2	30	66	0	0	0
S3	48	48	0	0	0
S4	40.5	40.5	0	0	5
S5	35.5	35.5	0	0	10
S6	0	77	19	0	0
S7	24	30	30	12	0

Tab. 2. Physicochemical characteristics of the docetaxel liposomes

Sample	Encapsulation Efficiency (%) ± SD	Drug Loading (%) ± SD	Mean Diameter (nm) ± SD	Zeta Potential mV± SD	PdI
S1	77.80 ± 0.06	3.11 ± 0.21	160 ± 16	−15.21 ± 0.21	0.190
S2	74.25 ±0.10	2.97 ± 0.40	179 ± 22	−16.11 ± 0.10	0.14
S3	65.00 ± 0.08	2.6 ± 0.32	156 ± 19	−17.5 ± 0.54	0.341
S4	85.00 ± 0.10	3.4 ± 0.31	139 ± 10	−9.66 ± 0.21	0.347
S5	82.40 ± 0.12	2.91 ± 0.25	145 ± 11	−8.12 ± 0.10	0.351
S6	67.50 ± 0.2	2.7 ± 0.52	328 ± 14	−14.12.± 0.31	0.325
S7	88.25 ± 0.01	3.53 ± 0.06	185 ± 11	13.14 ± 0.25	0.119

In addition to the sample formulation shown in Tab. 1, a lot of experiments were done on selection of the lipids type and ratio. The simplest lipid phase was the combination of the DSPC/cholesterol at ratio of 1:1 (S3), which liposome size was 156 ± 19 nm with EE of 65%. Decreasing the amount of cholesterol or addition of DPPC or ePG caused a significant increase in particles size and EE ($p < 0.05$). Docetaxel-unloaded liposome had the lowest particle size (data not shown). The best ratio of DSPE-PEG to other parts of the formulation in pegylated liposomes was 5% w/w (S4).

Zeta potential is another important index for the stability of the liposomal formulation. High absolute value of zeta potential indicates high electric charge on the surface of the drug-loaded liposomes, which can cause strong repellent forces among particles to prevent aggregation of the liposomes in buffer solution [19]. Zeta potential of liposomes was negative due to the presence of terminal carboxylic groups in the lipids [20]. The value of the zeta potential for pegylated liposomes was more negative, due to the negatively charged phosphate group of mPEG-DSPE, thus eliciting a reduced electrophoretic mobility, and a more negative potential value ensures a high-energy barrier that stabilizes the nanosuspension [19] which is also in accordance with the result reported before [21].

The particle size distribution of the liposomes prepared in this study showed a mono-modal distribution (data not shown) and there was no significant change in liposome particle size and zeta potential before and after freeze-drying ($p > 0.05$) suggesting that the freeze-drying cycle used was optimum and the formulation contained sufficient amount of lyoprotectant to preserve the integrity of the liposomes. According to the SEM photographs, the liposomes all had a fine spherical shape with a relatively monodispersed size distribution confirming the size distribution measurement studies (Fig. 2).

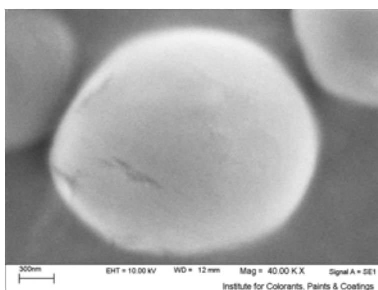

Fig. 2. SEM micrograph of liposomes (S2)

DSC studies were performed to investigate the physical state of the drug in the liposomes, because this aspect could influence the in vitro and in vivo release of the drug from the systems. Drug may exist in the liposomes in forms such as amorphous or crystalline phase. Moreover, a drug may be present either as a solid solution or solid dispersion in liposome. Fig. 3 shows the DSC thermograms of pure docetaxel, physical mixture of the DSPC/cholesterol/docetaxel, and docetaxel-loaded liposomes (S3).

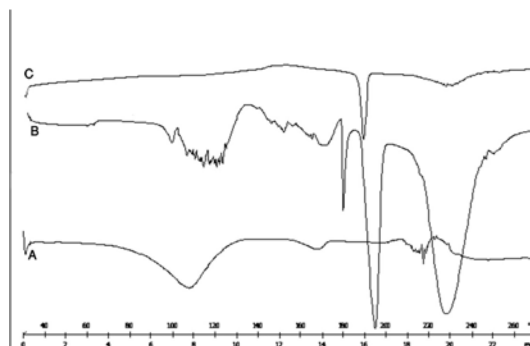

Fig. 3. Results from differential scanning calorimetry (DSC) on (A) docetaxel, (B) physical mixture of lipids and (C) liposomes containing docetaxel; (S3).

Pure docetaxel showed an endothermic melting peak (T_m) at 159 °C (Fig. 3A). Physical mixture of the DSPC/cholesterol showed two distinct peaks at 178 °C and 195 °C which are related to the melting peaks of the lipids (Fig. 3B). Docetaxel melting peak was depleted in the calorimetric curve of loaded liposomes (Fig. 3C), evidencing the presenting of amorphous part of the drug in the liposome samples. It might be hypothesized that the liposomization inhibited the crystallization of docetaxel during liposomal formation. Therefore, it could be concluded that docetaxel in the liposomes was in amorphous phase of a molecular dispersion or a solid solution state in the lipid matrix after the production [22].

In vitro release studies

The in vitro release behavior of the docetaxel-loaded liposomes, PEGylated and non-PEGylated, within 14 days is summarized in the cumulative percentage release shown in Fig 4.

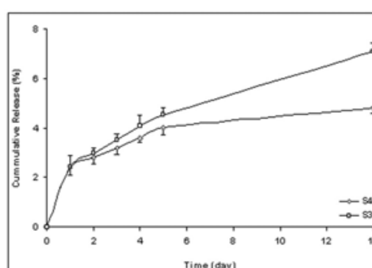

Fig. 4. In vitro release curves of docetaxel-loaded non-PEGylated (S3) and PEGylated (S4) liposomes in phosphate buffer (pH 7.4).

In fact both liposomal formulations produced an initial faster effect in which docetaxel release was more than 2.4% for both PEGylatded and non-PEGylated liposomes within the first sampling time (24 h). The burst release is related to docetaxel adsorbed on the liposome surface [23] and/or to the release of the drug encapsulated near to liposome surface. After this burst release, a constant docetaxel release was observed in such a way that after 14 days, about 5% and 7% of the loaded drug was released in PEGylated and non-PEGylated liposomes showing a typical sustained and prolonged drug release that depends on drug diffusion and matrix erosion mechanisms [24]. The faster release in

PEGylated liposomes could be because of the fast hydration process at presenting the PEG on the surface of the particles. This result suggests that it takes time for docetaxel 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 PEGylated liposomal formulation. The above results, which suggest that the drug would be stable in the blood circulation and would be released slowly at the tumor site, are indications that our PEGylated liposomal formulation meets the requirements for an effective drug delivery system [25]. Also the drug release data confirmed the drug entrapment efficiency results determined.

Stability study

Docetaxel liposomes (S4) were selected and their physical and chemical stability was evaluated at three different temperatures for up to 3 months. Initially, the mean vesicle diameter was 139 nm and EE was 85%, No significant changes in drug EE was observed during the course of stability study for formulations stored at −20 °C or 4 °C ($p > 0.05$) but there was a significant decrease in docetaxel EE for liposomes stored at room temperature ($p < 0.05$). The mean vesicle diameter showed an increase at all storage temperatures ($p < 0.05$) while particle size of the formulations was increased up to 3.25 folds after 3 months at room temperature. Results of the stability study are shown in Fig. 5.

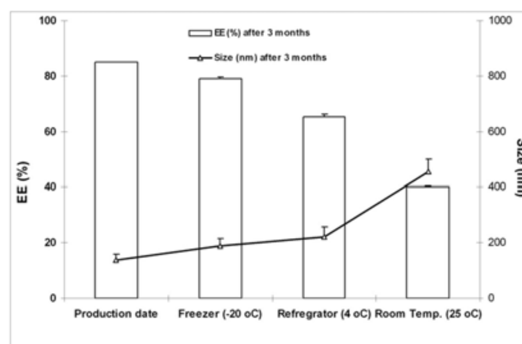

Fig. 5. Docetaxel liposomes' stability after 3 months storage at 3 different temperatures (S4).

There was no significant difference between stability results of the liposome storage at 4 °C or −20 °C. The results of the stability study revealed that the prepared liposomes are stable for more than 3 months storage at 4 °C or −20 °C. The most important finding of this study is that the prepared liposomes showed a significant increase in stability time in comparison with last reports on docetaxel liposomes by Immordino et al, which their formulation was stable for 15 days at 4 °C [13].

Conclusions

Stable sub-micron liposome-based formulation of docetaxel, were developed. The most significant finding of this study was that pegylated liposomes with high docetaxel content and good stability were successfully developed.

Experimental

Materials

Docetaxel was purchased from Cipla Pharma Co., India. Egg yolk phosphatidylglycerol (ePG), Dipalmitoylphosphatidylcholine (DPPC), Distearoylphosphatidylcholine (DSPC), Distearoylphosphatidylethanolamine (DSPE) were from Lipoid GmbH (Ludwigshafen, Germany). Methoxypolyethylene glycol succinate N-hydroxy succinimide ester (approx. 50%) and Cholesterol (CHOL) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Polycarbonate filters (diameter: 19mm, pore diameter: 100nm) were obtained from Avestin Inc. (Ottawa, Canada). Deionized water was used throughout the experiments. The in vitro release measurement was carried out at pH 7.4 at 37˚C in phosphate buffer. All the other chemicals used were of analytical grade.

Synthesis of mPEG-DSPE

mPEG-DSPE was synthesized according to the method described by Klibanov, et al with some modifications [26]. Briefly, an aliquot of compound **1** (Methoxypolyethylene glycol succinate *N*-hydroxy succinimide ester = α-({4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxobutan-oyl}oxy)-ω-(2-methoxyethyl)poly(ethane-1,2-diyloxy)) in chloroform was added to a solution of compound **2** (DSPE = 2-ammonioethyl 2,3-bis(icosanoyloxy)propyl phosphate) in chloroform, followed by the addition of triethylamine (TEA) (compound **1**/compound **2**/TEA: 3:1:3.5 m/m). The reaction mixture was incubated overnight at room temperature and chloroform was evaporated to yield compound **3**. The synthesis reaction is shown in Fig. 6.

The formation of compound **3** was monitored and confirmed by infrared spectra (Fig. 1).

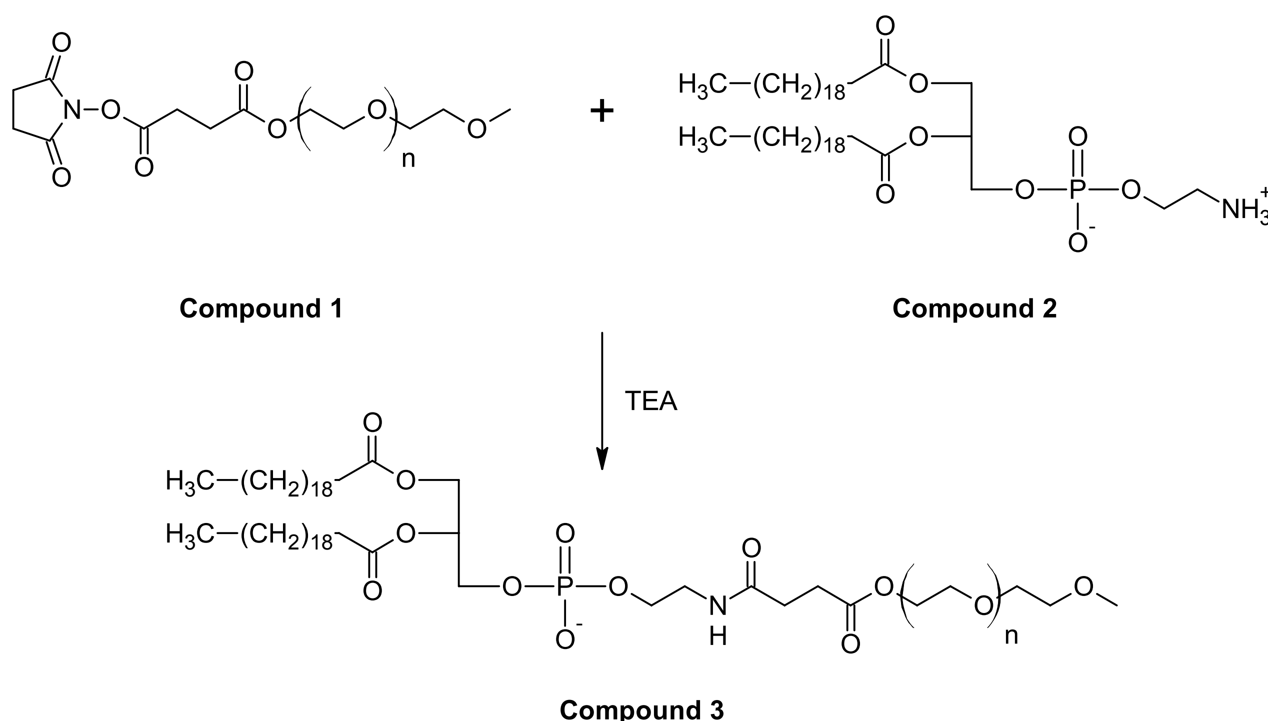

Fig. 6. Pathway for Synthesis of mPEG-DSPE.

Preparation of liposomes containing docetaxel

Different ratios of docetaxel, phospholipids, and cholesterol were mixed and dissolved in 10ml of a solution of methanol and chloroform (1:1) and dried in Roto-evaporator (Buchi Rotavapor R-124, Heidolph, Germany) at 65 ˚C for one hour to form a lipid film. The molar ratios of the mentioned materials are summarized in Tab. 1. In all formulations docetaxel content was 4 mol % (mol drug: mol lipid). The lipid film was then hydrated with 50 ml of 1% (w/v) sucrose solution and stirred for one hour. The liposome suspension was first homogenized by a high speed homogenizer (T18 basic Ultra-Turrax, IKA, USA) at 16,000 rpm for 4 min and then it was extruded through polycarbonate filters with pore size of 100 nm for 11 cycles (Liposofast® Extruder, Avestin Inc., Ottawa, Canada). The suspension was then freeze-dried at −40 ˚C for 48 h (Lyotrap Plus, LTE Scientific Limited, UK) to obtain a fine powder of liposomes.

Measurement of encapsulation efficiency (EE)

The concentration of docetaxel in the produced liposomes was determined by HPLC method. A reversed phase C_{18} Column (25×0.46 cm, pore size 5 µm) (Teknokroma, Spain) was used. The mobile phase consisted of a mixture of methanol and water (80:20, v/v) delivered at a flow rate of 1.00 ml/min with a pump (WellChrom K-1001, Knauer, Germany). 1 ml of acetonitrile was added to 1 ml aliquots of the extruded suspension in tubes and mixed followed by sonication for 2 min. The tubes were then centrifuged at 21,000 g for 10 min. 20 µl of the supernatant was analyzed by the HPLC system. Column elute was monitored spectrophotometrically at wavelength of 230 nm with a UV detector (WellChrom K-2600, Knauer, Germany). The calibration curve of docetaxel was linear over the range of standard concentrations of 0.1–10 mcg/ml with a correlation coefficient of R^2>0.999. The encapsulation efficacy was obtained as the mass ratio between the amount of each drug incorporated in liposomes and that used in the liposome preparation.

Size and size distribution

Particle size and size distribution of liposomes in the extruded suspension were determined by laser light scattering (Zetasizer ZS, Malvern, UK).

Morphology

Scanning electron microscopy (XL 30 scanning microscope, Philips, the Netherlands) was employed to determine the shape and surface morphology of the produced liposomes. A small amount of the lyophilized liposomes was stuck on a double-sided tape attached on a metallic sample stand, then coated under vacuum with a thin layer of gold before SEM.

Differential Scanning Calorimetry (DSC) measurements

The DSC thermograms were obtained from a Mettler Toledo DSC system (DSC-823E, Mettler Toledo, GmbH, Switzerland). A Mettler Stare software system, version 9.x was used for data acquisition and Indium was used to calibrate the instrument. 2–3 mg of liposomes were put into DSC aluminium pans and sealed. Samples were scanned from 30 to 270 ˚C at a rate of 10 ˚C/min under Nitrogen atmosphere. Each experiment was repeated three times.

In vitro drug release

20 mg samples of the lyophilized powder were suspended in tubes containing 2 ml of phosphate buffer (PH 7.4). The tubes were then placed in a shaker water bath (WB14, Memmert, Germany) at 37˚C and shaken horizontally at 90 cycles/min. At predetermined time intervals, the tubes were centrifuged (Sigma 3K30, Sigma, Germany) at 21,000 g for 7 minutes and 1 ml aliquots were taken from the supernatant which were substituted with the same amount of fresh phosphate buffer. 1 ml of acetonitrile was added to each aliquot to precipitate the lipids and dissolve the entrapped docetaxel and then centrifuged at 21,000 g for 10 minutes [27]. Samples were taken from the supernatant and analyzed by HPLC method. The HPLC method used was the same as described above. Drug release data was normalized by converting drug concentration in solution to a percentage of the cumulative drug release. The experiments were carried out in triplicates.

Stability study

The stability of the lyophilized docetaxel liposome was evaluated after storage at −20, +4 and 25 °C for 3 months storage. The particle size distribution and drug encapsulation efficiency of the samples were determined as a function of the storage time.

Statistical analysis

One-way analysis of variance (ANOVA) test was performed on the data to assess the impact of the formulation variables on the results. P values of < 0.05 were considered significant. All calculations were performed using a statistical software program (SPSS® 11.5, Microsoft).

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

[1] Han HD, Lee A, Hwang T, Song CK, Seong H, Hyun J, Shin BC.
Enhanced circulation time and antitumor activity of doxorubicin by comblike polymer-incorporated liposomes.
J Control Rel. 2007; 120:161–168.
doi:10.1016/j.jconrel.2007.03.020

[2] Onishi H, Machida Y.
Preparation and in vitro characteristics of lactoferrin-loaded chitosan microparticles.
Drug Dev Ind Pharm. 2007; 33: 641–647.
doi:10.1080/03639040500528954

[3] Vyas SP, Rawat M, Rawat A, Mahor S, Gupta PN.
Pegylated protein encapsulated multivesicular liposomes: a novel approach for sustained release of Interferon α.
Drug Dev Ind Pharm. 2006; 32: 699–707.
doi:10.2174/1389557023405873

[4] Metselaar JM, Mastrobattista E, Storm G.
Liposomes for I.V. Drug Targeting: Design and Applications.
Mini Rev Med Chem. 2002; 2: 319–329.
doi:10.2174/1389557023405873

[5] Fenske DB, Cullis PR.
Entrapment of small molecules and nucleic acid-based drugs in liposomes.
Methods Enzymol. 2005; 391: 7-40.
doi:10.1016/S0076-6879(05)91001-X

[6] Budai L, Hajdu M, Budai M, Grof P, Beni S, Noszal B, Klebovich I, Antal I.
Gels and liposomes in optimized ocular drug delivery: Studies on ciprofloxacin formulations.
Int J Pharm. 2007; 343: 34–40.
doi:10.1016/j.ijpharm.2007.04.013

[7] Ugwu S, Zhang A, Parmar M, Miller B, Sardone T, Peikov V, Ahmad
Preparation, characterization, and stability of liposome-based formulations of mitoxantrone.
Drug Dev Ind Pharm. 2005; 31: 223–229.
doi:10.1081/DDC-200047850

[8] Crosasso P, Ceruti M, Brusa P, Arpicco S, Dosio F, Cattel L.
Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes.
J Control Rel. 2000; 63: 19–30.
doi:10.1016/S0168-3659(99)00166-2

[9] Nishiyama N, Kataoka K.
Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery.
Pharmacol Ther. 2006; 112: 630–648.
doi:10.1016/j.pharmthera.2006.05.006

[10] Wu J, Liu Q, Lee RJ.
A folate receptor-targeted liposomal formulation for paclitaxel.
Int J Pharm. 2006 ; 316: 148–53.
doi:10.1016/j.ijpharm.2006.02.027

[11] Yang T, Cui FD, Choi MK, Cho JW, Chung SJ, Shim CK, Kim DD.
Enhanced solubility and stability of PEGylated liposomal paclitaxel: *In vitro* and *in vivo* evaluation.
Int J Pharm. 2007; 338(1-2): 317–326.
doi:10.1016/j.ijpharm.2007.02.011

[12] Zhang JA, Anyarambhatla G, Ma L, Ugwu S, Xuan T, Sardone T, Ahmad I.
Development and characterization of a novel Cremophor EL free liposome-based paclitaxel (LEP-ETU) formulation.
Eur J Pharm Biopharm. 2005; 59: 177–187.
doi:10.1016/j.ejpb.2004.06.009

[13] Immordino ML, Brusa P, Arpicco S, Stella B, Dosio F, Cattel L.
Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel.
J Control Rel. 2003; 91: 417–429.
doi:10.1016/S0168-3659(03)00271-2

[14] Musumecia T, Ventura C, Giannone I, Ruozi B, Montenegro L, Pignatello R, Puglisi G.
PLA/PLGA nanoparticles for sustained release of docetaxel.
Int J Pharm. 2006; 325: 172–179.
doi:10.1016/j.ijpharm.2006.06.023

[15] Bissery M, Guenard D, Gueritte-Voegelein F, Lavelle F.
Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue.
Cancer Res. 1991; 51: 4845–4852.
doi:10.1016/0165-6147(92)90048-B

[16] Horwitz SB.
Mechanism of action of Taxol.
Trends Pharmacol Sci. 1992; 13: 134–136.
doi:10.1158/1078-0432.CCR-04-0118

[17] Einhaus CM, Retzinger AC, Perrotta AO, Dentler MD, Jakate AS, Desai PB, Retzinger GS.
Fibrinogen-Coated Droplets of Olive Oil for Delivery of Docetaxel to a Fibrin(ogen)-Rich Ascites Form of a Murine Mammary Tumor.
Clin Cancer Res. 2004; 10: 7001–7010.
doi:10.1073/pnas.0601755103

[18] Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R.
Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo.
Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(16): 6315-6320.
doi:10.1073/pnas.0601755103

[19] Müller RH.
Charge determinations.
In: Colloidal Carriers for Controlled Drug Delivery and Targeting Modification, Characterization and In Vivo.
Boca Raton, Florida, USA: CRC Press, 1991.

[20] Mu L, Feng SS.
Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (Taxols).
J Control Rel. 2002; 80: 129–44.
doi:10.1016/S0168-3659(02)00025-1

[21] Hinrichs WLJ, Mancenido FA, Sanders NN, Braeckmans K, De Smedt SC, Demeester J, Frijlink HW.
The choice of a suitable oligosaccharide to prevent aggregation of PEGylated nanoparticles during freeze thawing and freeze drying.
Int J Pharm. 2006; 311: 237–244.
doi:10.1016/j.ijpharm.2005.12.032

[22] Dubernet, C.
Thermoanalysis of microspheres.
Thermochim Acta. 1995; 248: 259–269.
doi:10.1016/0040-6031(94)01947-F

[23] Magenheim B, Levy MY, Benita S.
A new in vitro technique for evaluation of drug release profile from colloidal carriers-ultrafiltration technique at low pressure.
Int J Pharm. 1993; 94: 115–123.
doi:10.1016/0378-5173(93)90015-8

[24] Holland SJ, Tighe BJ.
Biodegradable polymers.
In: Advanced Pharmaceutical sciences.
New York, USA: Academic Press, 1992.

[25] Song H, Zhang J, Han Z, Zhang X, Li Z, Zhang L, Fu M, Lin C, Ma J.
Pharmacokinetic and cytotoxic studies of pegylated liposomal daunorubicin. Cancer Chemother Cancer Chemother Pharmacol. 2006; 57: 591–598.
doi:10.1007/s00280-005-0076-6

[26] Klibanov AL, Maruyama K, Torchilin VP, Huang L.
Amphipathic polyethleneglycols effectively prolong the circulation time of liposomes.
FEBS Lett. 1990; 268: 235–237.
doi:10.1016/0014-5793(90)81016-H

[27] Denkbas EB, Kaitianm X, Tuncel A, Piskin E.
Rifampicin-carrying poly (D,L-lactide) microspheres: loading and release.
J Biomat Sci Polym Ed. 1995; 6: 815–825.
doi:10.1163/156856295X00201