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Creation and Characterisation of proliposomes to boost the oral bioavailability of Eppigallocatech in gallate for the treatment of cancer

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ABSTRACT:
Systems for delivering drugs are essential for improving the bioavailability and effectiveness of medicinal substances. Proliposomes, among them, provide an effective means of delivering normally dry, freely-flowing powders orally by enhancing their stability and absorption. The purpose of this work is to enhance oral bioavailability by the synthesis and characterisation of proliposomes loaded with EGCG (epigallocatechin gallate). The preparation of a stable and secure proliposome formulation with improved oral bioavailability is the main goal of this study. The goals are to assess the preformulation parameters, optimise the formulation procedure, and describe the stability, in vitro drug release, and physical and chemical characteristics of the proliposomes. The film-deposition on carrier approach was used to create the proliposomes. Analytical methods such as FTIR and UV-visible spectroscopy, as well as solubility, melting point, partition coefficient, and organoleptic characteristics were evaluated in preformulation experiments. During formulation, the drug-to-lipid ratio had to be optimised, and the physical characteristics, particle size, zeta potential, entrapment effectiveness, and in vitro drug release profiles had to be assessed. The proliposome formulation F4 had the best drug entrapment effectiveness, measuring 82.25±0.4%, and the ideal particle size, measuring 116.09 nm with a zeta potential of -30.3 mV, signifying strong stability, according to the data. The sustained release pattern seen in the in vitro drug release assays was in good agreement with the first-order kinetics model. The purity of the EGCG and its compatibility with the excipients were verified by the preformulation experiments. As a result of the study's effective development and characterisation of EGCG-loaded proliposomes, the bioavailability of EGCG may be improved. This strategy provides a flexible foundation for oral drug delivery systems and can be applied to Other poorly soluble drug.

Keywords: EGCG, nanotechnology, oral medication administration, liposomes, and bioavailability.

I. INTRODUCTION

By addressing issues with a medicine's solubility, permeability, and stability, nanotechnology improves oral drug administration [1]. The two main issues affecting the oral delivery of many current medications are poor water solubility and the intrinsic dissolution rate, which is the mass of the drug dissolved per time unit and area. Furthermore, low water solubility is present in more than 40% of the unique chemical entities produced by drug discovery screens. [2]. The Biopharmaceutical Classification System (BCS) lists inadequate intestinal permeability, variable absorption, and restricted bioavailability as problems that affect medications in classes II, III, and IV. Drugs in class III are poorly permeable to membranes but are well soluble [3]. To produce oral formulations for drugs with low water solubility, it is crucial to understand obstacles. The solubility of hydrophobic medications is one of the primary factors limiting their oral bioavailability [4]. Though proliposomes are normally dry, liquid proliposomes offer a simpler manufacturing method and spontaneously produce liposomes with a reduced mean particle size and unimodal distribution without the requirement for external forces [5]. When mixed with a 0.9% NaCl solution, these liquid proliposomes generate drug-loaded liposomes. Proliposomes are a cutting-edge method of drug administration via carrier mediated systems, with many benefits above conventional liposomes. Proliposomes are more stable than liposomes, which is why medication delivery is a better fit for them. They are a free-flowing, granular, dry material that, upon contact with physiological fluids such as water, rapidly changes into a liposomal dispersion [6]. Proliposomal formulations provide flexibility, stability, and ease for large-scale manufacture by improving the

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solubility and bioavailability of poorly soluble medicines. These flexible delivery systems work well as carriers for a range of active ingredients [7].

1.1 Techniques for Making Proliposomes

Different methods that affect vesicle size, encapsulation, and content retention can be used to prepare proliposomes. The method of choice should be compatible with the characteristics of the medication, reduce the amount of solvent used and mechanical stress, be economical, and be scalable for large-scale production [8–9].

1.1.1 The carrier method for film deposition

Lipid and a water-soluble carrier are mixed to create lipid-coated solid particles, which are then used in the creation of proliposomes. These particles disintegrate when hydrated, forming liposomes from lipids. A drug and phospholipid solution are applied dropwise onto a carrier in a rotary evaporator under vacuum, using the film deposition on carrier method. This procedure yields a loose powder that, when wet, readily condenses into liposomes. Sorbitol, mannitol, and microcrystalline cellulose are common transporters [10].

1.1.2 The spray-dry technique

Spray drying is a scalable method that produces proliposomes with consistent size and shape, making it suitable for large-scale production. It improves particle control by integrating drying and particle production into a single, continuous step. For both aqueous and non-aqueous environments, this technique is effective. Lipids or lipids with carriers are made into liquid dispersions, which are atomised, dried, and collected in a tank with the help of a spray nozzle and air flow [11].

1.1.3 The fluidised bed technique

Based on particle coating technology, the fluidised bed technique produces liposomes on a massive scale. An organic solvent and drug solution are sprayed onto carriers such as non-pareil beads or crystalline powder, and the solvent is then removed by vacuum. This smooths the surface for the phospholipid coating, which when hydrated produces small-sized liposomes and thin, homogeneous coatings. The last lipid-coated particles are vacuum-dried to remove any last traces of solvent [12].

1.1.4 The anti-solvent supercritical approach

Proliposomes are made using supercritical carbon dioxide (SCCO2) in the supercritical anti-solvent technique. A solution containing phospholipids, cholesterol, and medicine is put in a reaction vessel with a carrier, and SCCO2 is pumped in. On the carrier particles, a drug-phospholipid coating develops upon swirling and solvent removal. Proliposomes that are produced as a result are gathered and kept at 4°C [13–14].Complex changes in cell signalling and biological processes are involved in cancer, a major worldwide public health concern with a fast increasing death rate currently estimated at 9.55 million per year [15]. Although the precise causes of cancer are still unknown, carcinogens are widely believed to have a significant role in the development of this infamous illness. For most tumours, radiation and chemotherapy—including doxorubicin, cisplatin, 5-fluorouracil, and paclitaxel—are the standard forms of care. Because they have side effects, even normal cells can be cytotoxic, chemotherapeutic medicines disrupt physiological and biochemical processes as well as cell signalling pathways [16]. Natural compounds from medicinal plants have the potential to be useful in managing cancer because they can regulate key cell-signaling molecules with fewer side effects, enhance the efficacy of current drugs, and decrease toxicity [17]. The search for safe, affordable anti-cancer drugs is still ongoing.

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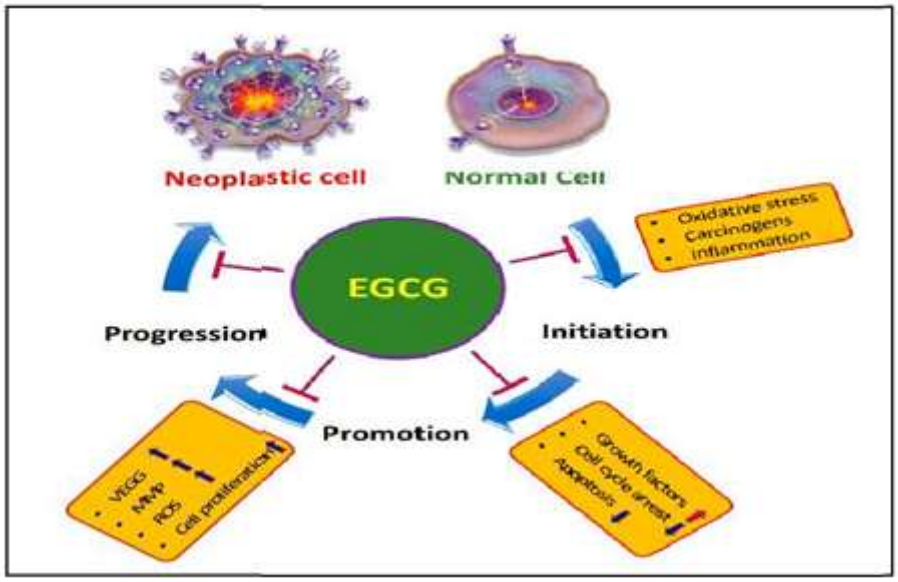

Figure 1: The function of epigallocatechin-3-gallate (EGCG) in blocking the carcinogenesis process is to inhibit the formation of cancer.

1.2Epigallocatechin-3-gallate [18]

(-)-Epigallocatechin-3-gallate (EGCG) is the primary polyphenolic component of green tea; other catechins, including ECG, EGC, EC, and (+)-catechin, are present in smaller amounts. More than half of the mass of catechins in green tea is made up of EGCG, which is primarily responsible for its health benefits. More than 8000 scholarly investigations examine the chemistry, bioactivity, and health benefits of green tea, with over 4000 of those studies concentrating on EGCG. Chemical analysis, epidemiology, antioxidant properties, biological potential, enzyme interactions, preparation patents, health advantages for animal models, and clinical trial results are all covered in these publications.

1.2.1 Principal Processes by Which EGCG Inhibits Cancer [19]

1.2.1.1 Inflammatory response

A key factor in the development of cancer is inflammatory response, which is mediated by molecules like TNF-α and NF-κB. Compounds found in green tea, such as EGCG, may have anti-inflammatory properties when used in cancer treatment. [20]. When paired with chemotherapy, EGCG can improve the treatment of prostate cancer by reducing MCP-1 synthesis, oxidative stress, and TNF-α activity [21].

1.2.1.2 Reactive Oxygen Species (ROS)

Oxygen species that react (ROS)are mostly formed as byproducts, have a wide range of negative effects, and are involved in the aetiology of many different diseases [22]. Homeostasis depends on a balanced ROS production/antioxidant defence system [23]. On the other hand, neutralising or eliminating ROS is a critical step in preventing pathogenesis. Antioxidant enzymes have a significant leading role in neutralising ROS or preventing their synthesis. Nonetheless, natural products' antioxidant qualities demonstrate a dynamic role in the inhibition of pathogenesis and the reduction of oxidative stress. A well-known antioxidant, EGCG scavenges the majority of free radicals, including ROS and RNS. Glutathione levels can be regulated and glutathione peroxidase's enzymatic activity can be restored by EGCG. Furthermore, H2O2 and NO generation in human skin are inhibited by EGCG [24]. Because it effectively scavenges hydroxyl and superoxide radicals, protects DNA from damage, induces apoptosis in colon cancer cells with low-dose H_2O_2 , and, at a dose of 25 μM, lowers TPA-mediated hydrogen peroxide production and normal cellular levels, EGCG has the potential to be used as a chemopreventive agent in conjunction with tamoxifen.

1.2.1.3 Angiogenesis

By lowering VEGF synthesis, EGCG inhibits angiogenesis and is a promising natural molecule for cancer treatment that can be used in place of medications like bevacizumab, which have negative side effects [25]. EGCG has been shown to suppress VEGFR-2 activity[26]. Moreover, via

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inhibiting the activation of HIF-1α, NFκB, and VEGF synthesis, EGCG reduces tumour angiogenesis and the progression of breast cancer. The results also showed that EGCG treatment dramatically reduced the weight of the tumor in relation to the control and the expression of VEGF in the tumor [27]. EGCG stops the creation of VEGF via decreasing the constitutive activation of Stat3 and NF-kappa B in cancer cells. EGCG may be helpful in the treatment of head and neck squamous cell carcinoma and breast cancer since it can generate both antiproliferative and antiangiogenic activities. [28]. Vascular endothelial growth factor-induced DNA synthesis, cell division, and autophosphorylation of vascular endothelial growth factor receptors-1 and -2 were all suppressed by epigallocatechin-3 gallate in a concentration-dependent manner [29].

II. MATERIALS AND METHOD

2.1Preformulation study

Pre-formulation studies, which include methods like IR and UV spectroscopy and melting point determination to assist formulation design and guarantee a safe, effective, and stable dosage form, are crucial to the development of new drugs because they evaluate a drug's physical and chemical properties [30].

2.1.1 Organoleptic properties

The drug sample's physical attributes, including look, colour, and odour, were assessed.

2.1.2 DSC study of the drug EGCG

The drug EGCG was thermally examined using a DSC 6000 Differential Scanning Calorimeter (DSC) to investigate its properties related to heat. Five milligrams of EGCG were added to metal trays and heated between thirty and four hundred degrees Celsius at a rate of ten degrees Celsius per minute. The nitrogen flow rate was kept constant at 20 milliliters per minute for the thermal testing. Pyris software was utilized to document the samples' DSC thermogram.

2.1.3 UV spectrum of EGCG in water

Depending on the kind of electronic transition involved in the absorption, molecules in solution can absorb light of a particular wavelength when exposed to visible or ultraviolet light. For this reason, it is typical practice to employ UV-visible spectrophotometers to get precise data regarding the chromophoric component of the molecules in solution. To record absorption, the UV spectrum is often plotted versus wavelength. [32].A double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) was used to measure the drug's λmax.

2.1.3.1 Preparation of Calibration Curve

- **Preparation of stock solution of EGCG in water [31]**

With water, the standard EGCG stock solution (1000µg/ml) was made. Water was added to this solution to create a range of concentrations, from 5 to 40 µg/ml. These solutions' absorbance was measured at 294 nm against water as a blank using a UV-visible spectrophotometer, and a standard curve was created against concentration. The calibration curve's intercept, slope, straight line equation, and correlation coefficient were ascertained.

2.1.4 Solubility

The spontaneous interaction of two or more chemicals that results in a uniform molecular dispersion is referred to as solubility. For a quantitative solubility analysis, extra medication was put in thoroughly cleaned culture tubes along with three milliliters of different solvents. After then, the culture tubes were tightly sealed. These culture tubes were shaken on a water bath shaker set at 25°C for a full day at room temperature. For 24 hours, each sample was centrifuged at 15,000 rpm, and the supernatant was taken out. The filtrates were then suitably diluted and quantified using spectrophotometry after the supernatant was filtered. [32]

2.1.5 FTIR study

To look for any potential medication interactions with excipients, EGCG and other ingredients were mixed together, and the FT-IR spectra was captured. One or two milligrammes of EGCg were added to the FT-IR chamber. The range of the infrared spectrum was observed between 4000 and 400 cm-1. [33]

2.1.5.1 Drug-excipients Compatibility Study by FTIR

The compatibility of the medication and excipients was assessed using FT-IR. FTIR was used as a tool to identify any physical or chemical interactions between the medication and the excipients. The drug was well mixed in a 1:1 ratio with several excipients. The samples were scanned in the 400–4000 cm-1 range using FTIR. The spectra of the medication without excipients and

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the medication with them were compared to check for any physical alterations or incompatibilities. [34].

2.1.6 X-ray diffraction (XRD)

The substance EGCG underwent X-ray diffraction (XRD) analysis to determine the crystalline structure of the samples. It was an X-ray diffractometer. Diffractograms were produced using a Cu Kα radiation source up to the final angle of 50°. The acquired data were gathered using a 0.02° step width.

2.2 Preparations of EGCG proliposomes

The film-deposition on the carrier approach was utilised to generate proliposomes using varying ratios of mannitol, cholesterol, and phospholipid. Phospholipid, cholesterol, and medication were weighed and placed in a vial. The solution was then supplemented with methanol and chloroform in a 9:1 ratio, respectively. Once a homogenous solution was formed, mannitol was added as a carrier. Proliposomes appeared on the flask's surface when the solvent was removed using a Rota evaporator. The solvent of choice was a methanol and chloroform combination [35].

Table4.3: Composition of different proliposomes formulations

Formulatio-ncode	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
EGCG(mg)	458	458	458	458	458	458
Soyalecithin(mg)	758	1137	1516	1895	2277	2653
Cholesterol (mg)	381	381	381	381	381	381
Mannitol (mg)	758	1137	1516	1895	2277	2653

2.3 Characterization of EGCG Proliposome

2.3.1Appearance

It was done by using visual observation of all formulation.

2.3.2Entrapment efficiency [40]

To calculate the Proliposome encapsulation efficiency, an ultra-filtration method was used. After diluting the prepared proliposome powder with water, 1 mL of the diluted proliposomes was added to the top of a centrifuge tube that had an ultrafilter attached, and the tube was spun for 30 minutes at room temperature at 6000 rpm. As previously indicated, the filtrate containing unencapsulated EGCG was collected and subjected to UV analysis at 294 nm. The percentage of encapsulation efficiency was calculated indirectly using the following equation:

$$EE\% = \frac{\text{Initial amount of drug} - \text{Final amount of drug}}{\text{Initial amount of drug}} \times 100$$

2.3.3 Percentage yield

The prepared proliposomes formula's percent production yield was computed by multiplying the formula's end weight by the raw material's beginning weight multiplied by 100.

$$\text{Percentage yield} = \frac{\text{Practical mass of proliposomes}}{\text{Theoretical mass}} X100$$

2.3.4 Particle size and zeta potential determinations

The Zeta sizer Nano device was utilised to measure the particle size (PS) and zeta potential of drug-loaded proliposomes, while photon correlation spectroscopy and electrophoretic mobility were also employed. Prepared proliposomes were 100 times diluted, added to the sample cell, and positioned inside it in order to

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measure the Zeta potential and particle size, respectively [41].

2.3.5 SEM

The proliposome compound's surface morphology was examined using SEM. After utilising a gold sputter module to coat the proliposomes on adhesive tape attached to an aluminium stub, the coated sample was scanned and SEM photomicrographs were captured.

2.3.6 FTIR of EGCG proliposomes formulation [42]

Infrared spectroscopy was performed using an FTIR spectrophotometer, yielding a spectrum with a wavelength range of 4000 to 400 cm-1. The procedure included distributing the sample.

2.3.7 In-vitro Drug Release Study

An in-vitro release study of EGCG proliposomes was conducted using a USP Type II paddle device. A proliposome formulation containing 458 mg of the drug was analyzed at 37±0.5°C with a paddle rotation speed of 50 rpm. The dissolution media used were 900 mL of phosphate buffer (pH 5.5) and 0.1N HCl. Samples (5 mL) were taken at 15, 30, 60, 180, 240, 300, and 600 minutes, and replaced with fresh media to maintain sink conditions. The aliquots were filtered and analyzed at 294 nm. The drug release from proliposomes was calculated as a percentage over time and compared to the release from the pure substance. The study was performed in triplicate.

2.6.8 In vitro drug release kinetic

The release profile was assessed using the Korsmeyer-Peppas, Higuchi, First Order, and Zero Order models, based on model-dependent approaches.

a. Zero order kinetics

It can be used to explain the dissolution of many pharmaceutical dosage forms with modulated release, such as osmotic systems, matrix tablets containing coated low-soluble medications, some transdermal systems, etc.. Zero order release can be expressed in the simplest form as:

Q0 - Qt = K0t.

The cumulative amount of drug released versus time was calculated using data from in vitro drug permeation assays. Q0 represents the initial drug concentration (usually Q0 = 0), Qt represents the amount dissolved at time t, and K0 is the zero-order release constant (concentration/time).

a. **First order kinetics**

The following formula, which represents the drug's release according to first-order kinetics: log C = log C0 - K.t / 2.303, where K is the rate constant, t is time, and C0 is the starting drug concentration. When the log cumulative percentage of medicine left is plotted against time, a straight line with a slope of K/2.303 is produced.

b.Higuchi's Model

The Higuchi model uses the formula Qt=KHt, where \(KH \) is the release rate constant, to describe drug release from an insoluble matrix based on Fickian diffusion. Making a plot

A straight line representing diffusion-controlled release with a gradient equal to \(KH \) can be obtained when plotting the cumulative drug released against the square root of time.

c.Korsmeyer-Peppas Model

Korsmeyer used a simple connection to illustrate the drug release from a polymeric system.The release rates from controlled release polymeric matrices can be described using the formula developed by Korsmeyer et al.

Q = K.tn

The Korsmeyer-Peppas model, with \(Q \) as the drug proportion released at time \(t \), \(n \) as the diffusional exponent (0.45 for Fickian, 0.45-0.89 for anomalous, 0.89 for zero order), and \(K \) as a kinetic constant, describes the relationship between log cumulative % drug release and log time.

III. RESULTS AND DISCUSSION

3.1 Preformulation studies

3.1.1 Organoleptic properties [44-45]

The medication EGCG's organoleptic qualities were determined to be in line with previous research. Table 5.1 presents the organoleptic features of EGCG.

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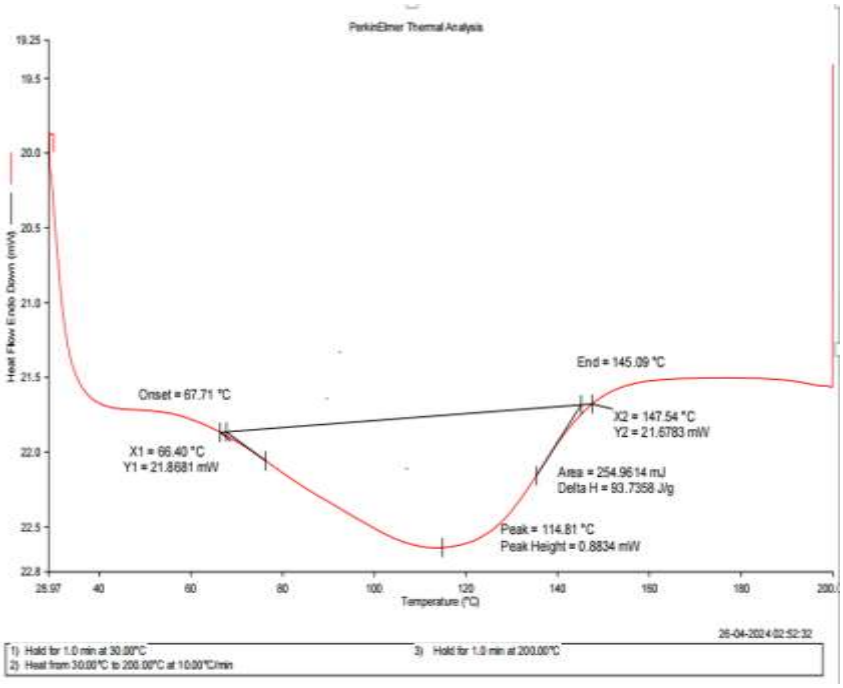
Table5.1: Organoleptic properties of ECGC

Sr.No.	Properties	Description
1	Odour	Odourless
2	Colour	Yellowish brown powder
3	Taste	bitter

3.1.2 Melting point



3.1.3 Determination of absorption maxima by UV spectroscopy

Figure 5.1 showed the results of the EGCG UV spectrum analysis.

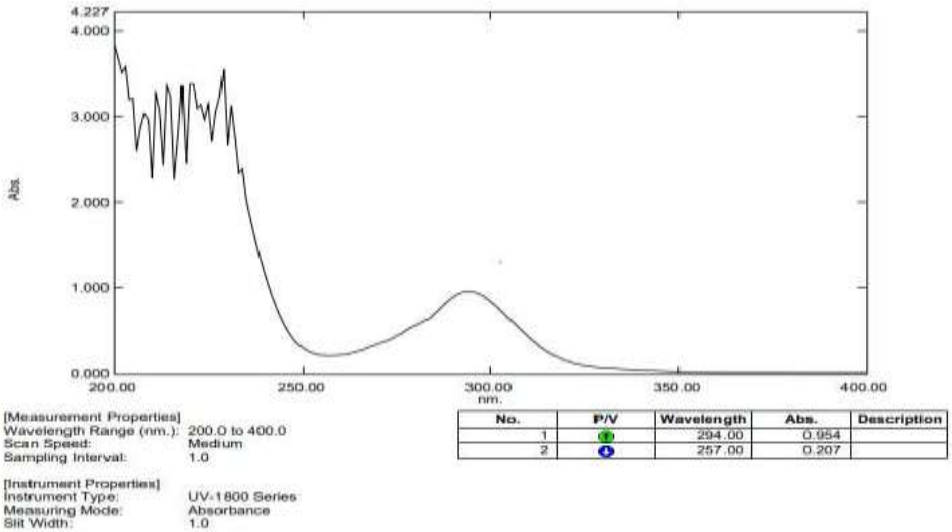


Figure 5.1: UV spectrum of EGCG

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Table 5.3: Absorption maxima (λmax) of EGCG

Nameof drug	Absorption maxima(λ_{max})	
	Observed	Reference
EGCG	294nm	296nm

3.1.4 Preparation of calibration curve of EGCG in Water

Water was used to create the 1000µg/ml EGCG standard stock solution. This solution was diluted with water to the proper dilutions (5to 40µg/ml) and then spectrophotometrically analysed at 294 nm. The results are shown graphically in the following Table 5.4 and Figure 5.2.

Table5.4: Calibration curve of EGCG in water

Sr. No.	Concentration(µg/ml)	Absorbance
1	0	0
2	5	0.146±0.002
3	10	0.260±0.002
4	15	0.372±0.002
5	20	0.514±0.002
6	25	0.655±0.002
7	30	0.746±0.001
8	35	0.861±0.002
9	40	0.954±0.002

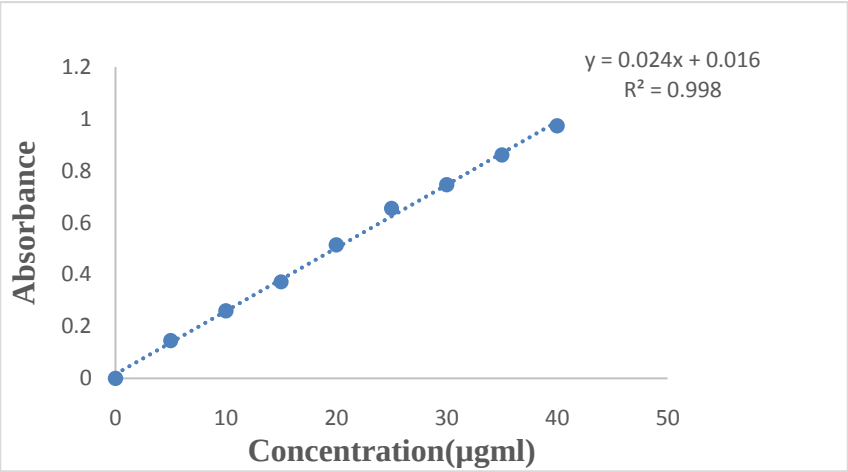

Figure5.2: Calibration curve of EGCG in water

3.1.5 Solubility studies

The observed solubility profile of the drug was shown in figure 5.3 and table 5.6

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Table 5.6: Solubility profile of EGCG in different solvent

Sr.No.	Solvents	Solubilitymg/ml
1	Methanol	15.235±0.288
2	Ethanol	14.974±0.619
3	Chloroform	12.601±0.082
4	pH7.4	11.435±0.104
5	Water	19.487±0.063

(Mean ±SD,N=3)

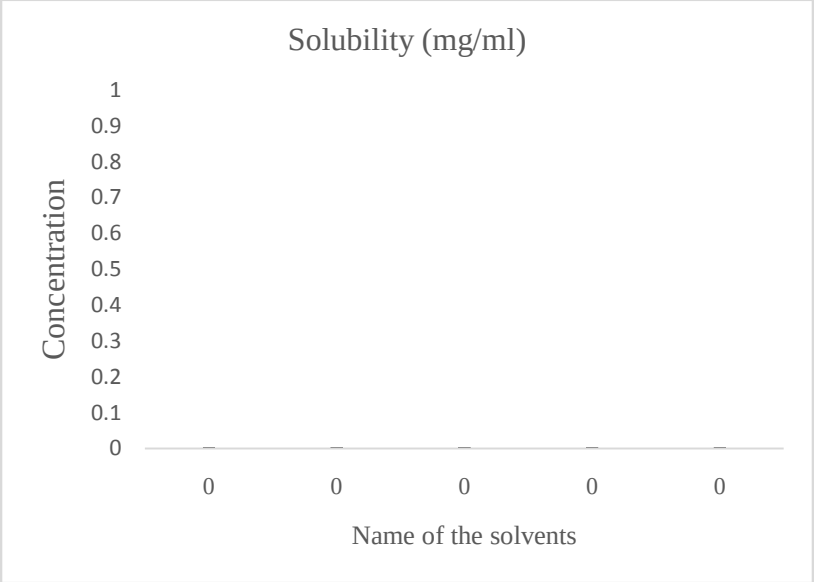

Figure5.3: Solubility profile of EGCG in different solvent

3.1.6 FTIR analysis of pure drug and excipients

3.1.6.1 FTIR of EGCG

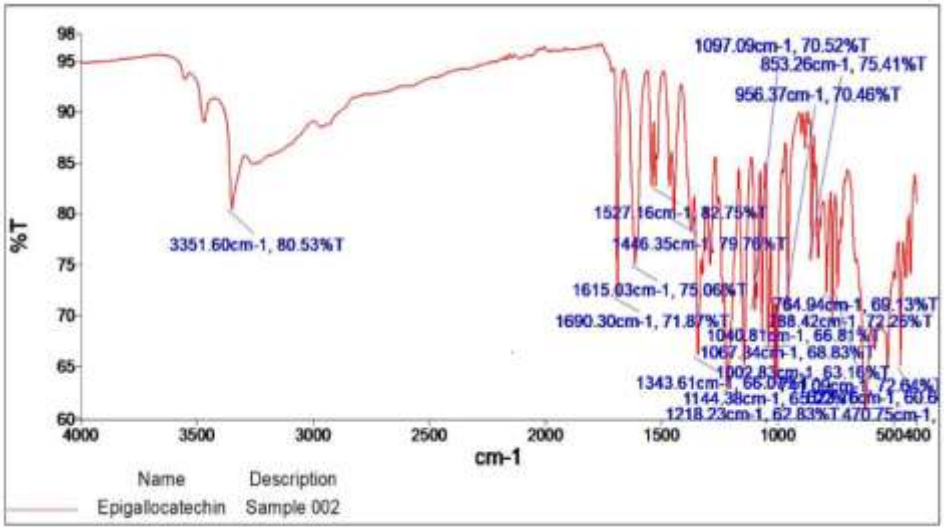

Figure5.4:FTIR spectrum of EGCG

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Table5.7: FTIR Spectrum interpretation of EGCG

Characteristics Peaks	Reported (cm^{-1})	Observed(cm^{-1})
O-H	3478-3355	3351
C=O	1690	1690.30
C-H	1526	1527.16

3.1.7 XRD of the drug EGCG

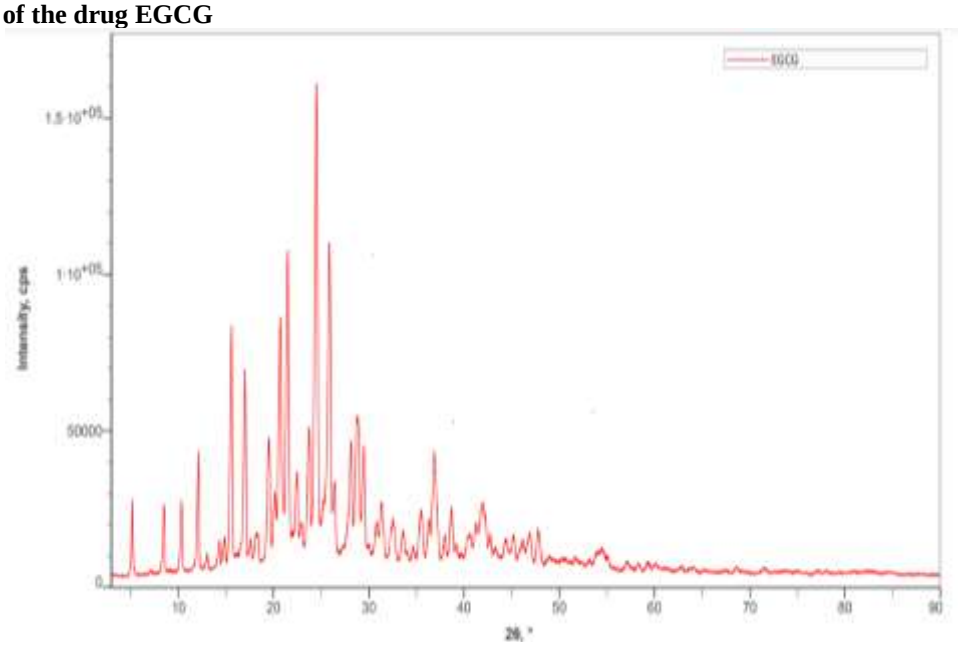

Figure 5.9: XRD of the drug

Table 5.12:Interpretation XRD of the drug

Sr. No.	Formulation	Observed 2θ	Reference 2θ
1	Pure drug	7.1	7.5
2		15.6	15.0
3		22.4	22.5

3.2Evaluationof EGCG loaded proliposomes[46]

Table5.13: Physical appearance of all the Formulation

Sr.No.	Formulation Code	Color	Appearance
1	F1	Lightbrown	Powder
2	F2	Lightbrown	Powder
3	F3	Lightbrown	Powder
4	F4	Darkbrown	Powder
5	F5	LightBrown	Powder
6	F6	LightBrown	Powder

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3.2.1 Entrapment Efficiency[47]

Table5.15: Entrapment Efficiency of EGCG loaded proliposomes

Sr.No.	Formulation	Mean±sd
1	F1	66.80±0.2
2	F2	74.38±0.2
3	F3	79.62±0.3
4	F4	82.25±0.4
5	F5	78.84±0.3
6	F6	76.89±0.2

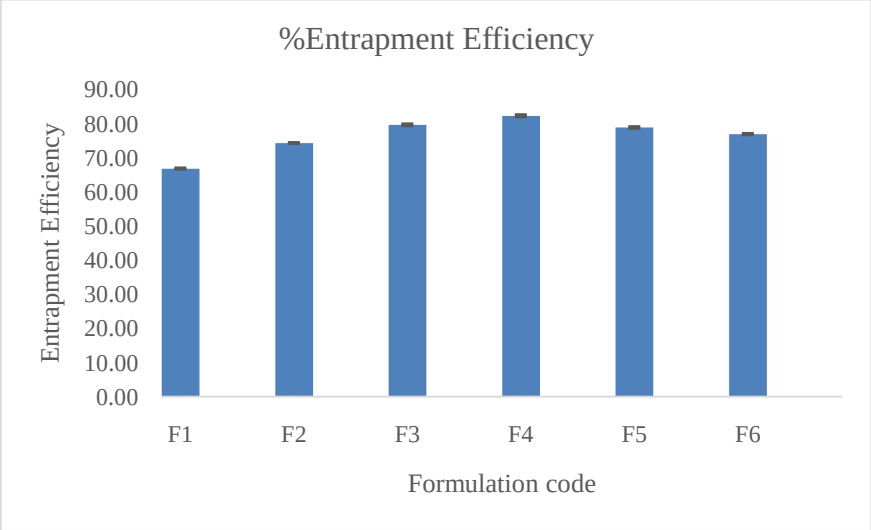

Figure5.8: DrugEntrapment offormulations(F1-F6**)**

3.2.2 IN VITRO DRUG RELEASE STUDIES

Table 5.16:In vitro drug release of pure drug & EGCG loaded proliposomes

Sr. No.	Time(min)	Drug Release of pure drug(%)	Drug Release of Formulation F4(%)
1	0	0	0
2	0.25	14.568±0.069	8.473±0.314
3	0.5	24.472±0.361	17.92±0.314
4	1	40.35±0.206	26.996±0.206
5	2	58.182±0.157	36.872±0.206
6	4	-	57.586±0.412
7	6	-	65.073±0.314
8	8	-	70.794±0.629
9	10	-	75.819±0.518
10	12	-	85.049±0.617
11	24	-	97.84±0.412

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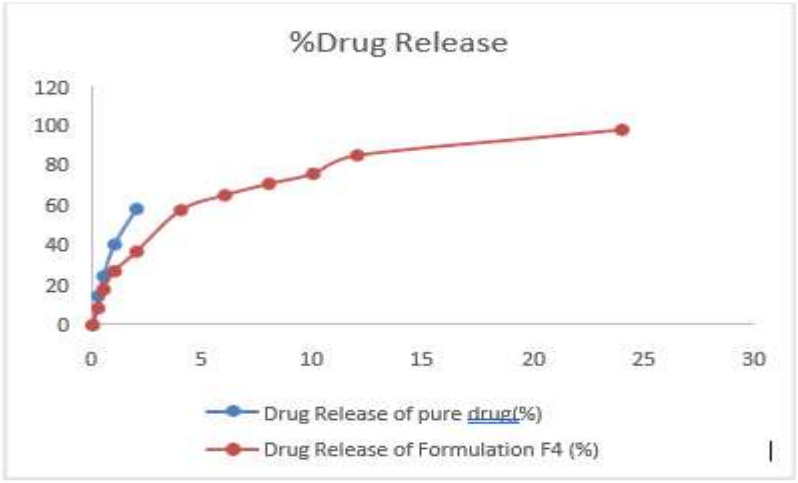

Figure 5.12: Percentage drug release of pure drug & EGCG loaded proliposomes (F4)

4.1. Drug Release Kinetic Studies

a. Zero order kinetics

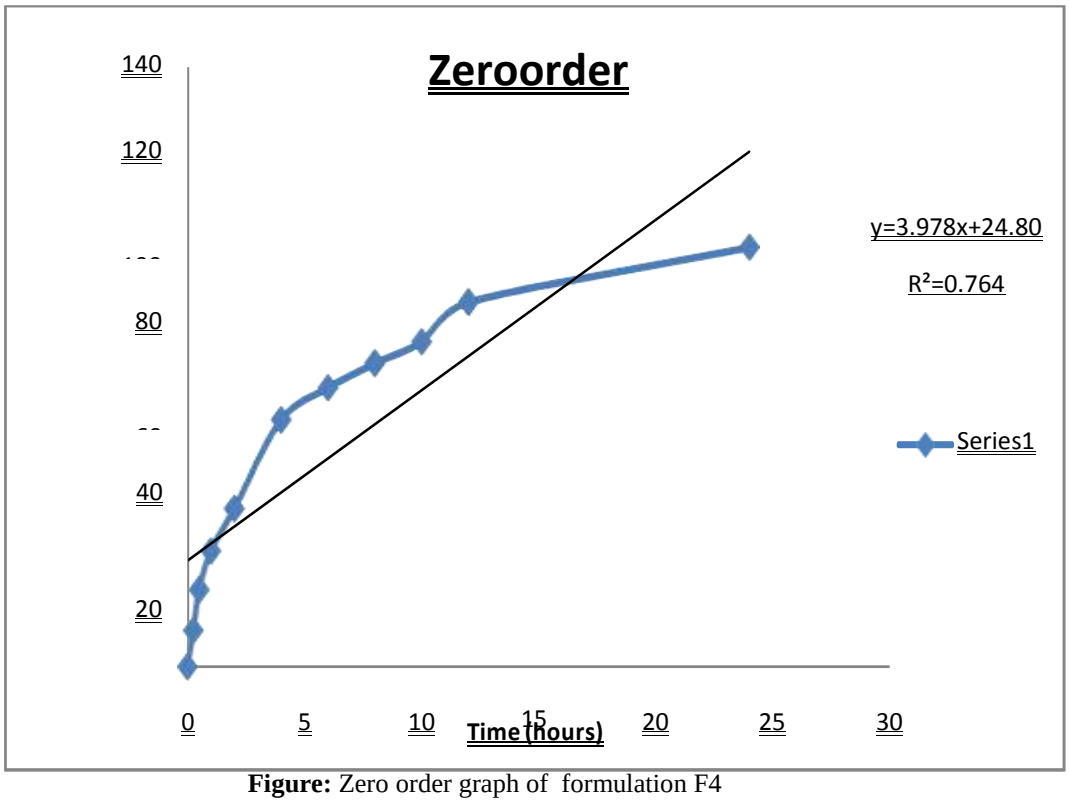

Figure: Zero order graph of formulation F4

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b. First order kinetics

Figure: Zero order graph of formulation F4

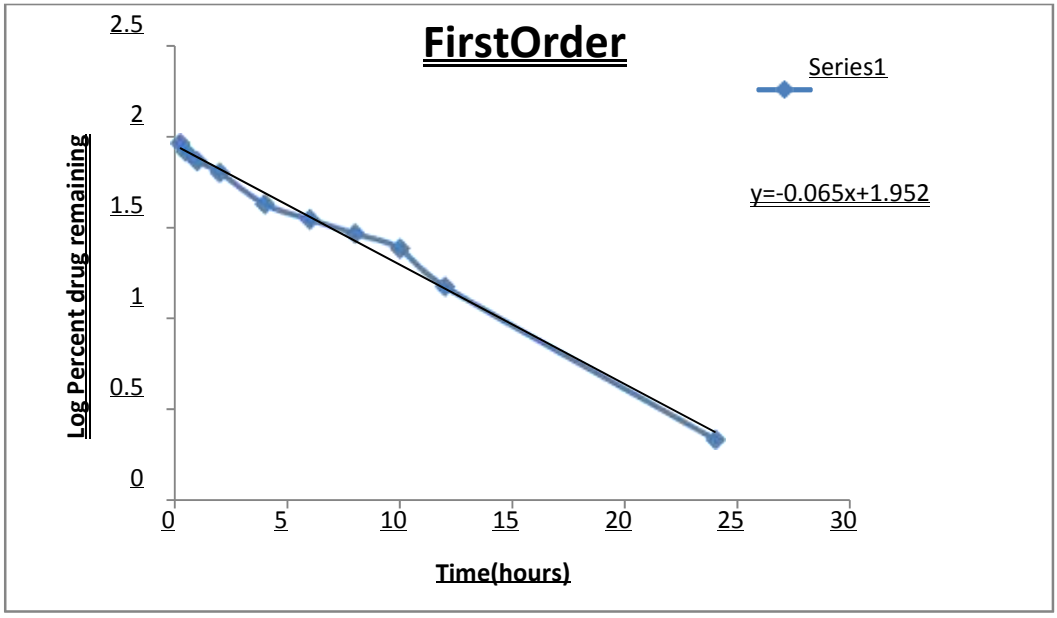

Figure: Zero order graph of formulation F4

c. Higuchi'sModel

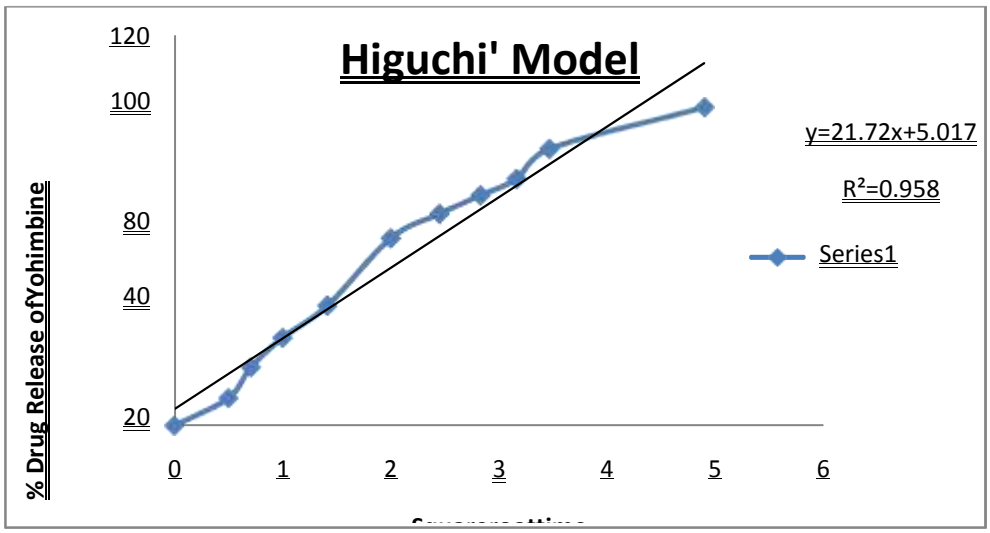

Figure5.15: HiguchiordergraphofformulationF4

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d. Korsmeyer-Peppas Model

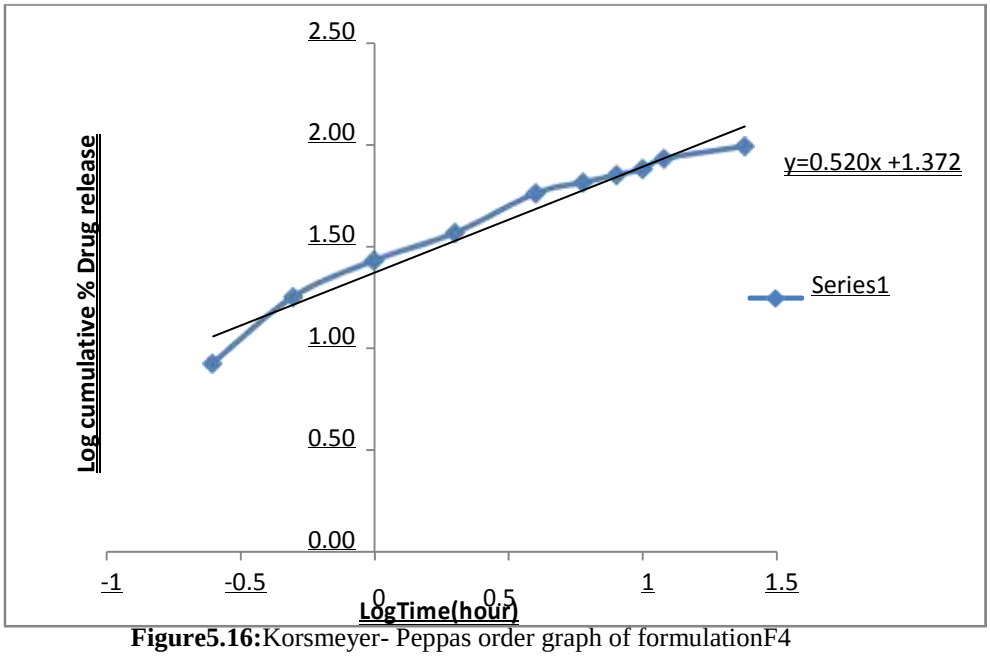

Figure5.16: Korsmeyer- Peppas order graph of formulationF4

Table5.17: Kinetic equation parameter of formulationF4

Formulation Code	Zeroorder		First order		Higuchi		K.Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F4	3.9789	0.7646	-0.0658	0.992	21.727	0.9587	0.5206	0.9616

IV. SUMMARY AND CONCLUSION

One of the main catechins in green tea, EGCG, makes up between 50 and 60 percent of the content and is well-known for its antioxidant qualities, which help prevent and treat tumours as well as help cancer cells overcome medication resistance. Through FT-IR, UV, and melting point investigations, preformulation studies verified the high purity, solubility, and hydrophilic nature of EGCG. Different polymer dosages were used to prepare the liposomes; formulation F4 produced the highest drug entrapment (82.25%) and the largest particle size (116.09 nm). By using Korsmeyer-Peppas kinetics, this formulation demonstrated a 99.012% drug release in a 24-hour period, indicating the possibility of using proliposomes to increase EGCG's oral bioavailability.

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