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Original Article

Formulation and evaluation of Phospholipid complex of Green Tea Polyphenol

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ABSTRACT:

OBJECTIVE: Phospholipid complexes are formulated to improve absorption, bioavailability and stability of herbal product. There are many herbal extracts having excellent *in-vitro* activity but less *in-vivo* activity because of their macromolecular size and poor aqueous/lipid solubility, which result in poor absorption and bioavailability. Green tea polyphenol has poor oral bioavailability due to many known and unknown reasons and is unstable in the gastrointestinal tract. There have been published reports that encapsulating green tea polyphenols in drug delivery carrier significantly delayed its degradation in simulated digestive fluids and leads to improved oral bioavailability.

METHODS: The present work aims in improving the GI dissolution and oral bioavailability of green tea extract rich in Epigallocatechin-3-gallate (EGCG) by formulating into phospholipid complex. EGCG-Phospholipid complex (EPC) was formulated by anti-solvent evaporation method using green tea extract and phosphatidylcholine (PC) in the ratio of 0.5:1, 0.75:1, 1:1, 1:0.75 and 1:0.5.

RESULT: EPC were characterized by solubility, particle size, drug content, % entrapment efficacy, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and *in vitro* dissolution study. The results showed that the average particle size of optimized EPC formulation was 201.96 nm. The Drug content and Entrapment efficiency was found to be 90.57 ± 1.187 and 95.41 ± 0.898 , respectively. *In vitro* drug release studies revealed that the cumulative % drug release of the optimized EPC was 98.41% at 3 hours.

CONCLUSION: Results of the physicochemical and drug release studies suggested that EPC would serve as useful novel drug delivery system and provide improved oral bioavailability.

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INTRODUCTION

Plant preparations or their parts have been widely used in medicine since ancient times and till today the use of phytomedicines is widespread. Most of the biologically active constituents of plants are polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, tannins, glycosidal aglycones, etc. are poorly absorbed due to macromolecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability [1].

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or oolong tea. Among these, however, the most significant effects on human health have been observed with the consumption of green tea [2].

The major catechins in green tea are (-)-epicatechin (EC), its hydroxyl derivative (-) epigallocatechin (EGC), and their respective gallic acid esters, (-)-epicatechin-3-gallate (ECg) and (-)-epigallocatechin-3-gallate (EGCG). Among green tea catechins, EGCG is abundant in green tea leaves, and has been shown to exhibit strong health-promoting activity [3].

The effectiveness of any herbal medication is dependent on the delivery of effective level of the therapeutically active compound. Severe limitation exists in their bioavailability when administered orally [4].

Epigallocatechin gallate is a type of catechin that has many therapeutic applications, however, its scope is limited due to its poor bioavailability. Epigallocatechin gallate is unstable in the gastrointestinal tract.⁷ It rapidly degrades in both acidic and neutral conditions. There are known and still unknown reasons for erratic bioavailability of EGCG. Most of the ingested EGCG apparently does not get into the blood, since absorption takes place in the small gut and substantial quantities pass from the small to the large intestine where it undergoes further degradation by the action of local microbiota [5].

The limited bioavailability of tea flavanols is dependent on their high molecular weight and effective molecular size due to the large number of hydrogen bond-donating hydroxyl groups, which form a large hydration shell [12]. The limited bioavailability of tea flavanols is dependent on their high molecular weight and effective molecular size due to the large number of hydrogen bond-donating hydroxyl groups, which form a large hydration shell [12].

It was reported that the limited oral bioavailability of green tea polyphenols is dependent on high molecular weight and effective molecular size due to the large number of hydrogen bond donating hydroxyl groups, which forms a large hydration shell [6]. The oral administration of pure EGCG in healthy human volunteers at a dose of 1.6 g produced physiologically-relevant peak EGCG concentrations (greater than 1 $\mu\text{mol/L}$) were reached between 1.3 to 2.2 h after the administration and the mean elimination half-life ranged from 1.9 to 4.6 hr [7]. Encapsulating epigallocatechin gallate into nanoparticles, significantly delayed its degradation in simulated digestive fluids and able to enhance the absorption leading to enhanced bioavailability [8-10]. Caibiao *et al.*, (2015) reported that uptake of EGCG incorporated in solid self-double emulsifying drug delivery system (SDEDDS) was much higher compared to pure drug. Solid SDEDDS also improved the storage stability of EGCG [11].

Table 1: Formulation of different phytosome complex

Formulation	Molar ratio of EGCG: PC	Drug (Green tea extract) gm	Phospholipid (Soya lecithin) in gm	Solvents
F1	0.5:1	0.67	2	Dichloromethane + n-hexane
F2	0.75:1	1.01	2	Dichloromethane + n-hexane
F3	1:1	1.35	2	Dichloromethane + n-hexane
F4	1:0.75	1.80	2	Dichloromethane + n-hexane
F5	1:0.5	2.71	2	Dichloromethane + n-hexane

Physicochemical evaluation of phytosomes:

UV-Spectroscopy:

A stock solution of Green tea (100 $\mu\text{g/ml}$) was prepared by dissolving 5 mg of drug in 50 ml of phosphate buffer pH 6.8 in a 50 ml volumetric flask. From the stock solution 0.5 ml was

As the most abundant green tea (GT) constituent, EGCG has been the focus of research in relation to the reduction of morbidity and mortality because of cardiovascular disease. Several mechanisms of action have been proposed as to why EGCG may benefit cardiovascular health in humans, such as the lowering of plasma cholesterol through a reduction of cholesterol absorption [12], a decrease in cholesterol synthesis [13] and/or an increase in the cholesterol clearance rate through an up regulation of the LDL receptor [14-16]. Phospholipid complexes exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts [17, 18]. The present work envisaged to develop phospholipid complex of green tea extract rich in Epigallocatechin 3-gallate (EGCG) to improve the *in vitro* dissolution and in turn the oral bioavailability.

MATERIALS AND METHODS:

Materials:

Fine powder of Green Tea extract containing 50.8% of EGCG was purchased from Green Heaven India (A Herbal Manufacturing Unit), Nagpur, Maharashtra; Soy lecithin (Phosphatidylcholine) from Otto Chemie Pvt Ltd, Chemika Biochemika Reagents. Potassium Dihydrogen phosphate, Sodium Hydroxide and solvents (Dichloromethane, Ethanol, Phosphate Buffer) used were of analytical grade.

Preparation of EGCG-Phospholipid complex (EPC) by anti-solvent precipitation technique:

The specific amount of Green tea extract and soya lecithin as 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 60°C for 2 hrs (Table-1). 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 colored glass bottle and stored at room temperature. The EPC was prepared by taking molar ratios of EGCG to PC of 0.5:1, 0.75:1, 1:1, 1:0.75 & 1:0.5 as mentioned in [19, 20].

taken and made up the volume with phosphate buffer pH 6.8 up to 10 ml. The λ_{max} for quantitative analysis of Green tea was determined after scanning the appropriate dilute solution between 200-400 nm using UV spectrophotometer (Shimadzu, UV 1800) and it was found to be at 275 nm. The absorbance of the dilute solutions of known concentrations was measured at 275 nm against the blank. The absorbance was plotted against the concentration and linear relationship was obtained.

The concentration of EGCG was calculated from the regression equations [21].

Differential scanning calorimetry (DSC):

Green tea extract, soya lecithin, physical mixture of Green tea and soya lecithin and phospholipid complex were placed in the aluminum crimp cell and heated at 10°C/min from 0 to 400°C in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer [22-24].

FTIR Spectroscopy:

FT-IR studies were performed for green tea extract, PC, and phospholipid complex in an Alpha FT-IR spectrophotometer (Bruker, Germany). 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 (cm⁻¹) [21].

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 80 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 upto the mark with distilled water. The absorbance was determined using water as a blank at the λ_{max} 275 nm.

Partition coefficient study:

Five numbers of separating funnels were thoroughly cleaned, dried and labelled as S₁, S₂, S₃, S₄ and S₅. To all separating funnel 10 ml n-octanol was added. Then, 10 ml aqueous solvent was added- in S₁ 10 ml of phosphate buffer p^H 6.8, in S₂ 10 ml of phosphate buffer p^H 6.8 in S₃, 10 ml of phosphate buffer p^H 7.4, in S₄ 10 ml of acetic buffer p^H 2, in S₅ 10 ml of acetic buffer p^H 4. Then 10 mg of prepared phospholipid complex was added to all the separating funnels. Then the funnels were shaken in mechanical shaker up to 24 hrs. After 24 hrs 1 ml aqueous sample was collected and added into a 10ml volumetric flask and then made up the volume with distilled water. Then the absorbance was measured at 275 nm [22].

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 analyzed in size analyzer (Malvern, Nano series, S90 Zetasizer) [21]

Determination of drug content:

Drug content of EPC was determined by dissolving accurately weighed 10 mg of complex in 10 ml of phosphate buffer pH 6.8

solutions. After suitable dilution absorbance was determined by UV Spectrophotometer at 275 nm and drug content was determine [23].

$$\% \text{ Drug content} = (\text{Amount of drug loaded/ amount claimed}) \times 100$$

Drug entrapment:

A weighed quantity of phytosomes 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 (CF10 Centrifuge, Daihan Scientific Co. Ltd, Korea) 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 257 nm (UV1800, Shimadzu, Japan). The drug entrapment (%) was calculated using the following formula: [23,24]

$$\text{Drug entrapment (\%)} = (\text{Total amount of drug}) - (\text{amount of free drug}) \times 100 / (\text{Total amount of drug})$$

In vitro release study:

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 and 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 275nm using UV-visible double beam spectrophotometer.

RESULTS AND DISCUSSION

Standard curve of EGCG:

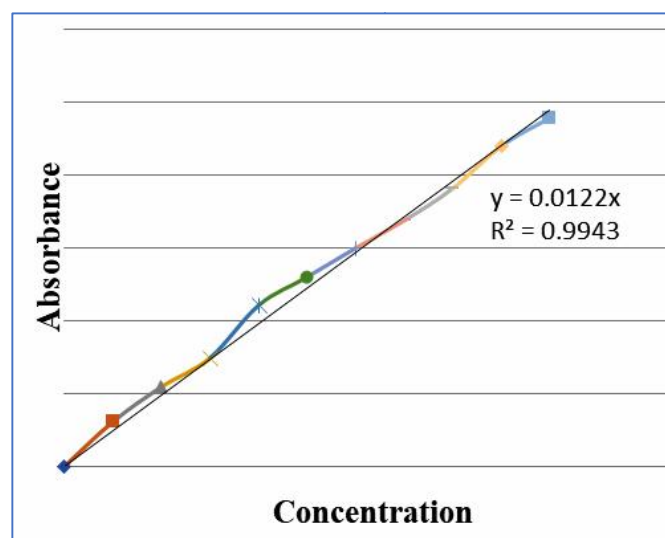


Figure 1: Standard curve of EGCG in phosphate buffer 6.8

Table 2: Data for Standard curve in phosphate buffer pH 6.8

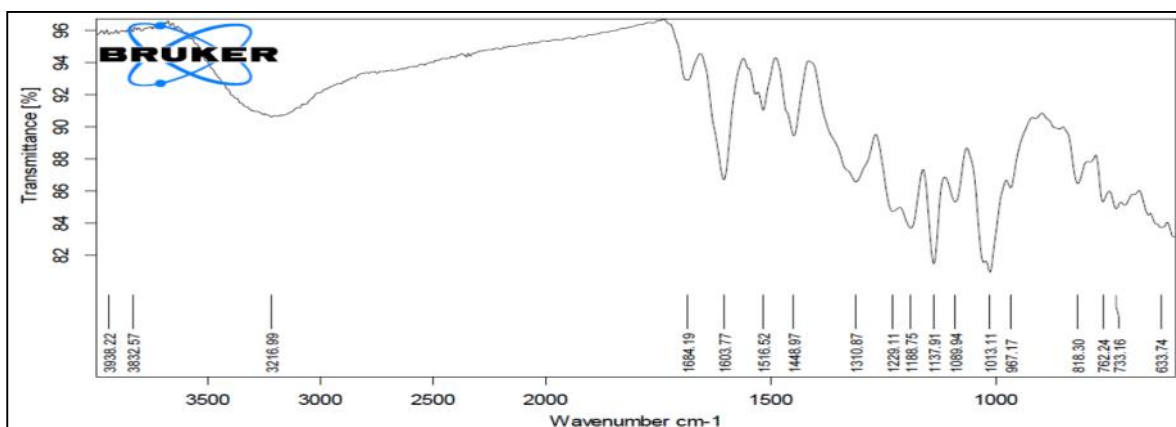
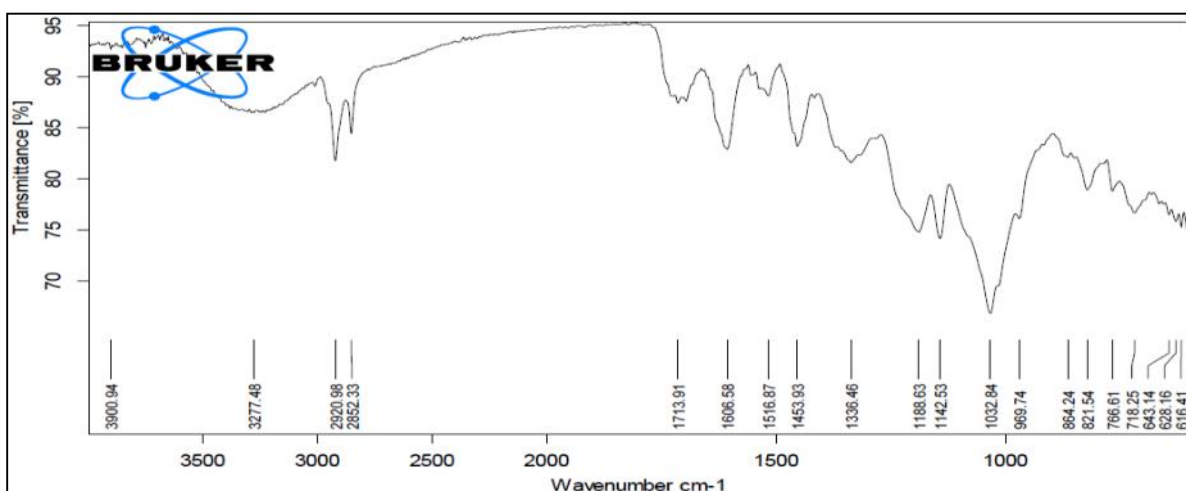
Concentration	Absorbance
0	0
2	0.0308 ± 0.69
4	0.0543 ± 0.76
6	0.0741 ± 1.03
8	0.1102 ± 0.96
10	0.1294 ± 0.85
12	0.1497 ± 1.27
14	0.1687 ± 0.98
16	0.1907 ± 0.71
18	0.2201 ± 0.63
20	0.2385 ± 0.82

FT-IR and DSC study:

The compatibility study of green tea extract and excipients was performed by DSC and FTIR study. The FTIR study of green tea extract rich in EGCG (**Figure 2**) 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 1137.91 cm^{-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} . In the IR study of physical mixture showed the peaks of pure drug with some variation in the same range indicating no chemical interaction. Whereas the IR study of formulation (**Figure 3**) 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.

The DSC thermogram of the pure drug (**Figure 4A**) showed an endothermic peak at 117.10°C with an onset of 101°C which is attributed to its melting point. While the thermogram of formulation (**Figure 4B**) exhibited with a sharp endothermic peak at 137.4°C with an onset of 129.03 °C. The thermogram of physical mixture showed two distinguished peaks similar to that of the ECGC and phosphatidylcholine. This result revealed that there may be interaction between phospholipid and drug in complex which is hydrophobic in nature. Also, contribution of hydrogen bond and aromatic ring in interaction could be involved. Such interactions are indicative of phospholipid complex formation.

**Figure 2: FTIR spectra of EGCG****Figure 3: FTIR spectra of Formulation**

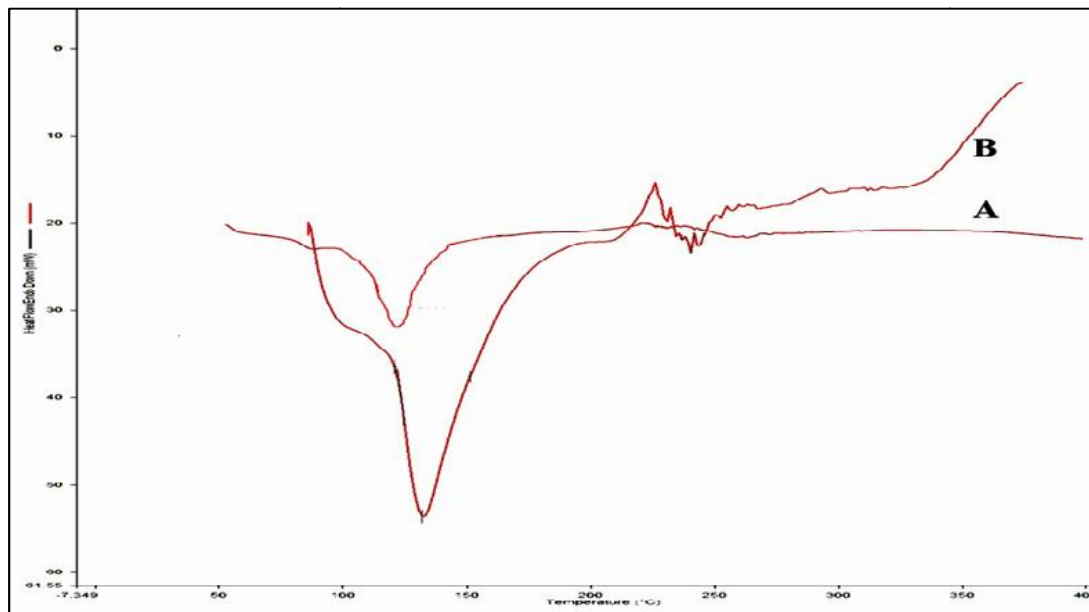


Figure 4: Comparative DSC study of (A) green tea extract and (B) EPC

Solubility Study:

EPC were found to be better soluble than pure green tea (Table 3). The amount soluble in the solvent significantly varied as the ratio of Green tea to phosphatidylcholine (PC) varied. Highest solubility was observed for F3 formulation 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 (13.28 ± 0.7067 mg/ml) than the other in water (10.13 ± 0.53 mg/ml) and acetate buffer pH 4.0 (9.89 ± 0.36 mg/ml). Compared to F3, in case of F1 and F2 formulations' solubility in basic medium were 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.

Table 3: Results of solubility study and particle size analysis

Formulation	Solubility			Average particle size (nm) \pm SD
	Water	Phosphate buffer pH 6.8	Acetate buffer pH 4	
F1	9.35 ± 1.06	12.14 ± 0.96	8.37 ± 0.86	182.7 ± 0.35
F2	7.98 ± 0.97	10.01 ± 0.83	9.12 ± 0.69	185.6 ± 0.92
F3	10.13 ± 0.53	13.28 ± 0.71	9.89 ± 0.36	211.9 ± 1.34
F4	5.34 ± 0.87	4.95 ± 0.99	6.71 ± 1.13	213.7 ± 1.69
F5	6.49 ± 0.65	7.33 ± 1.06	7.06 ± 0.95	215.9 ± 1.91

Particle size determination:

The particle size of all the formulations was analyzed and average particle size varied between 182.7 nm to 215.9 nm (Table 3). The results indicate that as the molar fraction of Green tea increased and polymer amount decreased in phytosomes from F1 to F5 formulations, the particle size gradually increased. The reason may be attributed to the availability of number of GT molecule as compared to phospholipid molecule in contact during complex formation. [25].

Partition coefficient study:

Aqueous solubility of drug as well as partition coefficient is an important factor in designing formulations in purpose of absorption of drug from an aqueous environment.

For oral absorption of the permeant (octanol/phosphate buffer) the partition coefficient must be in the range of -0.4 to $+5.6$ range. The results of the partition coefficient study are presented in Table-4 where the values of partition coefficient in all the solvent system were ranged between 0.0261 ± 0.0088 (octanol/Phosphate buffer or acetate buffer) to 0.0954 ± 0.0111 . The results revealed the lipophilic nature of formulation. Partition coefficient value when exceed 3 may retard drug absorption from intestinal surface due to difficulty in permeating.

Considering this fact, the most satisfactory partition coefficient value for EPC was exhibited by the solvent system containing phosphate buffer pH 6.8 as the aqueous medium (0.0954 ± 0.0111). Whereas, aqueous solubility of drug as well as n-octanol/phosphate buffer pH 6.8 partition coefficient are important factors in designing formulations for oral application and deciding the fate of permeant for oral absorption.

Table 4: Results of Partition coefficient study at different pH

Separating funnel	Solvent system	Partition coefficient
S1	n-octanol/phosphate buffer pH 5.8	0.0798 ± 0.0149
S2	n-octanol/phosphate buffer pH 6.8	0.0954 ± 0.0111
S3	n-octanol/phosphate buffer pH 7.4	0.0558 ± 0.0056
S4	n-octanol/acetate buffer pH 2	0.0261 ± 0.0088
S5	n-octanol/acetate buffer pH 4	0.0476 ± 0.0091

Table 5: Drug content and entrapment of drug particle

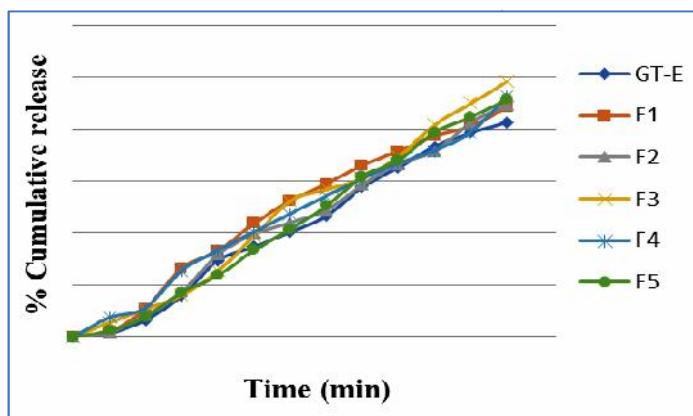
Formulation	Entrapment Efficiency	Drug content
F1	87.82 ± 1.152	89.38 ± 0.947
F2	90.44 ± 1.251	86.20 ± 1.294
F3	95.41 ± 0.898	90.57 ± 1.187
F4	91.35 ± 0.806	88.19 ± 0.997
F5	92.65 ± 1.668	89.82 ± 1.859

Drug Content and entrapment efficiency:

The drug content of the phytosome complexes were studied and presented in Table 5. The percentage of drug content was found in a range of 85-90%. 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 and where it gets the chance to interact more sufficiently which is compared with the finding of Ponnuraj *et al*, 2015 [25]. The entrapment efficiency of formulations from F1 to F5 were analyzed which is represented in Table 5.

Table 6: Modelling and release kinetics from different formulations

Model	Parameter	GT-E	F1	F2	F3	F4	F5
Zero order	R ² _{adjusted}	0.9856	0.9816	0.9887	0.9857	0.9916	0.9830
	K ₀	0.467	0.520	0.487	0.532	0.501	0.498
First order	R ² _{adjusted}	0.9232	0.9549	0.9242	0.8917	0.9496	0.8920
	K ₁	0.007	0.009	0.007	0.008	0.008	0.008
Higuchi	R ² _{adjusted}	0.8111	0.8722	0.8237	0.8062	0.8711	0.7873
	K _H	5.069	5.726	5.307	5.773	5.505	5.379
Hixson-Crowell	R ² _{adjusted}	0.9498	0.9771	0.9518	0.9281	0.9725	0.9264
	K _{KP}	0.002	0.002	0.002	0.002	0.002	0.002

**Figure 5: In- vitro drug release data of the EPC formulations**

The entrapment efficiency of F3 formulation showed better entrapment in which GT and PC were present in 1:1 ratio.

In-vitro release study:

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 and partition coefficient, 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% which contains drug and phospholipid in 1:1 ratio (Figure-5). 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. The free drug also showed less but nearly a similar pattern of release with that of the F5 formulation. The release of drug further studied for kinetic profile by plotting the values of cumulative release in different kinetic models as shown in Table 6. By plotting the cumulative percent release with time, a linear relationship [$r^2=0.98-0.99$] was found which depicted that the permeation followed the zero-order kinetics.

CONCLUSION

EGCG-phospholipid complex (EPC) was successfully prepared by anti-solvent evaporation method using soy lecithin, in five different ratios 1:0.5, 1.0:75, 1:1, 0.75:1 and 0.5:1. Results of drug content and entrapment efficacy studies, of the complex at 1:1 ratio showed better drug content and entrapment efficiency, compared to the other four ratios. The average particle size of the optimized EPC formulation was 201.96 nm. *In vitro* drug release study showed enhanced dissolution in sustained release manner of drug from 1:1 ratio of EPC, after 3 hours following diffusion and swelling mechanism. Many literatures claim that phyto-phospholipid complex has good GI stability and offer higher absorption through the gut mucosa.

From the present study, it is concluded that EGCG-phospholipid complex would serve as an effective carrier for EGCG due to high drug loading, smaller particle size and higher solubility and dissolution.

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