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RESEARCH ARTICLE

MODIFICATION OF SOLUBILITY CHARACTERISTICS OF LIPOPHILIC DRUGS BY VESICULAR SYSTEM: LIPOSOMES

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ABSTRACT

There is a vast time approach evolution in medication and there preparation methods which has brought into medicines which are even more effective and target the specific cells and not the entire body and produce more biological effect rather than producing adverse effects one of these is liposomal vesicles which has been evolve over time. Vesicular systems are highly ordered single or multiple assemblies Bilayer of concentrated lipids. Nonetheless, its physicochemical possessions such as sedimentation, hydrolysis, and oxidation, as well asstowingcircumstances have been restricted the consumption of liposomes. The soft gelatin-based capsules containing proliposomes of DCT were prepared using film deposition on carrier method.6 different formulations were prepared with varying concentrations of DCT and phospholipids. Particle Size, PDI and zeta potential of the formulations is given in table 2. The particle size was in the range of 212 ± 12 to 414 ± 18.3 nm following hydration of proliposomal formulations in 0.1 M HCl to obtained simulated conditions of gastric fluid. Liposomes usually have biphasic release pattern. A rapid release is observed in the first phase followed by a relatively slower and sluggish release that is usually sustained type for 12 h or more. The initial rapid release may be associated with the erosion of the outer surface due to presence of no entrapped drug there.

INTRODUCTION

Vesicular systems have been implemented in various scientific ways as extremely useful carrier systems. Vesicular systems are highly ordered single or multiple assemblies Bilayer of concentrated lipids. The oral route for liposome is favorable for its flexibility, safety, and patient consistency and its ability to locate drug at targeted site. However, its physicochemical properties such as sedimentation, hydrolysis, oxidation, and storage conditions has been limited the use of liposomes. Therefore, proliposomes has been formulated to resolve these issues of liposomes.By developing the proliposomal formulation, factors of solubility and bioavailability of poorly soluble drugs can be overcome. Proliposomes are originally defined as dry, free-flowing particles that on hydration develop liposomal suspension (Byeon et al., 2019). They offer the opportunity to form liposomes only at the delivery site, which is more stable during sterilization and storage (Aggarwal and Singh, 2011).

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Osteoarthritis is a major type of arthritis worldwide which affects both women and men. Inflammation of joints are more common in women. Injury can cause breakage of bone that can further damage the ligaments. Overweight, family history, age and past injury are risk factors. Age is the most common factor, and it becomes severe with increasing age due to inflammation, stiffness, progressive destruction of cartilages and degradation of joints.

Inflammation of joints are mild or moderate, they can be treated by management of pain and joint replacement (Glyn-Jones et al., 2015). To formulate diacerein (DCT)liquid proliposomes composed with phospholipid base, polyethylene glycol 400 and tween 80 to improve the solubility and release into the intestinal tract.Diacerein is sparingly soluble in water but soluble in organic solvents. Its physical appearance is yellowish powder. It is used in osteoarthritis.In the colon, the unabsorbed diacerein is metabolized to Rhein that induces a laxative effect by activating chloride secretion by exciting submucosal neurons and releasing acetylcholine and endogenous prostaglandin, not by releasing histamine or serotonin(Jain, Nagori and Yadav, 2018).

MATERIALS AND METHODS

Diacerein was generously donated as a gift sample from Pacific Laboratories (Pvt.) Ltd, Multan Road, Lahore. Phospholipid (Soy Lecithin) was purchased from ELMA, Belgium.

Polyethylene glycol 400, Polysorbate 80 and Ethanol from China. Double distilled water was freshly prepared in the Research Lab, Department of Pharmacy, The University of Faisalabad. All the chemicals used were of analytical grade and use without further modification.

METHODOLOGY

Soft gelatin-based capsules containing proliposomes of DCT were prepared using film deposition on carrier method. Briefly, a mixture of Phospholipids, DCT, PEG 400 and Polysorbate 80 were dissolved in absolute ethanol and mixed by using magnetic stirrer for 15 min at 2000 rpm until a transparent white solution was obtained. Proliposomes were filled into capsule shells following careful weighing using a syringe (BD, Malaysia).

Capsules were sealed using hot metal spatula. Prepared capsules were dried at ambient temperature and were placed in glass vials for further usage. 6 different formulations were prepared with varying concentrations of DCT and phospholipids as mentioned in Table 1(Aggarwal and Singh, 2011).

Table 1. DCT proliposomal formulation contents

Formulation Code	DCT (mg)	Phospholipid (mg)	PEG 400 (ml)	Tween 80 (ml)	Ethanol (ml)
DCT-1	80	80	1	0	9
DCT-2	80	200	1	0	9
DCT-3	80	400	1	0	9
DCT-4	80	800	1	0	9
DCT-5	80	800	1	2	9
DCT-6	80	800	1	4	9

Physico-chemical characterization of Proliposomes

Determination of Diacerein contents: DCT contents in proliposomes were determined using UV/VIS Spectrophotometer at 256 nm. DCT proliposomal formulations were taken with each containing an amount equivalent to 80 mg of DCT and contents of DCT in each formulation were determined by standard calibration curve.

Measurement of Zeta Potential, Particle Size and Polydispersity Index: Dynamic light scattering technique was used to measure the size, zeta potential and polydispersity index (PDI) of the hydrated liposomes using Zeta Sizer. Samples were hydrated using double distilled water for measuring the size.

Proliposomes were hydrated using distilled water and 0.1 M HCl following appropriate dilution to meet the requirements of measurement for the aforesaid instrument. Obtained results were mentioned as mean ± Standard deviation using three different formulations for each batch prepared (Khan, Madni and Peltonen, 2016).

Rate of Conversion from Proliposomes to Liposomes: Absorbance was measured during conversion of proliposomes to liposomes. For sample preparation, pre-weighed amount of DCT proliposomes was mixed with 0.1 M HCl using a 1 cm path length Quartz cuvette. Reference Cuvette was only filled using 0.1 M HCl to automatically null the buffer absorbance. Absorbance was determined using UV/VIS Spectrophotometer (Shimadzu, Germany) (Jain, Jain and Mahajan, 2014).

RESULT AND DISCUSSION

Particle size, zeta potential and polydispersity index: Particle Size, PDI and zeta potential of the formulations is given in table 2. The particle size was in the range of 212 ± 12 to 414 ± 18.3 nm following hydration of proliposomal formulations in 0.1 M HCl to obtained simulated conditions of gastric fluid. All sizes were in nanometer range whereas PDI for all the formulations was less than 0.5 indicating monodisperse formulations nature. Zeta potential is an important parameter related to stability as well as surface properties of the liposomes.

High zeta potential values indicate strong repellent action among the nanoparticles resulting in greater stability of the formulations. Zeta potential values for DCT formulations were in the range of 27.5 ± 1.9 mV to 33.6 ± 5.2 mV which was indicative of excellent stability of the formulations as it is evident that zeta potential with negative value and higher than 20 mV is sufficient to prevent the coalescence among the nanovesicles that minimizes the chances of aggregation and increase in size of the particles. Higher zeta potential values are also associated with prevention of sediments production as well as adsorption of proteins during blood circulation.

Table 2. Particle Size, Zeta Potential and PDI of DCT formulations

Formulation Code	Particle Size (nm)	Zeta Potential (mV)	PDI
DCT-1	212 ± 12	27.5 ± 1.9	0.39 ± 0.01
DCT-2	236 ± 16.3	29.3 ± 1.5	0.28 ± 0.01
DCT-3	315 ± 22.5	31.2 ± 2.6	0.47 ± 0.01
DCT-4	405 ± 13.5	30.6 ± 2.1	0.39 ± 0.02
DCT-5	412 ± 8.4	33.6 ± 5.2	0.44 ± 0.01
DCT-6	414 ± 18.3	33.4 ± 2.9	0.24 ± 0.02

Scanning Electron Microscopy: The electron micrographic image of the optimized formulations is shown in Figure 1. The image indicates that the particle size was different with the light scattering owing to the reason that both methods follow different sample preparation technique. The particles had spherical type structure in most of cases with the clear evidence of drugloading inside the vesicles. Particles showed discrete boundaries confirming the formation of liposomes from the proliposomal formulation

Entrapment efficiency: EE is causally related with the phospholipid type. EE % of DCT liposomes following hydration in distilled water was in the range of 84.8 ± 4.2 to 89.6 ± 3.5 % whereas there was a slight decrease in case of 0.1 M HCl where its value ranged from 76.3 ± 2.2 to 80.2 ± 4.9 %. A higher value of EE % is probably associated with the nature of drug being lyophobic.

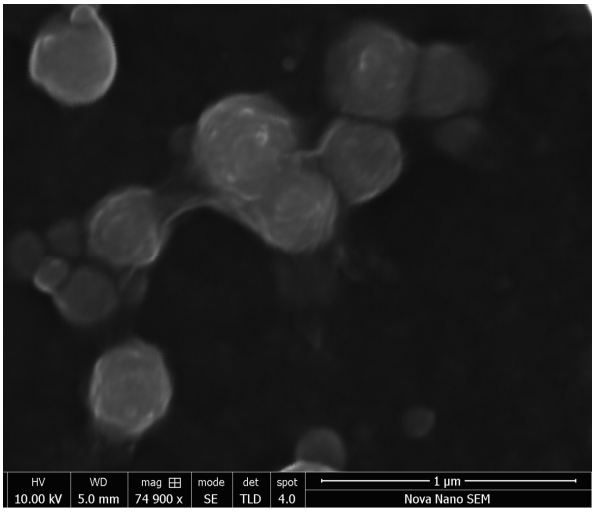

Figure 1. SEM image of DCT formulation

A decrease in the EE % was observed in case of 0.1 M HCl but still it was more than 75 % in case of all prepared formulations. Decrease can be associated with disruption for some liposomes due to low pH of acidic medium that can result indrug leakage from the liposomes. Phospholipid has also been known to increase the EE % owing to rigidity of the liposomal membranes. EE % for DCT formulations is given in Table 3.

Table 3. EE % of DCT formulations

Formulation Code	EE (%) in Distilled Water	EE (%) in 0.1 M HCl
DCT-1	84.8 ± 4.2	76.3 ± 2.2
DCT-2	85.3 ± 3.7	76.8 ± 4.1
DCT-3	86.4 ± 2.9	79.3 ± 2.6
DCT-4	88.4 ± 3.8	78.9 ± 2.9
DCT-5	85.1 ± 3.1	79.4 ± 4.5
DCT-6	89.6 ± 3.5	80.2 ± 4.9

Conversion Rate of Liposomes from Proliposomal Formulations: The prepared liposomes of DCT were transparent liquid before any hydration. Upon addition of distilled water, an evident change in the turbidity was observed. Highest absorption was observed at 30 sec and afterwards, no further increase was observed which was suggestive of a progressive and rapid conversion to liposomes. It can be assumed that the prepared proliposomes will be rapidly converted into liposomes upon contact with the physiological fluids of the human body on administration through oral route.

Diacerein contents in the Proliposomal formulations: Table 4. shows the DCT incorporated in the proliposomes was determined as more than 97.7 %. The drug was distributed uniformly in all the formulations. % of drug contents was in the range of 97.7 ± 0.4 to 99.4 ± 1.2 %.

Table 4. % contents of DCT in formulations

Formulation Code	% of DCT
DCT-1	98.7 ± 0.4
DCT-2	98.9 ± 0.3
DCT-3	97.7 ± 0.9
DCT-4	99.4 ± 1.2
DCT-5	98.4 ± 0.6
DCT-6	99.1 ± 1.3

Release Kinetics: Liposomes usually have biphasic release pattern. A rapid release is observed in the first phase followed by a relatively slower and sluggish release that is usually sustained type for 12 h or more. The initial rapid release may be associated with the erosion of the outer surface due to presence of no entrapped drug there. A decrease in release was observed with increasing the concentration of the phospholipid due to more stabilization of lipid bilayers showing a more probable depot effect. Important thing was increased dissolution of the insoluble drug in the form of proliposomes. This is attributed to increased solubility of DCT by phospholipids. It is also evident that drug remained stable and is released slowly at the site of requirement indicating that proliposomes prepared were suitable to sustain the release of the drug DCT. Release of DCT1 was nearly more than 96.4 ± 2.8 % whereas for DCT it was near to 76.4 ± 2.8 % following 12 h of dissolution study.

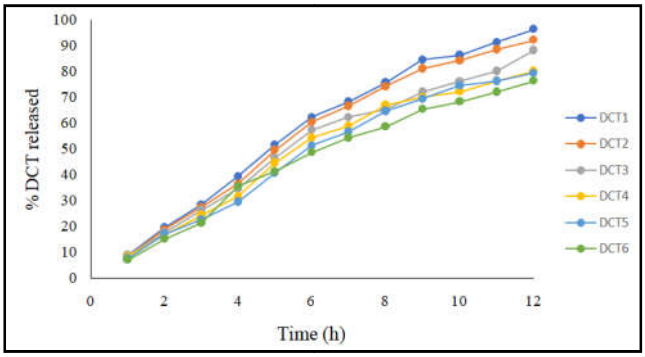

Figure 2. Dissolution data of DCT formulations following 12 h of release

Figure 2. shows the dissolution data of various DCT formulations. Table 5. clearly indicates Korsmeyer Peppas as the most suitable mathematical model with best fit where values of regression coefficient are 0.99 for almost all formulations of DCT. "n" values are also more than 0.45 for all proliposomal formulations that indicates both swelling as well as diffusion to be responsible for the drug release from the proliposomal formulations. As mentioned, aforesaid, proliposomes were successfully converted to liposomes in less than one min upon adding in distilled water so that results in swelling of the formulations followed by drug leakage from the liposomes obtained depending on the amount of phospholipids in the formulations. It is also evident from the data that encapsulation of the DCT in the proliposomes leads to a substantial improvement in the absorption of the drug. Several factors can play a pivotal role in this regard which include the uptake of the liposomal formulation from the GI track where particle size plays an important role. Liposomes of size 300 nm and above can be efficiently up taken in the small intestine especially in the lymphoid tissue and this can also result from escape from first pass effect occurring in the liver. Similarly, surfactants in the formulation also affect the permeability of the membrane as used Tween 80 in formulation DCT5 and DCT6 which is related directly with the solubility. Also, by incorporating the drug in the lipid bilayer of vesicles can prevent it from bacterial as well as enzymatic degradation during the process of absorption. So, it can be ascertained that DCT encapsulation in the proliposomes can result in an enhanced bioavailability of the drug besides increasing circulation time in the blood.

Table 5. Mathematical models for In vitro drug release of DCT proliposomal formulations

Formulation Code	Zero Order		First Order		Higuchi		Hixon Crowell		Korsmeyer Peppas		
	K_0	R^2	K_1	R^2	K_h	R^2	K_{hc}	R^2	K_{kp}	R^2	N
DCT1	9.15	0.845	0.236	0.92	28.63	0.765	0.036	0.96	14.26	0.99	0.843
DCT2	8.98	0.89	0.214	0.91	26.54	0.774	0.038	0.95	13.4	0.99	0.862
DCT3	8.64	0.84	0.168	0.94	24.48	0.832	0.034	0.93	12.44	0.98	0.834
DCT4	7.632	0.88	0.132	0.93	21.23	0.841	0.028	0.92	8.32	0.99	0.792
DCT5	6.235	0.79	0.198	0.90	19.25	0.792	0.021	0.91	8.56	0.98	0.816
DCT6	5.32	0.86	0.145	0.94	16.32	0.745	0.031	0.94	6.65	0.99	0.824

Table 6. Results of DCT formulations in stability testing at refrigerated temperature

Formulation Code	EE (%) in Distilled Water	EE (%) in 0.1 M HCl	Particle Size	Zeta Potential	PDI	% DCT Contents
DCT-1	82.1 ± 2.9	75.3 ± 2.8	226	28.1	0.34 ±0.01	96.4
DCT-2	83.2 ± 2.1	76.2 ± 4.1	262	30.2	0.41 ±0.01	97.4
DCT-3	80.2 ± 2.2	77.3 ± 2.9	313	28.6	0.39 ±0.01	94.2
DCT-4	83.3 ± 4.1	79.6 ± 5.2	342	30.2	0.35 ±0.02	96.9
DCT-5	84.2 ± 3.1	76.3 ± 3.9	368	31.2	0.24 ±0.01	95.5
DCT-6	81.2 ± 2.6	79.4 ± 4.2	426	34.2	0.39 ±0.01	97.1

Stability Testing of DCT Proliposomal formulations: Results of stability testing at both conditions are mentioned in Table 6. DCT proliposomes wrapped in soft gelatin capsules appeared as transparent and liposomal suspension was automatically revealed on contact with the water phase. No significant difference was observed in the EE % and particle size of the reconstituted liposomes after storage at the aforesaid period.

It was also revealed that soft gelatin capsules also enhanced the stability of DCT proliposomal formulations which are normally highly susceptible to various phenomenon like hydrolysis and oxidation. Similarly, the % DCT contents also remain almost same during the entire period of storage that also indicates no leakage of the drug from the proliposomes. This was also evident from the zeta potential and PDI values that no aggregation of the nanospheres occurred that maintained the size of the particles as well as the integrity of the formulations at refrigerated temperature.

CONCLUSION

Proliposomes containing DCT were successfully prepared using film deposition on carrier method by using varying ratios of phospholipid and surfactant. Particle size, PDI and zeta potential were found in the acceptable range. SEM confirmed the formation as well as entrapment of the DCT proliposomal structures with probably spherical shape. Storage stability resulted in negligible changes in the zeta potential, particle size, EE %, PDI and assay of the DCT in the formulations.

In vitro drug release showed Fickian drug release with the value of n as > 0.45 for all the formulations showing both swelling as well diffusion as the possible release mechanism and Korsmeyer-Peppas model was the best fit model for the formulations with sustained release of the drug for 12 h.

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