REVIEW ARTICLE

Comparison of *In-vitro* Release Study of PEGylated and Conventional Liposomes as Carriers for the Treatment of Colon Cancer

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ABSTRACT

Cancer is the main cause of death in economically developed countries and the second cause of death in developing countries. The main objective of this study is to develop and compare the *in vitro* release properties of conventional and PEGylated capecitabine liposomes for the treatment of colon cancer. The film hydration method was used for the preparation of conventional and PEGylated capecitabine encapsulated liposomes. The formulation for the release of capecitabine liposomes was optimized and examined by the dialysis method. *In vitro* drug release studies showed a significant difference in the percentage of the cumulative drug release pattern. The pegylated liposomes showed prolonged release due to the presence of a PEG coating on the surface, which slowly releases the active ingredient over an extended period of time. SL3-CAP formulations showed a longer release over 36 hours and gave a release of 95 ± 0.3% of the drug compared to the conventional liposomal formulation with 81% for 24 hours.The drug release from conventional liposomes followed zero order kinetics, and Korsmeyerpeppas and stealth liposomes followed the Higuchi pattern with an n-value greater than 1, indicating a diffusion of the super case II type when compared to conventional liposomes.

Keywords: Capecitabine, Colon cancer, *In-vitro* release, PEGylated liposomes.

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INTRODUCTION

Cancer is the world's biggest health problem; Colon cancer is the third most common cancer and causes the highest mortality and morbidity in the world; Cancer chemotherapy is often accompanied by side effects; at the right time, the cancer could be cured without side effects.[1] One of the main goals of nanomedicine is to develop a nanocarrier that can selectively deliver cancer drugs to attack tumors and affect as few cells as possible in a healthy way. Various types of drug carriers or "vehicles" have been developed for this task, including polymers, liposomes, dendrimers, and inorganic nanoparticles.The use of these vehicles has several advantages over conventional dosage methods, including decreased inactivation of the drug, increased potency, and decreased non-specific interactions.[2,3] Liposomes have been used successfully as a controlled drug release system. Liposome technology is the new approved tool for overcoming the disadvantages associated with liposomes. Liposomes are heterogeneous vesicular bilayer systems made up of phospholipids with or without cholesterol. In 1965, Bangham and his colleagues first used a liposomal structure as a model to study the effect of narcotics on the membranes of the lipid bilayer. Helpful in terms of biocompatibility, biodegradability and low toxicity, and can control biodistribution by changing size, lipid composition and physical properties.[4,5] In addition, liposomes can capture both hydrophobic and hydrophilic drugs and continuously release entrapped substrate, making them useful drug carriers.[6] Liposomes have the ability to release drugs by increasing circulation in the systemic circulation and avoiding opsonin adsorption. Protein molecules (improved in vivo stability) due to the property of stearin instability, which leads to improved patient compliance by lengthening the systemic circulation.[7]

Achieving dispersion stability of liposomes is a challenge; requires a repulsive interaction proportional to the area and size of the VanderWaals force. Steric stabilization can be achieved by covering the surface of liposomes with an adsorbed layer of bulky molecules such as polyethylene glycol (PEG).[8]

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Modifying the surface of liposomes with PEG adding has several advantages, such as the endothelium of the reticulum leads to prolongs blood circulation and also helps reduce vesicle aggregation and improves stability.[9] By incorporating PEG lipids, the liposomes stay longer in the bloodstream and are distributed relatively evenly throughout the body, with most of the dose remaining in the central compartment (i.e. in the blood) and only 10–15% of the dose delivered to the liver. Pegylated liposomes provide an attractive platform to improve the therapeutic index of a wide variety of drugs.[10,11]

Capecitabine is widely used to treat colon cancer. In most cases, capecitabine causes side effects such as myocardial infarction, angina pectoris, hand-foot syndrome, diarrhea, nausea, stomatitis, anemia, thrombocytopenia, hyperbilirubinemia in conventional dosage form.[12] Despite its promising anti-cancer potential, capecitabine has not previously been used as an antineoplastic drug due to its poor water solubility, which can be overcome by administration of capecitabine, as stealth liposomes can deliver the drug in a controlled manner with a much smaller dosage regimen for this study used the thin film hydration method to make anti-cancer drug-encapsulated liposomes and the formulation was optimized.[13] The dialysis method was used to study the release properties of capecitabine-containing liposomes. The aim of the present work was the formulation and characterization of capecitabine liposome suspensions in the form of conventional liposomes and stealth liposomes.

MATERIALS

For the preparation of liposomal formulation Capecitabine was a received gift sample from Mylon Laboratories, Bangalore, India. DSPC and PE- PEG 2000 were purchased from Lipoid Germany. Solvents and reagents used were of laboratory reagent (LR) grade.

METHODS

Preparation of Anticancer Drug-loaded Stealth Liposomes

Capecitabine-pegylated liposomes were prepared from various combinations of phospholipids by the thin film hydration process. The weighed amount of the drug, phospholipids and cholesterol was dissolved in a mixture of anhydrous ethyl acetate and ethanol (2:1) in a sterile round bottom flask, then evaporated on a rotary evaporator and evaporated vacuum applied to obtain a thin film of dry lipids. The film was allowed to hydrate using PBS pH 7.4 with manual shaking for 15 minutes and then kept at room temperature and sonicated. The drug was removed by centrifugation, this step is called liposome purification.The final dispersion of liposomal was filled in sterile glass vials covered with special stoppers for lyophilization.[14,15]

In vitro Release Study

The *In vitro* release of capecitabine from pegylated liposomes was examined by a dialysis method. The dialysis bags were soaked in distilled water at room temperature for 12 hours to remove the preservative prior to use, followed by a thorough rinse with distilled water. The liposomal dispersion was poured into a dialysis tube (donation chamber) (Himedia Laboratories Pvt. Ltd. Mumbai) with a molecular weight cutoff of 14,000 Da. The dialysis tube placed in a beaker with release medium, i. H. PBS (pH 7.4) immersed and stirred with a magnetic stirrer at one hundred rpm to preserve the sink condition. Sample (1 mL) was removed at predetermined time durations at 1, 2, 4, 6, 12, 24, 28, 30 and 36 hours. Fresh dissolution medium was replaced to maintain constant volume. The drug concentrations within the dissolution medium were determined by a UV spectrophotometric method.[16]

Drug Release Kinetic Study

In order to analyze the mechanism of the kinetics of the drug release from the dosage form, the data obtained were fitted to various kinetic equations of zero order, first order, Higuchi model and Korsemeyerpeppas model, taking into account the best model based on the maximum value of the correlation coefficient.[17]

RESULTS

In vitro Drug Release study

In vitro drug release profiles were performed for all liposomal formulations. In the first 4 hours of this liposomal formulation, an *In vitro* burst release was observed. This characteristic initial burst of release is common with liposomes and then follows a slower release rate. The initial rate of rapid release is generally due to the detachment of the drug from the liposomal surface, while the subsequent slow release is the result of sustained release of the drug from the inner lamellae. It is also observed that as the cholesterol concentration increases, the drug release increases and then, after the cholesterol concentration decreases, drug-releasing PEGylated liposomes exhibited prolonged release due to the presence of a PEG coating on the surface and gave 95 ± 0.3% drug release in 36 hours compared to the conventional liposomal formulation at 81.32 at 24 hours, suggesting that the drug would be more stable in the blood circulation with a slower rate of release. *In vitro* drug release was determined by UV spectrophotometric methods shown in Table 1 and the plots of release versus time from conventional and stealth liposomes are shown in Figure 1.

Release Kinetic

To understand the mechanism of drug release, all data from *In vitro* drug release studies from capecitabine liposomes were fitted to kinetic models. The drug release from conventional liposomes followed zero order kinetics and Korsmeyerpeppas with an R2 value of 0.7130.969 with n value greater than 1, indicating a diffusion of the super case II type. The *in vitro* drug release kinetics of stealth liposomes followed by the Higuchi model showed time-dependent drug release from the insoluble matrix and the correlation coefficient (*R*2) was used as a tool. The regression for the formulation ranged from 0.744 to 0.991. From the regression values of various models for all formulations, it was observed during the *In*

Table 1: *In-vitro* drug release profile of capecitabine stealth liposomes

Time in hr	*SL-1CAP*	*SL-2 CAP*	*SL-3CAP*	*SL-4CAP*	*SL-5 CAP*	*SL-6CAP*	*SL-7CAP*	*SL-8CAP*
1	4.0 ± 0.03	3.9 ± 0.7	9.3 ± 0.2	4.9 ± 0.5	7.3 ± 0.1	5.12 ± 0.5	9.7 ± 0.5	3.05 ± 0.2
2	10.2 ± 0.5	16 ± 0.01	21 ± 0.7	9.5 ± 1.2	11.5 ± 1.8	17 ± 0.26	20 ± 1.6	14 ± 1.4
4	22.5 ± 0.5	23.8 ± 0.04	28.2 ± 0.02	14.6 ± 0.5	24.5 ± 1.1	23.8 ± 0.0	26.2 ± 1	19.4 ± 0.2
6	30.0 ± 0.5	35.7 ± 0.5	31.6 ± 0.4	29.5 ± 0.6	28.6 ± 0.02	32.7 ± 0.5	30.6 ± 1.8	24.5 ± 0.6
12	47.1 ± 0.7	54 ± 0.8	52.6 ± 0.2	37 ± 0.4	51.6 ± 0.6	50 ± 0.1	54.6 ± 0.3	35 ± 1.0
24	53.5 ± 0.1	60 ± 0.54	70 ± 0.05	50.5 ± 0.5	65 ± 0.34	56 ± 0.22	68 ± 1.0	49.5 ± 0.3
28	65.2 ± 0.34	62.4 ± 0.2	77 ± 0.6	56 ± 0.7	72.3 ± 0.27	62.4 ± 1.2	75.3 ± 0.1	54 ± 0.1
30	74.3 ± 0.4	74.4 ± 0.02	90 ± 0.5	66.0 ± 0.6	86 ± 0.12	76.4 ± 0.0	88 ± 0.23	69.0 ± 0.1
36	79.4 ± 0.5	80 ± 0.5	95 ± 0.33	73 ± 0.1	92 ± 0.3	89 ± 0.04	94 ± 0.05	72 ± 0.01

Table 2: R^2 values of various kinetics models of optimized capectabine liposomal formulations

	R^2 values						
					Korsmeyer-peppas		
Formulation code	*Zero order*	*First order*	*Huguchi model*	*Hixson crowell*	R^2	n value	*Best fit model*
SL-7 CAP	0.947	0.909	0.987	0.959	0.745	0.868	Huguchi
CL-7 CAP	0.915	0.832	0.731	0.868	0.904	1.357	Zero order

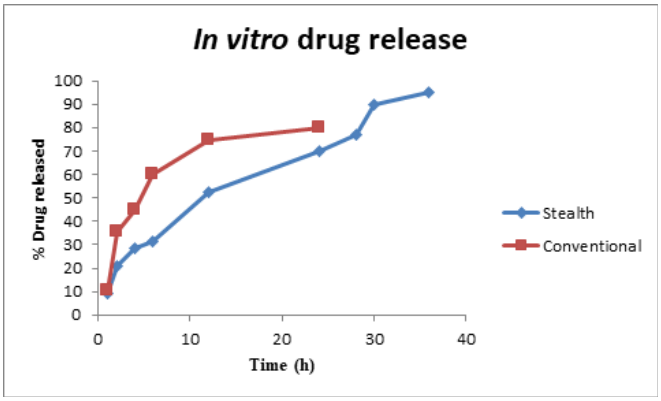

Figure 1: *In vitro* drug release comparison of conventional and PEGylated liposomes

vitro release of capecitabine from stealth liposomes that all formulations fit better into the Higuchi matrix.kinetic model with an n-value of 0.8680.964, indicating a non-Fickian release diffusion. The value of n was greater than 0.5 for the stealth liposomess containing capecitabine. *In vitro* drug release from capecitabine stealth liposomes followed by Higuchi described drug release as a diffusion process. liposomes, which help to release and diffuse capecitabine from liposomes. Kinetic data for all formulations are shown in Table 2.

DISCUSSION

Among a variety of targeted drug delivery systems, liposomes have been extensively studied for their ability to adapt to a variety of drugs, along with their good biocompatibility, low toxicity, and lack of activation or suppression of the immune system and comparison of capeitabine, which contains conventional capeitabine, and PEGylated liposomes. The *in vitro* release behavior of conventional capeciatbin-loaded liposomes and PEGylated liposomes was summarized. Comparing the results of the conventional and PEGylated liposome formulations, it is evident that the PEGylated liposomes exhibited a more sustained effect due to the presence of the PEG coating on the surface, which slowly releases the drug over a longer period of time. Conventional liposomes released more than 80% of the active ingredient within 24 hours, while pegylated liposomes extend the release of up to 36 hours. This result suggests that the release of capeciatbine, once capeciatbine is encapsulated in liposomes, takes time because the lipid bilayer is stabilized with cholesterol so that a stable depot effect could be achieved with liposomes, especially in the PEGylated liposomal formulation and stable in the bloodstream and be released slowly at the target site, this is evidence that our PEGylated liposomal formulation meets the requirements of an effective drug delivery system. The present study focused on the preparation of a new liposomal formulation using the lipid hydration method that contains conventional and PEGylated capecitabine liposomes. When comparing the results of conventional and PEGylated liposomal formulations, the PEGylated liposomes showed a longer lasting effect due to the presence of a PEG coating. on the surface, it slowly releases the drug over a long period of time.

CONCLUSION

Evaluation of the *in vitro* release profile of hydrophobic drugs from liposomal formulations could be problematic, but this could be manipulated through the use of an appropriate delivery medium that could provide sufficient immersion conditions without compromising the stability of the liposomal formulation. The results suggest that capeciatbine is released from pegylated liposome formulations over an extended period of time. Since they were nano-dimensions, a longer residence time in the systemic circulation could help them reach the target tissue. Nanosized pegylated liposomes would be a promising delivery system for capeciatbine in the treatment of colon cancer. Based on the above results, it can be surmised that the drug is stable in the bloodstream and released slowly

at the cancer site.

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