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Synthesis of Nitric Oxide Derivatives of Lumefantrine for Antimalarial Activity

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Keywords: Lumefantrine, Poly(dichlorophosphazenes), polyphosphazenes, Nitric oxide, an antimalarial drug.

ABSTRACT

The present research work entitled, “Synthesis of Nitric oxide derivatives of Lumefantrine For Antimalarial Activity", pertains to the synthesis and evaluation of novel poly(dichlorophosphazenes)-linked Prodrugs of Lumefantrine to have the desired delivery to the infected targets. Poly(dichlorophosphazenes) was synthesized from hexachlorocyclotriphosphazene (prepared by reacting ammonium chloride and phosphorus pentachloride) and linked with antimalarial drug Lumefantrine, through the spacer. These substituted polyphosphazenes can also be suitably modified to have the desired physiochemical properties. Therefore, the proposed polymer-linked antimalarial analogues are expected to have the targeted drug delivery with prolonged action. Nitric oxide (nitrogen oxide, nitrogen monoxide) is a molecular, chemical compound with chemical formula of NO. One of several oxides of nitrogen, it is a colorless gas under standard conditions. Nitric oxide (NO), a small endogenic gas molecule, plays an important role in regulating physiological functions, including the inhibition of platelet aggregation and thrombus formation in cerebrovascular and cardiovascular systems. Nitric oxide is secreted as free radicals in an immune response and is toxic to bacteria and intracellular parasites, including malaria; the mechanism for this includes DNA damage and degradation of iron sulfur centers into iron ions and iron-nitrosyl compounds.

1. Introduction

1.1 Incidence, Prevalence and Survival

Almost one-half of the population lives under the threat of malaria, and the disease is responsible for approximately 1.7-2.0 million deaths per year **(1)**.A large percentage of the fatalities occur in Africa; however, malaria is endemic throughout most of South East Asia, the Indian subcontinent, the South Pacific region, and Latin America. Malaria is caused by parasitic protozoans belonging to the *Plasmodium* .Five species of *Plasmodium* can infect and be spread by humans. Most deaths are caused by *P. falciparum*. The disease is most commonly transmitted by an infected female *Anopheles* mosquito and humans.

1.2Malaria Life Cycle

The life-cycle of malaria begins by the bites of an infected female mosquito by her prey, withdrawing blood and at the same time injecting sporozoite-containing saliva into the capillaries of the skin. The sporozoites enter liver cells and multiply to form about 30,000 merozoites each. After about 5 days, the merozoites are released into the blood stream. They enter into red blood cells and `develop through the so-called ring, trophozoite, and schizont stages. The erythrocyte provides the parasite with a safe haven from the host's immune system, but presents certain logistical problems with regard to access to nutrients and disposal of waste products **(2)**.

Parasite growth is supported by host hemoglobin ingestion. During a 48-hr (or 72-hr for *Plasmodium malariae*) cycle the parasite divides to produce 16–20 daughter merozoites. The merozoites burst from the mature schizont and release cell debris, which causes a febrile episode in the host. After that the merozoites invade new red blood cells and the cycle continues. After several cycles, some of the intra-erythrocytic parasites develop into sexual stage gametocytes. When a mosquito bites an infected individual the gametes are ingested. They mate in the gut of the insect and then pass through the gut wall, where they develop into poipoocysts that release sporozoites that migrate to the salivary glands to be passed on to another individual. This stage of the malaria parasite is intra-erythrocytic in which the disease pathology is produced as shown in **Figure1.1.**

Due to complications of infections with *Plasmodium falciparum* most of the deaths are occur, whereby erythrocytes infected with mature-stage parasites adhere to the vascular

endothelium of post-capillary venules, particularly in the brain. Vascular occlusion and/or an inappropriate hostimmune reaction can lead to coma **(3)**. Once a coma is established in malaria patient, the patient has only a 10–50% chance of survival, even with optimal medical care also. Whilst the blood forms of the parasite cause most of the pathology of the disease, they are also the stages that are most susceptible to attack by antimalarial drugs. Therefore, there is direct need for the novel effective antimalarial drugs and also various approaches that may not result into drug resistance.

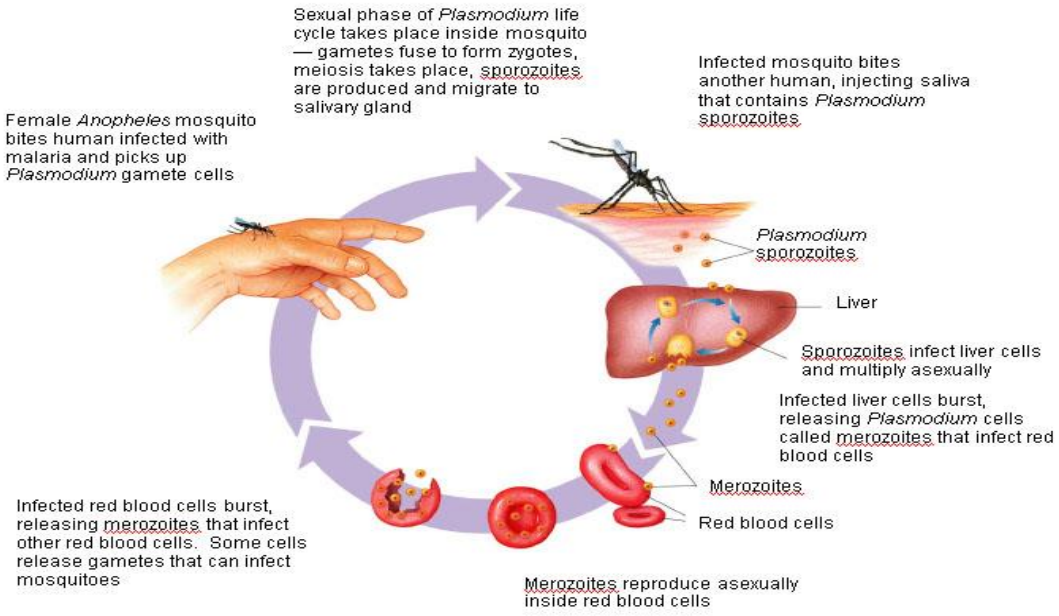


Fig.1.1Life cycle of malaria.

Time taken for infection to symptoms:

Plasmodium falciparum – 6-12 days.

Plasmodium vivax – 10-17 days.

Plasmodium ovale – 14 days.

Plasmodium malariae – 28-30 days

2. Material and method

2.1 Methodology

2.1.1Preformulation studies

The main goal of preformulation study is to investigate critical physiochemical factors which assure identity, purity, product performance and quality. It is the first stage of formulation development as it helps to determine the state as well as composition of pure drug substance. Furthermore, it also helps to provide information regarding physicochemical properties such as solubility, the effect of pH and predict sensitivities to environmental conditions. Some of these preformulation parameters include checking physical appearance, melting point determination, IR and UV analysis. Development of beneficial pharmaceutical dosage form requires various preformulation parameters as described in **Figure 4.1 (Sinha *et al.*, 2014;Gibson, 2009)**.

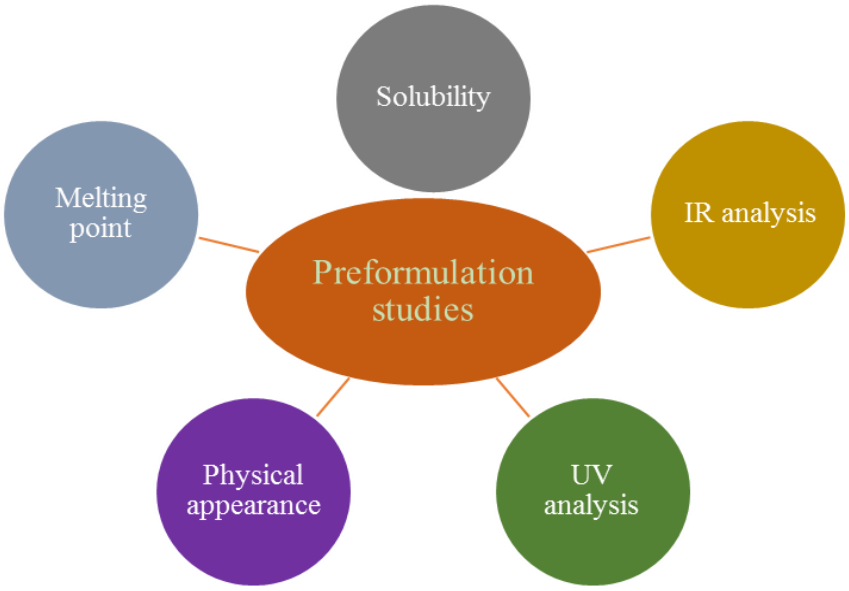


Figure 4.1: Common Screening Techniques for Preformulation

2.1.2 Drug identification

Antimalarial drug Lumefantrine was obtained as a gift sample from Alkem Laboratories limited Navi Mumbai- 410208 India. Drug sample was characterised according to standard procedures.

2.1.3 Physical appearance

Physical appearance of procured drug was noted by visual observation.

2.1.4 Melting point determination

Melting point of drug sample was determined by using digital capillary apparatus. A small amount of drug was filled into one sided sealed capillary and was placed in the melting point apparatus along with calibrated thermometer and the temperature at which drug melted was recorded. This test was performed in triplicate to observe the melting point range as shown in the result **(IP, 2014)**.

2.1.5 Drug-excipient interaction study (FTIR Spectroscopy)

Drug excipient compatibility study was performed for identification and structural analysis of the drug Lumefantrine using Fourier Transformed Infrared Spectrophotometry. The potassium bromide disc technique was employed using a drug (1.0 mg) in IR grade dried potassium bromide (100.0 mg). The mixture was triturated into a fine powder using an agate mortar/pestle and compressed into potassium bromide disc using a hydraulic press at 10,000 psi. Each potassium bromide disc was scanned 32 times at 4 mm s^{-1} at a resolution of 2 cm^{-1} over a pallet wave number region of 4000-400 cm^{-1} and characteristic bands were recorded. Further results were compared with standard peaks available in the literature. The spectrum obtained has shown identical peaks as reported in the reference sample of Lumefantrine as shown in results **(Karuna *et al.*, 2014).**

2.1.6 Ultraviolet absorption maxima (λ_{max}) (Arun *et al.*, 2011).

The solution of drug sample Lumefantrine was prepared in methanol and was scanned from wavelength 200-400nm using Shimadzu 1800 Spectrophotometer taking methanol as blank. The drug sample showed absorption maxima at λ_{max} 234 nm.

2.1.6.1 Development of calibration curves

Calibration curve of Lumefantrine was prepared by plotting absorbance of its various concentrations in methanol.

2.1.6.2 Standard curve of Lumefantrine in Methanol.

(a) Preparation of stock solution with Methanol

Lumefantrine (10 mg) was accurately weighed and transferred into a 100.0 ml volumetric flask. Methanol (50.0 ml) was added, sonicated for 15 min. for complete dissolution of drug and finally the volume was made upto 100.0 ml with methanol.

(b) Preparation of working solutions

From the stock solution 5 dilutions were prepared in the concentration range of 2, 4, 6, 8 and 10 µg/ml. Stock solution (0.2 ml) was withdrawn and transferred into 10.0 ml of volumetric flask and volume was made upto 10.0 ml with methanol. Similarly, all four dilutions were made in the concentration range of 4, 6, 8, 10 µg/ml following the same procedure. The absorbance of each solution was measured spectrophotometrically at λ_{max} 234 nm and a standard curve between the concentrations and their respective absorbance was plotted.

2.1.6.3 Standard curve of Lumefantrine in phosphate buffer pH 7.4

(a) Preparation of Phosphate buffer solution pH 7.4

Potassium dihydrogen phosphate (2.7 gm) was placed in 100.0 ml of volumetric flask and volume made up with water. Sodium hydroxide (0.8 gm) was placed in 100.0 ml of volumetric flask and volume was made up with water. Potassium dihydrogen phosphate solution (62.5 ml) and sodium hydroxide (48.8 ml) was placed in 250.0 ml of volumetric flask **(IP, 2014)**.

(b) Preparations of stock solutions

Lumefantrine **(8)** (10 mg) was accurately weighed and transferred into 100.0 ml of volumetric flask. Methanol (50.0 ml) was added and sonicated for 15 min. for the complete dissolution of drug and the volume was made up to 100.0 ml with phosphate buffer solution pH 7.4.

(c) Preparation of working solutions

From the stock solution 5 dilutions were prepared in the concentration range of 2, 4, 6, 8 and 10 µg/ml. Stock solution (0.2 ml) was withdrawn and transferred into 10.0 ml of volumetric

flask and volume was made up to 10.0 ml with Phosphate buffer of pH 7.4. Similarly, all the dilutions were made as followed by same procedure. The absorbance of each solution was measured spectrophotometrically at λ_{max} 234 nm and a standard curve between the concentrations and their respective absorbance was plotted as shown in the result.

2.1.6.4 Standard curve of Lumefantrine in phosphate buffer solution pH 6.8

(a) Preparation of Phosphate buffer solution 6.8

Potassium dihydrogen phosphate (2.7 g) was placed in 100.0 ml of volumetric flask and volume made up with water. Sodium hydroxide (0.8 g) was placed in 100.0 ml of a volumetric flask and volume made up with water. Potassium dihydrogen phosphate solution (62.5 ml) and sodium hydroxide (28.0 ml) were placed in 250.0 ml of volumetric flask. The pH was adjusted to 6.8.

(b) Preparations of stock solutions

Lumefantrine **(8)** (10 mg) was accurately weighed and transferred into 100.0 ml of volumetric flask. Methanol (50.0 ml) was added, sonicated for 15 min. for complete dissolution of the drug and the volume was made up to 100.0 ml with phosphate buffer solution pH 6.8.

(c) Preparations of working solutions

From the stock solution 5 dilutions were prepared in the concentration range 2, 4, 6, 8 and 10µg/ml. Stock solution (0.2 ml) was withdrawn and transferred into the 10.0 ml of volumetric flask and volume was made up with phosphate buffer solution pH 6.8. Similarly, other four dilutions were made in the concentration range of 4, 6, 8 and 10 µg/ml following the same procedure. The absorbance of each solution was measured spectrophotometrically at λ_{max} 234 nm and a standard curve between the concentrations and their respective absorbance was plotted as shown in the results.

3. Result And Discussion

3.1 Characterization of Drug Sample (Lumefantrine)

3.1.1 Drug Sample Identification

Lumefantrine drug sample was identified on the basis of its physical appearance, melting point, solubility, absorption maxima, and FT-IR spectra. Based on the results of characterization study of the drug sample was confirmed.

3.1.2 Physical Appearance

Drug sample of Lumefantrine was visually observed and was found to be yellow coloured powder.

3.1.3 Melting Point

The melting point was found to be 127-132°C for Lumefantrine drug samples respectively which is as per the reported melting point **(IP, 2014; Saini *et al.*, 2015)**.**Table 3.1** gives the details of the melting point observed.

Table 3.1 Melting Point of Lumifantrine

Parameter	Drug name	Observed	Standard
Melting point	Lumefantrine	127-132°C	128-132°C

3.1.4 IR Analysis

IR spectrum of Lumefantrine is shown in **Figure 3.1**. Observed peaks in IR spectrum were found to be concordant with functional groups present in structure of Lumefantrine and shows frequency of observed bands and its interpretation. Purity of procured sample was confirmed from its IR spectrum. All the results of identification and characterization confirmed identity and purity of both procured drug samples.

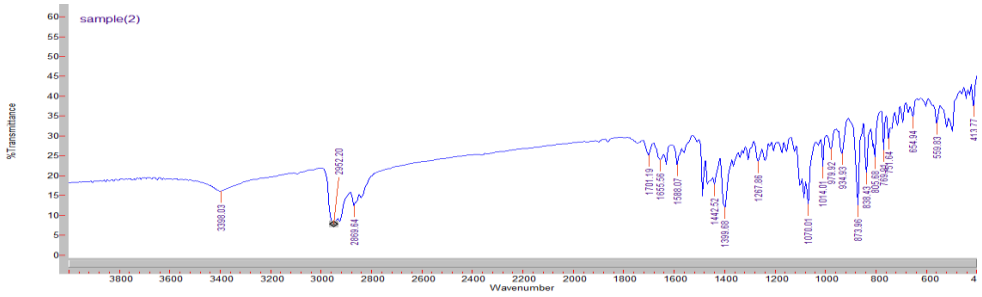


Figure 3.1: FT- IR Spectrum of Lumefantrine

IR (KBr) cm^{-1}: 3398 (O-H str. alcoholic), 2952 (-CH_2 str., aliphatic butyl), 2869 (-CH_2 str., methylene), 1442 (-C=C- str., aromatic) and 838 (C-Cl).

3.1.5 Standard Curve of Lumefantrine

To obtain calibration curve, different concentrations of Lumefantrine was dissolved in 0.1 N HCl (pH 1.2), PBS (pH 6.8), PBS (pH 7.4) and Methanol absorbance was measured at 234 nm. **Table 3.2, Table 3.3, Table 3.4** and **Table 3.5** shows absorbance values for concentration of Lumefantrine in 0.1 N HCl (pH 1.2), PBS (pH 6.8) and PBS (pH 7.4) and Methanol. **Figure 3.2, Figure 3.3 and Figure 3.4** and **Figure 3.5** explain the calibration curve of Lumefantrine in 0.1 N HCl (pH 1.2), PBS (pH 6.8), PBS (pH 7.4) and Methanol with correlation coefficientr^2 = 0.992 (pH 1.2), r^2 = 0.994 (pH 6.8), r^2 = 0.995 (pH 7.4), and r^2 = 0.996 (Methanol). Results inferred that Beer's law was obeyed in concentration ranges 0.2-1.0 µg/ml of Lumefantrine in 0.1 N HCl (pH 1.2), PBS (pH 6.8), PBS (pH 7.4) and Methanol respectively **(Arun *et al.*,2011).**

Table 3.2: Absorbance Values for Different Concentrations of Lumefantrine in 0.1N HCl (pH 1.2).

Sr. No	Concentration (µg/ml)	Mean Absorbance (nm)	Standard Deviation
1	0	0	0
2	2	0.218	0.004
3	4	0.333	0.007
4	6	0.477	0.007
5	8	0.670	0.005
6	10	0.783	0.004

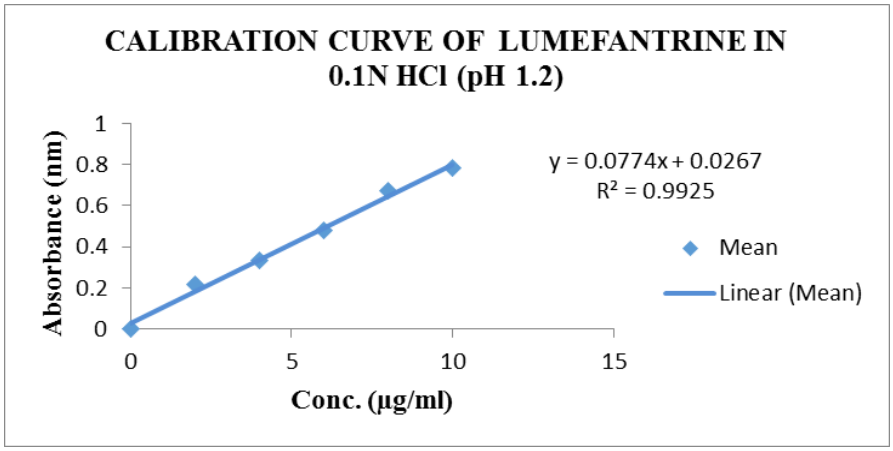


Figure 3.2 Calibration Curve of Lumefantrine in 0.1N HCl (pH 1.2)

Table 3.3: Absorbance Values for Different Concentrations of Lumefantrine with phosphate buffer solution (PBS) pH 6.8.

Sr. No	Concentration (µg/ml)	Mean Absorbance (nm)	Standard Deviation
1	0	0	0
2	2	0.225	0.002
3	4	0.355	0.003
4	6	0.514	0.052
5	8	0.662	0.005
6	10	0.807	0.002

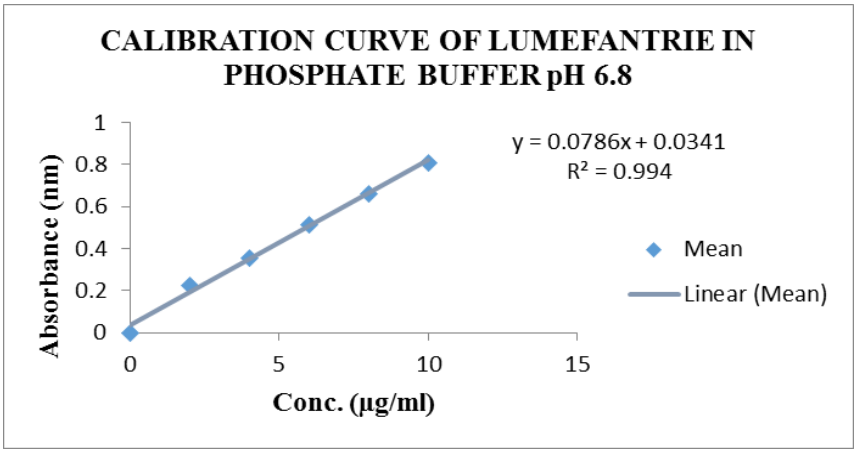


Figure 3.3 Calibration Curve of Lumefantrine in Phosphate Buffer pH 6.8

Table 3.4: Absorbance Values for Different Concentrations of Lumefantrine in Phosphate buffer pH 7.4.

Sr. No	Concentration (µg/ml)	Mean Absorbance (nm)	Standard Deviation
1	0	0	0
2	2	0.203	0.002
3	4	0.319	0.006
4	6	0.468	0.015
5	8	0.609	0.005
6	10	0.763	0.056

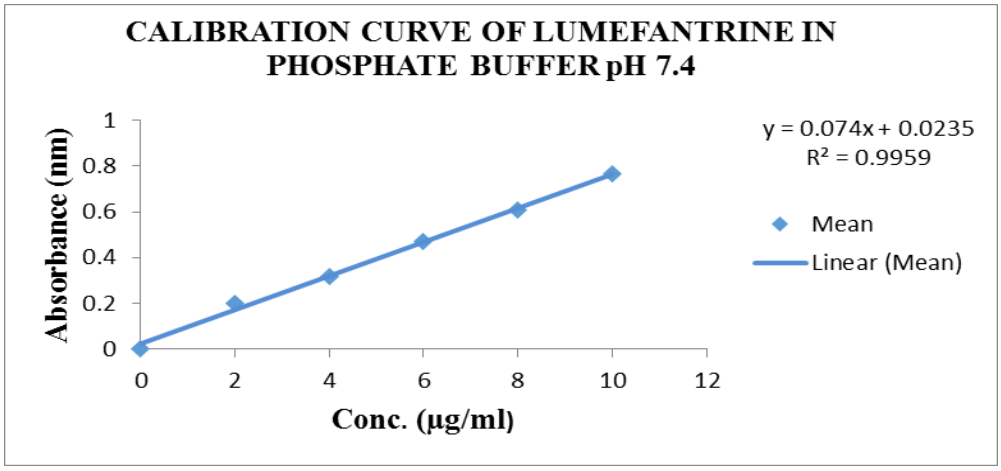


Figure 3.4 Calibration Curve of Lumefantrine in Phosphate Buffer pH 7.4.

Table 3.5: Absorbance Values for Different Concentrations of Lumefantrine in Methanol.

Sr. No	Concentration (µg/ml)	Mean Absorbance (nm)	Standard Deviation
1	0	0	0
2	2	0.203	0.001
3	4	0.336	0.003
4	6	0.475	0.002
5	8	0.629	0.001
6	10	0.768	0.003

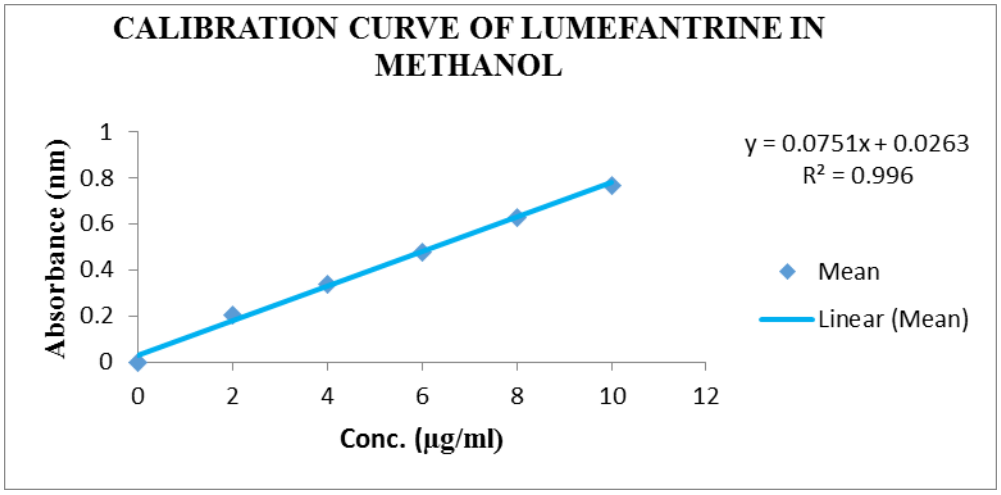


Figure 3.5 Calibration Curve of Lumefantrine in Methanol

3.1.6 Saturation solubility

The solubility profile of Lumefantrine in various solvents is given in **Table 3.6**. Solubility of Lumefantrine in water was found to be very low. This result is in compliance with reported value. However, it was found to be soluble in organic solvents like methanol, ethanol, chloroform and tetrahydrofuran. Results of solubility studies confirmed the high affinity of Lumefantrine towards organic solvents and poor solubility in aqueous media. This confirmed the lipophilic nature of the drug **(Gibson, 2009; Kotila *etal.*, 2013)**.

Table 3.6: Qualitative Solubility Studies of Lumefantrine

Solvent	Standard (mg/ml)	Experimental (mg/ml)
Tetrahydrofuran	15	13
Chloroform	6.8	7.5
Ethanol	2.8	2.3
Methanol	2.9	2.5
Distilled Water	0.1	0.05

3.2 The research work carried out is discussed under the heads:

1. Synthesis of ss2-(dibutylamino)-1-(9-benzylidene-2,7-dichloro-9,9a-dihydro-4a*H*-fluoren-5-yl) ethyl 4-aminobenzoate**(14)**

2. Synthesis of Polymer-Drug conjugates of Lumefantrine **(8)** substituted with Methyl 4-amino benzoate**16 (PDA-1)**, Glycine methyl ester **19 (PDA-2),** Glycine ethyl ester **21 (PDA-3)** and Anilino substituted polymer-drug conjugate **23 (PDA-4).**

3. Characterization of Polymer-Drug Conjugates of Antimalarial Drug.

4. Drug release profile Lumefantrine **(8)** from Polymer linked antimalarial Drug Conjugates.

3.3 Synthesis of 2-(dibutylamino)-1-(9-benzylidene-2,7-dichloro-9,9a-dihydro-4a*H*-fluoren-5-yl) ethyl 4-aminobenzoate (14)

Figure 3.6: FT-IR spectrum of Ethyl 4-amino benzoate (14)

IR (KBr) cm^{-1}: 3400.09 (N-H str.), 2927.86 (C-H-str., aliphatic), 1722.67 (-C=O str., ester), 1440.57 (-C=C- str. aromatic), 1174.90 (C-O str.), 833.84(-C-Cl str.).

^{1}H NMR (CDCl$_3$) δ (ppm):0.97-1.71 (m, 18H, aliphatic protons), 5.40-5.41 (S, 1H, HC=C-AR), 7.26-8.04 (m, aromatic protons) and 9.18--9.50 (br, 2H, NH protons).

3.4. Synthesis of Methyl 4-amino benzoate (15)

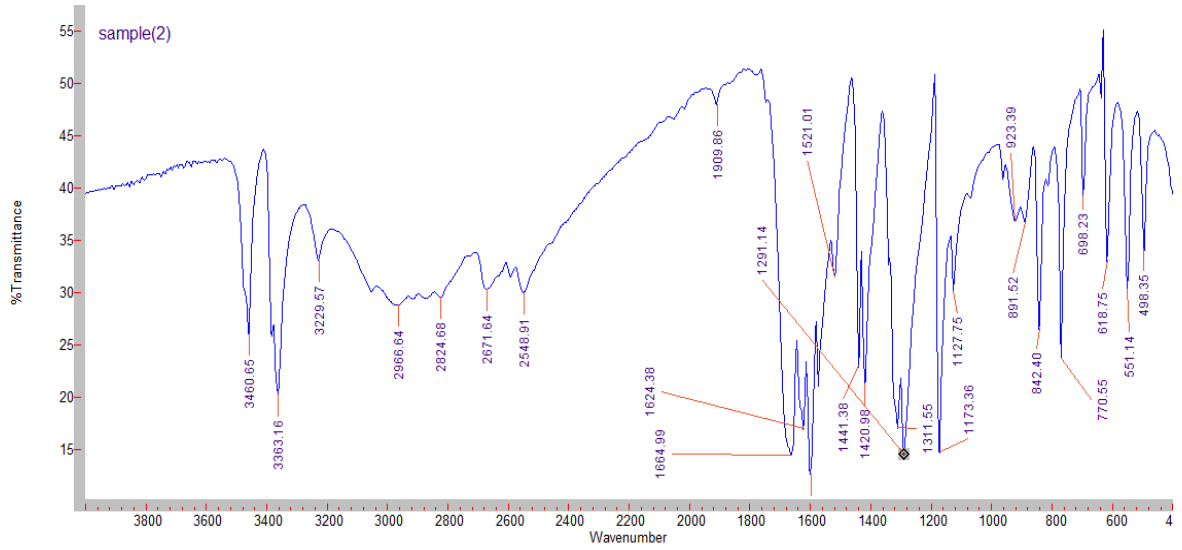


Figure 3.7: FT- IR spectrum *p*-aminobenzoic acid (13)

IR (KBr) cm^{-1}: 3460.05 (N-H str.), 3363.16 (O-H str., -COOH), 1664.99 (-C=O str., carboxylic), 1600 (C=C str., aromatic) and 1127.75 – 1311.55 (C-O str., carboxylic).

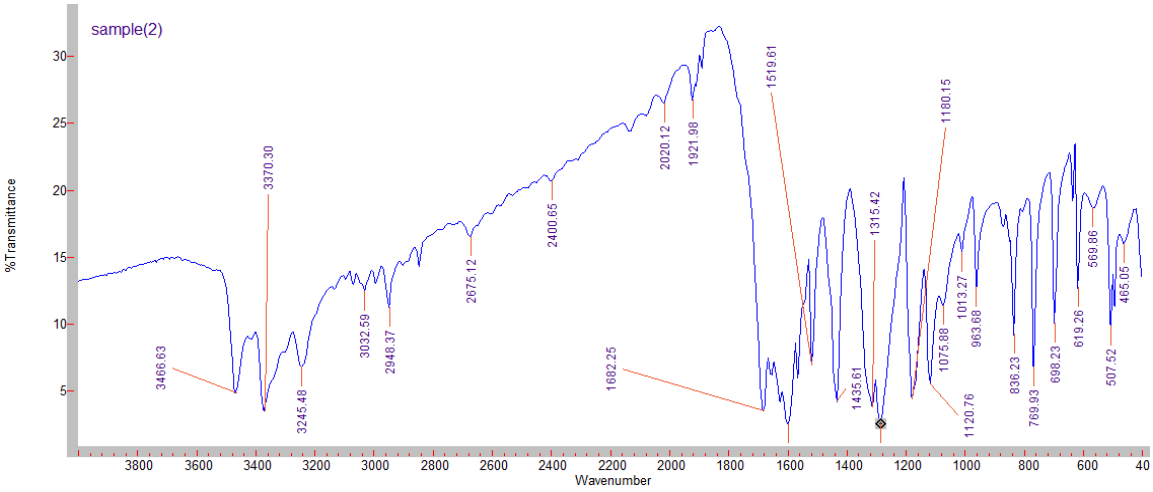


Figure 3.8: FT-IR spectrum of Methyl 4-amino benzoate (15)

IR (KBr) cm^{-1}: 3466.63 (N-H str.), 3370.30 (O-H str., -COOH), 3032.59 (C-H str., aromatic), 2948.37 (-C-H str., aliphatic), 1682.25 (-C=O str., ester), 1600 (C=C str., aromatic) and 1120.76 – 1315.42 (C-O str., carboxylic). **Yield:89. 67%.**

3.4.1 Synthesis of Polydichlorophosphazenes (12)

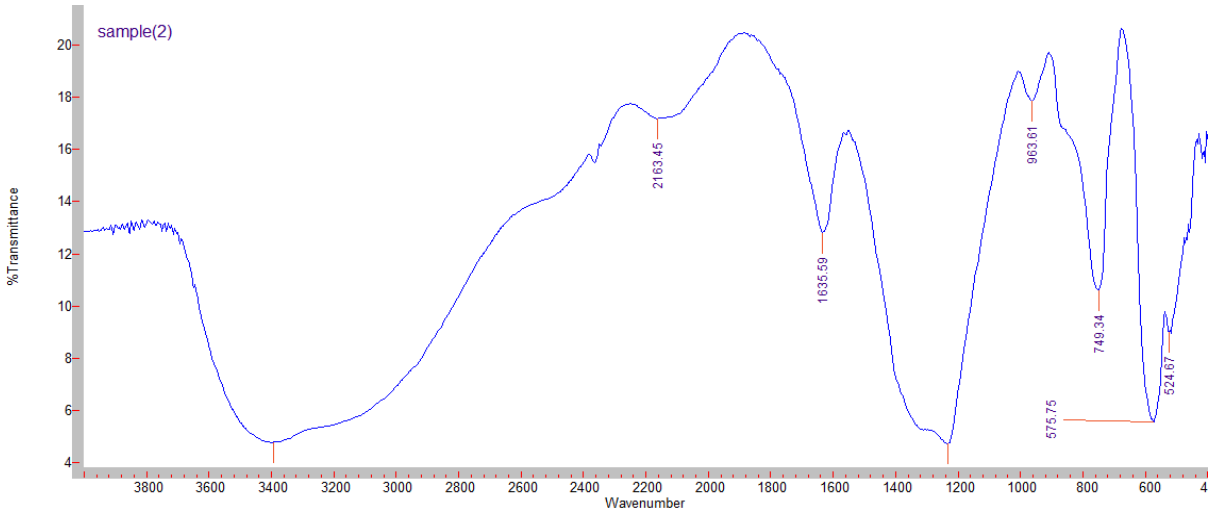


Figure 3.9: FT-IR Spectrum of dichloropolyphosphazenes (12)

IR (KBr) cm^{-1}: 1218 (P=N str.).**3.4.2 Synthesis of Polymer-Drug Conjugates of Lumefantrine substituted with methyl 4-aminobenzoate 16 (MDA-1)**

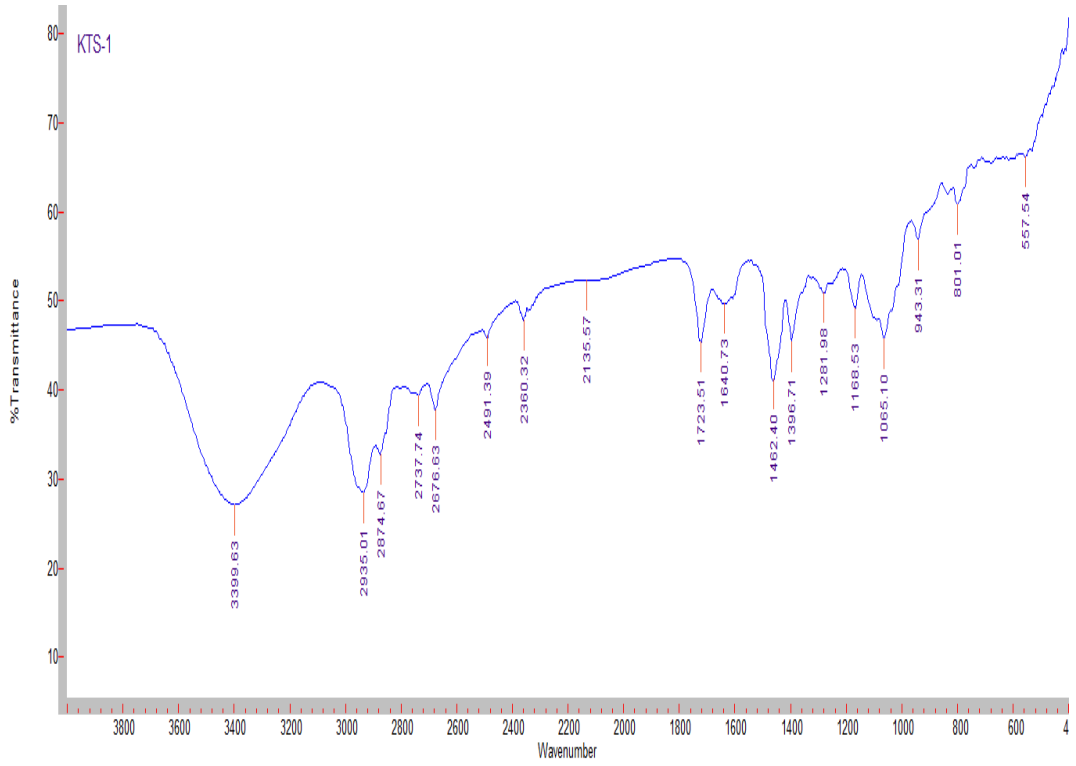


Figure 3.10: FT-IR spectrum of Polymer-Drug Conjugates of Lumefantrine substituted with Methyl 4-aminobenzoate 16 (PDA 1)

IR (KBr) cm^{-1}: 3399.63 (N-H str.), 2935.01 (C-H str., aliphatic), 2874.67 (C-H str. PEG), 1723.51 (-C=O str., ester), 1640.73 (-C=C- str. alkene) and 1462.40 (-C=C- str. aromatic), 1281.98 (-P=N- str.), 1168.53(C-O str.), 1065 (C-O str. PEG) and 801.01(-C-Cl str.).Yield: 92%.

^{1}H NMR ($CDCl_3$) δ (ppm):0.85-1.94 (m, aliphatic protons), 2.02-2.53 (m, benzylic proton of lumefantrine), 3.14-3.84 (m, oxymethyl protons of PEG), 4.06-4.39 (m, NH protons), 7.41-8.44 (m, aromatic protons).

3.5 Synthesis of Glycine methyl ester hydrochlorides (18)

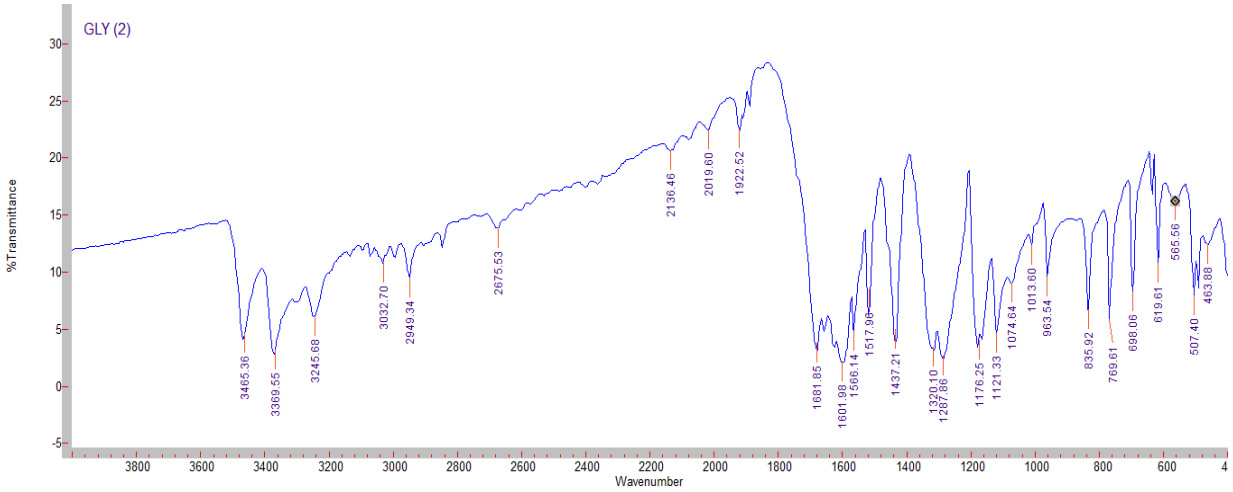
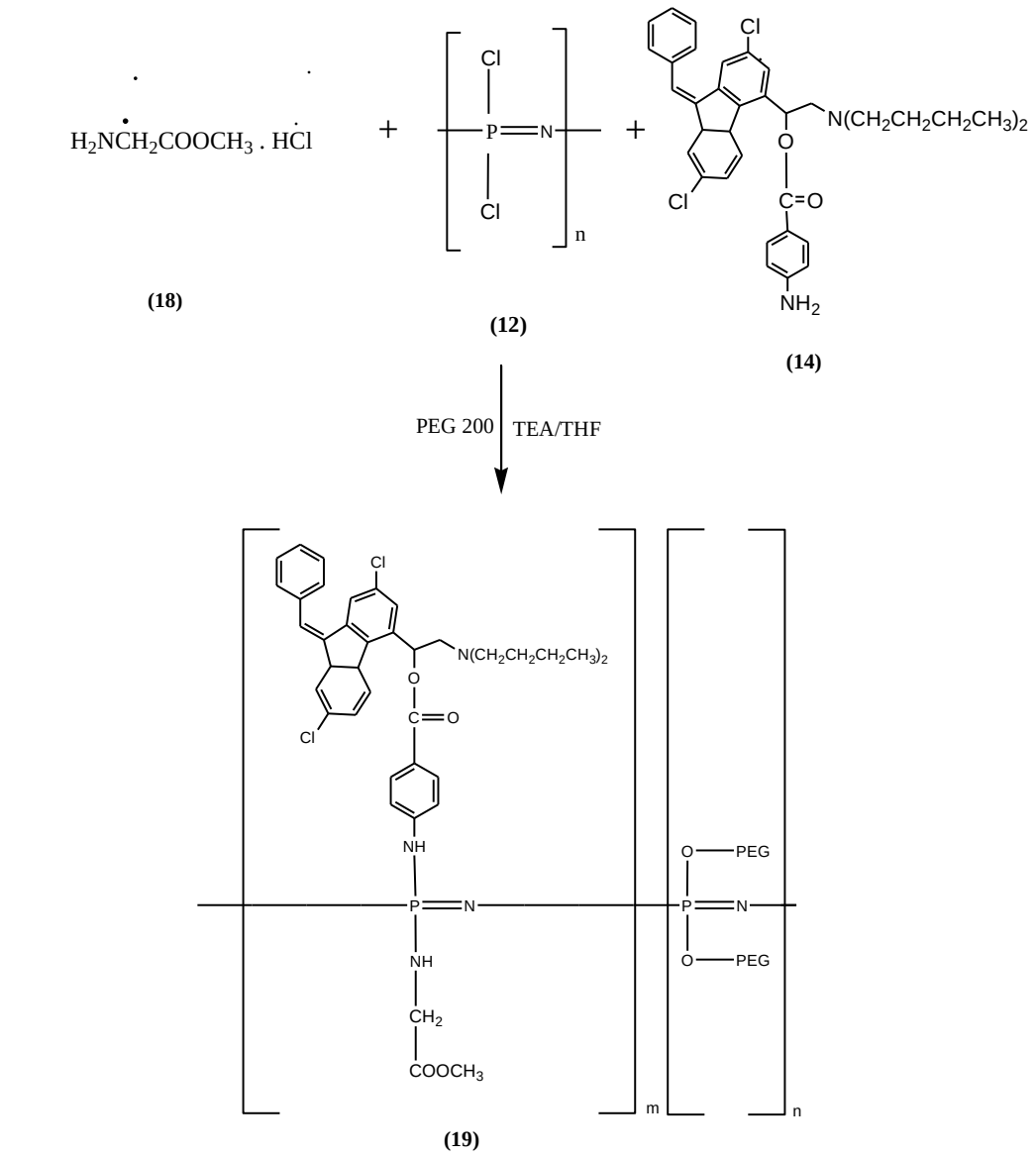


Figure 3.11: FT-IR Spectrum of Glycine methyl ester (18)

IR (KBr) cm^{-1}: 3465.36 (N-H str.), 2949.34 (C-H str., aliphatic), 1681.85 (-C=O str., ester) and 1121.33 (C-O str.). **Yield: 90.34%**

3.5.1Synthesis of Polymer-Drug Conjugates of Lumefantrine substituted with Glycine methyl ester 19 (PDA-2)

Polymer polydichlorophosphazene **(12)** (0.094 g) (0.00081 moles) was dissolved in dry tetrahydrofuran (100 ml) and placed in round bottom flask. The compound **14 (**0.5 g) (0.00081 moles) was transferred into thepolymer solution. Triethylamine (10.0 ml) and PEG 200 (0.1 ml) was added into the polymer solution. Thereafter, glycine methyl ester **(18)**(0.050 g) (0.0004 moles) was transferred to polymer solution. The reaction mixture was refluxed for 170 hr After refluxing, the solution was cooled at room temperature and the filtrate was concentrated under reduced pressure to yield the final product. The product was dried under vacuum desiccator for 2 days. Yield: 87%



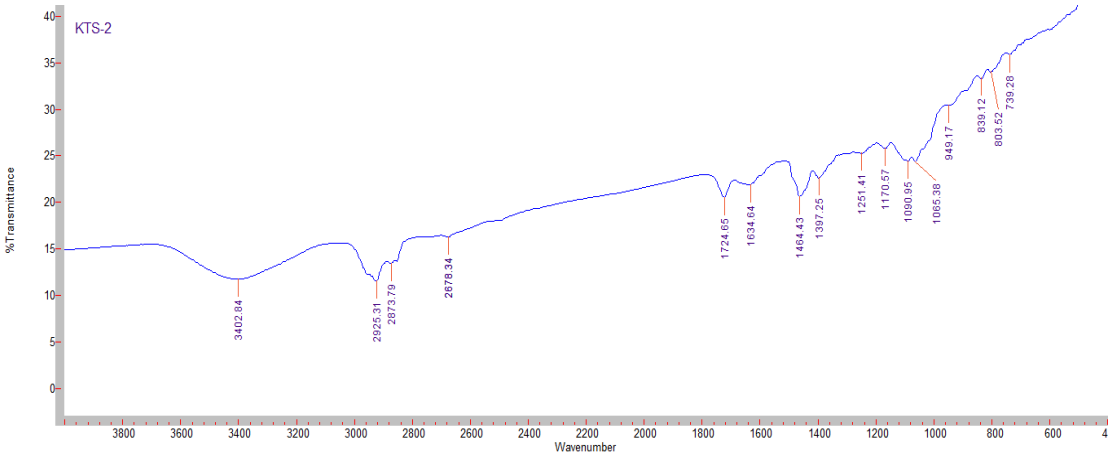


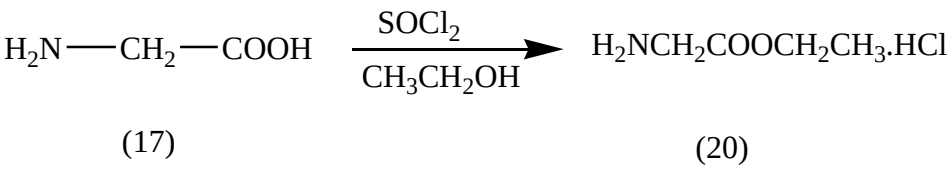
Figure 3.12: FT- IR Spectra of Polymer-Drug Conjugate of Lumefantrine substituted with Glycine methyl ester 19 (PDA 2)

IR (KBr) cm^{-1}: 3402.84 (N-H str.), 2925.31 (C-H str., aliphatic), 2873.79 (C-H str. PEG), 1724.65 (-C=O str., ester), 1634.64 (-C=C- str. alkene), 1464.43 (-C=C- str. aromatic), 1251.41 (-P=N- str.), 1090.95(C-O str.), 1065.38 (C-O str. PEG) and 949.17(-C-Cl str.).Yield: 87%.

^{1}H NMR ($CDCl_3$) δ (ppm):0.67-1.71 (m, aliphatic protons of lumefantrine & glycine), 2.03-2.52 (m, benzylic protons of lumefantrine), 3.00-3.99 (m, oxymethyl protons of PEG), 4.01-4.38 (N-H protons of aromatic & glycine moiety), 7.31-7.63 (m, aromatic protons).

3.6 Synthesis of Glycine ethyl ester hydrochlorides (20)

Thionyl chloride (1.4 ml) was added to ethanol (100.0 ml) slowly at 0 °C. Glycine **(17)** (2.0 g) was added to solution. The resulting mixture was refluxed for 8-10 hr at ambient temperature. Solvent was evaporated and the residue was triturated with ether at 0 °C until excess dimethylsulphite was removed. The crude product was crystallized from methanol andether at 0 °C to get pure Glycine ethyl ester hydrochloride **(20)**.Yield: 94.34%(**Ramesh*et al*., 2008)**.



(17) (20)

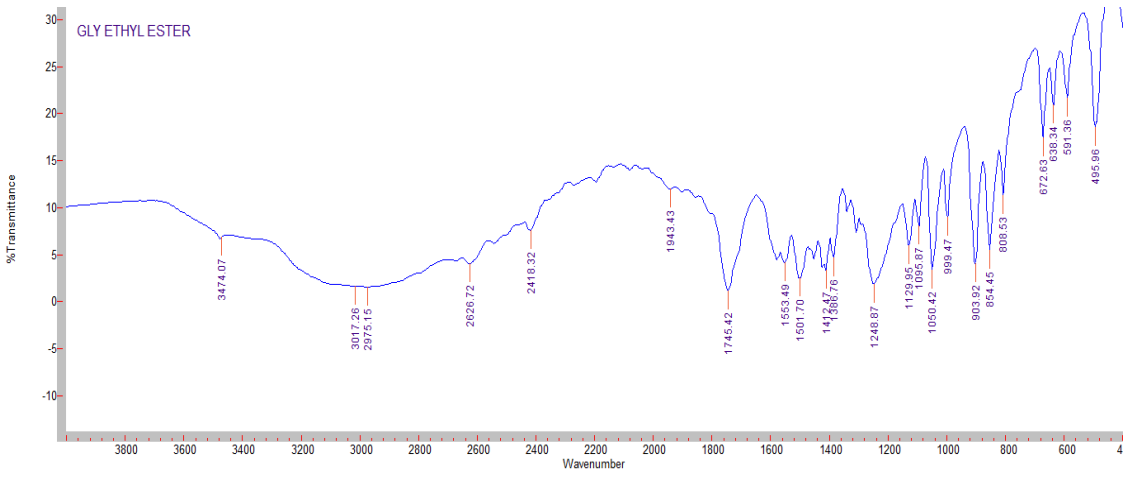
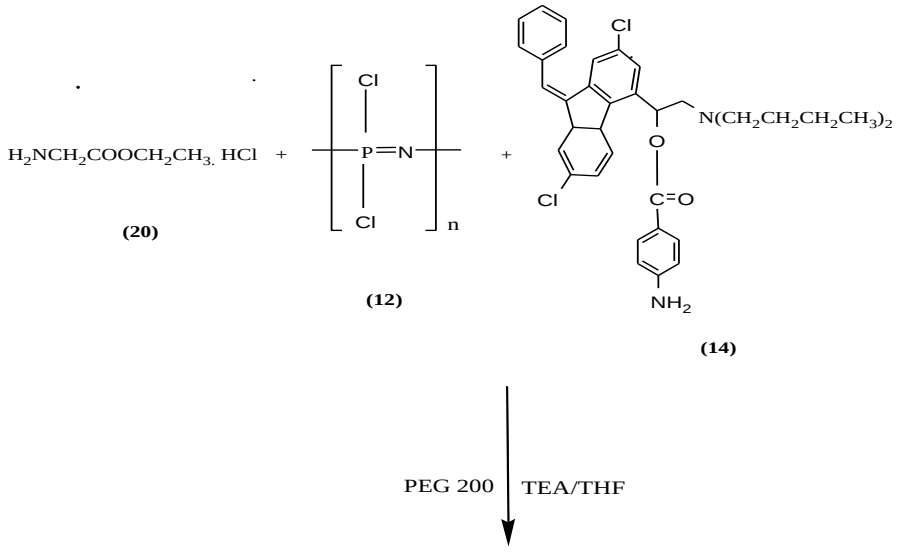


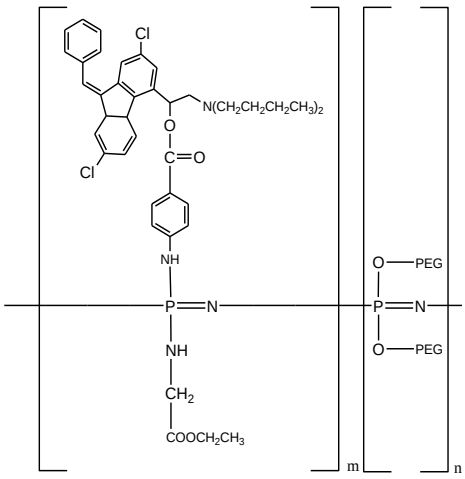
Figure 3.13: FT-IR Spectrum of Glycine ethyl ester (20)

IR (KBr) cm^{-1}: 3474.07 (N-H str.), 2975.15 (C-H str., aliphatic), 1745.42 (-C=O str., ester), 1129.95 (C-O str.). **Yield: 94.34%**

3.6.1 Synthesis of Polymer-Drug Conjugates of Lumefantrine substituted with Glycine ethyl ester 21 (PDA-3)

Polymer polydichlorophosphazene **(12)** (0.094 g) (0.00081 moles) was dissolved in dry tetrahydrofuran (100 ml) and placed in round bottom flask. The compound **14 (**0.5 g) (0.00081 moles) was transferred into thepolymer solution. Triethylamine (10.0 ml) and PEG (200) (0.1 ml) was added into the polymer solution. Thereafter, glycine ethyl ester **(20)**(0.0565 g)(0.0004 moles) was transferred to polymer solution. The reaction mixture was refluxed for 170 hr. After refluxing, the solution was cooled at room temperature and the filtrate was concentrated under reduced pressure to yield the final product. The product was dried under vacuum dessicator for 2 days. Yield: 85%





(21)

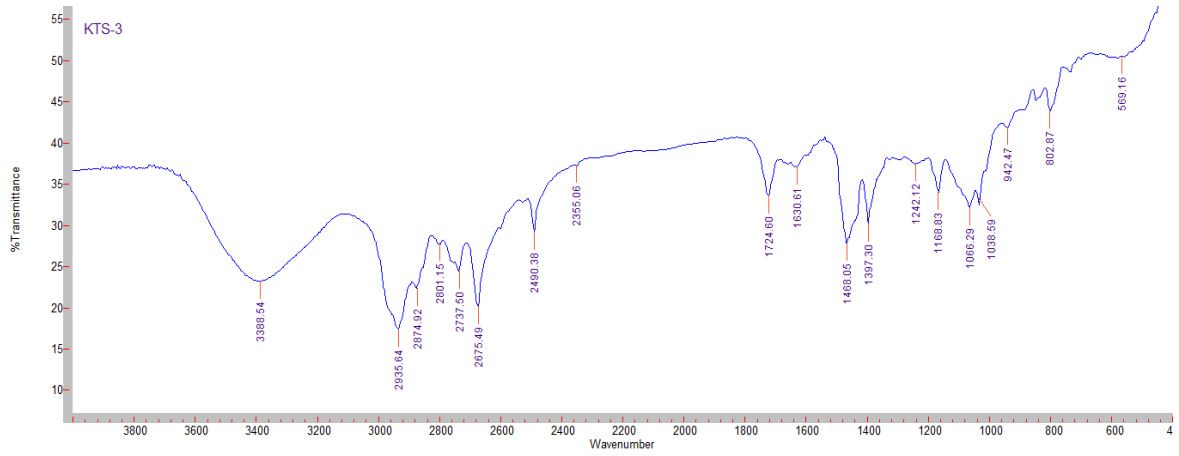


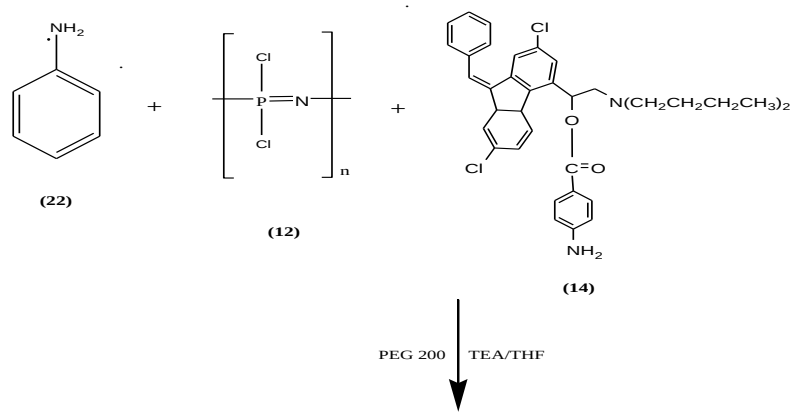
Figure 3.14: FT- IR spectra of Polymer-Drug Conjugate of Lumefantrine substituted with Glycine ethyl ester 21 (PDA 3)

IR (KBr) cm^{-1}: 3388.54 (N-H str.), 2935.64 (C-H str., aliphatic), 2874.92 (C-H str. PEG), 1724.60 (-C=O str., ester), 1630.61 (-C=C- str. alkene), 1468.05 (-C=C- str. aromatic), 1242.12 (-P=N- str.), 1168.29(C-O str.), 1066.29 (C-O str. PEG) and 802.87(-C-Cl str.).Yield: 85%.

^{1}H NMR ($CDCl_3$) δ (ppm):0.85-1.91 (m, aliphatic protons), 2.02-2.53 (m, benzylic protons of lumefantrine), 3.02-3.73 (m, oxymethyl protons of PEG), 4.35-4.38 (m, NH protons) and 7.38-8.49 (m, aromatic protons).

3.7 Synthesis of Anilino substituted Polymer-Drug Conjugates23 (PDA-4)

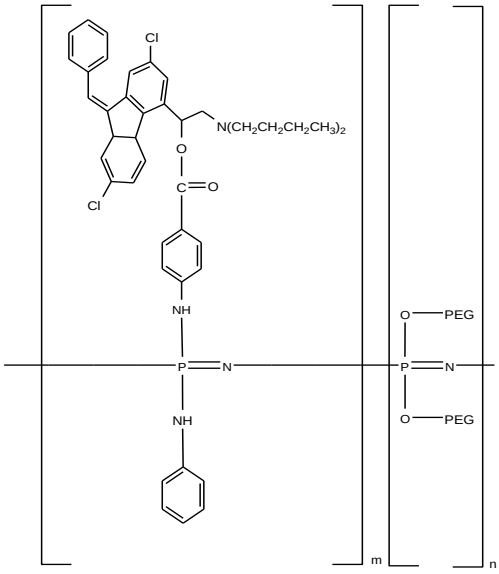
polymer (polydichlorophosphazene) **(12)** (0.094 g)(0.00081 moles) was dissolved in dry tetrahydrofuran (100 ml) and placed in round bottom flask. The compound **14 (**0.5 g) (0.00081 moles) was transferred into the polymer solution. Triethylamine (10.0 ml) and PEG (200) (0.1 ml) was added into the polymer solution. Thereafter, Aniline **(22)**(0.1 ml) was transferred to polymer solution. The reaction mixture was refluxed for 72 hr.

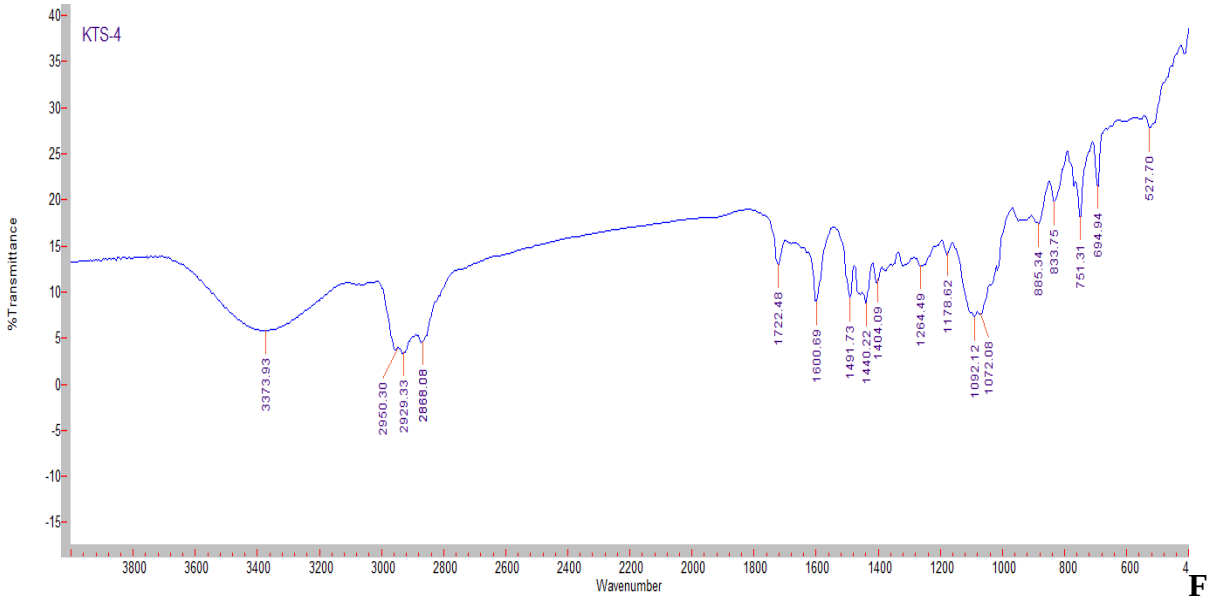


(22) (12) (14)

After refluxing the solution was cooled at room temperature and the filtrate was concentrated under reduced pressure to yield the final product. The product was dried under vacuum desiccator for 2 days. Yield: 89%.

(23)





igure 3.15 : FT- IR Spectra of Anilino substituted Polymer-Drug Conjugates 23 (PDA 4)

IR (KBr) cm^{-1}: 3373.93 (N-H str.), 2950.30 (C-H str., aliphatic), 2868.08 (C-H str. PEG), 1722.48 (-C=O str., ester), 1600.69 (-C=C- str. alkene), 1440.22 (-C=C- str. aromatic), 1264.49 (-P=N- str.), 1092.12 (C-O str.), 1072.08 (C-O str. PEG) and 885.34(-C-Cl str.).Yield: 88%.

^{1}H NMR ($CDCl_3$) δ (ppm):0.85-1.99 (m, aliphatic protons of lumefantrine), 2.06-2.74 (m, benzylic protons of lumefantrine), 3.03-3.99 (m, oxymethyl protons of PEG), 4.01-4.65 (m, NH protons) and 6.60-7.70 (m, aromatic protons).

3.8 Percent Drug Content

The percent drug content of Lumefantrine was determined by adding 5.0 mg of Polymer-Drug Conjugates in 10.0 ml of Methanol. The solution was centrifuged for 15 min. at 2000 rpm.The absorbance of each solution was measured spectrophotometrically at λ_{max} 234 nm. The percent drug content of Lumefantrine was found to be **90.19%, 86.21%, 84.27%, and 87.53%** in conjugates (Methyl 4-amino benzoate substituted polymer-drug conjugate, glycine methyl ester substituted polymer-drug conjugate, glycine ethyl ester substituted polymer-drug conjugate, and Anilino substituted Polymer-Drug Conjugate, respectively) as shown in **Table 3.7**. The Lumefantrine was found to be uniformly distributed in the Polymer-Drug Conjugates.

Table 3.7: Percent (%) Drug content of Polymer-Drug Conjugates of Lumefantrine

S.No.	Polymer-Drug Conjugates of Lumefantrine	Percentage (%) Drug Content
1.	Methyl 4-amino benzoate substituted polymer-drug conjugates**16 (PDA-1)**	90.19%
2.	Glycine methyl estersubstituted polymer-drug conjugates**19 (PDA-2)**	86.21%
3.	Glycine ethyl estersubstituted polymer-drug conjugates**21 (PDA-3)**	84.87%
4.	Anilino substituted polymer-drug Conjugates**23 (PDA-4)**	87.53%

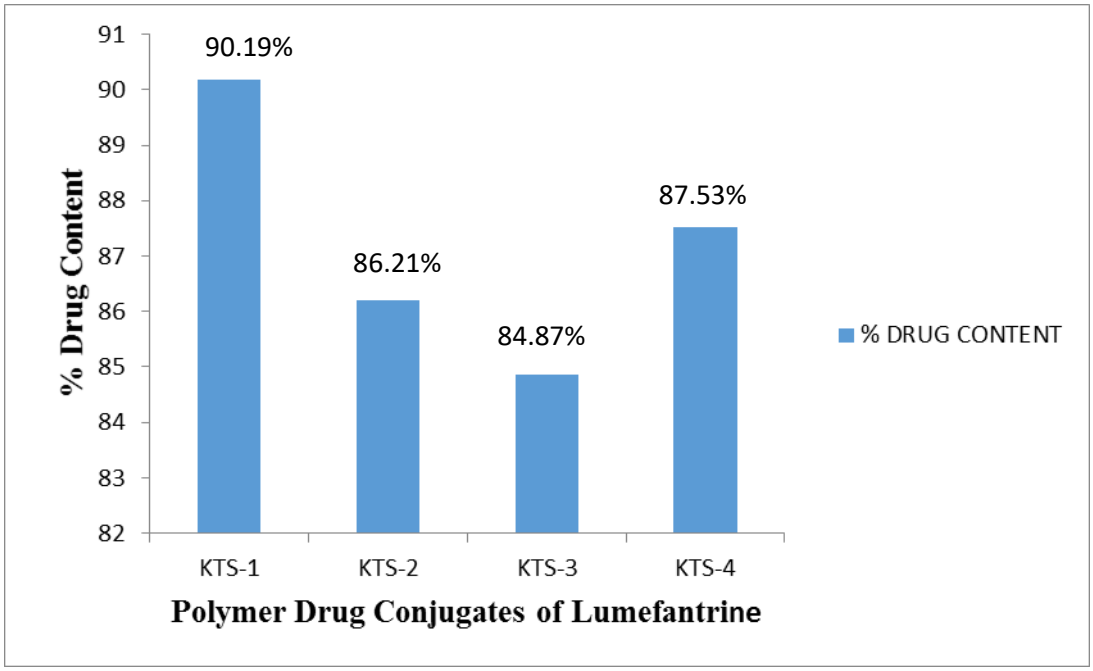


Figure 3.16: Percent Drug Content of Polymer-Drug Conjugates of Lumefantrine

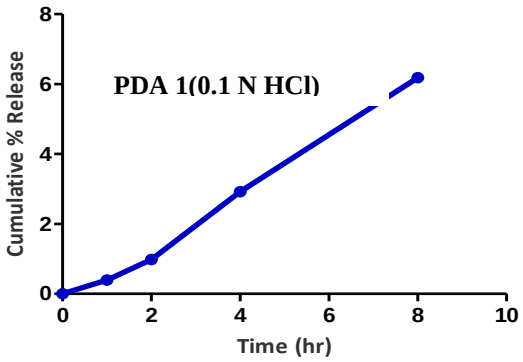
3.9 *In vitro* Drug Release Studies

The release study of Polymer-Drug Conjugates (Methyl 4-amino benzoate substituted polymer-drug conjugates **16 (PDA-1)**, glycine methyl ester substituted polymer-drug conjugates **19 (PDA-2)**, glycine ethyl ester substituted polymer-drug conjugates **21 (PDA-3)**, and Anilino substituted polymer-drug conjugates**23 (PDA-4)** were carried outby using

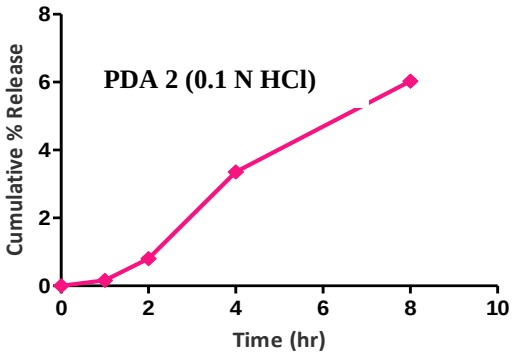
Dialysis bag membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 Dalton (HiMedia, India). The beaker was placed on a magnetic stirrer at 37 °C at 100 rpm with hot plate using receptor medium. Phosphate buffer solution pH 7.4, pH 6.8 and 0.1N HCl (pH 1.2) were used as recepter medium and during the release study and maintained sink condition at different interval of time. **Table 3.8, Table 3.9 and Table 3.10** shows the cumulative percent release of polymer linked Lumefantrine conjugates in 0.1N HCl (pH 1.2),Phosphate buffer solution pH 7.4, Phosphate buffer solution pH 6.8, respectively. **Figure 3.17, Figure 3.18,Figure 3.19,Figure 3.20,Figure 3.21** and **Figure 3.22** showed the cumulative percent drug release and comparison of cumulative percent release of Polymer-linked Lumefantrine conjugates in 0.1 N HCl (pH 1.2),Phosphate buffer solution pH 7.4 and Phosphate buffer solution pH 6.8, respectively.

Table 3.8: Cumulative percent release of polymer-linked Lumefantrine conjugates in 0.1N HCl (pH 1.2).

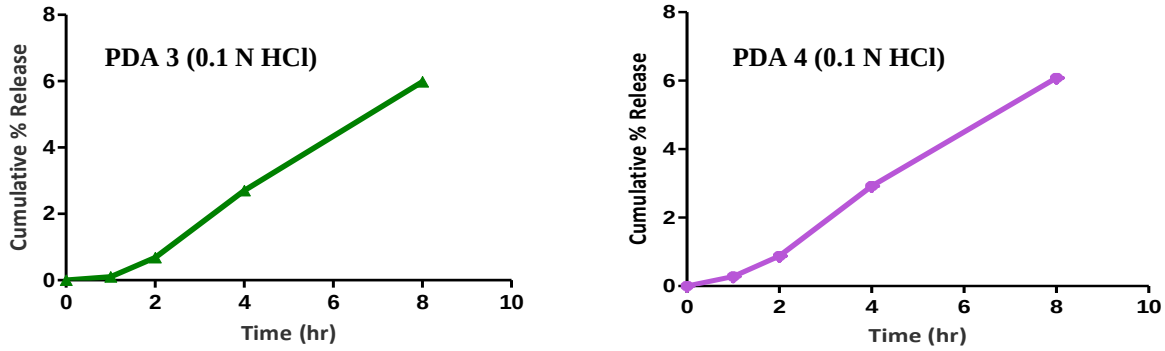
Time(hrs)	Cumulative % Release			
	PDA 1 (16)	**PDA 2 (19)**	**PDA 3 (21)**	**PDA 4 (23)**
0	0	0	0	0
1	0.394±0.004	0.161±0.003	0.101±0.004	0.281±0.005
2	0.984±0.003	0.800±0.005	0.682±0.003	0.882±0.003
4	2.924±0.006	3.360±0.007	2.702±0.007	2.922±0.001
8	6.184±0.010	6.032±0.009	5.982±0.010	6.082±0.009



(A)



(B)



(C) (D)

Figure 3.17: cumulative Percent release of polymer-linked Lumefantrine conjugates: PDA-1 (A), PDA-2 (B), PDA-3 (C) & PDA-4 (D) in 0.1N HCl (pH 1.2).

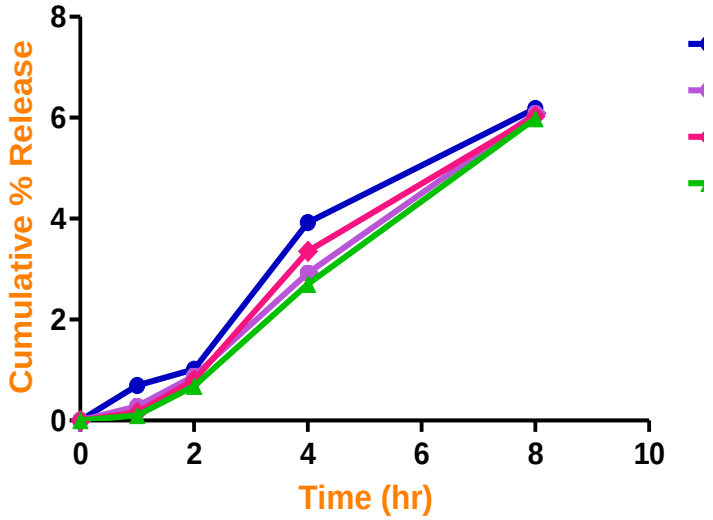


Figure 3.18: Comparison between cumulative Percent release of polymer-linked Lumefantrine conjugatesPDA-1, PDA-2, PDA-3 & PDA-4 in 0.1 N HCl (pH 1.2)

Table 3.9: Cumulative percent release of polymer-linked Lumefantrine conjugates in phosphate buffer solution pH 6.8.

Time(hrs)	Cumulative % Release			
	PDA 1 (16)	**PDA 2 (19)**	**PDA 3 (21)**	**PDA 4 (23)**
0	0	0	0	0
1	0.779±0.006	0.699±0.007	0.588±0.005	0.705±0.006
2	1.93±0.009	1.86±0.009	1.83±0.007	1.95±0.008
4	8.79±0.005	7.52±0.005	7.29±0.009	8.41±0.010
8	24.75±0.007	20.10±0.010	18.87±0.010	22.39±0.015
12	35.53±0.010	29.28±0.015	26.65±0.020	30.17±0.018
24	56.75±0.015	50.50±0.019	47.87±0.026	54.79±0.024
48	79.33±0.025	73.08±0.020	70.45±0.03	77.37±0.028
72	89.95±0.033	83.86±0.026	80.83±0.031	86.79±0.033

Table 3.10: Goodness of fit for the Comparison of Mechanism of Release of Polymer-linked Lumefantrine Conjugates (Phosphate buffer pH 7.4)

Polymer Drug conjugates of Lumefantrine	Zero Order	First Order	Higuchian	Korsmeyer and peppas	
	r^2	r^2	r^2	n	r^2
PDA1	**0.998**	0.989	0.987	1.33	0.973
PDA2	**0.989**	0.987	0.976	1.41	0.974
PDA3	**0.987**	0.983	0.971	1.48	0.971
PDA4	**0.989**	0.986	0.983	1.41	0.974

Table 3.11: Goodness of fit for the Comparison of Mechanism of Release of Polymer-linked Lumefantrine Conjugates (Phosphate buffer pH 6.8).

Polymer Drug conjugates of Lumefantrine	Zero Order	First Order	Higuchian	Korsmeyer and peppas	
	r^2	r^2	r^2	n	r^2
PDA1	**0.997**	0.988	0.988	1.43	0.969
PDA2	**0.987**	0.985	0.984	1.40	0.976
PDA3	**0.980**	0.981	0.982	1.42	0.974
PDA4	**0.984**	0.986	0.985	1.42	0.973

4. Conclusion

Malaria is the most fatal human parasitic infection and remains a major health problem and affects more than 400 million individuals, causing approximately 2 million deaths each year. *Plasmodium falciparum* is the most common causative parasite of malaria. Many antimalarial drugs which are in clinical use have liver toxicity and developed drug resistance. Therefore, taking into consideration the emergence of drug-resistant strains of malarial parasite, it was considered of interest to evolve antimalarial drug delivery system which will not lead to drug-resistance and in addition should be more effective on account of multi-targeted specificity against the malarial parasites located in blood, liver and brain.

The present study pertains to the delivery of antimalarial drug (Lumefantrine). In this approach, polyphosphazene has been used as polymeric backbondin the synthesis of polyphosphazene-linked conjugates of Lumefantrine. These polymer-linked conjugates have been synthesized and characterized by sophsticated modern analytical techniques such as U.V., I.R., ^{1}H-NMR. Their*in-vitro* release studies have been carried out in the phosphate buffer solution having pH 7.4, pH 6.8 and pH 1.2. The polymer-linked conjugates of Lumefantrine *viz*.Methyl 4-aminobenzoate substituted polyphosphazene**(16)**, Glycine methyl ester substituted polyphosphazene**(19)**, Glycine ethyl ester substituted polyphosphazene**(21)** and Anilino substituted polyphosphazene drug conjugates **(23)** have been found to have drug content 90.19%, 86.21%, 84.87% and 87.53%, respectively.

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