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Formulation and Evaluation of Gemcitabine HCl Loaded Phytosomes for Oral Drug Delivery

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Akanksha Choudhary*, Manjit Kaur, Prerna Upadhyay, Shaina Kujar, Nitan Bharti Gupta, Rajni Sharma

Sri Sai College of Pharmacy, Pathankot

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ABSTRACT

The objective of this study was to prepare the Gemcitabine HCL - phospholipid(S-100) complex (GEM HCL - PC) to enhance the oral bioavailability of GEM HCL. It is an antimetabolite prodrug. Gemcitabine Hydrochloride is a BCS class III drug of choice in the treatment of cancer, as a single or in combination chemotherapy. A novel phospholipid complex of GEMHCL was successfully prepared by the solvent evaporation method and optimized. Physical interaction of GEM HCL with phospholipid was evaluated byFT-IR, UV spectroscopy, water/n-Octanol solubility, partition co-efficient. TEM images of a diluted aqueous dispersion of GEM HCL -PC showed identified, spherical and discreet having large internal aqueous space. The percent entrapment efficiency of formulations was around 40% to 69%. In vitro release profile studies showed that around 87%–94% of the drug was released from formulations by using a dialysis bag method. This study aimed to develop an oral delivery system of gemcitabine HCL (GEM HCL) to supply a sustained-release profile, to prolong residence time, and to reinforce its efficiency in the treatment of cancer.

INTRODUCTION

Several novel drug delivery systems are emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery by encapsulation of the drug in circulation which reduces the tonicity and selective uptake of the drug. [1]

Most of the medicaments are administered by oral route due to high patient compliance. However, the ideal drug delivery system needs two basic components. Firstly, the drug should be delivered at a predetermined rate for a prolonged time, for the prevention of fluctuation in plasma concentration. [2] Secondly, the drug should bind solely to its selective receptor. Hence the time demands modification in conventional dosage form for the betterment of therapeutic efficacy and drug safety. [3] Manufacturing sustained release or controlled release drug delivery systems can help overcome the above-mentioned drawbacks. [4]

Since ancient times the therapeutic uses of traditional medicines and phytomedicines have proved very popular for health maintenance by various means. [5]

The term ‘Phyto’ means plant while ‘Some’ means cell-like. The phytosome is a unit of a few molecules bonded together. [6] Phytosome is a vesicular drug delivery system in which phytoconstituents of herb extract surround and bound by lipid. Phytosome technology may breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, without compromising nutrient safety. [7]

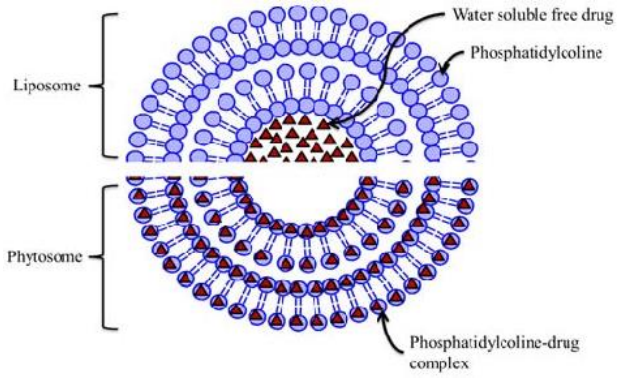

Figure no. 1: structure of phytosome

Gemcitabine Hydrochloride is that the hydrochloride salt of an analog of the antimetabolite nucleoside deoxycytidine with antineoplastic activity. Gemcitabine is converted

intracellularly to the active metabolites difluorodeoxycytidine di- and triphosphate (dFdCDP, dFdCTP). dFdCDP inhibits ribonucleotide reductase, thereby decreasing the deoxynucleotide pool available for DNA synthesis; dFdCTP is incorporated into DNA, leading to DNA strand termination and apoptosis. [8]

In the present study, we demonstrated the formation of a molecular complex of phospholipid with a water-soluble drug. The aim is to develop a molecular complex of phospholipid(S-100) and GEM HCL a sustained delivery platform by promoting lipid solubility and thus, plasma stability of drug molecules. The GEM HCL-PC was prepared by a solvent evaporation method. This formulation was further suitably characterized by different techniques like FT-IR spectroscopy, UV spectroscopy, water/n-Octanol solubility, partition coefficient, TEM (Transmission electron microscopy). In-vitro drug release kinetic study for GEM HCL-PC was also evaluated. We anticipate that the phospholipid complex of GEMHCL may be a useful platform for developing a new drug delivery system with sustained release behavior.

MATERIALS AND METHODS

MATERIALS

Gemcitabine HCL (received as a gift sample from Mac-Chem Products Pvt. Ltd., Mumbai, India), Soya phosphatidylcholine (Lipoid S 100) (received as a gift sample from Lipoid GmbH, Germany), Methanol were HPLC grade.

METHODS

Determination of Absorption Maxima of Gemcitabine HCL

Absorption maxima (λ max) of the drug were determined by UV Spectrophotometer (Shimadzu Pharma. Spec 1800). The stock solution was prepared by dissolving 10mg/ml in 10ml of water and then pipette out 5ml from the stock solution and make up the volume 10ml, the primary stock solution was prepared. Appropriate dilutions scanned for determining λ max from 200-400 nm through a UV spectrophotometer. [9]

Preparation of Calibration Curve of Gemcitabine-HCL in water

Stock solution was prepared by dissolving 10mg drug in 10ml of water. The primary stock solution was prepared by pipette out 5ml from the stock solution and make volume up to 50ml. Make dilutions in the range of 0.5µg/ml to 4µg/ml were prepared from the primary stock solution. Dilutions were scanned for determining λ max from 200-400nm through a UV spectrophotometer. [10]

Partition Coefficient of Drug

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

Shake flask method

The partition coefficient study was performed by using the shake flask method. Excess amounts of the drug dissolved in 5 ml of two solvents (n-octanol: Water) together (1:1) and placed for 24 hrs. After 24 hrs, the two layers were separated with the help of separating funnel and centrifuge for 15 minutes at 15,000 rpm. The absorbance was taken in a UV spectrophotometer at the respective λ max after appropriate dilution. [11]

Compatibility studies

Were performed by FT-IR of drug GEM HCL and lipid, and then interpretation was done to check compatibility. [12]

Preparation of Gemcitabine HCL loaded Phytosome

GEM HCL-PC was prepared by the solvent evaporation method with slight modifications. Briefly, GEM and Lipoid were co-dissolved in the selected ratio in 10 ml of optimized solvent and refluxed at 60°C for 4 hr. The solvent was then evaporated using a rotary

evaporator to get the GEM HCL-PC and then dried under vacuum overnight to remove traces of solvents. The resultant complex was stored in an airtight container at below 20°C. Then hydrate the mixture to break the layer. After that, the solution was centrifuged at 18,000 rpm for 15 min. by placed in a cooling centrifuge, then was suitably diluted and determined photometrically. [13]

Evaluation of phytosome formulation

Transmission Electron Microscopy (TEM) Analysis

Distribution of gemcitabine-loaded phytosome was observed under Transmission Electron Microscopy. One drop of diluted gemcitabine-loaded phytosome dispersion was deposited on a film-coated copper grid and it was stained with one drop of 2% (w/v) aqueous solution of phosphotungstic acid. Excess of the solution was drained off with a filter paper and then the grid was allowed to dry for contrast enhancement. The sample was then examined by Transmission Electron Microscopy. [14]

Particle Size Determination

The mean particle size distribution of selected phytosomal formulation phytosomes was determined by photon correlation spectroscopy technique. The particle size and zeta-potential of BPC were determined at 25ºC using photon correlation spectroscopy. Proper dilution of the suspension was prepared with double-distilled water before each analysis. [15]

FTIR Spectroscopy

Fourier transform infrared Spectroscopies of different compounds were performed for the identification of that particular compound. FT-IR Spectroscopy of optimized formulation was done using KBr pellets. [16,17]

Entrapment efficiency

The entrapment efficiency was studied using the dialysis method (candy method). 1ml of GEM HCL-lipid dispersion was filled in a dialysis bag, respectively. The bags were placed in 50 mL phosphate-buffered (pH 6.8) and shaked in platform shaker (100 rpm, 37 ◦C). At fixed time intervals, 1 mL was withdrawn from the release medium and GEM. HCL concentration was analyzed by UV spectrophotometry. All the operations were carried out in triplicate. The

cumulative drug released was calculated as the ratio of drug released at each time points to that of the initial used. [18]

Solubility

Apparent solubility was determined by adding an excess of GEM. HCL and GEM. HCL lipid complex to 5ml of water or n-octanol in sealed glass containers at room temperature (25-30º C). The liquid was agitated for 24 hours then centrifuged for 15 min. at 15,00 rpm. The supernatant was filtered through a 0.2 m membrane filter. Then 1 ml filtrate was mixed with 9 ml of distilled water and n-octanol to prepare suitable dilution and the samples were analyzed by spectrophotometrically. [19, 20]

***In-vitro* drug release studies**

For this purpose, a dialysis bag (Molecular weight cutoff 10,000 Da) method was used wherein 1ml of phytosome suspension was taken inside the dialysis bag which was then tied from both ends. This bag containing phytosomes was then placed in a beaker containing 50ml of release media having pH 6.8. These beakers were placed on magnetic stirrer maintained at 37°C/100 rpm. During incubation, gemcitabine was released from the phytosomes and crosses the dialysis membrane to reach into the external media. At regular time intervals, 1 ml of external release medium was withdrawn and replaced with the same amount of fresh medium. The amount of the drug released was then analyzed in the samples by using the UV method and the percent cumulative release was plotted v/s time. To elucidate the mechanism of gemcitabine release form the phytosome, the release curves were fitted into different kinetic models such as zero order, first order. Higuchi model, Hixon-Crowell, and Baker Lonsdale model. Best goodness-of-fit was determined from the linear regression co-efficient obtained from the model fitting of the data. [21]

Drug release kinetic studies [22]

To know the release kinetics, the data obtained from the in-vitro release profile was fitted into various models like:

- Zero-order kinetic model: cumulative percent drug release v/s time
- First-order kinetic model: log cumulative percent drug remaining v/s time

- Higuchi's model: Cumulative percent drug release v/s square root of time
- Korsmeyer – Peppas model: log cumulative percent drug release v/s log time

Zero-order kinetics

It describes the system in which the drug release rate is independent of its concentration.

$$Q_t = Q_0 + K_{0t} \text{ ..... (1)}$$

Where Qt = Amount of drug dissolved in time t

Q0 = Initial amount of drug in the solution, which is often 0

K0 =Zero order release constant

If the release pattern obeys zero-order, then the plot of Qt v/s t will give a straight line with a slope of K0 and an intercept at 0.

First-order kinetics

It describes the drug release from the systems in which the release rate is concentration-dependent.

$$\log Q_t = \log Q_{0 + kt/2.303} \text{ ..... (2)}$$

Where,

Qt = Amount of drug released in time t

Q0 = Initial amount of drug in the solution

K = First order release constant

If the release pattern obeys first order, then the plot of log (Q0-Qt) v/s t will be a straight line with a slope of kt/2.303 and an intercept at t = log Q0.

Higuchi model

According to this model, the fraction of drug from the system is proportional to the square root of time.

$$M_t/M_\alpha = kH_{t1/2} \ldots\ldots\ldots (3)$$

Where,

Mt & Mα = Cumulative amounts of drug release at time t and infinity

kH = Higuchi dissolution constant (reflects formulation characteristics)

If the Higuchi model of drug release is obeyed, then a plot of Mt/M ∞ v/s t ½ will be a straight line with a slope of a.

Korsmeyer – Peppas model (power-law)

The power law describes the drug release from the polymeric system in which the release deviates from Fickian diffusion. It is expressed using the following equations:

$$M_t/M_\alpha = k_{tn} \ldots\ldots\ldots (4)$$

$$\log [M_t/M_\alpha] = \log k + n \log t \ldots\ldots\ldots (5)$$

Where,

Mt & Mα = Cumulative amounts of drug release at time t and at infinity

k = Constant incorporating structural and geometrical characteristics of the system

n = Exponent determining the mechanism of drug release to characterize the release mechanism, the dissolution data (Mt/Mα < 0.6) are evaluated.

Stability studies

Formulation stability was studied by storing samples at three different temperatures and relative humidity (RH; 5°C±2°C, 25°C±5°C/60% RH, 40°C±5°C/75% RH). They were inspected visually for organoleptic properties and the formulation was analyzed over 6 months for entrapment efficiency, solubility. [25]

RESULTS AND DISCUSSION

Determination of absorption maxima in water

Absorption maxima of Gemcitabine-HCL were found to be at 268 nm similar to literature as shown in **Figure 2. [3]**

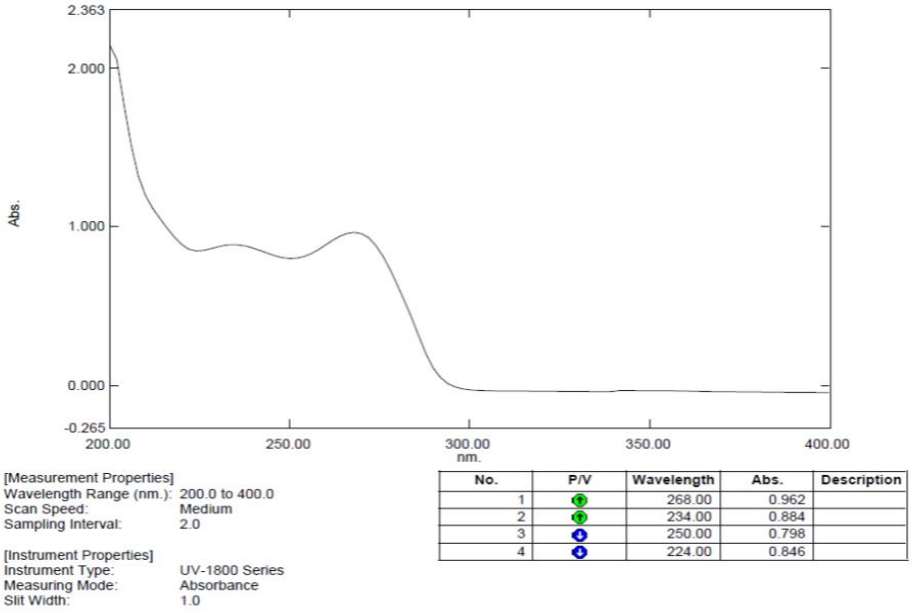

Figure No. 2: UV spectrum of Gemcitabine-HCL in water

Preparation of standard curve of Gemcitabine-HCL in water

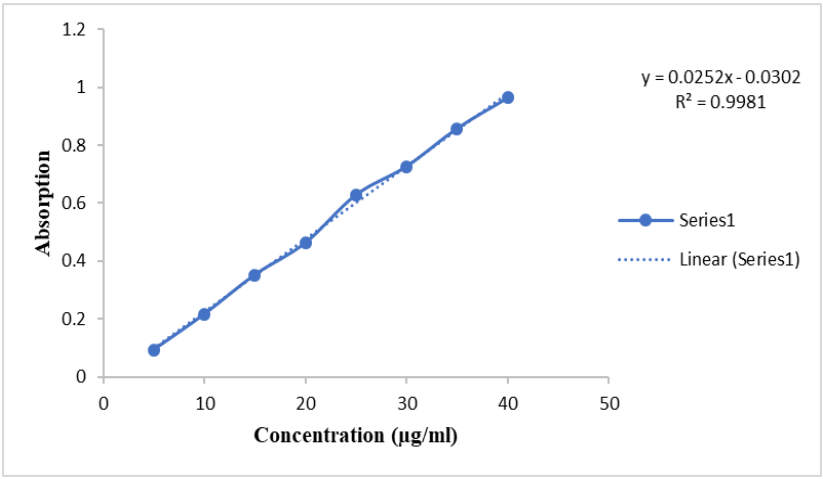

Figure No. 3: Graph of standard calibration curve of Gemcitabine-HCL in water

Table No. 1: Result of regression analysis of the UV method for the estimation of Gemcitabine-HCL

Statistical parameters	Results
λ max	268nm
Regression equation ** Y=mx+C	Y= 0.0252x-0.0302
Slope (b)	0.0252
Intercept (C)	0.0302
The correlation coefficient (r^2)	0.9981

The calibration curve for Gemcitabine-HCL was obtained by using the0.5µg/ml to 4µg/ml concentration of Gemcitabine-HCL in water. The absorbance was measured at 268 nm. The calibration curve of Gemcitabine-HCL as shown in the graph indicated the regression equation Y= 0.0252x-0.0302 and R^2 value 0.9981, which shows good linearity as shown in **Table 1** and **Figure 3.**

Partition coefficient determination

Table No. 2: Partition coefficient determination of Gemcitabine-HCL

The partition coefficient of Drug	Solvent System	Log P-Value
Gemcitabine HCL	water: n-octanol	-3.306±0.001

Value is expressed as mean ± SD; n = 3.

The partition coefficient of Gemcitabine HCL in n- Octanol: Water was found to be -3.306±0.001 this indicates that the drug is hydrophilic.

Compatibility studies

The study was performed with the help of FTIR Method.

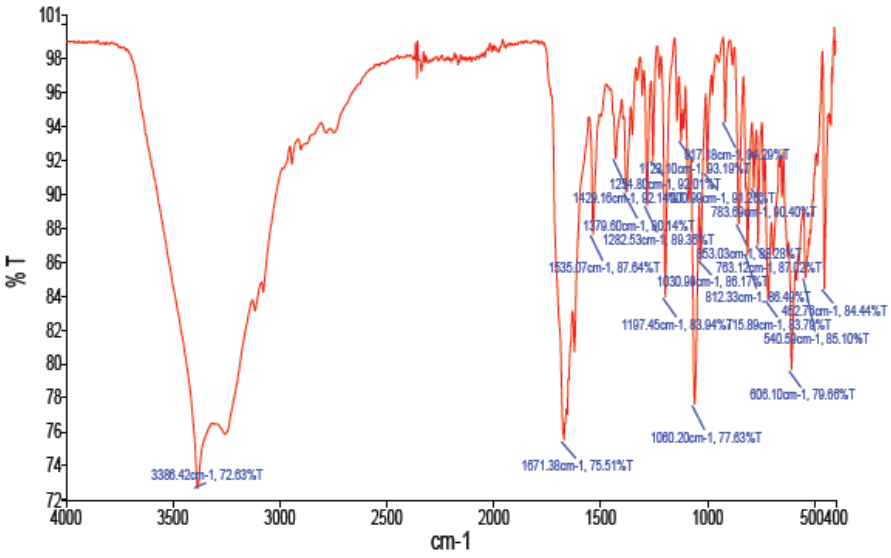

Figure No. 4: FTIR spectrum of drug GEM HCL

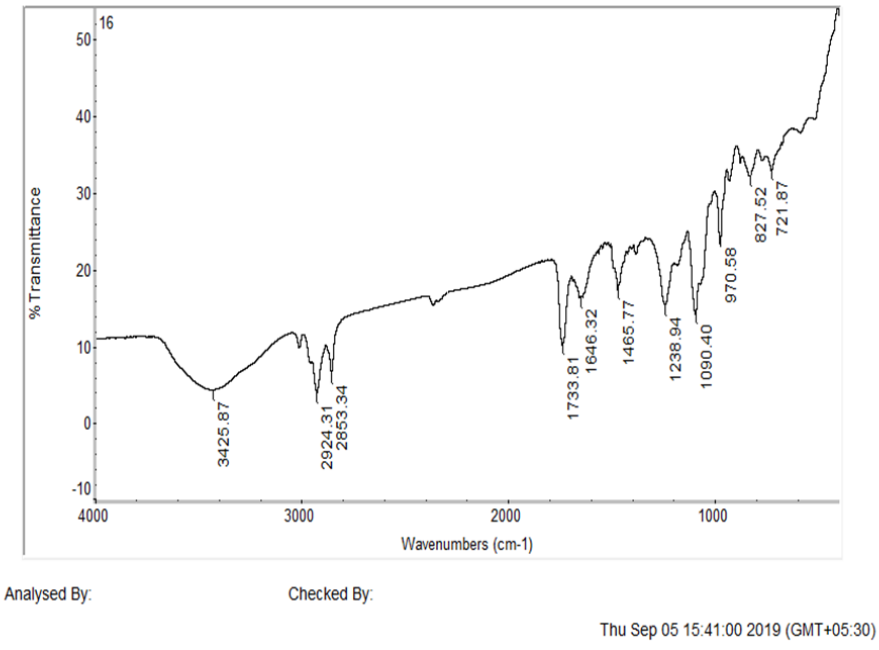

Figure No. 5: FTIR spectrum of phospholipid S-100

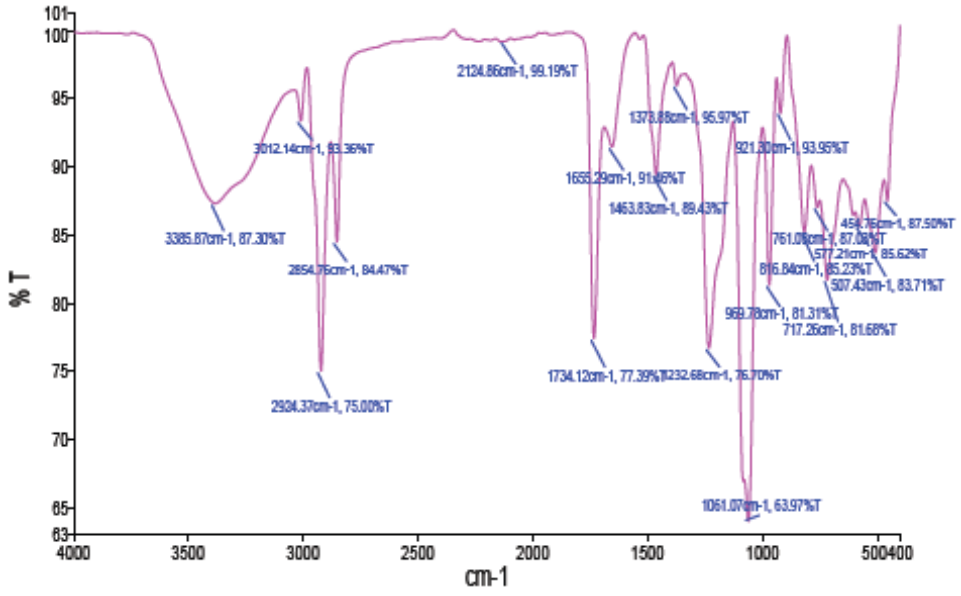

Figure No. 6: FTIR spectrum of PM

Drug compatibility was studied with FTIR to check whether there is any reaction between drugs and lipids. FTIR of the standard drug i.e. GEMHCL is shown in **figure 4**, FTIR of lipid i.e. S-100 is shown in **Figure 5** and the FTIR spectrum of PM is shown in **Figure 6.** When the drug was mixed with the lipid, no interaction was found.

Preparation of Gemcitabine HCL loaded phytosome

Phytosomes loaded Gemcitabine-HCL with were successfully prepared using the solvent evaporation technique with slight modifications. Various formulations were prepared using different types of lipids. This Gemcitabine-HCL loaded phytosome was characterized by %EE. Based on % EE, S-100 has been selected for further optimization of formulation and proceed for further studies. Briefly, different molar ratio (0.01:0.01, 0.01:0.02, 0.01:0.04, 0.01:0.06, 0.01:0.08 and 0.01:0.10) of GEM. HCL: Phospholipids were studied for the preparation of GEM HCL-PC. GEM and Lipoid S-100 were co-dissolved in the selected ratio in 10 ml of optimized solvent and refluxed at 60°C for 4 hr. The solvent was then evaporated using a rotary evaporator to get the GEM HCL-PC and then dried under vacuum overnight to remove traces of solvents. The resultant complex was stored in an airtight container at below 20°C. Then hydrate the mixture to break the layer. After that, the solution was centrifuged at 18,000 rpm for 15 min. by placed in a cooling centrifuge, then was suitably diluted and determined photometrically [9]. The ratio of 0.01:0.08 showed optimum EE%. Hence formulation N5 has been selected for the final batch.

EVALUATION OF PHYTOSOME FORMULATION

Transmission Electron Microscopy (TEM) Analysis

It appears as a well defined, uniform micellar shape vesicles with an inner dark core surrounded by lighter striations, probably composed of the phospholipid.

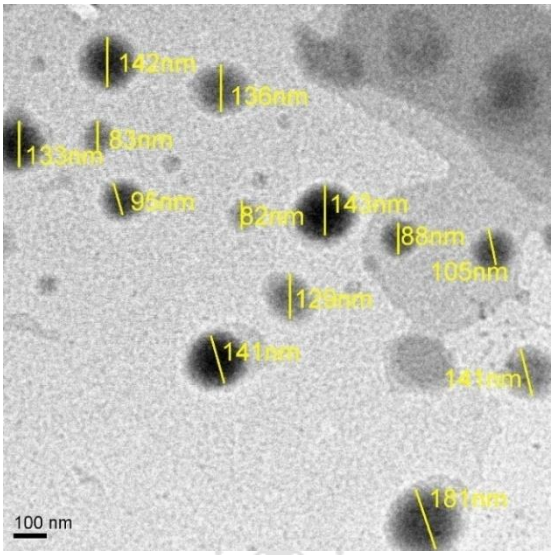

Figure No. 7: TEM Images of N5 formulation

Particle size distribution analysis

The small particle size, the monodisperse and good particle size distribution have a major impact on the fate of a nanoparticle system. Additionally, monodisperse size distribution is essential for excellent physicalstability. [23, 24]

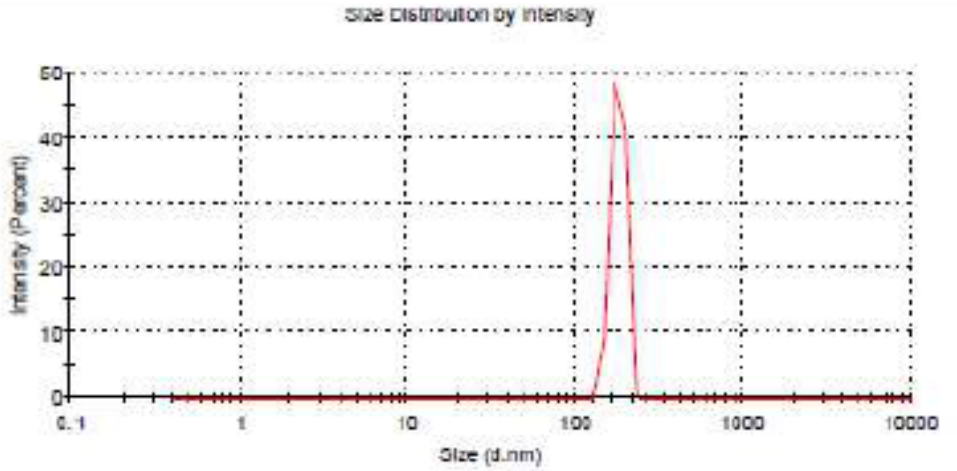

Figure No. 8: Particle size distribution of phytosomal formulation

The average particle size (nm) is 173.1±53.05 and PDI is 0.487±0.

Values are mean ± SD, n = 3.

FTIR spectra of final phytosome formulation (N5)

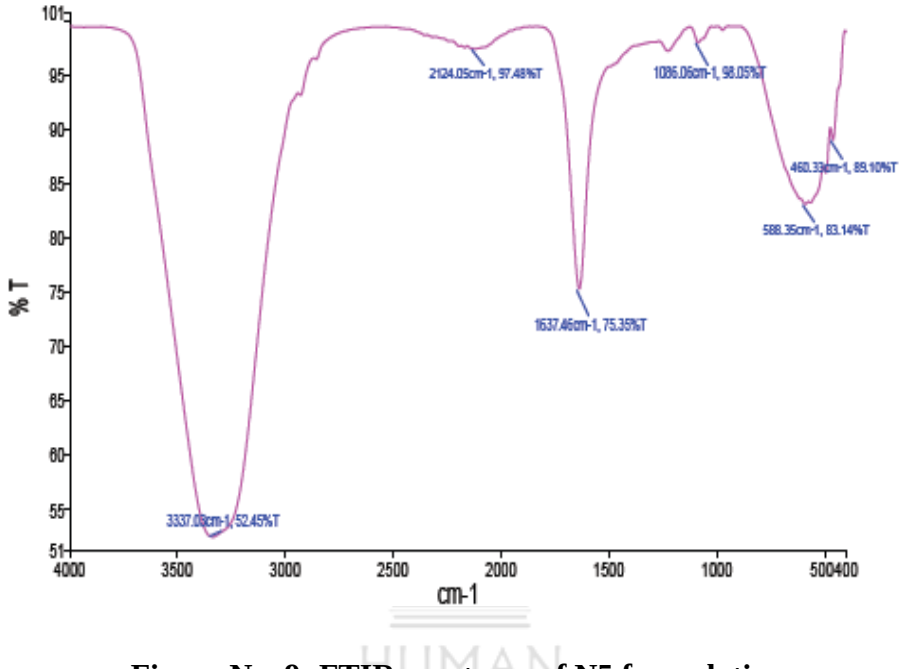

Figure No. 9: FTIR spectrum of N5 formulation

Entrapment efficiency

Upon incorporation of edge activator in low concentration, growth in vesicle size occurred whereas; further, an increase in the content of edge activator may have led to pore formation in the bilayers. The entrapment efficiency was studied using the dialysis method (candy method). Hence formulation N5 has been selected for the final batch which shows high EE% i.e. 69%.

Solubility

Liposolubility of phospholipid complex in n-octanol was significantly increased as compared with pure drug i.e. 6.938±0µg/ml. This indicated the enhancement of liposolubility of the hydrophilic drug, following complexation with phospholipid.

In-vitro drug release studies

For this purpose, a dialysis bag method was used for drug release purposes. From **Figure 10** we can see that the drug release time of phytosome formulation has been increased as compared to the pure drug.

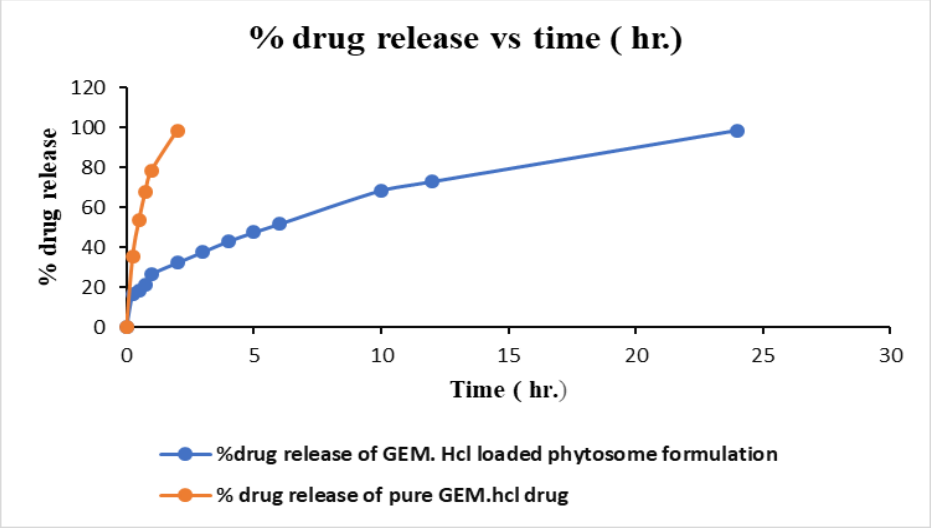

Figure No. 10: *In-vitro* drug release data of N5 Phytosome formulation

In-vitro drug release kinetic

In-vitro drug release kinetic study data of N5 Formulation was given below.

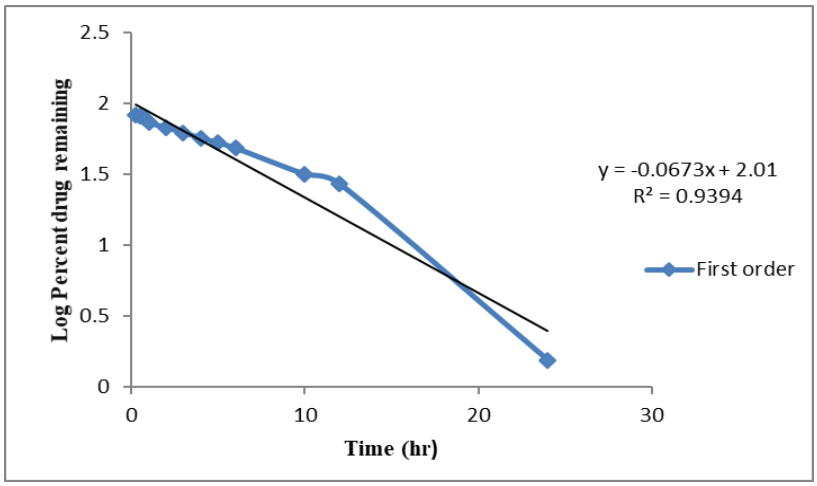

Figure No. 11: First-order graph for N5 formulation

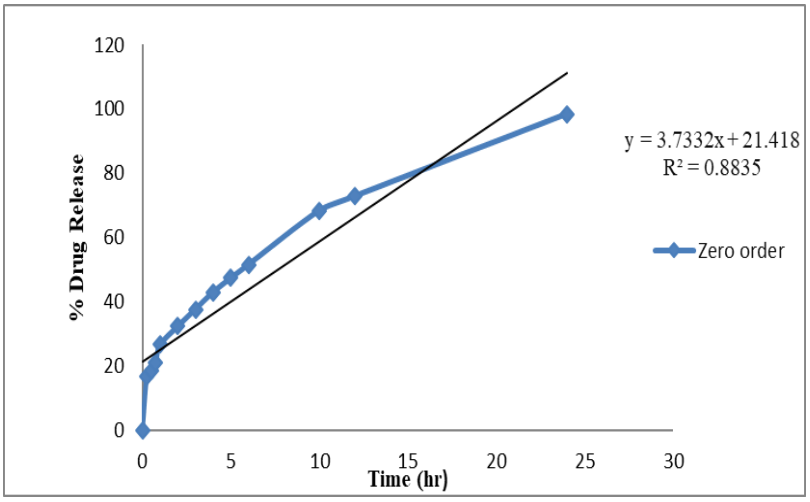

Figure No. 12: Zero-order graph for N5 formulation

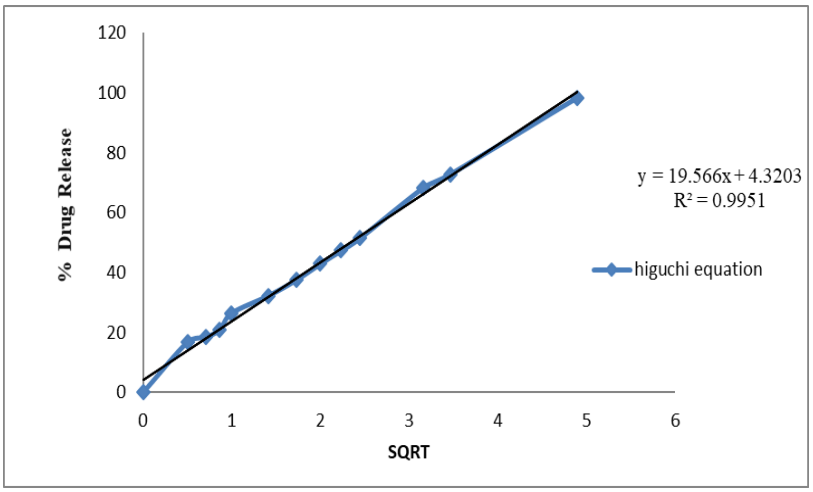

Figure No. 13: Higuchi order graph for N5 formulation

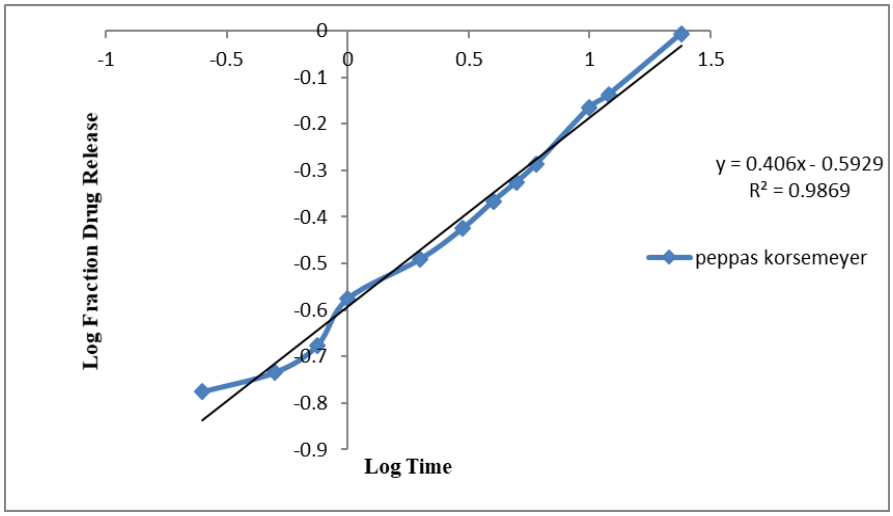

Figure No. 14: Korsmeyer –Peppas order graph for N5 formulation

Considering the determination coefficients i.e. R^2, Higuchi order was found (R^2=0.9951) to fit the release data best. It could be concluded from the results that the drug was released from Phytosome by a sustained mechanism.

Stability study analysis:

Stability studies of formulation N5 were examined over 6 months period by storing samples at three different temperatures and relative humidity for % EE and solubility. The organoleptic properties of Gemcitabine-HCL were not changed. [25]

Table No. 3: Stability study data of N5 Formulation

Time (6 months)	5°C±2°C		25°C±5°C/60% RH		40°C±5°C/75% RH	
	Solubility	**%EE**	**Solubility**	**%EE**	**Solubility**	**%EE**
Before	6.938±0.006	69.144±0.220	6.938±0.006	69.144±0.220	6.938±0.006	69.144±0.220
After	5.954±0.000	62.912±0.660	5.934±0.006	60.113±0.220	4.581±0.013	48.285±0.220

CONCLUSION

We have successfully developed a Phospholipid complex of GEM-HCL using the solvent evaporation method primarily to enhance lipid solubility. The complexation phenomenon was able to transform the physicochemical and pharmacological properties of the drug. Our findings based on in vitro studies demonstrated the superiority of GEM HCL-PC over the free drug. The optimum phytosomal preparation with the most satisfying characteristics was obtained when the GEM HC-PC mixture was used at a ratio of 0.01:0.08. This formula was able to produce phytosome with sufficient vesicle size, size distribution, entrapment efficiency. It can be concluded that the molar ratio of gemcitabine HCL to phospholipid are the key factors that influenced the successful approach.

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Image *Author -1*	***AKANKSHA CHOUDHARY*** *M. Pharmacy Student* *Sri Sai College Of Pharmacy, Badhani, Pathankot*
Image *Author -2*	***MANJIT KAUR*** *M. Pharmacy Student* *Sri Sai College Of Pharmacy, Badhani, Pathankot*
Image *Author -3*	***PRERNA UPADHYAY*** *M. Pharmacy Student* *Sri Sai College Of Pharmacy, Badhani, Pathankot*
Image *Author -4*	***SHAINA KUJAR*** *M. Pharmacy Student* *Sri Sai College Of Pharmacy, Badhani, Pathankot*
Image *Author -5*	***DR. NITIN BHARTI GUPTA*** *Associate Professor* *Sri Sai College Of Pharmacy, Badhani, Pathankot*
Image *Author -5*	***RAJINI SHARMA*** *Assistant Professor* *Sri Sai College Of Pharmacy, Badhani, Pathankot*