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FORMULATION AND EVALUATION OF DOCETAXEL LOADED NANO STRUCTURED LIPID CARRIER SYSTEM

Vipin K.V * [1], Dr. Sarath Chandran C[2], Ann Rose Augusthy [3]

[1]PhD Scholar, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

[2]Department of Pharmaceutics, Academy of Pharmaceutical Sciences, Pariyaram, Kerala, India.

[3]PhD Scholar, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India.

Abstract:

The objective of the research work was to design and characterize Nanostructured Lipid Carriers (NLC) incorporated with anticancer agent docetaxel. NLC were prepared by hot homogenization followed by ultrasonication method using stearic acid as the solid lipid and oleic acid as the liquid lipid. Soya bean lecithin was used as surfactant and poloxamer 188 as the cosurfactant. Optimization of the formulations was done based on sedimentation volume, particle size, polydispersity, % entrapment efficiency and zeta potential. The optimized batch of NG9 exhibited a particle size of 180µm, polydispersity of 0.25, %EE of 78% and zeta potential of 41mV. Based on this it was decided that concentration of lipid at 20%, solid lipid: liquid lipid ratio of 80:20, surfactant and cosurfactant at 4%, mannitol at 3% was the optimal proportion. The process parameters was further optimized and the results revealed that at homogenization RPM of 6000, homogenization time of 20min and sonication time of 10min resulted in NLC with ideal characteristics. The in vitro release of Docetaxel from the optimized batch, NG9 was found to be almost complete with a release of 98.80 ±0.08 at 4 hours. The drug release of this batch when subjected to pharmacokinetics studies using various models showed that the data best into zero order and Higuchi release kinetic model with a coefficient of determination (R^2) value of 0.9972 and 0.9347. Linearity to Higuchi kinetic model indicated a diffusion controlled drug release. The release component (n) from peppas model was found to be 0.42, which indicated Fickian diffusion from which it can be assumed that the drug delivery system under study is a matrix device.

Key words: *Nano structured lipid carriers (NLC), Docetaxel, Poloxamer 188, soya bean lecithin, stearic acid, and oleic acid.*

Corresponding author:

Vipin K.V,

PhD Scholar,

Pacific Academy of Higher Education and Research University,

Udaipur,Rajasthan, India.

E-mail: vipinkv99@rediffmail.com,

Phone no: 9846896141

QR code

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INTRODUCTION:

Nanostructured Lipid Carriers

NLC are a new generation of lipid nanoparticles that were developed to overcome some limitations of solid lipid nanoparticles (SLN)[1]. Nanostructured lipid carriers consist of a blend of solid and liquid lipids. The particle diameter of nanostructured lipid carriers ranges from 10 nm to 1000 nm[2]. The limitations associated with SLN such as drug expulsion during storage caused by lipid polymorphism, risk of gelation and low drug loading are reported to be overcome by formulating as NLC[3]. In view of this, NLC may be regarded as a superior lipid carrier system and an alternative to SLN for improving the delivery of both hydrophilic and lipophilic drugs. Presence of liquid lipids of higher drug solubility should have lead to improved drug payload capacity, minimization of drug expulsion, enhancement of chemical stability and improved long term stability of NLC[4].

Docetaxel is a semisynthetic side-chain analogue of paclitaxel with antineoplastic property[5]. It is a taxoid antineoplastic agent[6]. They inhibit the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Docetaxel binds specifically to the beta-tubulin of microtubules and antagonizes the disassembly of the microtubule proteins. This results in the persistence of aberrant microtubule structures and results in cell-cycle arrest and subsequent cell death[7].

MATERIALS AND METHODS :

Materials

Docetaxel was obtained as a gift sample from MacChem Labs Pvt. Ltd, Mumbai. Chitosan was a kind gift sample from Zydus cadila, Ahmedabad. Stearic acids, Oleic acid, Poloxamer 407 were purchased from Fizmerk India chemicals, Hapur (U.P).

Methods

Selection of Ingredients of NLC Formulation

The main ingredients required for formulation NLCs are drug, solid lipid (fat), liquid lipid (oil), stabilizer and an aqueous phase vehicle. Prior to the development of NLC formulation, lipid screening should be done to select the most suitable lipids for the NLC. Lipids like Stearic acid, Glyceryl monostearate, Cholesterol were screened as solid lipids while Oleic acid, Castor oil and Olive oil were screened as liquid lipids.

Screening of solid lipid for NLC [8,9]:

Docetaxel in excess was dispersed in 1g of melted solid lipid. Solid lipids were melted at a temperature of 10°C above its melting point. To the above melted drug-lipid matrix, added 10 ml of hot distilled water and shake for 24 hours. Above mixture was centrifuged for 15 minute at 3000 rpm and aqueous phase was separated. 0.5 ml of aqueous phase was diluted to 10 ml with methanol and analyzed by UV spectrophotometric method at 230 nm.

Screening of liquid lipid for NLC[8,9]:

Excess of drug was added individually to liquid lipid (1ml) in screw capped tubes and shaken for 24 hours. After 24 hours each sample was centrifuged for 15 minute at 3000 rpm and 0.5 ml of the clear supernatant layer was diluted with methanol to 10 ml and analyzed by UV spectrophotometric method at 230 nm.

Selection of surfactant and co-surfactant [10]:

Surfactant and cosurfactant are required to stabilize the NLC. The same are selected based on required HLB value for the selected lipid. For example, stearic acid has a required HLB value of 15 and hence requires a surfactant of HLB value equal or more than 15 (preferably nearer to 15) for formation of stable biphasic system.

Preparation of NLC [11, 12, 13]

NLC were prepared using hot homogenization followed by ultrasonication method. As per the formula given in Table No. 1.

Preparation of oil phase:

The lipid phase was prepared by melting solid lipid at suitable temperature (10 °C above its melting temperature). Into this the warmed liquid lipid was added and mixed. After both lipids has been uniformly mixed, drug is slowly added into this and mixed to uniformly disperse.

Preparation of aqueous phase:

Poloxamer 188 was dissolved in required quantity of water. Into this mixture added soya bean lecithin and heat to 75°C.

Mixing process:

Oil phase was poured into aqueous phase and mixed with a stirrer for 30 minute. This was followed by homogenization at 75°C at 6000 rpm for 20 minute. The oil in water primary emulsion obtained was subjected for ultrasonication for 10 minute. Finally emulsion is cooled to to form docetaxel loaded NLC dispersion. Mannitol was used in the freeze-drying process as a cryoprotectant at a concentration of 3 % (w/w). The NLC dispersions were frozen in an aqueous mannitol solution at -20 °C overnight. This sample was transferred to freeze dryer for lyophilization at -70 °C. After 72 hours, the lyophilized DOCETAXEL-NLC powder was subjected for physicochemical characterization and *in vitro* drug release studies.

Optimization of lipids:

Considering the cost of Docetaxel, optimization processes wherever possible are conducted as blank formulations without incorporating the drug.

Blank formulations with lipid concentration in the range of 5, 10 and 15% were tried in preparing NLC. The optimized lipid concentration was

selected based on sedimentation volume and stability.

Optimization of proportion of solid lipid and liquid lipid:

Stearic acid (solid lipid) and Oleic acid (liquid lipid) was taken in various proportions of 70:30, 80:20 and 90:10 in the prepared NLC. The optimum proportion was selected based on sedimentation volume and stability.

Optimization of surfactant and co-surfactant:

Poloxamer 407 (surfactant) and Tween 80 (co-surfactant) were tried out in various proportions based on required HLB value to provide a stable dispersion. Initially the concentration of surfactant and co-surfactant were fixed at 2%. Further trials on the optimal formulation with inclusion of 4% and 6% of surfactant: cosurfactant mixture were tried out after the lipid concentration, cosurfactant and proportion of liquid to solid lipid has been optimized. The optimal concentration was selected based on sedimentation volume and stability.

Table No: 1. Trials with stearic acid and Oleic acid as Lipids

Formulation Code	Ingredients (%)*						
	Lipid	Stearic acid	Oleic acid	Surfactant	Soya bean Lecithin	Poloxamer 188	Water (q.s)
BSO-1	10	7.00	3.00	2.00	0.99	1.01	100 ml
BSO-2		8.00	2.00		1.01	0.99	100 ml
BSO-3		9.00	1.00		1.04	0.96	100 ml
BSO-4	20	14.00	6.00	2.00	0.99	1.01	100 ml
BSO-5		16.00	4.00		1.01	0.99	100 ml
BSO-6		18.00	2.00		1.04	0.96	100 ml
BSO-7	30	21.00	9.00	2.00	0.99	1.01	100 ml
BSO-8		24.00	6.00		1.01	0.99	100 ml
BSO-9		27.00	3.00		1.04	0.96	100 ml
BSO-10	40	28.00	12.00	2.00	0.99	1.01	100 ml
BSO-11		32.00	8.00		1.01	0.99	100 ml
BSO-12		36.00	4.00		1.04	0.96	100 ml

Table No: 2. Trials with stearic acid and Isopropyl myristate (IPM) as Lipids

Formulation Code	Ingredients (%)*						
	Lipid	Stearic acid	IPM	Surfactant	Soya bean Lecithin	Poloxamer 188	Water (q.s)
BSI-1		7.00	3.00		1.18	0.82	100 ml
BSI-2		8.00	2.00		1.14	0.86	100 ml
BSI-3	10	9.00	1.00	2.00	1.1	0.9	100 ml
BSI-4		14.00	6.00		1.18	0.82	100 ml
BSI-5		16.00	4.00		1.14	0.86	100 ml
BSI-6	20	18.00	2.00	2.00	1.1	0.9	100 ml
BSI-7		21.00	9.00		1.18	0.82	100 ml
BSI-8		24.00	6.00		1.14	0.86	100 ml
BSI-9	30	27.00	3.00	2.00	1.1	0.9	100 ml
BSI-10		28.00	12.00		1.18	0.82	100 ml
BSI-11		32.00	8.00		1.14	0.86	100 ml
BS-I2	40	36.00	4.00	2.00	1.1	0.9	00 ml

Table No: 3. Trials with Glyceryl Behenate and oleic acid as Lipids

Formulation Code	Ingredients (%)*						
	Lipid	Glyceryl Behenate	Oleic acid	Surfactant	Soya bean Lecithin	Poloxamer 188	Water (q.s)
BGO-1		7.00	3.00		1.84	0.16	100 ml
BGO-2		8.00	2.00		1.83	0.17	100 ml
BGO-3	10	9.00	1.00	2.00	1.71	0.29	100 ml
BGO-4		14.00	6.00		1.84	0.16	100 ml
BGO-5		16.00	4.00		1.83	0.17	100 ml
BGO-6	20	18.00	2.00	2.00	1.71	0.29	100 ml
BGO-7		21.00	9.00		1.84	0.16	100 ml
BGO-8		24.00	6.00		1.83	0.17	100 ml
BGO-9	30	27.00	3.00	2.00	1.71	0.29	100 ml
BGO-10		28.00	12.00		1.84	0.16	100 ml
BGO-11		32.00	8.00		1.83	0.17	100 ml
BGO-12	40	36.00	4.00	2.00	1.71	0.29	100 ml

Table No: 4. Trials with Glyceryl behenate and Isopropyl myristate (IPM) as Lipids

Formulation Code	Ingredients (%)*						
	Lipid	Glyceryl Behenate	IPM	Surfactant	Soya bean Lecithin	Poloxamer 188	Water (q.s)
BGI-1	10	7.00	3.00	2.00	1.80	0.20	100 ml
BGI-2		8.00	2.00		1.73	0.27	100 ml
BGI-3		9.00	1.00		1.68	0.32	100 ml
BGI-4	20	14.00	6.00	2.00	1.80	0.20	100 ml
BGI-5		16.00	4.00		1.73	0.27	100 ml
BGI-6		18.00	2.00		1.68	0.32	100 ml
BGI-7	30	21.00	9.00	2.00	1.80	0.20	100 ml
BGI-8		24.00	6.00		1.73	0.27	100 ml
BGI-9		27.00	3.00		1.68	0.32	100 ml
BGI-10	40	28.00	12.00	2.00	1.80	0.20	100 ml
BGI-11		32.00	8.00		1.73	0.27	100 ml
BGI-I2		36.00	4.00		1.68	0.32	100 ml

Once the concentration of lipids, surfactant and co surfactants are finalized based on evaluation of sedimentation volume and physical stability, formulations containing the drugs are prepared with the optimized formula. Docetaxel loaded NLC formulation is further optimized for the following.

Optimization of speed of homogenizer

The optimized formulation from the above studies will be subjected to further studies for optimization of speed of homogenization. The effect of homogenization on particles size (PS), polydispersity index (PI), encapsulation efficiency (EE) and zeta potential (ZP) were evaluated at 4000 rpm, 5000 rpm and 6000 rpm. Based on results speed of homogenizer will be fixed.

Optimization of duration of homogenization

The selected formulation with the optimized homogenization speed will be evaluated for the duration for homogenization. For this the prepared formulation will be homogenized at the constant homogenization speed for 10, 15, 20, 25 and 30 min and evaluated for particles size (PS), polydispersity index (PI), encapsulation efficiency (EE) and zeta potential (ZP).

Optimization of duration of sonication

The homogenized mixture was ultra-sonicated for different durations (5, 10 and 15 min). The duration of sonication was finalized on the basis of its effects on particle size (PS), polydispersity index (PI), encapsulation efficiency (EE) and zeta potential (ZP) of NLC.

Optimization of cryoprotectants[14]

The stress generated during freezing and dehydration process can destabilize the NLC system. To stabilize the NLC during lyophilization, cryoprotectants are to be added. In the optimization trials, 1, 2, 3 and 4% of mannitol were included in the formulations and evaluated for of its effects on particle size (PS), polydispersity index (PI), encapsulation efficiency (EE) and zeta potential (ZP) of NLC.

Evaluation of the Developed Nanostructured Lipid Carriers [15]

Particle size:

The prepared nanostructured lipid carriers were evaluated for particle size, particle size distribution and poly dispersity index. The average particle size and polydispersity index measured using a particle size analyzer, Zetasizer (Malvern, UK) based on the principle of dynamic light scattering. In this method, NLC suspension (0.5mL) was diluted 10 folds by redispersing in 5mL of filtered distilled water and the diluted suspension was analyzed for particle size, particle size distribution and polydispersity index.

Zeta potential:

The zeta potential of NLC was measured by using the Zetasizer Nano ZS (Malvern, UK) based on electrophoretic light scattering. The NLC dispersion was diluted with distilled water (1:100) and mixed to get a uniform dispersion. The zeta

potential of this diluted sample NLC was measured at 25ºC.

Transmission Electron Microscopy (TEM)

The TEM equipped with combination of bright field imaging, digital imaging and a photography system of 35 mm were used to reveal the morphology and size of the NLCs. To perform the TEM observations, the lyophilized NLCs samples were suspended directly into the distilled water and examined by TEM.

Drug entrapment efficiency

2 ml of drug loaded NLC sample was centrifuged at a speed of 8000 rpm for 45 minutes to separate the lipid and the aqueous phase. The supernatant was then taken, filtered through 40 µm filter paper, diluted with methanol, and the drug content was determined by UV-visible spectrophotometer at 230 nm. The entrapment efficiency of NLC was calculated by:

$$\% EE = [(Wa-Ws)/Wa]\times 100.$$

Where, EE = Entrapment efficiency.

Wa = Amount of Docetaxel added to the formulation.

Ws = Amount of free drug in supernatant.

***In vitro* drug release studies**

The dialysis membrane used in the study was Visking tube semipermeable membrane Dialysis Tubing 14 mm dia x 1 meter, with capacity 200 ml/meter volume was pretreated with glycerol before the test.

In vitro release studies were performed using the dialysis bag method

A volume of NLC containing equivalent amount (10mg) of Docetaxel was taken for the study. NLC was diluted with 10ml of phosphate buffer solution. Dialysis membrane is cut into required length and tied at one end with thread. Docetaxel NLC was added into the membrane and other end was fixed by thread and placed into the release media. The release media taken was 250 ml of phosphate buffer solution (pH 6.0). It was stirred using magnetic stirrer and maintained at 37^0 C using a thermostat. Set stirrer at a speed that will allow dialysis bag to slowly float at the top of the solution. Sample of volume of 1 ml was withdrawn from the beaker at fixed time intervals of 1, 2, 3, 4, 5, 6, 7 and 8hrs and analyzed using UV spectrometry at 230 nm. To maintain sink condition, withdrawn sample was replaced with buffer.

RESULT AND DISCUSSION:

Optimization of proportion of solid lipid and liquid lipid:

Based on solubility studies the ideal solid lipid and liquid lipid were selected. Glyceryl behenate and stearic acid were selected as the solid lipid and isopropyl myristate and Oleic acid as the liquid lipid for further trials. Since the concentration of lipids were found to affect the formulation characteristics like particle size, polydispersity, zeta potential and drug entrapment, trials with various concentrations of 10%, 20%, 30% and 40% were attempted. It has been reported in literature that apart from total concentration of lipids, the ratio of solid: liquid lipid also affects the characteristics of NLCs. Therefore to select the optimum solid lipid: liquid lipid ratio, trials with 70:30, 80: 20 and 90: 10 ratios at each concentration level of lipids were also tried out.

Effect of lipid concentration on physical stability of NLC

It was observed during the study that NLCs with 20% lipid composition of stearic acid and oleic acid in proportions of 70:30, 80:20 and 90:10 exhibited high stability without phase separation. For all other lipid combinations, stability was found to be only at 80:20 proportion of solid and liquid lipid. The physical stability of the blank formulations are reported in Table no: 5, 6, 7 and 8. Based on results, it was decided to incorporate docetaxel into these formulations and further evaluate for particle size, polydispersity and entrapment efficiency.

Effect of lipid concentration on particle size and entrapment efficiency

As learnt from the literature review, higher the lipid concentration, higher should be the solubilization capacity of a lipophilic drug inside NLC. Thus the lipophilic Docetaxel is expected to dissolve easily in the NLC at higher lipid concentration. This solubilization should result in a higher drug entrapment in the NLCs. This is evident from the results as entrapment efficiency was found to increase with an increase in lipid concentration. In formulation F1, where combination of stearic acid and oleic acid were used as the lipids at 10%, it was observed that the %EE was found to be low at 32%. But as lipid concentration increased to 20% (F2, F3 and F4), entrapment efficiency raised to the range of 50%, 54% and 52% respectively. %EE was found to be at 72% with 30% lipid concentration (F5) and with 40% concentration (F6) a very high %EE of 84% was observed.

But the concentration of lipids has a negative effect on particle size of NLC. It was observed that as lipid concentration increased particle size decreased. The NLCs formulated with 10% lipid concentration (F1) showed a particle size of 276µm. When lipid concentration was increased to 20%, 30% and 40% in F3, F5 and F6, the particle size increased to 298µm, 465µm and 660µm respectively. Among the all batches, it was decided to select a formulation that possessed optimal characteristics of particle size and %EE with scope of further improvement. Hence F3, with 20% lipid concentration and solid and liquid lipid in 80:20 ratio with particle size 298µm and %EE 54% was selected for further optimization trials.

A similar pattern of results was observed across other formulations formulated with lipid combinations of stearic acid: Isopropyl myristate, Glyceryl behenate: Isopropyl myristate and Glyceryl behenate: Oleic acid with particle size and %EE increasing with increase in lipid concentration.

The results are tabulated in table No: 9.

Optimization of surfactant and co-surfactant:

To stabilize NLCs, which contains a mixture of liquid lipid and solid lipid a combination of surfactants is needed to achieve the required HLB value. Generally a combination of lipophilic surfactant and hydrophilic surfactant in an optimal ratio should result in a stable NLC. Soya bean lecithin (lipophilic in nature with HLB value of 7) and poloxamer 188 (hydrophilic in nature with HLB value of 24) was selected as surfactant and cosurfactant respectively. The concentration of surfactant and co-surfactant was initially fixed at 2% and further studied at 4%, 5% and 6%. At each concentration, the ratio of surfactant and cosurfactant that should be used was calculated by allegation method.

Effect of Surfactant: Cosurfactant mixture on particle size and entrapment efficiency

The initial batches were prepared with a fixed surfactant concentration of 2%. At this surfactant concentration, F3 formulation with 20% lipid concentration and solid and liquid lipid in 80:20 ratio was found to possess optimal characteristics with particle size of 298µm and %EE of 54%. Hence it was decided to improve these characteristics with aid of surfactants. For the same further trials were taken with 4%, 6% and 8% of surfactant and co-surfactant.

It was observed that as the concentration of surfactant mixture increased from 2% to 4%, particle size decreased from 298µm to 172µm and %EE increased from 54% to 76% in F25. But further increase of surfactant concentration showed a negative effect on particle size and entrapment efficiency. At 6% concentration (F26), the particle size and %EE was found to be 212µm and 65% whereas at 8% concentration (F27), the particle size further increased to 260µm and %EE reduced to 60%.

The reason for the positive response on particle size should be that, at optimal concentration, surfactant prevents interfacial tension and aggregation of particles resulting in decreased particle size. But as surfactant concentration increased above optimum concentration of 4%, it is expected that surfactant molecules arranged on the surface of the particles to form loops with surfactant head attached to one particle and tail on to another particle. This bridge formation of surfactant between particles may lead to formation of aggregates and resulted in an increase in particle size. Also at higher surfactant concentration it is expected that micellar solubilization must have occurred. This resulted in increased solubilization of docetaxel into the aqueous vehicle phase rather than into lipophilic core of NLCs. This is evident from the low entrapment efficiency of 65% and 60% at surfactant concentrations of 6% and 8%. The low polydispersity of 0.21 indicated that particles are almost monodispersed at 4% surfactant concentration. Zeta potential of 42 mV for formulations with 4% surfactant concentration indicated that there is absence of aggregation and the formulation can be considered to be stable. Hence formulation F25 was selected to be the optimized NLC formulation.

The results of particle size, polydispersity, %EE and zeta potential and the results are reported in table no: 10.

Optimization of homogenization process parameters

Homogenization process parameters like homogenization time and speed can induce large mechanical force of shear and are reported to affect particle size, PI, % EE and Zeta potential. Hence various trials to optimize these parameters are taken up.

Optimization of homogenization speed

It was observed that, as homogenization speed increased from 5000 to 6000 rpm, particle size decreased and %EE increased. PI was also lower at 6000 rpm indicating existence of monodispersed particles. But when speed was further increased to 7000 rpm no significant improvement in particle size or %EE was observed. On the contrary, PI increased above 0.52 indicating polydispersity. Thus it was concluded that 6000 RPM is the optimum homogenization speed. Results are tabulated in Table No: 11.

Optimization of homogenization time

As the homogenization time increased from 10 min to 20 min, particle decreased and %EE increased with homogenization speed of 5000 and 6000rpm. But increase of homogenization time to 30 min did not significantly improve the particle size and %EE.

The results of optimization trials are tabulated in Table No:11. From the results it can be concluded that NLCs prepared at a homogenization speed of 6000 for 20 min, exhibited a particle size of 222µm, PI of 0.28, %EE of 72% and zeta potential of 42Mv. Hence the same parameters were considered to be as optimal.

Optimization of sonication time

The process of sonication can also provide a mechanical shear on NLCs and could result in particle size reduction. Hence trials were planned with different sonication time of 5, 10 and 15 min. As the sonication time increased from 5min to 10min, particle size reduced from 222 µm to 170µm. The PI also improved from 0.28 to 0.19 indicating good monodispersity. %EE was not been

significantly affected by sonication time and remained high at 79%. Further increase of sonication time to 15min could not provide any positive effects on particle size, polydispersity and %EE. Hence it was concluded that 10 min should be the optimum sonication time. The results of optimization trials are tabulated in Table No: 12
Based on the above trials, homogenization at 6000rpm for 20min followed by sonication for 10 min was concluded to be the ideal processing parameters for preparation of NLCs.

Screening of cryoprotectants

The stress generated during freezing and dehydration process can destabilize the NLC system. To stabilize the NLCs during lyophilization, cryoprotectants are added. Mostly sugars like mannitol are preferred. Mannitol is reported to decrease the osmotic activity of water during crystallization process and also said to prevent particle aggregation which generally occurs during lyophilization. Mannitol provided a smooth and bright texture to lyophilized NLCs. The ease of reconstitution was also better with the presence of mannitol as cryoprotectant. In the trials for optimization of cryoprotectant 1%, 2%, 3% and 4% of mannitol were included in the formulations. Among these batches, formulation F29, with 3% mannitol concentration exhibited better characteristics with particle size of 180µm, PI of 0.25, %EE of 78% and zeta potential of 41Mv. Hence it has been decided that 3% of mannitol is the optimal concentration as cyroprotectant for the formulation.

Table No: 5. Trial formulations with stearic acid and oleic acid as lipids

Blank Formulation	Sedimentation	Sedimentation Volume	After centrifugation
BSO-1	Unstable	0.80	Creaming
BSO-2	Stable	0.95	Stable
BSO-3	Unstable	0.85	Creaming
BSO-4	Stable	1.0	Stable
BSO-5	Stable	1.0	Stable
BSO-6	Stable	1.0	Stable
BSO-7	Stable	0.95	Creaming
BSO-8	Stable	0.95	Stable
BSO-9	Stable	0.95	Creaming
BSO-10	Unstable	0.85	Creaming
BSO-11	Stable	0.95	Stable
BSO-12	Unstable	0.75	Creaming

Table No: 6. Trial formulations with stearic acid and isopropyl myristate as lipids

Blank Formulation	Sedimentation	Sedimentation Volume	After centrifugation
BSI-1	Unstable	0.80	Creaming
BSI-2	Stable	0.95	Stable
BSI-3	Unstable	0.85	Creaming
BSI-4	Stable	1.0	Stable
BSI-5	Stable	1.0	Stable
BSI-6	Stable	1.0	Stable
BSI-7	Stable	0.95	Creaming
BSI-8	Stable	0.95	Stable
BSI-9	Stable	0.95	Creaming
BSI-10	Unstable	0.85	Creaming
BSI-11	Stable	0.95	Stable
BS-I2	Unstable	0.75	Creaming

Table No: 7. Trial formulations with glyceryl behenate and oleic acid as lipids

Blank Formulation	Sedimentation	Sedimentation Volume	After centrifugation
BGO-1	Unstable	0.80	Creaming
BGO-2	Stable	0.95	Stable
BGO-3	Unstable	0.85	Creaming
BGO-4	Stable	1.0	Stable
BGO-5	Stable	1.0	Stable
BGO-6	Stable	1.0	Stable
BGO-7	Stable	0.95	Creaming
BGO-8	Stable	0.95	Stable
BGO-9	Stable	0.95	Creaming
BGO-10	Unstable	0.85	Creaming
BGO-11	Stable	0.95	Stable
BGO-12	Unstable	0.75	Creaming

Table No: 8. Trial formulations with glyceryl behenate and isopropyl myristate as lipids

Blank Formulation	Formulation	Sedimentation Volume	After centrifugation
BGI-1	Unstable	0.80	Creaming
BGI-2	Stable	0.95	Stable
BGI-3	Unstable	0.85	Creaming
BGI-4	Stable	1.0	Stable
BGI-5	Stable	1.0	Stable
BGI-6	Stable	1.0	Stable
BGI-7	Stable	0.95	Creaming
BGI-8	Stable	0.95	Stable
BGI-9	Stable	0.95	Creaming
BGI-10	Unstable	0.85	Creaming
BGI-11	Stable	0.95	Stable
BGI-12	Unstable	0.75	Creaming

Table No: 9. Characterization of NLC

Blank Formulation	Formulation	PS (µm)	PI	%EE
BSO-2	**F1**	276	0.151	32.0
BSO-4	**F2**	316	0.370	50.0
BSO-5	**F3**	298	0.330	54.0
BSO-6	**F4**	325	0.360	52.0
BSO-8	**F5**	465	0.452	72.0
BSO-11	**F6**	660	0.535	84.0
BSI-2	**F7**	432	0.166	28.0
BSI-4	**F8**	410	0.380	43.0
BSI-5	**F9**	450	0.355	45.0
BSI-6	**F10**	480	0.378	47.0
BSI-8	**F11**	560	0.433	68.0
BSI-11	**F12**	690	0.598	75.0
BGO-2	**F13**	429	0.171	26.0
BGO-4	**F14**	435	0.378	40.0
BGO-5	**F15**	468	0.366	46.0
BGO-6	**F16**	489	0.379	45.0
BGO-8	**F17**	510	0.177	66.0
BGO-11	**F18**	575	0.389	70.0
BGI-2	**F19**	456	0.188	30.0
BGI-4	**F20**	478	0.390	38.0
BGI-5	**F21**	480	0.379	41.0
BGI-6	**F22**	498	0.399	45.0
BGI-8	**F23**	648	0.588	59.0
BGI-11	**F24**	708	0.597	68.0

Table No: 10. Effect of surfactant and cosurfactant concentration

Formulation code	Surfactant: Cosurfactant	Particle size (µm)	Polydispersity	%EE	Zeta potential
F3	2	298	0.33	54.0	26
F25	4	172	0.21	76.0	42
F26	6	212	0.28	65.0	34
F27	8	260	0.32	60.0	19

Table No: 11. Optimization of homogenization speed and RPM

Homogenization speed (RPM)	Homogenization duration (min)	Particle size (µm)	PI	%EE	ZP
5000	10	172	0.21	76.0	42
	20	154	0.14	78.0	40
	30	158	0.15	65.0	39
6000	10	165	0.16	77.0	42.0
	20	151	0.18	79.0	41.0
	30	166	0.10	72.0	40.0
7000	10	164	0.32	64.0	36.0
	20	160	0.38	62.0	34.0
	30	184	0.25	60.0	32.5

Table No: 12. Optimization of Sonication time

Sonication Time (min)	Particle size (µm)	PI	%EE	ZP
0	151	0.18	79.0	41.0
5	143	0.28	78.0	40.0
10	127	0.19	80.0	42
15	182	0.24	75.0	39

Table No: 13. Optimization of cryoprotectant

Before Lyophilization					
Formulation	Mannitol Concentration	Particle size (µm)	PI	%EE	ZP
F25	0	127	0.19	80.0	42
	After Lyophilization				
	Mannitol Concentration	Particle size (µm)	PI	%EE	
F27	1	422	0.81	68.0	
F28	2	280	0.61	76.0	
F29	3	180	0.25	78.0	41
F30	4	205	0.36	76.0	

Based on the various optimization trials done, it was concluded that the following are the optimal concentrations of ingredients and process parameters adopted during formulation. The same are summarized in table no. 14 and 15.

Table No. 14. Optimized composition of Docetaxel NLC

Sl.No:	Composition	Optimized Concentration
1	Docetaxel	2%
2	Lipid	20%
3	Solid Lipid : Liquid Lipid	80:20
4	Stearic acid	2.02%
5	Oleic acid	1.98%
6	Mannitol	3%

Table No. 15. Optimized process parameters for preparation of Docetaxel NLC

Sl.No:	Process parameters	Optimized condition
1	Homogenization RPM	6000
2	Homogenization time	20 min
3	Sonication time	10 min

***In Vitro* Drug Release**

The optimized NLC formulation F29 was further formulated as a nasal gel with Poloxamer 407 as the thermoresponsive gelling agent and chitosan as the mucoadhesive polymer. The invitro drug release studies were done. The results of in vitro diffusion studies across nasal mucosal tissue indicated that drug release occurred in a sustained manner. The concentration of Poloxamer 407 remained constant at 16%w/w in all the formulations while mucoadhesive polymer concentration was varied at 0.5%, 1%, 1.5% and 2% to evaluate its effect on drug release from in-situ gels. The drug release from a control formulation containing plain nasal gel without mucoadhesive polymer (NG0) was also studied. The drug release from in-situ gels was found to be dependent on concentration of mucoadhesive polymer incorporated. In vitro diffusion study of NG0 without mucoahesive polymer exhibited a incomplete drug release of 69.8±0.04 in 8 hours. In vitro diffusion study of NG7 with 0.5%w/v of Chitosan resulted in drug release which extended up to 8 hours (89.50±0.06%). Since the objective was to provide a rapid and prompt drug release, the Chitosan concentration was increased to 1%w/v in NG8. It was observed that, as Chitosan concentration increased, a comparatively better drug release could be achieved within 6 hours (97.90±0.03%). In NG9 formulation, Chitosan concentration was further increased to 1.5%w/v, which resulted in a complete drug diffusion within 4 hours. The drug release was also found to be almost complete with 98.80±0.08% in 4 hours. Based on the above study, it was concluded that 1.5%w/v of Chitosan is the optimum concentration as a permeation enhancer and mucoadhesive polymer. The drug release values of formulations NG7, NG8 and NG9 are shown in Table No: 16 and fig No. 1

Table No: 16. Cumulative percentage drug release

TIME (hrs)		% DRUG RELEASE		
	NG0	NG7	NG8	NG9
0	00.00	00.00	00.00	00.00
1	18.34±0.05	22.48±0.12	26.22±0.05	26.24±0.05
2	22.45±0.10	34.16±0.24	48.50±0.04	54.20±0.03
3	32.50±0.05	55.35±0.15	58.40±0.09	77.30±0.05
4	38.25±0.06	61.50±0.18	67.21±0.23	98.80±0.08
5	48.30±0.03	72.25±0.06	80.58±0.18	98.10±0.06
6	54.40±0.05	80.65±0.08	97.90±0.03	97.80±0.05
7	60.25±0.07	84.99±0.20	97.80±0.21	97.30±0.02
8	69.80±0.04	89.50±0.06	97.22±0.22	97.00±0.04

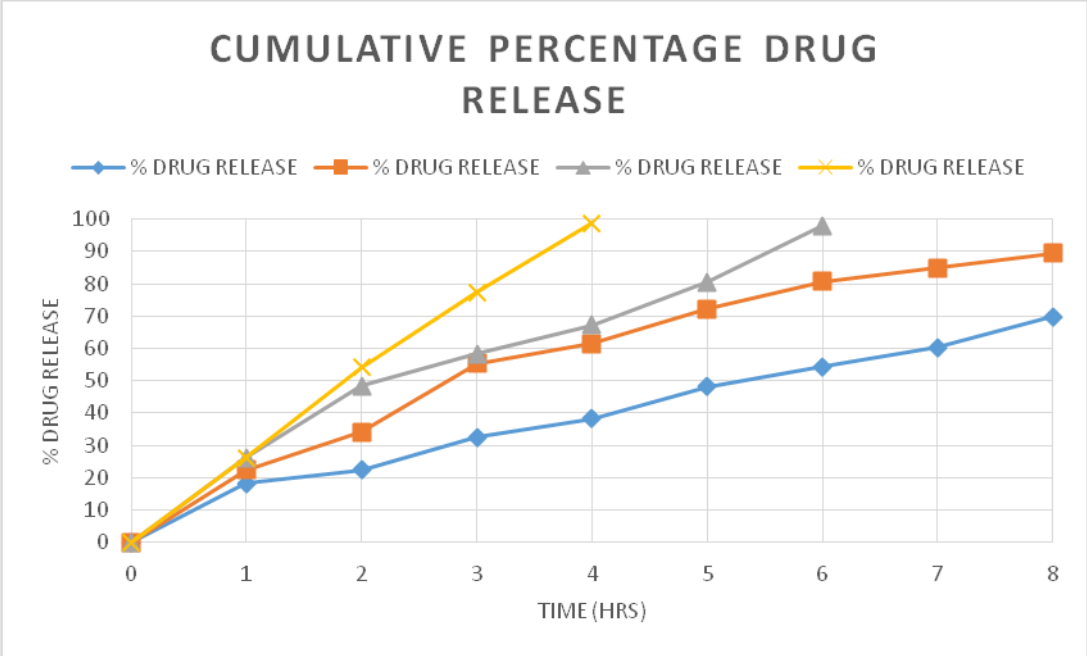

Fig No. 1. Cumulative percentage drug release

Release Kinetics

The release data of the formulation NG9 was fitted to various models like Zero order, First order, Higuchi model, Hixson crowell and Peppas model to determine the release mechanism. The results of the same are reported in Table no. 17. Plots of zero order, first order, Higuchi, Hixon Crowell and Peppas model are depicted in Figure No: 2, 3 and 4. The coefficient of determination (R^2) values of zero order, first order, Higuchi model and Peppas model are tabulated in Table No: 18. From the data, it is evident that drug release shows best fit in zero order pharmacokinetic model (R^2=0.9972). This indicates that the rate of drug release is independent of drug concentration. The drug diffusion data of NG9 formulation has been found to best fit with Higuchi model having R^2 value of 0.9347, which confirm that the drug release occurred by diffusion. The release exponent (n) values in Peppas model for NG9 is 0.42. It indicates that the drug transport mechanism is Fickian diffusion. This indicated that drug release by diffusion is driven by concentration gradient, diffusion distance, degree of polymer swelling etc. Based on this we can assume that the developed drug delivery system is a matrix based device.

Table No: 17. Kinetic study *of in-vitro* release from NG9

Time (hrs)	Log Time	Square Root of Time	% Cumulative Drug Release	Log % Cumulative Drug Release	% Drug remaining	Log % Drug remaining	Log M_t / M_o	$W^{1/3}$ - $W^{1/3}$
0	0	0	00.00	00.00	100.00	2.00	0	0
1	0.000	1.000	26.24±0.05	1.422	73.76	1.868	-0.57	0.10
2	0.301	1.414	54.20±0.03	1.709	45.80	1.661	-0.26	0.160
3	0.477	1.732	77.30±0.05	1.888	22.70	1.356	-0.11	0.236
4	0.602	2.000	98.80±0.08	1.995	1.20	0.079	-0.01	0.469
5	0.699	2.236	98.10±0.06	1.992	1.80	0.255	-0.01	0.757
6	0.778	2.449	97.80±0.05	1.990	2.20	0.342	-0.01	1.117
7	0.845	2.646	97.30±0.02	1.988	2.70	0.431	-0.01	1.559
8	0.903	2.828	97.00±0.04	1.987	3.00	0.477	-0.01	2.13

Table No: 18. Determination of correlation in linear regression

Formulation Code	Zero order	First order	Higuchi model	Hixson crowell	Peppas model	
	R^2	R^2	R^2	R^2	R^2	n
NG9	0.9972	0.7919	0.9347	0.9096	0.5829	0.42

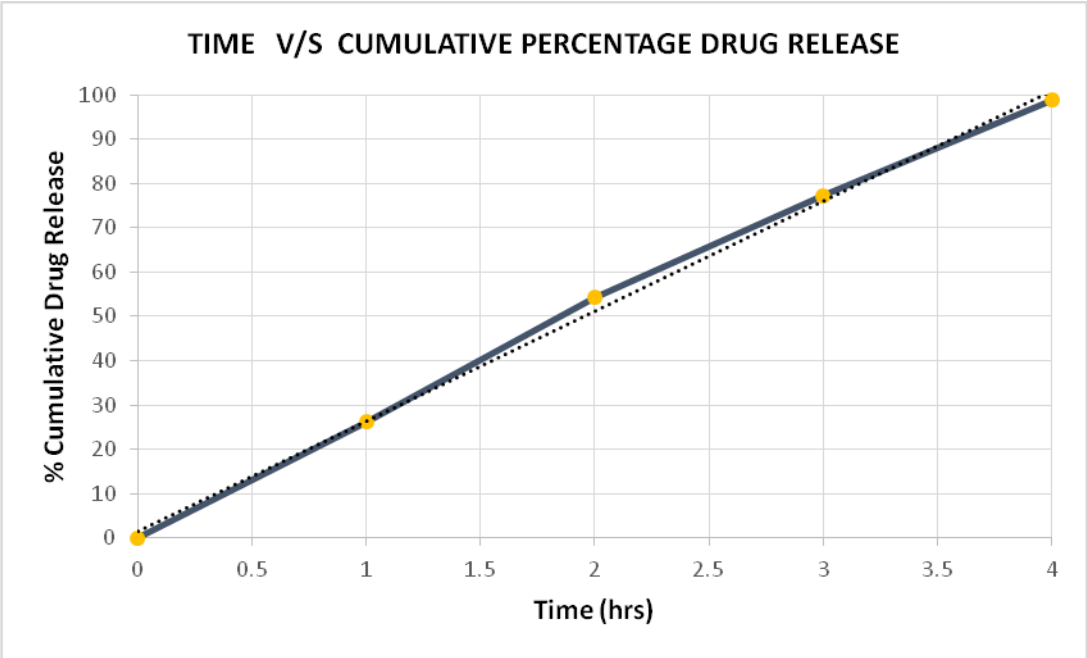

Fig No. 2. Zero-order release model of NG9

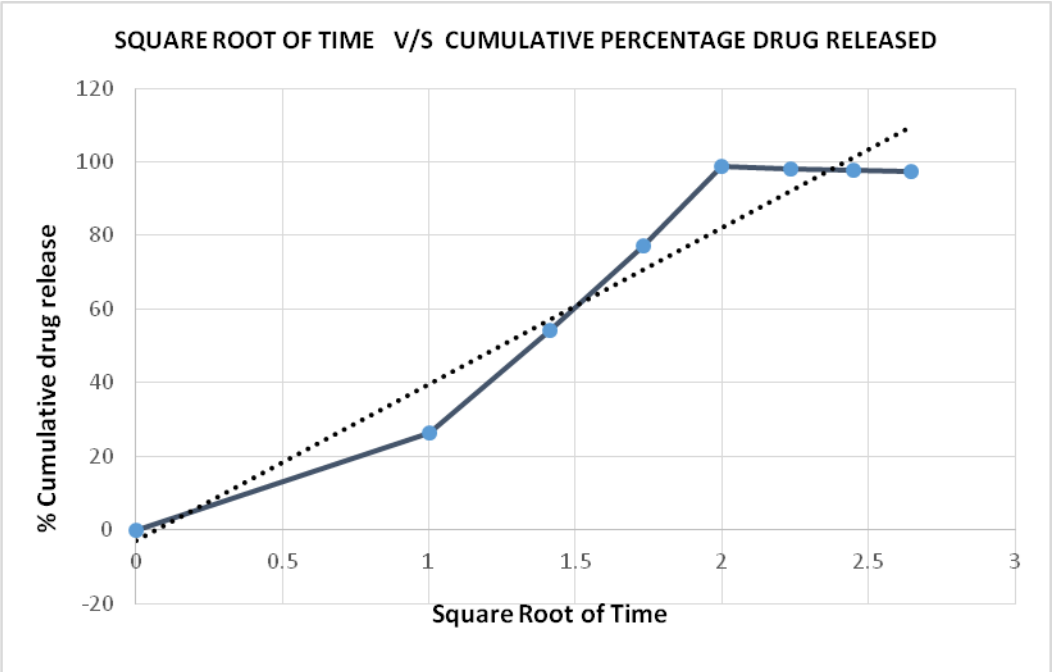

Fig No. 3. Higuchi release model of NG9

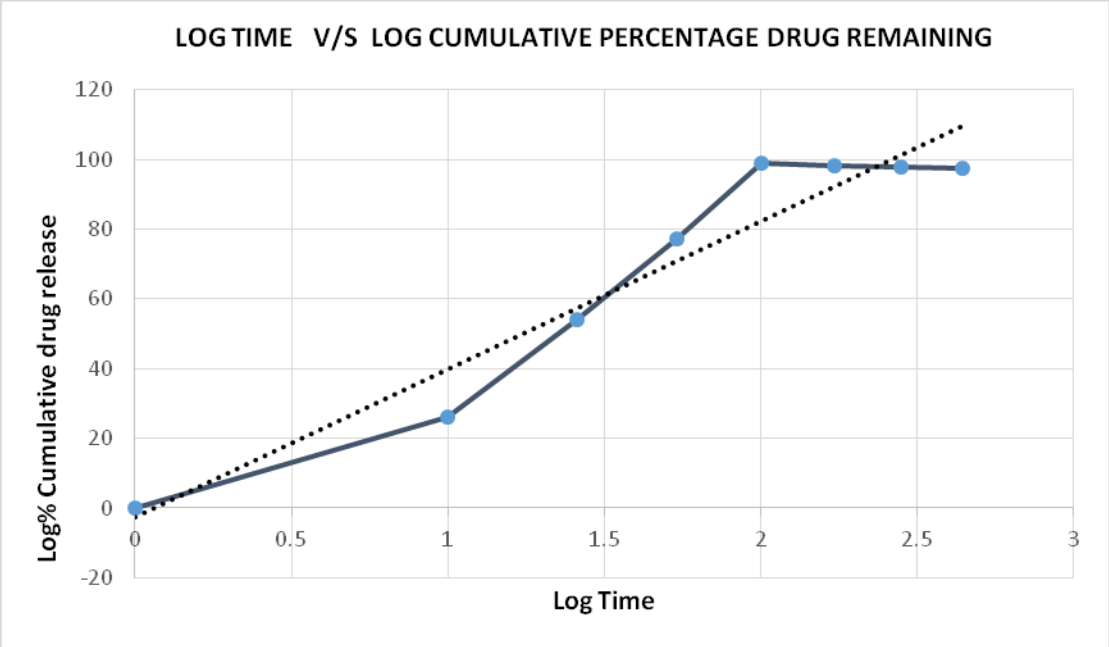

Figure No: 4. Peppas model plot for F9

CONCLUSION:

The study proved that NLC formulations can result in preparation of nanoparticulate delivery systems of Docetaxel for better clinical efficacy treatment of cancer. But further *in vivo* animal studies and human clinical studies will be required to establish the safety and efficacy of the formulation for use in humans.

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