FORMULATION AND EVALUATION OF SIMVASTATIN LOADED POLYMERIC NANOPARTICLES

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ABSTRACT

AIM:-To formulate and evaluate polymeric nanoparticles of simvastatin. The aim of the present study is 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 Gelatin and sodium alginate as polymers. Gelatin and 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) with Gelatin and (S1-S6) with Sodium alginate. 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 88.58% drug content, 79.46% entrapment efficiency and the In-vitro release was found to be 64% and S5 was found to have 75.04% entrapment efficiency, 92.89% drug content and 68% drug release in 12hrs.

KEYWORDS: Simvastatin, Anti-hyperlipidemic agent, Polymeric nanoparticles, Gelatin, sodium alginate, surfactants.

1. 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.

MATERIALS AND METHODS
2.1 Materials
Simvastatin was a obtained as gift sample from Aurobindo Pharmaceutical Ltd. Sodium alginate, 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.

METHOD
Emulsification Solvent Evaporation Technique For Preparation Of Polymeric Nanoparticles
In this method, Simvastatin nanoparticles were prepared by using solvent evaporation technique which is an effective technology in preparation of Simvastatin 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 are nanoparticles.

Formulations of Polymeric nanoparticles by Solvent Evaporation method by altering polymer to surfactant ratio

I.   Formulation Table Using Gelatin

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

II. Formulation Table Using Sodium Alginate

Ingredients	S1	S2	S3	S4	S5	S6
Simvastatin	5mg	5mg	5mg	5mg	5mg	5mg
Sodium Alginate	0.05g	0.1g	0.15g	0.1g	0.1g	0.1g
Pva	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g
Tween-80	0.05ml	0.05ml	0.05ml	0.1ml	0.15ml	0.2ml
Span-80	0.05ml	0.05ml	0.05ml	0.1ml	0.15ml	0.2ml

Characterization and Evaluation of Polymeric 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 is calculated form the following formula:

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

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 pH65.8 buffer and kept at 50rpm at 37°C ±0.5° 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 290nm 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. 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) and drug +sodium alginate(S5). The study was carried out using a DSC. The samples were heated in a hermetically sealed aluminum pans in the temperature range of 25-300°c at heating rate of 10°c /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.
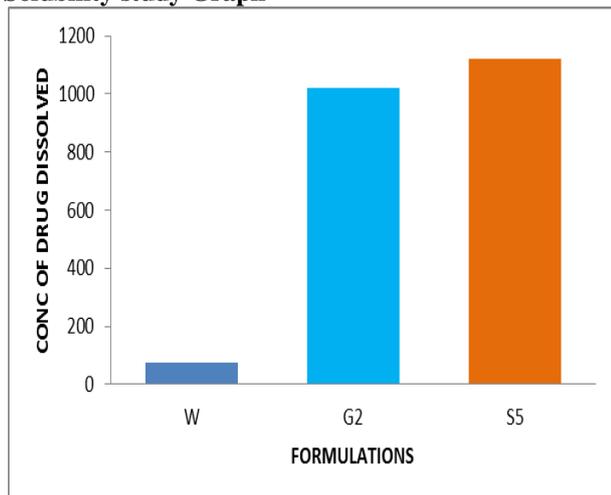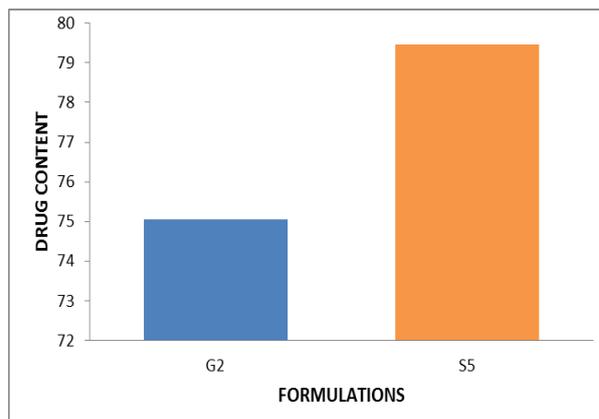
RESULTS AND DISCUSSIONS

STANDARD PLOT FOR SIMVASTATIN

$y = 0.0166x$
$R^2 = 0.9991$
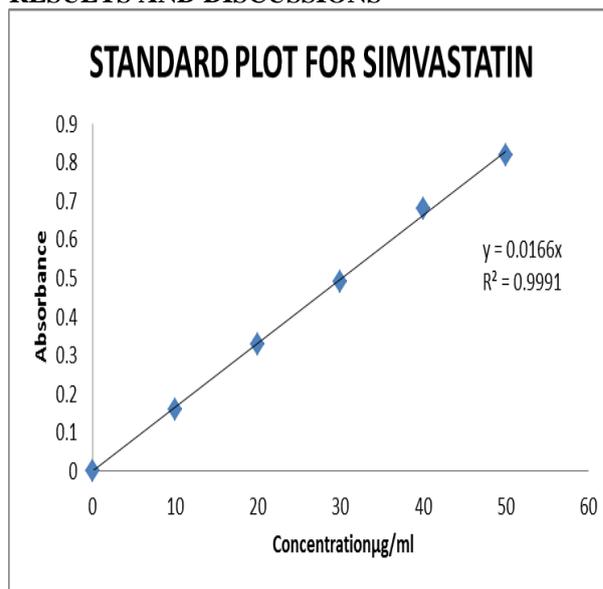
Solubility Studies
Table Solubility studies.

Polymer/Formulation	Concentration of drug dissolved in (µg/ml)
Water	75 µg/ml
Formulation(G2)	1021.5 µg/ml
Formulation(S2)	1119.2 µg/ml

Solubility study Graph

Solubility Graph

CHARACTERIZATION AND EVALUATION STUDIES
The five formulations were Prepared by using Gelatin and six formulations were Prepared by using Sodium Alginate. The prepared formulations were characterized and evaluated for Drug content, Entrapment efficiency, Drug release studies, Measurement of Particle size, Measurement of Zeta potential, DSC and Stability Studies.

DRUG CONTENT
Drug Content of Simvastatin loaded Gelatin Nanoparticles.

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

Drug Content of Simvastatin loaded Sodium alginate Nanoparticles.

FORMULATIONS	DRUG CONTENT
S1	
S2	88.84%
S3	87.05%
S4	50%
S5	92.89%
S6	69.87%

All the Prepared formulations were analysed for drug content. The Drug content values of Gelatin ranges from 41.02% to 88.58% and for Sodium alginate Ranges from 50% to 92.89%. Among all the formulations S5 has shown higher drug content value(92.89%).

Comparision of drug content values from both the polymers(g2 and s5).

ENTRAPMENT EFFICIENCY
Entrapment efficiencies of Simvastatin loaded Gelatin Nanoparticles

| Formulations	Entrapment efficiency
G1	50.6%
G2	75.04%
G3	69.7%
G4	72.17%
G5	50.89%

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.

Entrapment efficiencies of Simvastatin loaded Sodium alginate Nanoparticles.

Formulations	Entrapment efficiency
S1	46.94%
S2	75%
S3	65.78%
S4	55.12%
S5	79.46%
S6	34.05%

All the prepared formulations were analyzed for entrapment efficiency and the values were found to be 49.64%, 75%, 65.78%, 55.12%,79.46% and 34.05%. Among all the formulations, S5 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.

From the above, The Entrapment efficiencies of Gelatin ranges from 50.6% to 75.04% and for Sodium alginate Ranges from 34.05% to 79.46%. Among all the formulations S5 has shown higher Entrapment efficiency value(92.89%).
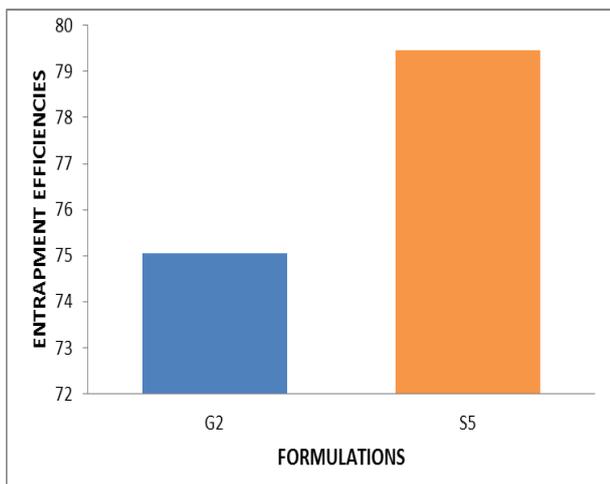
Comparision of entrapment efficiencies of both the polymers(g2 and s5).

Drug Release Studies
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.

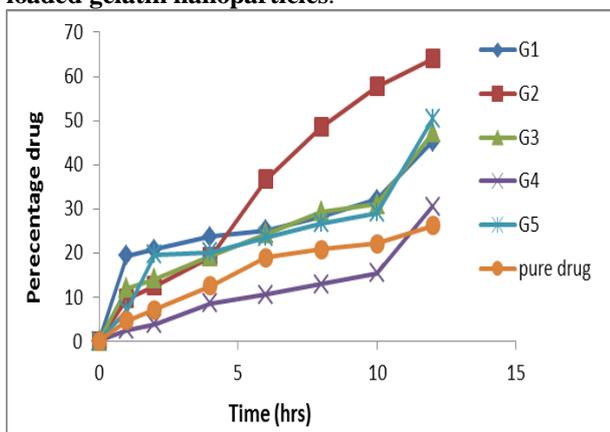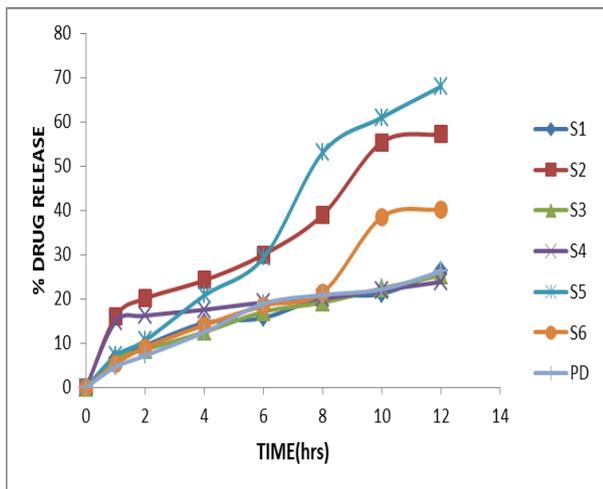**Invitro Drug Release Of Simvastatin Loaded Gelatin Nanoparticles**

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.

Comparison of In vitro drug release of simvastatin loaded gelatin nanoparticles.

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

Invitro Drug Release Of Simvastatin Loaded Sodium Alginate Nanoparticles With Pure Drug

TIME	S1	S2	S3	S4	S5	S6	PURE DRUG
1HR	6.46%	16%	6.46%	14.76%	7.15%	5.30%	4.6%
2HR	9.46%	20.07%	8.53%	16.16%	10.6%	9%	7.15%
4HR	14.53%	24.18%	12.48%	17.53%	20.76%	14.07%	12.46%
6HR	15.69%	30%	17.07%	19.15%	29.30%	18.46%	18.9%
8HR	20.3%	39%	19.14%	20.07%	53.07%	21.46%	20.86%
10HR	21.16%	55.38%	22.38%	21.92%	61%	38.5%	22.13%
12HR	26.28%	57.23%	25.28%	23.81%	68%	40.20%	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 from26.28% to 68%. Among all, S5 formulation has shown 68% drug release in 12hrs which is in accordance with entrapment and drug content values.

Comparison of Invitro drug release of pure drug and formulations of simvastatin loaded Sodium alginate nanoparticles by solvent evaporation method
From the above Formulations, the GelatinG2 shows higher value(64%) and Sodium alginate S5 shows higher value(68%) were selected and compared respectively, and S5 shows higher drug release when compared to GelatinG2.
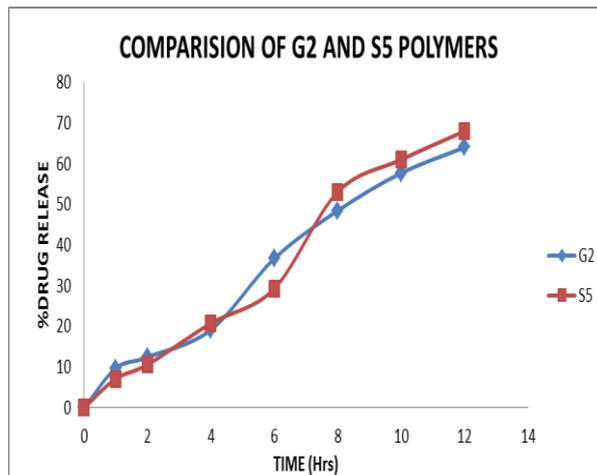
Characterization Of Optimised Formulations For Instrumental Analysis
Among all the prepared formulations, the optimized formulation was found to be S5 Formulations as it has shown better results for drug content, entrapment efficiency and Percentage drug release than other formulations.
It was further 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
The particle size of the S5 was considered as the best formulation with the particle size of 766.8nm. Particle size was determined by Malvern nanoparticle analyzer. Thus it was observed that formulation was found to be in nano range.
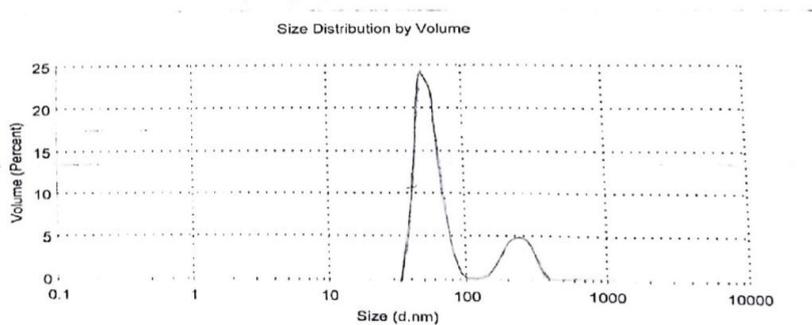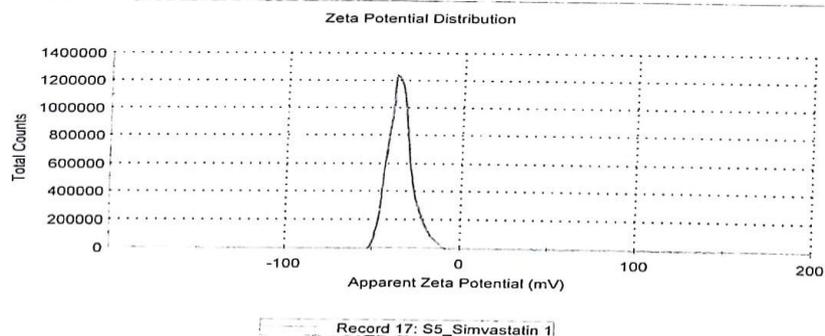

Particle size of S5 formulation of simvastatin loaded nanoparticles
The average Particle size was found to be 149.3nm with PDI of 1.000

Determination Of Zeta Potential
The zeta potential value indicated the stability of nanoparticles. It was determined by Malvern nanoparticle analyzer. And the best formulation S5 showed the zeta potential value of -35.6mV.Thus it was found that the formulation was stable.

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -35.6	Peak 1:	-35.6	100.0	6.72
Zeta Deviation (mV): 6.72	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0744	Peak 3:	0.00	0.0	0.00
Result quality : Good				

Zeta Potential Distribution

Record 17: S5_Simvastatin 1

Zetasizer Ver 7.12
Serial Number : MAL500978

**Zeta potential report of S5 formulation of Simvastatin loaded Sodium alginate**

From the above Zeta Potential value, the S5 Was found to be -35.6mV, which indicates that Stable Formulation.
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.
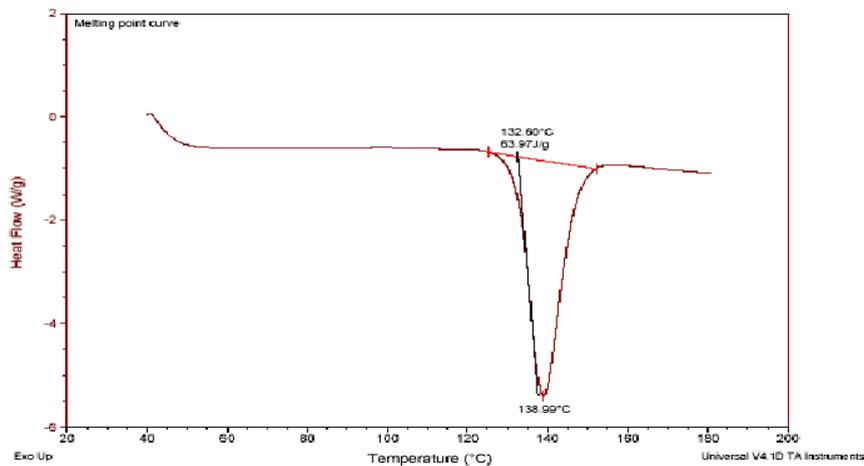
**DSC thermogram of Pure Drug**
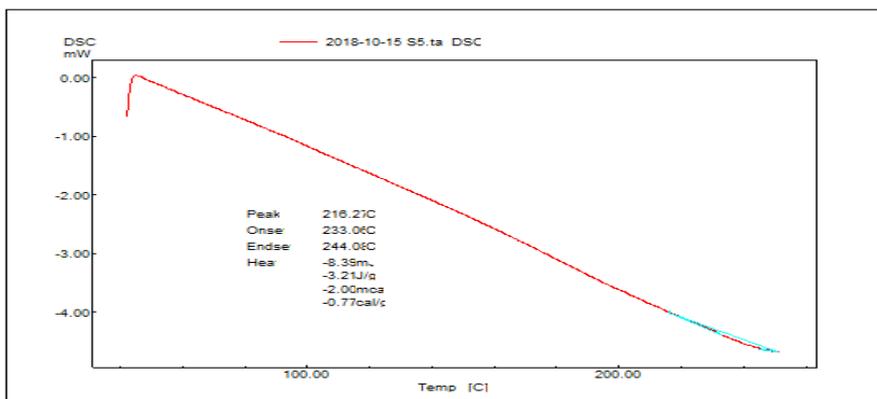
DSC Thermogram of Formulation S5

While DSC thermograms of sodium alginate doesn't 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 sodium alginate(S5) nanoparticles by solvent evaporation method**

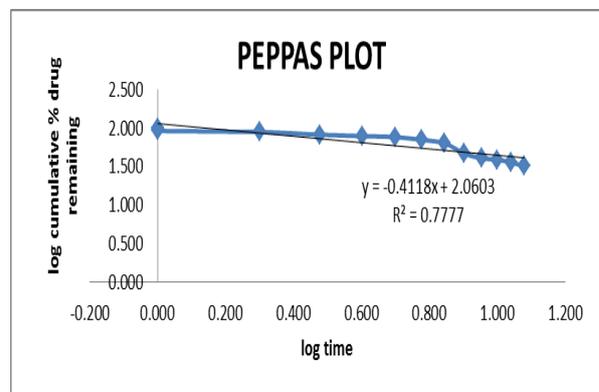| Formulation	Zero order plot (r^2)	First order plot (r^2)	Higuchi plot (r^2)	Peppas plot (n)
S5	0.972	0.947	0.8758	0.777
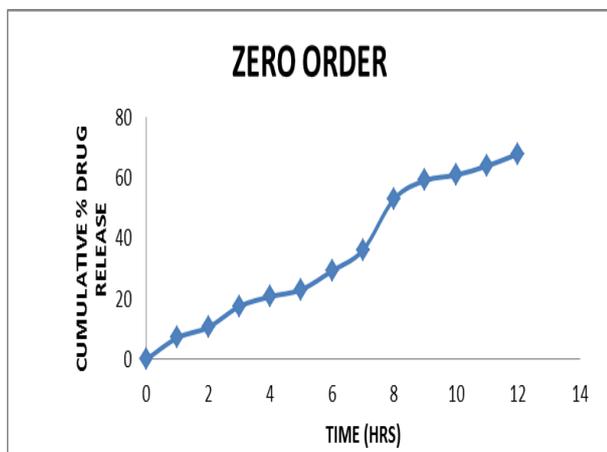
Zero order Plot of S5 simvastatin loaded Sodium alginate nanoparticles
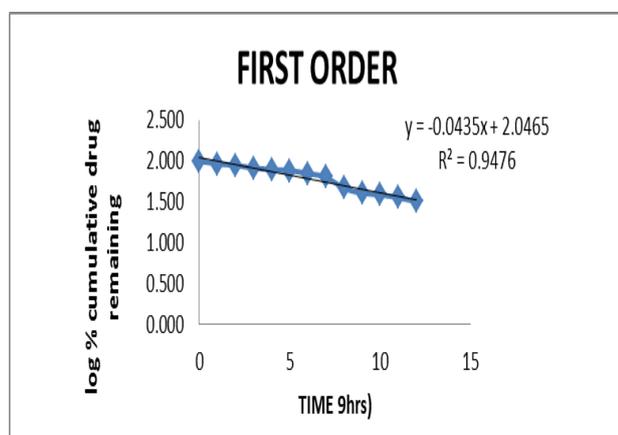
First order plot of S5 simvastatin loaded Sodium alginate nanoparticles
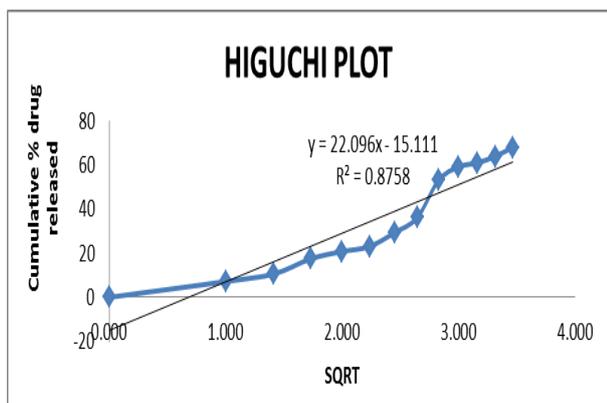
Higuchi plot of S5 simvastatin loaded Sodium alginate nanoparticles

Peppas Plot of S5 simvastatin loaded Sodium alginate 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 and Sodium Alginate, By employing Solvent evaporation method, it can be concluded that simvastatin can be successfully Prepared as Nanoparticles. Among all the formulations S5 has shown the better results in terms of Drug content, Entrapment Efficiency, Drug release, Particle size and Zeta potential when Compared to the other polymer formulations.

The entrapment efficiency was found to be 79.46%, Drug content 92.89%, In-vitro drug release was 68% 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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