FABRICATION AND INVITRO EVALUATION OF ROSUVASTATIN LOADED SOLID LIPID NANOPARTICLES

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Abstract

This investigation utilizes quality-by-design approach to develop the Rosuvastatin Calcium (Rst)-loaded solid lipid nanoparticles (SLN). Effect of formulation variables such as amount of lipid (200-500 mg stearic acid) and surfactant concentration were studied. Design of Experiment (DoE) was used to quantify the extent of impact of lipid amount and surfactant concentration on the physicochemical properties of the SLN and to identify optimized SLN formulation. It was observed that interplay of formulation variables had significant effect on particle size, %EE and In vitro release Based on the results, point optimization was carried out to obtain the SLN with minimum particle size maximum %EE and sustained In vitro release within the design space. In vitro drug release data fitted well in Korsmeyer-peppas model indicating the fickian diffusion mechanism. Ex vivo studies indicated sustained permeation of the Ropt compared to the control. Furthermore, stability studies indicated Ropt formulation exhibited no significant physical or chemical change under accelerated conditions.

Keywords: *SLN, DoE, Rosuvastatin, Zetapotential, Entrapment efficiency*

Introduction

Nanoparticles are known as drug carriers that are solid, submicron sized and that may or may not be biodegradable. Nanoparticles are colloidal polymeric structures that are submicron size. Nanoparticles, nanospheres, or nanocapsules can be created through this process [1]. Drug nanoencapsulation requires the creation of substances with diameters varying from 1 to 1000 nm filled with drugs. Nanoparticles' submicron scale has several benefits over microparticles. The 100-400 nm nanoparticle scale demonstrated 6 times greater absorption relative to 10μm microparticles in the caco-2 cell line. The 100 nm particle size uptake performance was 15-250-fold higher than microparticles larger in size (1-10μm) [2- 3]. In the pharmaceutical and medical sectors, polymeric nanoparticles act extensively as particulate carriers because, due to their regulated and maintained release characteristics, and they act as drug delivery mechanisms. Negatively charged, hydrophilic polymer-based nanoparticles display a significant improvement in bio adhesive properties and are ingested by enterocytes. Chemical modification of these hydrophilic polymer nanoparticle systems is the way to manage the system of opsonization and enhance the modulation of substrate and processing with polymers. Classification according to the danger of human health by the International Conference on Harmonization (ICH) and division into three groups. Category 1(solvents to be prevented), Category 2 (solvents to be restricted) and Category 3 (low harmful potential solvents). According to this, we took purified water as a solvent for human health protection preparation [4-6] Page 9 The use of nanoparticles in biological applications containing water soluble drugs is limited by low encapsulation efficiency quality and rapid release of the drug substance. Nanoparticles may enter narrow capillaries, increasing the aggregation at the targeted cells of nanoparticle-encapsulated medicines. Nanoparticles can passively target the tissues of tumours and increase the effects of permeability and durability. Nanoparticles developed utilizing synthetic polymers; poor quality of drug entrapment demonstrated and fast drug release profile in water E.g. PLGA nanoparticles deliver dexamethasone at 8 o'clock and both polycaprolactone and PLGA nanoparticles discharge Propranolol hydrochloride, a water-soluble medication, at 8 o'clock. So, we developed a new surfactant-polymer drug delivery system showing a water-soluble drug with continuous release. E.g. Nanoparticles formulated utilizing hydrophilic polymers often demonstrate fast release of drugs such as alginate-bovine serum albumin nanoparticles released within 72 hours for 5-fluorouracil 84 percent of the drug substance [7-8]. 1.1. Applications of Nanoparticles: • Controlled release of encapsulated drugs. • Tissue targeting in cancer therapy by pH specific targeting ligand to Nanoparticle surface. • Carrier action for drug distribution. • Rise in drug solubility • Enhanced permeation and retention effect etc., [1-9] Biodegradable nanoparticles have proven successful systems for drug delivery. Different polymers were used for this in drug delivery as they distribute the active drug safely to a target site and thereby improve the clinical value and minimize the side effect. In increasing drug / protein stability, nanoparticles are stronger than liposomes in tissue binding and have valuable controlled release properties. E.g. In parenteral and implantation drug delivery applications, PLA and PLGA polymers that are tissue compliant together with alginate provide controlled release formulation. E.g. For the delivery of ophthalmic medicines, PCL (poly caprolactone) is used. Page 10 Recently, natural hydrophilic / biodegradable polymers are often used because the manufactured nanoparticles are readily retrieved,

providing high trapping ability if the process of surfactant polymer is being used [10-13]. Prodrugs were developed and found to be helpful in regulating drug release, adjusting biodistribution, excretion, enhancing half-life, bioavailability, and achieving drug targeting. Biological evidence on the safety and pharmacokinetics of collected and very healthy macromolecules are being used as n2on-toxic drug delivery systems E.g. PLGA, alginate, dextran and albumin etc. Macromolecules must be water soluble and its conjugates and polymers used ought to be water soluble, acidic and weakly anionic macromolecules must not be prone to bio macromolecules interaction/More than 4 nm polymers and anionic macromolecules tested as micro-nanoparticles carriers demonstrated elevated systemic circulation, increased permeability persistence impact on solid tumour tissues, and even transported drugs to infected locations. This depends on pH / enzymatic hydrolysis [14-16]. The prodrugs have two major strengths, the drug treating the appropriate diseased region in the cancer therapy and the controlled release of medications to minimize harmful effects. But often the harmful impacts of conjugate macromolecules rise. Thus, macro molecular conjugates converted to micro-nanoparticles because size, shape, charge and surface characteristicslike lipophilicity/hydrophilicity and charge are depend with pharmacodynamic and pharmacokinetic properties to decrease side effects of prodrugs, so we used only surfactant (charge for stability of nanoparticles) - polymer based carrier system for prodrug to increase bioavailability, half-life, and sustained release with nanoparticle system [17]. The simplest and most effective method for non-invasive treatment is the oral route. The oral drug delivery is perhaps the most cost-effective system and dominates the global market for drug delivery. The optimal route for persistent opioid therapy is the oral route. Due to their weak aqueous solubility, various potent lipophilic drugs show low bioavailability. Around 35-40 percent of new applicants for medications have low Page 11 solubility in water. The very first step in achieving solubility when a medication is delivered via oral route is the first step it to solubilized and then absorbed [18-19].

METHODOLOGY

Preformulation Studies

The aim of preformulation studies is to determine the appropriate characteristics of the

drug like solubility and also to study the compatibility of drug and excipient through FTIR

and DSC studies.

Solubility studies:

Drug solubility has been checked by dissolving the drug in increasing concentration in water and in numerous lipids such as Dynasan 114, Stearic acid, Compritol 888 ATO, Witepsol H 32, Cholesterol. After that, it's permitted to dissolve in 10 mL of lipid until it's saturated. The drug's lipid solubility was calculated in milligrams per milliliter.

FTIR analysis

The surfactants used in the formulation have been used in FTIR experiments to determine the compatibility profile between both the Rosuvastatin and other components such as lipids. For pure drug (Rosuvastatin) SLN dispersion, studies were conducted using the potassium bromide (KBr) palletization technique.Along with the KBr, 0.2 percent opioids are ground, and the mixture is then pressed with a mini KBr pellet press at a pressure of about 7 MT by repeatedly pressing the press handle. The SLN dispersion was put in a centrifuge tube and centrifuged at 15000 RPM for 40 minutes. The excess volume of water was extracted by syphoning the supernatant solvent from the centrifugal tube and collecting the residue, which was then dried at 50oC. The dry SLN dispersion was sandwiched between a plane KBr pellet and screened over a wave number range of 4000 to 500 cm-1.

Solid Lipid Nanoparticle formulation techniques

4.2.1. SLN preparation by process of micro emulsification

The solid lipid nanoparticle (50-500 nm) was prepared using a micro emulsification process by heating the weighed solid lipid mixture (stearic acid) above its melting point. Beaker containing the appropriate volume of purified water was taken, and the weighed amount of surfactant (Span 80) has been allowed to dissolve gradually with the aid of magnetic stirrers. This results in formation of translucent, thermodynamically stable microemulsion. The weighed medication was slowly distributed into the microemulsion by homogenization using High Speed Homogenizer, CAT, Germany. The formulated hot microemulsion is then distributed under extreme homogenization at 10,000 RPM with a specific time under 2-4°C. Then the formulated emulsion wasdissolved in water in a 1:20 ratio for further use.

Formulation of SLN by Hot Homogenization method

The SLN formulations were prepared using a hot homogenization process. By modifying the concentrations of formulating parameters such as solid lipid in SLN, surfactant and process variables (homogenization time), eight compositions were developed, as seen in Table 4.1. According to the composition, the volume of lipid weighed in the ratio, i.e. the ratio between the proportion of drug and lipid, as shown in the table, is taken in the China dish. In order to have a transparent viscous liquid, melt the lipid mixture at 75°C. Then, with constant stirring, the Rosuvastatin drug (10 mg) was spread into the molten lipid to achieve a homogeneous solution. This lipid mix is then poured into a beaker containing 0.25-0.5 percent of Span 80 surfactant during varying homogenization. periods in drop wise, as seen in formulations. A milky white suspension of SLN has been produced.

Process of Optimization

QbD Plan for SLN formulation

In the ongoing study, for the optimization experiment, 2^3 mathematical models have been used, using 3 stages, 2 factor and 8 run, which are performed with the aid of JMP QbD tools. Critical material attributes (CMA) such as lipid concentration (A in mg), surfactant concentration (B in ml) and critical process parameter (CPP) such as homogenization period (C in min) are

chosen and set at high or low levels on the basis of the Quality attributes (CQA) result.8 For CQAs such as particle size (Y1), zeta potential (Y2), which was selected as the response variable, SLN compositions are formulated and defined according to this model. The principal effect of CMA and CPP on CQAs is demonstrated by these models.

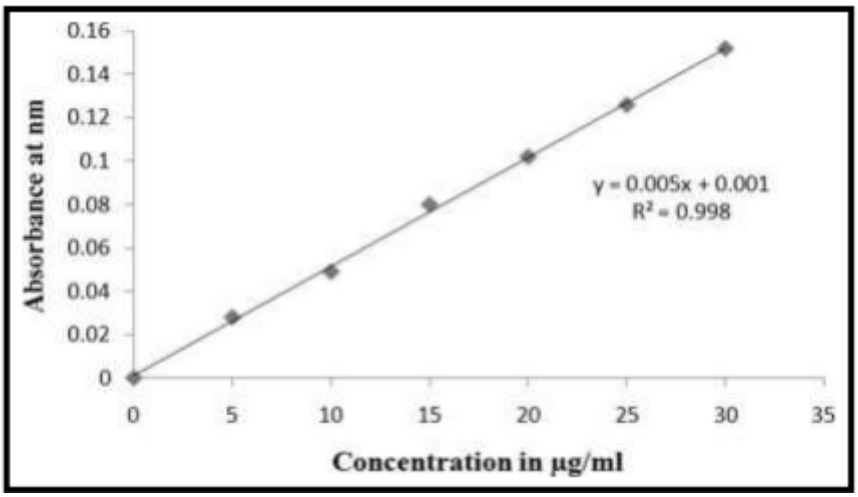
In-vitro drug release studies

The percentage of SLN dispersion drug release achieved by the dialysis membrane system is measured as in-vitro drug release. 1 ml of SLN dispersion was filled into the 0.45 m pore size dialysis membrane after one end of the dialysis membrane was firmly shut. After the dialysis membrane was filled, both of their ends were tightly bound. Ascertain that the tied dialysisdoes not leak SLN dispersion.The compartment portion of the dialysis membrane is filled. A 100 mL pH 7.4 Phosphate Buffer Solution was deposited in a 100RPM magnetic stirrer after the dialysis membrane was filled. The phosphate buffer solution yielded 5 ml of sample after a daily duration of 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24 hours.Then the same 5 ml of fresh PBS solution was substituted in the receptor compartment to preserve the state of the sink. Using a UV Spectrophotometer at 248 nm for Rosuvastatin, the released drug absorbance was measured at each time interval. The triplicate experiment was performed (n=3).

Results and Discussions

Preparation of Calibration Curves

Rosuvastatin 's absolute wavelength (λmax) has been found to be 252 nm. By using the pH 7.4 phosphate buffer, the calibration curve for Rosuvastatin was plotted. Within the concentration, it exhibits a strong association and linearity from 3 to 18µg / ml rangeconcentration and indicates an 0.998 of R2 valuein the phosphate buffer pH 7.4. It indicates that it complies with the Beers Rule.

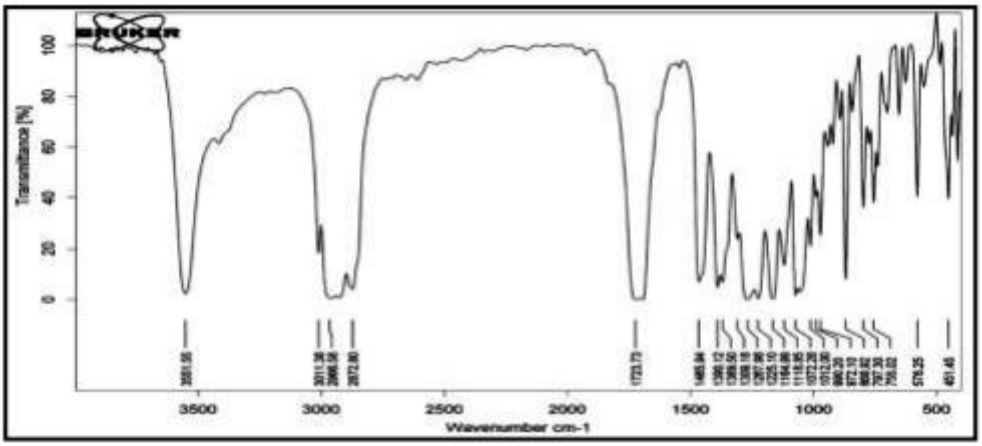


Rosuvastatin Calibration curve in pH 7.4 PBS at 252 nm

FTIR Analysis: FTIR spectra were shown as follows.

In the Rosuvastatin drug, the main functional groups have wave numbers of 3551.55 cm-1, 2966.58 cm-1, 2872.80 cm-1, -C=O stretching of 1723.73 cm-1, -C-O stretching of 1118.85

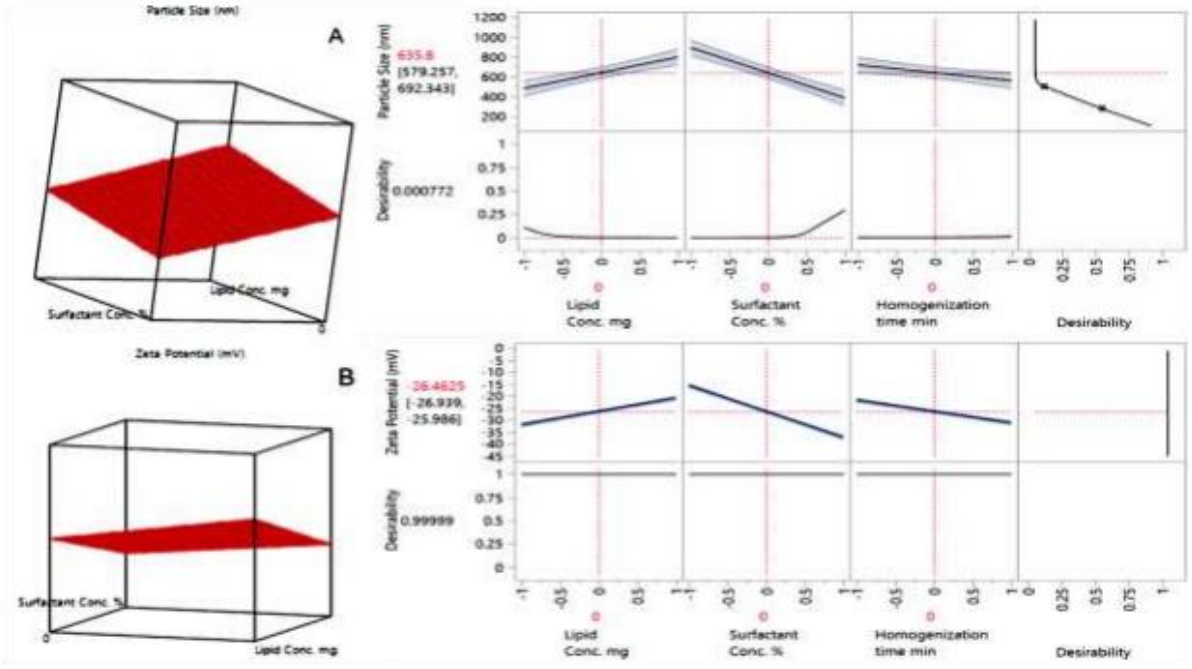
cm-1, and -C-H bending of 990.20 cm-1; –OH stretching as 3340 cm-1, 2944.50 cm-1, 2832.87 cm-1; –OH stretching as 3340 cm-1.Same functional groups are reproducible in optimized Rosuvastatin SLN formulation with stearic acid (SLN7). Fig 5.3 -5.4 the results.



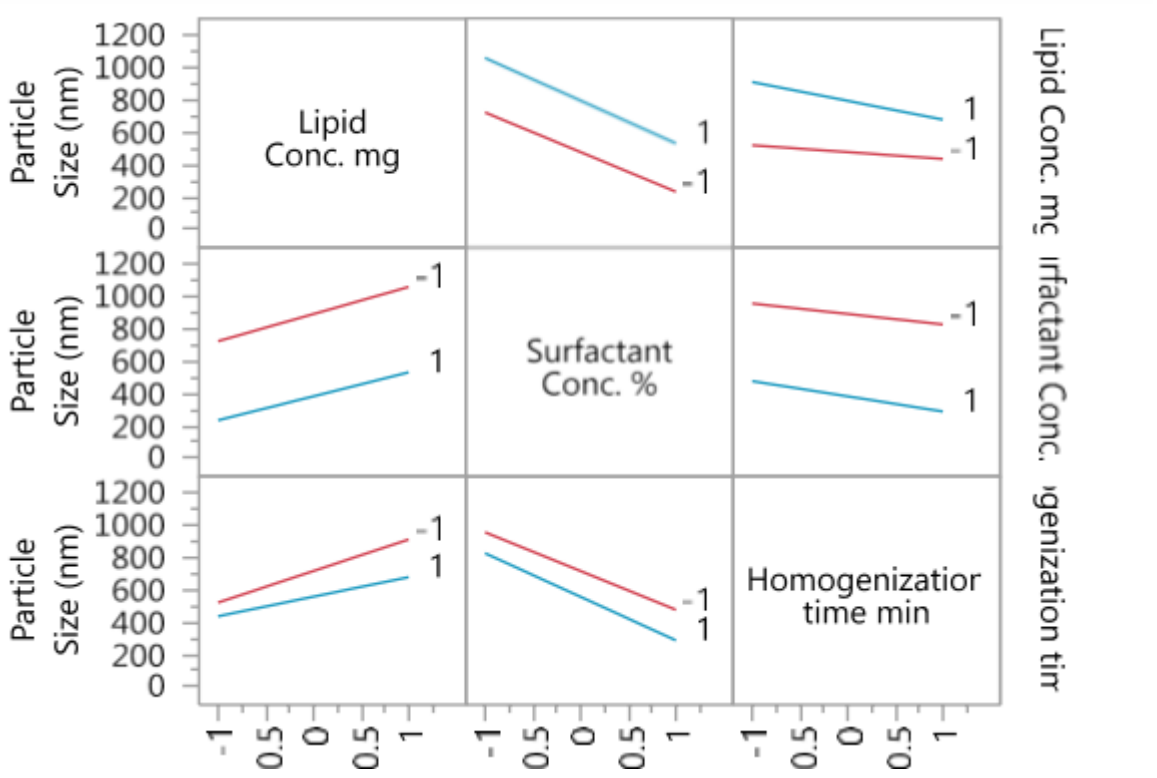
Rosuvastatin pure drugs FTIR spectra

Optimization of Rosuvastatin Solid Lipid Nanoparticle (SLN) Formulation

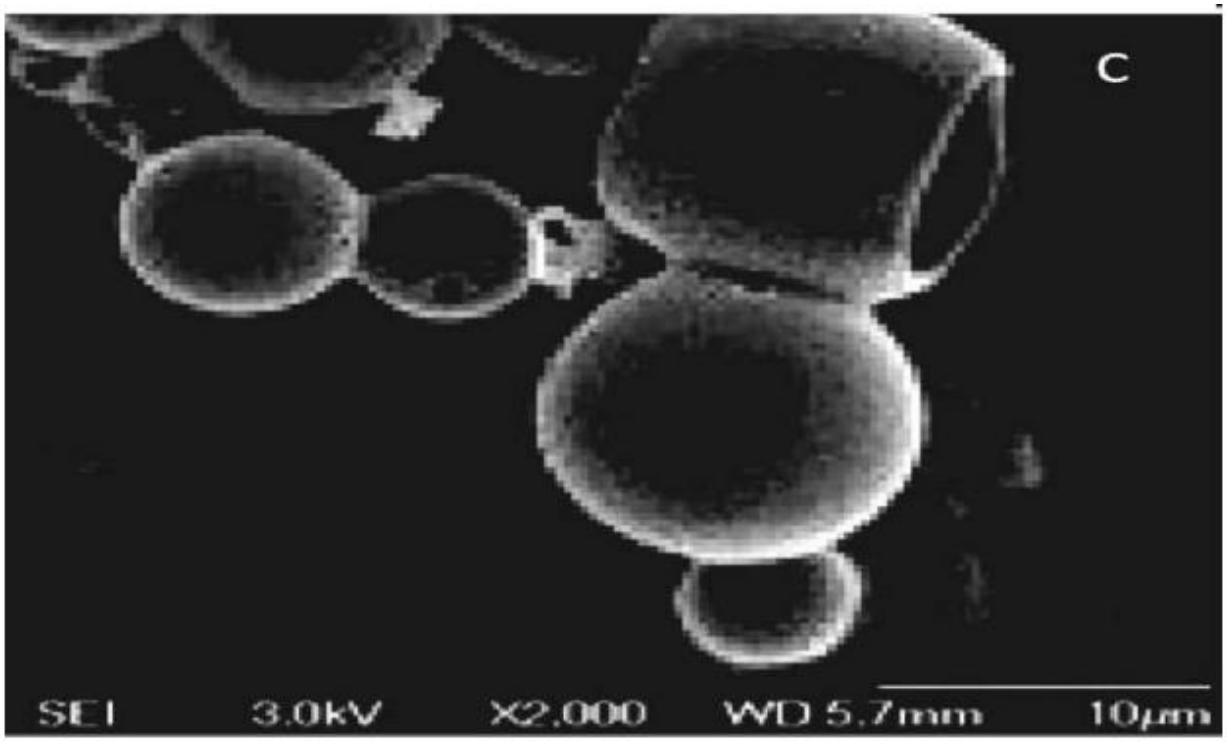
Run	CMA and CPP (Level Codes and its concentration)			CQA		Other variables		
	Factor A: Lipid Conc. Stearic acid mg	Factor B: Surfactant Conc. Span 80 %	Factor C: Homogenization time(min).	PS* Y1 nm	ZP * Y2 mV	PI*	%EE *	%Yield*
SLN1	-1 / 2.0	-1 / 1.5	-1 / 15	124.6 ± 20.2	-21.6 ± 1.12	1.248 ± 0.10	82.14 ± 2.02	82.40 ± 2.38
SLN2	1 / 5	-1 / 1.5	-1 / 15	164.4 ± 18.6	-2.4 ± 1.24	1.246 ± 0.10	84.90 ± 2.60	80.96 ± 2.34
SLN 3	-1 / 2.0	1 / 1.0	-1 / 15	215.8 ± 22.4	-39.6 ± 1.22	0.834 ± 0.12	70.24 ± 2.42	82.10 ± 2.40
SLN 4	1 / 5	1 / 1.5	-1 / 15	165.3 ± 20.2	-25.4 ± 1.26	0.728 ± 0.12	72.34 ± 2.40	72.54 ± 2.42
SLN 5	-1 / 2.0	-1 / 0.5	1 / 30	231.1 ± 24.6	-24.4 ± 1.24	0.686 ± 0.12	84.66 ± 2.42	86.42 ± 2.46
SLN 6	1 / 5	-1 / 0.5	1 / 30	158.2 ± 26.2	-15.2 ± 1.26	0.674 ± 0.10	70.44 ± 2.50	80.64 ± 2.78
SLN 7	-1 / 2.5	1 / 1.0	1 / 30	120.9 ± 30.2	-42.5 ± 1.22	0.424 ± 0.12	96.42 ± 2.84	91.42 ± 2.18
SLN 8	1 / 5	1 / 1.0	1 / 30	295.6 ± 10.2	-40.6 ± 1.32	0.542 ± 0.10	86.42 ± 2.84	74.64 ± 2.64



Contour profiler and Lipid concentration effect, concentration of surfactant and time of homogenization on (A) particle size and (B) potential Zeta



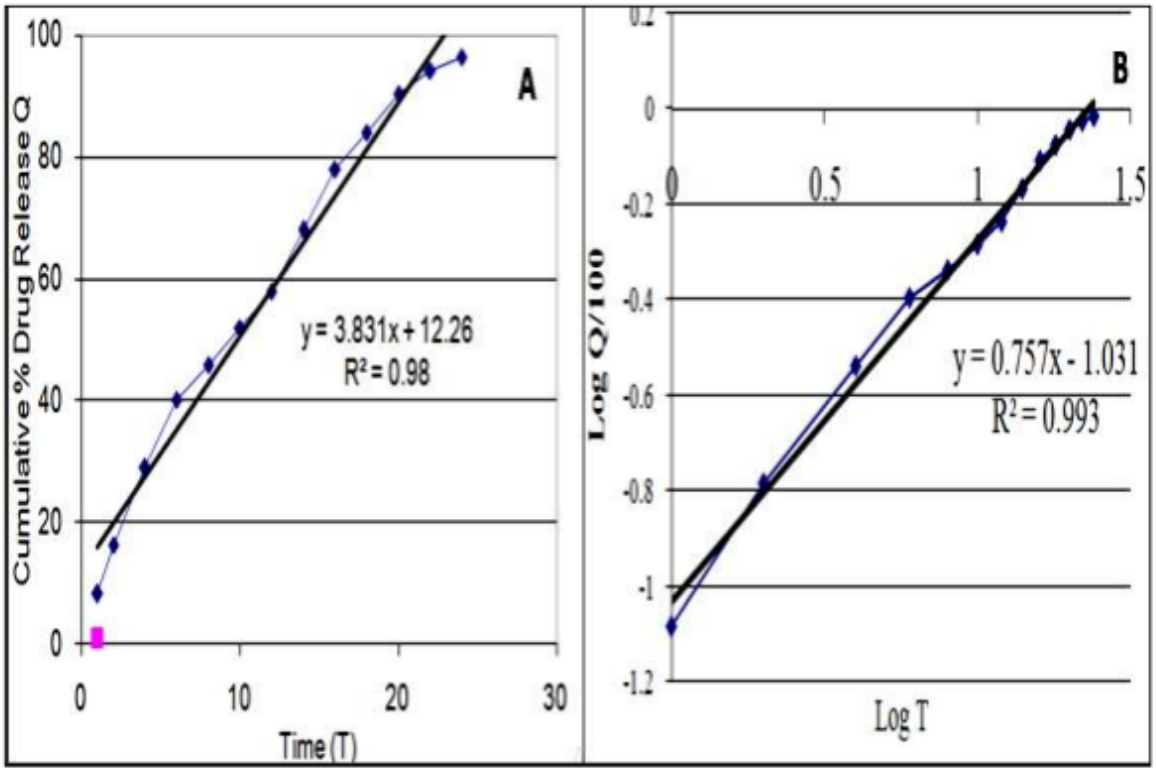
Interaction Profile which shows the interation of CMAs and CPPs on CQA (PS)



SEM images of Optimized Solid Lipid Nanoparticle (SLN7)

Invitro drug release and invitro release kinetics studies

The percentage amount of studies on drug release performed for the optimized formulation of SLN7 (Fig 5.10-5.11). The drug release percentage of SLN7 was found to be 96.48± 2.40 percent in 24 hours with controlled and predetermined release pattern compared to conventional Altoprev tablet (20 mg) which was found to be 88.42± 2.83 percent at 16 hours further indicate a decrease in the pattern of drug release. The release pattern shows a best linear fit for zero order and korsmeyer peppas kinetic model with strong regression value (R2) like 0.98, 0.993 respectively, and release exponent value "n" is 0.757, falls between 0.45-0.89 for peppas kinetic model i.e. The drug diffusion mechanism is based on lipid relaxation.



***In-vitro* drug release kinetics studies of Optimized SLN7 formulation Zero order release kinetics graph; (B) Peppas release kinetics graph**

Conclusion

The outcome of the research is as follows: The objective of this research is must to improve the bioavailability of low bioavailable drugs (< 5 percent) such as Rosuvastatin in the form of an SLN carrier and also to choose the best optimized SLN formulation technique. The chosen and reproducible method for the formulation of SLN was concluded as microemulsification technique for Rosuvastatin SLN. Therefore, for poorly bioavailable BCS class II Antihyperlipidemic drugs such as Rosuvastatin Solid Lipid Nanoparticle would be a promising drug delivery system.

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