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DEVELOPMENT AND CHARACTERIZATION OF AN ANTIMICROTUBULAR TAXANE LOADED MPEG-b-PCL NANOPARTICLES

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ABSTRACT

The main objective of the work was to design and characterize MPEG-b-PCL nanoparticles containing Antimicrotubular Taxane and then the prepared nanoparticles were freeze dried to increase the stability during storage condition. Paclitaxel was an Antimicrotubular Taxane prevents the uncontrolled cell division of tumor cells. From the prepared formulations with varying drug to polymer concentrations, optimised formulation was selected based on the higher EE and in vitro drug release and then the formulation was subjected to freeze drying , after that EE%, release studies, particle size and Zeta potential analysis were carried out to find out the significant change between freeze dried and normal formulation. The average particle sizes of Nanoparticles before and after lyophilisation were found in the size range of nanomicellar level (10-200 nm).The drug loading content or entrapment efficiencies of nanoparticles increases with increasing polymer concentration up to particular value. Release kinetics of selected formulation showed zero order with mechanistic Fickian diffusion release from nanoparticles. Nanoparticles were stored at refrigerated and normal room temperature condition as per WHO guidelines to check the stability.

KEY WORDS

Nanoparticles, Paclitaxel, MPEG-b-PCL copolymer and Lyophilisation.

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INTRODUCTION

Nanotechnology based therapeutics in the cancer chemotherapy have the advantages of the increased therapeutic drug efficacy, reduced adverse effects or toxicity to normal healthy cells and tissues, and improved patient compliance. It plays an important role in satisfying the delivery of anticancer drugs particularly to the site of action with a minimum effective concentration over a period of time[1].

Nanosize based drug carriers like polymeric nanomicelles, polymer-drug conjugates[2], solid lipid nanoparticles and polymeric nanoparticles have numerous benefits over the conventional dosage forms like injections and oral solids for delivering anticancer drugs.

A successful formulation for the delivery of chemotherapeutic agents should have the features of targeted delivery, longer circulation time, smaller size to escape from the scavenging effect of phagocytes, avoid the opsonisation, enhanced tumor uptake of drug and Promote endocytosis of drug loaded carrier. The above features were hold good by delivering the anticancer drugs through the polymeric micellar nanotechnology, because they were longer time circulating nanoparticles with stealth properties and nano size ranged particles[3].

The primary function of micellar drug delivery was to improve the solubility and stability of hydrophobic drugs. This was achieved by entrapping the drug in the hydrophobic core of polymeric micelle and the long circulation time, reduced plasma protein binding, uptake by phagocytes and RES cells were removed because of the presence of hydrophilic shell on the outer surface of nanomicelles[4].

The most commonly used nanomicelles in the cancer drug delivery were derived from the amphiphilic block copolymers like MPEG-b-PCL copolymer, MPEG-b-PLA copolymer, MPEG-b-PLGA copolymer and MPEG-b-PEGMA copolymer. These polymeric micelles have capacity to encapsulate the various anticancer drugs such as Paclitaxel, Docetaxel and Doxorubicin. So, the polymeric micellar nanotechnology has a very good capability of loading and targeting the anticancer drugs in the treatment of various cancer conditions in the human body.

MATERIAL AND METHODS

Materials

Paclitaxel and MPEG-b-PCL copolymer (Gift samples from Celon Laboratories Pvt, Ltd, AP), Dichloromethane (procured from E-Merck, Mumbai), Cellulose Acetate membrane filters 0.2 microns purchased from Ray labs, New Zealand. Other reagents and solvents used were of analytical grade.

Fabrication of Nanoparticles

Nanoparticles with different drug to polymer concentrations (1:3.5,1:4,1:4.5,1:5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5 and 1;8) were prepared by Emulsion solvent evaporation method[5]. Required quantities of drug and polymer were dissolved in 2ml of Dichloromethane and sonicated for 15 min to completely solubilisation. Then, the organic solution was added drop wise into 10 times the volume of 2% aqueous PVA solution under high shear homogenizer at 12,000 rpm for 15 min for the better entrapment of drug. After that, the homogenized solution was stirred at 8000 rpm with mechanical stirrer for 1hour at room temperature. During this step the formed nanoparticles were hardened and organic solution is slowly evaporated. For removing the traces of Dichloromethane in the aqueous solution, the solution was evaporated by using vaccum Rotary evaporator (Buchi) at 25^0C with 40 rpm for 30 min by applying a vaccum of about 25 mm Hg.

Filter the Nanoparticle solution through cellulose acetate membrane filter, under vaccum to get the sterile formulation. Then, the collected nanoparticles were redispersed in 20 ml of Water for injection for lyophilisation. Here, the PVA over the particles was dissolved in to the solution and act as a lyoprotectant for protecting the formulation against the freezing and drying stress during freeze drying[6].

Finally, the Nanoparticle suspension was transferred into a 50 ml glass vial and sealed with a rubber stopper followed by an over seal (Aluminium) and then wrapped in Aluminium foil to protect from light.

The best formulation was selected based on the amount of the drug loaded or percentage entrapment efficiency and in vitro drug release behaviour. Then, the selected or optimised formulation was further lyophilised by freeze drying method to increase the stability of nanoparticle formulation during storage.

Lyophilisation of Nanoparticle formulation was carried out in three stages

a. Freezing (solidification).
b. Primary drying (ice sublimation)
c. Secondary drying (desorption of unfrozen water)

Freezing

During this step the Nanoparticle suspension was freezed at -45^{0}C for 60 min, then the liquid suspension is cooled and ice crystals are formed and the suspension becomes concentrated. At the end of freezing step, the concentrated suspension was in an amorphous form or combined amorphous-crystalline form.

Primary Drying

In the primary drying, the ice crystals that were formed in the frozen product were sublimated at 25^{0}C under controlled vaccum of 60-65 MT for 220 min. At the end of drying, porous plugs were formed those represents the spaces occupied by ice crystals.

Secondary drying

In the secondary drying, bound water present in the product was removed at 35^{0}C under controlled vaccum of 65 MT for 60 minutes, which was not separated out as ice during the freezing was removed.

Reconstitution of lyophilised Nanoparticle formulation

Finally, the formed lyophilised cake was reconstituted slowly with 20 ml of pH 7.4 phosphate buffer saline for 1min to prevent foaming and allowed to settle for 5 minutes for proper wetting of the lyophilized cake and then gently swirl or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs.

Characterization of nanoparticles

Size and Zeta potential analysis of nanoparticles

The prepared nanoparticles were subjected to particle size and zeta potential analysis before and after lyophilisation by MALVERN ZETASIZER V 6.01 in Indian institute of chemical technology, Hyderabad.

Entrapment Efficiency and Drug loading content

The Entrapment efficiency of Nanoparticle preparation was determined by UV absorbance of drug at 227 nm by UV spectrophotometry[5]. Nanoparticle suspension was subjected to the ultracentrifugation at 14,000 rpm for 30 min to remove the supernatant containing excess of Paclitaxel, the sediment was formed at the bottom of the centrifuge tube. It was vortexed with 2 ml Dichloromethane for 15 min to extract the entrapped drug from the core of the Nanoparticles and add the solvent media (PBS pH7.4) to the solution of the released drug then purged with nitrogen gas to evaporate the Dichloromethane[7], until the clear solution is obtained. After that, the solution is filtered through the 0.2 micron syringe filter to remove the polymer aggregates.

The 1ml of filtered solution was taken and suitably diluted with solvent media. Then, the concentration of diluted solution was measured spectrophotometrically.

The following equation was used to find out entrapment efficiency of paclitaxel in Nanoparticle formulation

$$\text{Entrapment efficiency(\%)} = \frac{\text{Amount of Drug entrapped}}{\text{Amount of drug used for preparation}} \times 100$$

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

By UV Spectrophotometric method[5]

In vitro release of Paclitaxel from Nanoparticle formulation was carried out by using dialysis bag diffusion technique[8, 9].

The prepared Nanoparticle formulation was placed in a dialysis bag membrane having molecular weight cut off 14,000 daltons, which was previously soaked for overnight, both the ends were tied tightly and dropped into a beaker containing 200 ml of diffusion solvent medium pH 7.4 PBS, maintained at 37^{0}C and stirred with the help of a magnetic stirrer. Aliquots (1ml) of release medium were withdrawn at different time intervals (8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 and 96 hr) and the sample was replaced with same amount of fresh solvent medium to maintain the constant volume.

Then, the samples were suitably diluted and analysed spectrophotometrically at 227 nm. The release study was also performed for the lyophilised formulation F7 after reconstitution with 20 ml pH 7.4 PBS.

Drug release kinetics

To analyze the mechanism of drug release from the optimised lyophilised formulation, the data obtained from *in vitro* release studies were subjected to Zero order model, First order, Higuchi's model and Korsmeyer's models[10, 11, 12].

Stability study

The stability studies were carried out by using optimised lyophilised formulation as per WHO guideline[13]. The lyophilized Nanoparticle formulations were kept for three months at 5^0C and 25^0C/65% RH in a stability chamber (Osworld). The stability of drug loaded nanoparticles was evaluated in terms of its EE%, % of drug released.

RESULTS AND DISCUSSION

Entrapment Efficiency and Drug loading content

The entrapment efficiency (%) of the Paclitaxel loaded Nanoparticle formulations (F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10) and an optimised freeze dried formulation F7 were tabulated in Table No.1 and 2.

The % entrapment efficiency was determined by ultracentrifugation method and it was found that the formulations F1, F2 and F3 has showed entrapment efficiencies of 40.76 ± 0.28 %, 42.81 ± 0.32%, 50.34 ± 0.80 % with drug loading contents of 16.31 ± 0.17mg, 17.12 ± 0.13mg and 20.13 ± 0.42mg respectively. The low % entrapment efficiencies were due to lower drug loading contents because of lesser availability of PCL carboxyl groups of copolymer to entrap the drug.

The formulations F4, F5 and F6 has showed the entrapment efficiencies of 68.72 ± 0.73 %, 75.19 ± 0.60 %, 83.26 ± 0.56 % with drug loading contents of 27.73±0.45 mg, 30.07 ± 0.24 mg and 33.30 ± 0.19 mg respectively. The % entrapment efficiencies were increased with increase in loading contents because of increased copolymer concentrations with respect to the drug and also due to availability of PCL carboxyl groups of copolymer to entrap the drug.

The F7 formulation has showed entrapment efficiency of 92.0 ± 0.36 % with a higher drug loading content of 36.79 ± 0.17mg. This was because of maximum availability of PCL carboxyl groups to entrap the drug. Then, the formulations F8, F9 and F10 has showed % entrapment efficiencies of 87.6 ± 1.08 %, 82.57 ± 0.78 % and 77.78 ± 1.08 % with drug loading contents of 35.07 ± 0.32 mg, 33.02 ± 0.36mg and 31.09 ± 0.33mg respectively. The gradual decrease in the % entrapment efficiency then compared to formulation F7 was due to increased copolymer concentration because of higher hydrophobic or steric interactions between the carboxyl groups of PCL copolymer and drug.

Hence the F7 formulation has shown highest % entrapment efficiency with higher drug loading content when compared with other formulations. So, it was selected as a best formulation and then it was freeze dried by lyophiliser.

The entrapment efficiency of lyophilised F7 formulation was found to be 91.96 ± 0.18 % with drug content of 36.76 ± 0.14mg. This signifies that there was no change in drug loading content and entrapment efficiency before and after lyophilisation of formulation F7.

***In vitro* Drug release profile of Paclitaxel Nanoparticles**

The *in vitro* drug release characteristics for prepared Nanoparticle formulations were evaluated with the help of release profiles shown in Figure No.1.

The formulations F1, F2 and F3 showed a drug release of 35.6 %, 42.87 % and 50.46 % at 96 hr respectively. The drug release was increased from F1 to F3, because of the increased % entrapment efficiencies due to the availability of increased carboxyl groups of copolymer.

The formulations F4, F5 and F6 showed a drug release of 66.15 %, 73.23 % and 84.77 %, at 96 hrs respectively. The percentage drug releases of formulations were increased due to increased entrapment efficiencies because of the increased copolymer concentration with respect to the drug or due to increase in carboxyl groups of copolymer to entrap the drug.

The formulation F7 has showed a drug release of 89.84 % in 96 hours. The release of the drug was suddenly increased with a drug loading content of 36.79 mg due to the availability of maximum

carboxyl groups of copolymer to entrap the drug. Then, the F8, F9 and F10 have showed a drug release of 83.06 %, 79.54 % and 78.34 % respectively. The gradual decrease in drug release when compared with the F7 formulation was found to be due increased hydrophobic or steric interactions between drug and carboxyl groups of copolymer and also in between the carboxyl groups of copolymers. Hence, the F7 formulation was selected as the best formulation with highest percent drug release and also having the higher % encapsulation efficiency then compared to the other formulations.

So, it was selected for the freeze drying to increase the stability during storage. This freeze stabilized formulation F7 has showed drug release of 89.82 % with a drug loading content of 36.76 mg. It was found that there was no significant change in percentage drug release for selected formulation F7 before and after lyophilisation. So, it was selected further to study the release kinetics

Particle size and zeta potential

The optimised Paclitaxel loaded MPEG-b-PCL Nanoparticle formulation before and after the lyophilisation was subjected to study particle size, Zeta potential and poly dispersive index analysis. The results were shown in the Table No.3.

The average particle size of the nanoparticle formulation F7, before and after lyophilisation was found to 169.7 nm and 187.0 nm respectively. The observed particle sizes were fall in the range of nano micellar level (10 nm-200 nm) and also the calculated ratio value of nanoparticle size before and after lyophilisation was nearer to the value of one which was an important indication of conservation of the nanoparticle size after the freeze drying.

The zeta potential values of the formulation F7, before and after freeze drying were -19.6 mV and -15.9 mV respectively. The decrease in the zeta potential was due to the effect of PEG in the outer shell of nanoparticles and also probably due to the adsorption of Paclitaxel on the nanoparticle surfaces during preparation process. The lower negative zeta potential results in a reduction of cell repulsion to the nanoparticles up to a certain degree and also contributes to the stability of nanosuspension.

Poly dispersity index (PDI) values for the formulation F7, before and after lyophilisation were found to be 0.195 and 0.17. This lower PDI values indicates that the nanoparticle formulation before and after freeze drying has mono dispersive characteristics and narrow particle size distribution.

Release kinetics

In kinetics data, the plots had showed the correlation coefficients of different kinetic models for Paclitaxel optimized freeze dried formulation (F7). Higuchi plot (R^2=0.995) (Figure No. 4) were found to be of highest linearity with correlation coefficient greater than that of the zero order kinetics plot (R^2=0.987) (Figure No.2) and corresponded to that of the first order kinetics plot (Figure No.3) indicating that the drug release mechanism from nanoparticles was by diffusion method.

Studies revealed that the release of Paclitaxel was found to be very close to zero-order kinetics indicating that the concentration was nearly independent of drug release. Moreover, *in-vitro* release Paclitaxel was best explained by Korsmeyer-Peppa's plot (Figure No.5) also indicated a good linearity. The release exponent 'n' was 0.408, which indicates Fickian diffusion.

Stability study

The results of the stability studies of the prepared Paclitaxel nanoparticles, reveals that the freeze dried formulation was stable in the refrigerated condition for a period of 3 months. The entrapment efficiency and drug release of the formulation at 5^0C did not showed any significant change during storage condition when compared with the same formulations stored at 25^0C/65 % RH which has shown significant changes.

Table No.1: Entrapment efficiency of formulations with drug and copolymer Before lyophilisation (n=3), values were the mean of 3 experiments ± S.D

S.No	Formulation code	Amount of drug loaded(mg)	Percentage Entrapment efficiency
1	F1	16.31±0.17	40.76±0.28
2	F2	17.12±0.13	42.81±0.32
3	F3	20.13±0.42	50.34±0.80
4	F4	27.73±0.45	68.72±0.73
5	F5	30.07±0.24	75.19±0.60
6	F6	33.30±0.19	83.26±0.56
7	F7	36.79±0.17	92.0±0.36
8	F8	35.07±0.32	87.6±1.08
9	F9	33.02±0.36	82.57±0.78
10	F10	31.09±0.33	77.78±1.08

Table No.2: Entrapment efficiency of formulation F7 with drug and copolymer After lyophilisation (n=3). Values were Mean ± S.D

S.No	Formulation code	Amount of drug loaded(mg)	Percentage Entrapment efficiency
1	F7	36.76±0.14	91.96±0.18

Table No.3: Physicochemical characteristics of an optimised Paclitaxel Nanoparticle formulation before and after Lyophilisation

S.No	Formulation F7 (1:6.5)	Average particle Size (nm)	Zeta potential (mV)	Poly dispersive Index
1	Before Lyophilization	169.7	- 19.6	0.195
2	After Lyophilization	187.0	- 15.9	0.170

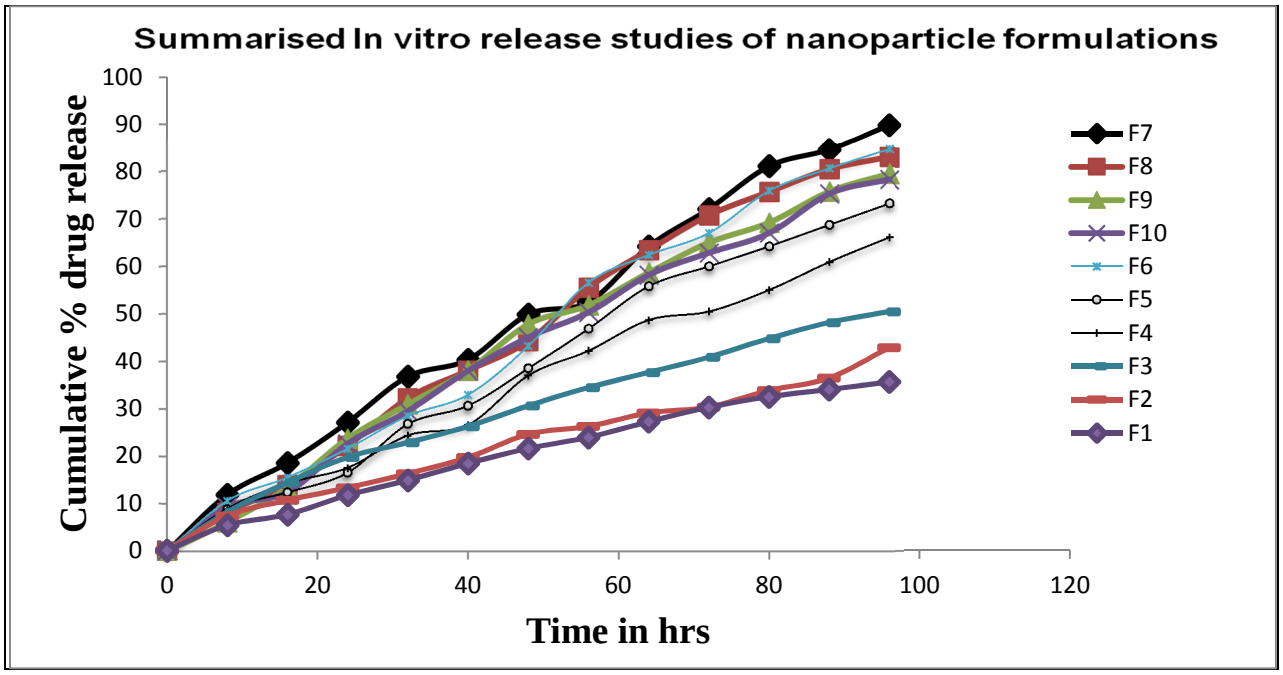

Figure No.1: Summarised Invitro drug release profiles of all Paclitaxel nanoparticle formulations

Figure No.2: Zero-Order Kinetics Plot

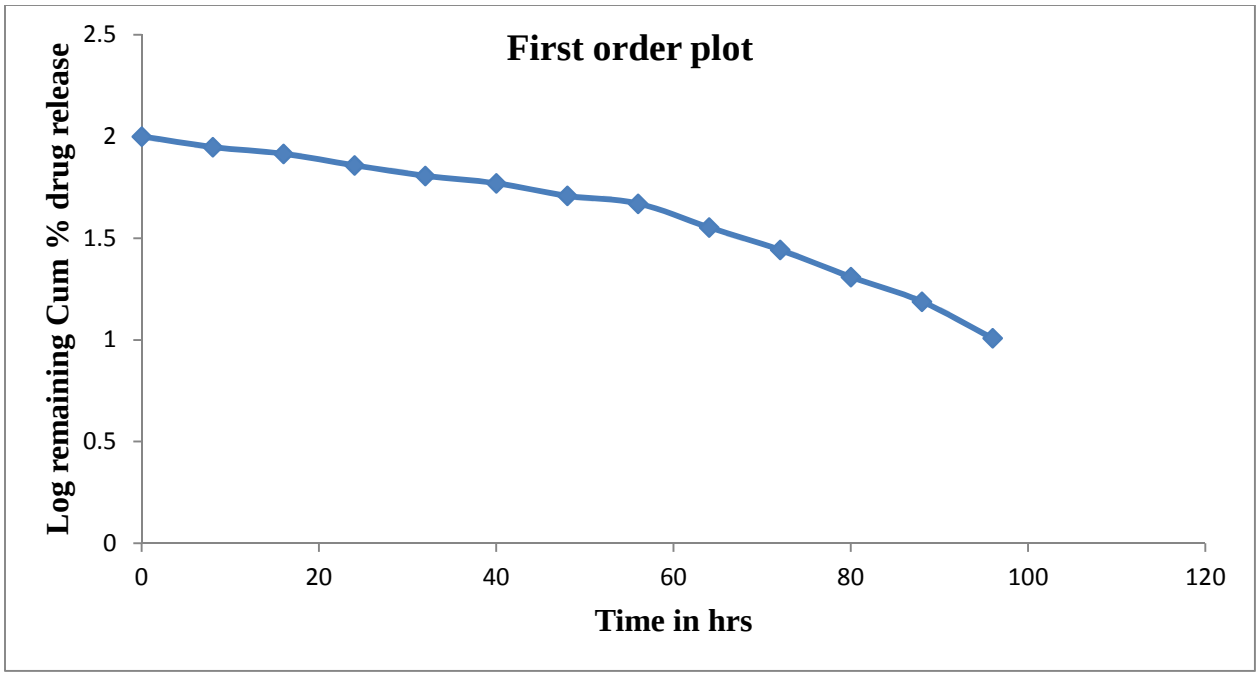

Figure No.3: First-Order Kinetics Plot

Figure No.4: Higuchi Plot

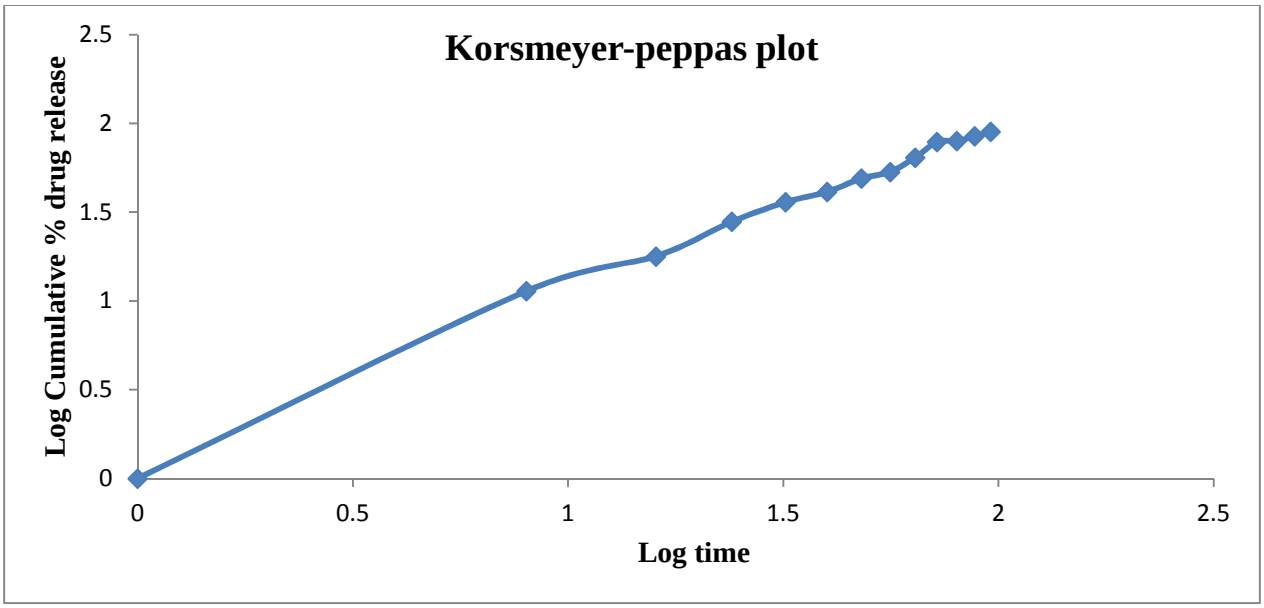

Figure No.5: Korsemeyer – Peppa's model Kinetics Plot

CONCLUSION

Thus from Nanoparticle research work, it can be concluded that the objective of proposed work has been fulfilled and polymeric Nanoparticles of paclitaxel has been prepared using blend of MPEG-b-PCL copolymer. Formulation F7 having particle size, zeta potential, PDI, % EE and % drug release were 169.7 nm, -19.6 mV, 0.195, 89.84%, 92% respectively. On the basis of experimental data this formulation was considered as optimised formulation and lyophilised to increase stability during storage and evaluated particle size, zeta potential, % EE and release studies, it was found that there was no significant change before and after lyophilisation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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