

Research Article



Sustained Release of Green Tea Polyphenols from Liposomal Nanoparticles; Release Kinetics and Mathematical Modelling

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Background: Green tea polyphenols (GTP) are known to have several health benefits. In spite of these benefits, its application as a therapeutic agent is limited due to some of its limitations such as stability, bioavailability, and biotransformation. To overcome these limitations, liposomal nanoparticles have been used as a carrier of the GTP.

Objective: Encapsulation of GTP to the liposomal nanoparticles in order to achieve a sustained release of the GTP and to determine the drug release kinetics and the mechanism of the release.

Materials and Methods: GTP encapsulated liposomal nanoparticles were prepared using phosphatidyl choline and cholesterol. The synthesized particles were characterized for their particle size and morphology. *In vitro* release studies were carried out, followed by drug release kinetics, and determining the mechanism of release. *In vitro*, antioxidant assay was determined following 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

Results: Atomic force microscope (AFM) and high resolution scanning electron microscope (HR SEM) images showed spherical particles of the size of 64.5 and 252 nm. An encapsulation efficiency as high as 77.7% was observed with GTP concentration of 5 mg.mL⁻¹. *In vitro* release studies showed that the loading concentrations of GTP were independent to the cumulative percentage of the drug release. GTP release by varying the pH and temperature showed a direct correlation between the release parameter and the percentage of drug release. The higher the pH and temperature, the higher was the percentage of the drug release. The release data showed a good correlation with Zero order kinetics and the mechanism of the release being anomalous mode. Radical scavenging activity of the released GTP showed a potent scavenging activity.

Conclusion: GTP encapsulated liposomal nanoparticles could be used as a delivery vehicle for achieving a sustained release.

Keywords: Anomalous transport, Diffusion, Erosion, Sustained release, Zero order kinetics.

1. Background

Liposomes are considered to be an ideal drug carrier as they consist of an aqueous core encapsulated by the natural or synthetic phospholipids and can deliver both hydrophobic and hydrophilic drugs. Hydrophobic drugs are likely to be entrapped within the lipid bilayer, while the hydrophilic drugs have a tendency to be entrapped within the core (1). Several liposomal formulations have been approved for delivery of the drugs like amphotericin B (2), doxorubicin (3), vincristine sulfate (4), cytarabine (5), etc. Few of the recent advancements in the liposomal nanoparticles based drug delivery system include the work of Ong *et al.* (6), where liposomal ciprofloxacin have been explored to predict the enhanced lung residence time of the formulation.

Zhou *et al.* (2015) developed a novel liposomal formulation for the cisplatin to study the potent antitumor activity *in vitro* and *in vivo* and achieved significantly decreased the incidence of the side effects with cisplatin loaded liposomal nanoparticles than that of free cisplatin (7).

The green tea polyphenols (GTP) are known to have a wide range of health benefits. The major constituents of the tea polyphenols, known as catechins constitute about 30-42% of the dry weight (8). Several reports are available on the applications of GTP in the preventive effects of cancer (9), cardiovascular diseases (10), and neuroprotective effects (11). In spite of several health benefits, its use as a therapeutic agent is limited due to a few limitations such as bioavailability, stability,

and biotransformation (12, 13). To overcome these limitations and improve the efficiency of GTP, liposomal nanoparticles have been developed and used as a carrier for the delivery of GTP.

2. Objectives

The present study deals with the encapsulation of GTP to the liposomal nanoparticles to achieve sustained release of the GTP, to determine the drug release kinetics, and the mechanism of the release.

3. Materials and Methods

The green tea polyphenols (GTP) was purchased from the Sigma Chemical Co., USA. Other chemicals such as cholesterol, phosphatidylcholine, and tween 80 were procured from Himedia, India.

3.1. Synthesis of the GTP Loaded Liposomal Nanoparticles

Phosphatidyl choline and cholesterol in a ratio of 20:1 was dissolved in 2 mL ethanol to form a lipid phase. This mixture was heated in a water bath at 60 °C. Tween 80 (0.1%) was dissolved in 10 mL PBS (pH 6.5) to form an aqueous phase. The obtained aqueous phase was dropped into lipid phase upon stirring. The mixture was placed on a magnetic stirrer for 10 min followed by sonication at 30% amplification for 10 min. The final liposomal nanoparticles were obtained after being cooled in the ice bath, diluted to 20 mL, and filtered. For GTP loaded liposomal nanoparticles, different quantities (1, 2.5, 5 and 10 mg) of the GTP were dissolved in 1 mL ethanol prior to the dissolution of the phosphatidyl choline and cholesterol (14).

3.2. Characterization of Liposomal Nanoparticles

The hydrodynamic diameter and the surface charge of the nanoparticles were determined by dynamic light scattering (DLS) and ζ potential analysis using Nanopartica, Nanoparticle analyzer SZ-100. The morphology and polydispersity of the particles were analyzed through atomic force microscope (AFM, Nano Surf Easy Scan2, Switzerland) and high resolution scanning electron microscope (HR SEM, FEI Quanta FEG 200).

3.3. Determination of Encapsulation Efficiency (EE)

The encapsulation efficiency was determined by separating the unloaded GTP by centrifugation. The amount of unloaded GTP was quantified by photometric Folin-Ciocalteu assay as described by Swain and Hillis (15). About 0.5 mL of sample was added to 0.5 mL of 1 M Folin - Ciocalteu reagent and 0.5 mL of 35%

sodium carbonate. The mixture was incubated for 30 min in dark and the absorbance was measured at 700 nm applying a spectrophotometer. EE was calculated using the formula:

$$EE (\%) = \frac{\{(Total\ amount\ of\ GTP - Amount\ of\ free\ GTP)\}}{Total\ amount\ of\ GTP} \times 100$$

3.4. Release of GTP from the Liposomal Nanoparticles

Release studies were carried out in the phosphate buffer saline (PBS) at a physiological pH of 7.4 at 37 °C through dialysis method. One mL of the encapsulated GTP nanoparticles was transferred to a dialysis bag with both ends sealed. The bag was placed in a beaker containing 50 mL of PBS. At appropriate time intervals (1, 2, 3, 4, 5, 6, 7, 8, 12, 24, and 48 h), 1 mL of the sample from the beaker was withdrawn. The collected samples were used to determine the amount of drug released. Quantification of the GTP was carried out by the method developed earlier (15). The drug release profiles were studied for the different loading concentrations of GTP (1, 2.5, 5, and 10 mg.mL⁻¹) at different pH (3, 7.4, and 9) and temperatures (room temperature (RT), at 37 and 45 °C, respectively).

3.5. In vitro Antioxidant Assay

The radical scavenging activity of the released GTP was determined *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay following the method of Williams *et al.* (16). About 0.1 mM DPPH was used for the study and was prepared in the absolute ethanol. To 0.5 mL of DPPH, 100 μ L of the sample and 550 μ L PBS was added. The mixture was incubated for 30 min in dark and the absorbance was measured at 517 nm using a spectrophotometer.

3.6. Mathematical Modelling

In vitro release data was fitted to various mathematical models to study the drug release kinetics and the mechanism of the drug release was studied as well.. The different models that were used in this study are Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models (17). The correlation coefficient (R²) and the release exponent (n) were used to determine the best-fit kinetic model and the mechanism of the drug release.

3.7. Statistical Analysis

All the data were subjected to the analysis of variance (ANOVA) and presented as mean \pm SD. The means were separated by Duncan's multiple range test (DMRT) at $p \leq 0.05$.

4. Results

GTP at different concentrations was encapsulated into the liposomal nanoparticles. The mean hydrodynamic diameter of the particles was observed to be 322.1 nm with a polydispersity index of 0.921. The AFM and HR SEM images revealed spherical particles with sizes of 64.5 and 252 nm, respectively (Fig. 1). The particles were negatively charged with a zeta potential value of -24.2 ± 2.75 mV.

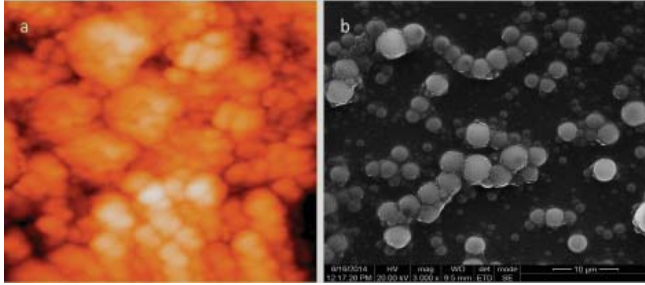


Figure 1. AFM (a) and HR SEM (b) images of the liposomal nanoparticles. The morphology of the particles was observed to be spherical.

The encapsulation efficiency (EE) of the GTP loaded liposomal nanoparticles are presented in Table 1. The maximum EE (77.78%) was observed when GTP was loaded at a concentration of 5 mg.mL⁻¹.

Table 1. The encapsulation efficiency (EE) of the GTP loaded liposomal nanoparticles upon loading different concentrations of the GTP. Means followed by the same letter at the column are not significantly different at $p \leq 0.05$ according to DMRT.

S. No	Concentration of GTP (mg.mL ⁻¹)	EE (%)
1	1	69.39±2.418, c
2	2.5	75.40±0.056, b
3	5	77.78±0.300, a
4	10	64.49±1.379, d

In vitro drug release of the GTP from liposomal nanoparticles were studied at different concentrations of the GTP (1, 2.5, 5, and 10 mg.mL⁻¹), at different pH (3, 7.4, and 9), and temperatures (RT, 37 °C, and 45 °C) (Fig. 2). At a GTP concentration of 1 mg.mL⁻¹, 68% of the drug was released during the initial 8 h, and as the release was prolonged up to 48 h, 78% of the drug was released. With the increase in the concentration of GTP in the liposomal nanoparticles, the cumulative percentage of drug release decreased (61% and 60% was observed for 2.5 and 5 mg.mL⁻¹ respectively in 48 h). No significant difference was observed in the release pattern for 2.5 and 5 mg.mL⁻¹ of the loaded GTP. As

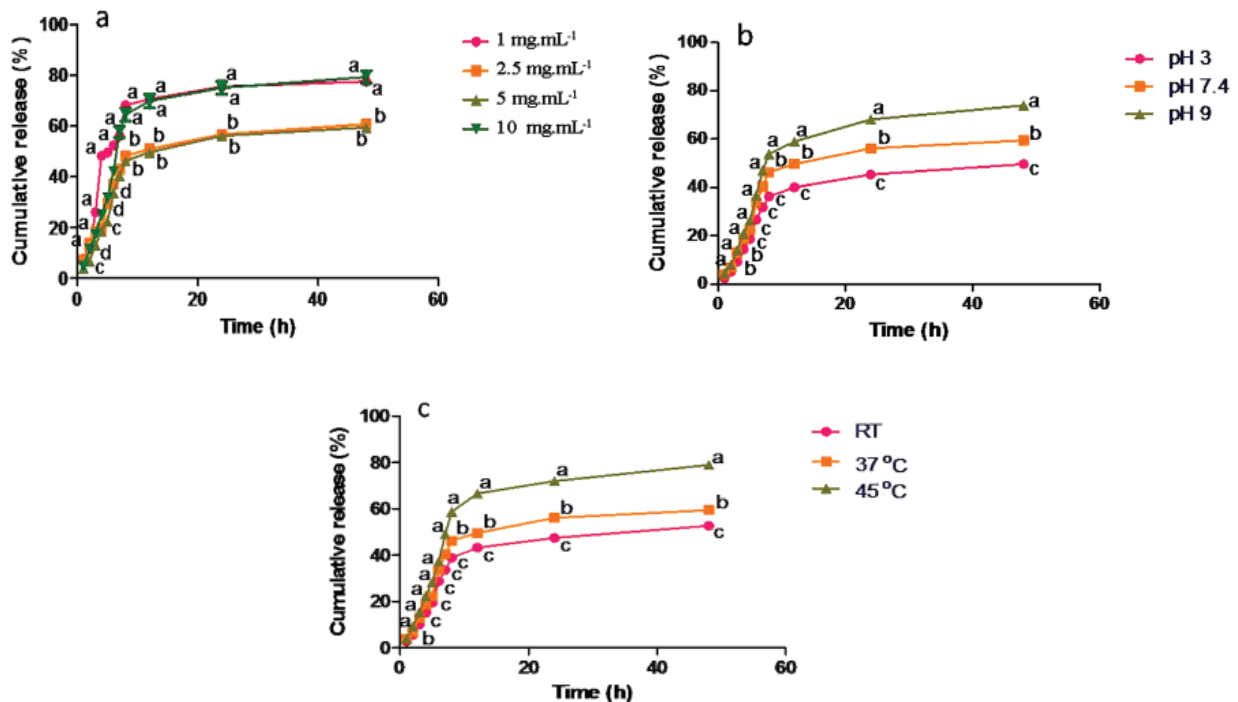


Figure 2. *In vitro* release of the GTP from liposomal nanoparticles at different concentrations of the GTP (a); different pH (b) and the different temperatures (c) at the different time intervals. Different letters at a particular time indicate significant difference according to DMRT, $p \leq 0.05$ ($n=3$).

the GTP concentration was increased to 10 mg.mL⁻¹, a significant increase in the cumulative percentage of the drug release was observed, where 83% of the drug was released in 48 h. The release of GTP was monitored by varying pH of the release medium. It was observed that, as pH of the medium shifted from acidic to basic, there was an increase in the cumulative percentage of the drug release. About 50% of the drug was released at pH 3 in 48 h, while at pH 7.4 and 9 it was 60 and 74%, respectively. As the temperature was increased from RT to 45 °C, an increase in the cumulative percentage of the drug release was observed. At RT, about 53% of the drug was released within 48 h. As the temperature was increased to 37 °C and 45 °C, 60% and 79% of the drug was released within 48 h.

The obtained *in vitro* release data was fitted to various kinetic models. The regression parameters (*i.e.*, the regression coefficients (R²), the release kinetic constants (k), and the release exponent (n) are presented

in Table 2 for the different parameters. All the release data showed a good correlation with Zero order kinetics. Predominance was observed in the anomalous mode of the drug transport, as the release exponent (n) for all the release data were observed to be in the range of 0.43-0.89. The release mechanism here is characterized by coupling of the diffusion and erosion mechanisms.

All the released samples showed potent antioxidant activities. The free radical scavenging activity of GTP loaded liposomal nanoparticles is shown in Figure 3. The highest scavenging activity was observed to be 75% for GTP concentration of 10 mg.mL⁻¹ for the sample released in 7 h, while the activity was observed to be 72% for the GTP concentration of 5 mg.mL⁻¹. No significant difference in the activity was observed for the GTP concentrations of 2.5 and 1 mg.mL⁻¹.

5. Discussion

Several studies have been carried out using liposomes

Table 2. The GTP release profiles with various models and mechanism of release at different conditions.

Condition	Zero order		First order		Higuchi model		Hixson-Crowell model		Korsmeyer-Peppas model		Drug transport mechanism
	R ²	k ₀	R ²	k ₁	R ²	k _H	R ²	k _{HC}	R ²	N	
1 mg.mL ⁻¹ GTP	0.9337	0.615	0.9782	0.006	0.6543	9.528	0.9724	0.014	0.7436	0.6125	Anomalous
2.5 mg.mL ⁻¹ GTP	0.9882	0.617	0.9873	0.007	0.750	10.698	0.990	0.022	0.8461	0.5739	Anomalous
5 mg.mL ⁻¹ GTP	0.9896	0.889	0.9855	0.012	0.7543	13.783	0.9891	0.040	0.8260	0.7617	Anomalous
10 mg.mL ⁻¹ GTP	0.9783	1.401	0.9580	0.009	0.7154	24.277	0.9720	0.036	0.8223	0.7720	Anomalous
pH 3	0.9951	0.829	0.9876	0.012	0.7854	12.846	0.9918	0.041	0.8252	0.8576	Anomalous
pH 7.4	0.9896	0.889	0.9855	0.012	0.7543	13.782	0.9891	0.040	0.8260	0.7617	Anomalous
pH 9	0.9926	0.692	0.9612	0.009	0.7939	11.986	0.9758	0.032	0.8511	0.7799	Anomalous
RT	0.9935	0.816	0.9878	0.012	0.7741	12.653	0.9912	0.041	0.8333	0.7903	Anomalous
37 °C	0.9896	0.889	0.9855	0.012	0.7543	13.782	0.9891	0.040	0.8260	0.7617	Anomalous
45 °C	0.9923	0.768	0.9545	0.009	0.7815	13.302	0.9865	0.032	0.8419	0.8099	Anomalous

R: Correlation coefficient; k: Constant; n: Release exponent

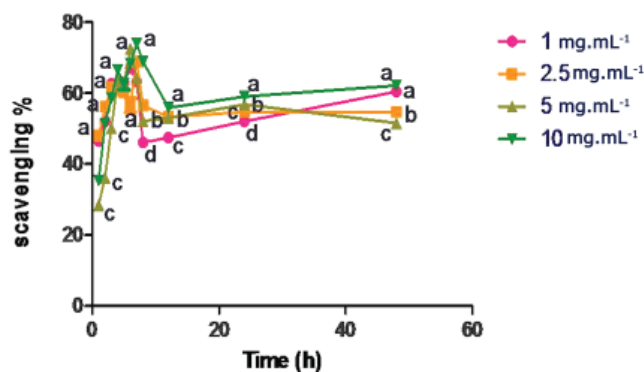


Figure 3. The free radical scavenging activity of the released samples upon loading different concentrations of the GTP to the liposomal nanoparticles. Different letters at a particular time indicate significant difference according to the DMRT, $p \leq 0.05$ ($n=3$).

for the drug delivery to the target cancer (18-20), for the treatment of the Alzheimer's disease (21), and various biomedical fields including immunoassay and targeted drug delivery (22). To the best of our knowledge, there are no articles available on the encapsulation of GTP to liposomal nanoparticles. Our work emphasizes on the encapsulation of the GTP to liposomal nanoparticles and developing a sustained release system. Liposomal nanoparticles have been synthesized and the results of our study showed particles with a diameter of ~ 250 nm. As well, the particles are being stable (ζ potential value of -24.2 ± 2.75 mV), while Wang *et al.* (23) have reported the surface charge of the liposomal nanoparticles to be -15 mV. The high ζ potential (positive or negative) provides a good dispersion stability by preventing aggregation and fusion of the liposomes due to electrostatic repulsion (24). The DLS results showed a higher hydrodynamic diameter of the particles compared to that of AFM and HR SEM (64.5 and 252 nm, respectively). The higher size of the particles could be due to the aggregation of the particles within the dispersion medium.

To enhance the efficiency of the GTP, liposomal nanoparticles were used as carriers. Upon loading different concentrations of the GTP to the liposomal nanoparticles, with an increased concentration of the GTP up to 5 mg.mL^{-1} , there was an increase in the EE and on further increasing, the GTP concentration, EE was decreased (Table 1). Therefore, 5 mg.mL^{-1} GTP was considered as an optimal loading concentration where EE was 77.7%, while Ramasamy *et al.* (2014) have reported the EE of MTX hydrochloride to the liposomal nanoparticles to be $\sim 60\%$ (25). Analyzing the release pattern, by applying different loaded concentrations of

the GTP showed no particular trend in the cumulative percentage of the drug release. The pH of the release medium was shown to play a role in the release of GTP from liposomal nanoparticles. A maximum of 60% of the GTP was released in 48 h at a pH of 7.4. In a similar study carried out by Ramasamy *et al.* (25), a maximum of 80% of the drug was released from the liposomal nanoparticles in 48 h when the release was carried out at pH 7.4. As the release medium shifted from acidic to basic, there was an increase in the cumulative percentage of the drug release. One of the limitations for the orally developed formulations is its metabolism in the gastrointestinal tract (GIT) which is mostly due to the harsh pH conditions of the GIT. In our study, to simulate the conditions of GIT, the release was carried out in a medium with acidic pH (pH 3). In an acidic pH (pH 3), a lower percentage of the drug was released, while the maximum drug was retained within the nanoparticles. In basic pH (pH 9), the percentage of drug release was increased. The basic pH might have affected the structure or the complexity of the liposomes, thereby releasing more amount of the drug. The temperature of release medium was directly proportional to the cumulative percentage of the drug release. As the temperature was increased, there was an increase in the drug release percentage. The higher temperatures could influence the structure of the liposomal nanoparticles or may affect the binding between liposomes and GTP thereby releasing a higher percentage of the drug (Fig. 2).

In vitro, drug release data was fitted into various kinetics models to evaluate the drug release mechanism (Table 2). The ideal model of the drug release for nanoparticulate dosage forms or sustained release formulations is the Zero order kinetics (17). All our release data showed good correlation with Zero order kinetics, therefore, liposomal nanoparticles could be used as an ideal carrier for the delivery of GTP, achieving a prolonged or a sustained release. Korsmeyer-Peppas stated that for a Fickian release, n value is constrained to 0.5, 0.45, and 0.43 for the release from slabs, cylinders, and spheres (26). For spheres, values of n ranging from 0.43-0.89 are regarded as anomalous and the release is characterized by diffusion and erosion mechanism and that > 0.89 is characterized by the polymer erosion and relaxation and is termed as super case II transport. In our study, n values ≤ 0.43 was regarded as a pure Fickian release, as the release of GTP was from spherical nanoparticles and the release was through diffusion. Predominance in anomalous transport was observed, which involves a coupling of erosion and diffusion.

Antioxidant assay revealed the potent scavenging

activity of the GTP with respect to the released GTP at various times (Fig. 3). It has proved the retention of the antioxidant activity of GTP even after release from the liposomal nanoparticles. The highest scavenging activity was observed between 5-7 h which corresponds to the higher amounts of drug released. The potent scavenging activity was observed for all the released samples and GTP encapsulated liposomal nanoparticles could be used as an effective antioxidant source.

To conclude, for achieving a prolonged release of the GTP, 5 mg.mL⁻¹ loading concentration was ideal in the acidic pH at 37 °C. The *in vitro* release data showed a good correlation of the GTP released with the release parameters such as pH and temperature, suitable for an ideal oral formulation where the release was minimum in the lower pH and temperature. The predominance was observed in Zero order kinetics with anomalous mode of transport. The antioxidant potential was observed for all the released samples. On further evaluation, the GTP encapsulated liposomal nanoparticles would serve as a potent source for oral delivery.

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