

Formulation and in-vitro evaluation of long circulating coated vitamin ETPGS phytosomes of docetaxel

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ABSTRACT:

Docetaxel (DCX) is a highly effective chemotherapeutic drug used in the treatment of different types of cancer, including non-small cell lung cancer (NSCLC). The drug is known to have low oral bioavailability due to its low aqueous solubility, poor membrane permeability and susceptibility to hepatic first-pass metabolism. To mitigate these problems, DCX is administered via the intravenous route. Coated vitamin ETPGS loaded phospholipid complex (phytosomes) was formulated by solvent evaporation method. A new, simple, rapid, economical and sensitive UV - Spectrophotometric method has been developed for the estimation of docetaxel. The formulations were characterized for particle size, zeta potential, entrapment efficiency, FTIR (Fourier transformed infrared), TEM (transmission electron microscopy) and drug release properties. In-vitro drug release studies were carried out in phosphate buffer saline using dialysis bag method. Thus, the results indicate that the formulation of coated vitamin ETPGS phytosomes of docetaxel could be developed as safe and beneficial.

Keywords: docetaxel, phytosomes, vitamin, ETPGS, cancer.

1.1 INTRODUCTION:

Cancer is the leading cause of mortality worldwide. Overall, the prevalence of cancer has actually increased; just in the United States alone, approximately 1,665,540 people suffered from cancer, and 585,720 of them died due to this disease (1). Therefore, cancer is a serious problem affecting the health of all human societies. Unfortunately, it is a variety disease at the tissue level and this variety is a major challenge for its specific diagnosis, followed by efficacy of treatment (2, 3). Parenteral drug administration is carried out directly through the skin, in or towards systemic circulation. It is the route of choice for drugs that cannot be absorbed orally and/or that are unstable in the gastrointestinal tract (e.g. insulin, heparin). These routes of administration are also used for the treatment of unconscious patients or under circumstances that require a rapid onset of action. Parenteral routes of administration exhibit higher bioavailability than other routes and are not subjected neither to first-pass metabolism nor to the sometimes extreme conditions of the gastrointestinal environment, while offering the greatest control over the real drug amount that accesses systemic circulation. As main drawbacks, drug administration by these routes is irreversible and can cause fear, pain, tissue damage and/or infections. Parenteral administration can be performed by injection (small volumes), infusion (large volumes) or implant, and while its typical goal is to achieve systemic effects, it can also be used locally on a specific organ or tissue by injecting the pharmaceutical active ingredient directly on the site of action, in order to minimize systemic adverse effects. The three main parenteral routes are intravenous (IV), intramuscular (IM) and subcutaneous (SC). (4,5)

Docetaxel (DTX) is a semi-synthetic analog of paclitaxel which is an extract from a rare Pacific yew tree *Taxus brevifolia*. DTX is more water-soluble than paclitaxel owing to its chemical structure in which there is a tertbutyl carbamate ester in its phenylpropionate side chain and a hydroxyl functional group on carbon. By binding to and stabilizing tubulin, DTX prevents physiological microtubule depolymerization and disassembly, leading to cell cycle arrest at the G2/M phase and cell death. DTX also inhibits expression of the antiapoptotic gene Bcl2 and promotes the expression of the cell cycle inhibitor p27. These effects confer DTX antitumor activities against a broad range of tumors, including breast, non-small cell lung cancer, ovarian, gastric and prostate carcinomas. However, DTX has not gained clinical use owing to its poor solubility, low selective distribution and fast elimination. In addition, currently available commercial formulations of DTX (Taxotere and Duopaferi) have been reported to cause serious side effects which include neutropenia, musculoskeletal toxicity, peripheral neuropathy and hypersensitivity reactions, attributable to either DTX itself or the solvent (polysorbate 80). Therefore, overcoming the side effects of DTX and improving its anticancer effects have been a focus of studies of nanocarriers. (6)

Phytosomes are a novel drug delivery system that has the advantages of delivering herbal drugs at a predetermined rate, delivering drugs at the site of action, minimising toxic effects, increasing drug bioavailability, and controlling drug distribution by incorporating the drug in a carrier system or changing the drug's structure at the molecular level. (7) Phytosomes transfer more smoothly from a hydrophilic environment to the lipid-friendly environment of the enterocyte cell membrane, then inside the cell, and finally into the bloodstream. Pharmacokinetic and pharmacological parameters have been enhanced by phytosomes. (8)

2.1 MATERIALS & METHODS:

Docetaxel was gifted by Sanofi Aventis Pharma, India, Lipoid S 100 by Lipoid koshmetik, Germany, Vitamin ETPGS by pmciscochem, France. Methanol and Sodium Chloride purchased by Fisher Scientific India Pvt. Ltd. New Delhi, Potassium Dihydrogen orthophosphate, Disodium hydrogen orthophosphate purchased by Thomas Baker, New Delhi. All other materials were of analytical grade.

3.1 PRE-FORMULATION STUDIES:

Pre-formulation is an integral part of the entire development process. It is the study of the physical and chemical properties of the drug prior compounding process. These studies focus on those physicochemical properties of the drug that could affect its performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. In the simplest case, these preformulation investigations may merely confirm that there are no significant barriers to the compound's development. These studies are indispensable protocol for development of safe, effective and stable dosage form. The obtained drug sample was identified by various analytical techniques such as IR spectroscopy, UV spectroscopy, melting point etc. (9)

3.1.1 Organoleptic Characteristics: The drug sample was characterized for the physical characterization like appearance, color and odor. (10)

3.1.2 Melting point: The melting point of the solid is defined as the temperature at which solid and liquid are at equilibrium at a total pressure. Melting point apparatus is used for the determination of melting point of the drug. A few amounts of the drug were placed in a thin walled capillary tube 10-15 mm long, about 1 mm inside diameter, and closed at one end. The capillary, which contains the sample, was suspended to heat the samples slowly and evenly and thermometer placed to check the temperature. The temperature range over where the sample is observed to melt is taken as the melting point of the drug. (11)

3.1.3 UV spectrum of Docetaxel: UV-visible spectrophotometer is generally used for structural information of various drugs to obtain specific information on the chromophoric part of the molecules in solution when exposed to light in the visible/ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption. The UV spectrum is generally recorded as a plot of absorbance versus wavelength Double beam UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) was used to know the $\lambda_{\max}$ of drug. A 32 $\mu\text{g/ml}$ solution of Docetaxel was scanned in the range of 200-400 nm. (12)

3.1.4 Estimation of Ascorbic acid by UV-visible spectrophotometer:

3.1.4.1 Preparation of Stock Solution:

Standard stock solution of ascorbic acid was prepared by dissolving 10mg of ascorbic acid in 10ml of water which gives 1000 $\mu\text{g/ml}$. 10ml of this stock solution was taken and was diluted up to 100ml by using water to produce a concentration of 100 $\mu\text{g/ml}$ solution.

3.1.4.2. Preparation of Working Solution:

The standard stock solution of ascorbic acid (100 $\mu\text{g/ml}$) was prepared in water. This solution was diluted with water, to obtain various dilutions (2-18 $\mu\text{g/ml}$). Absorbance of these solutions was recorded at 260nm against water as blank using UV-visible spectrophotometer and standard curve was plotted against concentration. From the calibration curve intercept, slope, straight line equation and correlation coefficient were obtained.

3.1.5 Solubility Studies: The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called solubility. For quantitative solubility study, excess amount of drug was taken in thoroughly cleaned culture tubes containing 3 ml of different solvents (Methanol, Ethanol, Chloroform, Water, PBS (pH 7.4) and were tightly closed. These culture tubes were shake on water bath shaker for 24 h at room temperature. After 24 hr each sample was centrifuged 10,000 rpm and supernatant was withdrawal. After that supernatant was filtered and filtrates was suitably diluted and determined spectrophotometrically. (13)

3.1.6 Partition Coefficient of Drug: Partition coefficient (oil/water) is a measure of a drug's lipophilicity/hydrophilicity and an indication of drug's ability to cross cell membranes. It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. Partition coefficient provides a means of characterizing the lipophilic/hydrophilic nature of the drug. Drugs having values of P much greater than 1 are classified as lipophilic, where as those with values much less than 1 are indicative of a hydrophilic drug. The partition coefficient is commonly determined using an oil phase of n-octanol and water. In the case n-octanol and water:

$$P_{o/w} = \frac{C_{(n-octanol)}}{C_{(water)}}$$

The partition coefficient ($P_{o/w}$) therefore is the quotient of two concentrations of drug in n-octanol ($C_{n-octanol}$) and water (C_{water}) respectively and is usually given in the form of its logarithm to base 10 (log P).

➤ Shake flask method:

The partition coefficient determination study was performed by using shake flask method. Excess amounts of the drug (Docetaxel) dissolved in 10 ml of two solvents (n-octanol: Water) together (1:1) and placed for 24 h. After 24 h, the two layers were separated and centrifuge for 15 mins at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective $\lambda_{\max}$ after appropriate dilution. (14)

3.1.7 FTIR of Docetaxel and Excipients: FT-IR (Fourier Transform Infrared) spectrum of any compound or drug gives information about the groups present in that particular compound. FT-IR Spectroscopy was used for structure analysis. The potassium bromide (KBr) disc technique was employed. Since the KBr has no absorption in the fundamental region of IR spectrum, only the spectrum of sample is obtained. An FT-IR spectrum of Docetaxel and drug plus excipients mixture was recorded for the determination of drug interaction with excipients. (15)

3.1.8 Drug-excipients Compatibility Study by FTIR: The compatibility of drug with excipients was ascertained by FT-IR. FTIR was used as tool to detect any physical and chemical interaction between drug and excipients. Drug and various excipients were mixed thoroughly in ratio of 1:1. Samples were scanned by FTIR under the range of 400-4000 cm^{-1} . The spectra of pure drug and drug with excipients were compared to check any incompatibility and physical changes.

3.2 Preparations of docetaxel loaded Phytosomes:

The Docetaxel-phospholipid Complex were prepared by reflux method. Docetaxel, Vitamin ETGS and phospholipid S100 was taken in different millimolar ratios. Briefly, accurately weighed amounts of Docetaxel and phospholipid S100 were placed into a 100 mL round bottom flask and dissolved in 10 mL of methanol. The reaction temperature of the reflux was controlled at 60 °C using a water bath for 2 h. The resultant clear solution was dried at 60°C under vacuum to remove traces of solvents in order to obtain the Docetaxel-phospholipid complex. The prepared thin layer had been kept overnight in room temperature prior to hydration. This dried film was hydrated with 10ml PBS 7.4 in a rotary at 60°C. The phytosome was finally sonicated for 4 minutes in a probe sonicator, with 60% amplitude and 5 seconds on-off interval. All phytosome was stored in the refrigerator. (16,17)

S. No.	Formulation Code	Drug: Phosphatidylcholine S100 Molar Ratio (g)	Methanol (ml)
1	F1	1:0.5	10
2	F2	1:1	10
3	F3	1:1.5	10
4	F4	1:2	10

Table 1: Composition of docetaxel loaded phytosomes formulation containing molar ratios of docetaxel and S100 Phosphatidylcholine

S. No.	Formulation Code	Drug : Phosphatidylcholine S100: Vitamin ETPGS Molar Ratio (g)	Methanol (ml)
1	F5	1:1:0.01	10
2	F6	1:1:0.03	10
3	F7	1:1:0.05	10

Table 2: Composition of coated vitamin ETPGS docetaxel loaded phytosomes formulation containing molar ratios of Docetaxel, S100 Phosphatidylcholine & Vitamin ETPGS

3.3 Evaluation of Docetaxel loaded phytosomes:

3.3.1 Visual appearance: All the batches of phytosomes and coated vitamin ETPGS phytosomes were evaluated by visual appearance. (18)

3.3.2 Optical microscopy: Optical microscopic study of docetaxel loaded Phytosomes formulation was observed under microscopy. One drop of formulation was deposited on a glass slide and it was then examined by optical Microscopy at 100x magnification. (19)

3.3.3 pH: The pH measurement of the phytosomes formulation was carried out using a digital pH meter by dipping the glass electrode completely into the phytosomes formulation system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded. (20)

3.3.4 Determination of Entrapment efficiency:

The entrapment efficiency of docetaxel loaded phytosome was determined by calculating the amount of entrapped docetaxel in the phytosomes. To determine the entrapment efficiency of docetaxel in phytosome, an appropriate amount of dispersion was transferred in culture tube. The dispersion was centrifuge for 15 min at 15000 rpm. After centrifugation the supernatant was collected and Percentage Drug Entrapment amount of free docetaxel was determined spectrophotometrically ($\lambda_{\text{max}} = 228 \text{ nm}$). The entrapment efficiency has been determined according to the following equation:

$$\text{EE \%} = \frac{W_{(\text{Added drug})} - W_{(\text{free drug})}}{W_{(\text{Added drug})}} \times 100$$

Where, W (added drug) is the amount of drug added during the preparation of phytosomes, W (free drug) is the amount of free drug measured in the lower chamber of the culture tube after centrifugation. (21)

3.3.5 Particle size and zeta potential determinations:

The particle size and zeta potential were analyzed by dynamic light scattering system spectroscopy using a Particle Size Analyzer. To perform the measurement, the sample was introduced into the disposable cell and the both particle size and zeta potential were determined. (22)

3.3.6 TEM: Coated vitamin ETPGS was analyzed using transmission electron microscope (TEM). One drop of sample was put on a carbon-coated grid sized 400nm and dried at room temperature. After completely dried, the sample was analyzed under the microscope. (23)

3.6.7 In-Vitro Drug Release Study: In vitro release kinetics of phytosome was determined in this work using dialysis method. In brief, phytosome formulation equivalent to dose or drug solution with the equivalent drug concentration was enclosed in a dialysis bag and then placed in 100 mL of phosphate buffer saline (PBS) pH 7.4 used as release media. The entire system was kept at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with continuous magnetic stirring. At selected time intervals (0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 72 hour), 3 mL of solution was withdrawn from the release medium and replenished with the same volume of release medium. The collected samples were suitably diluted and analyzed by UV-visible spectrophotometer at 228nm. (24)

3.6.8 Drug release kinetics: Model dependent methods are based on different mathematical functions, which describe the release profile. Once a suitable function has been selected, the release profiles are evaluated depending on the derived model parameters. (25,26)

The data obtained from ex vivo permeation studies were plotted in different models of data treatment as follows;

- Zero Order model
- First Order model
- Higuchi's Model
- Korsmeyer-Peppas model

I. Zero order kinetics: It can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc. In its simplest form, zero order release can be represented as:

$$Q_0 - Q_t = K_0 t$$

Where, Q_t is the amount of drug dissolved in time t , Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug permeation studies were plotted as cumulative amount of drug released versus time.

II. First order kinetics: It can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices. The release of the drug which followed first order kinetics can be expressed by the equation:

$$\log C = \log C_0 - K.t / 2.303$$

Where, C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of $K/2.303$.

III. Higuchi's Model: This model expected to pronounce drug release from a matrix system. Primarily regarded for planar systems, it was then extended to different geometrics and porous systems. This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible), (iii) drug particles are much smaller than system thickness, (iv) matrix swelling and dissolution are negligible, (v) drug diffusivity is constant, and (vi) perfect sink conditions are always attained in the release environment.

Higuchi was the first to derive an equation to describe the release of a drug from an insoluble matrix as the square root of a time-dependent process based on Fickian diffusion. Simplified Higuchi equation is following;

$$Q_t = K_H (t)^{0.5}$$

Where, Q_t is the amount of drug released in time t and K_H is the release rate constant for the Higuchi model. When the data is plotted as cumulative drug released versus square root of time, it yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to ' K_H '.

IV. Korsmeyer-Peppas Model: Korsmeyer derived a simple relationship which described drug release from a polymeric system. (27) The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer et al.

$$Q = K.t^n$$

Where, Q is the percentage of drug released at time 't' K is a kinetic constant incorporating structural and geometric characteristics of the tablets and 'n' is the diffusional exponent indicative of the release mechanism.

For Fickian release, $n=0.45$ while for anomalous (Non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero order release, $n = 0.89$. The Korsmeyer-Peppas model was plotted between log cumulative % drug releases versus log time.

4.0 RESULTS & DISCUSSION:

4.1 Result of Preformulation studies:

4.1.1 Organoleptic properties: The organoleptic properties of Docetaxel was found to be (28) colour (white), form (amorphous) & taste (bitter) . (28)

4.1.2 Melting Point:

The melting point of docetaxel was found to be in range 230-232°C which is of the pure drug. Hence drug sample was free from any type of impurities. (29)

4.1.3 UV Spectroscopy:

4.1.3.1 Determination absorption maxima by UV spectroscopy of Docetaxel:

The result of UV spectrum of Docetaxel is shown in **Fig.1.** (30)

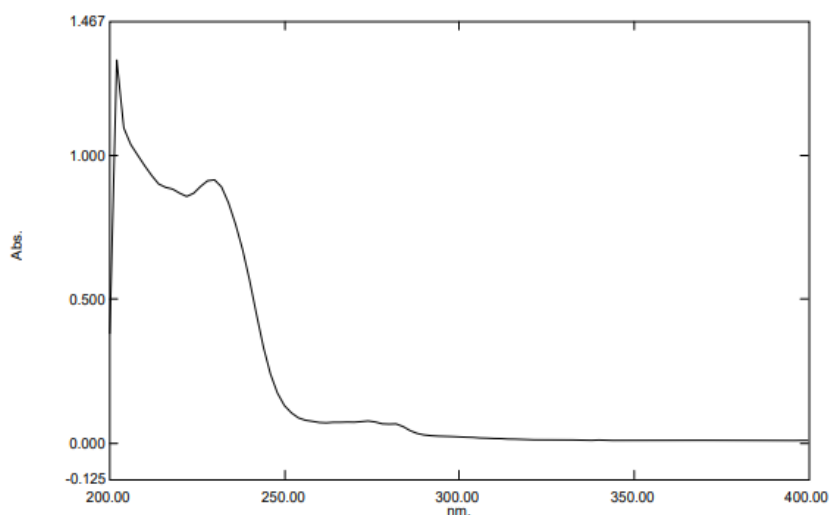


Fig.1: UV Spectrum of Docetaxel in methanol

The maximum wavelength of Docetaxel was observed at 228 nm.

4.1.3.2 Preparation of Standard Calibration Curve of Docetaxel in methanol

Sr.No.	Concentration($\mu\text{g/ml}$)	Absorbance $\pm$ SD
1	4	0.111 $\pm$ 0.002
2	8	0.219 $\pm$ 0.001
3	12	0.329 $\pm$ 0.001
4	16	0.453 $\pm$ 0.001
5	20	0.560 $\pm$ 0.001
6	24	0.663 $\pm$ 0.001
7	28	0.784 $\pm$ 0.001
8	32	0.902 $\pm$ 0.002

Table 3: Calibration Curve of Docetaxel in methanol

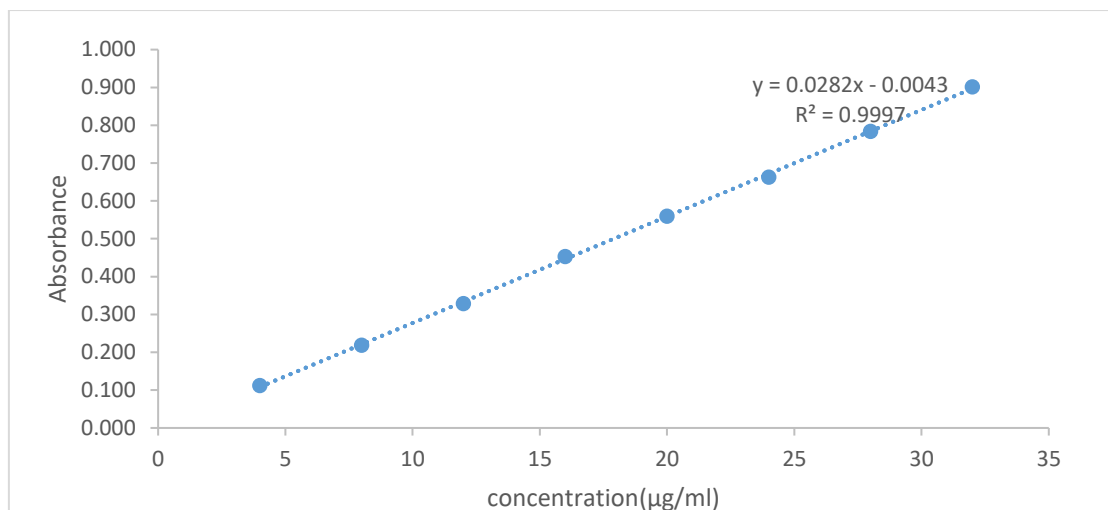


Fig.2: Graph of Standard calibration curve of Docetaxel in methanol

The calibration curve for Docetaxel was obtained by using the 4 to 32 µg/ml concentration of Docetaxel in methanol. The absorbance was measured at 228nm. The calibration curve of Docetaxel as shown in graph indicated the regression equation $Y=0.0282x-0.0043$ and R^2 value 0.9997, which shows good linearity as shown in **Table 3** and **Fig.2**, respectively.

4.1.4 Solubility studies:

Solubility of drug in various solvents, were carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 228 nm.(31)

Sr.No.	Solvent	Solubility (mg/ml)
1	Methanol	33.615±0.054
2	Ethanol	30.849±0.054
3	Chloroform	27.28±0.035
4	Water	7.351±0.035
5	PBS 7.4	6.393±0.035

Table 4: Solubility studies of Docetaxel for different solvents

From the above data, it is clearly seen that Docetaxel is highly soluble in methanol, ethanol and chloroform shown in **Table 4**.

4.1.5 Partition coefficient determination (32):

The partition coefficient of Docetaxel in n-octanol: water was found to be 2.687 ± 0.002 this indicates that the drug is lipophilic in nature. The partition coefficient of Docetaxel as shown in **Table 5**.

Table No.5: Partition coefficient determination of Docetaxel

Partition coefficient of drug	Solvent system	Log P Values
Docetaxel	n-octanol:water	2.687 ± 0.002

Value is expressed as mean $\pm$ SD: n=3

4.1.6. FTIR Studies:

4.1.6.1 FTIR spectrum of Docetaxel (33) :

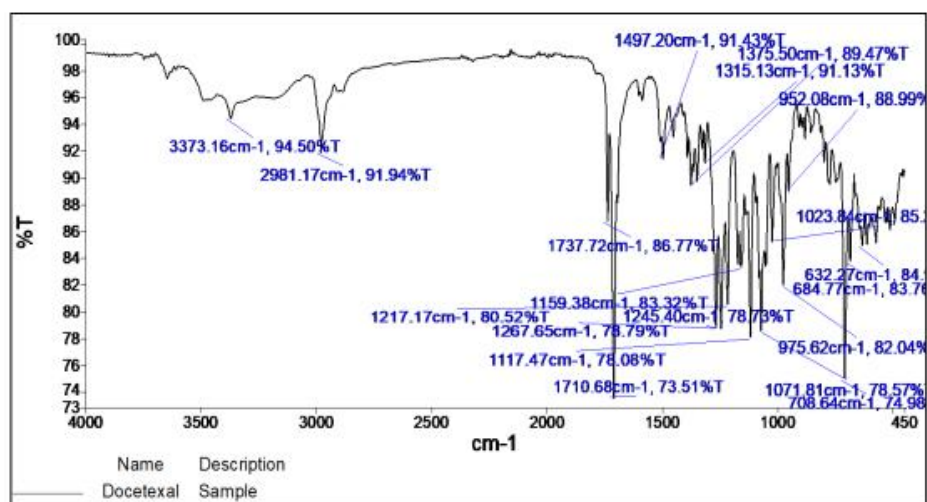


Fig.3: FTIR spectrum of Docetaxel

The FTIR spectra of Docetaxel were shown in the **Fig.3**. The spectra of DTX shows characteristic peaks at 3373.16 cm^{-1} and 2981.17 cm^{-1} which may be ascribed to N H stretching and O H stretching of alkanes, respectively. Peaks at 1737 cm^{-1} and 1710 cm^{-1} can be attributed to C O stretching. This observation confirmed the purity and authenticity of the Docetaxel.

4.1.6.2 FTIR spectrum of Phosphatidylcholine S100 (34):

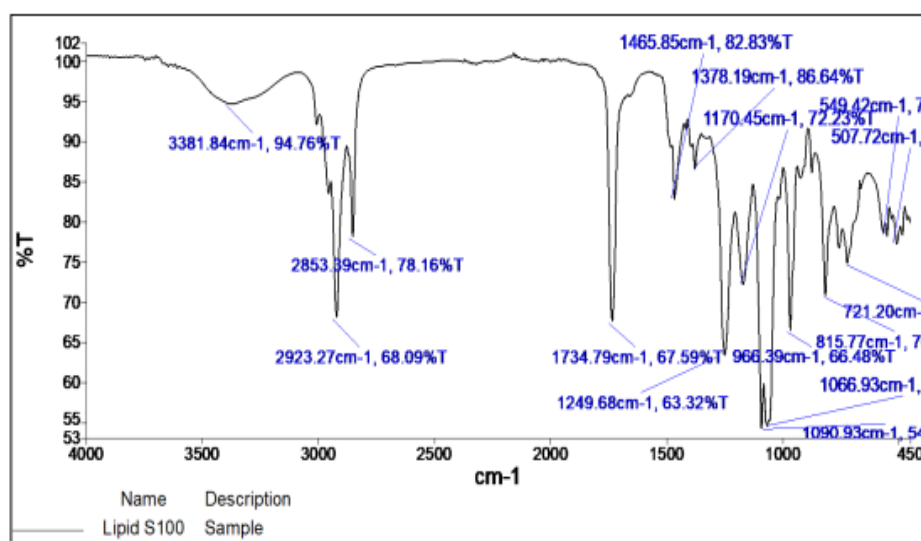


Fig.4: FTIR spectrum of Phosphatidylcholine S100

The FTIR spectra of phosphatidylcholine S100 were shown in the **Fig.4**. The principal IR absorption peaks of phosphatidylcholine S100 at 2923.27 and 2853.39 cm^{-1} (C–H stretching band of long fatty acid chain), 1734.79 cm^{-1} (Carbonyl stretching band in the fatty acid ester), 1249.68 cm^{-1} (P=O stretching band), 1090.93 cm^{-1} (P–O–C stretching band) and 966.39 cm^{-1} ($\text{N}^+(\text{CH}_3)_3$ stretching) were all observed in the spectra of phosphatidylcholine S100. These observed principal peaks. This observation confirmed the purity and authenticity of the phosphatidylcholine.

4.1.6.3 FTIR spectrum of vitamin ETPGS (35):

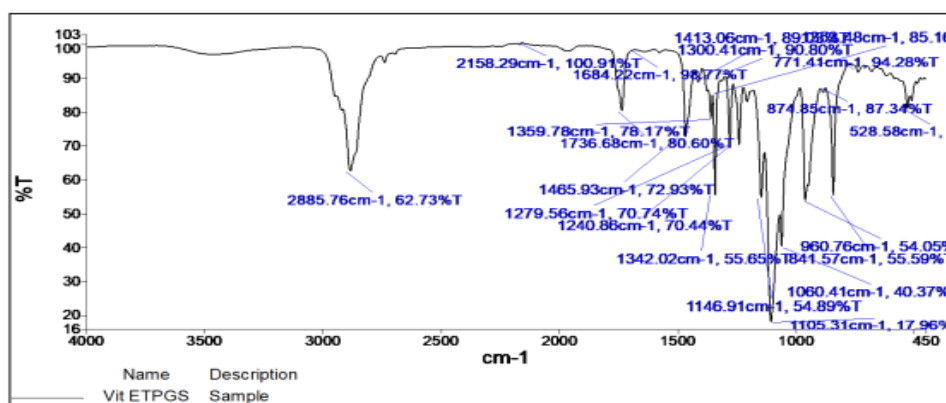


Fig.5: FTIR spectrum of vitamin ETPGS

The FTIR spectra of vitamin ETPGS were shown in **Fig.5**. The absorption peaks of vitamin ETPGS observed at 1736.68 cm⁻¹ Carbonyl band (-C=O). Overlapping of the -CH Stretching band of TPGS observed at 2885.76 cm⁻¹. The peaks at 1146.91 cm⁻¹ & 1240.88 cm⁻¹ were observed for (-C-O-) stretching of TPGS. The peaks for -C-C- stretching in aromatic ring was appeared at 1465.93 cm⁻¹. The peaks of -CH₂ group of PEG chain was observed at 1359.78 cm⁻¹.

4.1.6.4: FTIR spectrum of physical mixture

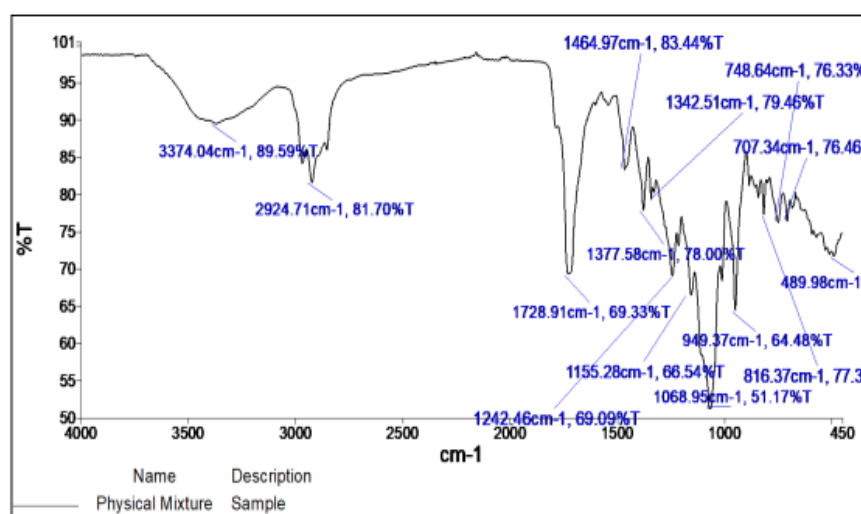


Fig.6: FTIR spectrum of physical mixture

The FTIR spectra of physical mixture studies were shown in **Fig.6**. were carried out to eliminate the possibility of interaction between drug and excipients used with analytical method of drug estimation. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipients peaks. Hence no interaction was observed in this mixture.

4.2 Evaluation of Docetaxel loaded phytosomes formulation:

4.2.1 Visual appearance:



Fig.7: Docetaxel loaded Phytosome Formulation

Visual Appearance		
Sr.No.	Formulation code	Appearance
1	F1	Milky white homogenous dispersion with no phase separation
2	F2	Milky white homogenous dispersion with no phase separation
3	F3	Milky white homogenous dispersion with no phase separation
4	F4	Milky white homogenous dispersion with no phase separation

Table 6: Composition of Docetaxel loaded Phytosome Formulation

Freshly prepared docetaxel loaded phytosome formulations, were found to be milky white homogenous dispersion with no phase separation as shown in Fig.7 & Table 6.

4.2.2 Optical Microscopy:



Fig.8: Optical microscopy of Docetaxel loaded phytosome

The optical microscopy of docetaxel loaded phytosomes was uniform, regular and rigid vesicles were observed in optical microscopic view as shown in Fig.8.

4.2.3 pH of Docetaxel loaded phytosome:

Sr. No.	Formulation Code	pH $\pm$ S.D
1	F1	7.44 $\pm$ 0.01
2	F2	7.45 $\pm$ 0.01
3	F3	7.43 $\pm$ 0.006
4	F4	7.43 $\pm$ 0.01

Table 7: pH of Docetaxel loaded phytosome

The pH values of all formulations was found in range of 7.44 $\pm$ 0.01 to 7.43 $\pm$ 0.01 respectively as shown in Table 7.

4.2.4 Drug Entrapment Efficiency: Percentage Drug Entrapment Efficiency of all formulation was given in a Table 6.

S. No.	Formulation Code	Entrapment Efficiency (%)
1	F1	72.948 $\pm$ 0.0443
2	F2	87.93 $\pm$ 0.0443
3	F3	82.64 $\pm$ 0.0677
4	F4	76.228 $\pm$ 0.0443

Table 8: Percentage Entrapment efficiency of docetaxel loaded Phytosomes formulation

From the Table 8. It was found that percentage drug entrapment of all formulation was found to be in range of 72.948 $\pm$ 0.0443% to 87.93 $\pm$ 0.0443%. These results explain that there is a significant effect on percentage entrapment efficiency of phytosome was observed with lipid concentration increased. On the basis of percentage entrapment, F2 was selected as optimized formulation. Thus it has been carried further study.

4.3. Evaluation of coated vitamin ETPGS Docetaxel loaded phytosomes:

4.3.1 Visual appearance:

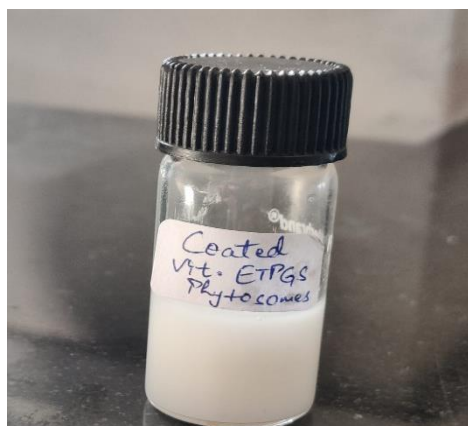


Fig .9: Coated vitamin ETPGS Docetaxel loaded Phytosome Formulation

Visual Appearance		
Sr.No.	Formulation code	Appearance
1	F5	Milky white homogenous dispersion with no phase separation
2	F6	Milky white homogenous dispersion with no phase separation
3	F7	Milky white homogenous dispersion with no phase separation

Table 9: Percentage Entrapment efficiency of docetaxel loaded Phytosomes formulation

Freshly prepared coated vitamin ETPGS Docetaxel loaded phytosome formulation, was found to be milky white homogenous dispersion with no phase separation as shown in **Fig.9 & Table 9**.

4.3.2 Optical microscopy: The optical microscopy of coated vitamin ETPGS Docetaxel loaded phytosome of formulation was determined by optical microscope at 100X magnification .

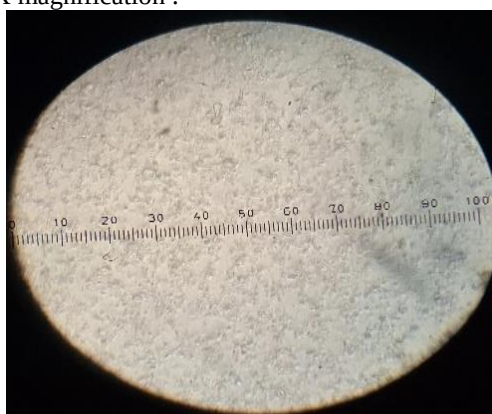


Fig.10: Coated vitamin ETPGS Docetaxel loaded phytosome Formulation

The optical microscopy of coated vitamin ETPGS Docetaxel loaded phytosomes was uniform, regular and rigid vesicles were observed in optical microscopic view as shown in **Fig.10**.

4.3.3 pH of coated vitamin ETPGS Docetaxel loaded phytosomes:

Sr. No.	Formulation code	pH $\pm$ S.D
1	F5	7.39 $\pm$ 0.006
2	F6	7.41 $\pm$ 0.01
3	F7	7.40 $\pm$ 0.01

Table 10: pH of coated vitamin ETPGS Docetaxel loaded phytosomes

The pH values of all formulations was found in range of 7.39 ± 0.006 to 7.4 ± 0.01 respectively as shown in **Table 10**.

4.3.4 Percentage drug entrapment of coated vitamin ETPGS Docetaxel loaded phytosomes:

The Percentage Drug Entrapment Efficiency of all formulation was given in a **Table 10**

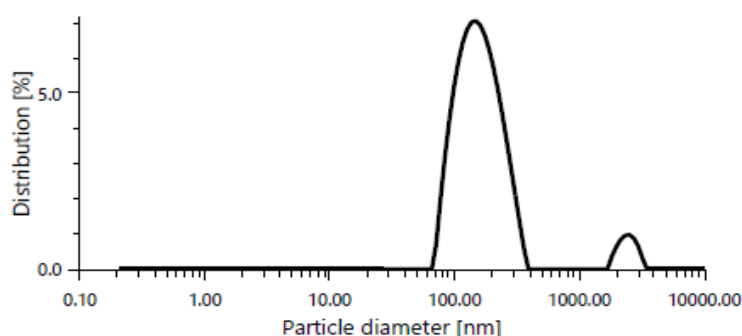
S.No.	Formulation code	Entrapment efficiency%
1	F5	79.773 ± 0.0443
2	F6	86.6 ± 0.0443
3	F7	65.53 ± 0.0677

Table 11: Percentage Entrapment efficiency of coated vitamin ETPGS Docetaxel loaded phytosomes:

From the **Table 11**, it was found that percentage drug entrapment of all formulation was found to be in range of $79.773 \pm 0.0443\%$, $86.6 \pm 0.0443\%$ and $65.53 \pm 0.0677\%$. These results explain that there is a significant effect on percentage entrapment efficiency of phytosome was observed with lipid concentration increased. On the basis of percentage entrapment, F6 was selected as optimized formulation. Thus it has been carried further study.

4.3.5 Particle size and zeta potential determinations Size:

Particle size distribution (intensity)



Results

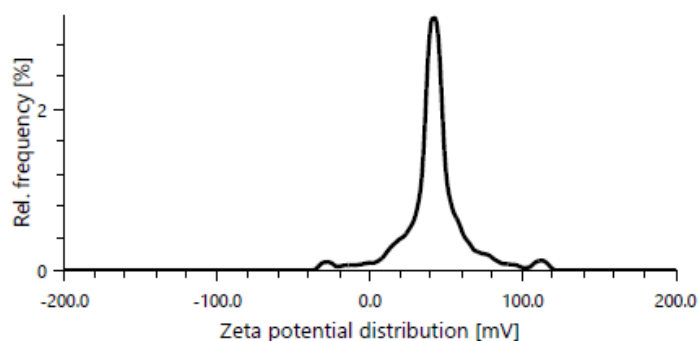
Hydrodynamic diameter	166.23 nm	Mean intensity	305.3 kcounts/s
Polydispersity index	18.7 %	Absolute intensity	323119.3 kcounts/s
Diffusion Coefficient	$3.0 \mu\text{m}^2/\text{s}$	Intercept $g1^2$	0.8547
Transmittance	57.4 %	Baseline	1.017

Fig. 11: Particle size peak of coated vitamin ETPGS Docetaxel loaded phytosome formulation

Particle size peak of coated vitamin ETPGS Docetaxel loaded phytosome formulation was 166.23nm with PDI 305.3.

4.3.6 Zeta Potential:

Zeta potential distribution



Results

Mean zeta potential	40.3 mV	Mean intensity	482.8 kcounts/s
+/- Standard deviation	0.8 mV	Filter optical density	3.7624
Distribution peak	41.9 mV	Conductivity	2.147 mS/cm
Electrophoretic mobility	3.1416 $\mu\text{m}^2\text{cm/Vs}$	Transmittance	57.8 %

Fig.12: Zeta potential peak of coated vitamin ETPGS Docetaxel loaded phytosome formulation

Zeta potential peak of coated vitamin ETPGS docetaxel loaded phytosome formulation was 40.3mV with SD 0.8mV.

4.3.7 TEM

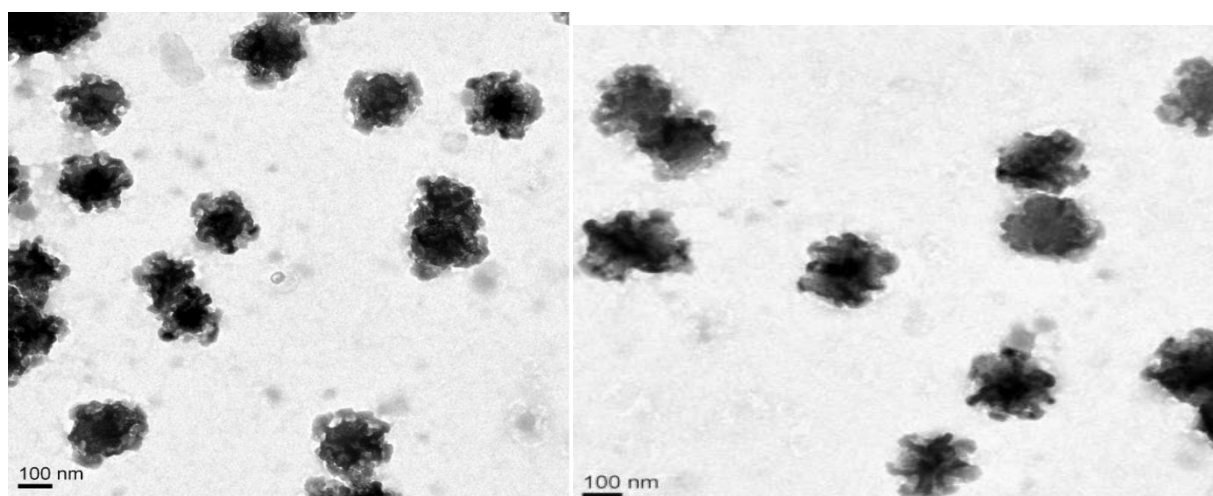


Fig.13: TEM of coated vitamin ETPGS Docetaxel loaded phytosome formulation

Coated vitamin ETPGS Docetaxel loaded phytosomes, was examined by using (TEM) transmission electron microscopy was shown as discrete spherical structures without aggregation in **Fig.13**. It was observed that the particle sizes were of uniform shape with narrow size distributions (average 100 nm).

4.3.8 FTIR of formulation:

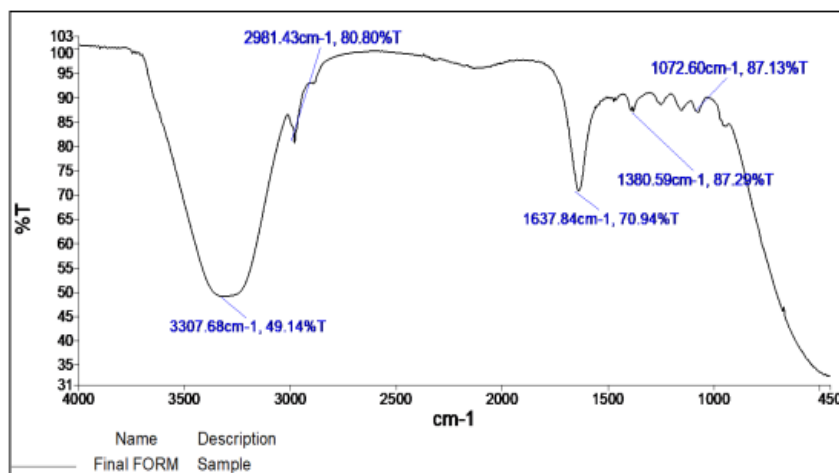


Fig.14: FTIR spectrum of formulation

Spectrum of formulation (F6) peak were observed at 3307.68cm^{-1} (N-H stretching), at 2981.43cm^{-1} (O-H stretching), at 1380.59cm^{-1} (P=O stretching band), at 1072.60cm^{-1} (P-O-C stretching band). The FTIR spectra of final formulation are maintained pure drug peaks with slighting shifting **Fig. 14**.

4.4 In-vitro drug release study:

The In-vitro % drug release of formulation and pure drug was given in a **Table 12**

Time(hr)	% Drug release of F6	% Drug release of Pure Drug
0	0	0
0.5	3.720 ± 0.271	0.056 ± 0.009
1	8.951 ± 0.185	0.077 ± 0.005
2	13.413 ± 0.235	0.086 ± 0.005
3	17.993 ± 0.051	0.095 ± 0.005
4	20.594 ± 0.000	0.103 ± 0.005
6	24.672 ± 0.266	0.115 ± 0.005
8	27.863 ± 0.089	0.242 ± 0.009
10	31.853 ± 0.235	0.366 ± 0.009
12	33.853 ± 0.089	0.543 ± 0.009
24	41.427 ± 0.089	1.120 ± 0.009
36	46.746 ± 0.089	2.140 ± 0.009
48	51.563 ± 0.358	3.107 ± 0.011
72	62.172 ± 0.177	4.152 ± 0.018

Table 12: In-vitro drug release study of % Drug release of formulation (F6) and Pure Drug

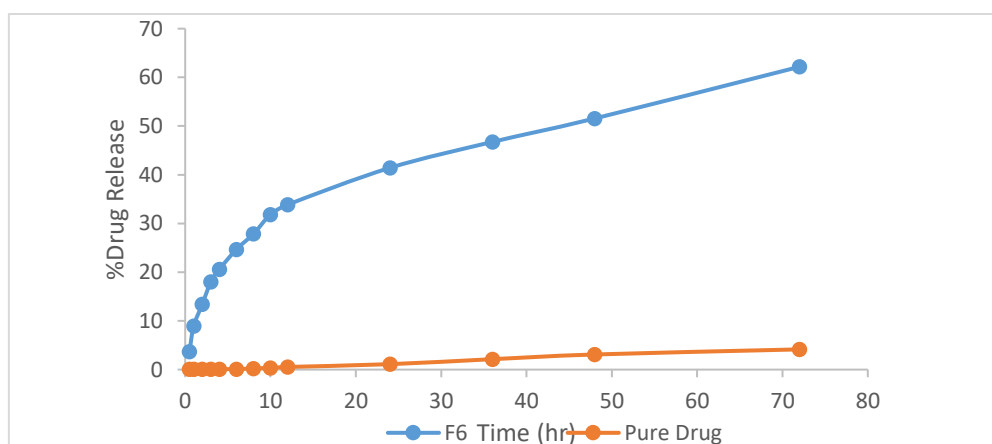


Fig. 15 In-vitro drug release study of formulation (F6) and pure drugs

Drug release graphs for pure drug & formulation (F6) was shown in **Fig.15 and Table 11** were significantly different from the profile of drug alone. In the pure drug solution, % drug release was released within 2hr 0.086%. On the other hand, the release of formulation (F6) was considerably reduced with a maximum 13.413% release within 2hr. After that drug was released upto 1.120% within 24 hr release. After that formulation was released with a maximum upto 41.427 % within 24 hr release. Then, after that the drug was released upto 4.152% within 72 hr release and the release of formulation (F6) was considerably reduced with a maximum 62.172 % within 72 hr release followed by sustained manner.

4.4.1 In vitro drug release kinetic study:

- Zero order

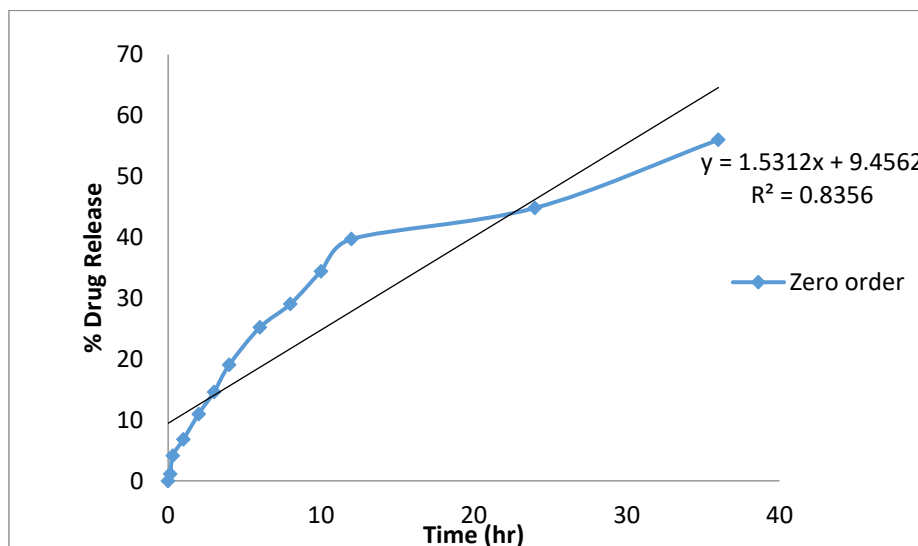


Fig. 16 Zero order graph for coated vitamin ETPGS docetaxel loaded phytosome formulation

- First Order

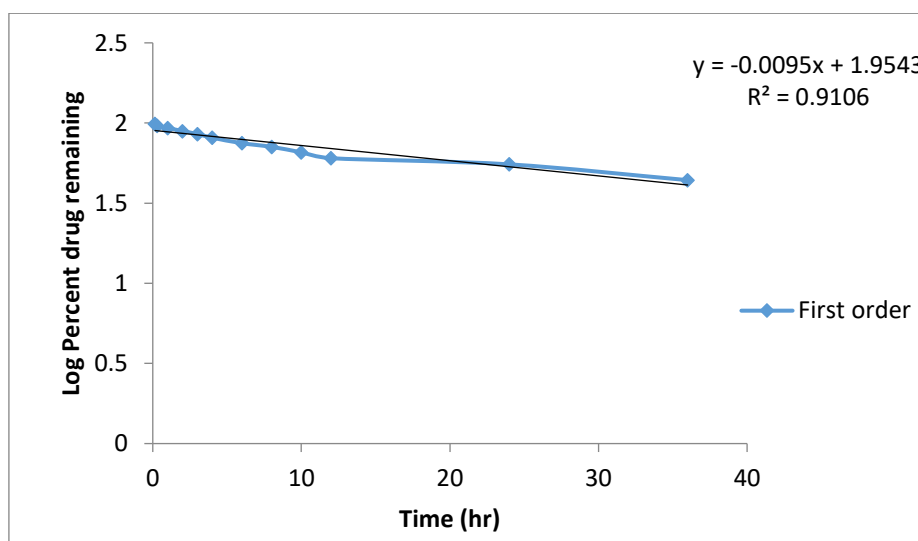


Fig.17 First order graph for coated vitamin ETPGS docetaxel loaded phytosome formulation

- Higuchi order

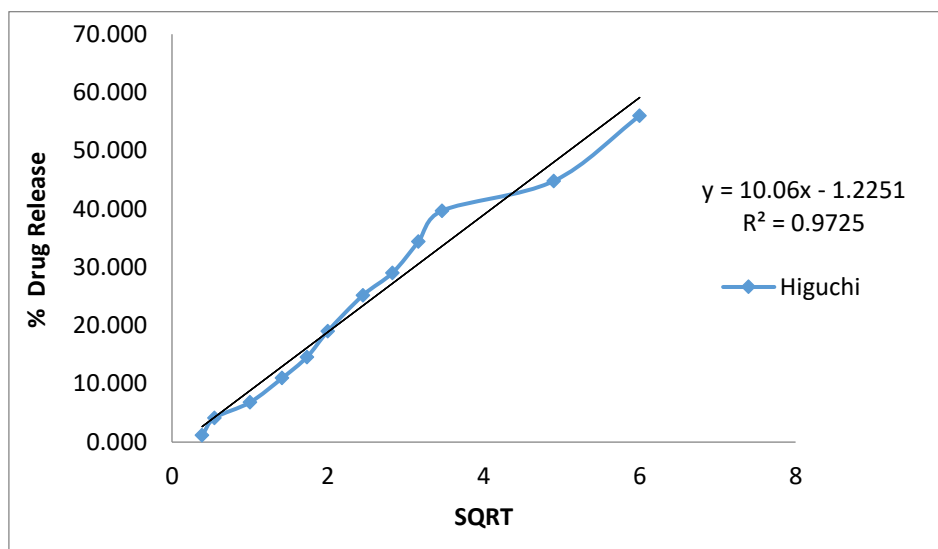


Fig. 18 Higuchi order graph for coated vitamin ETPGS docetaxel loaded phytosome formulation

- Korsmeyerpeppas:

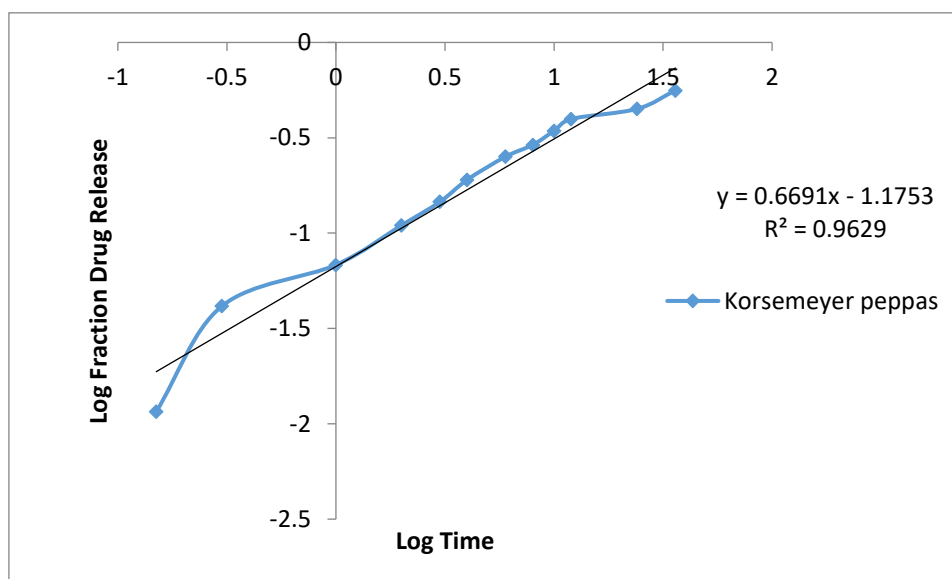


Fig. 19 Korsmeyerpeppas graph for coated vitamin ETPGS docetaxel loaded phytosome formulation

Table 13: Kinetic equation parameter of formulation F6

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	R ²	K ₀	R ²	K ₀	R ²	K ₀	R ²	K ₀
F6	0.8356	1.5312	0.9106	0.0095	0.9725	10.06	0.9629	0.6691

Mathematical models are commonly used to predict the release mechanism and compare release profile. For all the optimized formulations, the % drug release vs time (zero order), log percent drug remaining vs time (first order), log per cent drug release vs square root of time (Higuchi plot), and log of log % drug release vs. log time (Korsmeyer and PeppasExponential Equation) were plotted. In each case, R^2 value was calculated from the graph and reported in **Table 13** and **Figure 19**. Considering the determination coefficients, Higuchi model was found ($R^2=0.9725$) to fit the release data best. It could be concluded from the results that the drug was released from coated vitamin ETPGS docetaxel loaded phytosome by a sustain mechanism.

CONCLUSION:

Any drug delivery system's purpose is to deliver a therapeutic dose of drug to the right location in the body, as well as to attain and maintain the target drug plasma concentration for a particular period of time. Inadequate drug release, shorter dosage form residence time in the gastrointestinal tract, and a strong hepatic first pass impact all contribute to lower bioavailability. Because of the limits of traditional dosage forms, a new era of controlled and unique drug delivery systems has emerged. Docetaxel (DTX) is a powerful chemotherapeutic drug that has been used to treat a variety of malignancies. However, due to the use of an organic solvent in the injection and its low selectivity for tumour cells, DTX clinical chemotherapy has a number of negative side effects. With the advancement of pharmaceutical technologies, a lot of work has gone into developing novel DTX formulations to address these issues. Drug-phospholipid complexes increase the bioavailability of medicines with a low lipid solubility or a low water solubility. Pharmacokinetic and pharmacological parameters have been enhanced by phytosomes. Because of their increased ability to permeate lipid-rich biomembranes and eventually reach the blood, phytosomes have a higher bioavailability than their active pharmaceutical ingredient. Phospholipids are phospholipids. It's an important part of the phytosomes process. Phospholipids are used as digestion aids and carriers for both water and lipid soluble nutrients. As a result, drugs can be complexed into phytosomal complexes to improve biopharmaceutical characteristics and increase medication absorption in the human body. Drug-phospholipid complexes have the following advantages in addition to increasing drug absorption: they can potentially improve drug entrapment and provide medications with a longer duration of action.

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