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Formulation and Evaluation of Phytosome© Drug Delivery System of *Camellia sinensis* (Polyphenols)

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Abstract

Phytochemicals found in medicinal plants have become a great treatment choice for a wide range of illnesses these days. Poor solubility and bioavailability, however, may restrict their clinical use. Consequently, bioavailability is regarded as a significant obstacle to enhancing bio-efficacy in the transportation of phytochemicals found in food. Various techniques have been suggested to produce efficient carrier systems that increase phytochemical bioavailability. This study aim to formulate phyto-phospholipid complex of *Camellia sinensis* using antisolvent precipitation method of five different batches varying the phospholipid and extracts of *Camellia sinensis* ratio and evaluate for solubility, drug excipient compatibility study, drug content, particle size, drug entrapment, DSC, in vitro drug release, release kinetics, stability, SEM etc. The result shows that batch F3 having molecular ratio 1:1 shows good result in all the aspect. The solubility of both the extracts formulations increases as compared to the raw extracts. In dissolution study, shows better result in F3 formulation for both the extracts as compared to other formulations .In release kinetics, the optimized formulation (F3) follow Zero-order kinetics of drug release. It has been described that the release of the active substance from the matrix is concentration independent.

Keywords: Phyto-phospholipid complex, drug entrapment, solubility, bioavailability, drug release, release kinetics.

INTRODUCTION

Natural items, especially plants, have been used by humans throughout history to promote and preserve good health and to fend off illness, discomfort, and disease. An increase in the number of doctors and public health special lists around the world who are reconsidering an alternative generally and traditionally plant-based drugs in particular has been evidenced by the fact that the allopathic medicines are being replaced by the herbal traditional drugs in many ways as a result of greater awareness to traditional knowledge of these medicinal plants (Hoareau and Dasilva1999; Cordell 2000; Kamboj 2000). Today, there is a worldwide search for environmentally acceptable, non-toxic, and Products made from plants that are commercially viable to treat various ailments. Several reasons have influenced the current, rapid rise in herbal remedy consumption that is being seen throughout the world (Arora et. al.2005; Farnsworth et. al. 1985) Medicinal plants continue to the quality of life only through rapid and essential participation in the majority of the world's population. Plant extract dosage formulation is a complex process that cannot be viewed as being solely a pharmaceutical technology issue. Numerous strategies must be used to address the issues with the existing goods, which have poor absorption, a quick metabolism, and a quick systemic disposal. Research is being done on the development of products based on plant extracts (Soejarto 1989). The majority of a plant's biologically active components are water soluble molecules. Water-soluble

phytoconstituents, however, are poorly absorbed either because of their large molecular size, which prevents passive diffusion from taking place, or because of their decreased lipid solubility. More than a typical liposome, phospholipid complex are the type of molecule that can traverse lipid-rich biomembranes (Yang , Sang et. al 2008) Camellia sinensis (polyphenols) easily penetrates the hydrophobic stratum corneum, but because the lower epidermal and dermal layers are hydrophilic, its solubility, bioavailability and ability to transfer from stratum corneum are constrained (Patel et al. 2009) The present objective of the study is to prepare and evaluate *Camellia sinensis*(polyphenols) loaded phospholipid complex.

MATERIAL AND METHODS

Materials

Camellia sinensis extracts were purchase from vital herbs Mohan garden, Uttam Nagar, Delhi- 110059 and other chemicals and reagents used were of analytical grade.

Preliminary screening and Solubility studies:

The preformulation studies were performed for organoleptic properties, solubility, melting point, moisture content, absorption maximum and pH. Solubility studies of Camellia sinensis extract were performed in Phosphate buffer pH 6.8, acetate buffer pH 4 and water.

Drug excipient compatibility study:

Drug excipient compatibility study was carried out by FT-IR spectra. FT-IR studies were performed for green tea extracts and phospholipid complex in an Alpha FT-IR spectrophotometer (Bruker). A small quantity of sample was placed just below the probe on to which the probe was tightly fixed and scanned in the wave number region 4000-500 cm^{-1}. The obtained IR spectra were interpreted for functional groups at their respective wave number.

DSC studies were performed using a Perkin Elmer DSC 6000. Green tea extract, a physical blending of soybean lecithin and green tea and Green tea extract, a physical blending of soybean lecithin complexes and green tea and phytosomal complexes, is heated and placed in an aluminium crimp cell from 0°C to 400°C at a rate of 10°C/min in a dry nitrogen atmosphere. Melting points of samples were determined from thermograms.

Preparation of phospholipid complex:

By antisolvent precipitation technique:

The specific amount of Green tea extract and soya lecithin a source of phosphatidylcholine (PC) were taken into a 100 ml round bottom flask and refluxed with 20 ml of dichloromethane at a temperature not exceeding 60ºC for 2 hrs (Table-1 & Table-2). The mixture is concentrated to 5-10ml. Hexane (20ml) was added carefully with continuous stirring to get the precipitate which was filtered and collected and stored in vacuum desiccators overnight. The dried precipitate is crushed in mortar and sieved through #100 meshes. Powdered complex was placed in amber coloured glass bottle and stored at room temperature.

Evaluation of phospholipid complex:

***Solubility Study*:**

Excess amount of prepared formulations was taken and added to 5ml solvents water, phosphate buffer 6.8 and acetate buffer 4 in tightly capped glass vial. To mix properly sample were constantly agitated at 100 rpm at room temperature for 24 hrs in REMI rotary shaker. At the end of 24hrs, the samples were centrifuged in REMI centrifuge at 1000 rpm. The supernatant liquid was separated and from that 0.5ml transfer to 100ml volumetric flask. The volume was made up to the mark with distilled water. The absorbance was determined using water as a blank at the λmax 279 nm.

***Determination of particle size*:**

The prepared EPC were dispersed in phosphate buffer pH 6.8 by stirring on a magnetic stirrer for 10 minutes. The dispersion was analysed in size analyzer (Zetasizer)

***Determination of drug content*:**

Drug content of phospholipid complex was determined by dissolving accurately weighed 10 mg of complex in 10 ml of phosphate buffer pH 6.8solutions. After suitable dilution absorbance was determined by UV Spectrophotometer (UV1900, Shimadzu) at 279 nm and drug content was determine.

% Drug content = (Amount of drug loaded/ amount claimed) ×100

***Drug entrapment*:**

A weighed quantity of phospholipid complex equivalent to 10 mg Green tea was added to 50 ml phosphate buffer pH 6.8 in a 100 ml beaker. The contents were stirred on a magnetic stirrer for 4 hours and then allowed to stand for one hour. Clear liquid was decanted and centrifuged at 5000 rpm for 15 minutes. After centrifugation the supernatant was filtered through 0.45µ whatman filter paper and after suitable dilution absorbance was measured in UV-Visible spectrophotometer at 279 nm. The drug entrapment (%) was calculated using the following formula: [23, 24]

Drug entrapment (%) = (Total amount of drug) – (amount of free drug) × 100 (Total amount of drug)

% drug release:

The prepared EPC was loaded (a quantity equivalent to 100 mg of green tea extract) in zero size capsules. *In vitro* dissolution studies for all the prepared formulations were carried out using USP Type-II dissolution apparatus at 50rpm in 900 ml of phosphate buffer 6.8 pH as dissolution media, maintained at 37±50C. 5ml samples were withdrawn at the specified time intervals & assayed spectrophotometrically. An equal volume of fresh media was replaced after each sampling to maintain the constant volume. The samples after appropriate dilution with distilled water were analyzed at 279nm using UV-visible double beam spectrophotometer.

Drug Release Kinetics:

In order to assess the drug release pattern of phospholipid complex, the release data were further analyzed for their kinetic profiles and replaced with Zero, First, Higuchi and Korsemeyer-Peppas models. By plotting the cumulative percent release versus time, the release constant and regression coefficient (R2) were calculated from the slope of the corresponding plot.

Stability testing:

One of the most important elements in determining the suitability and safety of formulations and products is *in vitro* stability. Individually packed the optimised phospholipid complexes in nitrogen-purged glass vials with rubber stoppers and aluminium seals. In order to conduct stability tests, the samples were divided into 3 batches and kept at:

- 25˚ C ± 2˚ C/ 60% RH ± 5% RH for 6 month
- 30˚ C ± 2˚ C/ 65% RH ± 5% RH for 6 month
- 40˚ C ± 2˚ C/ 75% RH ± 5% RH for 6 month

RESULT AND DISCUSSION

Preliminary screening and Solubility studies:

In preliminary screening the organoleptic properties of extracts were observed and found to be dark brown in colour, odour & taste is Characteristic and appears as fine powder.

In solubility, it was found that extracts were more soluble in phosphate buffer pH 6.8 (41.28 mg/ml) than Acetate buffer pH 4 (33.64 mg/ml) than water (27.48 mg/ml).

The melting point were found in the range of 142º to 145º c

The pH of sample is 6.2

The analytical wavelength of Green tea extract was found to be 277 nm.

The moisture content of Green tea extract was found to be 2%.

Drug excipient compatibility:

The compatibility study of green tea extract and excipients was performed by FTIR study. The FTIR study of green tea extract showed peaks at 3216.99 cm-1 due to O-H stretching vibration of aromatic ring. The peaks appeared at 1603.77 cm-1 and 1516.87 cm-1 is attributed to C=C stretching of alkene. The band appearing at 1448.97 cm^{-1} due to C-H bending of alkane and a peak was observed at 1229.11 cm-1 due to C-O stretch of alkyl aryl ether.

The C-O stretching of aliphatic ether was observed at 1137.91cm-1 and 1089.94 cm-1. Another C-O stretch due to presence of alkyl aryl ether was observed with characteristic broad peak at 1013.11 cm-1. The IR study of formulation showed no appearance of bands appeared previously in pure drug spectra at 1229.11 $cm^{-1}$and 1089.94 cm^{-1}. But the appearance of new peaks at 292.98 cm^{-1}, 2852.33 $cm^{-1}$and 1713.91 cm^{-1}due to N-H stretching of amine group and O-H stretching respectively confirmed the modification in drug molecular structure, indicating the formation of phospholipid complex.

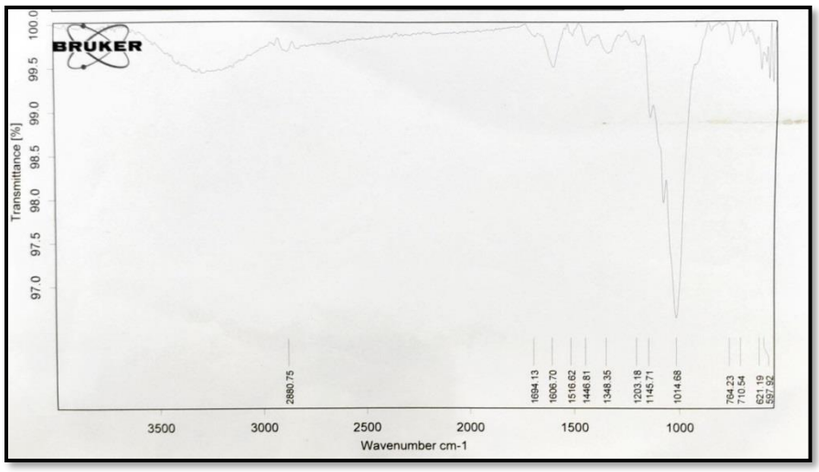

Figure 4: FTIR graph of Green tea extract

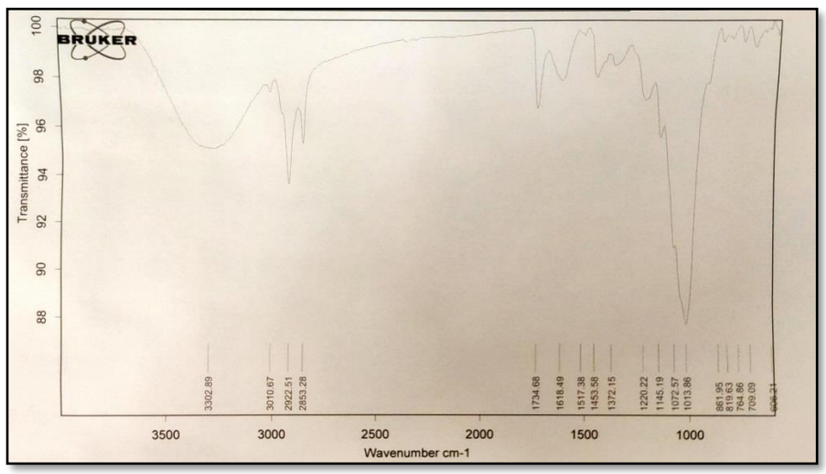

Figure 5: FTIR graph of Physical Mixture

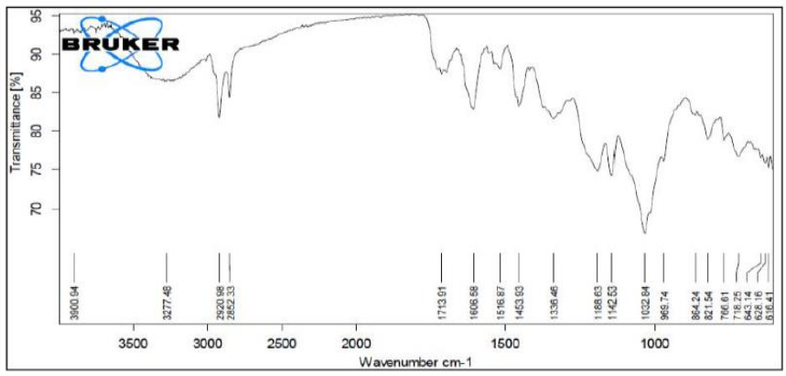

Fig6: FT-IR spectra of formulation

DSC analysis

Figures 7, 8, and 9 show the DSC thermograms of free green tea extract, physical blend, and optimized phospholipid complex preparation, respectively. The pure drug's DSC thermogram displayed a clear endothermic peak at about 97.83°C, which corresponds to its melting point. Physical mixtures of drugs and polymers exhibit endothermic peaks with sharp or broad peaks at various temperature ranges. On the other hand, the formulation thermograms show several sharp peaks with similar temperature ranges for EGC and phosphatidylcholine (physical mixture). These results indicated that there may be interactions between the phospholipids in the complex and the drug, possibly due to their hydrophobic character. Hydrogen bonding and aromatic ring interactions may also play a role. Such interactions indicate the formation of phospholipid complexes (EPCs).

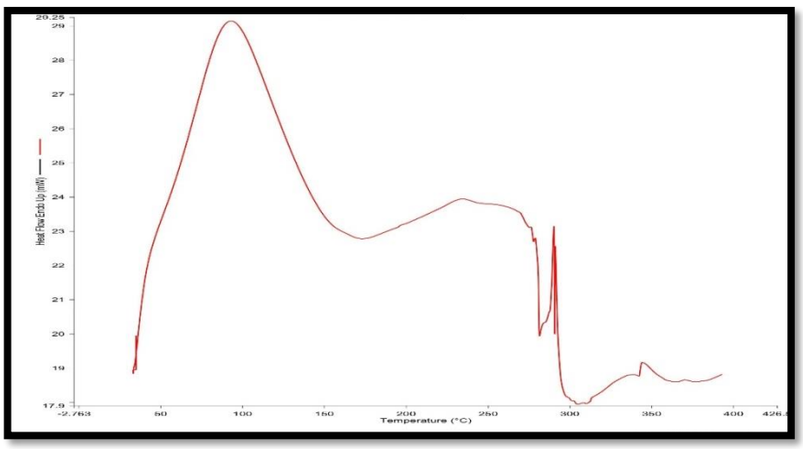

Figure 7: DSC thermograms of pure Green tea

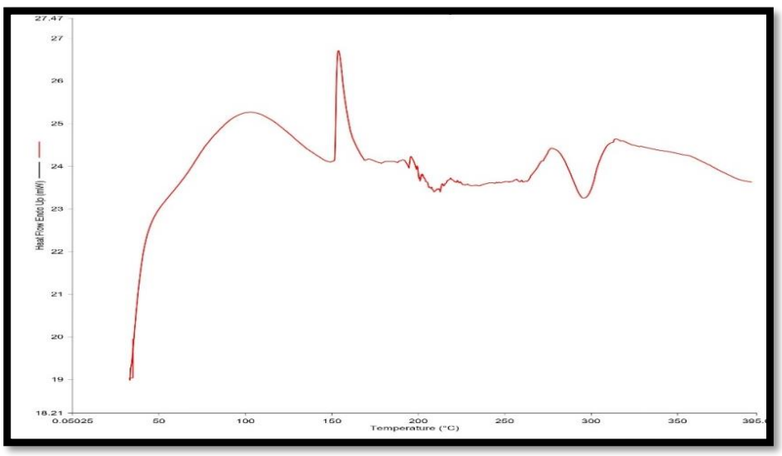

Figure 8: DSC thermograms of Physical mixture of Green tea and Soya lecithin

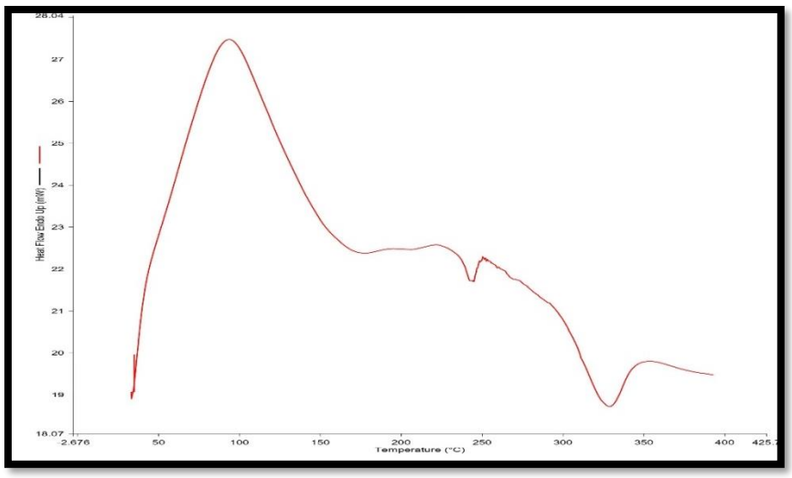

Figure 9: DSC thermograms of Formulation

Preparation phospholipid complexes:

By antisolvent precipitation technique:

Different batches of phospholipid complexes were prepared by using the following formula.

Table1 - For Sample 1: Green tea Polyphenols (80% EGCG)

Sl no	Molar ratio of EGCG : PC	Drug (Green tea extract) gm	Phospholipid (Soya lecithin) gm	Solvents
F1	0.5:1	1.25	2	Dichloromethane + n-hexane
F2	0.75: 1	1.86	2	Dichloromethane + n-hexane
F3	1:1	2.5	2	Dichloromethane + n-hexane
F4	1:0.75	2.5	1.5	Dichloromethane + n-hexane
F5	1: 0.5	2.5	1	Dichloromethane + n-hexane

Table2 - For Sample 2: Green tea Polyphenols (50% EGC)

Sl no	Molar ratio of EGC : PC	Drug (Green tea extract) gm	Phospholipid (Soya lecithin) gm	Solvents
F1	0.5:1	2	2	Dichloromethane + n-hexane
F2	0.75: 1	3	2	Dichloromethane + n-hexane
F3	1:1	4	2	Dichloromethane + n-hexane
F4	1:0.75	4	1.5	Dichloromethane + n-hexane
F5	1: 0.5	4	1	Dichloromethane + n-hexane

***Solubility Study*:**

The prepared formulations were found to be better soluble than pure green tea (Table3). The amount soluble in the solvent significantly varied with the ratio of Green tea to phosphatidylcholine (PC). Highest solubility was observed for F3 formulation in all the three solvents, where the molar ratio of Green tea to Phosphatidylcholine is 1:1. Results show that F3 formulation solubility in phosphate buffer pH 6.8 is much higher than the other in water and acetate buffer pH 4.0. Compared to F3, in case of F1 and F2 formulations' solubilitywere found less. The reason may be the higher quantity of PC that remained unbound where EGCG: PC ratios were 0.5:1(F1) and 0.75:1(F2). The unbound PC might form extra layers surrounding the vesicles. On the other hand, less solubility also observed in case of F4 and F5 in comparison to F3.

Table3 - For Sample 1 (Green tea Polyphenols containing 80% EGCG)

Formulations	Solubility (mg/ml)		
	Water	Phosphate Buffer pH 6.8	Acetate buffer pH 4
F1	8.76	11	10.23
F2	7.54	10	8.98
F3	9.14	14	10.31
F4	5.23	5.76	7.23
F5	6.76	8.96	8.43

Table4 - For Sample 2 (Green tea Polyphenols containing 50% EGC)

Formulations	Solubility		
	Water	Phosphate Buffer pH 6.8	Acetate buffer pH 4
F1	7.84	10.45	9.34
F2	6.72	10.21	8.82
F3	8.83	13.45	10.13
F4	7.65	7.66	8.03
F5	6.54	8.16	8.64

***Determination of particle size*:**

Average particle size varied between 185.7 nm to 216.7 nm for sample 1 and 182.4 nm to 211.3 nm for sample 2. The results indicate that as the molar ratio of Green tea increased and polymer amount decreased in phospholipid complexes from F1 to F5 formulations, the particle size gradually increased. The reason may be attributed to the availability of number of green tea molecule as compared to phospholipid molecule in contact during complex formation.

Table5 – Results of particle size

Sl No.	Formulation	Average particle size (nm)	
		For sample1	For sample 2
1	F1	185.7± 0.23	182.4± 0.35
2	F2	189.9± 0.31	186.7± 0.61
3	F3	209.8±0.97	203.8±0.69
4	F4	212.8± 1.12	207.8± 1.02
5	F5	216.7± 1.87	211.3± 1.11

***Determination of drug content*:**

The drug content of the phospholipid complexes were studied and presented in Table 6. The percentage of drug content was found in a range of 82-90% (for sample 1) and 81 – 89% (for sample 2). The results showed less percentage of drug content which may be attributed with the unbound amount of the GT with PC. But F3 formulation showed highest percentage of drug presence where the GT and PC were in same molecular ratio for the sample.

Table6 – Drug content of phospholipid complexes

Sl no.	Formulation	% Drug content	
		For Sample 1	For sample 2
1	F1	82.75± 0.56	81.23± 0.45

2	F2	84.34± 0.97	83.78± 0.56
3	F3	90.76± 1.23	89.99± 1.84
4	F4	85.94± 0.99	84.97± 0.95
5	F5	87.65± 1.45	85.94± 1.13

***Drug entrapment*:**

The entrapment efficiency of formulations from F1 to F5 were analyzed and represented –

Table7 – Entrapment efficiency of phospholipid complexes

Sl no.	**Formulation**	**Entrapment efficiency**	
		For Sample 1	**For sample 2**
1	F1	82.72 ± 1.152	80.67 ± 1.352
2	F2	84.44 ± 0.251	81.87 ± 0.465
3	F3	92.47 ± 0.798	89.64 ± 0.596
4	F4	85.53 ± 0.806	83.45 ± 0.806
5	F5	88.75 ± 1.868	84.25 ± 0.9684

Phytosome© morphology:

Scanning Electron Microscopy (SEM) Scanning electron microscopy of the optimized Phytosomal suspension was performed by Scanning Electron Microscope for determining the surface morphology, size and shape of formulation. The SEM of optimized Phytosomal suspension is shown in Figure indicates that particles are spherical in shape with 10 µm in size.

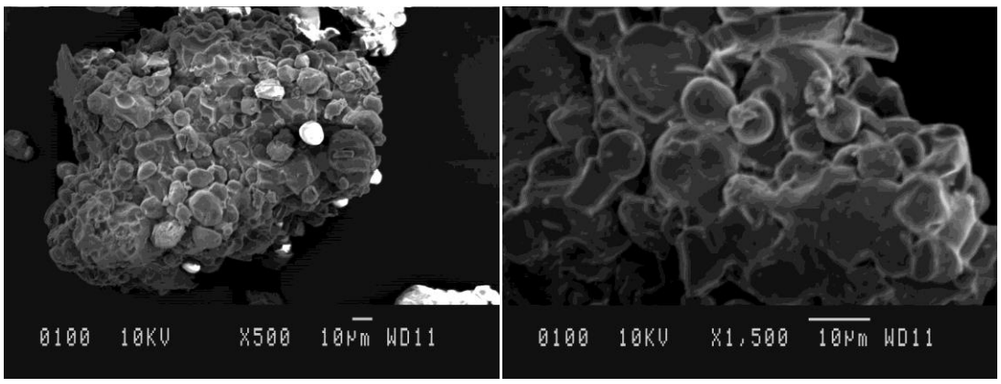

Figure1: SEM view of phytosomes

% drug release:

The in-vitro release study of EPC formulations was carried out for 3 h in phosphate buffer pH 6.8. Based on the results of solubility study, phosphate buffer pH 6.8 was chosen as dissolution medium. After 3 h of release, the F3 formulation showed a better release of 98.41% and 94.67% for sample1 and sample 2 respectively, which contains drug and phospholipid in 1:1 ratio. The release of drug increased with the increase of drug concentration in the complex but again decreased in F4 and F5 formulations with decrease of phospholipid amount.

Table 8- % drug release of prepared phospholipid complexes

Formulation	**% Drug release**											
	For sample 1						For sample 2					
	30 min	60 min	90 min	120 min	150 min	180 min	30 min	60 min	90 min	120 min	150 min	180 min
F1	27.43	56.35	64.59	72.56	78.96	84.35	25.76	49.45	62.34	70.56	74.56	82.98
F2	28.21	57.34	65.47	71.36	80.46	88.45	27.45	53.34	62.98	69.90	78.87	85.43

F3	19.34	38.97	64.37	81.34	91.34	98.41	23.45	36.89	62.87	78.98	86.78	94.67
F4	13.76	35.46	67.87	79.43	86.89	91.34	10.76	33.23	64.67	74.98	85.76	89.97
F5	15.78	23.78	42.66	62.87	79.53	86.78	13.96	20.89	39.67	60.34	76.85	83.56

Drug Release Kinetics:

The drug release pattern of phospholipid complex, the release data were further analyzed for their kinetic profiles and replaced with Zero, First, Higuchi and Korsemeyer-Peppas models.

Table 09: Kinetic Model Parameters of optimized formulation

Kinetic Model	**F1**
Zero order	**y= 0.4027x – 0.9036** **R^2=0.9911** **K=0.4027**
First order	**y= -0.01x + 4.9036** **R^2= 0.9174** **K= -0.01**
Higuchi	**y= 6.4139x-16.267** **R^2=0.9038** **K=6.4139**
Korsemeyer-Peppas	**y= 0.8376x – 0.1605** **R^2=0.9875** **n=0.8379** **K=0.1605**

From the R^2 values, it can conclude that the phospholipid complexes follow Zero-order kinetics of drug release. It has been described that the release of the active substance from the matrix is concentration independent. To explain the mechanism of drug release from phospholipid complexes, the Korsemeyer-Peppas equation showed good linearity with a release exponent 'n' of 0.828 (range 0.5 to 1), suggesting non-fickian transport-type release is showing. Considering this, it can be understood that drug release from phospholipid complexes follows diffusion and erosion mechanisms.

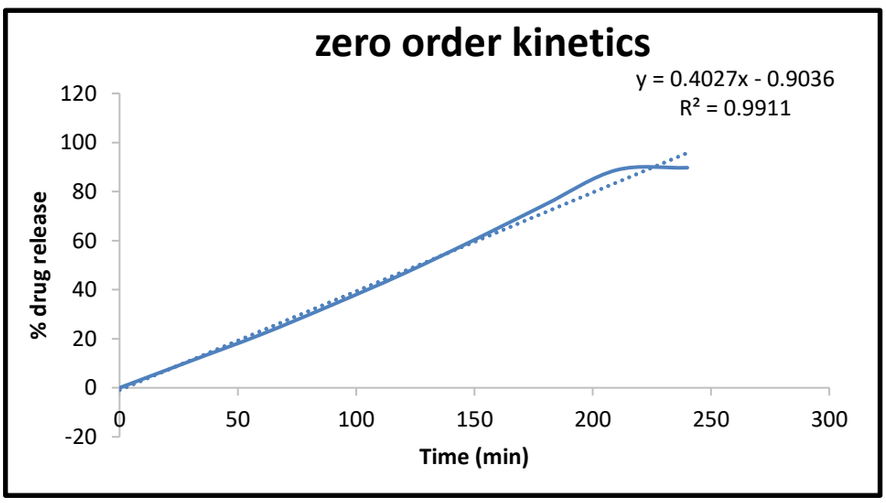

Figure 2: Release plot in Zero order

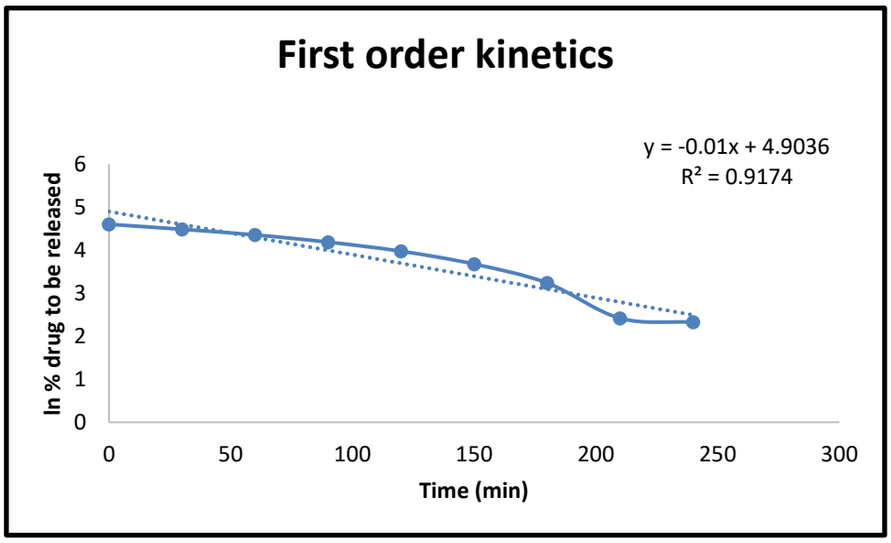

Figure 3: Release plot in First order

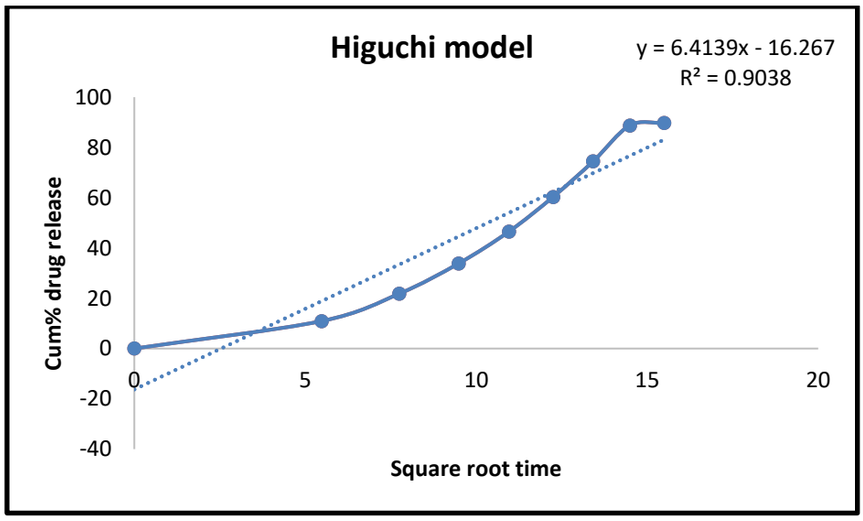

Figure 4: Release plot in Higuchi model

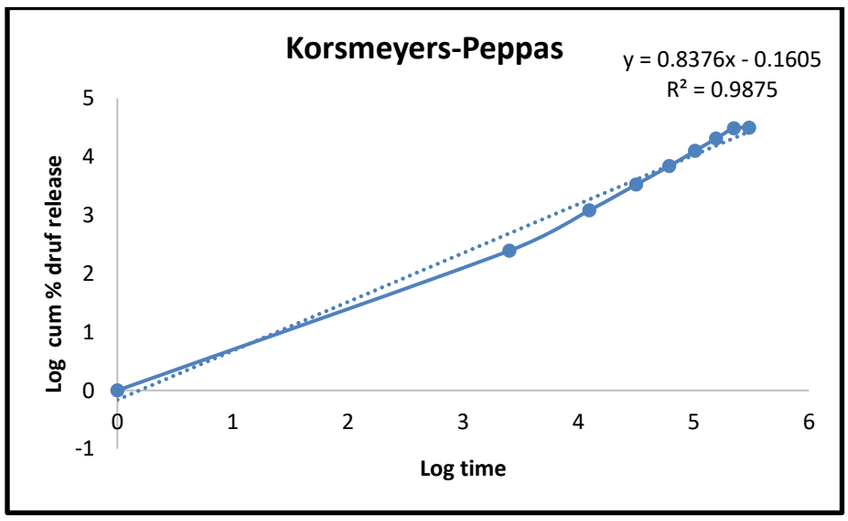

Figure 5: Release plot in Korsemeyer-Peppas model

Stability study:

The optimized phospholipid complex were stored at different conditions (shown in Table 10). During predetermined intervals (0, 15, 30,60, 90,120,150 and 180 days), the optimized formulation had no significant effect on the solubility, drug content, or rate of drug release, showing that the optimised phospholipid

complexes were stable. These outcomes demonstrated that green tea extract and the polar groups of soybean lecithin are compatible.

Table 10: Stability study data of Phospholipid complexes

Time (days)	At 25±2ºC			At 30±2ºC			At 40±2ºC		
	Solubility (mg/ml)	% drug content	% drug release	Solubility (mg/ml)	% Drug content (mg/ml)	% drug release	Solubility (mg/ml)	% drug content	% drug release
0	14	90.76	98.41	14	90.76	98.41	14	90.76	98.41
15	14	90.76	98.41	14	90.76	98.41	14	90.76	98.41
30	14	90.76	98.41	14	90.76	98.41	14	90.76	98.41
60	14	90.76	98.41	14	90.76	98.41	14	90.76	98.40
90	14	90.76	98.41	14	90.76	98.37	13	90.73	97.06
120	14	90.56	98.23	13	90.03	98.34	13	88.06	97.89
150	14	90.64	98.31	12	89.48	97.98	12	86.96	97.98
180	14	90.76	98.41	10	89.21	97.41	11	85.57	96.42

CONCLUSION:

Prior to the development of phospholipid complex, pre-formulation studies were performed to characterize the chemical and physical properties of the drug substance. The results were satisfactory, and it can conclude that there was no interaction between the active pharmaceutical ingredient and the excipients and that it could be used for formulation development.

Green tea phospholipid complexes were prepared using soybean lecithin as the phospholipid at five different ratios such as 0.5:1, 0.75:1, 1:1, 1:0.75 and 1:0.5 by using the anti-solvent evaporation method. Results of studies on the drug content and entrapment efficiency of the complex at 1:1 ratio showed superior potency and entrapment efficiency compared to the other four ratios. The average particle size of the optimized EPC formulation was 209.8±0.97 nm. The results of solubility studies showed that the phospholipid complex is more soluble than pure green tea and the highest solubility was observed for the F3 formulation with the molar ratio of 1:1. *In vitro* drug release was studied in phosphate buffer pH 6.8 and this study confirmed that the formulation indeed followed a sustained release pattern. The results showed that the F3 formulation had better release compared to the other formulations. Fitting the in vitro data to Zero order, First order, Higuchi and Korsemeyer-Peppas models, the results showed that optimized formulation followed Zero order kinetics. phospholipid complexes were created with the goal of overcoming the problem of poor bioavailability associated with drugs. Further research is required on this new formulation to explore more.

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