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# PREPARATION & EVALUATION OF AZITHROMYCIN-PHOSPHOLIPID BASED PHYTOSOME FOR ORAL DRUG DELIVERY

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## ABSTRACT

In the present study, Azithromycin-phospholipid complex was developed and evaluated for its impact on solubility and bioavailability of Azithromycin. Azithromycin phospholipid complex was prepared by solvent evaporation method and characterized. FTIR revealed the disappearance of the characteristic peaks of Azithromycin in the complex, which can be due to weakening, removal or shielding by the phospholipid molecule. This phenomenon could be due to packing of Azithromycin in the hydrophobic cavity of phospholipid and being held by van der Waals forces and hydrophobic interactions. Azithromycin phospholipid complex exhibited increased solubility, dissolution rate with decreased distribution coefficient indicating its increased hydrophilicity. Oral bioavailability of Azithromycin and Azithromycin phospholipid complex were evaluated in Sprague-Dawley (SD) rats. Azithromycin-PLC exhibited considerable enhancement in the bioavailability with an increase in concentration maxima. This enhancement can be attributed to the improvement of the aqueous solubility of the complex and a probable decrease in its extent of intestinal and hepatic metabolism. Thus, phospholipid complexation holds a promising potential for increasing oral bioavailability of Azithromycin.

**KEYWORDS:** Sustain release, In vitro release.

## INTRODUCTION

Azithromycin is an antibiotic used for the treatment of a number of bacterial infection.[1] This includes middle ear and certain other intestinal infection.[1] It may also be used for a number of sexuall transmitted infection, including gonorrhea infections. Along with other

medications, it may also be used for malaria. It can be taken by mouth or intravenously with doses once per day.

Over the last century, phyto-chemical science and phyto-pharmacological science established various plant compounds with varied biological activities and health promoting advantages like anti-mutagenicity, anti-carcinogenicity and anti-oxidative activity, for age-related diseases particularly cognitive state, pathology, diabetic wounds, immune and liver disorders, etc.

The term "phyto" means that plant and "some" means that cell like. thanks to the creation of Associate in Nursing H- bond between phospholipids and also the phyto-constituents, phytosomes show higher physical stability, enhancing absorption of deliquescent polar phyto-constituents leading to increased bioavailability and larger therapeutic advantages.[2]

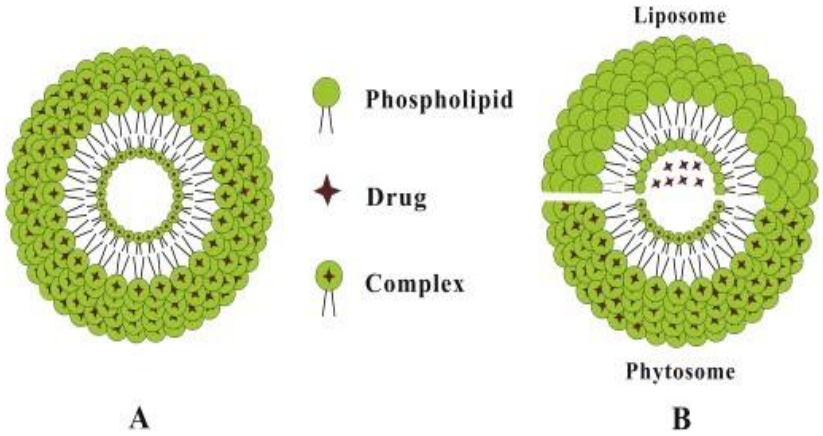



**Figure 2: Diagrammatic representation of phytosome.**

### 1.2 Advantages of phytosomes

- There may be a sweetening of botanic extract because of their colour with lipoid and improved absorption within the enteric path.
- Phytosome permits higher absorption from the enteric lumen.[3]
- Using of Phytosome in medical applications is not harmful so we can use in pharmaceutical and cosmetic use.

Phytosome are accustomed deliver liver protective flavonoids as a result of they will be created simply obtainable by phytosomes. additionally to the present, Phosphatidylcholine is additionally hepatoprotective.[4]

Using in cosmetics helps to protecting skin as shield against endogenous and exogenous effects.[5]

- Phytosomes are often additionally help to increase permeation of drug through skin for transcutaneous and dermal extract.[6]
- These helps to delivery of enormous and numerous cluster of medication.[7]

## MATERIAL AND METHODS MATERIALS

The following materials was from *Azithromycin (*Pharmaceuticals, Hyderabad), Phytosome (Signet Chemical Corp. Pvt. Ltd., Mumbai), Ethanol (Changshu Yanguan Chemical, China), Methanol (Fisher Chemical Ltd., Ahmedabad), Acetone (Fisher Chemical Ltd., Mumbai), Di-chloro methane (Fisher Chemical Ltd., Mumbai), Chloroform (Fisher Chemical Ltd., Mumbai), Di-methyl sulphur oxide (Fisher Chemical Ltd., Mumbai), Di- methyl foramide (Fisher Chemical Ltd., Mumbai), n-octanol (Fisher Chemical Ltd., Mumbai).

### Equipments

The following equipments were used UV Spectrophotometer (Shimadzu, Japan), Hot air oven (NSW India 143,New Delhi), Hot metal plate (REMI), pH meter (Ohaus, USA), Digital Balance (Shimadzu, Japan), Orbital shaker (REMI Equipment, Vasai India), Melting Point Apparatus (Remi Equipment, Mumbai), Vortex mixer (REMI Equipment, Mumbai), Cooling centrifuge (REMI Equipment), Sonicator (PCI Analytic), Eppendorf tubes (Tarsons Products Pvt.Ltd), FTIR spectroscopy (Brucker), Dissolution (Orchid Scientific).

## METHODS

### Pre-formulation studies

### 1.1. Organopleptic Characteristics

The drug sample was determined for the physical characterization like appearance, color and odor.

### Melting Point

For determination of melting point USP method was followed. Melting point of drug was determined by capillary fusion method. A small amount of drug was filled in capillary and it was placed in melting point apparatus. Then the temperature at which drug crystals started melting and turned into liquid was noted down.[8]

### 1.3. UV spectrum of Azithromycin

UV-visible photometer is usually used for structural data of assorted medication to get specific data on the chromophoric a part of the molecules in answer once exposed to lightweight within the visible/ultraviolet region of the spectrum absorb lightweight of specific wavelength looking on the kind of electronic transition related to the absorption. The ultraviolet spectrum is usually recorded as a plot of absorbance versus wavelength.[9]

Double beam UV-visible photometer (Shimadzu, UV-1800, Japan) was accustomed apprehend the λmax of drug. A a hundred µg/ml resolution of Azithromycin in grain alcohol was scanned within the vary of 200-400nm.[10]

### 1.4. Estimation of Azithromycin

#### 1.4.1. Estimation of Azithromycin by UV-visible spectrophotometer

The standard stock answer of Azithromycin (1mg/ml) was ready in grain alcohol. This answer was diluted with grain alcohol, to get varied dilutions from 100-500 µg/ml. Absorbance of those solutions was recorded at 208 nm against grain alcohol as blank victimization UV-visible photometer and customary curve was premeditated against concentration. From the standardisation curve intercept, slope, line equation and parametric statistic were obtained**.**

### 1.5. Dissolvability Studies

The unconstrained connection of 2 or a ton of substances to make the equivalent sub-atomic scattering is named solvency.

For quantitative dissolvability think about, overabundance amount of medication was taken in totally clean culture cylinders containing 5ml of different solvents, oils surfactants and co-surfactants and Culture cylinders were firmly shut. These take a look at tubes were shaking on water tub shaker at 25°C for twenty-four h at temperature. once twenty four h every sample was centrifuged fifteen,000 revolutions per minute and supernatant was withdrawal. at the moment supernatant was filtered and filtrates was befittingly diluted and determined spectrophotometrically.[11]

### 1.6. Partition Coefficient of Drug

Parcel consistent (oil/water) might be a live of a medication's lipophilicity/hydrophilicity and an indication of medication's capacity to cross cell films. it's plot in light of the fact that the

greatness connection of unionized medication disseminated between the natural and fluid stages at harmony. Parcel steady gives a method for portraying the lipophilic/hydrophilic nature of the medication. prescription having estimations of P a ton of bigger than one territory unit named oleophilic, any place as those with qualities a ton of however one zone unit demonstrative of a hydrophilic medication. The segment consistent is normally decided exploitation partner oil some portion of n-octanol and water. inside the case n-octanol and water:

$$P\ o/w = C\ n\text{-octanol}/C\ water$$

The parcel consistent (Po/w) in this way is the remainder of 2 groupings of medication in n-octanol (Cn-octanol) and water (Cwater) severally and is regularly given inside the assortment of its list to base ten (log P).

- **Shake flask method**

The partition constant determination study was performed by victimisation shake flask methodology. Excess amounts of the drug (Azithromycin) dissolved in five millilitre of 2 solvents (n-octanol: Water) along (1:1) and placed for twenty-four h. After 24 h, the 2 layers were separated and centrifuge for fifteen min's at fifteen, 000 rpm. The absorbance was taken in ultraviolet light photometer at the individual λ 208 GHB when acceptable dilution.[12]

### 1.5 FTIR of Azithromycin and Excipients

FT-IR (Fourier rework Infrared) spectrum of any compound or drug offers info concerning the teams gift in this specific compound. FT-IR spectrometry was used for structure analysis. The restrainer (KBr) disc technique was utilized. Since the KBr has no absorption within the elementary region of IR spectrum, solely the spectrum of sample is obtained. Associate in Nursing FT-IR spectrum of Azithromycin and drug and excipients mixture was recorded for the determination of drug interaction with excipients.[13]

#### 1.5.1. Medication excipients Similarity think about by FTIR

The similarity of medication with excipients was seen by FT-IR. FTIR was utilized as apparatus to find any physical and concoction connection among medication and excipients. Medication and fluctuated excipients were blended totally in quantitative connection of 1:1. Tests were checked by FTIR underneath the change of 400-4000 cm-1. The spectra of unadulterated medication and medication with excipients were contrasted with inspect any incongruence and physical changes.

### 6.3 Preparations of Azithromycin phospholipid Complex[14]

The Azithromycin lipide progressed was prepared by refluxing the Azithromycin and phosphatidylcholine in milli molar quantitative connection (5:1,5:2,5:3,5:4,5:5,5:6,5:7,5:8). each the reactants were set in a hundred cc round base jar containing twenty cc grain liquor.

| S. No. | Formulation Code | Drug Ratio: Phosphatidylcholine S100 Ratio(milimolar) | Ethanol (ml) |
|---|---|---|---|
| 1 | F1 | 5:1 | 20 |
| 2 | F2 | 5:2 | 20 |
| 3 | F3 | 5:3 | 20 |
| 4 | F4 | 5:4 | 20 |
| 5 | F5 | 5:5 | 20 |
| 6 | F6 | 5:6 | 20 |
| 7 | F7 | 5:7 | 20 |
| 8 | F8 | 5:8 | 20 |

### 6.4 Evaluation of Phytosome

**6.4.1 Visual Appearance:** Phytosome can range from translucent to milky, depending on the composition and particle size.

### 6.4.2 Optical microscopy

Optical Microscopy of drug loaded phytosome formulation was determined by optical microscopy at 100x magnification.

### 6.4.3 Molecule size and alphabetic character potential conclusions

Vesicle properties, molecule measure breadth and alphabetic character potential, were resolved at temperature by alphabetic character Potential/Molecule Sizer instrument (Malvern). Phytosome definitions were weakened with phosphate supported saline, pH 7.4, for alphabetic character potential and molecule measure assurance, severally.[15]

### 5.4.5 Drug Content[16]

Drug Content of phytosome loaded is determined by dissolving accurately weighed 100mg of phytosome loaded in 10ml alcohol. once acceptable dilution absorbance could also be determined by UV- photometer (ʎmax= 239 nm). The drug content was calculated.

### 6.4.4 Determination of Entrapment efficiency

The demurrer potency of phytosome make up my mind by calculative the quantity of entrapped Kabolin decanoate within the phytosomes. to work out the demurrer potency of

Kabolin decanoate in phytosome, associate applicable quantity of dispersion was transferred in culture tube. The scattering was axis for fifteen min at 15000 rate. when action the supernatant was gathered and extent Medication protest amount of free Kabolin decanoate decide spectrophotometrically (ʎmax= 239 nm). The challenge strength has been resolved with regards to the ensuing condition:

EE nothing = W (Included medication) −W(free drug) ×100 W (Included medication)

Where, W (included medication) is that the amount of medication extra all through the arrangement of phytosomes, W (free medication) is that the amount of free medication estimated inside the lower assembly of the way of life tube once movement.[17]

### 6.5 In-Vitro Medication unharness Study

In vitro unharness dynamics of phytosome was firm during this work victimisation qualitative analysis technique. In brief, phytosome (2.0 cubic centimetre) or drug answer with the equivalent drug concentration was fenced in a very qualitative analysis bag so placed in one hundred mL of acid (0.1N) and phosphate buffer saline (PBS) hydrogen ion concentration vi.8 used as unharness media. the complete system was unbroken at 37°C ± zero.5°C with continuous magnetic stirring. At choose time interims (0.5,1,2,3,4,5,6,7,8 and twenty four hour), three cubic centimeter of answer was pulled back from {the unharness|the discharge} medium and renewed with a comparable volume of discharge medium. The gathered examples were fittingly weakened and examined by UV–obvious photometer at 239 nm.[18]

#### 6.5.1 Drug kinetics release

Model dependent strategies area unit supported completely different mathematical functions, that describe the discharge profile. Once an appropriate operate has been chosen, the discharge profiles area unit evaluated reckoning on the derived model parameters.[19-20] the info obtained from ex vivo permeation studies were planned in numerous models of knowledge treatment as follows.

**Zero Request model First Request model Higuchi's Model Korsmeyer-Peppas model**

##### 6.5.1.1 Zero request mechanics

It are regularly wont to depict the medication disintegration of numerous types of changed release pharmaceutical uncertain amount shapes, as inside the instance of some transdermic frameworks, besides as network tablets with low dissolvable drug in covered structures, dispersion frameworks, and so on. In its most straightforward sort, zero request release are regularly painted as:

$$Q0 - Qt = K0t$$

Where, Qt is that the amount of medication broke down in time t, Q0 is that the underlying amount of medication inside the goals (most occasions, Q0 = 0) and K0 is that the zero request release steady communicated in units of focus/time. to check the release mechanics, learning acquired from in vitro medication penetration studies were planned as collective amount of medication released versus time.

**6.5.1.2 First request mechanics**

It will be wont to portray the medication disintegration in pharmaceutical uncertain amount structures like those containing solvent drug in permeable frameworks. the release of the medication that pursued starting request elements will be communicated by the condition:

$$\log C = \log C0 – K.t/2.303$$

Where, C0 is that the underlying grouping of medication, k is that the underlying request rate consistent, and t is that the time. the data got territory unit planned as log aggregate offer of medication remaining versus time which may yield a line with a slant of K/2.303.

**6.5.1.3 Higuchi's Model**

This model expected to articulate medication unharness from a grid framework. Principally respected for smoothed frameworks, it had been then reached out to totally unique geometrics and permeable frameworks. This model is predicated on the theories that (I) beginning medication fixation inside the network is far more than medication dissolvability; (ii) sedate dispersion happens exclusively in one measurement (edge result ought to be unimportant), (iii) tranquilize particles ar a great deal of littler than framework thickness, (iv) grid swelling and disintegration ar immaterial, (v) sedate diffusivity is consistent, and (vi) phenomenal sink conditions ar constantly earned inside the unharness environment.

Higuchi was the essential to determine Partner in Nursing condition to clarify the release of a medication from Partner in Nursing insoluble grid in light of the fact that the foundation of a period subordinate strategy bolstered Fickian dispersion. Disentangled Higuchi condition is following;

$$Qt = KH (t) \text{ zero.5}$$

Where, Qt is that the amount of medication free in time t and KH is that the unharness rate consistent for the Higuchi model. when the data is planned as aggregate medication free versus base of your time, it yields a line, demonstrating that the medication was free by dissemination system. The incline is competent 'KH'.

#### 6.5.1.4 Korsmeyer-Peppas Model

Korsmeyer determined a direct relationship that portray medication unharness from a compound framework. {the unharness|the discharge} rates from controlled discharge compound frameworks are regularly portray by the condition arranged by Korsmeyer et al.

$$Q = K.tn$$

Where, alphabetic character is that the offer of medication free at time't' K could be a dynamic steady fusing basic and geometric attributes of the tablets and 'n' is that the diffusional type characteristic of the release system.

For Fickian unharness, n=0.45 though for irregular (Non-Fickian) transport, n extends somewhere in the range of zero.45 and 0.89 and for zero request unharness, n = 0.89. The Korsmeyer-Peppas model was aforethought between log inclination offers medication discharges versus log time.

## 1. RESULT AND DISCUSSION

### 1.1. Result of Preformulation

The point of preformulation studies is to look into the physical and compound properties of a medication substance. The picked medication Azithromycin was oppressed for examination of physical portrayal parameters, for example,

- Organoleptic properties
- UV-visible spectra
- FT-IR spectra
- Melting point
- Solubility
- Partition coefficient

### 1.1.1. Organoleptic properties[21]

Organoleptic properties of drug Azithromycin found to be as per USP treatise. The Organoleptic properties of Azithromycin were found to the given **Table 1**.

**Table 1: Organoleptic Properties of Azithromycin.**

| S. No. | Properties | Inferences |
|---|---|---|
| 1. | Colour | White |
| 2. | Odour | Odourless |
| 3. | Form | Powder |

### 1.1.2. Melting Point[22]

The temperature given to a substance at which the strong stage gets changed over to fluid stage The dissolving point assurance suggests the virtue of medication. Dissolving purpose of Azithromycin was dictated by slender cylinder technique and was observed to be very like the revealed liquefying point as appeared Table 2.

**Table 2: Melting Point of Azithromycin.**

| Drug | Observed melting point | Reference melting point |
|---|---|---|
| Azithromycin | 110-116°C | 114°C |

**Discussion**: Azithromycin dissolving point was observed to be in range 110 -1160C which is of the unadulterated medication. Consequently medication test was free from any types of impurities.

### 1.1.3.UV Spectroscopy

#### 1.1.3.1. Determination of absorption maxima in ethanol

Absorption maxima of Azithromycin were found to be at 208 nm similar to literature as shown in

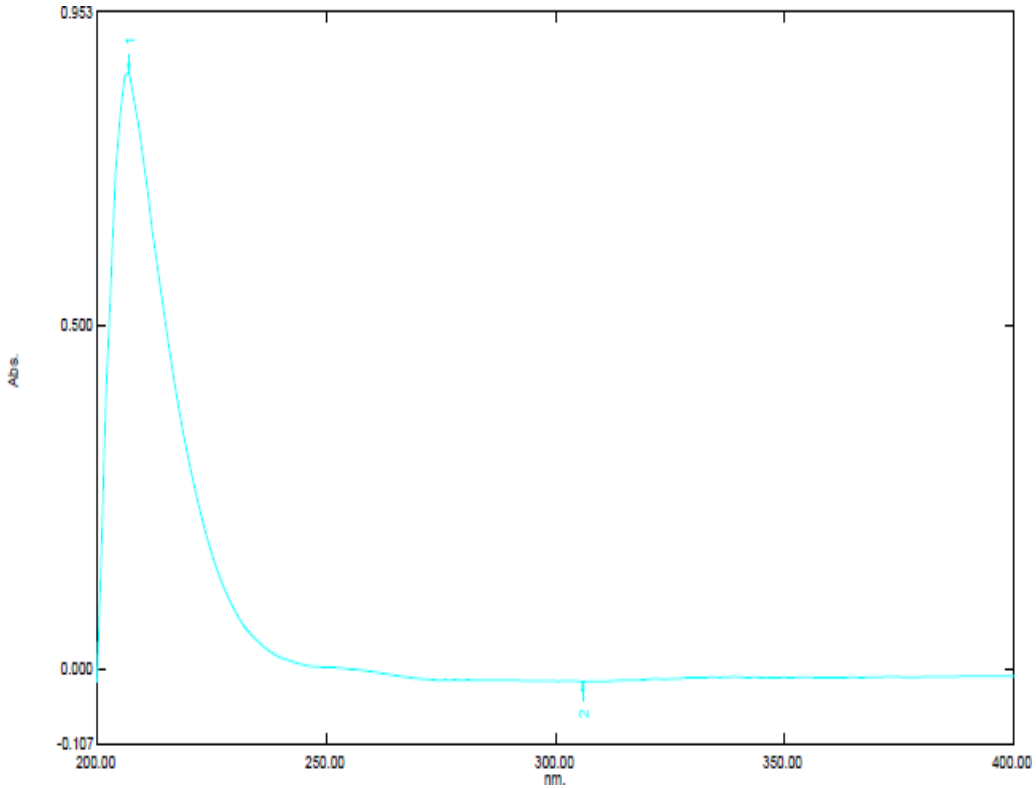



**Figure 1.**

### 1.1.3.2. Preparation of standard curve of Azithromycin in ethanol

**Table 3: Calibration curve of Azithromycin in ethanol ($\lambda_{max}$ = 208nm).**

| S.NO. | Concentration µg/ml | Absorbance |
|---|---|---|
| 1 | 100 | 0.111±0.001 |
| 2 | 150 | 0.187±0.003 |
| 3 | 200 | 0.285±0.001 |
| 4 | 250 | 0.400±0.001 |
| 5 | 300 | 0.522±0.001 |
| 6 | 350 | 0.613±0.003 |
| 7 | 400 | 0.699±0.001 |
| 8 | 450 | 0.816±0.001 |
| 9 | 500 | 0.920±0.001 |

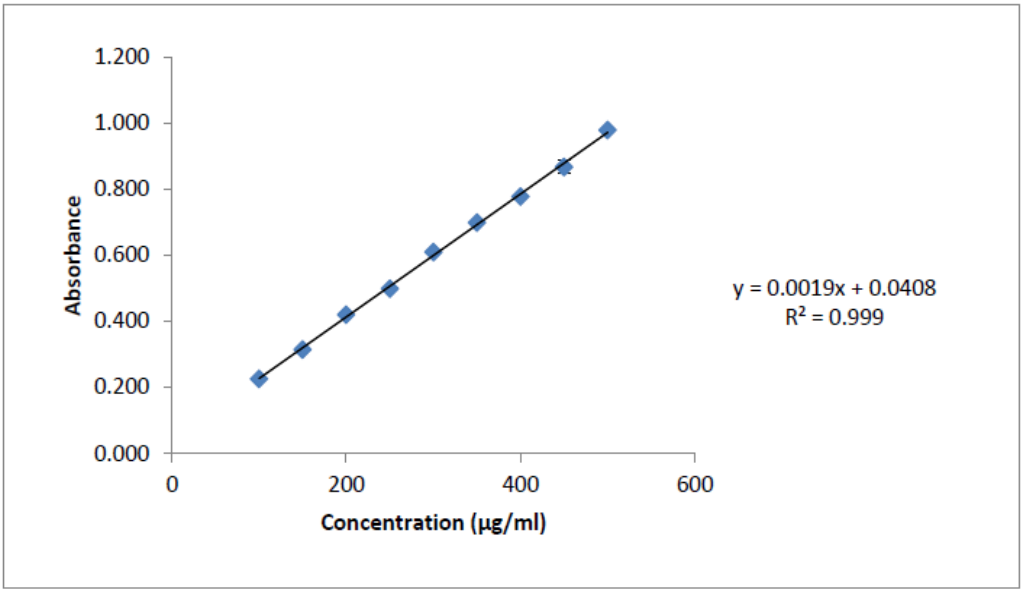



**Figure 2: Chart Ploted adjustment bend of Azithromycin in Ethanol.**

**Table 4: Result in regression analysis of UV method for estimation of Azithromycin.**

| Statistical parameters | Results |
|---|---|
| λ max | 208nm |
| Regression equation | y = 0.0019x + 0.0408 |
| Slope (b) | 0.055 |
| Intercept (C) | 0.0408 |
| Correlation coefficient ($r^2$) | 0.999 |

**Discussion:** The standard curve for Azithromycin were obtained by using the 100 to 500µg/ml concentration of Azithromycin in ethanol. The absorbance was measured at 208 nm. Calibration curve of Azithromycin as shows in graph indicated the regression equation y=0.0019x+0.0408 R2=0.999 which shows good linearity as shown in **Table 4** and **Figure 2**.

### 1.1.4 Solubility studies

Solubility of drug in solvents was disbursed so as to screen for the parts to be used for formulation development. Analysis of the drug was disbursed on ultraviolet illumination photometer at 208 nm.

#### 1.1.4.1 Solubility Studies of Azithromycin in various Solvents.

**Table 5: Solubility studies of Azithromycin for different solvents.**

| S.no. | Solvent | Solubility in (mg/ml) (mean±SD) |
|---|---|---|
| 1 | Water | 2.453±0.028 |
| 2 | Chloroform | 1176.491±3.039 |
| 3 | Methanol | 2195.789±5.263 |
| 4 | N-octanol | 2276.491±25.963 |
| 5 | ethanol | 2567.719±8.040 |
| 6 | THF | 3057.192±3.039 |
| 7 | Acetone | 3587.017±8.040 |
| 8 | DMF | 10343.85±132.450 |

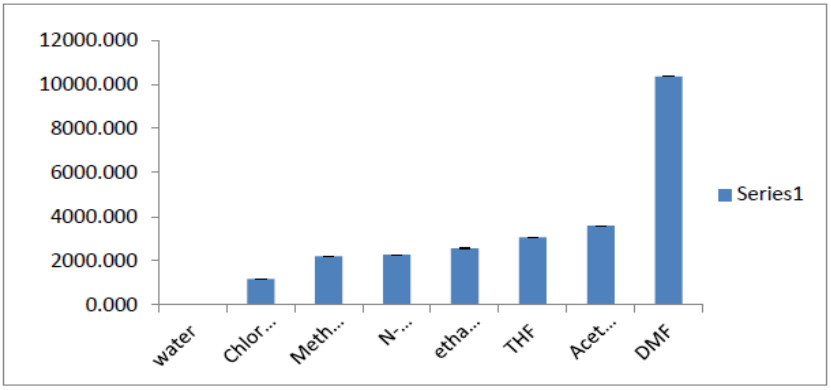



**Figure 3: Solubility study of drug in different solvents.**

**Discussion:** Azithromycin is highly soluble in DMF, Acetone, ethanol followed by DMSO and slightly soluble in water (**Figure 3 and Table 5**).

### 7.1.5 Partition constant determination

Partition constant of the Azithromycin make up my mind mistreatment n-octanol and water. Log P larger than one indicates that the drug is oleophilic in nature, whereas those with partition coefficients but one square measure indicative of a deliquescent drug. This indicated the lipophilicity and purity of drug.

**Table 9: Partition constant determination of Azithromycin.**

| Partition constant of Drug | Solvent System | Log P Value |
|---|---|---|
| Azithromycin | Water:n-octanol | 2.383±0.008 |

Worth is explained as mean ± SD; n = 3

**Discussion:** The segment consistent of Azithromycin in n-Octanol: Water was observed to be two.383±0.008 this implies the medication is lipotropic in nature.

### 5.1.6. Characterization of Azithromycin and excipients by FT-IR spectroscopy[23-24]

The spectra obtained from FT-IR spectroscopy studied at wavelength from 4000cm$^{-1}$ - 400cm$^{-1}$.

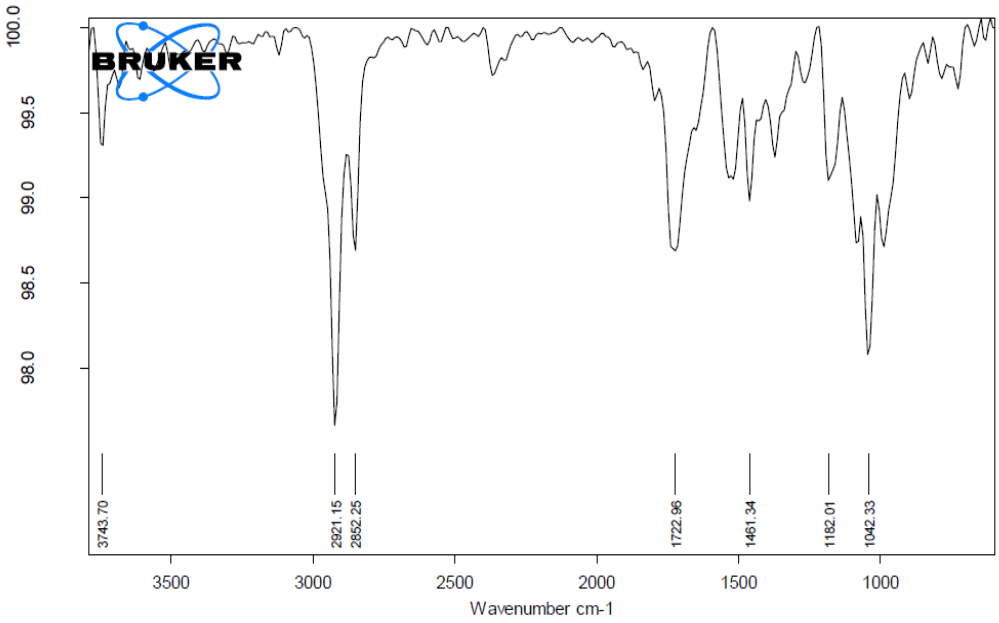



**Figure 8: FT-IR spectra of unadulterated Azithromycin.**

**Table 15: Interpretation of FTIR of Pure Azithromycin.**

| Reference Peaks | Observed Peaks | Functional Peak (Vibration) |
|---|---|---|
| 1052 | 1042.33 | (symmetrical aliphatic ether) |
| 1721 | 1722.96 | (C O stretch of the ester structure), |
| 2950 | 2921.15 | (C-H extend), |
| 3496 and 3561cm | 3743.70 | (O-H stretch) |

The principal IR absorption peaks of Azithromycin at 1042.33cm$^{-1}$ (symmetrical aliphatic ether)' 1722.96 cm$^{-1}$(C O stretch of the ester structure), 2921.15 cm$^{-1}$ (C-H extend), 3743.70 cm$^{-1}$ (O-H stretch) were all observed the spectra of Azithromycin. These observed principal peaks. This observation confirmed the purity and authenticity of the Azithromycin.[26]

### 7.1.6 FTIR of Excipient Phosphatidylcholine S100

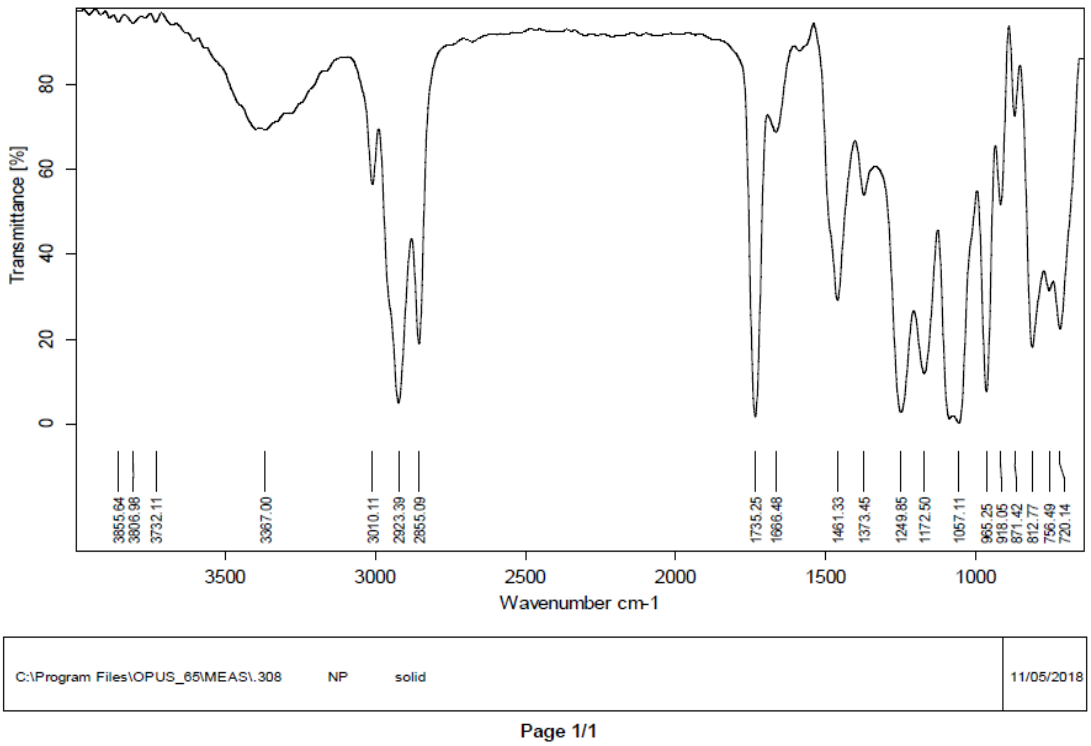



**Figure 13: FTIR spectrum of Phosphatidylcholine S100.**

**Table 15: FTIR interpretation of Phosphatidylcholine.**

| Reported ($cm^{-1}$) | Observed($cm^{-1}$) | Characteristics Peaks |
|---|---|---|
| 2918.3 and 2854.96 | 2923.39 and 2856.09 | C-H extending band of long unsaturated fat chain |
| 1728.22 | 1735.25 | Carbonyl extending band in the unsaturated fat ester |
| 1710–1665 | 1666.48 | C=O stretch α,β–unsaturated aldehydes, ketones |
| 1236.37 | 1249.85 | P=O stretching band |
| 1093.65 | 1057.11 | P–O–C stretching band |
| 966.34 | 985.25 | N+(CH3)3 stretching |

The FTIR spectra of phosphatidylcholine S100 were appeared inside the Figure 13; Table fifteen. The chief IR retention pinnacles of phosphatidylcholine S100 at 2923.39 and 2856.09cm-1 (C–H extending band of long carboxylic corrosive chain), 1735.25cm-1 (Carbonyl extending band inside the carboxylic corrosive ester), 1249.85cm-1 (P=O extending band), 1057.11cm-1 (P–O– C extending band) and 985.25cm-1 (N+(CH3)3 extending) were altogether decided inside the spectra of phosphatidylcholine S100. These decided main pinnacles. This perception affirmed the immaculateness and believability of the phosphatidylcholine S100.[25]

- **FTIR of Pure drug and physical mixtures**

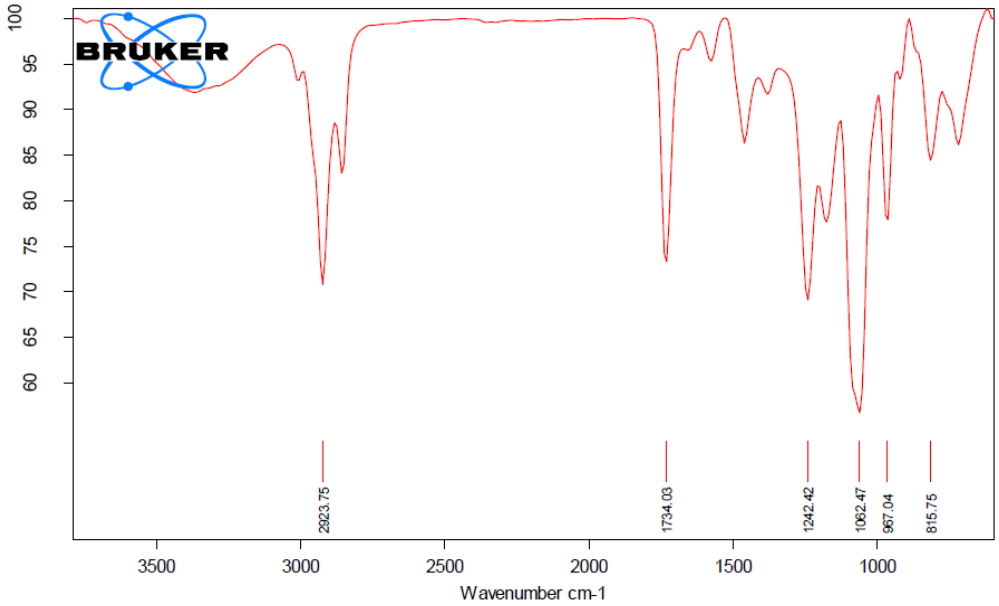



**Figure 14: FT-IR spectra of Azithromycin in phosphatidylcholine S100.**

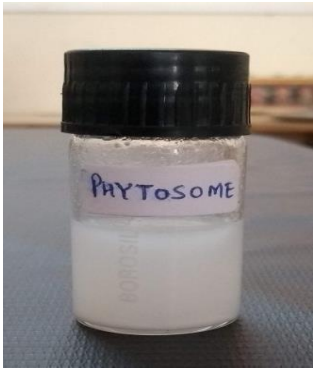
**Table 16: FTIR interpretation of Azithromycin and phosphatidylcholine S100.**

| Reference Peaks | Observed Peaks | Functional Peak (Vibration) |
|---|---|---|
| 1042.33 | 1062.47 | (symmetrical aliphatic ether) |
| 1722.96 | 1734.03 | (C O stretch of the ester form), |
| 2921.15 | 2923.75 | (C–H stretch), |
| 1249.85 | 1242.42 | P=O stretching band |
| 985.25 | 967.04 | N+(CH3)3 stretching |

FTIR of Pure drug and physical mixture studies **(Figure 12-14**; **Table 14-16)** were carried out to eliminate the possibility of interaction between drug and excipients. 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.

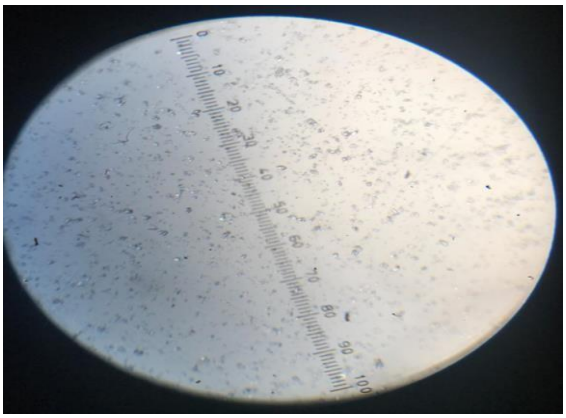
### 5.3 Evaluation of phytosome

### 7.3.1.Appearance of phytosome



### 5.3. Optical microscopy

The photomicrograph of F7 formulation revealed that particles present in uniform shape without any aggregation.



### 7.3.2.Precentage of drug content

Drug ingredient of all formulation was given in a **Table 15**.

**Table 15: Precentage drug content of different Azithromycin phospholipid complex phytosome.**

| S.No. | Formulation Code | Percentage ingredient |
|---|---|---|
| 1 | F1 | 97.610±0.284 |
| 2 | F2 | 88.601±0.434 |
| 3 | F3 | 98.416±0.296 |
| 4 | F4 | 95.476±0.376 |
| 5 | F5 | 91.872±0.357 |
| 6 | F6 | 89.217±0.284 |
| 7 | F7 | 95.998±0.457 |
| 8 | F8 | 96.993±0.296 |

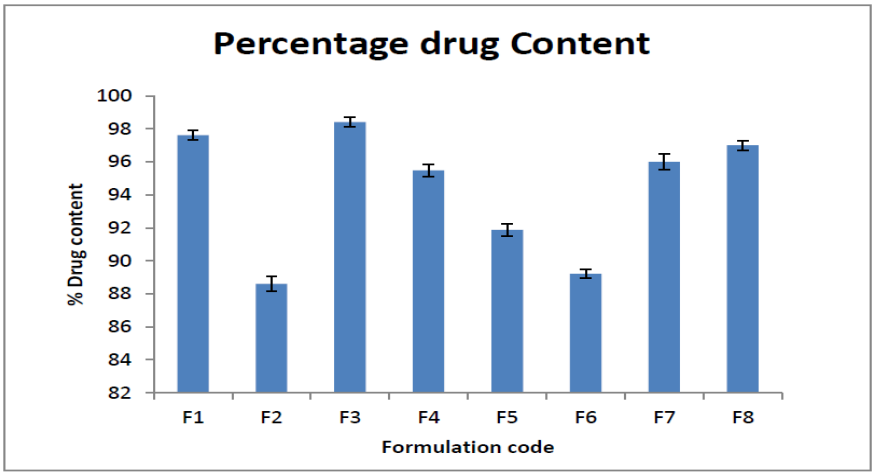



**Figure 19: Percentage drug content in Azithromycin phospholipid complex phytosome**

**Percentage yield was found in a range of 88.601±0.434 to 98.416±0.296.**

### 7.3.3.Percentage Drug Entrapment

Percentage Drug Entrapment of all formulation was given in a **Table 16**.

**Table 16: Percentage Drug Entrapment of different Azithromycin phospholipid complex phytosome.**

| S.No. | Formulation Code | Entrapment efficiency (%) |
|---|---|---|
| 1 | F1 | 86.697±0.037 |
| 2 | F2 | 87.123±0.037 |
| 3 | F3 | 87.446±0.035 |
| 4 | F4 | 89.020±0.021 |
| 5 | F5 | 89.148±0.057 |
| 6 | F6 | 90.035±0.220 |
| 7 | F7 | 91.152±0.142 |
| 8 | F8 | 86.455±0.014 |

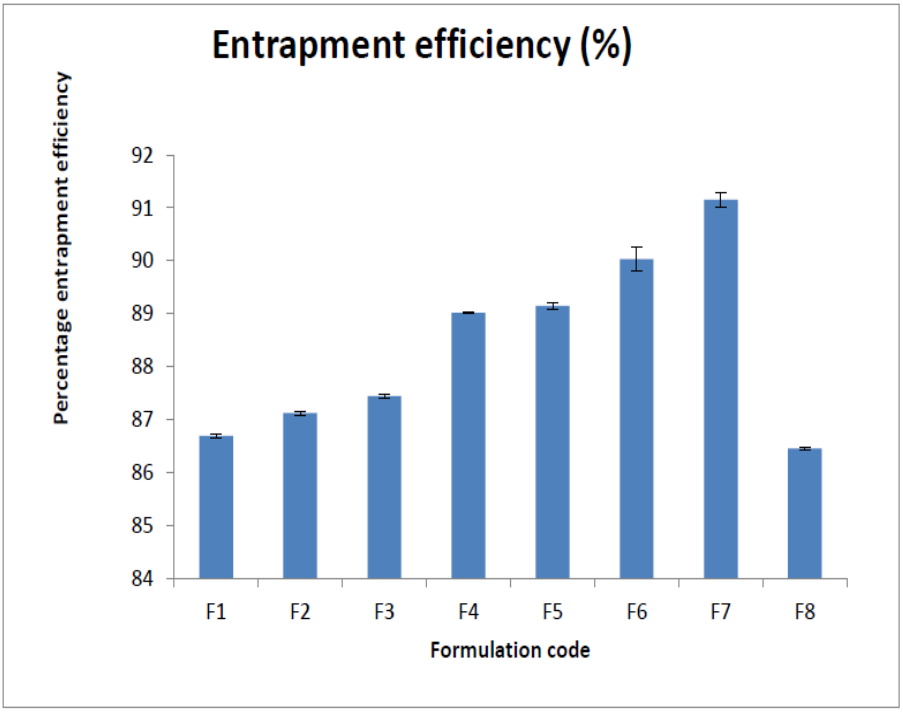



**Figure 20: Percentage drug denial of Azithromycin lipide advanced phytosome.**

From the Table sixteen, it absolutely was found that proportion drug denial of all formulation was found to be in a very vary eighty six.455±0.014 to 91.152±0.142. These results justify that there's a big impact on % denial potency of phytosome was discovered with supermolecule concentration.[26-27]

### 7.3.4. Molecule shape and zeta potential conclusions Molecule Estimate

| | | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 305.1 | Peak 1: | 355.9 | 86.1 | 102.4 |
| PdI: | 0.633 | Peak 2: | 34.95 | 7.4 | 9.123 |
| Intercept: | 0.972 | Peak 3: | 73.35 | 6.5 | 16.91 |
| Result quality : | Refer to quality report | | | | |

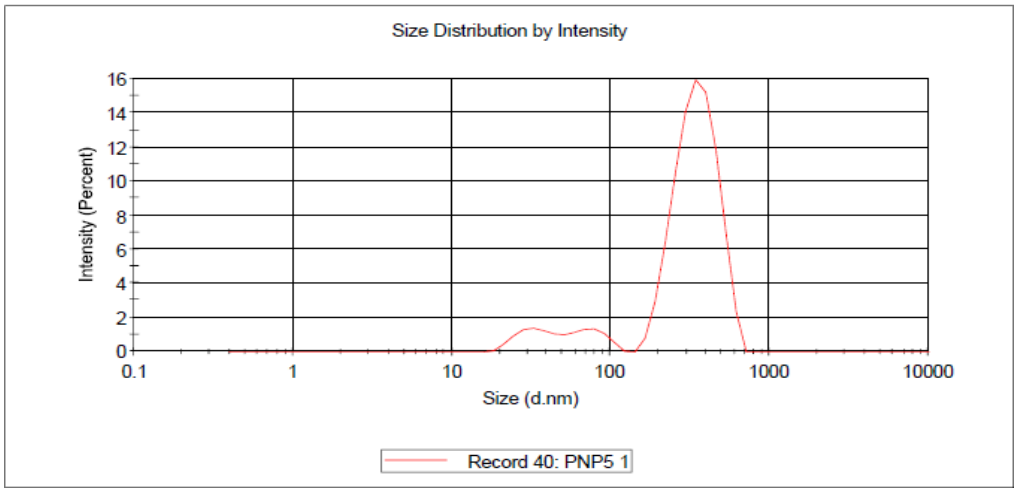



**Figure 21: Particle size peak of phytosomal formulation(F7).**

Figure 21 demonstrated particle size of phytosome formulation was 305.1nm with PDI 0.633.

### Zeta Potential

| | | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|---|
| Zeta Potential (mV): | -26.2 | Peak 1: | -26.2 | 100.0 | 5.64 |
| Zeta Deviation (mV): | 5.64 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): | 0.0125 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality : | Good | | | | |

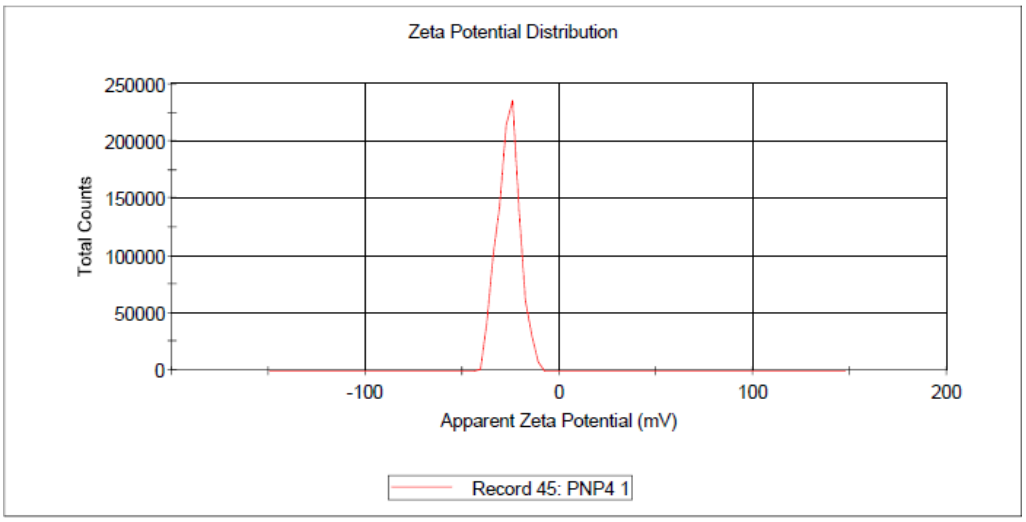



**Figure 22: Zeta potential graph of phytosomal formulation (F7).**

**Discussion:** Figure 22 demonstrated zeta potential of phytosomal formulation was -26.2mV represents stability of formulation.

### 7.3.5.FTIR spectral analysis

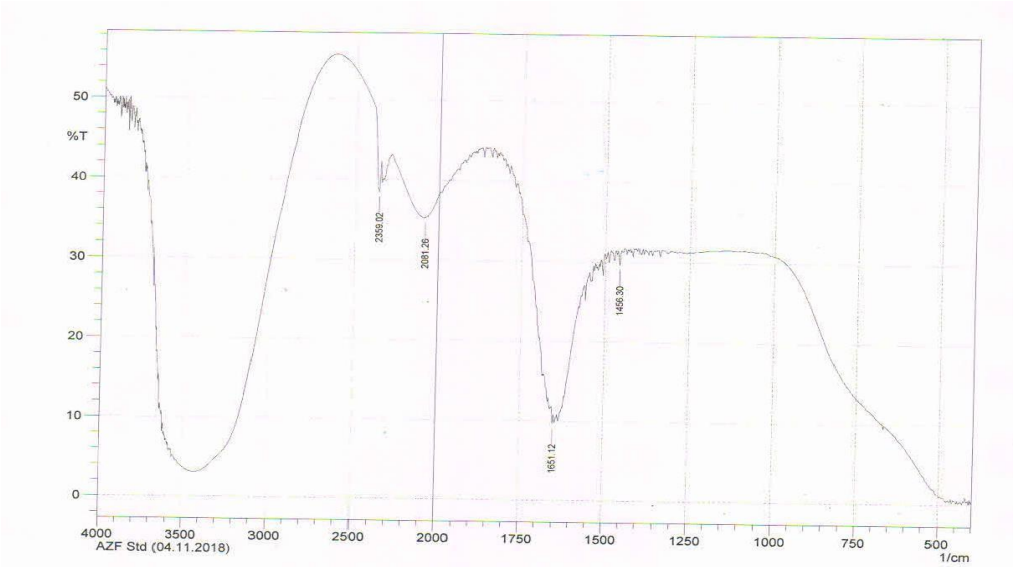



**Figure 23 IR spectrum of Final formulation (F7).**

As seen from **Figure 23** the spectrum of formulation (F7), peaks were obtained at 1651.12 $cm^{-1}$ C O stretch of ester form), 1647.2 $cm^{-1}$ (C=O stretch α,β–unsaturated aldehydes, ketones) and 2360.95 $cm^{-1}$ (O–H stretch).

**Discussion:** The FT-IR spectra of ultimate formulation (F7) indicate that characteristic peak of drug wasn't visible within the phytosomal formulation spectra and Ohio bond gift because of presence of water in formulation that indicates that drug was utterly encapsulate within the formulation.

### 7.4. Investigation of In-vitro Medication discharge

**The in-vitro medication release of Detailing F7 and Unadulterated medication was given in an exceedingly Table 19**

**Table 19: proportion drug unleash of Formulation F7 and Pure drug.**

| Time (Hr) | Pure drug (%) | F7 Formulation (%) |
|---|---|---|
| 0.25 | 14.129±0.914 | 8.819±1.422 |
| 0.50 | 25.557±0.968 | 14.983±0.821 |
| 1.00 | 39.639±0.821 | 22.048±1.603 |
| 2.00 | 57.183±1.422 | 32.574±0.739 |
| 3.00 | 69.748±1,480 | 41.109±0.865 |
| 4.00 | 77.192±1.566 | 47.368±1.865 |
| 6.00 | 86.628±0.865 | 56.187±0.752 |
| 8.00 | 89.947±1.351 | 62.778±0.672 |
| 10.00 | 90.848±0.821 | 66.761±0.783 |
| 12.00 | 91.417±1.282 | 69.653±0.821 |
| 24.00 | 92.508±1.789 | 75.912±1.452 |

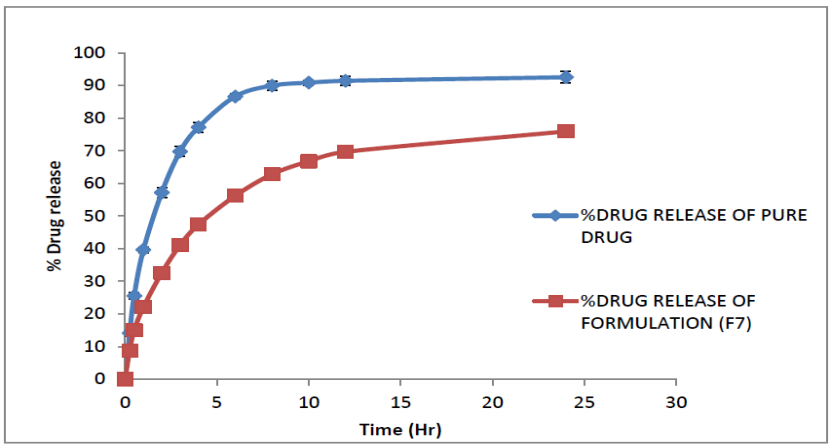



**Figure 24: In-Vitro Drug unleash of Azithromycin phospholipid complex phytosome of pure drug.**

### 7.4.1. In-vitro drug release kinetic

In-vitro drug unleash kinetic study data of formulation F7 was given below.

#### 7.4.1.1. Zero order

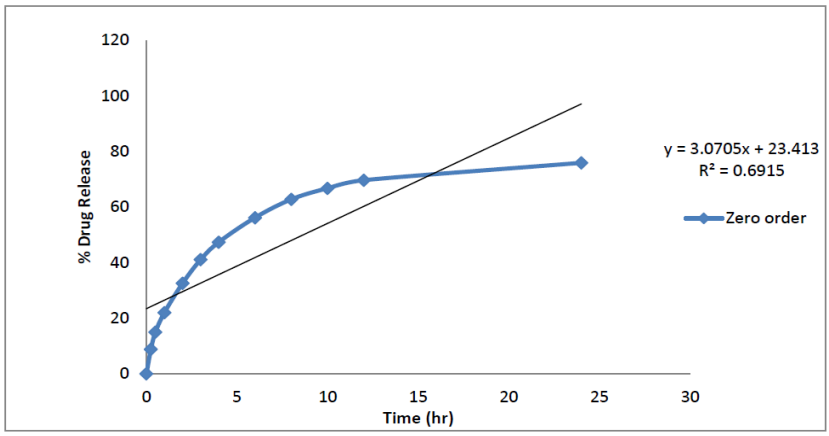



**Figure 25.Graph of formulation F7.**

#### 7.4.1.2 First Order

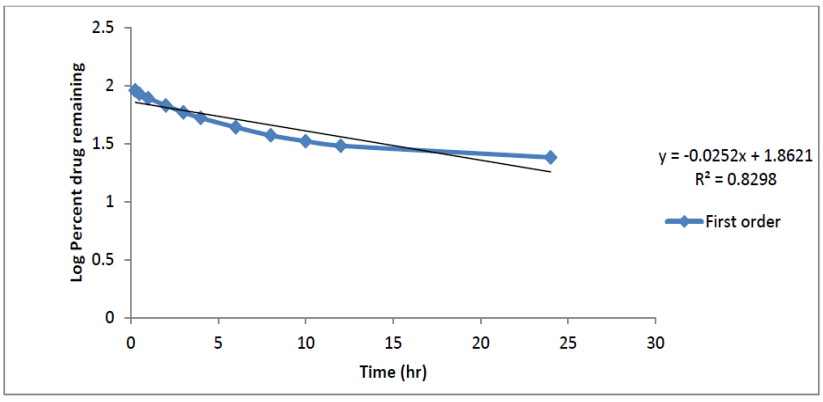



**Figure 26: Graph of formulation F7.**

### 7.4.1.3 Higuchi

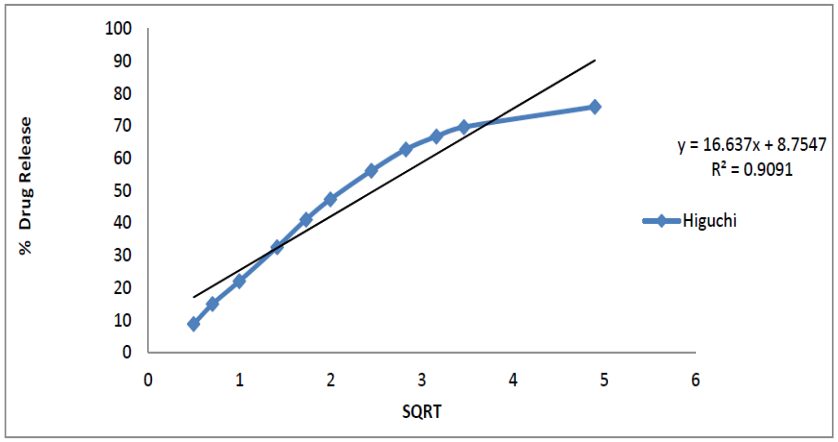



**Figure 27: Higuchi order graph of formulation F7.**

### 7.4.1.4 Korsmeyer peppas

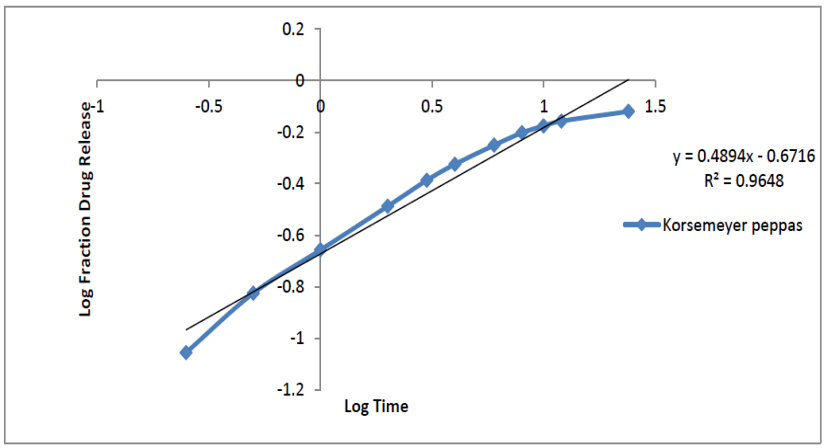



**Figure 28: Korsmeyer peppas order graph of formulation F7.**

**Table 20: Kinetic equation parameter of formulation F7.**

| Formulation name | Zero mechanics | | First mechanics | | Higuchi | | Peppas | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $K_0$ | $R^2$ | $K_0$ | $R^2$ | $K_0$ | $R^2$ | $K_0$ |
| F7 | 0.691 | 3.070 | 0.823 | 0.025 | 0.909 | 16.63 | 0.964 | 0.489 |

Scientific models ar regularly wont to foresee unleash|the discharge} component and look at discharge profile. For all the upgraded details, the half medication unharness versus time (zero request), log p.c medication remaining versus time (first request), log percent medication unharness versus base of your time (Higuchi plot), and log of close medication unharness versus log time (Korsmeyer and Peppas Exponential Condition) were planned. For each situation, R2 cost was determined from the chart and concurring in Table twenty and Figure twenty five to work twenty eight. Thinking about the assurance coefficients, Korseymer peppas model was found (R2=0.964) to suit the release learning best. It likely

could be finished from the outcomes that the medication was released from phytosome by a controlled system.

## CONCLUSION

The goal of drug delivery system is to provide therapeutic amount of drug to the proper site in the body and also to achieve and maintain the beloved plasma concentration of drug for a particular period of time. However, incomplete release of drug, shorter residence time of dosage form in the gastrointestional tract and high hepatic first pass effect leads to lower bioavailability. Such limitations of the conventional dosages forms have paved to an era of controlled and novel drug delivery systems.

Azithromycin is a macrolides antibiotics. It has a good effect on infections of the respiratory tract, eyes and so on. However, when applied systematically, it stimulates the gastrointestinal tract, and because of blood - aqueous humor barrier, the drug concentration stays low when absorbed into orbital tissue. Like other lipophilic drugs with poor solubility, Azithromycin has typical problems of low bioavailability and instability of absorption.

Drug-phospholipid complexes improve the bioavailability of drugs which have either very low lipid solubility. Therefore, drug can be complexed for improving biopharmaceutical properties. To improving the drug absorption, drug-phospholipid complexes also have the following advantages: 1) increasing the drug stability, 2) prolonging the drug duration of action.

Before phytosome development, preformulation studies were carried out to characterize the chemical and physical properties of drug substance. The FT-IR spectrum of drug samples was found to be in concordant with the reference chemical groups present in the structure of the Azithromycin. The UV spectrum of Azithromycin in Ethanol exhibited a broad band at 208 nm. The melting point was determined by capillary method which complies with the melting point given in reference. The solubility results showed that Azithromycin is soluble in DMF, Acetone, ethanol and DMSO. The solubility profile of drug in different solvents shows that drug is lipophilic in nature which is further confirmed by the partition coefficient study.

The standard curves of Azithromycin were prepared ethanol and the absorbance data obtained subjected to linear regression. The correlation coefficients were found to be 0.999 for Azithromycin which is closed to one indicated for good linearity.

The preformulation study (FT-IR spectrum, UV spectrum and melting point) results suggested that the Azithromycin was pure and good in quality and the estimation procedure was found to be quite reliable, accurate and suitable for formulation development.

Phytosomal formulation of Azithromycin was prepared by using the reflux technique method.

For optimization of Phytosome, different formulations (F1 to F8) were prepared using the various quantities of lipid. Formulation (F7) with maximum entrapment efficiency, No phase separation and optimum size considered as optimized formulation.

The shape and size of the optimized F7 formulation was confirmed through microscope and particle size and found that most of the particles were well identified.

Optimized formulation *in vitro* drug release was studied in Hydrochloric acid (0.1N) and phosphate buffer saline (PBS) pH 6.8 using dialysis method. To know precisely, the rate and mechanism of drug release, the *in vitro* data was fitted to zero order, first order, Higuchi and Korsmeyer-Peppas model. The results showed that the drug release of F7 formulation followed Korsmeyer-Peppas order which describes that the Azithromycin follows a controlled mechanism for release from phytosome.

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**REFERENCES**

1. Aungst, B.J. and Rogers, N.J., Site dependence of absorptionpromoting actions of Laureth-9, Na salicylate, Na2EDTA, and Aprotinin on rectal, nasal, and buccal insulin delivery, Pharm. Res., 1988; 5: 305-308.
2. Amit P., Tanwar Y. S., Rakesh S. & Poojan P., Phytosome: Phytolipid Drug Delivery System for Improving Bioavailability of Herbal Drug, J. Pharm. Sci. Biosci. Res., 2013; 3(2): 51–57.
3. Valenzuela A, Aspillaga M, Vial S, Guerra R. Selectivity of silymarin on the increase of the glutathione content in different tissues of the rat. Planta Med., 1989; 55: 420-422.
4. Saraf S, Kaur CD Phytoconstituents as photoprotective novel cosmetic formulations. Pharmacogn Rev., 2010; 4: 1-11.
5. Pandey S. Phytosome: Technical Revolution in Phytomedicine. International Journal of

Pharm Tech Research, 2010; 2: 627-631.

6. Amin T, Bhat S. A Review on Phytosome Technology as a Novel Approach to Improve the Bioavailability of Neutraceuticals. International Journal of Advancements in Research and Technology, 2012; 1: 1-15.
7. Saha S, Sarma A, Saikia P, Chakrabarty T. Phytosome: A Brief Overview. Scholars Academic Journal of Pharmacy, 2013; 2: 12-20.
8. Mahajan RT, Chaudhari GM. A novel approach towards phytosomal flavonoids. Int J Pharm Sci., 2012; 3(3): 1-15.
9. Singh Devendra, Rawat M. S. M., Semalty Ajay, Semalty Mona. Chrysophanol–phospholipid complex: A drug delivery strategy in herbal novel drug delivery system (HNDDS). Journal of Thermal Analysis and Calorimetry, 2013; 111: 2069–2077.
10. Ali J, Khar R, Ahuja A. A textbook of dosage form design. Birla publications Pvt Ltd, Delhi; 3rd edition, 2008; 100-107.
11. A Pandey, Rath B, AK. D. Pharmaceutical Preformulation Studies with Special Emphasis on Excipients Compatibility Chem Inform, 2012; 43(23): 20-5.
12. G. Casay, O. Quattrocchi, W. Hauck, A. Hernandez-Cardoso, J. Belsky, USP melting point reference standards: Evaluation of parameters that affect the melting point, USP Pharmacopeial Forum, PF39(4).
13. S Sharma, A Diwan, R Kalra, V Arora. Physico-chemical characterization, analytical meyhod development and Solubility studies for progesterone, Indian J. Pharm. Biol. Res., 2017; 5(2): 29-35.
14. Pavia DL, Lampman GM, Kriz GS. Introduction to Spectroscopy. Harcourt college publishers, Orlando, Florida, USA. 3rd edition, 2001; 13–27.
15. Baka E, Comer JE, Takacs-Novak K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. J Pharm Biomed Anal, Jan 22, 2008; 46(2): 335-41.
16. Xia XR, Baynes RE, Monteiro-Riviere NA, Riviere JE. Determination of the partition coefficients and absorption kinetic parameters of chemicals in a lipophilic membrane/water system by using a membrane-coated fiber technique. Eur J Pharm Sci., Jan, 2005; 24(1): 15-23.
17. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Himalaya publishing house, Delhi. 5th edition., 2002; 2.149-2.159.
18. Singh Devendra. Rawat M.S.M. Semalty Ajay. Semalty Mona. Rutin-Phospholipid Complex: An Innovative Technique in Novel Drug Delivery System- NDDS. Current

Drug Delivery, 2012; 9: 305-314.

19. Lars I, Martin B. Determination of the size distribution of liposomes by SEC fractionation, and PCS analysis and enzymatic assay of lipid content. AAPS Pharm Sci Tech., 2002; 3(2): 9–15.
20. Zhang J, Tang Q, Xu X, Li N. Development and evaluation of novel phytosome-loaded chitosan microsphere system for curcumin delivery. International Journal of Pharmaceutics, 2013; 448: 168-174.
21. Solmaz Rasaie, Saeed Ghanbarzadeh, Maryam Mohammadi, Hamed Hamishehkar. Nano Phytosomes of Quercetin: A Promising Formulation for Fortification of Food Products with Antioxidants. Pharmaceutical sciences, 2014; 20: 96-101.
22. Zhou, Chunsheng He, Kun Chen, Jieren Ni, Yu Cai, Xiaodi Guo, Xiao Yu Wu. A New Yousheng Method for Evaluating Actual Drug Release Kinetics of Nanoparticles inside Dialysis Devices via Numerical Deconvolution. Journal of Controlled Release, 2016; 243: 11-20.
23. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm., 2010; 67(3): 217-23.
24. Islam M, Reza S, Rahman H. In vitro Release Kinetics Study of Diltiazem Hydrochloride from Wax and Kollidon SR Based Matrix Tablets. Iranian Journal of Pharmaceutical Research, 2010; 7: 101-8.
25. Kaushik Dinesh, Kumar Pankaj, Sardana Satish Design Development and Evaluation of Nanosuspension of Azithromycin International Journal of Pharmaceutical Sciences and Drug Research, 2015; 7(5): 384-394.
26. Kaushik Dinesh, Kumar Pankaj, Sardana Satish Design Development and Evaluation of Nanosuspension of Azithromycin International Journal of Pharmaceutical Sciences and Drug Research, 2015; 7(5): 384-394.
27. Mohammadi Ghobad. Valizadeh Hadi. Barzegar-Jalali Mohammad. Lotfipoura Farzaneh. Adibkia Khosro. Morteza Milania. Azhdarzadeha Morteza. Kiafar Farhad. Nokhodchid Ali. Development of azithromycin–PLGA nanoparticles: Physicochemical characterization and antibacterial effect against Salmonella typhi. Colloids and Surfaces B: Biointerfaces, 2010; 80: 34–39.
28. Parul A. Ittadwar, Prashant K. Puranik. Novel umbelliferone phytosomes: development and optimization using experimental design approach and evaluation of photo-protective and antioxidant activity. International Journal of Pharmacy and Pharmaceutical Sciences, 2017; 9(1): 218-228.