# DENDRITIC NANOPARTICULATED CARRIERS FOR THE DELIVERY OF RIFAMPICIN

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## ABSTRACT

*The present study was aimed at developing and exploring the use of PPI dendrimers for delivery of anti-tuberculosis drug, Rifampicin (RMP). RMPwas selected for incorporation into dendrimers based on its antitubercular activity, short biological half-life and solubility characteristics. For this study,poly(propyleneimine) dendritic architecture was loaded with Rifampicin.Various physicochemical and physiological parameters like UV, FT-IR, TEM, DSC, zeta potential, drug entrapment and drug release of formulations were determined. By constructing drug release kinetics, the release mechanism followed was a non fickian diffusion. In this, the system was found to have increased their drug-loading capacity, reduced their drug release rate. Therefore the system was found to be suitable for prolonged delivery of Rifampicin.*

***Key Words:*** *PPI dendrimers, drug delivery system, Nanoparticles, Rifampicin, Prolonged release.*

## 1. INTRODUCTION

Tuberculosis (TB) is a highly contagious persistent infection caused by *Mycobacterium tuberculosis* and *Mycobacterium Bovis* and has the highest mortality rate of any other infectious disease. There are several problems in the treatment of TB i.e. it essentially gets backs to the unique structure of the TB that could cause drug resistance [1] [2]. Rifampicin and isoniazid are among the most frequently used drugs in the treatment of tuberculosis that should be administered for about 6 to 24 months. Thus, strategies like the design and development of novel formulations along with novel antimicrobial compounds that could reduce the length of treatment and reduce the resistance are inevitable. Nanotechnology has evolved to be an integral part of the twenty-first century. Nanotech-enabled products find applicability in almost everything we touch on a day to day basis, such as medicine, pharmaceuticals, chemicals, biologics and information technology [3] .In particular, the pharmaceutical industry has been energized with breakthroughs in nanoengineering, especially in the field of drug delivery and formulation development. Over the last few decades, there has been an explosion of research at both academic and industrial levels- pertaining to nanoformulations: liposomes, nanoparticles,nanoemulsions and dendrimers [4][5].

Dendrimers are synthetic macromolecules with a well-defined, highly branched molecular structure that are synthesized in an algorithmic step-by-step fashion with every repeated sequence of reactions producing a higher generation (G) molecule that has a practically doubled molecular weight and a doubled (discrete) number of functional end-groups. They are highly branched synthetic polymers. It consists of a centre core, integral region and numerous functional end groups. The growth of polymer occurs in the outward direction from the central core by stepwise polymerization. It is characterized by numerous cavities in the core structure creating channels and cages. Precise control of size can be achieved by the extent of polymerization. Dendrimer represents a novel type of polymeric material. Compared with linear polymers, the dendrimers provide several advantages for drug delivery applications. The controlled multivalency of dendrimers can be used to attach drug molecules, targeting moieties, and solubilizing groups on the surface of the dendrimers in a well-defined manner. There are also possibilities of non-covalent encapsulation of drugs into the dendrimers [6][7][8][9].

With the well defined 3D structure and many functional surface groups, drug molecules can be loaded both in the interior of the dendrimers as well as attached to the surface groups. Dendrimers can function as drug carriers either by encapsulating drugs within the dendritic structure or by interacting with drugs at their terminal functional groups via electrostatic or covalent(prodrug)[10][11]. There are broadly two mechanisms for drug delivery. A) First, is by invivo degradation of drug dendrimer conjugate (covalent bonding of drug to dendrimer), which depends on the presence of suitable enzymes or an environment capable of degrading bonds. B) The second one is by releasing the drug due to changes in the physical environment such as pH, and temperature. This approach is independent of the external factors and takes place in cavities of the core (endo-receptor) or outer shell of the receptor (exo-receptor [12][13][14].

Dendrimer as a drug delivery agent is a reliable, safe and selective drug delivery method. Its essential property is selectively targetting the desired tissue. It is having promising future for the treatment of several disorders. Other properties like very small in size, polyvalency, monodispersity, and good stability make it a good carrier for delivering drugs with precision and selectivity. Dendrimer as a drug delivery system is based on the approach of injecting a nanoparticle (10-9) into the body, loaded with a drug. By encapsulating drugs within the dendritic structure, or by interacting with drugs at their terminal functional groups[15][16]. Dendrimers are generally used to increase bioavailability especially sustained, controlled and targeted release of drug can be achieved. The present study was aimed at developing and exploring the use of PPI dendrimers for delivery of the anti-tuberculosis drug, Rifampicin (RMP). RMP was selected for incorporation into PPI dendrimers based on its antitubercular activity, short biological half-life and solubility characteristics[17][18][19][20]. We expect that this approach will improve the management of drug therapy in tubercular patients by delivering the drug at a controlled rate for a prolonged period.

# 2. MATERIALS AND METHODS

## 2.1.MATERIALS

Potassium dihydrogen phosphate (SD fine chemicals, Mumbai), sodium hydroxide (Loba chem. products, Mumbai), HPLC grade methanol (Merk, Mumbai), cellulose dialysis bag (MWCO 12-14 Kda, Himedia, India), PPI dendrimers (Shasum chemicals, Chennai), Rifampicin was a benevolent gift from Lupin laboratories, Aurangabad, India. All other chemicals were reagent grade and used without further modification.

## 2.2. METHODS

### 2.2.1.Drug loading in formulation

Drug loading was carried out by the dialysis method. The known molar concentrations of 5.0G PPI dendrimer and RMP were dissolved in 25ml of methanolic solution. The mixed solution was incubated with slow magnetic stirring (50 rpm) using Teflon beads for 24 h. This solution was dialysed twice in a cellulose dialysis bag (MWCO 12-14Kda, Himedia, India) against 50ml of methanol under strict sink conditions for 1h to remove the free drug from the formulations, which were then estimated spectrophotometrically at 475nm (UV-VISIBLE spectrophotometer) to determine indirectly the amount of drug-loaded within the system. The dialyzed formulations were lyophilized and used for further characterization

### 2.2.2.PREFORMULATION STUDIES

It is very important to conduct the drug excipient interactions in the preformulation studies to overcome the experimental hurdles in the formulation stage.

**Compatibility Studies**

One of the requirements for the selection of suitable excipients or carriers for the pharmaceutical formulation is its compatibility. Therefore in the present work, a study was carried out by using an FTIR spectrophotometer and Differential scanning calorimeter (DSC) to find out if there is any possible chemical interaction.

**a) Fourier Transform Infrared Spectrophotometer (FTIR)**

By using Alpha-FTIR, compatibility studies of the drug were conducted with the excipients. In this study, individual samples of Rifampicin, the carrier (dendrimer), the physical mixture of Rifampicin and dendrimer in a 1:1 ratio, and the prepared formulation. A formulation of RMP loaded PPI dendrimers was analyzed by IR spectroscopy using FT–IR module.The pellets were prepared at high compaction pressure by using KBr and the sample to KBr ratio was 1:100.The pellets thus prepared were examined and the spectra of drug and dendrimer in the formulation were compared to those of the original formulation.

**b) Differential Scanning Calorimetry**

We investigated the thermal stability and changes in crystallinity over a range of temperatures using differential scanning calorimetry (DSC).Study was conducted on RMP (the drug), PPI dendrimers (carrier) and RMP loaded dendrimers. An aluminum pan was filled with powder or sample, and the lid was crimped onto the pan. The pan was then placed in the sample cell of the DSC module (DSC Q20 V9.0 Build 275, TA Instruments, USA). The temperature of the DSC module was equilibrated at 35°C and then increased at a rate of 10°C/min under a N2 gas

purge until the material began to degrade. Temperatures were determined for each peak of the resulting curve, which provided indications of phase transitions and stability of temperature.

## 2.3.EVALUATION

### 2.3.1.Morphology of the Dendrimers

To analyse particle size and obtain information on nanoparticle morphology, transmission electron microscopy (TEM) was used. Transmission electron microscopy experiments were performed on RMP loaded dendrimer formulations that had been prepared and dialyzed. The TEM studies were carried out on Philips CM-10 TEM and Fei-Philips Morayagni 268D with digital TEM image analysis system at 50-60 Kv using 3mm Forman (10.5 percent plastic powder in amyl acetate) coated copper grid (300 mesh) at 60 Kv using negative staining with 2 percent phosphotungstic acid (PTA) for whole generation of dendrimers at 150,000X magnification.

### 2.3.2.Drug loading and entrapment efficiency

The unentrapped drug found in the dialysis medium on dialysis (MWCO 12-14Kda) after 1 hrwas used to indirectly estimate the amount of drug entrapped during loading of RMP (soluble drug) by adsorption, binding, or complexation. The amount of free drug present in the clear supernatant was estimated by UV-VISIBLE spectrophotometer at 475 nm and pH7.4 phosphate buffer as blank. Using the calibration curve the amount of free drug were calculated and then subtracted from the total amount of drug added during the loading process.

Efficiency of drug entrapment was calculated in terms of percentage drug entrapment as per the following formula.

$$\%EE = \frac{(W-w)}{w} * 100 \qquad (1)$$

W= total amount of drug added during the loading process

W= amount of free drug in the supernatant

(W-w)= the amount of drug entrapped in the PPI dendrimers

### 2.3.3.Particle size and zeta potential

The size and the zeta potential of formulation were determined by Zeta sizer (PALS-phase analysis light scattering). The samples were diluted with distilled water and measured at 25°c. Results were automatically calculated by the analyser using the following equation.

$$m = \frac{ez}{h} \qquad (2)$$

Where z is zeta potential, m is mobility, e is the dielectric constant and h is the viscosity of electrolyte solution.

### 2.3.4.*In- vitro* drug release profile

*In–vitro* release kinetics study across diffusion membrane (MWCO 12-14 Kda, Himedia, India) precluding drug loaded PPI dendrimers was performed in a Franz diffusion chamber consisting of two compartments separated by a diffusion membrane. 10mg of the drug loaded dendrimer complex was placed in the donar compartment and the receptor compartment was filled with pH 7.4 phosphate buffer to determine the amount of Rifampicin diffused through the diffusion membrane. At regular prefixed time intervals 1ml of the sample was withdrawn and replaced

with fresh buffer solution in the receptor compartment. The amount of drug present in the sample was estimated by UV-Spectrophotometry at 475 nm.

### 2.3.5.Drug release kinetics

To describe the kinetics of drug release from dendrimer loaded formulations, mathematical models such as zero order, first order, Higuchi and Korsmeyer Peppas models were used. The interpretation of the data was based on the value of the resulting regression coefficients.

### 2.3.6.Hemolytic test

Human blood was collected in Hi Anticlot blood collection vials. The blood was washed with 0.1M phosphate buffered solution (PBS)($p^H$-7.4), centrifuged at 600rpm for 5min, and the supernatant was pippeted off repeatedly (3 times). The RBC suspension was diluted with 0.1M PBS to obtain a concentration of 5%W/V.RBC suspension. 0.5ml of drug solution/ 0.5ml of dendrimer solution and 0.5 ml of suitably diluted RMP encapsulaeddendrimer were mixed with 4.5ml of normal saline and incubated for 1hr with RBC suspension at 37°C. The drug and dendrimers in separate tubes were taken in such amount that the resultant final concentrations of drug and dendrimer were equivalent in all the cases. This gives comparative results of the hemolysys data of drug,dendrimer and RMP loaded dendrimers which helps to understand the effect of dendrimers on hemolysis. After incubating the mixtures were centrifuged at 3000rpm for 10min to remove non-lysed RBCs. The supernatant were collected and analyzed to determine the amount of released hemoglobin by UV spectrophotomter at 540nm. To obtain 0 and 100% hemolysis, RBC suspension were added to 5ml of 0.9% NaCl solution (normal saline) and 5 ml distilled water, respectively. The degree of hemolysis was determined by the following equation.

$$\text{Hemolysis (\%)} = \frac{Abs - Abs0}{Abs100 - Abs0} * 100 \qquad (3)$$

Where Abs, Abs100 and Absoare the absorbance of sample, a solution of 100% hemolysis and a solution of 0% hemolysis respectively

## 3. RESULTS AND DISCUSSION

### 3.1.Drug Loading and entrapment efficiency

The entrapment efficiency percentage of RMP loaded PPI dendrimer (F3) was increased when compared to other formulations (F1&F2) table 1. Since non-covalent interactions among RMP and 5.0 G PPI dendrimer, such as hydrophobic interaction and hydrogen bonding were responsible for binding of drug molecules inside dendritic crevices and at surfaces [21] [22]. Moreover significant increase in entrapment efficiency of F3 with respect to other formulations may be due to more interactions between the drug moieties and peripheral amino surface groups $(NH_2)_{64}$.

### 3.2.Morphology of the Dendrimers

TEM micrograph ( figure 1) showed that the drug loaded dendrimers were more or less spherical in shape and that the dendrimers were agglomerated. The mean diameter of the drug loaded PPI dendrimer was found to be 7nm.

### 3.3.Drug – Excipient compatibility study (IR spectra)

From the figure 2, Rifampicin spectra, PPI dendrimer, RMP and dendrimer physical mixture, Rifampicin loaded dendrimers The combined spectrum revealed that all of Rifampicin's distinctive peaks were present. There was no shift in the peak and no change in the frequency range of the respective functional groups of the drug and carrier, indicating that there was no interaction between the drug and the carrier and that the formulation was appropriate. As a result, the compatibility of Rifampicin and dendrimers was established.

### 3.4.Differential Scanning Calorimetry

Curves of DSC (figure 3) showed that RMP loaded dendrimers was not a phsical mixture by endothermic and exothermic transition. The DSC graph of rifampicin showed their characteristic peak at 272˚C. Absence of characteristic peak of Rifampicin in the DSC graph of Rifampicin loaded dendrimer confirmed the encapsulation of drug in PPI dendrimer.

### 3.5.Zeta potential

Zeta potential was significantly affected by amount of Rifampicin and PPI dendrimers. In the dialysis method Rifampicin compete for the primary amino groups of PPI dendrimers. After getting cross linked with Rifampicin still few amino groups will remain on the PPI dendrimers which contributes to the surface charge. This surface charge is responsible for the Zeta potential on which the stability of the formulation depends. Higher zeta potential (± 30mV) value indicates the stability of formulation. Zeta potential measurement of the prepared formulations (figure 4) indicated positive zeta potential value (F1,+29.6 ; F2,+25.6 ; F3, +23.1)

### 3.6.*In- vitro* drug release profile:

Rifampicin spectra, PPI dendrimer, RMP and dendrimer physical mixture, Rifampicin loaded dendrimers The combined spectrum revealed that all of Rifampicin's distinctive peaks were present. There was no shift in the peak and no change in the frequency range of the respective functional groups of the drug and carrier, indicating that there was no interaction between the drug and the carrier and that the formulation was appropriate. As a result, the compatibility of rifampicin and dendrimers was established.

### 3.7.Drug release kinetic analysis by using different release models:

The formulations *in vitro* release kinetics were fit to a variety of common release equations, including zero order, first order, higuchi model, and korsmeyer – peppas equation. Table 3 displays the results collected. The highest regression values for zero order release kinetics were discovered in *in-vitro* drug release, showing that the dendrimer demonstrated controlled drug release and the slope n value of Peppa's suggests that the mechanism of drug release was determined to be non-fickian diffusion. The F3 formulation was the greatest fit for extended drug release for 48 hours, with drug release of not more than 10% in the first hour and not less than 90% in the 48th hour.

### 3.8.Hemolytic toxicity

Rifampicin (10.5+ 1.3 percent) and 5.0 G PPI dendrimer (16.7+ 2.2 percent) both have hemolytic toxicity. Due to drug moieties attaching to more amino surfaces of dendrimer, rifampicin loaded PPI dendrimer lowered toxicity to 8.8 + 1.5 percent. The toxicity of PPI dendrimers is mostly related to their polycationic character, particularly in the case of a whole generation of amine ended charged dendrimers. As a result, dendrimers should be modified further to lessen toxicity.

## 4. CONCLUSION

The present study concluded the dendrimer as drug delivery system for Rifampicin. Due to its particle size and hemolytic studies, it can be administered safely through I.V route. The formulations showed constant release of drug from dendrimeric system throughout the study period, around 95.6% of drug was released upto 48 hrs. From the drug release kinetics, dendrimers showed controlled release of Rifampicin through non-fickian diffusion. Futher *in-vivo* studies opens the oppurtunity for management of drug therapy in tubercular patient by delivering the drug at a controlled rate for prolonged period of time by minimizing fluctuations in plasma drug concentration.

### LIST OF ABBREVIATIONS

PPI- Poly (propyleneimine)

5.0 G - Fifth generation

MWCO- Molecular weight cut off

RMP- Rifampicin

**TABLE 1 : Entrapment efficiency of drug loaded formulations**

| FORMULATION CODE | MOLAR RATIO (DENDRIMER: DRUG) | DRUG ENTRAPPED (mg) | ENTRAPMENT EFFICIENCY (%) |
|---|---|---|---|
| F1 | 1:8 | 2.08 | 31.69 |
| F2 | 1:32 | 13.14 | 49.58 |
| F3 | 1:64 | 38.47 | 68.78 |

**TABLE 2 : Cumulative percentage release of formulations**

| Formula Code | Molar ratio | Drug loaded(mg) | Cumulative % release | Extended release (hrs) |
|---|---|---|---|---|
| Pure drug | - | - | 96.1 % | 3 hrs |
| F1 | 1:8 | 2.08 | 95.77% | 24 hrs |
| F2 | 1:32 | 13.14 | 95.81% | 36 hrs |
| F3 | 1:64 | 38.47 | 93.67% | 48 hrs |

**TABLE 3 : In-vitro kinetic data of drug loaded formulations**

| Formula code | Zero-order Plots | First- order Plots | Higuchi's Plots | Koresmeyer- Peppa's plot | | Possible Drug Release mechanism |
|---|---|---|---|---|---|---|
| | Regression Coefficients ($R^2$) | Regression Coefficients ($R^2$) | Regression Coefficients ($R^2$) | Slope (n) | Regression Coefficients ($R^2$) | |
| F1 | 0.956 | 0.901 | 0.993 | 0.635 | 0.985 | Zero order Non-Fickian release |
| F2 | 0.963 | 0.955 | 0.986 | 0.614 | 0.983 | Zero order Non-Fickian release |
| F3 | 0.986 | 0.945 | 0.971 | 0.622 | 0.981 | Zero order Non-Fickian release |

**FIGURE 1 : TEM image of Rifampicin loaded dendrimers**

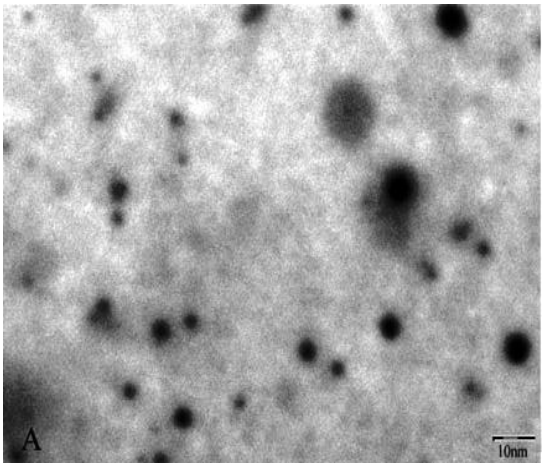
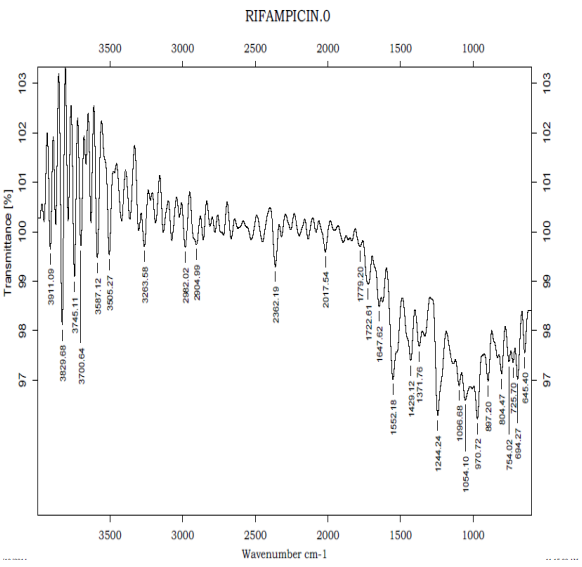
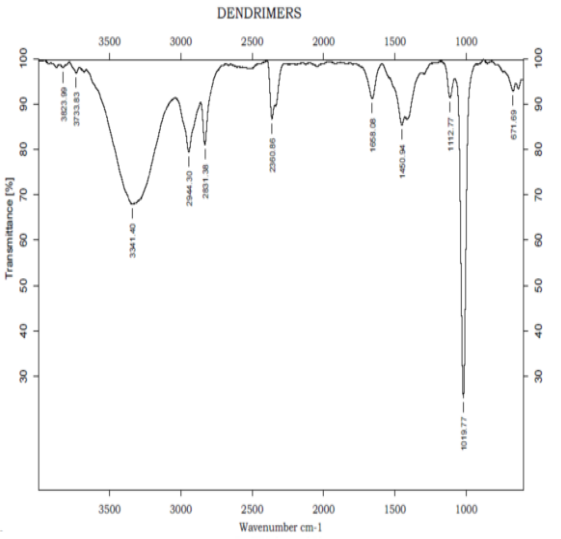



**FIGURE 2 : Fourier Transform Infrared Spectrophotometer (FTIR)**

**FTIR spectra of Rifampicin**




**FTIR spectra of dendrimers**




**FTIR spectra of phsical mixture of Rifampicin+dendrimerdendrimers**

**FTIR spectra of Rifampicin loaded**

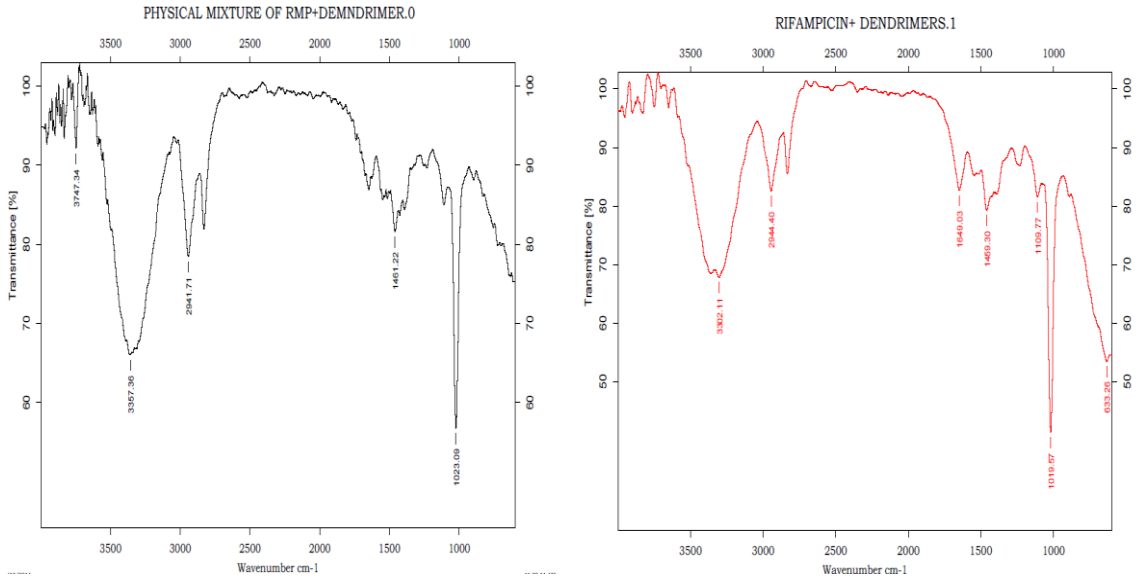
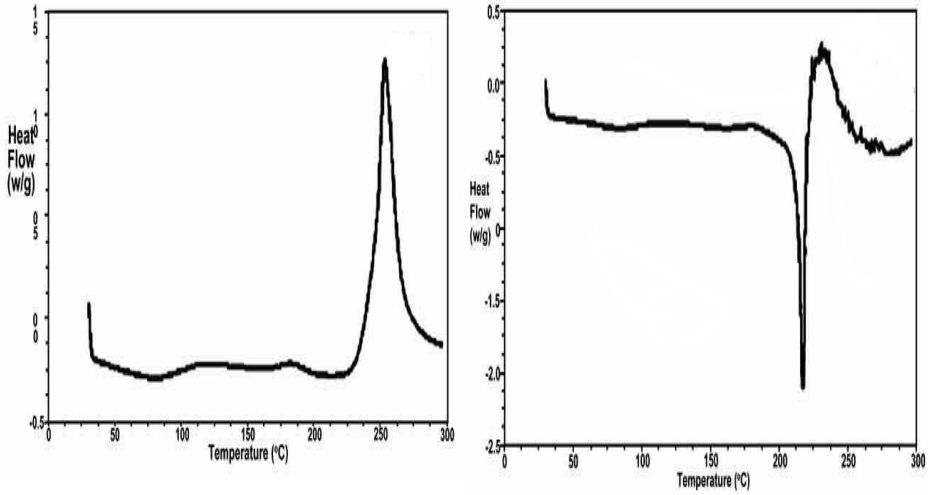



**FIGURE 3 : 3a. DSC spectra of pure drug , 3b.DSC spectra of RMP loaded dendrimer**




3a 3b

**FIGURE 4 : Zeta potential of drug loaded dendrimers**

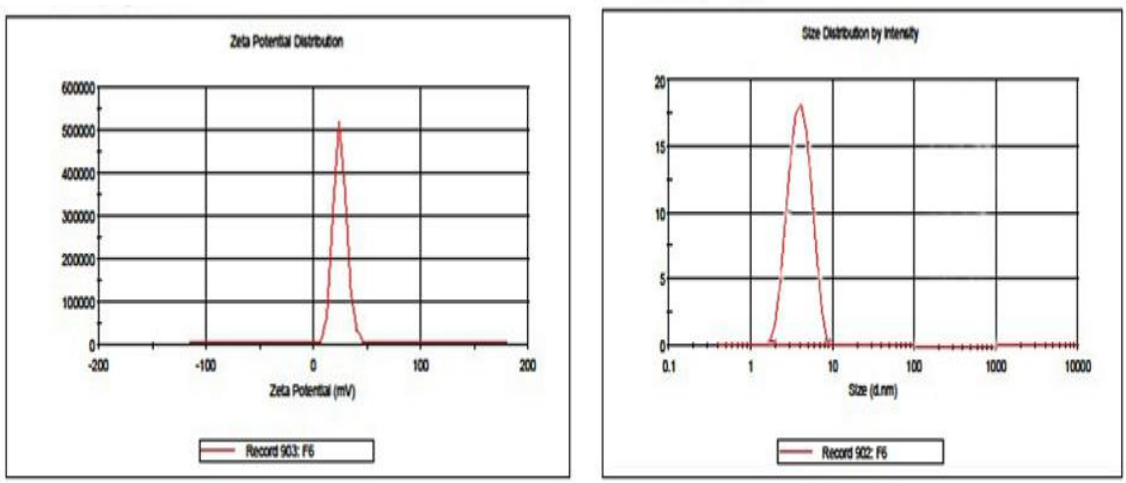


**FIGURE 5 : Comparative in-vitro cumulative percentage drug release of formulations**

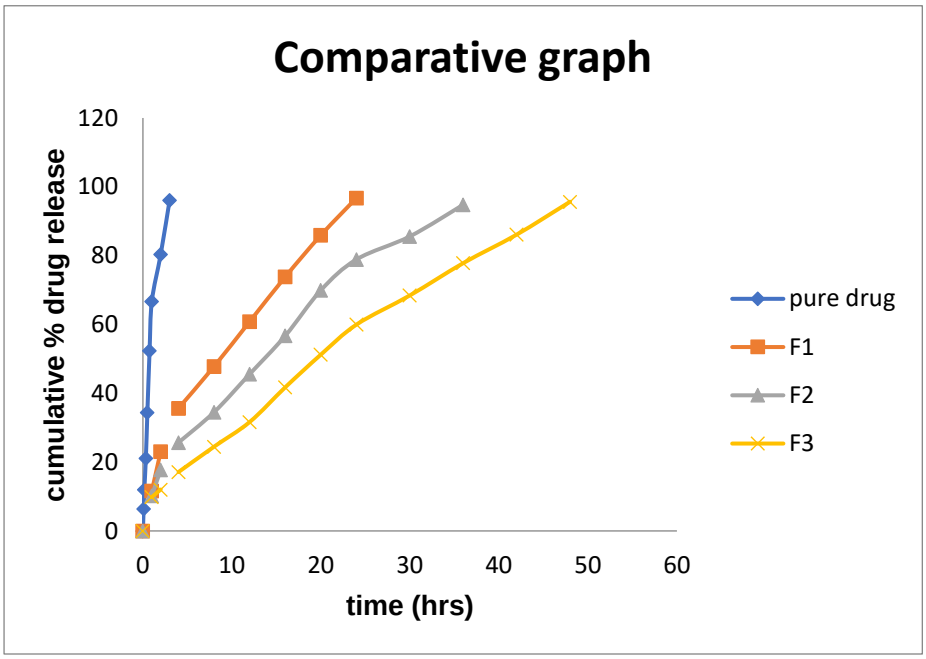
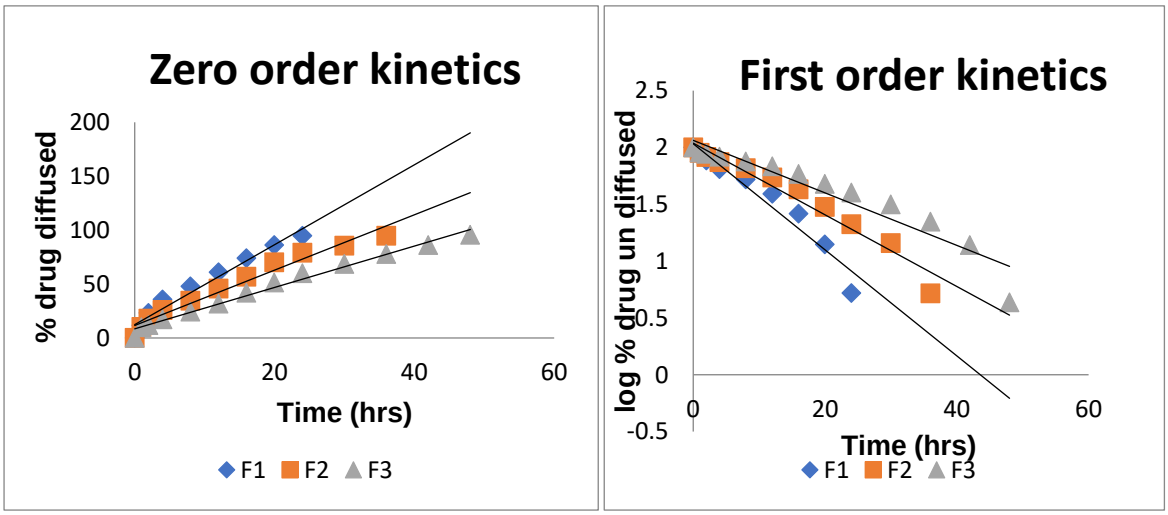
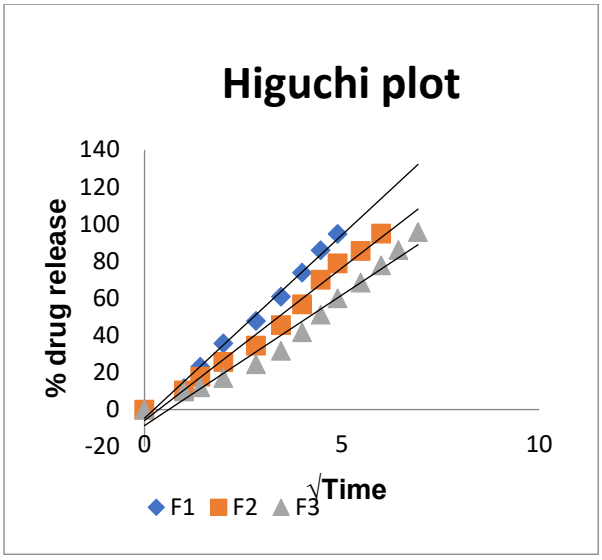
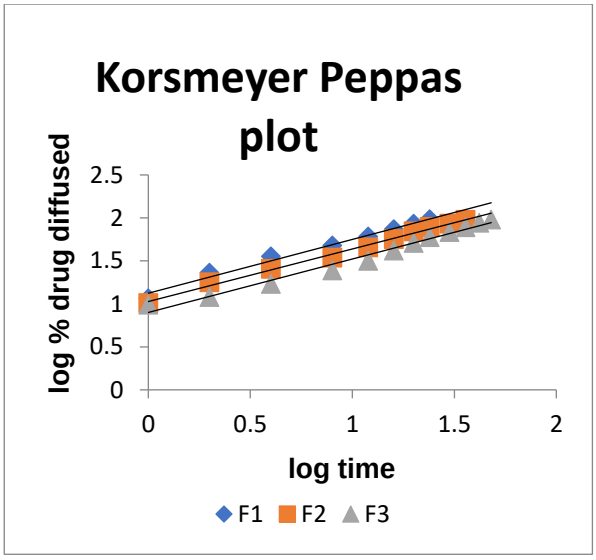



**FIGURE 6 :Drug release kinetics of formulations by different models**



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