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Ursodiol Loaded Polymeric Nanoparticle: Formulation, Optimization And Invitro – Invivo Pharmacodynamic Evaluation

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Abstract:

The present research is to formulate and evaluate the in-vivo pharmacokinetic studies on Ursodiol polymeric nanoparticle. The polymeric nanoparticles were optimized by 2^3 factorial design and the best formulation will be selected based on the effect of independent variable on dependent variable. The optimized polymeric nanoparticle will be subjected to in-vivo pharmacokinetic and pharmacodynamics studies. The Ursodiol polymeric nanoparticle was formulated by homogenization cum ultra-sonication method by investigating the effect of variables like polymer concentration (HPMC), homogenization time (min) and ultra sonication time (min) using a factorial design. The formulated Ursodiol polymeric nanoparticle was evaluated for particle size (nm), zeta potential (mV), polydispersity index, entrapment efficiency (%), drug content, in-vitro drug release, in-vitro release kinetic studies and stability studies as per ICH guidelines criteria. The optimized polymeric nanoparticle will be subjected to in-vivo pharmacokinetic and Pharmacodynamic studies. From the derived data U4 formulation showed predicted particle size (168.0 ± 3.24 nm), maximum zeta potential (-34.1 ± 2.34mV), less polydispersity index (0.320 ± 0.24), increased entrapment efficiency (98.62 ± 3.68%), and good release properties (92.22 ± 3.14 at 24h) among the eight formulations tested. From the in-vivo pharmacokinetic evidence, it was concluded that the polymeric nanoparticle with Ursodiol showed improved bioavailability than the marketed dosage form Ursocol SR®, by enhancing the plasma drug concentration profile like AUC and C_{max}. The findings suggest that PNs have controlled drug release and can be used as a drug delivery carrier for Ursodiol to improve bioavailability.

Keywords: Ursodiol; Polymeric Nanoparticle; 2^3 factorial design; Homogenization; Ultra sonication; Pharmacokinetic.

Introduction

Polymeric nanoparticles (NPs) are small particles with a diameter of 1 to 1000 nm that can be loaded with active chemicals or surface-adsorbed onto the polymeric nucleus. Polymeric NPs have showed considerable promise in the delivery of pharmaceuticals to specific locations for the treatment of a variety of ailments. Polymer nanotechnology is one of the most promising drug delivery technologies for addressing issues like limited solubility and permeability in drug distribution [1]. Advances in nanotechnology have facilitated the development of novel polymeric nanoparticle compositions that can alter the pharmacological, biopharmaceutical, and pharmacokinetic aspects of medications [2]. Polymeric nanoparticles (PNs) are one-dimensional particulate substances. Polymeric nanoparticles (NPs) are one of the most often used nanomaterial in nanomedicine because they may deliver a drug to a particular region of an organ at a lower dose, resulting in increased drug bioavailability at the targeted site [3]. Polymeric NPs are employed in drug administration for a variety of applications, including medicine conjugation and entanglement, prodrugs, stimuli sensitive systems, imaging modalities, and theranostics [4]. Analysis, imaging, sedative delivery, aesthetic agents, organ embeds, and tissue design are just a few of the therapeutic applications for biodegradable polymeric nanostructures that have showed great promise [5].

Polymeric NP is recognized as one of the most ideal drug delivery techniques to solve drug delivery issues such as low solubility, permeability, and bioavailability of BCS class II and III drugs [6]. The pharmacokinetics and pharmacodynamics of several pharmacological compounds have been altered and improved employing particle systems such as nanoparticles [7]. Nano capsules and nanospheres, which have different morphological structures, are both referred to as "nanoparticles." Polymeric NPs have shown potential in the administration of anticholesteremia medicine [8].

Ursodeoxycholic acid (UDCA), also known as ursodiol, is a secondary bile acid generated by gut bacteria in humans and most other species. In some animals, it is synthesised in the liver, and it was first discovered in bear bile, hence the name Ursus. It has been used to cure or prevent numerous illnesses of the liver and bile ducts in pure form. The bulk of ursodiol is absorbed by passive diffusion after oral administration, and this absorption is incomplete. In the absence of liver illness, ursodiol undergoes hepatic extraction to the level of around 50% once absorbed [9-12]. Ursodeoxycholic acid (UDCA) is classified as class II in the Biopharmaceutical Classification System (BCS), which means it has a low water solubility and a high permeability. This drug's solubility is extremely poor, resulting in a low dissolution rate and, as a result, a low bioavailability after oral administration [13].

Hence, to improve Ursodiol's bioavailability and dissolution profile, Ursodiol was developed into polymeric nanoparticles using the homogenization cum ultra-sonication technique by modifying formulation variables such as polymer concentration (hydroxyl propyl methyl cellulose concentration) and process variables such as homogenization time (min) and ultra-sonication time (min). Further in-vivo pharmacokinetic investigations will be conducted using the best optimized formulation and enhancement of bioavailability will be proved.

Materials and methods

Aurobindo Pvt. Ltd. in India provided Ursodiol. Himedia Labs Ltd in Chennai provided the hydroxyl propyl methyl cellulose. High Speed Homogenizer, Ultra Sonicator, Brukers FT-IR Spectrophotometer, Horiba Nanoparticles Size Analyzer, and Zeiss Scanning Electron Microscopy are some instruments utilised in the creation and evaluation of polymeric nanoparticles. Excipients and solvents of analytical grade are employed in the production and evaluation of polymeric nanoparticles.

Drug and excipients compatibility studies

FTIR studies

FTIR analyses were used to identify the chemical interactions between the drugs (Ursodiol) and other components in the mixture, such as polymer and surfactants. The potassium bromide (KBr) pelletization procedure was used to investigate ursodiol and a physical combination. The drugs (0.2 %) were ground with the KBr, and the mixture was then crushed at a pressure of around 7 tonnes using a compact KBr pellet press by repeatedly spinning the press handle. Prepared KBr pellets are scanned in an FTIR instrument (Bruker, Germany) equipped with the OPUS Spectrum software over a wave number range of 4000 to 500 cm–1 with a resolution of 4 cm–1. Using a force gauge of 100 N, samples were put on the sample stage, maintaining regular contact between the specimen and the sample stage for scanning [13, 14].

Differential scanning calorimetry (DSC) studies

DSC tests were used to determine the melting point of the samples. It aids in the reporting of drug purity, drug-excipient compatibility, and polymeric nanoparticle formulation crystalline quality. Ursodiol and drug-loaded polymeric nanoparticles were studied using the DSC-70, a Schimadzu model equipment. The samples were weighed at 5 mg and roasted at a rate of 20 °C/min in aluminium pans with dry nitrogen as the effluent gas at a temperature of 20-200 °C. An exothermic or endothermic peak was used to determine the melting point [15, 16].

High-speed homogenization followed by ultrasonication method - preparation of polymeric nanoparticles (PNS)

The needed amount of Ursodiol was dispersed uniformly in various concentrations of polymeric solution (varying from 80 to 5%), which was prepared by dissolving various concentrations of surfactant and co-surfactant in deionized water and heating if necessary. In a High Speed Homogenizer, the aqueous phase was homogenised for 10 minutes at 15000 RPM before slowly dispersing the medication into the aqueous phase. Polymeric nanoparticles precipitated in the form of an emulsion as a result. The resultant emulsion was ultrasonicated for 5 minutes at a 2 sec pulse rate using a Probe Ultrasonicator to produce uniformly dispersed stable polymeric nanoparticles. Keep the nanoemulsion at room temperature while continuing the lyophilisation process. To improve the above formulation procedure, 23 statistical factorial designs were applied. Its eight formulation runs were made by modifying the limitations and raising the level by three levels (low, medium and high). Polymer concentration (A in mg), homogenization time (in rpm) for 10 minutes, and ultrasonication duration (C in min) are all fixed product and process factors. This design is used to prepare and analyse 8 PNs formulations for response characteristics such as particle size (Y1), zeta potential (Y2), and polydispersity index (Y3) (Y3). These designs clarify the principal result of the independent variable over the dependent variable. Table 1[17-20] shows the formulation design.

Table 1. Design of Optimization of Ursodiol Polymeric Nanoparticle by 2^3 factorial design

Evaluation parameters of PNs

Particle size and particle size distribution

The Horiba Nanoparticle size analyzer (SZ-100 Nanopartica series) was used to determine the particle size distribution, mean particle size (PS -Z average in nm), and Polydispersity Index (PI) of polymeric nanoparticles. The samples were made with the necessary dilution of polymeric nanoparticles and distilled water twice deionized. Filtering the aforesaid solution using a 0.45 membrane filter was used for the analysis. The equipment automatically adjusted the dynamic light scattering intensity dependent on the viscosity of the medium, with 90^o light scattering for low viscous samples and 170^o light scattering for high viscous samples. Polymeric nanoparticles should have a particle size of 10 to 100 nm and a PI of less than 0.5, indicating a unimodel or uniform monodisperse size distribution. All measurements were done in triplicate (n=3) [21, 22].

Zeta potential (ζ)

The Horiba Nanoparticle size analyser (SZ-100 Nanopartica series) was used to measure the Zeta Potential, or surface charge potential (SZ-100 nanopartica series). An electrophoretic cell with an 80 mV electric field was used to transport the diluted polymeric nanoparticles into the probe. At 25 °C, all measurements were made in triplicate. The amplitude of zeta potential polymeric nanoparticles should be >30mV, indicating the colloid's durability. Using the Smolochowski equation, the Zeta potential was then directly calculated from the eqn. [23].

Run	Independent variables (Level code)			Independent variables (conc. / range)		
	Product variable	Process variable		Product variable	Process variable	
	Factor A: Polymer (HPMC) Conc.(mg)	Factor B: Homogenization time (min)	Factor C: Ultra Sonication Time (min)	Factor A: Polymer Concentration (mg)	Factor B: Homogenization time (rpm)	Factor C: Ultra Sonication Time (min)
U 1	-1	-1	-1	5	5000	5
U 2	1	-1	-1	10	5000	5
U 3	-1	1	-1	5	10000	5
U 4	1	1	-1	10	10000	5
U 5	-1	-1	1	5	5000	10
U 6	1	-1	1	10	5000	10
U 7	-1	1	1	5	10000	10
U 8	1	1	1	10	10000	10

$$\zeta = \varepsilon\mu/\eta$$

Where, ζ - Zeta Potential, μ - Electrophoretic mobility; Ɛ- Electric permittivity of the liquid; η is the viscosity of the liquid

Surface morphology studies - Scanning electron microscope (SEM) studies

The Scanning Electron Microscope was used to examine the surface morphology of the Polymeric nanoparticles for the selected optimum Ursodiol polymeric nanoparticles (Hitachi S-3000 N). Lyophilized Polymeric nanoparticles powder sections were stained with 600 platinum using a sputter coater and analysed using a scanning electron microscope (SEM). After that, the polymeric nanoparticles were put on a sample holder and scanned with an electron beam. The surface morphology picture of polymeric nanoparticles is created when an electron beam contacts the

polymeric nanoparticles particles and releases secondary electrons dependent on the nature of the surface. Then consider the average particle size of polymeric nanoparticles acquired by SEM with the average particle size of polymeric nanoparticles obtained by Horiba Nanoparticle size analyzer [24, 25].

Encapsulation efficiency studies

The centrifugation method was used to determine encapsulation efficiency. In this investigation, 1 ml of polymeric nanoparticles dispersion was placed in a dialysis bags a pore size of 2.4 nm was placed in dialysis bags (Himedia). The dialysis membrane bag was placed in the centrifuge tube once it had been prepared. To extract the free drug from the polymeric nanoparticles carrier, this centrifuge tube was previously filled with 9 ml of pH 7.4 phosphate buffer and centrifuged at 15,000 rpm for 1 hour in a REMI centrifuge. 5 cc of sample was taken from the phosphate buffer saline after 1 hour. The concentration of Ursodiol in the withdrew sample was measured using a UV Spectrophomotometer set to 216 nm. The blank solution was made using the same method and ingredients as the medication solution, but without the drug. The experiment was repeated three times (n=3). The below equation was used to calculate percentage entrapment efficiency.

$$\%\mathbf{EE} = \frac{\mathbf{Xs - Xt}}{\mathbf{Xs}} \mathrm{X}\ \mathbf{100}$$

Where, Xs - Total amount of drug used for formulation; Ext - Amount of drug in 5 ml saline [26, 27].

In-vitro drug release studies

The percentage amount of the drug released from polymeric nanoparticles dispersion performed out using the dialysis membrane technique is referred to as in-vitro drug release. 1 ml of polymeric nanoparticles dispersion was put into the dialysis membrane with 0.45 m pore size after one end of the dialysis membrane was closed or tied firmly. Both ends of the dialysis membrane were tightly knotted after it was filled. Ascertain that the tied dialysis membrane does not leak polymeric nanoparticle dispersion. A donor compartment is formed by a dialysis membrane that has been filled. The dialysis membrane was then immersed in a 100 ml pH 7.4 Phosphate Buffer Solution, which was maintained at 100 rpm in a magnetic stirrer. At regular intervals of 0, 1, 2, 4, 8, 12, 16, 20, 24 hours, 5ml of the sample was taken from the phosphate buffer solution phase. To establish a sink state, the same 5 ml of fresh PBS solution was replenished in the receptor compartment. A UV spectrophotometer set to 216 nm was used to detect the released drug absorbance at each sampling span. The experiment was performed in triplicate (n=3) [28, 29].

In-vitro release kinetic study

The drug release survey of PNs was fixed in various release kinetic parameters such as first order (time vs. log percent drug remaining); zero order (time vs. percent cumulative release); Higuchi's model (square root of time vs. cumulative percent drug release); Peppa's model (Time Vs. log of drug concentration) and their regression (r2) and k values were determined in order to acquire a linear regression analysis to verify the impact and process of release over time.

Stability studies

This study used an optimised polymeric nanoparticles dispersion. Each formulation was split into two batches for testing. Three lots of samples were collected in test tubes for each batch. Each test tube

was labelled with the months 3rd, 6th, and 12th. An aluminium foil layer is carefully covered and placed over these test tubes to shield them from light deterioration. One batch was kept at 2–6 °C in the refrigerator. Another batch was kept at room temperature for 60 percent of the time at 25°C±2 °C. Particle size (nm), zeta potential, polydispersity index (PI), and entrapment efficiency were assessed in each sample from both storage conditions over a period of time (percent). The findings of each formulation were examined for consistency [32, 33].

In-vivo Pharmacokinetic studies of Ursodiol loaded PN

The pharmacokinetic (PK) performance of PN following oral administration was studied by using PK solver software. Healthy male adult albino Wistar rats weighing between 180-250 gm were used. A single dose study in 2 groups comprising of 6 animals in each has been divided. One group is administered with CMC with Ursocol ® SR (4mg/kg/oral) and another group is administered with CMC with Ursodiol Polymeric Nanoparticles (U4) (4mg/kg/oral)
in oral feeding needle. Animals were fasted 24hrs prior to the administration of drug formulations but had free access to water. The test samples was administered orally with a help of oral feeding needle. Blood samples volume of about 0.5ml were collected at 1, 2,3, 4, 5,6 and 8 h time interval, after oral and transdermal administration by retro-orbital puncture. The samples were collected with the help of capillary tubes from retro orbital puncture into a heparinized glass tubes containing anticoagulant Ammonium oxalate (1% solution). The plasma was separated immediately with the help of micro centrifugation at 5000 RPM and stored at -20ºC until the analysis done in HPLC technique to determine the drug concentration in each time interval [34-38].

Results and discussion

Drug excipients compatibility studies

FTIR studies

On comparing pure Ursodiol to the data collected from FTIR spectra, as shown (Fig. 1) and Table 2, it was determined that the appropriate frequencies of fingerprint regions were replicable in an Ursodiol PNs. It was determined that the drug and excipients included in the formulations were compatible with one another.

XRD Study

HPMC can transform the properties of polymeric nanoparticle. Hence the melting and crystallization depends only on polymeric component. The pure drug presented sharp peaks at 2Ɵ of 2.5, 9.8, 14.0, 15.2, 18.9, 22.5 and 25.0 augurs that the pure drug Ursodiol is crystalline. The Ursodiol loaded polymeric nanoparticle crooked 9.7, 14.1, 15.2, 22.5 and 25.0 augurs that the pure drug ursodiol remains to be in crystalline form. The Ursodiol loaded polymeric nanoparticle crooked peak exhibiting polymeric nanoparticle are in amorphous nature.

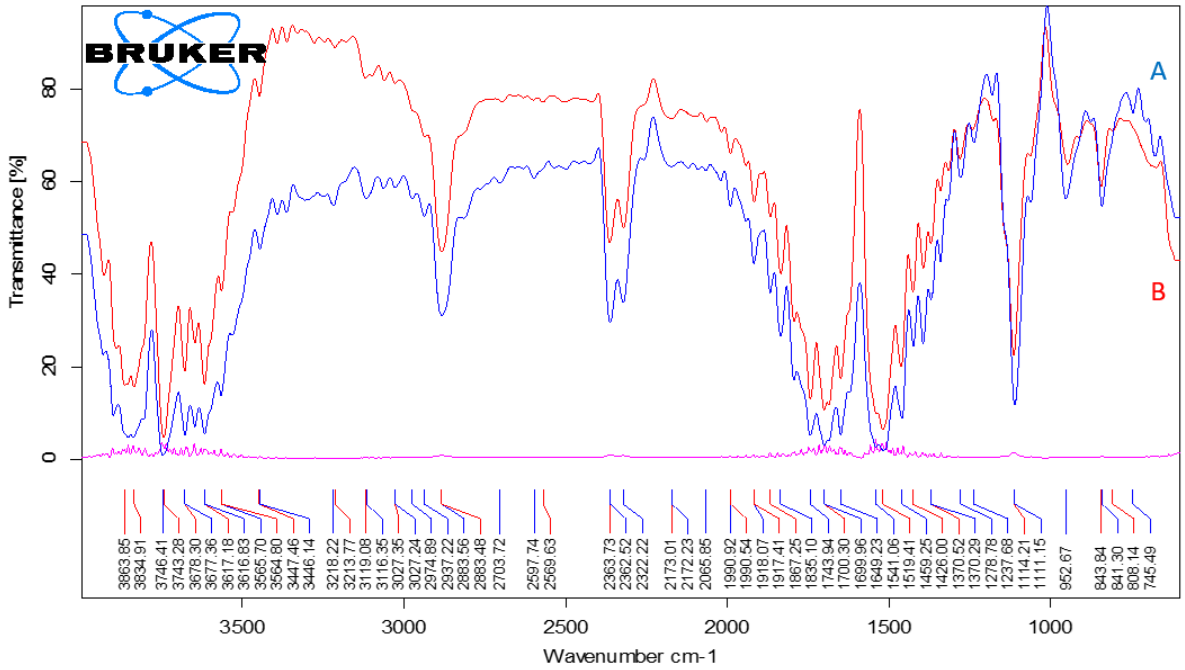

Figure 1: Drug excipients compatibility studies - FTIR studies of (A) Ursodiol pure drug and (B) Optimized Ursodiol PN

Table 2: FTIR spectrum interpretation of Ursodiol formulation

Functional group	Wave number (cm^{-1})	
	Ursodiol	Optimized Ursodiol PN
Aromatic compound	841.30	843.84
CH aliphatic bending group	1370.29	1370.52
CH2 bending	1459.25	1426.00
Aromatic Polyphenol	1541.06	1519.41
Aromatics	1699.96	1700.30
Aromatics	1835.10	1867.35
C=O Stretching bond of alkynes molecules	2172.23	2173.01
C-O bond	2362.52	2383.73
C=C conjugated group	2597.74	2569.63
CH and CH2 stretching aliphatic group	2883.56	2883.48
CH Stretching alkene group	3027.24	3027.36
Alcohol O-H ; O-H stretching vibration of hydrogen- bonded hydroxyl groups	3446.14	3447.46
Alcohol O-H	3746.41	3743.28

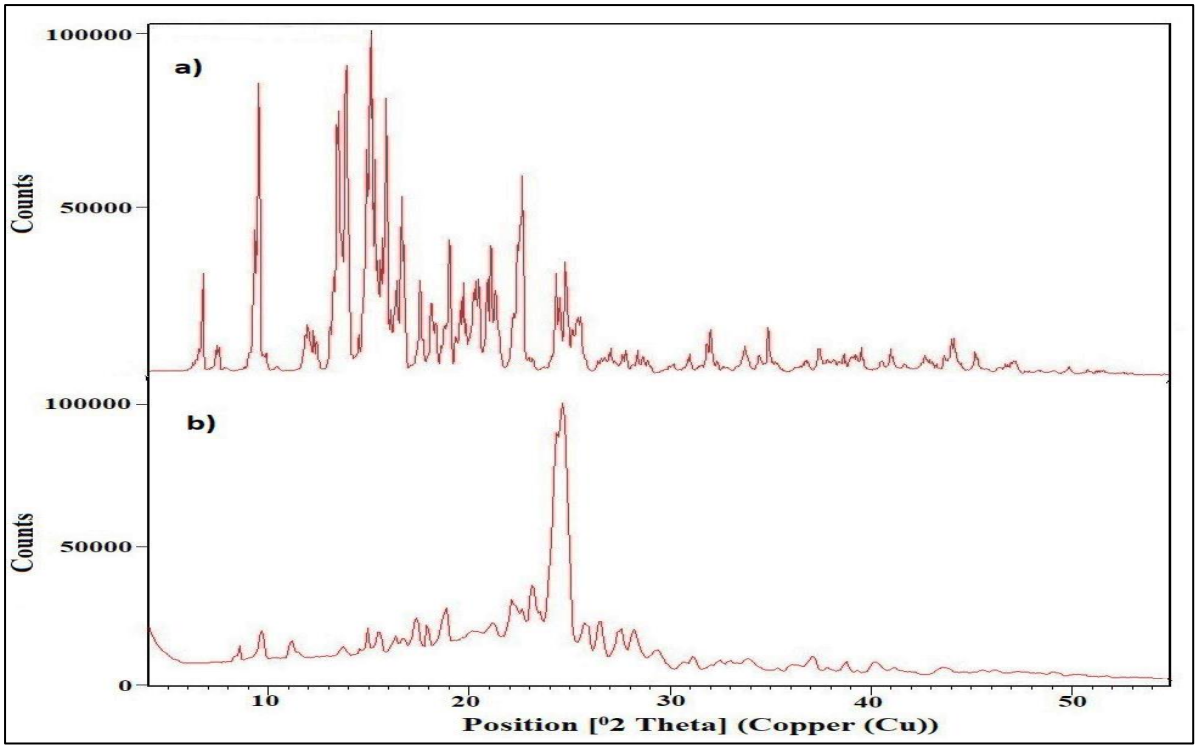

Figure 2: (a) XRD pattern of Ursodiol; (b) XRD pattern of Ursodiol polymeric nanoparticle

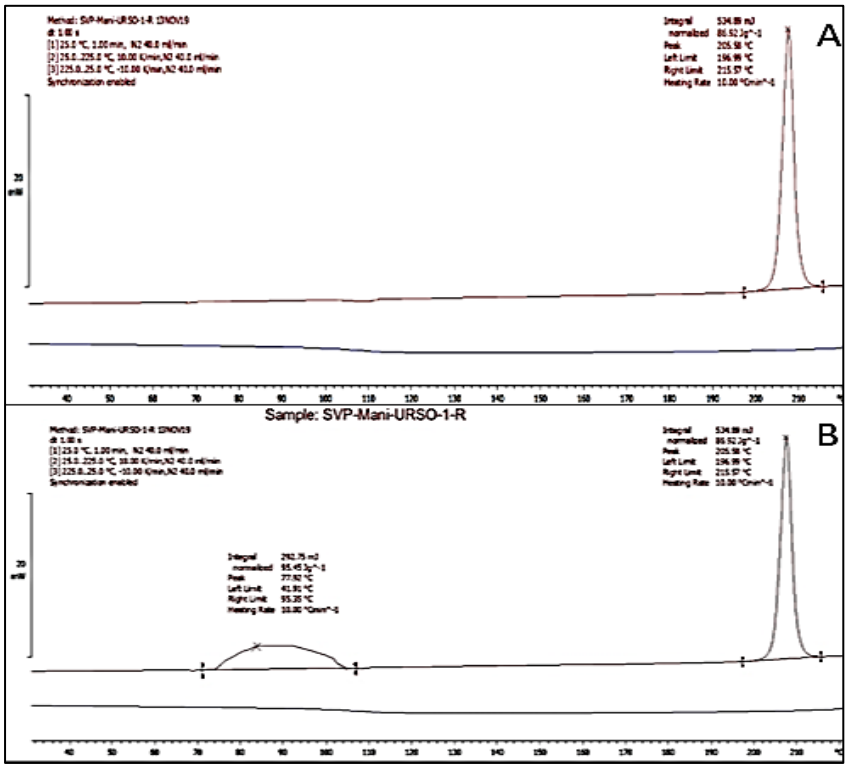

Figure 3: Drug excipients compatibility studies - DSC studies of (A) Ursodiol pure drug and (B) Optimized Ursodiol PN

DSC studies

As endothermic peak values in a DSC thermogram, the relevant melting points were observed: Ursodiol at 205.58 degrees Celsius; Ursodiol polymeric nanoparticle at 205.58 degrees Celsius. The polymer melted first, followed by the drug as shown (Fig. 3 B) ensuring that the drug was successfully encapsulated within the polymer during the DSC investigations for PNs formulation. The fact that the

Table 3: Optimization design showing the effect of independent variables on dependent variable in formulation of polymeric nanoparticle (mean ± SD, n=3).

Formulation Run	Independent variables			Dependent variables		
	Factor A: Polymer Conc. (mg)	Factor B: Homogenization time (min)	Factor C: Ultra Sonication Time (min)	Particle size (Y1)	Zeta potential (Y2)	Polydispersity index (Y3)
U1	1	1	-1	473.3 ± 6.94	-31.4 ± 2.16	0.353 ± 0.16
U2	-1	1	-1	361.1 ± 3.36	-33.7 ± 2.34	0.526 ± 0.14
U3	-1	-1	-1	665.0 ± 7.24	-25.1 ± 2.28	0.455 ± 0.12
U4	-1	1	1	168.0 ± 3.24	-34.1 ± 2.34	0.320 ± 0.24
U5	1	-1	1	279.4 ± 3.62	-42.7 ± 2.82	0.356 ± 0.22
U6	1	-1	-1	821.0 ± 6.64	-28.5 ± 2.38	0.576 ± 0.08
U7	1	1	1	299.4 ± 3.62	-34.4 ± 2.22	0.216 ± 0.20
U8	-1	-1	1	462.4 ± 6.60	-38.5 ± 2.24	0.391 ± 0.18

medications are amorphous or molecularly dispersed in nature is confirmed by their thermal behaviour. Fig. 3 (A) and (B) depicts the DSC thermogram.

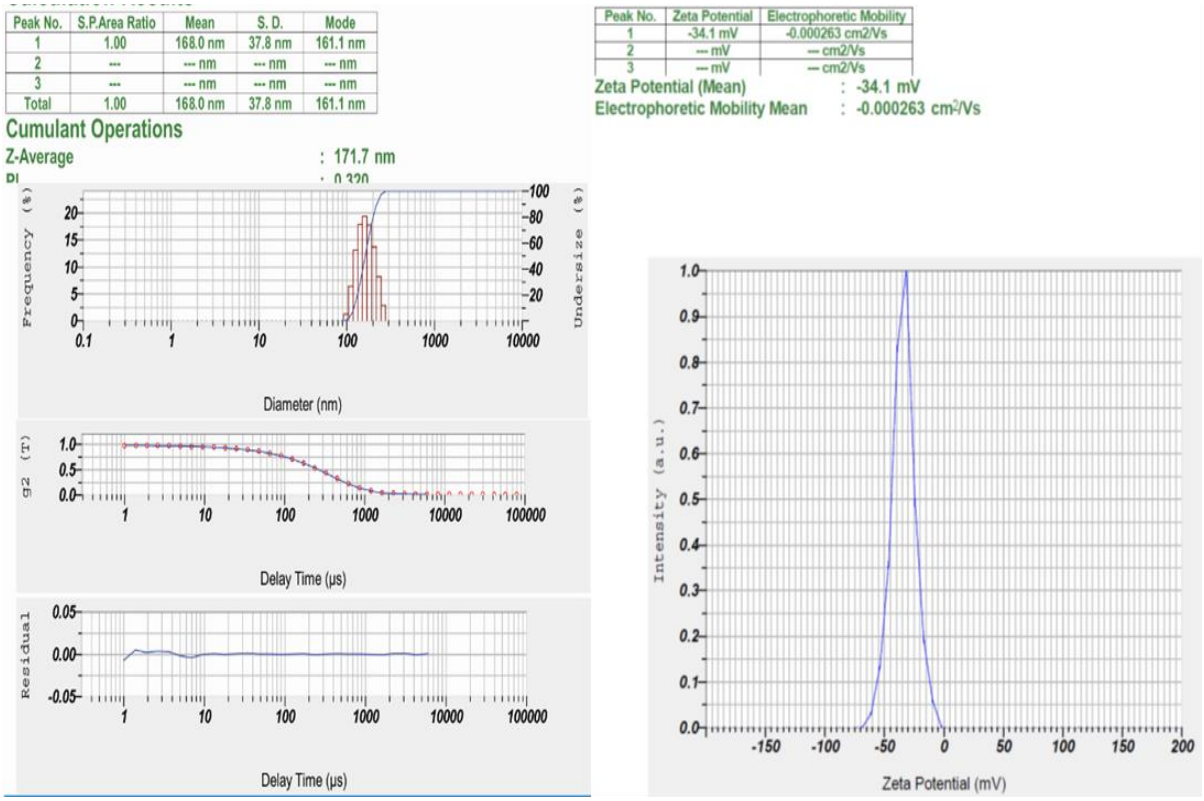

Figure 4: Ursodiol polymeric nanoparticle (A) Particle Size & Polydispersity index report; (B) Zeta potential report

Formulation Run	Independent variables			Dependent variables		
	Factor A	Factor B	Factor C	%EE*	%Yield*	% drug release at 24 h
U1	1	1	-1	78.76 ± 3.82	78.46 ± 3.08	63.06 ± 4.34
U2	-1	1	-1	82.42 ± 3.78	88.64 ± 3.72	76.44 ± 3.24
U3	-1	-1	-1	68.48 ± 3.68	64.80 ± 2.84	63.44 ± 4.22
U4	-1	1	1	98.62 ± 3.68	97.46 ± 2.12	90.64 ± 3.66
U5	1	-1	1	94.66 ± 3.82	93.44 ± 3.64	78.90 ± 3.64
U6	1	-1	-1	86.74 ± 3.60	82.80 ± 3.80	87.36 ± 3.24
U7	1	1	1	92.80 ± 3.60	89.62 ± 2.80	78.56 ± 3.44
U8	-1	-1	1	70.64 ± 3.74	78.60 ± 2.92	73.82 ± 3.68

Table 4: Evaluation of effect of independent variables on other dependent variable in formulation of polymeric nanoparticle (mean ± SD, n=3)

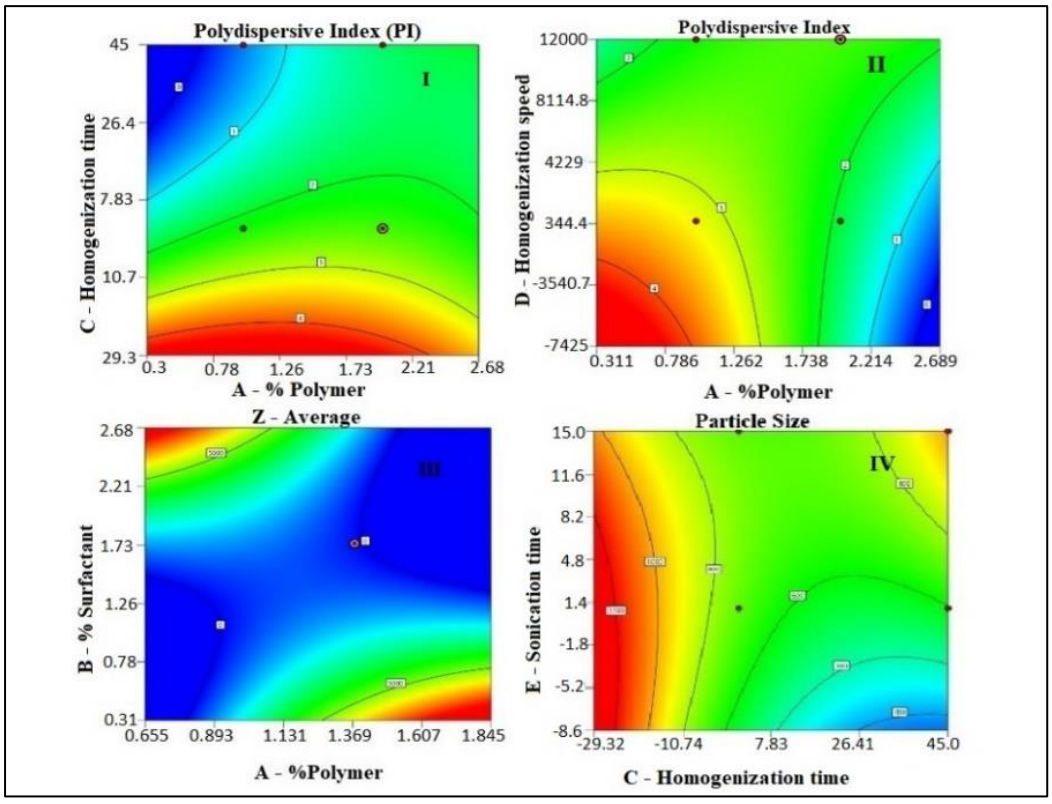

Figure 5: Contour profile graph showing the response of independent variable on dependent variable

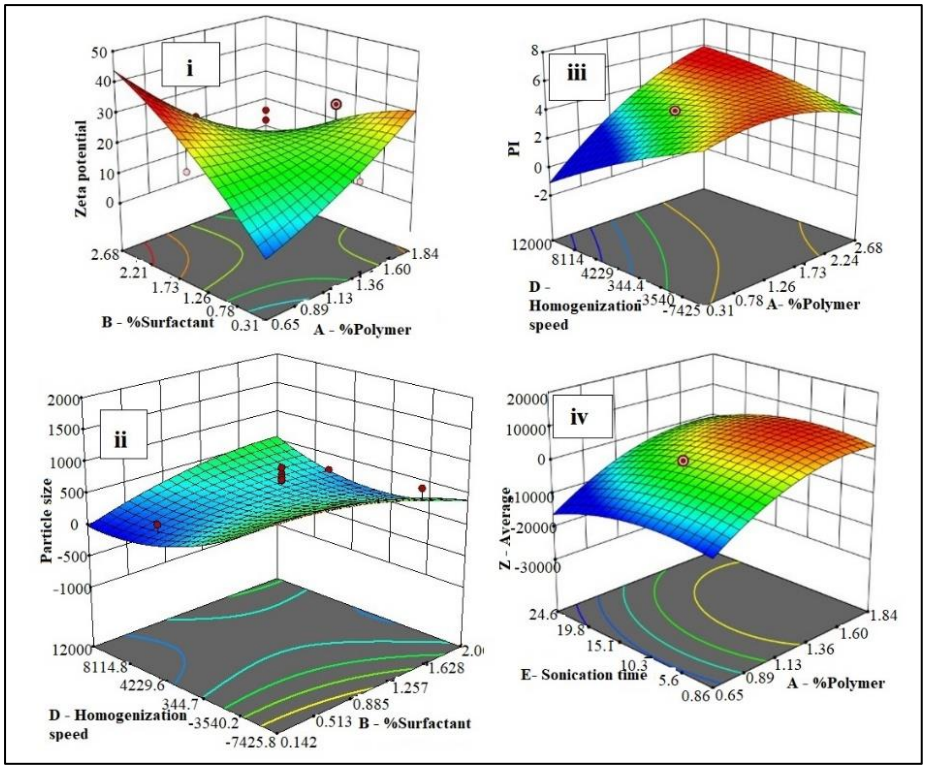

Figure 6: 3D surface response graph showing the response of independent variable on dependent variable

Particle size

Table 3 and Fig. 3 show the average particle sizes for all formulations. Based on the impact of the independent variable in the formulation process, particle sizes for all Ursodiol PNs formulations were determined to be in the range of 168.0 ± 3.24 to 821.0 ± 6.64 nm; zeta potential was in the range of -25.1 ± 2.28 to -42.7 ± 2.82mV; polydispersity index was in the range of 0.576 ± 0.08 to 0.216 ± 0.20. However, the particle size of polymeric nanoparticles should be <500 nm to meet the approval standards i.e., 168.0 ± 3.24mV, polydispersity index of 0.320 ± 0.24 which shows all the particle are dispersed uniformly throughout the phase and zeta potential of -34.1 ± 2.34mV, which shows all the particle having uniform particle surface charges and in good kinetic energy. The formulation U4 (5mg polymer concentration, 10000 rpm homogenization time, 10 min ultrasonicator time) has a particle size of 168.0 ± 3.24 nm, according to the approval criteria.

Zeta potential

The zeta potential of all Ursodiol PNs was determined to be in the range of -25.1 ± 2.28 mV to -42.7 ± 2.82 mV, owing to the influence of surfactant during the formulation process. However, the ZP of polymeric nanoparticle acceptability criteria must be determined between 30 and 60 mV. Based on the decrease in particle size, the formulation U4 (5mg polymer concentration, 10000 rpm homogenization time, 10 min ultrasonicator duration) has a maximum ZP of -34.1 ± 2.34mV, which meets the approval criteria i.e., >±30mV. The remaining formulation fell short or more of the target than the range, these may leads to aggregation or sedimentation of polymeric nanoparticle. Table 3 and Fig. 4 show the zeta potential data for prepared polymeric nanoparticle.

Polydispersity index

The polydispersity index for prepared Ursodiol PNs was reported to be between 0.576 ± 0.08 to 0.216 ± 0.20, owing to the effect of homogenization speed or ultra sonication time in the formulation process. However, for monodisperse nanoparticles, the PI acceptance requirement should be less than 0.7. The formulations U1, U3, U4, U5, U7, U8 have good polydispersity indexes of 0.353 ± 0.16, 0.455 ± 0.12, 0.320 ± 0.24, 0.356 ± 0.22, 0.216 ± 0.20, 0.391 ± 0.18 respectively, according to the acceptance requirements i.e., <0.5. The above said formulation are more stable, it leads to good stability i.e., it maintains the nanoparticle in phase with good movement. The other two formulations were found to be >0.5, which will leads to sedimentation and aggregation of particle in U2 and U6 formulation. Table 3 and Fig. 3 show the polydispersity index for all formulations.

Optimization of polymeric nanoparticle

The results of independent variables on dependent variables on Ursodiol PNs were shown by the 2^3 optimization design Table 3, 4 and Fig. 5-7. Based on the foregoing data, it was determined that there was a strong link among particle size and polymer concentration, i.e., increasing the polymer concentration increased the particle size of PNs. At low -1 level polymer, U4 formulation showed required particle size of around 168.0 ± 3.24 nm between all formulations (U1-U8) (5 mg). The reduction in particle size was achieved by combining a low polymer content with a high homogenization rpm and ultra sonication period as shown in table 3. Particle size reduction was also achieved as a result of increased homogenization speed and ultrasonication time, which separated large particles and particle aggregates into small dispersed particles, resulting in particle size reduction. In the preparation of PNs, increasing the homogenization speed and ultrasonication time resulted in a concomitant increase in the zeta potential with a decrease in the particle size, confirming the good phase stability of PNs and achieving the highest conductance of the particle. The charge distribution will be dispersed evenly on split tiny particles when the surfactant concentration increased, which may lead to a rise in zeta potential or surface charge potential, high nanoparticle stability, and particle mobility without sedimentation. At a high -1 level of surfactant concentration, +1 level of homogenization speed, and ultra sonication time, U4 formulation demonstrated the requisite zeta potential of about -34.1 ± 2.34 mV. With a rise in ultrasonication time and homogenization speed, the ZP in mV increased in lockstep with a reduction in polydispersity index of approximately 0.320 ± 0.24. The surface morphology of the Optimized Ursodiol PNs, U4 was studied using SEM, as illustrated in Fig. 7, where the PNs were observed as smooth spherical surfaced particles. Due to its spherical smooth nanometric surface, it was discovered that it will boost drug loading efficiency, entrapment efficiency, and simple diffusion of the drug into physiological barriers. The greatest percent yield and percent entrapment efficiency for the Ursodiol PNs (U4) formulation were 98.62 ± 3.68 and 97.46 ± 2.12 %, respectively. It is also possible to conclude from the above-mentioned findings that the medication concentration was distributed uniformly in the PNs.

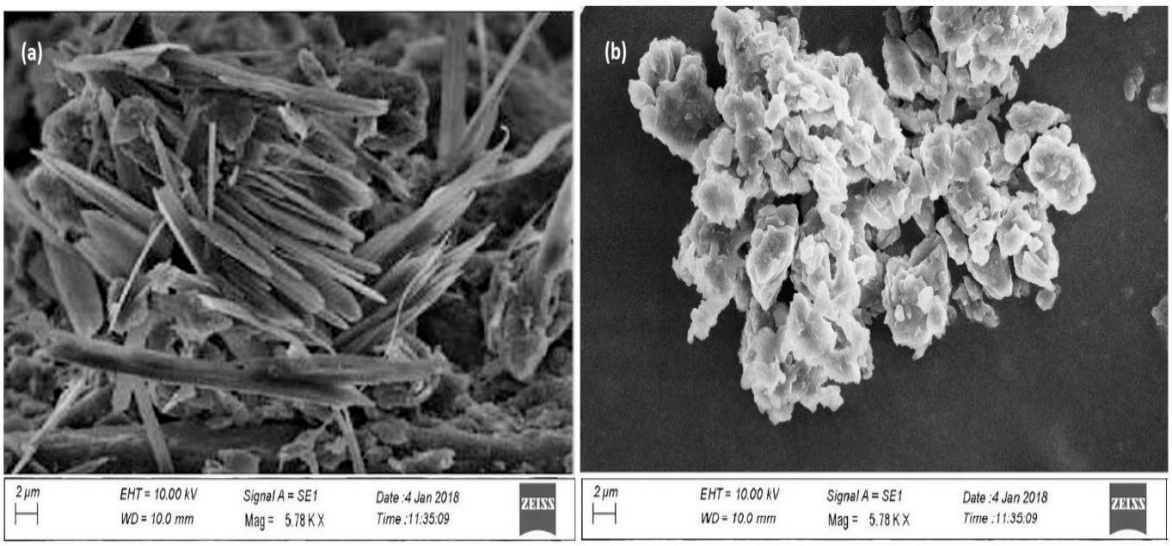

Figure 7: SEM images of polymeric nanoparticle

Percentage entrapment efficiency and percentage yield

For polymeric nanoparticles, the required percentage entrapment efficiency and yield should be greater than 85%. The effective entrapment efficiency of polymeric nanoparticle was found to be 68.48 ± 3.68 to 98.62 ± 3.68 %, and the percent yield was found to be 64.80 ± 2.84 to 97.46 ± 2.12%, according to the results provided in table 4. U4 displays the estimated amount of percentage entrapment efficiency and percentage yield by comparing all of the formulations. Based on the reduction in particle size the entrapment efficiency and % yield of nanoparticle will enhance. Simultaneously there will be increase in percentage amount of drug release.

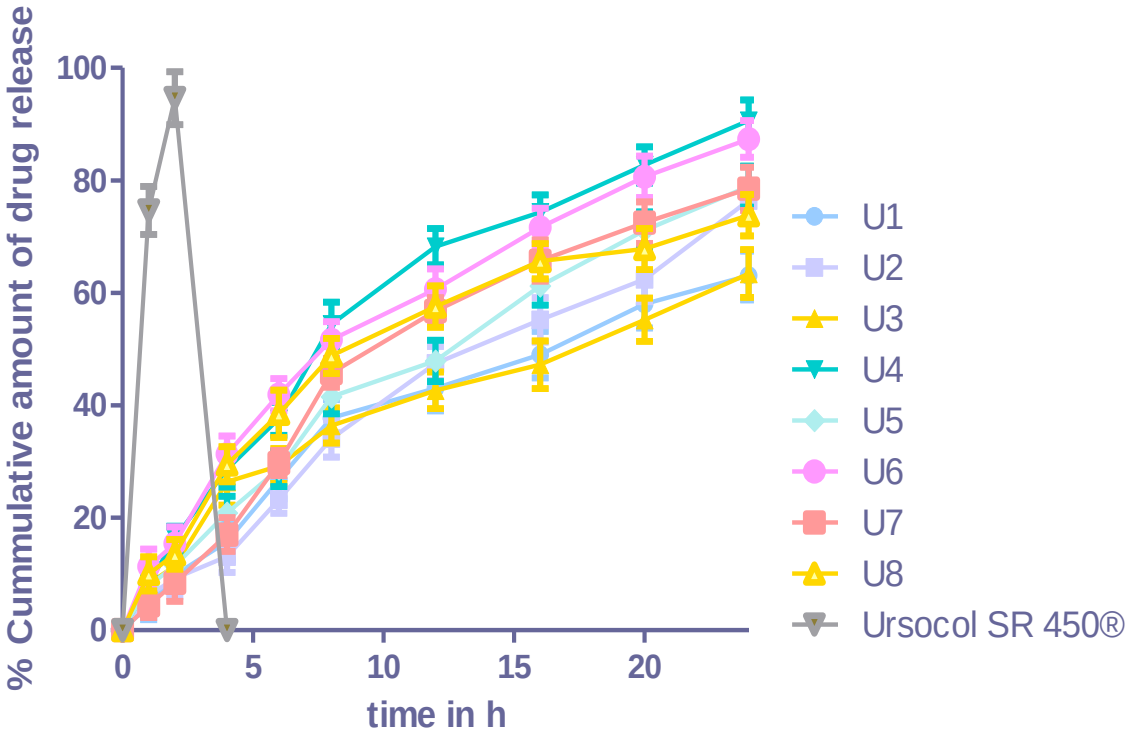

Figure 8: Comparative In-vitro drug release studies between Polymeric Nanoparticle vs. marketed Ursocol SR ® tablet (mean ± SD, n=3)

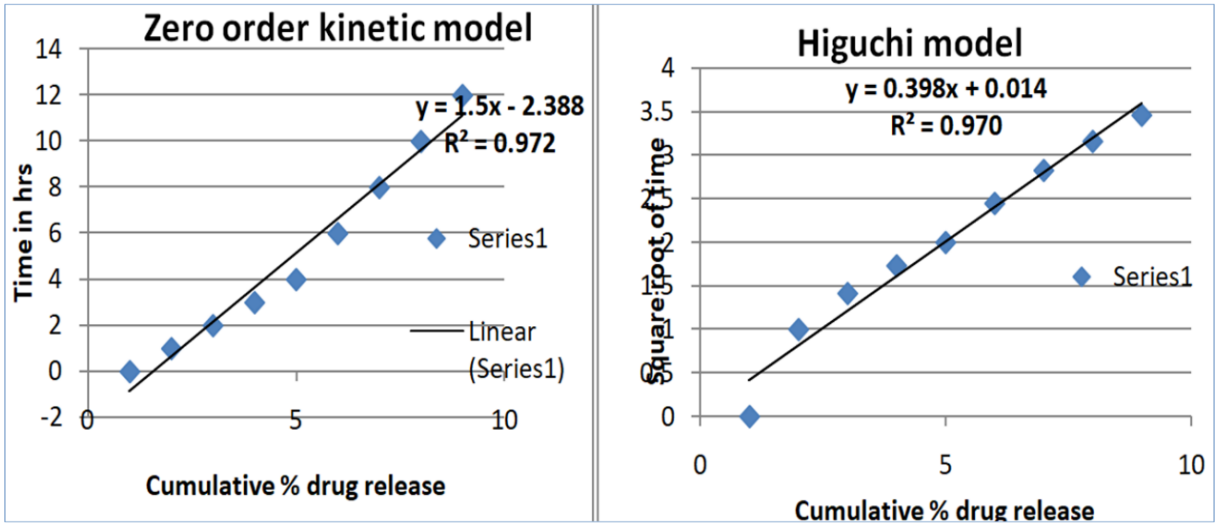

Figure 9: Release kinetic graph showing (a) Zero order kinetic graph (b) Higuchi kinetic graph for optimized U4 Ursodiol Polymeric nanoparticle.

Invitro drug release and invitro release kinetics studies

For the U4 optimised formulation, a percentage quantity of drug release experiments was conducted (fig. 8). As demonstrated in Fig. 8, in-vitro drug release studies for the Ursodiol PNs (U4) formulation revealed a better-controlled drug release of 90.42 ± 3.56% in 24 h when compared to the marketed available Ursodiol tablet dosage form Ursocol SR® tablet 450mg formulation. In 24 h, the percentage amount of drug released by U4 was discovered to be 90.64 ± 3.66%. Zero order, First order, Higuchi model, Hixson crowell model, and Korsmeyer Peppas model regression values (r^2) were discovered to be 0.972 ± 0.02, 0.642 ± 0.02, 0.970 ± 0.02, 0.826 ± 0.02 and 0.986 ± 0.02. The zero order release kinetic model was used in the in-vitro release kinetics experiments of Ursodiol Polymeric Nanoparticles (U4), and the regression values (r^2) were determined to be 0.972, indicating good linearity. The drug was delivered in a predefined and controlled manner from Ursodiol loaded PNs (U4), which matched zero order kinetics. It was validated as the best model for releasing the medicine in order to achieve the desired therapeutic effect without causing any side effects. Higuchi's release kinetic pattern had an r^2 of 0.970, indicating that the medication was released by diffusion. It meant that drug release from PNs was governed by a non-fickian diffusion process, in which the drug was discharged from the polymer by polymer relaxation and diffusion.

Stability studies

The stability data of optimised polymeric nanoparticles (U4) are tested for short-term stability at 4˚C±2˚C for 6 months. At three-month intervals, the parameters were assessed. The data shows the comparative stability study data for U4 before and after conducting stability experiments. U4's PS nm, ZP mV, and PI during preparation were 168.0 ± 3.24nm, -34.1 ± 2.34mV, 0.320 ± 0.24 and U4 after performing stability investigations, i.e. after 6 months of storage at 4˚ ± 2˚C, was 169.0 ± 4.14nm, -36.8 ± 2.64mV and 0.320 ± 0.22. After 6 months of storage at room temperature the particle size was found to be 172.0 ± 3.12nm, -30.2 ± 2.12mV, 0.388 ± 0.12. The PS, ZP, and PI of U4 did not vary much,

according to the results of stability experiments. The drug-loaded U4 was verified to be stable at refrigerated temperature (4˚C±2˚C) based on the results (Table 5).

Table 5: Comparison of physiochemical properties of optimized polymeric nanoparticle after stability studies

Evaluation parameters	Optimized Polymeric nanoparticle (U4)	After storage at 25ºC ± 2ºC / 60% RH ± 5% RH for 6 months	After storage at 4°C±2°C for 6 months
Particle size in nm	168.0 ± 3.24	172.0 ± 3.12	169.0 ± 4.14
Zeta potential mV	-34.1 ± 2.34	-30.2 ± 2.12	-36.8 ± 2.64
Polydispersity index	0.320 ± 0.24	0.388 ± 0.12	0.320 ± 0.22
Entrapment Efficiency (%)	98.62 ± 3.68	94.12 ± 3.10	98.12 ± 4.18
Yield (%)	97.46 ± 2.12	94.22 ± 2.10	98.14 ± 2.24
%CDR at 12 h	90.64 ± 3.66	88.26 ± 2.22	92.02 ± 3.12

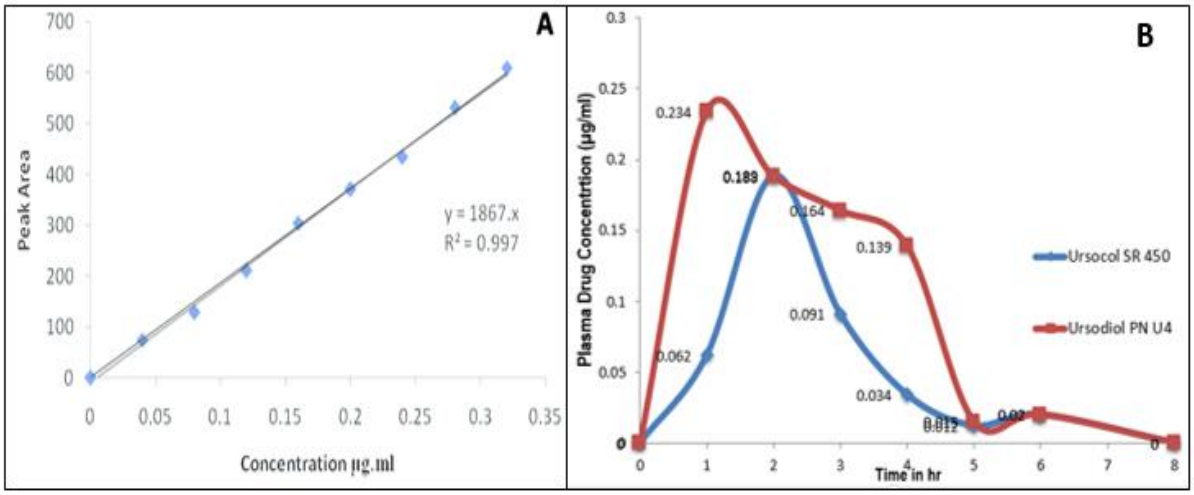

Figure 10: (A) Calibration curve for Ursodiol (HPLC); (B) Graph of Comparative in-vivo Pharmacokinetic study data between Ursodiol treatment groups

Table 6: Comparative in-vivo pharmacokinetic studies data between Ursodiol treatment groups

Parameter	CMC with Ursocol ® SR (4mg/kg/oral)	CMC with Ursodiol Polymeric Nanoparticles (U4) (4mg/kg/oral) in oral feeding needle
T_{max} (h)	2	1
C_{max} (µg/ml)	0.189	0.216
AUC 0-α (µg/ml/h)	68.126	246.124

Note: Increase in $AUC_{0-\infty}$; Increase in Cmax shows better enhancement of bioavailability

Invivo Pharmacokinetic Studies

To determine the unknown plasma drug concentration a calibration curve was designed by using different concentration of Ursodiol. The linearity of the calibration curve was determined by plotting the peak area and nominal concentration of Ursodiol. For linearity study, eight different concentrations of Ursodiol were analyzed (0, 0.04, 0.08, 0.12, 0.16, 0.2, 0.24, 0.28, 0.32µg/ml). The peak area response was found to be linear over the concentration range studied. The coefficient of

correlation 'r^2' was found to be 0.997 as shown in Fig.10 (A). The HPLC calibration curve has been successfully used to determine the pharmacokinetic data from the unknown plasma drug concentration followed by single dose administration of CMC with Ursocol SR 450® Tablet and CMC with Ursodiol Polymeric Nanoparticle (U4). From the peak area of the injected sample the unknown concentration was determined. The mean plasma concentration of Ursodiol as a function of time has been plotted as shown in Fig.10 (B) and the comparative studies on In-vivo plasma drug concentration profile between CMC with Ursocol SR 450®; CMC with Ursodiol polymeric nanoparticle (U4) was tabulated in Table 6. It was observed that CMC with Ursodiol polymeric nanoparticle (U4) enhances the drug release as well as the desired pharmacokinetic parameters when compared to the Ursocol SR 450®. There was a significant difference in 'p' value as < 0.05 between the pharmacokinetic parameters of Ursocol SR 450® and Ursodiol polymeric nanoparticle (U4) with T_{max} of 2 h and 1 h; and the maximum peak plasma concentration (C_{max}) of 0.189µg/ml and 0.216µg/ml respectively. Area Under Curve ($AUC_{0-\alpha}$) was found to be 68.126 µg/ml/h and 246.124µg/ml/h respectively. From the in-vivo pharmacokinetic data it was concluded that increase in $AUC_{0-\infty}$, T_{max} and C_{max} in Ursodiol polymeric nanoparticle treatment when compared to marketed Ursocol SR 450® tablets. In calculating the relative bioavailability by keeping marketed formulation as standard, it has been confirmed that the Ursodiol loaded Polymeric Nanoparticle showed the enhancement of bioavailability of about 3 folds.

Conclusion

By reducing Ursodiol's dose-dependent unfavourable side effects, PNs will significantly improve its bioavailability of Ursodiol. According to the findings, PNs have a good controlled drug release pattern and can be used as a drug delivery carrier for BCS Class II drug like Ursodiol to improve its bioavailability.

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