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Synthesis and *in vitro* drug release studies on substituted polyphosphazene conjugates of lumefantrine.

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Abstract

The present study pertains to the delivery of antimalarial drug (Lumifantrine). In this, polyphosphazene has been used in the synthesis of polyphosphazene-linked conjugates of Lumifantrine. These polymer-linked Conjugates have been synthesized and characterized by modern analytical techniques. The *in-vitro* drug release of Lumifantrine drug conjugates: *p*-Amino benzoic acid ester substituted polyphosphazene drug conjugate (15) and Glycine methyl ester substituted polyphosphazene drug conjugate (21) have been found to be 6.00 % and 5.96% (pH 1.2), 88.52% and 79.86% (pH7.4), respectively. These drugs conjugate may prove an effective delivery system for the treatment of malaria.

Keywords: polyphosphazene, lumefantrine, conjugate, malaria, *p*-amino benzoic acid ester.

Introduction

Incidence, Prevalence and Survival

Almost one-half of the world's population lives under the constant threat of malaria, and the disease is responsible for about 2 million deaths each 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 protozoan parasites of the genus *Plasmodium.* There are four species of *Plasmodium* that infect humans, the most deadly of these being *Plasmodium falciparum*. The parasite requires two hosts, a female *Anopheles* mosquito and a human. There were an estimated 0.881 million deaths worldwide in 2006, of which 90% were in the African region and 4% in each of the South-East Asia and the Eastern Mediterranean regions. The individual most at risk of significant morbidity and mortality owing to malaria are the children under the age of 5 years and the pregnant women [2, 3]. New empirical estimates put the number of episodes of clinical *Plasmodium falciparum* malaria in the region of half a billion per year [4]. The mortality rate from malaria has been estimated at approximately 2.7 million per year, with over 75% of these deaths occurring in African children [5]. Unfortunately, these staggering figures are on the increase largely as a result of parasite multi-drug resistance [6]. The introduction of chloroquine in the 1940s had a marvelous impact on global health; however, today resistance to the drug has been observed in every region where *Plasmodium falciparum* occurs [7].

Malaria 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 [8].

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 releasing 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 oocysts that release sporozoites that migrate to the salivary glands to be passed on to another individual. Due to complication of infections with *Plasmodium falciparum* most of the deaths are occur, where by 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 host immune reaction can lead to coma. 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

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drugs and also various approaches which may not result into drug resistance. *Plasmodium falciparum* is the most common causative parasite of malaria [9]. Taking into consideration emergence of drug-resistant strains of malarial parasite, it was considered of interest to evolve antimalarial drug delivery systems which will not lead to drug-resistance and expected to be more effective on account of multi-targeted specificity against the malarial parasite located in blood, tissue and brain [10].

Polyphosphazenes

Polyphosphazenes are polymers with an inorganic backbone composed of alternating nitrogen and phosphorus atoms linked by alternating single and double bonds, with two substituent's at each phosphorus atom. Although polyphosphazene chemistry has been intensively investigated only in mid 1950's the preliminary groundwork was laid down during the 19th century. In 1834, Liebig and Wohler obtained a small amount of hexachlorocyclotriphosphazenes (11) from the reaction of ammonium chloride with phosphorus pentachloride [11]. This is the most versatile class of inorganic polymers because a tremendous variety of substituent's can be attached to the backbone phosphorus atoms, thus resulting in a very broad spectrum of chemical and physical properties which make them suitable for many applications. This widespread tailor ability of properties has led to many potential applications such as specialty elastomers, vibration shock mounts, solid phase electrolytes, biostructural materials and polymeric drug-delivery systems [12]. The main advantages of these polymers come mainly from variable properties and can be designed and prepared by nucleophilic substitution of polydichlorophosphazene with various pendent groups [13]. This include from hydrophobic groups that confer water insolubility and protect the backbone against hydrolysis through groups that generate water solubility together with hydrolytic stability to side groups that provide a facile pathway to hydrolytic breakdown of polymers to innocuous, excretable or metabolizable small molecules [14]. It is colorless, with glass transition temperature (Tg) of 63°C [15]. It is amorphous at room temperature in an unstretched state showing rubbery characteristic [16]. but crystalline at low temperature or when stretched [17].

Material and Method

Material used

Antimalarial drug Lumifantrine was obtained as a gift sample from Alkem Laboratories limited Navi Mumbai- 410208 India. Drug sample was characterized according to standard procedures. All other chemicals were used without further purification.

Methodology

Preparation of Polymer -Drug Conjugate of *p*-Amino benzoic acid ester

Procedure for esterification of *p*-Amino benzoic acid

P-Amino benzoic acid (11) (1.2 g) was added into methanol (12.0 ml) and stirred the mixture until the solid dissolved. Slowly added 1.0 ml concentrated H_2SO_4 to mixture and a precipitate was formed. A reflux condenser was attached to the flask, and the mixture was heated at a gentle reflux for 60-75 min. The solid was dissolved as it undergoes reaction. After completion of the reflux, the reaction mixture was allowed to cool to room temperature. Poured the reaction mixture into ice water (30.0 ml). While stirring this mixture, 10% sodium carbonate solution (10.0 ml) was added.Gas evolution was observed as the acid was neutralized, the final pH was approximately 8. Vacuum filtered the resulting precipitates, and washed the product with water. The product was recrystalised with n-hexane and again vacuum filtered the resulting precipitates and allowed it to dry. Yield: 89. 67%.

Synthetic Procedure for Polymerization of low molecular weight Polydichlorophosphazene

The Pyrex glass tubes were soaked in chromic acid solution for 24 hr, washed by using distilled water and dried at 140 ˚C in an oven. The vacuum line was attached to dried tube, evacuated and flamed out. Thereafter, hexachlorocycotriphosphazenes (9) (5.0g,) was loaded in the tube using 5 % anhydrous aluminium chloride as catalyst. The tube was then sealed after evacuation for 1 hr. The sealed tube was then heated in a muffle furnace at 250 ± 5 ˚C and agitated at regular intervals to stir the melted reactant. After completion of polymerization for the required time period, the tube was then cooled and shifted to dry box filled with nitrogen gas.

Synthesis of Substituted Polyphosphazenes with *p*-Amino benzoic acid ester

The tube was broken, and polymer (polydichlorophosphazenes) (10) (0.58 g) was dissolved in dry tetrahydrofuran to carry out further substitution reactions. Thereafter, *p*-aminobenzoic acid ester (12) (0.765 g) was transferred to solution of polmer. Freshly distilled triethylamine (10.0 ml) was added into the polymer solution followed by drop wise solution of ethyl 4-aminobenzoate (0.172 g) in tetrahydrofuran (200 ml). The reaction mixture was refluxed for 170 hr, cooled to room temperature and filtered to remove hydrochloride salts. The clear filtrate was concentrated under vacuum and precipitation by petroleum ether (60-80 ˚C) to get the product. Yield: 62% [18].

Hydrolysis of *p*-Aminobenzoic acid ester

P-Amino benzoic acid ester (14)(1.0 g) was added into 10% aqueous sodium hydroxide solution (15.0 ml). Boiled the mixture under reflux until the molten ester has disappeared completely for about one hour. Poured 10% sodium hydroxide solution (3.0 ml) down the condenser to dislodged the ester and continued the boiling for further 10 min. until the solution was cleared. The solution was cooled in ice cold water and dilute sulphuric acid was added with continue stirring until a faint but permanent precipitates of benzoic acid was produced and it was tested with litmus - paper to ensured that solution was acidic. Thereafter, dilute sodium carbonate solution was added with vigorous stirring until the precipitates just redissolved and again was tested with litmus - paper to ensure that solution was basic. The solution was extracted twice with ether (tetrahydrofuran), both aqueous and ether layers were separated into beaker and ether layer was distilled off. Pour the remainder of whilst hot into evaporating – basin, the phenol was crystallised when the residual ether was evaporated. Hydrochloric acid was added on to the aqueous solution, the sodium carbonate was first neutralised and then benzoic acid was precipitated. The solution was filtered and precipitates were washed with water and then recrystallized with boiling water. Yield was found to be 78.43% [19].

Procedure for Preparation of Polymer- Drug Conjugate of *p*-Amino benzoic acid ester

Substitued polyphosphazenes (14) (1.0 g) was dissolved in dry tetrahydrofuran (100.0 ml) and placed in round bottom flask. Lumifantrine (8) (0.05 g) was transferred into the polymeric solution containing tetrahydrofuran and triethylamine (1.0 ml) was added on it, and then refluxed the solution for 72 hr. Yield: 66.92%.

Preparation of Polymer-Drug Conjugate of Glycine Methyl Ester

Synthesis of Glycine methyl ester hydrochlorides

Thionyl chloride (1.4 ml) was added to methanol (100.0 ml) slowly at 0 C. Glycine methyl (16) (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 dimethyl sulphite was removed. The crude product was crystallized from methanol and ether at 0 C to get pure Glycine methyl ester hydrochloride (17).Yield: 94.34% [20].

Synthesis of Polyphosphazene Conjugated Glycine methyl ester

Polydichlorophosphazene (2.0 g) was dissolved in tetrahydrofuran (200.0 ml). Glycine methyl ester hydrochloride (17) (5.0 g) was suspended in tetrahydrofuran (100.0 ml) and triethylamine (20.0 ml) was added. This suspension was refluxed for 24 hr, and then filtered and added to the polymer solution. The resultant solution was stirred at room temperature for 24 hr, and then refluxed for 48 hr. The solvent was removed under reduced pressure to yield a yellow solid. Yield: 72% [21].

Hydrolysis of Polyphosphazene Conjugated Glycine methyl ester

Polyphosphazene Conjugated Glycine methyl ester (20) (1.0 g) was transferred in 10% aqueous sodium hydroxide solution (15.0 ml).Boiled the above mixture uder reflux until the molten ester has disappeared completely for about 1hr. Poured 10% sodium hydroxide solution (3.0 ml) down the condenser to dislodged the ester and continued the boiling for further 10 min. until the solution was cleared. The solution was cooled in ice cold water and dilute sulphuric acid was added with continue stirring until a faint but permanent precipitates of benzoic acid was produced and was tested with litmus - paper to ensured that solution was acidic. Thereafter, dilute sodium carbonate solution was added with vigorous stirring until the precipitates were just redissolved and again was tested with litmus - paper to ensured that solution was basic. The solution was extracted twice with tetrahydrofuran, both aqueous and ether layers were separated into beaker and ether layer was distilled off. Pour the remainder of whilst hot into evaporating – basin, the phenol was crystallised when the residual ether was evaporated. Hydrochloric acid was added on to the aqueous solution, the sodium carbonate was first neutralised and then benzoic acid precipitated. The solution was filtered and precipitates were washed with water and was recrystallized with boiling water. Yield: 81.87% [6].

Procedure for Preparation of Polymer-Drug Conjugates of Glycine methyl ester

Substituted polyphosphazenes (20) (1.0 g) was dissolved into dry tetrahydrofuran (100.0 ml). Lumifantrine (8) (0.05 g) was transferred into the polymeric solution containing tetrahydrofuran and triethylamine (1.0 ml) was added on it, and then refluxed the solution for 72 hr. After refluxing, the solution was filtered and was evaporated to remove out the solvent. Yield: 69.32%.

Evaluation Parameters

Drug Content Percentage

Polymer- Drug Conjugates of *p*-Amino benzoic acid ester

Polymer-Drug Conjugate of *p*-Amino benzoic acid ester (15) (5.0 mg) was transferred into centrifuged tube and methanol (1.0 ml) was added on it. The solution was centrifuged for 15 min. at 2000 rpm in centrifuged apparatus. After the completion of centrifugation, 1.0 ml solution was removed from tube and transferred into volumetric flask (10.0 ml) and volume was made up with methanol. Again 1.0 ml solution was removed from above volumetric flask and transferred into another 10.0 ml flask and again the volume was made up to 10.0 ml with methanol. The absorbance of each solution was measured spectrophotometrically at 234 nm.

Polymer-Drug Conjugates of Glycine methyl ester

Polymer-Drug Conjugate of Glycine methyl ester (21) (5.0 mg) was transferred into centrifuged tube and methanol (1.0 ml) was added on it. The solution was centrifuged for 15 min. at 2000 rpm in centrifuged apparatus. After the completion of centrifugation 1.0 ml solution was removed from tube and transferred into volumetric flask (10.0 ml) and volume was made up with methanol. Again 1.0 ml solution was removed from above volumetric flask and transferred into another 10.0 ml flask and again the volume was made upto 10.0 ml with methanol. The absorbance of each solution was measured spectrophotometrically at 234 nm.

In-vitro drug release study

In-vitro drug release study of Lumefantrine (Polymer- Drug Conjugates of *p*-Amino benzoicacid ester and Glycine methyl ester) was performed in phosphate buffer of pH 7.4 and 0.1 N HCl by using dialysis bag method, dialysis membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 Dalton (HiMedia, India) were used. The dialysis membrane was pre-treated with sodium bicarbonate and was washed with distilled water prior to use. Formulation of Lumefantrine (5.0 mg) was placed in dialysis bag and was immersed in phosphate buffer pH 7.4 (100.0 ml) and bag membrane was sealed and placed into 100.0 ml of phosphate buffer solution pH 1.2 in a beaker. The beaker was placed on a magnetic stirrer at 37 C at 100 rpm. At predetermined time intervals, 5 ml of the sample was taken out and replaced with fresh phosphate buffer solution pH 7.4 and the same procedure was repeated for 0.1 N HCl. The drug concentration was determined by UV–Visible spectro photometer (Shimadzu UV-1800) at 200-400 nm and percent release drug was measured by using formula as given below [5].

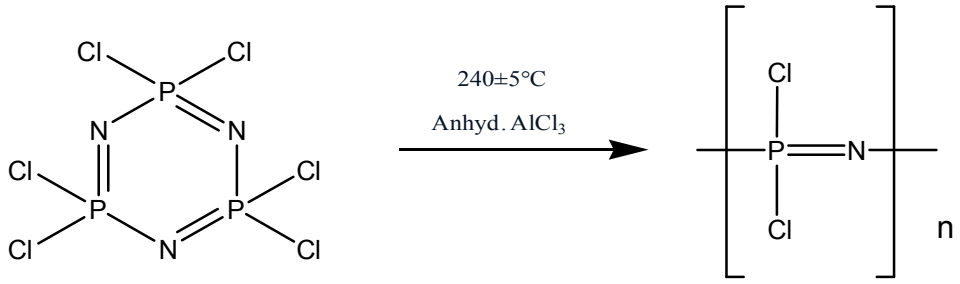
$$\% \text{ Release of Lumifantrine} = \frac{\text{Mass of Lumifantrine in releasing media}}{\text{Total mass of Lumifantrine}} \times 100$$

Result and Discussion

Synthesis, Characterization and Drug Release Profile of Polymer-Drug Conjugates of Lumifantrine (8) by using p-Amino benzoic acid ester

Synthesis of Polydichlorophosphazenes

Polyphosphazenes used as a polymeric backbone has been synthesized by thermal polymerization ofhexachlorocyclotriphosphazene (9) (prepared by reacting ammonium chloride and phosphorus pentachloride). The sealed tube was then heated in a muffle furnace at 240 ± 5 ˚C and agitated at regular intervals to stir the melted reactant. IR (KBr) cm^{-1}: 1218 (P=N str.).



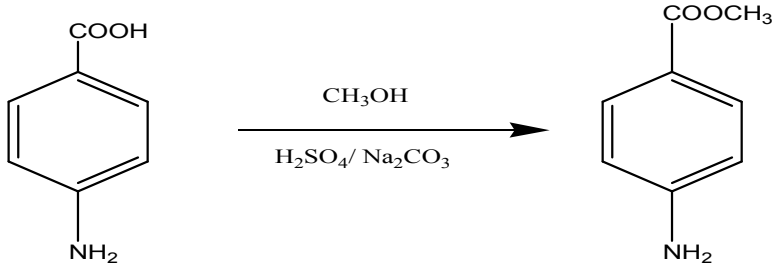
(9) **(10)**

Synthesis and Characterization of Polymer-Drug Conjugates *p*-Amino benzoic acid ester

Esterification of *p*-Amino benzoic acid ester

P-Amino benzoic acid (11) (1.2 g) was added into methanol (12.0 ml) and stirred the mixture until the solid dissolved. Slowly added 1.0 ml concentrated H_2SO_4 to mixture and a precipitate was formed. A reflux condenser was attached to the flask, and the mixture was heated at a gentle reflux for 60-75 min. 10% sodium carbonate

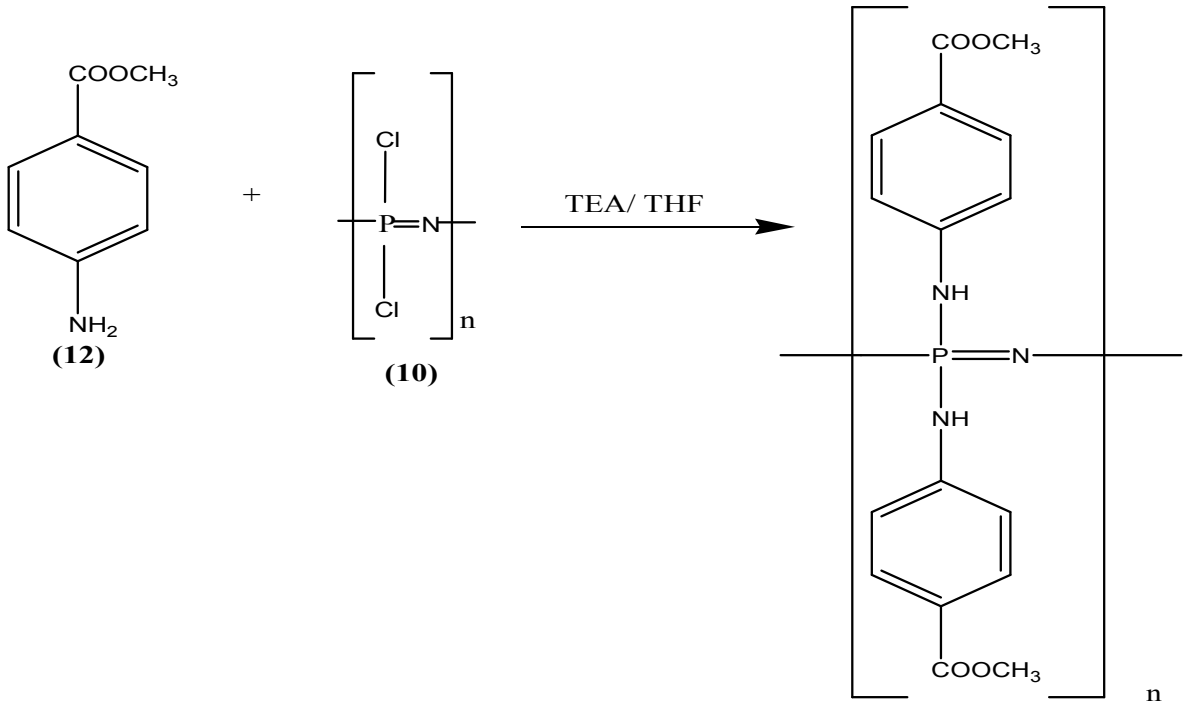
solution (10.0 ml) was added. IR (KBr) cm^{-1}: 3460.05 (N-H str.), 3363.16 (O-H str., -COOH), 1664.99 (-C=O str., carboxylic), 1600 (C=C str., aromatic), 1127.75 – 1311.55 (C-O str., carboxylic). Yield of the product was found to be 90.21%.



(11) (12)

Synthesis of Substituted Polyphosphazenes with *p*-Amino benzoic acid ester

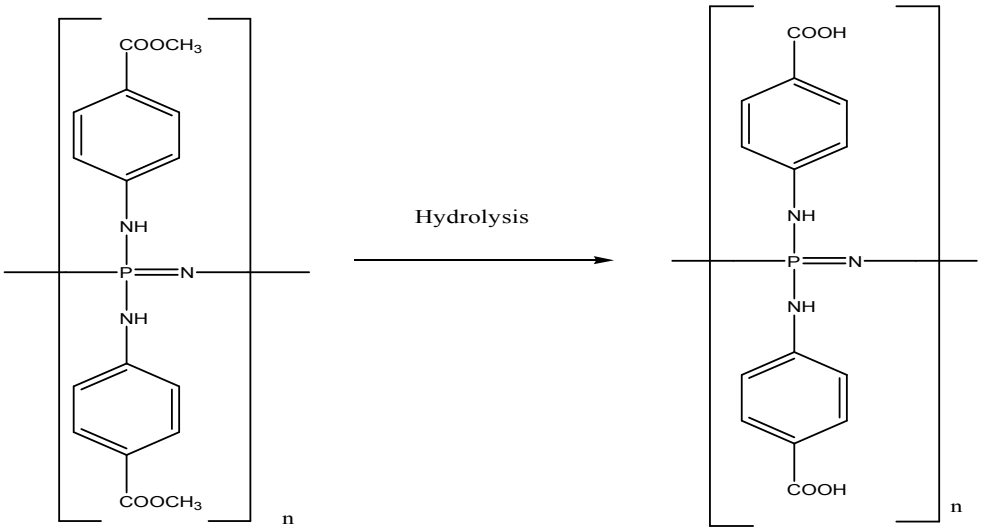
Polydiclorophosphazene(10) (0.58 g) was dissolved in dry tetrahydrofuran to carry out further substitution reactions. Thereafter, *p*-amino benzoic acid ester (12) (0.765 g) was transferred to solution of polmer. Freshly distilled triethylamine (10.0 ml) was added into the polymer solution followed by dropwise solution of ethyl 4-aminobenzoate (0.172 g) in tetrahydrofuran (200 ml).IR (KBr) cm^{-1}: 3350 – 3400 (N-H str.), 1286 (P=N str.), 1719 (C=O str.), 1175 (C-O str.), 852 (C-H str., bending). Yield: 62%.



(13)

Hydrolysis of Substituted Polyphosphazenes with *p*-Amino benzoic acid ester

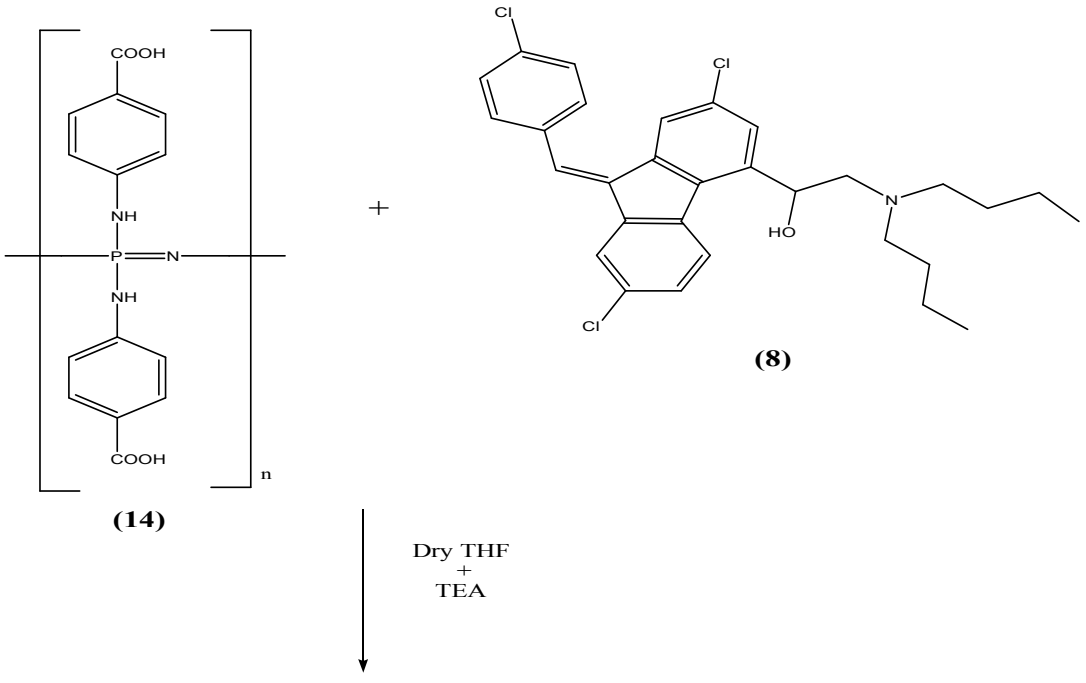
Substituted Polyphosphazenes with *p*-Amino benzoic acid ester (14)(1.0 g) was added into 10% aqueous sodium hydroxide solution (15.0 ml). Dilute sodium carbonate solution was added and the solution was extracted twice with ether (tetrahydrofuran) and both the layers were separated. Hydrochloric acid was added on to the aqueous solution.



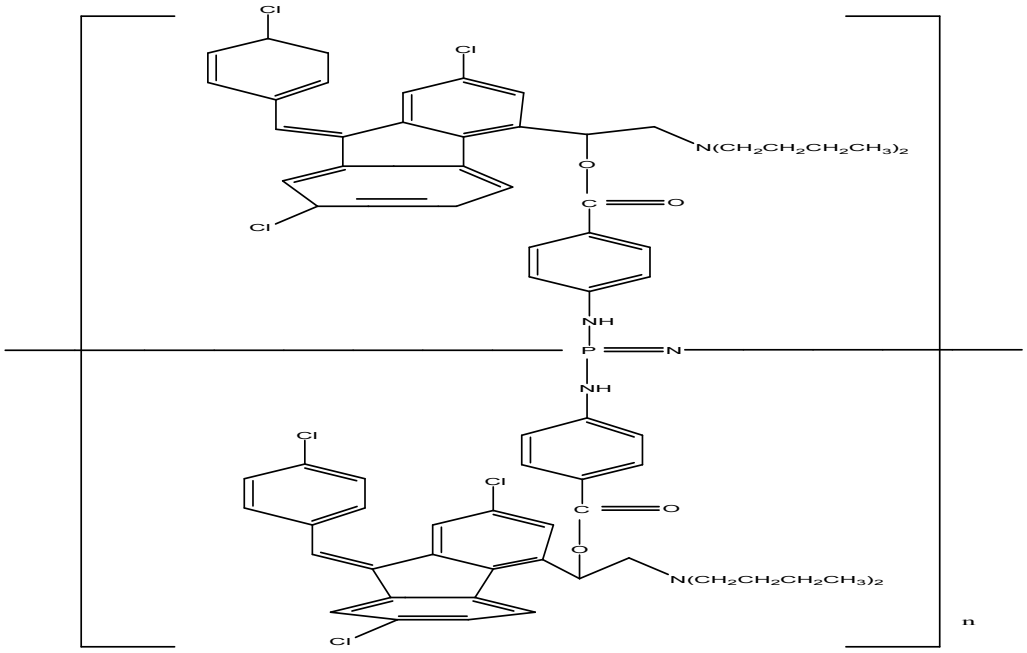
(13) **(14)**

Synthesis of Polymer-Drug Conjugates of Lumifantrine *p*-Aminobenzoic acid ester

Substituted polyphosphazenes (14) (1.0 g) was dissolved in dry tetrahydrofuran (100.0ml) and placed in round bottom flask. Lumifantrine (8) (0.05 g) was transferred into the polymeric solution containing tetrahydrofuran and triethylamine (1.0 ml) was added on it. IR (KBr) cm^{-1}: 3359.41 (-NH str.), 2921.76 (C-H str., aliphatic), 1736.73 (-C=O str., ester), 1492.24 (-C=C- str., aromatic), 1379.74 (C-Cl), 1235 (P=N, str.), 1063.80 (C-O str., ester).Yield: 66.92%.



(14) **(8)**



(15)

^{1}H NMR ($CDCl_3$) (ppm):
1.87-2.51(6H, m, br, -CH_3), 3.34-4.36(4H, m, br, -CH_2), 7.69(2H, NH).

Drug release profile Lumifantrine from Polymer linked antimalarial Drug Conjugates

Drug release profile Polymer-Drug conjugates of antimalarial drug was carried out by doing drug content percent and *in-vitro* drug release studies.

Drug Content Percentage

The drug content percentage of Lumifantrine was determined by adding (5.0) mg of Polymer-Drug Conjugates (*p*-Amino benzoic acid ester) in 10.0 ml of Methanol. The solution was centrifuged for 15 min. at 2000 rpm. The absorbance of solution was measured spectrophotometrically at 234 nm. The percent drug content of Lumifantrine was found to be 89.32% in *p*-Amino benzoic acid ester Polymer-Drug Conjugate, respectively. Lumifantrine was found to be uniformly distributed in the Polymer-Drug Conjugate.

In vitro Drug Release Studies

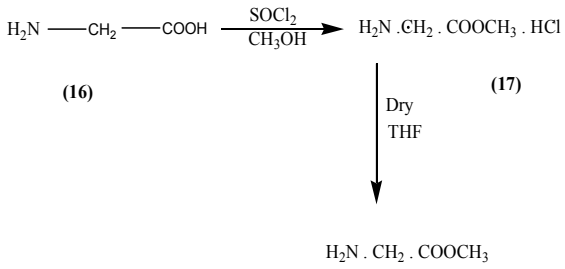
Drug release studies have been known to be important for ensuring the sustained release performance and reproducibility of rate and duration of drug release. The release study of Polymer-Drug Conjugate (*p*-Amino benzoic acid ester) was carried out in phosphate buffer solution pH 7.4 and 0.1 N HCl solution by using Dialysis bag membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 Dalton (Hi Media, 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 was added to maintained sink conditions during release study and 1% (w/w) drug was loaded and drug release was checked. Same procedure was repeated for 0.1 N HCl solutions. The percent cumulative release of drug in 72 hr 88.52% of polymer-Drug Conjugate of *p*-Amino benzoic acid ester release and in 8 hr 6.001% in 0.1 N HCl.

Synthesis, Characterization and Drug Release Profile of Polymer-Drug Conjugate of Lumifantrine (8) by using Glycine methyl ester

Synthesis and Characterization of Polymer-Drug Conjugate Glycine methyl ester (21)

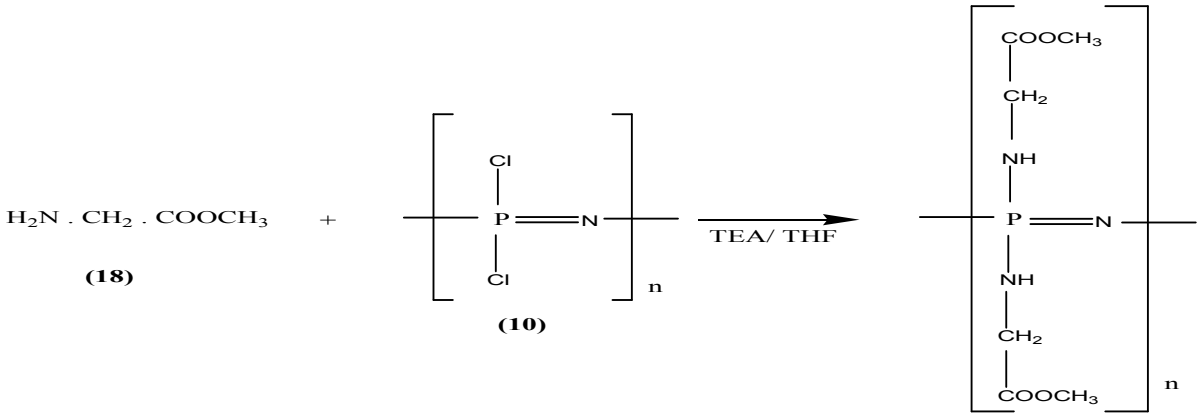
Esterification of Glycine methyl

Thionyl chloride (1.4 ml) was added to methanol (100.0 ml) slowly at 0 C. Glycine methyl (18) (2.0 g) was added to solution and refluxed for 8-10 hr. IR (KBr) cm^{-1}: 3391.83 (-NH str.), 2969.34 (-CH- str., aliphatic), 1742.42 (-C=O str., ester), 1052.45 (C-O str.).
Yield: 94.34%



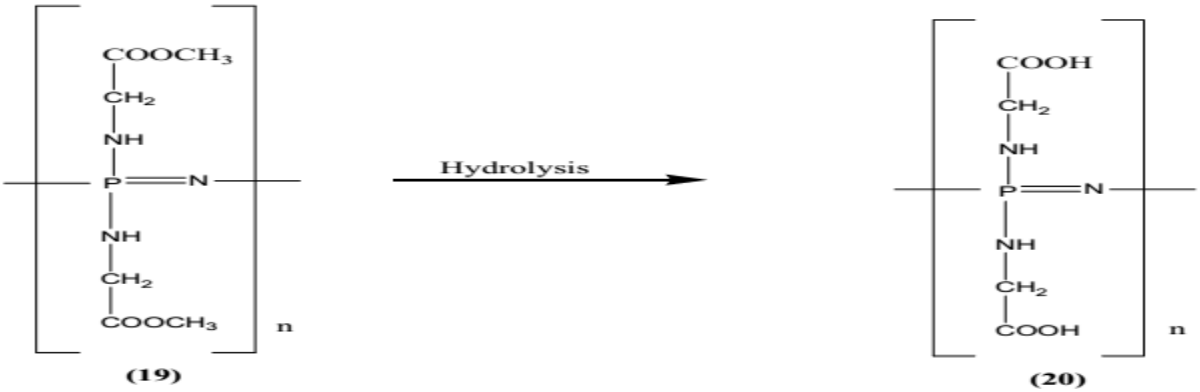
Synthesis of Substituted Polyphosphazenes with Glycine methyl ester

Polydichlorophosphazene (2.0 g) was dissolved in tetrahydrofuran (200.0ml). Glycine methyl ester (18) (5.0 g) was suspended in tetrahydrofuran (100.0 ml) and triethylamine (20.0 ml) was added and refuxed for 48 hrs.IR (KBr) cm^{-1}: 2896.52 (-CH str., aliphatic), 1744.11 (-C=O str., ester), 1235.68 (P=N str.), 1433.96 (-NH bending). Yield: 72%.



Hydrolysis of Substituted Polyphosphazenes with Glycine methyl ester

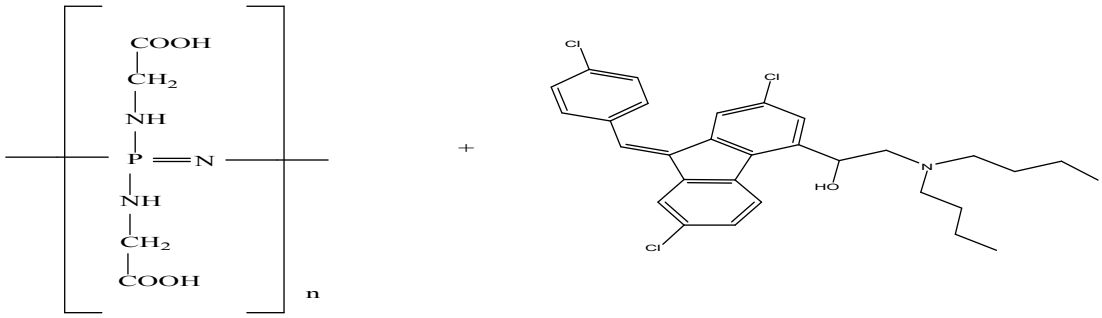
Substituted Polyphosphazenes with Glycine methyl ester (20) (1.0 g) was added into 10% aqueous sodium hydroxide solution (15.0 ml). Dilute sodium carbonate solution was added and the solution was extracted twice with ether and both the layers were separated. Hydrochloric acid was added on to the aqueous solution. IR (KBr) cm^{-1}: 3493.01 (-NH str.), 2979.31 (-CH_2- str., aliphatic), 1735.76 (-C=O str., carboxylic), 1440.91 (-NH, bending), 1232.69 (-P=N, str.), 1025.10 (C-O STR.). Yield: 81.87%.



Synthesis of Polymer-Drug Conjugates of Lumifantrine Glycine methyl ester

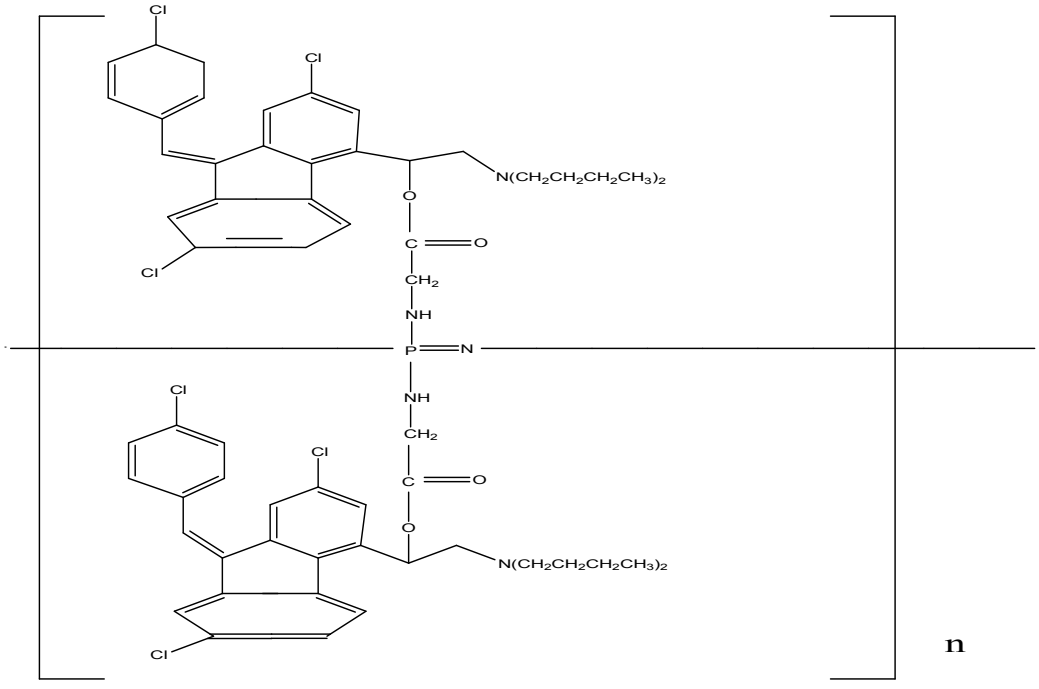
Substituted polyphosphazenes (20) (1.0g) was dissolved into dry tetrahydrofuran (100.0ml). Lumifantrine (8) (0.05 g) was transferred into the polymeric solution containing tetrahydrofuran and triethylamine (1.0ml) was added on it, and then refluxed the solution for 72 hr. IR (KBr) cm^{-1}: 3375.44 (-NH str.), 2979.83 (-CH str., aliphatic), 1767.77 (-C=O str., ester), 1614.16 (-C=C- str., aromatic), 1392.66 (-C-Cl str.), 1259.01 (P=N str.), 1069.82 (C-O str., ester). Yield: 69.32%.

^1H NMR ($CDCl_3$) (ppm) : 0.85-4.12 (4H, m , br, aliphatic protons, 6.69-6.79 (26H, m, br, -Ar*H*) and 9.15 (2H, br, -NH).



(20) (8)

Dry THF
Triethylamine



(21)

Drug release profile of Lumifantrine (8) from Polymer linked antimalarial Drug Conjugates

Drug Content Percentage

The drug content percentage of Lumifantrine was determined by adding 5.0 mg of Polymer-Drug Conjugates (Glycine methyl ester) 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 234 nm. The % drug content of Lumifantrine was found to be 87.55% in Glycine methyl ester Polymer-Drug Conjugates, respectively. The Lumifantrine was found to be uniformly distributed in the Polymer-Drug Conjugates.

In-vitro Drug Release Studies

The release study of Polymer-Drug Conjugate (Glycine methyl ester) was carried out in phosphate buffer solution pH 7.4 and 0.1 N HCl solution by using Dialysis bag membrane having pore size 2.4 nm and a molecular weight cut off 12000-14000 Dalton (Hi Media, 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 was added to maintained sink conditions during release study and 1% (w/w) drug was loaded and drug release was checked. The percent cumulative release of drug in phosphate buffer pH 7.4 is shown in Figure-1.

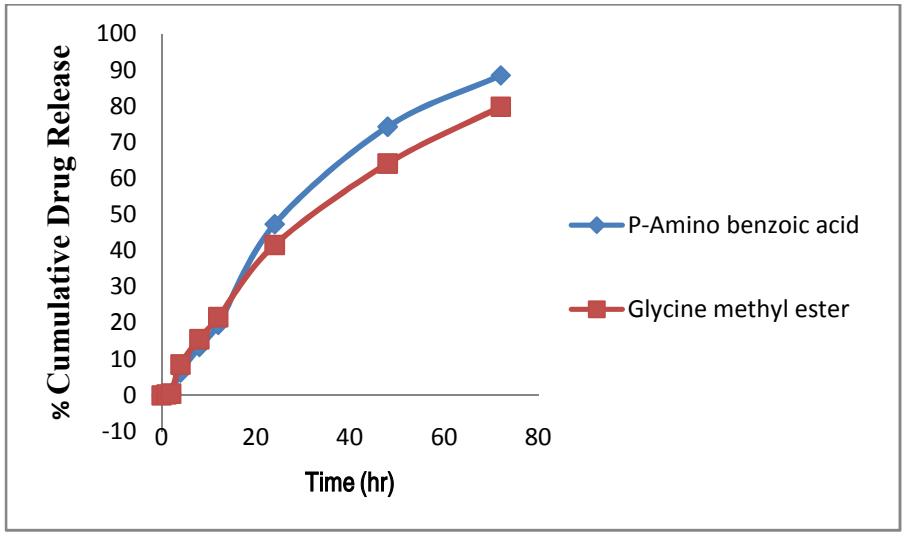


Figure 1: Comparison between *In-vitro* Drug Release of Lumifantrine *p*-Amino benzoic acid ester and Lumifantrine Glycine methyl ester in pH 7.4

The release study showed the drug release increased with the increasing time in hrs by using the formula cumulative % drug release was calculated. From the release curve of *p*-Amino benzoic acid and Glycine methyl ester clearly observed that *p*-Amino benzoic acid showed better drug release per hr. as well as % cumulative release in 72 hrs in phosphate buffer pH 7.4 as comparison to 0.1 N HCl. This may be due to higher dissolution of the formulation due to which more amount of drug escapes into the outer environment.

Conclusion

The polymer-linked Conjugates of Lumifantrine: *p*-Amino benzoic acid ester and Glycine methyl ester substituted polyphosphazene drug conjugates have been found to have drug content 89.32% and 87.55% , respectively.

Drug release profile of polyphosphazene-linked conjugates of Lumifantrine has been studied at pH 7.4 at the time intervals of 1, 2, 4, 8, 12, 24, 48 and 72 hr and at pH 1.2 at time intervals of 1, 2, 4 and 8 hr. The *in-vitro* drug release of Lumifantrine drug conjugates: *p*-Amino benzoic acid ester substituted polyphosphazene drug conjugate (15) and Glycine methyl ester substituted polyphosphazene drug conjugate (21) have been found to be 6.00 % and 5.96% (pH 1.2), 88.52% and 79.86% (pH 7.4), respectively. Therefore, from these studies, it could be concluded that *p*-Amino benzoic acid ester substituted polyphosphazene drug conjugate (15) has slightly better drug release profile as compared to the Glycine methyl ester substituted polyphosphazene drug conjugate (21).

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