FORMULATION AND EVALUATION OF SIMVASTATIN LOADED GELATIN NANOPARTICLES

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Abstract- To formulate and evaluate polymeric nanoparticles of simvastatin. Simvastatin is used as Anti-Hyperlipidemic agent. It is chemically 3-hydroxy-3-methylglutaryl (HMG) coenzyme A reductase. It has poor solubility and 5% bioavailability. It belongs to BCS Class-II. In the present work attempts were made to prepare polymeric nanoparticles of simvastatin with 12hrs drug release rates. The nanoparticles were prepared by employing sodium alginate as polymer. Sodium alginate is naturally occurring polymer.Tween-80 and span-80 were used as hydrophilic and lipophilic surfactant respectively. Different formulations were prepared at different ratios of polymer and surfactant. The prepared at different ratios of polymer and surfactant. The prepared formulations were coded as G1-G5. All the prepared formulations were analyzed for entrapment efficiency, drug content, microscopic examination and drug release studies. By comparing the results of all formulations, G2 was found to have 75.04% entrapment efficiency, 92.89% drug content and 64%drug release in 12hrs.

IndexTerms- Simvastatin, Anti-Hyperlipidemic agent, Polymeric nanoparticles, sodium alginate, surfactants.

I. INTRODUCTION

Oral Administration of the drug is most convenient way and commonly used route for the delivery of the drugs because its advantages like of administration, High Patient compliance, Cost effectiveness etc. The major challenge in Designing the oral dosage form lies with their Poor bioavailability depends on several factors like aqueous solubility, dissolution rate, first pass metabolism, drug Permeability, Pre systemic metabolism etc. But most often cause of the low oral bioavailability is due to Poor solubility and low Permeability. Thus to overcome this problem of bioavailability the solubility of drug must be enhanced.

There are several techniques to enhance solubility there by bioavailability of the poorly soluble drugs. Novel techniques possess advantages over traditional techniques. Various novel techniques that are used to enhance the solubility are like nanoparticles, self-emulsifying drug delivery systems, solid dispersions etc. Depending upon the problems associated with the drug, the techniques that is to be employed must be chosen. Here we chose Nanoparticle drug delivery for the study of Simvastatin.

Simvastatin is used for the treatment of anti-hyperlipidemia and also used to prevent atherosclerosis-related aggravations such as heart stroke and congestive heart failure in those who are at high risk of primary hypercholesterolemia. It is effective in reducing total LDL-cholesterol as well as plasma triglycerides. Simvastatin is a potent inhibitor of 3-hydroy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase which converts HMG CoA into mevalonate, a precursor in cholesterol synthesis. Simvastatin has widely used statin for the maintenance of the Hyperlipidemia. Simvastatin is derived synthetically from the fermentation of Aspergillus terreus. Simvastatin is a BCS class II drug. It has a very low aqueous solubility, approximately Water solubility of Simvastatin is very low, approximately 75µg/ml practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract.

Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability. Improvement of the aqueous solubility in such a case is a valuable goal that leads to enhancing therapeutic efficacy. It is reported that the absolute bioavailability of simvastatin is 5% after a 40 mg oral dose. It has a short half-life of 2hrs and is practically insoluble in water . So, there is need for some novel carriers which could improve the above problems by reaching to its target site without making any adverse effects to body and can carry the drug easily and safely to its destination.

Nanoparticles are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000nm in diameter. The major advantages of nanoparticles are improved bioavailability by enhancing aqueous solubility, increasing resistance time in the body(increasing the half life for clearance/increasing the specificity for its associated receptors and targeting drug to specific location in the body).This is why nanoparticles are increasingly used in variety of applications that includes drug carrier systems to pass organ barriers such as the blood-brain barrier, cell membrane etc. Here, the solubility of Simvastatin is increased by the addition of Polymer and surfactants and reduction of particle size. However, it has a short half-life of 3hrs and is practically insoluble in water and it can be said that nanoparticles are now a day's acting as a Prolific device for drug delivery system.

The NP's were manufactured using solvent evaporation method. The NP's were optimized for the particle size, PDI, Zeta potential and Drug content. entrapment efficiency and Invitro drug release studies.

II. MATERIALS AND METHODS:

2.1 Materials

Simvastatin was a obtained as gift sample from Aurobindo Pharmaceutical Ltd. Gelatin, PVA and dialysis bag (cellophane membrane, molecular weight cut off 10000-12000 Da), purchased from Hi-Media, Mumbai. India. All other reagents and chemicals used in this study were of analytical Grade.

2.2 Preparation of Simvastatin loaded nanoparticles

In this Solvent evaporation method is best suited for the Preparation of Nanoparticles.Accurately, weighed amount of Simvastatin was completely dissolved in Acetone. The above obtained drug solution containing surfactant span-80 was injected to polymer solution containing stabilizer PVA and tween-80 under stirring at 800 rpm. Precipitation of solid drug particles occurred immediately upon mixing. And the solvent present in the formulation gets evaporated. The obtained resultant were nanoparticles.

Formulations of Polymeric nanoparticles by Solvent Evaporation method by employing Gelatin

Tab 2.1 FORMULATION TABLE

INGREDIENTS	G1	G2	G3	G4	G5
SIMVASTATIN	5MG	5MG	5MG	5MG	5MG
GELATIN	0.25gm	0.25gm	0.25gm	0.25gm	0.25gm
PVA	0.05gm	0.05gm	0.05gm	0.05gm	0.05gm
TWEEN-80	0.01ml	0.025ml	0.05ml	0.10ml	0.15ml
SPAN-80	0.01ml	0.025ml	0.05ml	0.10ml	0.15ml

Analytical method for Estimation of Simvastatin

Determination of λ max for Drug

λ max of drug was determined with drug solution in methanol. Stock solution of Simvastatin was prepared by weighing 10mg of pure drug which was transferred into 100ml Volumetric flask and dissolved in small amounts of methanol and the volume was further made up to the mark with methanol. From the above solutions appropriate dilutions were made with pH 6.8 phosphate buffer and scanned at the range of 200-400nm using UV-Spectrophotometer.

Standard graph was constructed in methanol.

Construction of calibration curve of simvastatin in pH 6.8 phosphate buffer:

Preparation of pH 6.8 phosphate buffer:

9g of potassium dihydrogen orthophosphate (KH_2PO_4) and 6.3g of di sodium hydrogen orthophosphate (NaH_2Po_4) were weighed and transferred into 1000ml volumetric flask and volume was made up to 1000ml and mixed thoroughly.

CHARACTERIZATION AND EVALUATION OF NANOPARTICLES

The prepared Polymeric nanoparticles were characterized and evaluated for the various tests,

ENTRAPMENT EFFICIENCY:

Entrapment efficiency indicates the amount of drug encapsulated in the prepared formulation. The method of choice for knowing entrapment efficiency is by separation of free drug by Ultra centrifugation, followed by quantitative analysis of the drug from the formulation. The samples was centrifuged by using ultracentrifuge at 1000rom for 40mins. The sample was collected from the supernatant liquid. The collected liquid was filtered to measure the free drug concentration after suitable dilution with a fresh phosphate buffer pH 6.8. The absorbance was measured at 238nm in UV Spectrophotometer to calculate the entrapment efficiency.

Percentage entrapment efficiency may be calculated form the following formula:

$$\textit{ENTRAPMENT EFFICIENCY} = \frac{\textit{Amount of total drug-Amount of drug in aq.phase} \times 100}{\textit{Amount of total drug}}$$

DRUG CONTENT:

1ml of prepared simvastatin loaded nanoparticles were made with 10ml of methanol and was homogenously dispersed. The further dilutions were made with phosphate buffer of pH 6.8 and the concentration of the drug was analyzed using UV-Visible spectrophotometer at 238nm to calculate the drug content.

DRUG RELEASE STUDIES:

The in-vitro drug release of Simvastatin from different nanoparticle dispersions was determined using the dialysis membrane. A Known amount of nanoparticles sample was transferred into the dialysis membrane bag and sealed. The sealed bag was them tied to the paddle and then suspended into the dissolution media pH6.8 buffer and kept at 50rpm at 37̊C ±0.5̊ C. Aliquots of 5ml were withdrawn at predetermined intervals like 1hr,2hr, 4hr,6hr, 8hr,10hr,12hr and the same was replaced with fresh buffer. Then the drug content was determined spectrophotometrically by measuring the absorbance at 238nm using the respective pH6.8 buffer as a blank, to calculate the amount of drug release from nanoparticles.

Measurement of Particle size:

The mean diameter of polymeric nanoparticles in the dispersion was determined by Malvern Nanoparticle Analyser. Before the measurement one drop of sample was taken from each selected formulation and diluted in 10ml of dispersion medium (double distilled water). Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy, is a common technique for measuring the size of particles in sun micron range. It measures Brownian motion of particles, suspended in a liquid through the changes in the intensity of light scattered from particles through time. Consequently, the slower the motion the larger the particle will be, since smaller particles are more affected by interactions with the solvent. Considering this motion, and the temperature and viscosity of the sample throughout the analysis, DLS can calculate the hydrodynamic diameter of the particle.

Measurement of Zeta Potential:

The zeta potential is a physical property, exhibited by all particles in the preparation. It was analyzed by MALVERN instrument. It is an important factor to be considered in understanding the electric double layer repulsion and it can be measured by phase analysis light scattering when the electric field is applied across an electrolyte, charged particles in preparation are attracted towards the electrode of opposite charge while viscous forces acting on the particle tend to oppose the movement. When equilibrium is reached, the particles move with constant velocity, also known as electrophoretic mobility, and the zeta potential can be measure. The magnitude of the zeta potential gives and indication of the potential stability of the system. If modulus of zeta-potential is large, the particles in preparation will tend to repel each other. Hence, there will be no tendency to agglomerate. Contrastingly, when zeta potential values are low, it means that there will be no force acting to prevent the particles coming together and agglomerate.

Differential scanning calorimetry (DSC)

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, drug+ Gelatin(G2). The study was carried out using a DSC. The samples were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min

Kinetic studies: Mathemetical Models

Different Release kinetic equations (zero-order, First order, Higuchi's equation and Korsemeyer-Peppas equation) were applied to intercept the release rate of the drug from matrix systems for the optimized formulation. The best fit with higher correlation(R^2) was calculated.

Fitting Data Into Kinetic Models

The obtained drug release data was fitted into various kinetic plots for the optimized formulation G2(zero order, First order, Higuchi and Peppas) in order to determine the order and mode of drug release from the formulation NP's.

III. RESULTS AND DISCUSSION

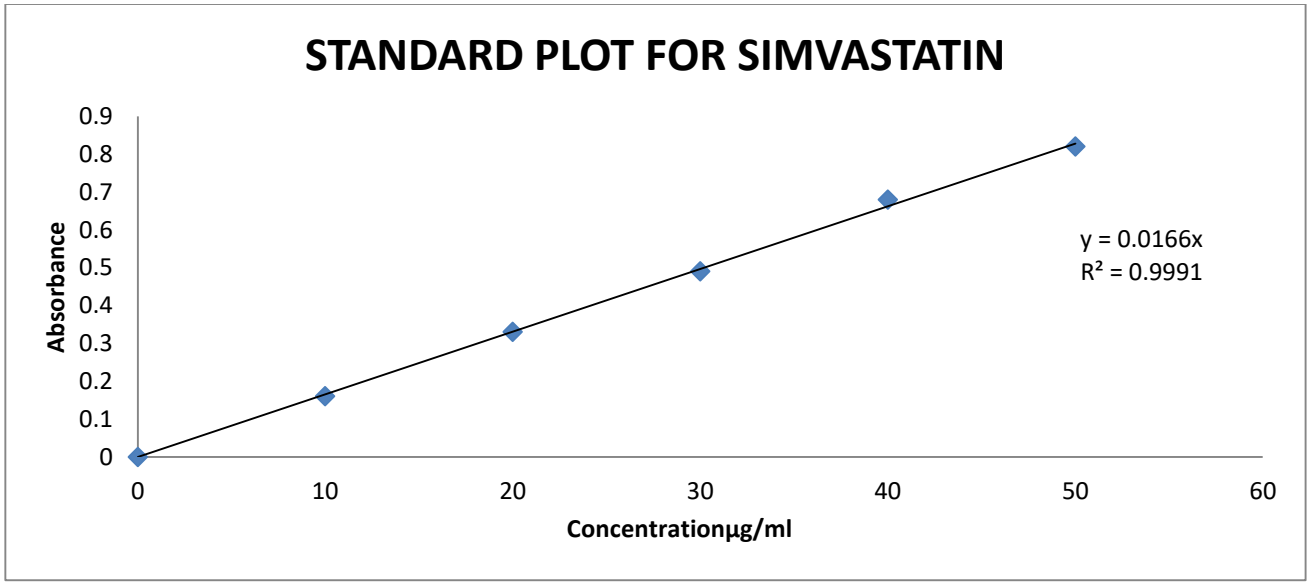

Fig 3.1 Standard Plot

SOLUBILITY STUDIES

Table 3.1: Solubility studies

Polymer/Formulation	Concentration of drug dissolved in (µg/ml)
Water	75 µg/ml
Formulation(G2)	1021.5 µg/ml

Solubility study Graph

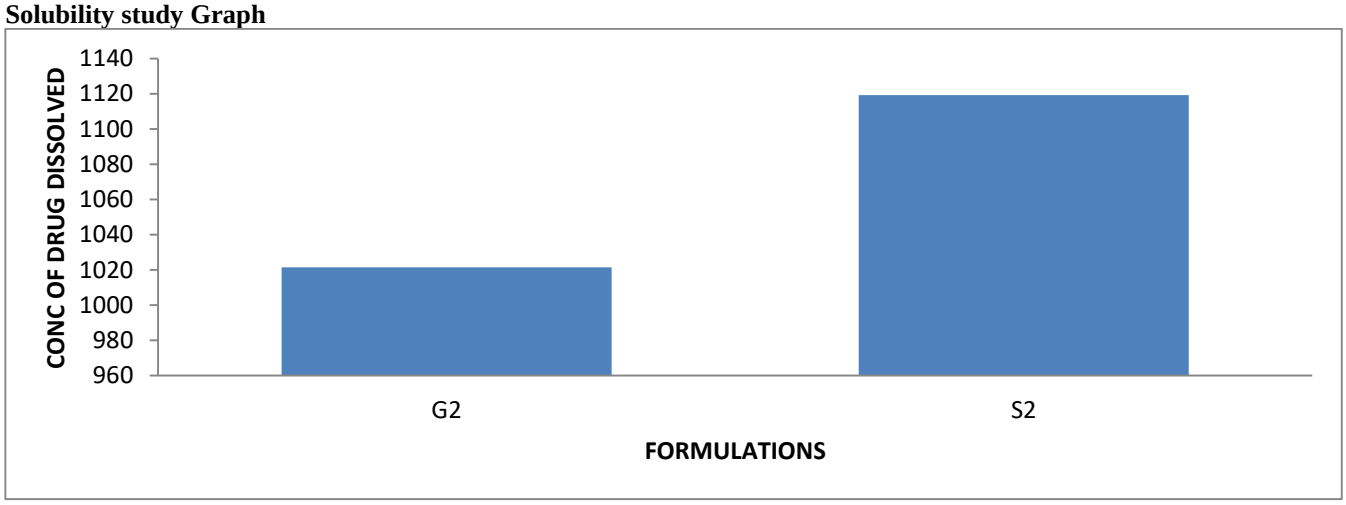

Fig 3.2 solubility graph

CHARACTERIZATION AND EVALUATION STUDIES OF SIMVASTATIN LOADED GELATIN NANOPARTICLES BY SOLVENT EVAPORATION METHOD

The five formulations were prepared by using this technique. The prepared formulations were characterized and evaluated for Drug content, Entrapment efficiency, Drug release studies, Measurement of Particle size, Measurement of Zeta potential and Stability Studies

Tab 3.2 Drug Content of Simvastatin loaded Gelatin Nanoparticles by Solvent Evaporation method

FORMULATIONS	DRUG CONTENT
G1	41.02%
G2	88.58%
G3	67.05%
G4	72.17%
G5	50.89%

All the Prepared formulations were analysed for drug content. The Drug content values range from 41.02% to 88.58%. Among all the formulations G2 has shown higher drug content value(88.58%).

Tab 3.3 Entrapment efficiencies of Simvastatin loaded Gelatin Nanoparticles by Solvent Evaporation method

FORMULATIONS	ENTRAPMENT EFFICIENCY
G1	50.6%
G2	75.04%
G3	69.7%
G4	72.17%
G5	50.89%

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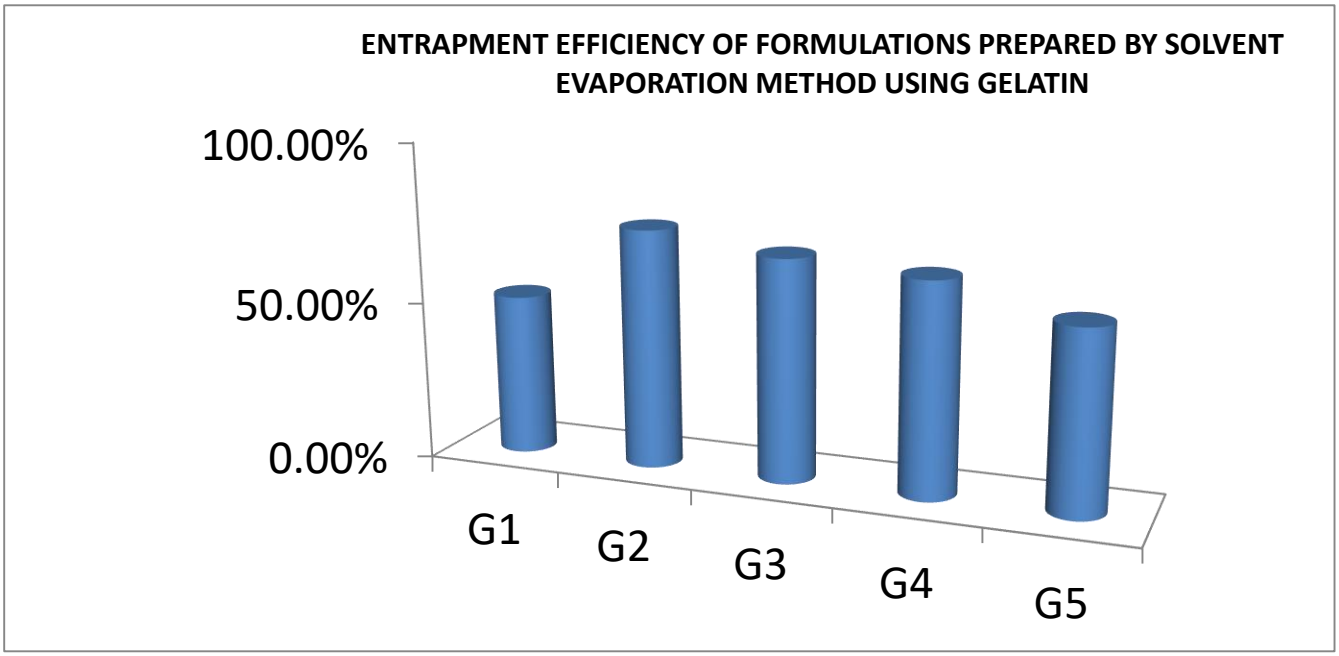


Fig 3.3 Comparison of entrapment efficiencies oh G1, G2, G3, G4 and G5 formulations of Simvastatin loaded Gelatin Nanoparticles by Emulsification Solvent Evaporation method.

All the prepared formulations were analyzed for entrapment efficiency and the values were found to be 50.6%, 75.4%, 69.7%, 72.17% and 50.89%. Among all the formulations, G2 formulation maximum amount of the drug was found to have 75.04% entrapped in the Polymer. The Polymer and the Surfactant concentrations were found to be sufficient to entrap the drug into it.

Invitro drug release studies of Simvastatin loaded gelatin nanoparticles.

Invitro drug release studies were carried by dissolution apparatus using USP II (paddle). Samples were collected at time intervals like 1,2,3,4,5,6,7,8,9,10,11,12 hours. Medium used for dissolution was pH 6.8, Temperature 37±2° C, rpm of 50 at wavelength of 238 nm.

Tab 3.4 INVITRO DRUG RELEASE OF SIMVASTATIN LOADED GELATIN NANOPARTICLES

TIME	G1	G2	G3	G4	G5	PURE DRUG
1HR	19.3%	9.69%	12.0%	2.6%	7.0%	4.6%
2HR	20.76%	12.46%	14.09%	3.9%	19.6%	7.15%
4HR	23.71%	19.15%	19.20%	8.6%	20.2%	12.46%
6HR	25.15%	36.69%	24.4%	10.6%	23.6%	18.9%

8HR	28.16%	48.46%	29.38%	12.92%	26.72%	20.86%
10HR	32.12%	57.69%	31.10%	15.4%	29.03%	22.13%
12HR	45.23%	64%	47.23%	30.50%	50.32%	26.16%

In vitro drug release studies were performed for a period of 24 hrs. The Percentage drug release among all the prepared formulations was calculated. It ranges from30.50% to 64%. Among all, G2 formulation has shown 64% drug release in 12hrs which is in accordance with entrapment and drug content values.

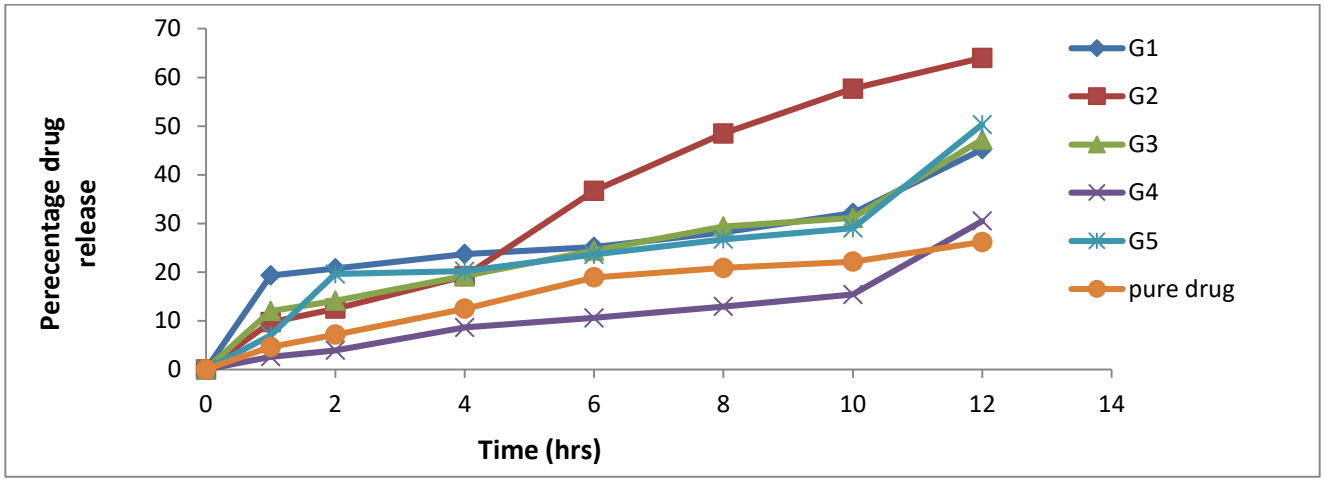


Fig 3.4 Comparison of Invitro drug release of pure drug and formulations of simvastatin loaded gelatin nanoparticles by solvent evaporation method

CHARACTERIZATION OF OPTIMISED FORMULATIONS FOR INSTRUMENTAL ANALYSIS

Among all the prepared formulations, the optimized formulation was found to be G2 Formulation as it has shown better results for drug content, entrapment efficiency and Percentage drug release than other formulations.

It was further analysed by instrumental methods including Particle size determination, zeta potential, DSC studies and These were further applied to the kinetic models.

DETERMINATION OF PARTICLE SIZE:

Among the five Prepared formulations, the particle size of the G2 was considered as the best formulation with the particle size of 149.3 nm. Particle size was determined by Malvern nanoparticle analyzer. Thus it was observed that formulation was found to be in nano range.

Results D(0.1): 15.7 nm D(0.5): 23.8 nm D(0.9): 107 nm

		Diam. (nm)	% Volume	Width (nm)
Z-Average (d.nm):	149.3			
PdI:	0.289	Peak 1:	140.5	21.7	109.5
Intercept:	0.890	Peak 2:	23.44	78.3	8.177
Result quality :	Good	Peak 3:	0.000	0.0	0.000

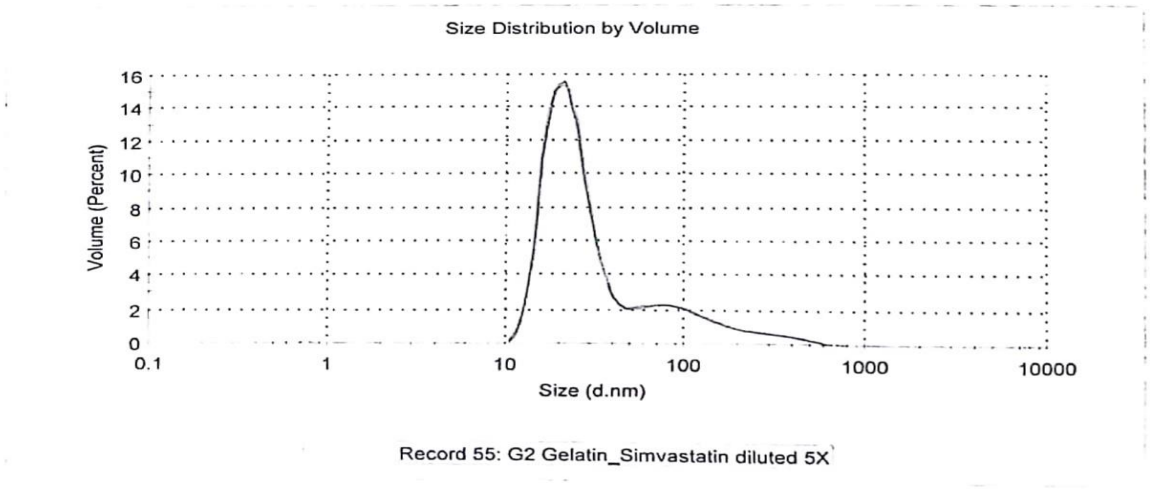


Fig 3.5 Particle size of G2 formulation of simvastatin loaded nanoparticles

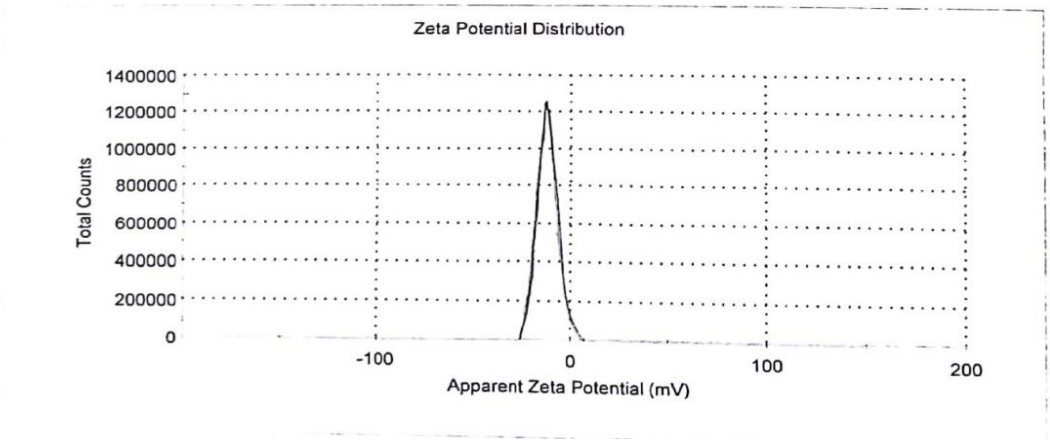
The average Particle size was found to be 149.3nm with PDI of 0.289.

DETERMINATION OF ZETA POTENTIAL

The zeta potential value indicated the stability of nanoparticles. It was determined by Malvern nanoparticle analyzer. And the best formulation G2 showed the zeta potential value of -11.6mV.Thus it was found that the formulation was stable.

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-11.6			
Zeta Deviation (mV):	4.85	Peak 1:	-11.6	100.0	4.85
Conductivity (mS/cm):	0.154	Peak 2:	0.00	0.0	0.00
Result quality :	Good	Peak 3:	0.00	0.0	0.00



Zeta potential report of G2 formulation of Simvastatin loaded gelatin

DSC: Thermal analysis of DSC gives information on the processes of melting, crystallization, decomposition or change of crystalline phase. DSC thermogram of simvastatin has an endothermic peak at 138.9 °C which corresponds to the melting point of simvastatin.

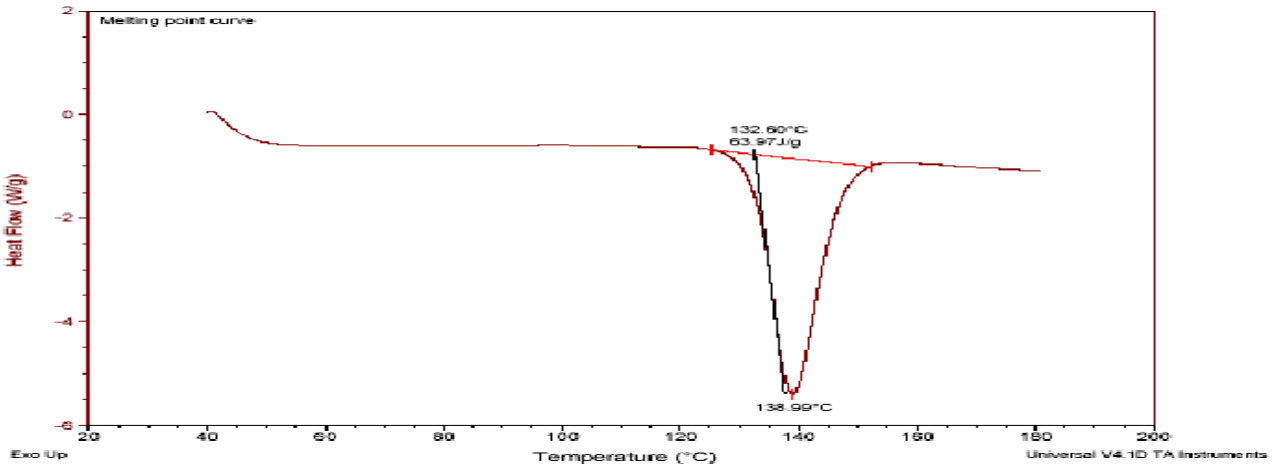


Fig 3.5 DSC thermogram of Pure Drug

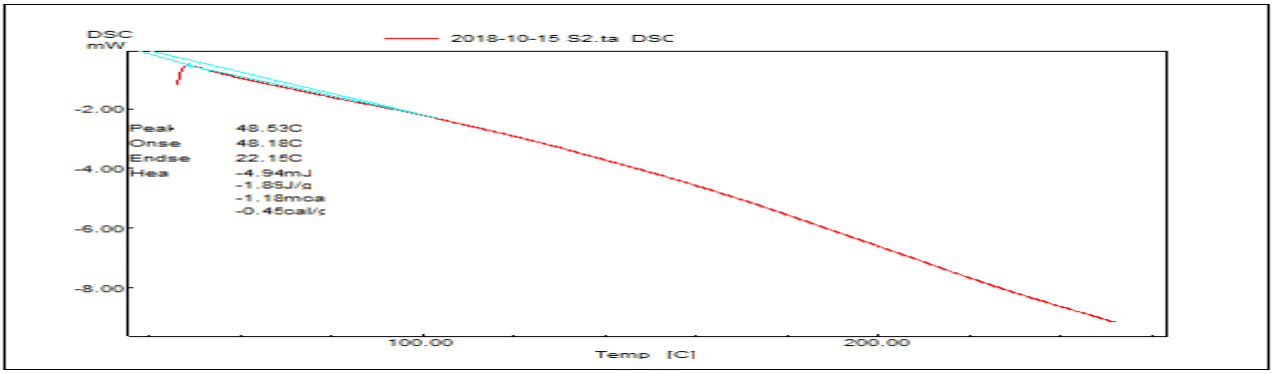


Fig 3.6 DSC Thermogram of Formulation G2

While DSC thermograms of Gelatin and sodium alginate does not exhibit an endothermic peak, In the above thermogram, the endothermic peak was disappeared, which suggest that the drug was entrapped entirely in the polymer.

In Vitro Release Kinetic data of Simvastatin loaded Gelatin(G2) nanoparticles by solvent evaporation method

Tab 3.5 Kinetic Data

FORMULATION	ZERO ORDER PLOT (R^2)	FIRST ORDER PLOT (R^2)	HIGUCHI PLOT(R^2)	PEPPAS PLOT (n)
G2	0.981	0.975	0.935	0.822

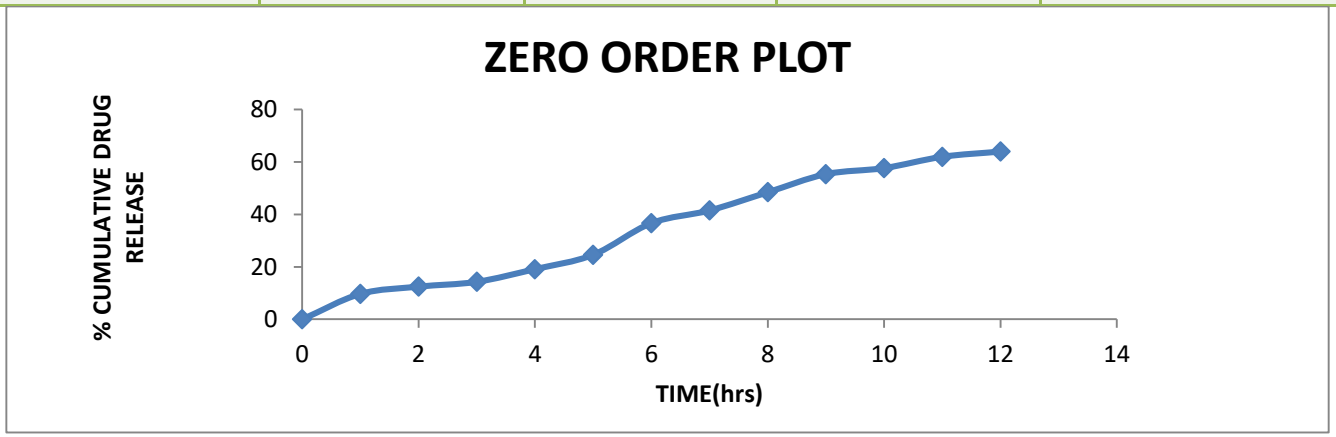


Fig 3.7 zero order Plots of simvastatin loaded gelatin G2 nanoparticles

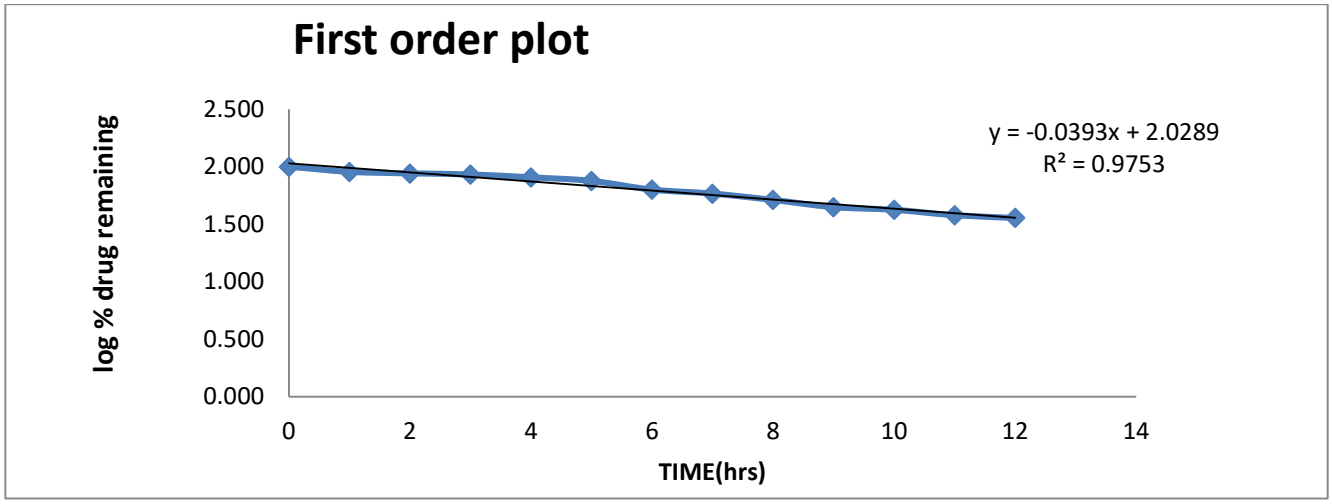


Fig 3.8 First order plots of G2 of simvastatin loaded gelatin nanoparticles

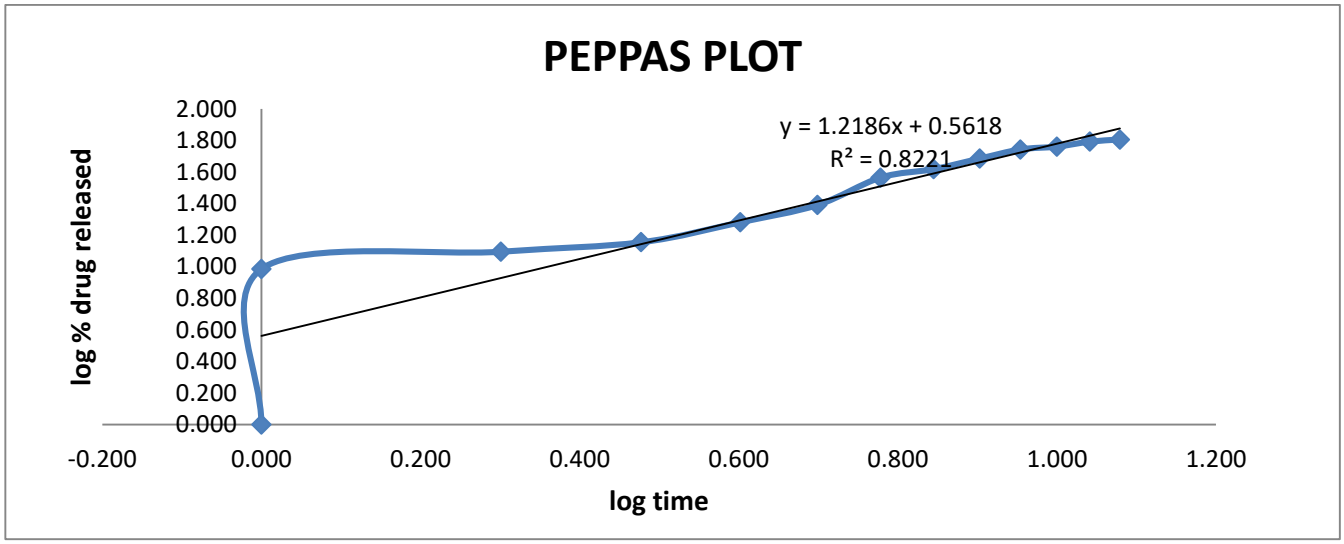


Fig 3.9 Peppas plot of G2 of simvastatin loaded gelatin nanoparticles

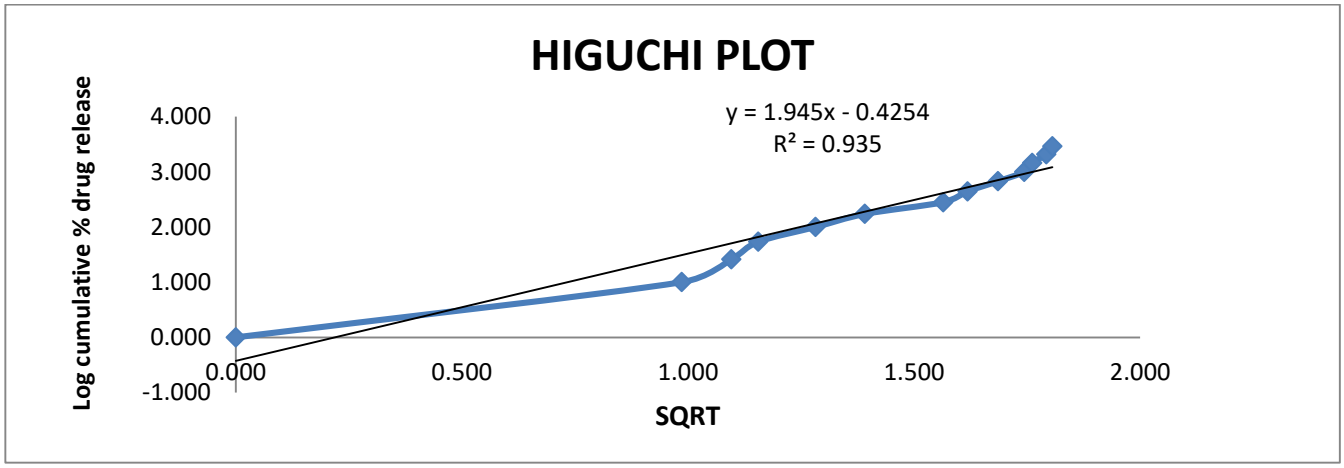


Fig 3.10 Higuchi Plot of G2 of simvastatin loaded gelatin nanoparticles

According to the data fit in the kinetic plots, it revealed that the formulation were following Zero order kinetics with non-fickian diffusion.

CONCLUSION

In the Present research, the different formulations were prepared by using Gelatin, By employing Solvent evaporation method, it can be concluded that simvastatin can be successfully Prepared as Nanoparticles. Among all the formulations G2 has shown the better results in terms of Drug content, Entrapment Efficiency, Drug release, Particle size and Zeta potential when Compared to the other formulations.

The entrapment efficiency was found to be 75.04%, Drug content 88.58% , In-vitro drug release was 64% and the formulation was found to have zero order release kinetics with non-fickian diffusion.

Solvent evaporation method was found to be the best method as Particle size obtained was small, with high entrapment efficiency value which may be because of better association of surfactant with Polymer Particles. This method was found to be simple, cost effective, easy and suitable to Produce NP's. Furthermore it could be Presumed that the obtained nanoparticles might increase the bio-availability. Hence we can conclude that Polymeric nanoparticles with sodium alginate Provide controlled release of drug and these systems are used as drug carriers for lipophilic drugs, to enhance the solubility there by oral bio-availability of Poorly water soluble drugs through these nanoparticles, as a drug delivery system.

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