



INFLAMMATORY BOWEL DISEASE



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Development and characterization of colon targeted gel core liposomes for treatment of inflammatory bowel diseases

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Abstract

Curcumin with its anti-inflammatory properties is an ideal drug candidate to treat colonic diseases. Conventional dosage forms of curcumin are available but have limitations like low bioavailability of curcumin due to its degradation in stomach and short duration of action. Hence current work was carried out with an objective to design oral colon targeted drug delivery of curcumin in the form of Gel Core Liposomes (GCL) as liposomes have property to get concentrated in inflamed areas, further gel core containing curcumin would serve as depot for controlled release of encapsulated curcumin. Gel core liposomes are polymer based liposomal formulation of drug that will gel following administration and thus will protect the drug as well as prolong its release. GCL were prepared by thin film hydration method with slight modification in which the dried lipid film containing drug was hydrated with aqueous solution of gel forming polymer i.e. 0.2%w/v Carbopol 974P. GCL were optimized by 2³ factorial design to study effect of concentration of Egg phosphatidylcholine, Cholesterol and Carbopol on entrapment efficiency and drug release. These liposomes were characterized for vesicle size, shape, entrapment efficiency, zeta potential and in vitro release. Colon targeting of optimized GCL was achieved by coating with Eudragit S100. Uncoated and Eudragit coated GCL were subjected to animal studies carried out on acetic acid induced colitis in male Albino Wistar rats. Results indicated that colon targeted GCL could be a novel drug delivery with potential for increased bioavailability of drug, decreased GI degradation, site targeted drug delivery which is desirable for treatment of Inflammatory Bowel Disease (IBD).

Published Online: May 15, 2020

eBook: Inflammatory Bowel Disease

Publisher: MedDocs Publishers LLC

Online edition: <http://meddocsonline.org/>

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Keywords: Curcumin; Gel core liposomes; Carbopol; Eudragit S100; Colon targeting; Inflammatory bowel diseases.

Introduction

Inflammatory bowel disease is a lifelong disease usually found in last part of small intestine and first part of large intestine. The mildest form of inflammatory bowel disease results in patches of inflammation and small ulcers whereas in moderate or severe inflammatory bowel disease, these ulcers become much larger and deeper the inflammation can make the intestine become thickened, blocking the passage of digested food [1]. Inflammatory bowel disease has affected more than 396/100,0000 persons worldwide. Treatment options are re-

stricted to controlling symptoms, maintaining remission, and preventing relapse. Treatment regimen presently available like aminosaliclates, corticosteroids, biologics and immunosuppressant have therapeutic limits and undue side effects [2]. Hence, present work focus on use of herbal drug, curcumin in the form of novel drug delivery system for the treatment of this disease. Curcumin have therapeutic potential in gastrointestinal diseases and shows site specific anti inflammatory activity in colon.



Capsules and liposomes of curcumin are available in market but, administration of capsules often results in poor bioavailability and therapeutic response due to rapid metabolism of the drug in upper GI and larger dose is required to give the therapeutic effects, the frequent periodic administration of capsules becomes necessary to maintain a level of medication [3]. Multi particulate drug delivery offer advantages over most colonic delivery systems. In multi particulate drug delivery, vesicular drug delivery systems like liposomes are more beneficial as they are completely biodegradable and relatively non-toxic. Another potential advantage of liposomes is their ability to come in an intimate contact with the inflamed site of colon and they are attracted towards the inflamed area, thereby increasing the probability of site specific action to the colon [4]. Liposomes are effective than capsules but they have some limitations like they do not show longer effect and they are difficult to reach the colon because they get degraded due to bile salts present in small intestine. In colonic inflammatory diseases long term treatment is needed. Hence there is a need to formulate colon targeted, controlled release drug delivery system.

Colon targeted (coated with pH sensitive polymer Eudragit S 100) gel core liposomes would be beneficial which would gel after administration when they reach to the colonic pH [5]. Gel formation in liposomes prolongs the release of drug from formulation gives longer period of action also minimize leakage of drug from liposomes during storage. Gel core liposomes have higher entrapment efficiency and greater stability than conventional liposomes [6]. No gel core liposomal system of curcumin has been formulated so far for inflammatory bowel diseases. So gel core liposomes of curcumin can be prepared which will help in increasing residence time as well as providing controlled and targeted release of curcumin for treatment of IBD. The schematic representation of Colon targeted gel core liposomes loded with curcumin is shown in **Figure 1**.

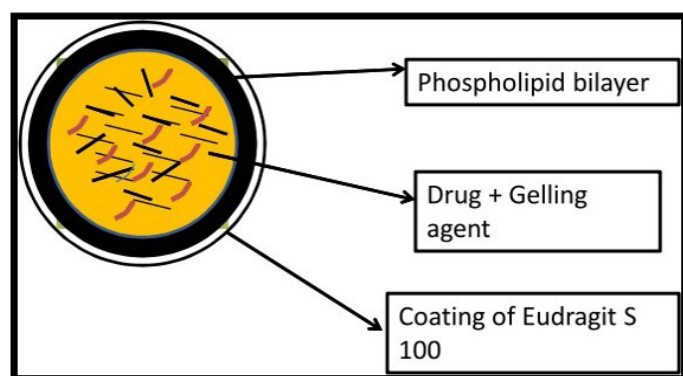


Figure 1: Schematic representation of Colon targeted gel core liposomes loded with curcumin.

Materials and methods

Materials

Curcumin (Cu) was kindly gifted by Amser Pvt. Ltd. Goa. Egg Phosphotidylcholine (Egg PC) lipova E-120 was gifted by Vav life sciences Mumbai and Eudragit S100 was gifted by Evonic India Pvt. Ltd. Cholesterol(CH), Ethanol, Methanol, t-butanol, acetone were purchased from Research Lab India, Carbopol 974P (CP) was purchased from Lubrizol Mumbai.

Methods

Preparation of gel core liposomes(GCL)

GCL were prepared using thin film hydration method [7,8]

with slight modification. Briefly Egg PC, CH and curcumin (100:50:10 mg) were dissolved in 10 mL of methanol-t-butanol mixture (1:2) to form clear solution in 250 mL round bottom flask. Then by using rotary evaporator (Heidolph, Germany) organic solvent was evaporated at 37°C for 30 min at 100 rpm. After complete removal of organic solvent, dried thin lipid film was hydrated with 10 mL distilled water containing (0.2% w/v) of carbopol 974P as gelling agent to form dispersion. During this process temperature was maintained at 37°C and speed at 60 rpm for 30 min. Prepared dispersion was sonicated for 10 min in bath sonicator (Spectralabs, Navi Mumbai). Then dispersion was kept overnight at 2-8°C for complete vesiculation.

Preparation of Eudragit S 100 coated GCL

Eudragit S100 is pH sensitive polymer which is used for colon targeting. Prepared GCL were coated with Eudragit by incubation method [9]. Eudragit S100 solution was prepared in mixture of acetone and water (7:3) and was added drop wise to the formulation of GCL in 1:1 ratio under magnetic stirring at constant speed for 1 hour, followed by incubation in refrigerator overnight.

Optimization of GCL by 2³ factorial design

Prepared GCL formulations were optimized using 2³ factorial design using Design Expert software for studying the effect of ratio of lipids and gel forming agents i.e. Egg Phosphatidyl Choline (Egg PC): Cholesterol(CH): Carbopol (CP) on Entrapment Efficiency (EE) and Drug Release(DR) [10,11] as seen in **Table 1**.

Table 1: Optimization of gel core liposomes by changing lipids and carbopol ratio.

Batch Code	Egg PC	Cholesterol	Carbopol	Drug
G1	100	45	0.2	10
G2	100	50	0.15	10
G3	95	45	0.2	10
G4	95	50	0.15	10
G5	95	45	0.2	10
G6	95	50	0.15	10
*G7	95	45	0.15	10
G8	95	50	0.2	10

Characterization of gel core liposomes

Uncoated and Eudragit S100 coated optimized GCL were characterized for various parameters. The mean vesicle size and vesicle size distribution was obtained by dynamic light scattering particle size analyzer (Sympatec-Nanophox (NX0088)) by diluting vesicle dispersions with deionized water [12,13]. Microscopic observation of vesicular formulation was done by Transmitting Electron Microscope (TEM). Samples for TEM were prepared by adding 20µl of formulation and 20µl of 1% phosphotangustic acid in Eppendorf tube by micro pipette and mixed gently. A drop of the mixture was placed on to the carbon-coated grid and excess was drained off. The grid was allowed to dry, and it was observed under transmission electron microscope (Philips TEM Model CM 200 SUPERTWIN STEM resolution 0.23 nm, voltage 200 kV) [14,15]. The zeta potential of uncoated and coated formulation measured in deionized water using Zetasizer version 3.94 (Brookhaven instrument corporation) [16].

Determination of entrapment efficiency

The GCL were centrifuged (Remi) at 2000 rpm for 45 min. Supernatant was removed and methanol was added to residue and sonicated in bath sonicator (spectralab Mumbai) for 10 min to disrupt the vesicles. The vesicles were broken to release the drug, which was then estimated for the drug content [9]. The absorbance of the drug was noted at 425 nm using UV spectrophotometer (Shimadzu V-630, Japan). The entrapment efficiency was then calculated using following equation,

$$\text{Entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug added}} \times 100$$

Confirmation of gelling of the core of GCL

Confirmation of gelling in inner compartment of GCL was done by treating the vesicles with organic solvent like methanol which dissolves lipids. The gel core liposomes formulation was centrifuged to remove un-entrapped polymer. Then it was divided into two parts one part was kept as such and other was dialyzed against PBS (pH 7.4) for gelling in the core. Both of the preparations treated with methanol and were evaluated for morphological characterization by motic microscope (Labomed) [8].

In vitro drug release studies

The release of curcumin from optimized formulations was determined using the membrane diffusion technique [8]. Liposomal dispersion (5mL) was sealed in dialysis bag (Himedia) which was kept in flask containing 200 mL dissolution media. This flask was placed in orbital incubator shaker (Chromous biotech # OS-02) maintained at $37 \pm 0.5^\circ\text{C}$ at 50 rpm.

The samples were withdrawn at scheduled intervals (replaced with equivalent amount of dissolution medium to maintain sink condition) and analyzed for drug content by UV spectrophotometer (Shimadzu V-630, Japan) at wavelength 425nm. Simulated GI track conditions respective to pH are maintained throughout the drug release study.

Drug release kinetics

To investigate the mode of drug release from the formulation, the release data was analyzed with different mathematical kinetic models like Zero order, First order, Higuchi, Hixson-Crowell, Korsmeyer Peppas, using PCP-DISSO-v2 Software [17].

In vivo studies on wistar rats

In vivo study was carried out to compare efficiency of plain drug suspension, uncoated and coated GCL to treat colitis. The in vivo study was performed using a model of diffuse colitis in white Albino Wistar rats induced by intra-luminal colonic instillation of dilute acetic acid. All the animal experiments were conducted according to the rules and guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The study was approved by Institutional Animal Ethical Committee of Smt. Kashibai Navale College of Pharmacy, Pune, registered under CPCSEA, India (Registration No: 1154/ac/07/CPCSEA). The rats were acclimatized at a temperature of 20°C at humidity of 60% and were fed on standard diet for 10 days prior to the commencement of the experiment at animal care facility of Smt. Kashibai Navale College of Pharmacy, Pune University (MH) India. Rats were divided into five groups each group contains three rats.

Table 2: In vivo studies on male wistar rats: Grouping of animals.

Sr. No	Group (n = 3)	Dose
1	Negative control(saline water)	-
2	Positive control(10% acetic acid)	0.2 mL
3	Drug suspension(curcumin)	100 mg/kg
4	Gel core liposomes(curcumin)	1mL (curcumin equivalent to 100mg/Kg)
5	Enteric coated gel core liposomes (curcumin)	1mL(curcumin equivalent to 100 mg/kg)

For instillation of acetic acid into colon of rats 2cm length of soft polyethylene tubing was used. Open end of tube was sealed with glue. The whole length of tube was perforated with a needle at 0.5mm length by four holes. 10% acetic acid in saline was filled to 1 mL syringe. Tubing was inserted intra-rectally to colon of rats & 0.2mL was injected into lumen. After 10s contact in situ, the remaining acid was withdrawn & lumen was washed 3 times with normal saline (0.5 mL). Test formulations were administered during following days. Histopathology of colonic region of rats was done for studying the effect of different formulations [18].

Stability studies

Coated and uncoated GCL were stored at different temperature conditions for 30 days. Formulations were evaluated for their physical and the chemical stability. The physical stability was assessed by vesicle size determination and visual observation for sedimentation. The chemical stability was determined by measuring the drug content by UV Spectroscopy [19,20].

Results and discussion

Anti-inflammatory activity of Curcumin is well proven and hence it is used in treatment of IBD. Curcumin gets degraded in acidic pH of stomach and therefore its colon targeting is essential. Also, IBD require long treatment regimen (for 3-5 days). This research work was carried out with an objective to prepare colon targeted site specific (i.e. liposomes are attracted to inflamed sites) drug delivery and to have controlled release of curcumin for prolong period. In this study colon targeted GCL of curcumin were also compared with uncoated GCL. The results are mentioned hereafter.

Optimization of GCL by 2³ factorial design

From Figure 2 it can be seen that entrapment efficiency and drug release are affected by lipid (Egg PC and CH) and CP concentration. A positive correlation was found between all the factors. As concentration of Egg PC, CH and CP increased, entrapment efficiency increased and drug release decreased. This is due to the increase in lipid concentration and gelling agent causes drug to be entrapped immobilized within core and as these GCL come in contact with colonic pH, CP subsequently gels further retarding the release of drug.

Entrapment efficiency of optimized formulation (G7) was found to be 81.21% which is better than conventional liposomes (usually have entrapment upto 50-60%)^[6]. These findings suggest that GCL can be potential carriers for drugs which are potent.

Characterization of GCL

Vesicle size of uncoated and coated GCL was found to be 495.88, 843.34 nm respectively. Coated gel core liposomes were larger in size than uncoated formulation. Transmission electron microscopy was used for surface morphological examination of prepared GCL. The gel core and coated gel core liposomes were almost spherical and surface was smooth (Figure 3). Increase in vesicle size and TEM studies confirmed coating was done successfully. Polydispersity index of less than 0.2 was recorded for both the formulations indicating narrow particle size distribution of vesicles (Table 3). Zeta potential of gel core liposomes was found higher than that of coated formulation (Table 3) which indicates that coating of liposomes was done with Eudragit S100 which gives negative charge to the surface of GCL, also these vesicles would remain stable without aggregation.

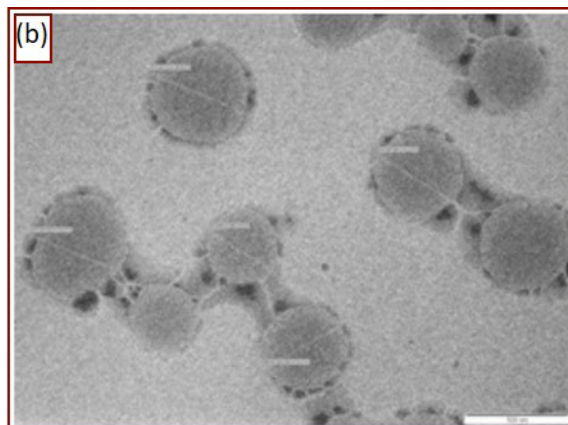


Figure 3: TEM image of (a) Gel core liposomes (b) Coated gel core liposomes.

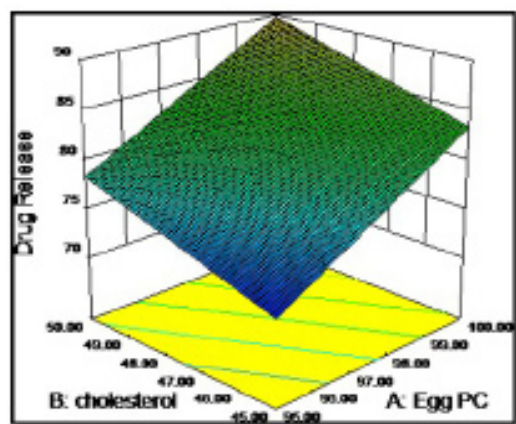
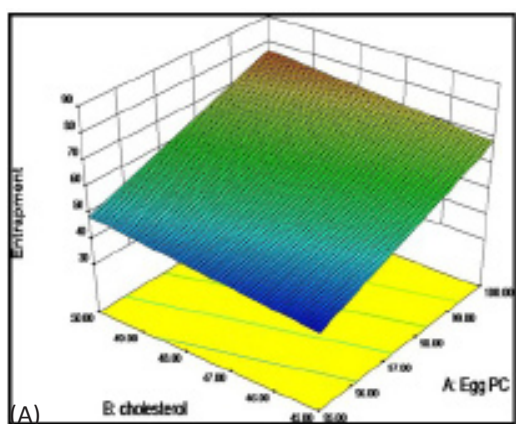


Table 3: Characterization of gel core liposomes.

Formulation	Zeta potential	Vesicle size	PDI
Gel core liposomes	-18.87 mV	495.88	0.28
Coated gel core liposomes	-24.04 mV	843.34	0.09

Confirmation of gelling

Spherical structure of gel core liposomes was observed in Figure 4(A) the formulation treated with methanol after gelling but on treatment with methanol before gelling the vesicular structure completely disappears as seen below in Figure 4(B). Since the addition of methanol to the gel core liposomes dissolves lipid layer but after gelling the spherical structure of gel core remains unaffected as seen in Figure 4(A). However, before gelling the polymer is in sol form and hence cannot retain its spherical shape after removal of lipid layer.

Figure 2: Effect of concentration of lipids and carbopol on (a) Entrapment and (b) Drug release of GCL (3D surface plots).

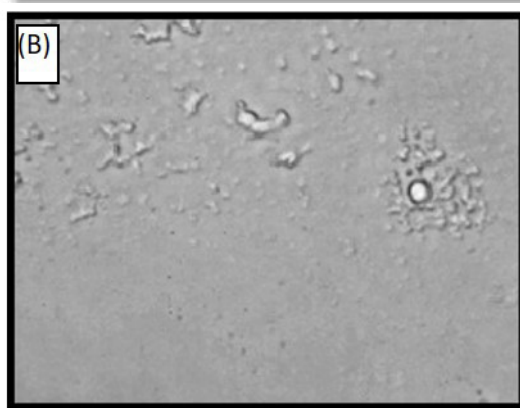
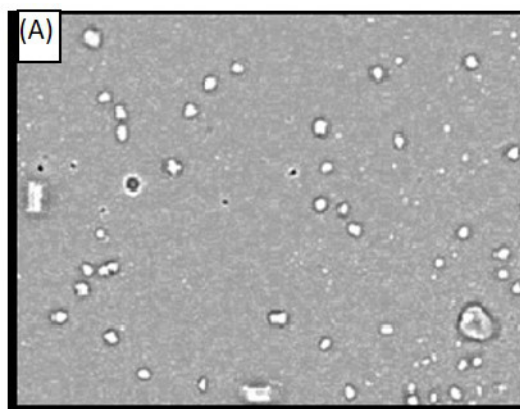
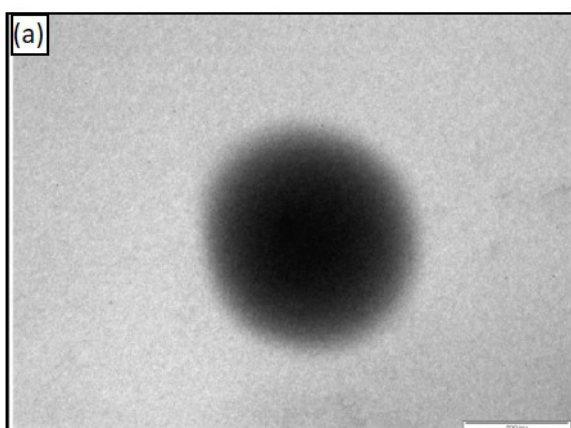


Figure 4: Formulation treated with methanol (A) After gelling (B) Before gelling.

In vitro drug release studies

Different release media representing each stage of the GI tract were used to evaluate release of curcumin from coated and uncoated GCL. At pH 1.2 and 6.8 the amount of drug released was significantly lower at all time points on addition of pH sensitive polymer (Eudragit S100). In **Figure 5 (a)**, at pH 1.2, over a period of 2 hours, uncoated GCL released three times more amount of curcumin in contrast to coated formulation. Similarly for a transit time representative of the small intestine (4 hours) the release for uncoated gel core liposomes is approximately 1.31% while for the same time period only 0.27 % release is observed for the coated formulations (**Figure 5(b)**).

Further, in simulated ileo-cecal junction i.e. dissolution media of pH 7.4, similar release rates for both uncoated and coated formulations were observed as seen in **Figure 5(c)**. This may be attributed to erosion of Eudragit coating once it reaches colonic pH.

Sustained release of curcumin from uncoated as well as coated GCL is due to gelling of the core only at colonic pH as well as lipid coating of the vesicle which further retards the drug release.

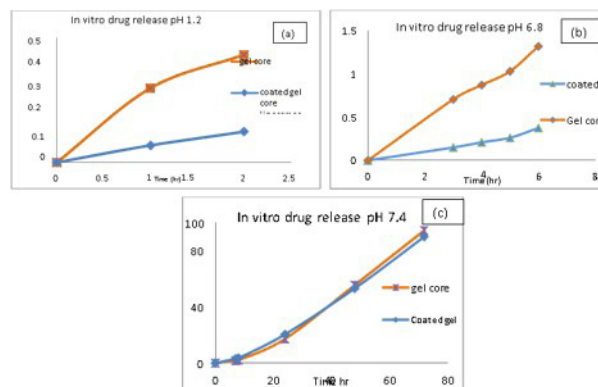


Figure 5: In vitro drug release study (a) At pH 1.2 (b) At pH 6.8 (c) At pH 7.4.

Drug release kinetics

The formulation of GCL was best fitted for the Korsmeyer Peppas equation as the formulation coefficient of correlation values predominates over zero-order and first-order kinetics (**Table 4**). This indicates the drug release mechanism by diffusion, as proposed by Peppas. On the basis of the Korsmeyer Peppas model, the best fitting was obtained with n value 1.1235 indicating super case II type drug transport mechanism for gel core liposomes.

Table 4: Summary of release kinetics for gel core liposomes.

Model	Zero Order	T-test	1st order	T-test	Matrix	T-test	Peppas	T-test	Hix.Crow	T-test
R	0.9841	19.998	0.9009	7.486	0.8702	6.367	0.9443	10.351	0.9469	10.621
K	1.3015	Passes	-0.0358	Passes	7.6942	Passes	0.8375	Passes	-0.0077	Passes

In vivo studies on Wistar rats

Liposomal formulation significantly reduces symptoms of colitis and inflammation of the colon. From histopathology reports of rat colon as seen in Figure 6 rats were treated with acetic acid suffered severe ulceration with necrosis and rats treated with coated GCL formulation showed mild colitis as compared to those treated with plain curcumin suspension or uncoated GCL. This may be due to degradation of curcumin in gastric environment when given as suspension or uncoated GCL form whereas the coating of Eudragit protected the drug until it reached colon and then the drug was released slowly from gelled core.

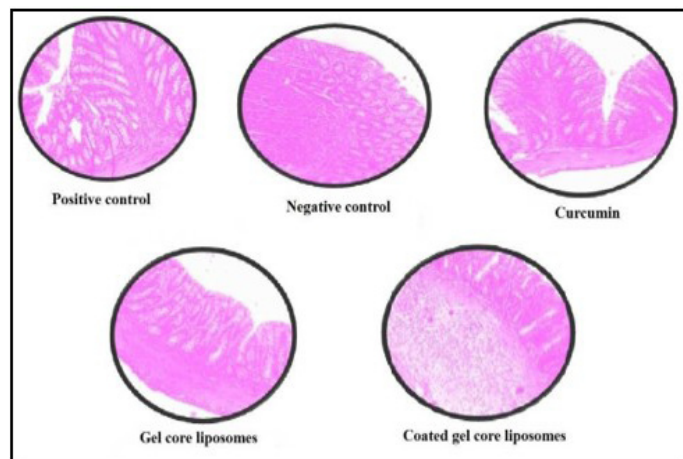


Figure 6: Histopathology reports of rat colon treated with different formulations of curcumin

Stability studies

Stability during storage of liposomal formulation was evaluated at three different temperature conditions 2-8°C, room temperature and 45°C. Both formulations were stable at refrigeration temperature and showed less drug leakage, slight change in particle size and drug release but at room temperature and at 45°C both the formulations were not stable due to aggregation and sedimentation and of vesicles at higher temperature.

Conclusion

Present research work was undertaken with an objective to have liposomal drug delivery of curcumin for treatment of IBD. Hence, uncoated GCL and GCL coated with pH sensitive polymer Eudragit S100 were prepared, optimized evaluated and compared with each other. Uncoated GCL were degraded in intestinal pH because of bile salts and Eudragit coated GCL could successfully reach colon. In IBD, treatment regimen is for longer duration which was possible for GCL as they released curcumin for 72 h. In vivo studies on male Albino Wistar rats also suggested that GCL proved to be potential dosage form having good entrapment efficiency, longer duration of action which would reduce frequency of dosage administration and thus increased patient compliance.

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