World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Development and evaluation of long circulating Vitamin E TPGS coated docetaxel liposomes for I.V. administration

Shaina Kujar, Akanksha Choudhary, Manjit Kaur, Prerna Upadhyay, Nitan Bharti Gupta, Neha kumari

Sri Sai College of Pharmacy, Badhani, Pathankot-145001, India

Received: 02-02-2020 / Revised Accepted: 31-03-2020 / Published: 01-04-2020

ABSTRACT

In the preparations of Docetaxel liposomes which are coated with p-alpha-tocopheryl polyethylene glycol succinate (TPGS),which is a good targeting agent for tumor, amphiphilic vitamin E TPGS which is a quite stable under normal condition as compared to PEG coated liposomes. Docetaxel coated liposomes were prepared by solvent hydration method and they are characterized for their entrapment efficacy, zeta potential and pharmacokinetic studies. Docetaxel coated liposomes were also observe under microscope which observed very clear, small, vascular like liquid, long circulating liposomes Vitamin ETPGS Coated liposomes were used because they directly target the tumor and protect the engulfment through Endoplasmic reticulumn and prevent the binding through protein and albumin. While performing the study preformulation studies were carried out such as spectrophotometric studies, solubility and p-ka studies were obtained. Long circulating liposomes coated with PEG, increase the stability of the liposomes in the blood & protect its engulfment through EPR protein binding. In the development of vitamin ETGPS coated liposomes various chemical were used like lipoid E80,cholesterol, methanol, chloroform were used and at the end liposomes were coated with ETPGS. Docetaxel were extracted from taxoid family of anti-neoplastic drug. TPGS coated liposomes showed great advantages in vitro than PEG coated liposomes. Study confirms that solvent hydration method is suitable for the docetaxel liposomes.

Key Words: Docetaxel (DOCE), p-alpha-tocopherol polyethylene glycol succinate (TPGS), Amphiphilic, vitamin ETPGS, spectrophotometric.

Address for Correspondence: Shaina Kujar, Sri Sai College of Pharmacy, Badhani, Pathankot-145001; Email: Shainabhatti22@gmail.com

How to Cite this Article: Shaina Kujar, Akanksha Choudhary, Manjit Kaur, Prerna Upadhyay, Nitan Bharti Gupta, Neha kumari. Development and evaluation of long circulating Vitamin E TPGS coated docetaxel liposomes for I.V. administration. World J Pharm Sci 2020; 8(4): 13-18.

INTRODUCTION

The size of the nearly spherical lipid vesicles can range from a few nanometers to several micrometers However, liposomes used for medical purpose range from 50 to 450 nm. Liposomes are a foam drug delivery system, since their morphology is similar to that of cellular membranes and because of their ability to incorporate various substances. Therefore, the liposomes have been widely investigated and they continue to be the subject of intense research. They are important for their biological and technological advantages as optimal delivery systems for biologically active substances, both in vitro and in vivo, and are considered to be the most successful drug-carrier system and several biomedical applications of liposomes are either in clinical trials or are about to be put on the market, while others have already been approved for public use.(1-5)

Definition: A liposomes is a spherical vesicles having at least one lipid bilayer. Liposomes can be used as substituent for the administration of nutrients and pharmaceutical drugs. Liposomes can be prepared by disrupting biological membranes.

The capacity of liposomes as a carrier system for drugs strictly depends on the physicochemical properties of their membranes, on the nature of their components, on their size, surface charge, and lipid organization Liposomes are mainly composed of phospholipids amphiphilic molecules that have a hydrophilic head and two polar hydrophobic chains.

When phospholipids are dispersed in aqueous solutions, due to their amphipathic nature they have a strong tendency to form membranes. On the one hand, their polar heads prefer to interact with the aqueous environment; on the other, their long non-polar aliphatic chains promote interaction with one another. The hydrophobic ends of each layer face each other and constitute a lipophilic inner side that acts as a permeability barrier, both inward and outward.

Hydrophobic interactions lies behind the formation of these lipid bilayers, and vander Waals forces keep the long hydrocarbon tails together, thus strengthening this architecture. Lastly, hydrogen bonds and polar interactions between the water molecules of the aqueous environment and the polar heads of lipids stabilize this organization. The final formation of lipids depends on their nature, concentration, temperature, and geometric form. If ions or molecules are present during the formulation process, they can be encapsulated inside these membranes.(6-8)

Liposomes are classified as

1. Multilamellar large vesicles (MLV 0.1-6m),
2. Small unilamellar vesicles (SUV 0.02-0.05m),
3. Large unilamellar vesicles (LUV 0.06 m),
4. Giant liposomes (10,000- 1, 00,000 nm) (9).

For prolongation of liposomes in-vivo, liposome circulation time which constitute hydrophilic carbohydrates or polymers, such as monosialoganglioside (GM1) and PEG in liposome composition. GM1 decreases the blood proteins absorbed on the liposomal surface and improves the half-life of liposomes in the blood.(10-11) Similarly, the PEGylation of the liposomal carrier proved to extend the blood-circulation time and diminishing the uptake of liposomes by the retoculoendothelial system (RES). Further, by modifying the PEG-molecule terminus.

D-α-Tocopheryl polyethylene glycol 1000 succinate (*TPGS)* is an amphiphilic molecule useful in chemotherapeutic delivery of drugs via nanoparticles as emulsifier, stabilizer, bioavailability enhancer, solubiliser, additive, P-glycoprotein (Pgp) inhibitor [13-15]. TPGS having hydrophilic lipophilic balance (HLB) value 13.2 and a relatively low critical micelle concentration (CMC) of 0.02% w/w. Therefore, TPGS is suitable to serve as an effective TPGS coated liposomes of docetaxel was reported for enhanced cellular uptake and cytotoxicity of in cancer cells than that of Taxotere®. IC50 value of TPGS coated liposomes was found to be in between 6.46-5.23 which is higher than that of Taxotere® and uncoated liposomes, respectively.

Moreover, TPGS coated liposomes shows significantly higher cytotoxicity and lower IC50 value than that of DSPE PEG 2000 coated liposomes. Folic acid receptor targeted multifunctional liposomes by MCF-7 cells and cytotoxicity was assessed .Multifunctional TPGS liposomes showed approximately 41.47 and 6.78 times lower IC50 than Taxotere® and non-targeted liposomes.

Multifunctional liposomes are significantly higher than that of non-targeted liposomes. Trastuzumab-conjugated vitamin E TPGS liposomes were used for sustained and targeted delivery of docetaxel in HER2 over expressing breast cancer cells. TPGS molecule consists of hydrophilic poly ethylene glycol (PEG) and lipophilic alkyl chain. PEG chains of TPGS minimise the adsorption of opsonin proteins and thereby reduce the uptake of nanoparticles by reticular endothelial system and prolong the systemic circulation d-α-Tocopheryl

polyethylene glycol 1000 succinate (TPGS or Vitamin E TPGS) can prepared by the esterification of Vitamin E succinate with polyethylene glycol 1000.

As novel nonionic surfactant, it shows amphipathic properties and can form stable micelles in aqueous vehicles at concentration as low as 0.02 wt%. It has been used for its emulsifying, dispersing, gelling, and solubilizing effects on poorly water-soluble drugs. Liposomes also act as a P-glycoprotein (P-gp) inhibitor and have been used as an excipient for overcoming multidrug resistance (MDR). TPGS has been approved by FDA as a safe pharmaceutic excipient, many TPGS-based drug delivery systems (DDS) have been developed. TPGS can be used as a Pgp inhibitor, solubiliser /absorption and permeation enhancer in drug delivery and TPGS-related formulations such as nano-crystals, nanosuspensions, tablets/solid dispersions, adjuvant in vaccine systems, nutrition supplement, plasticizer of film, anticancer reagent and so on .d-α-Tocopheryl polyethylene glycol 1000 succinate (simply TPGS or Vitamin E TPGS) is formed by the esterification of Vitamin E succinate with polyethylene glycol 1000.

Objective of the study

1. To enhance drug encapsulation efficiency.
2. To improve the stability of long circulating liposome in the blood by PEGylation.
3. To inhibit P-glycoprotein mediated multi-drug resistance and increase bioavailability of anti-cancer drugs.
4. Prolonged systemic circulation of nanoparticle and to reduce drug liposome surface binding with tissue protein.

METHODS

Preparation of Docetaxel liposomes: T5PGS coated liposomes loaded with docetaxel have been prepared using thin-film hydration method. The liposomes were comprised of cholesterol, lipoid E 80 (Egg phospholipid with (80% Phosphatidylcholine), Docetaxel Vitamin E TPGS in round bottom flask. Briefly TPGS, phospholipids and cholesterol were mixed in different ratios and were dissolved in minimum quantity of a mixture of methanol and chloroform in a ratio of 9:1. The Dry lipid film has been obtained by evaporating this mixture in a rotary evaporator. Organic solvents were removed completely by rotary (Quick kvap and popular India) at temperature (60 °C) at 51 rpm for 5 to 10min to obtain a uniform thin lipid film on the wall of the flask. The vacuum was initially set at 500 mbar and slowly reduced to 25 mbar to prevent eviction of organic solution. The film was dried to remove residual organic solvent and vacuum-dried overnight Finally, the lipid film was hydrated with 10ml phosphate-buffered saline (PBS, pH 7.4) by rotating the flask at about rpm at 60 ^{0}C.then observe vitamin ETPGS coated liposome under the microscope.(17-18)

In vitro evaluation studies

Drug entrapment efficiency of liposomes: volume of Docetaxel LPs theoretically equivalent to 20 mg of Docetaxel (total, entrapped and unentrapped) 1ml was filled in dialysis bag [70(high media)] and suspended in 50ml of phosphate buffer saline 7.4, 37±0.5 °C, for 1 hour. concentration in dialyses was measured spectro-photometricaly at 229nm (UV Shimadzu, Japan).1ml sample was carried out at 10,20,30,40,50 and 60 min for 1 hour And sample concentration was measured immediately in UV after each sample withdrawn from dialysis bag.(19)

In vitro drug release: In vitro release of Docetaxel and optimized batch of docetaxel-TPGS- lipo was carried out using dialysis membrane [70(high media)] in pH 7.4 phosphate buffer saline and liposomal suspensions equivalent to 20mg were filled in presoaked dialysis tubes and placed in 50 ml of release medium maintained at 37±0.5 °C [20-21]. The medium kept for stirring at 100 rpm using a magnetic stirrer. An aliquot of 1ml was Withdrawn at pre-determined time intervals (0, 5, 10,15, 30,60,90, 2, 4, 6, 8, 9, 12 and 24h replaced with equal volume of fresh release medium. The samples were analyzed by UV spectrophotometric method. The mean of triplicate drug release and standard deviation (mean ± SD, n=3) was used to plot the release profiles.

RESULT

Drug entrapment efficiency of docetaxel liposomes

Table 1: Drug entrapment efficiency of docetaxel liposomes

No Formulation	%EE
F1	97.117±0.013
F2	98.326±0.056
F3	90.532±0.116
F4	95.461±0.275
F5	95.461±0.275

(Mean ± SD, N=3)

Discussion: % EE of Docetaxel formulation has obtained**,** from which F2 has highest formulation of 326±0.056, so we had selected it for final formulation.

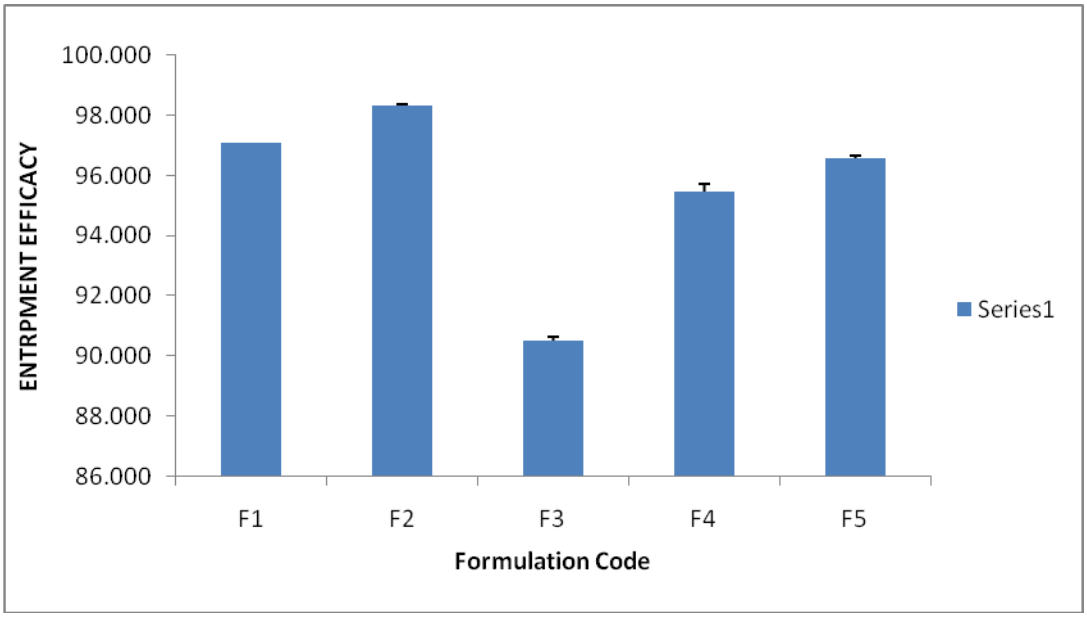

Figure 1: % Entrapment efficacy of docetaxel liposomes

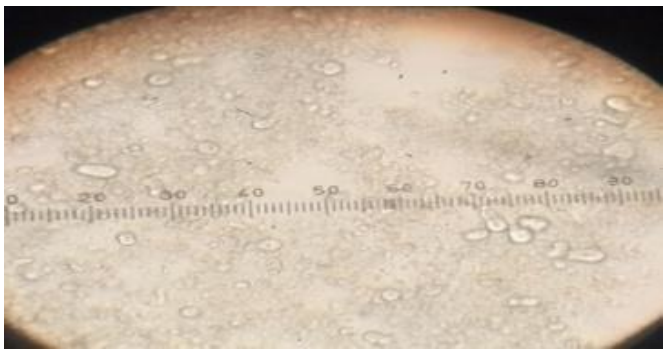

Figure 2: MICROSCOPIC VIEW OF F2 FORMULATION

Discussion: Microscopic view of % Entrpment Efficacy of F2 formulation which shows that liposomes are prepared before Vitamin ETPGS coating so we can select it as a final formulation for Vitamin ETPGS coating.

Table 2: % Entrapment Efficiency of final formulation of vitamin ETPGS docetaxel liposomes.

Formulation	% EE
F6	89.844±0.056
F7	84.338±0.258

(Mean ± SD, N=3)

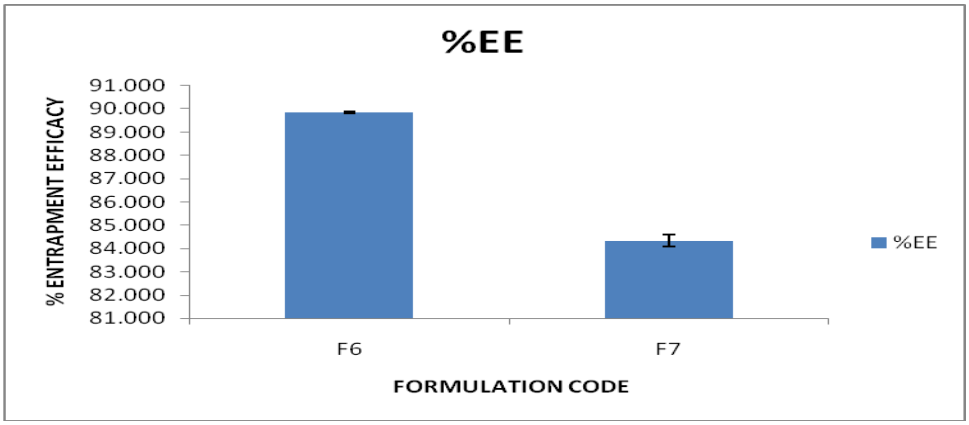

Figure 3: % Entrapment efficacy Final formulation of Vitamin ETPGS Coated of -docetaxel liposomes.

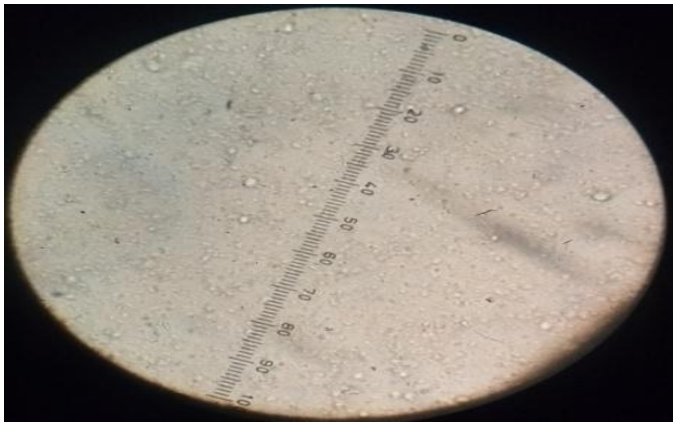

Fig 4: Microscopic view of F6 formulation

Discussion: After the selection of highest % EE of F2 formulation of Docetaxel liposomes are coated with Vitamin ETPGS .F6 is selected as final formulation because its %EE IS 89.844±0.056 which is highest than the % EE of F7 84.338±0.258 & Fig: 4. Shows exact formulation of liposomes coated with vitamin E TPGS.

In vitro release study: Fig 5. Shows the accumulated percentage release of docetaxel pure drug and TPGS coated liposomes in phosphate buffer saline (pH7.4). The Docetaxel and Docetaxel TPGS coated liposomes showed controlled release formulation. After 24hr study the percentage drug released from Docetaxel pure drug and Docetaxel TPGS coated liposomes was 43% and 63%.

Table 3: In vitro release profile of pure drug and TPGS coated liposomes formulation (F6)

Time (hr)	% Drug release of pure drug dispersion	% Drug release of final formulation
0	0.000±0.000	0.000±0.000
5	3.37±0.322	10.88±0.056
15	8.426±0.056	11.97±0.032
20	10.100±0.056	12.22±0.056
25	133.188±0.032	29.2±0.064
30	17.913±0.056	32.4±0.064
45	21.447±0.032	37.64±3.190
60	25.391±0.056	41.090±0.112
90	31.138±0.056	47.510±0.387
120	35.100±0.056	53.348±0.591
180	37.44±0.056	56.910±0.112
240	43.750±0.056	63.160±0.387

(Mean ± SD, N=3)

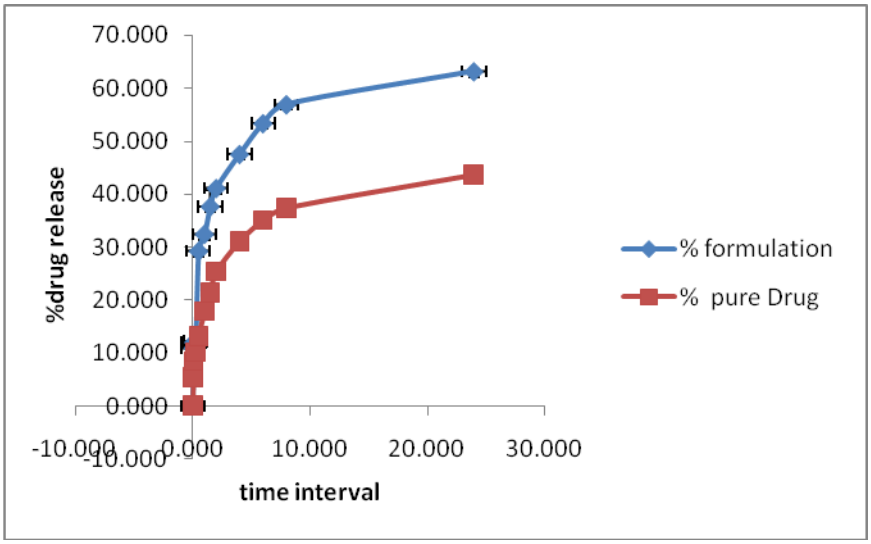

Fig 5: Comparative in vitro release profile

Conclusion: In the development of the TPGS coated liposome for better administration in the Drug delivery than the conventional liposomes (uncoated) and PEG for cancer therapy. Our main aim is to develop suitable anticancer liposomes for drug delivery system .Our study confirms that solvent hydration method is suitable for docetaxel loaded liposomes. In the preparation of docetaxel liposomes, the TPGS coated docetaxel liposomes shows entrapment efficacy up to 89%.Drug release profile of docetaxel TPGS coated liposomes shows controlled release profile. Moreover, TPGS coated liposomes improves the liposomal stability and thus protected the docetaxel from retoculoendothelial system than the PEG coated liposomes. These are the best pharmaceutical formulation than the other conventional formulation, in the drug delivery they considered as potential for targeted drug delivery by ligand conjugation and multifunctional nanocarriers.sss

REFERENCES

1. Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol. 1964; 8: 660–668.
2. Bangham AD et.al. Preparation and use of liposomes as models of biological membranes. In: Korn ED, editor. Methods in Membrane Biology, Volume 1. New York: Plenum; 1974:1–68.
3. Etheridge ML et.al. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine. 2013; 9: 1–14.
4. Felice B et al. Drug delivery vehicles on a Nano - engineering perspective. Mater Science Eng C Mater Biol Appl. 2014; 41: 178–195.
5. Fanciullino R, Ciccolini J. Liposome-encapsulated anticancer drugs: still waiting for the magic bullet, Curr Med Chem. 2009; 16: 4361–4373.
6. Euliss LE et al J. Imparting size, shape, and composition control of materials for nanomedicine. ChemSoc Rev. 2006; 35: 1095–1104.
7. Papahadjopoulos D, Kimelberg HK. Phospholipid vesicles (liposomes) as models for biological membranes: their properties and interactions with cholesterol and proteins. In: Progress in Surface Science. Vol. Oxford: Pergamon.1973:141–149.
8. Deamer D, Bangham AD. Large volume liposomes by an ether vaporization method. Biochim Biophys Acta. 1976; 443:629–634.
9. Milla P et al. PEGylation of proteins and liposomes: a powerful and flexible strategy to improve the drug delivery. Curr Drug Metab. 2012; 13: 105–109.
10. Gabizon A, Papahadjopoulos D. Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sky U S A. 1988; 85: 6949–6953.
11. Zhang Z et.al Vitamin E TPGS as a molecular biomaterial for drug delivery, Biomaterials. 2012; 33: 4889-4906.
12. Collnot E M et al. Vitamin E TPGS P-glycoprotein inhibition mechanism: influence on conformational flexibility, intracellular ATP levels, and role of time and site of access, Molecular pharmaceutics. 2010; 7: 642-651.
13. Immordino M L et al. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential , International Journal of Nanomedicine 2006:1(3): 297–315.
14. Vijayakumar M R et al. Copolymers of poly-lactic acid and D- alphatocopheryl polyethylene glycol 1000 succinate-based nanomedicines: versatilemultifunctional platforms for cancer diagnosis and therapy, Expert opinion on drug delivery. 2013; 10: 529-543.
15. Gadzag, M et al. Stable parenteral compositions of Vinblastine or Vincristine. US.1995; 5: 397-784.
16. Beecher P. (Ed.) Encyclopedia of emulsion technology, basic theory. Marcel Dekker: New York.1983; 1.
17. Collins Gold LC et.al. Parenteral emulsions for drug delivery. Adv Drug Delivery Rev 1990. 5:189-208.
18. Singh M, Ravin L. Parenteral emulsions as drug carrier systems. J Parenteral Science Technol. 1986; 40: 34-44.
19. Remington, the Science & Practice of Pharmacy, Parenteral Preparation, 20th ed. Philadelphia: ISE publication 2000; 1.
20. Uhumwangho MU, Okor RS. Current trends in the production and biomedical applications of liposomes: A review. J Medicine Biomedical Res. 2005; 4(1): 9-21.