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DEVELOPMENT AND CHARACTERIZATION OF NANOPARTICLES LOADED WITH PRAVASTATIN FOR THE TREATMENT OF ATHEROSCLEROSIS

S. Inchara*[1], A. R. Shabaraya[1], T. Bhavyashree[1]

[1*]Srinivas College of Pharmacy, Valachil, Mangalore, Karnataka, India.

ABSTRACT

Nanoparticles are described as particulate dispersion, or solid particles with a 10-1000nm size range. Nanoparticles are colloidal sub nanosized structures composed of synthetic and semisynthetic polymers. In the present work designing a control release formulation for the drug pravastatin prolongs the drug's therapeutic concentration in the blood and decreases the dosing frequency and also improves the drug's effectiveness and compliance with patients. The main aims of this work were to design and develop Pravastatin nanoparticle for the cholesterol treatment. The formulations were evaluated for various physiochemical parameters. FT-IR study confirmed the drug-polymer compatibility. Zeta potential of formulation was found negative indicating the stability of the nanoparticles. SEM photographs of formulations F3 revealed that nanoparticles were discrete particles with rough, rigid surface. The drug content was found to be in the range of 36.29±0.48 to 44.70±0.74 and the entrapment efficiency in the range of 72.41±0.62 to 89.59±0.41. The % cumulative drug release of the formulation ranged from 71.36% to 89.49%. Stability study was performed on the optimized formulations by storing the samples at 25±2ºC and 65 ± 5% RH and 40±2ºC and ± 5% RH with 96.32±5% RH for 60 days and was found to be stable.

KEYWORDS

Ionotropic gelation method, Pravastatin, Chitosan and Anti-cholesterol.

Author for Correspondence:

Inchara S,
Srinivas College of Pharmacy,
Valachil, Mangalore, Karnataka, India.

Email: incharashetty22@gmail.com

INTRODUCTION

To improve the efficacy of the treatment during last two decades, considerable attention has been given to the development of novel drug delivery system (NDDS). Nanoparticles have been used as a functional tool to modify and improve the pharmacokinetic and pharmacodynamic properties of different types of drug molecules. They were made from a different polymer, which improves the

therapeutic effect and decreases the side effect. Nanoparticles in the range of 10-1000nm are known as particulate dispersions or solid particles. The drug is dissolved, trapped in, encapsulated or bound to a layer of nanoparticles. Nanoparticles, nanospheres, or nano capsules can be collected, depending on the preparation process[1]. The system may be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc[2].

Pravastatin is an HMG-CoA reductase inhibitor used to lower lipid levels and to reduce the risk of cardiovascular events, including atherosclerosis, myocardial infarction and stroke. Pravastatin is a specific inhibitor of the hepatic HMG-CoA reductase in humans[3]. The inhibition of this enzyme produces a reduction in cholesterol biosynthesis as HMG-CoA reductase activity is an early-limiting step in cholesterol biosynthesis[4].

MATERIAL AND METHODS

Pravastatin was obtained from Yarrow Chem Products, Mumbai. Chitosan was obtained from Yarrow Chem. Products, Mumbai. Sodium tripolyphosphate was obtained from Loba Chemie, Mumbai.

All the other reagents and chemicals used are of analytical grade.

Methodology

Different concentration of chitosan ranging from 0.5g to 3g of chitosan was dispersed in 25ml of dilute acetic acid solution (0.2%v/v) and stirred for 2hours continuously to obtain chitosan gel. Then it was stabilized overnight to obtain clear chitosan gel. Pravastatin is added to the prepared chitosan solution. Sodium tri polyphosphate solution was prepared in 50ml distilled water to get 0.3% solution. Sodium tri polyphosphate solution was added drop wise with a syringe to chitosan solution while stirring (800rpm) and allowed to stand for 30 minutes. The nanoparticles formed were collected and dried at room temperature.

Evaluation of nanoparticles

Percentage yields[5]

Dried nanoparticles were collected and weighed to determine percentage yield (PY) from the following equation:

Percentage yield = Practical yield *100/ Theoretical yield

Particle size analysis[6]

The particle size was determined by dynamic light scattering, using a Malvern system, with vertically polarized light supplied by an argon-ion laser (Cyonics) operated at 40mW. Experiments were performed at a temperature of 25.0 ± 0.1ºC at a measuring angle of 90º to the incident beam.

Zeta potential[7]

Zeta potential was performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano ZS (Malvern instruments, UK) at 25°C. The diluted nanoparticle dispersion was poured into the disposable sizing cuvette which was then placed in the cuvette holder of the instrument and analyzed. Air bubbles, if any are removed from the capillary before measurement.

Surface morphology study[8]

Scanning electron microscopy (SEM) of drug loaded chitosan nanoparticle was performed to examine the surface morphology. The nanoparticles were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope under magnification of 20kv×25000.

Drug content[9]

Prepared nanoparticles were accurately weighed (equivalent to 40mg of Pravastatin) and suspended in 6.8 pH Phosphate buffer. After appropriate dilution with 6.8 pH Phosphate buffer, the drug content was analyzed by measuring absorbance in UV spectrophotometer at 239nm using 7.4 pH Phosphate buffer as blank. Amount of drug present in the formulation can be determined by the following formula;

Amount of drug = Concentration X DF /1000

Entrapment efficiency

The entrapment efficiency was determined by centrifugation method. Prepared nanoparticles were dispersed in 10ml of 6.8 pH phosphate buffer. The sample dispersion was centrifuged at 50,000rpm for 30min at 4ºC. The supernatant was collected and properly diluted using buffer and assayed spectrophotometrically.

$$EE\% = \frac{\text{Total drug - free drug}}{\text{Total drug}} \times 100$$

***In-vitro* Drug Release study**[10]

In-vitro dissolution study on solid nanoparticles (equivalent to 40mg) filled in capsules were carried out using dissolution test apparatus type I by using pH 6.8 phosphate buffer solution at 37 ± 0.20°C with 50rpm rotating speed. Samples of 5 ml were withdrawn at predetermined interval and equal volume of respective dissolution medium was added to maintain the sink condition. The rate of drug release was analyzed using UV spectrophotometer.

Drug release kinetics studies[11]

The dissolution profile of all the batches was fitted to zero order, first order, higuchi and peppas to ascertain the kinetic modeling of the drug release. The results were obtained from *in-vitro* release studies were plotted in four kinetics models of data treatment as follows:

Cumulative percentage drug release Vs. Time (Zero order rate kinetics)

Log cumulative percentage drug retained Vs. Time (First order rate kinetics)

Cumulative percentage drug release Vs. √T (higuchi)

Log of cumulative percentage drug release Vs. Log time (Peppas exponential equation)

Stability of Nanoparticles[12]

Stability studies of prepared nanoparticles determined by storing optimized formulation at 25°C±2°C and 65 ± 5% RH and 40°C ± 2°C and 75 ± 5% RH in stability chamber for 90 days. The samples were analyzed after a time period like at 0, 1, 2, and 3 months for their drug content and drug release rate.

RESULTS AND DISCUSSION

Pre-formulation Studies of Pravastatin.

Percentage Yield

The results of Percentage Yield are shown in Table No.2. The Percentage Yield of the Pravastatin nanoparticles were found to be in the range of 74.12% to 87.27%.

All the characteristic IR peaks related to pure drug Pravastatin also appeared in the FTIR spectrum of drug mixed with polymer, so there was no chemical incompatibility between drug and polymer.

Shape and Surface morphology

The shape and surface morphology of the prepared nanoparticles were observed by scanning electron microscopy. SEM photographs of formulations F3 revealed that nanoparticles were discrete particles with rough, rigid surface.

Drug content and Entrapment efficiency

The obtained results are reported in Table No.8. The drug content was found to be in the range of 36.29±0.48 to 44.70±0.74mg and Entrapment efficiency in the range of 71.36±0.64 to 88.49±0.35%.

Stability studies

The stability studies were carried out for all the formulation at 25±2ºC with 65±5% RH and 40±2ºC with 75±5% RH for 60 days. The samples were tested for any changes in drug content, and *in-vitro* drug release studies at monthly intervals. The results of stability studies did not show any significant change in the drug content and *in-vitro* dissolution studies.

S.No	Ingredients	F1	F2	F3	F4	F5	F6
1	Pravastatin(g)	40mg	40mg	40mg	40mg	40mg	40mg
2	chitosan(g)	0.5g	1g	1.5g	2.0g	2.5g	3.0g
3	Sodium tripolyphosphate (0.3%w/v)	50ml	50ml	50ml	50ml	50ml	50ml
4	Acetic acid	25ml	25ml	25ml	25ml	25ml	25ml

Table No.1: Organoleptic characteristics, Solubility and melting point of Pravastatin

S.No	Properties	Reported		Observed	
1	Appearance	White to off white, fine or crystalline powder		Powder	
2	Odour	Odourless		Odourless	
3	Solubility	Water	Freely soluble	Water	Freely soluble
		Methanol	Soluble	Methanol	Soluble
		Isopropanol	Slightly soluble	Isopropanol	Slightly soluble
4	Melting Point	171.2ºC		171.2ºC	
5	Identification UV)	239nm		239nm	

Table No.2: Percentage yield of Pravastatin nanoparticles

S.No	Formulation code	% Yield (%)
1	F1	77.14
2	F2	87.27
3	F3	86.33
4	F4	74.12
5	F5	84.99
6	F6	86.81

Table No.3: Interpretation of IR Spectra

S.No	Description	Reported Frequency (cm-1)	Pure Drug (cm-1)	Pure Drug + chitosan (cm-1)
1	C=O (Stretch)	1760-1690	1724.36	1720.50
2	O-H (Stretch)	3300-2500	3032.99	3273.20
3	C=C Aromatic (Stretch)	1600-1400	1564.27	1562.34
4	CH bending alkanes	1350-1480	1396.46	1379.10

Table No.4: Drug content and Entrapment efficiency of the prepared nanoparticles

S.No	Formulation code	Drug content(mg)	Entrapment efficiency (%)
1	F1	36.29±0.48	71.36±0.64
2	F2	44.21±0.82	74.59±0.41
3	F3	44.70±0.74	88.49±0.35
4	F4	44.56±0.52	84.41±0.62
5	F5	43.50±0.35	82.74±0.43
6	F6	37.83±0.40	72.34±0.20

***In-vitro* drug release**

Table No.5: *In-vitro* drug release data of all formulations (F1-F6)

S.No	Time (hours)	Percentage Cumulative Drug Release (%)					
		F1	F2	F3	F4	F5	F6
1	0	0	0	0	0	0	0
2	1	12.41	7.91	9.69	12.85	12.56	9.27
3	2	23.86	12.48	21.55	13.61	19.35	11.74
4	3	31.54	13.96	24.09	23.45	13.34	12.52
5	4	38.27	30.22	27.33	33.97	22.92	20.66
6	5	42.75	45.31	35.28	42.50	33.19	34.99
7	6	46.86	48.01	43.39	55.33	41.53	50.91
8	7	50.65	56.80	63.76	64.72	54.07	61.29
9	8	58.05	60.05	70.68	75.31	63.25	63.69
10	9	64.72	66.93	76.04	76.50	73.60	73.61
11	10	72.45	68.34	77.24	78.01	75.69	76.16
12	11	73.98	78.43	79.85	84.10	76.91	75.52
13	12	80.48	87.21	89.39	86.25	83.44	81.76

Table No.6: Kinetics release study of various formulations

S.No	Formulation code	R2 value			Peppas plot	
		Zero order	First order	Higuchi matrix	R^2 value	n value
1	F1	0.982	0.975	0.973	0.865	0.170
2	F2	0.933	0.916	0.915	0.897	0.160
3	F3	0.939	0.924	0.914	0.918	0.223
4	F4	0.953	0.944	0.923	0.925	0.223
5	F5	0.946	0.910	0.885	0.908	0.209
6	F6	0.961	0.902	0.884	0.947	0.201

Table No.7: Drug Content and *In-vitro* drug release of Pravastatin nanoparticle formulations after Stability Studies

S.No	Time (Days)	Conditions	Drug content (mg)	Cumulative drug release (%)
1	0	-	44.70	89.39
2	30	At 25 ± 2°C/ 65 ± 5% RH	44.69	89.37
		At 40 ± 2°C/ 75 ± 5% RH	44.62	89.35
3	60	At 25 ± 2°C/ 65 ± 5% RH	44.60	89.33
		At 40 ± 2°C/ 75 ± 5% RH	44.58	89.31

Drug Excipient Compatibility Study by FTIR

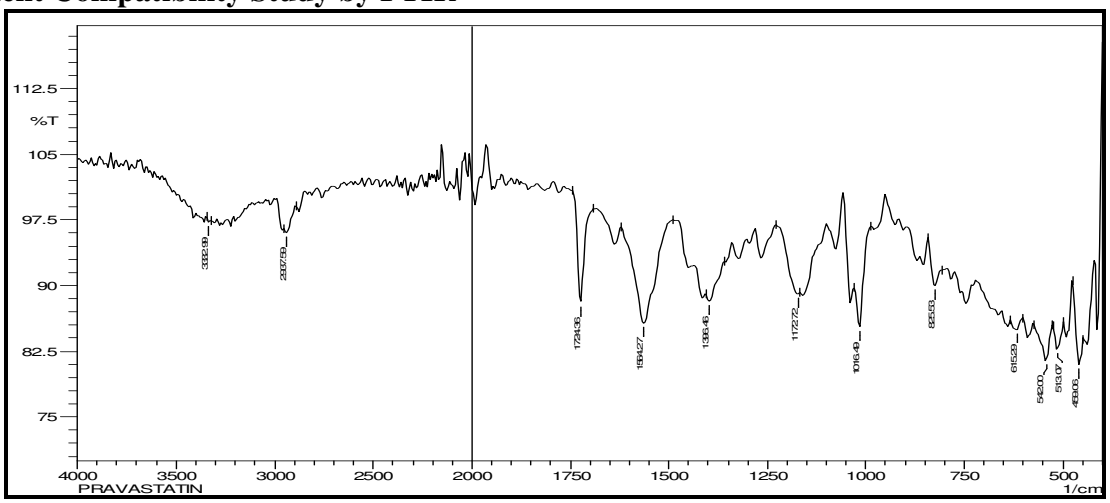


Figure No.1: FTIR of Pravastatin

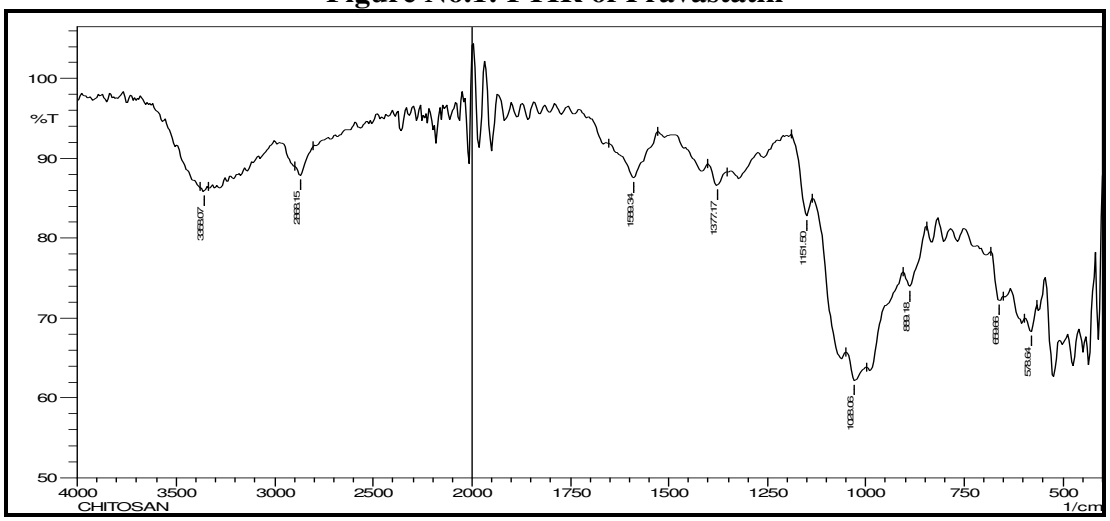


Figure No.2: FTIR of Chitosan

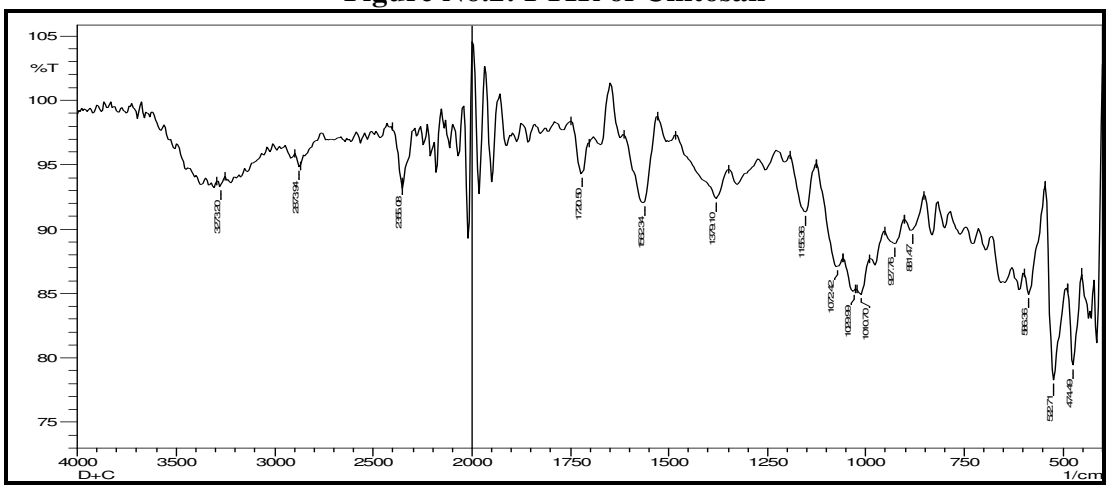


Figure No.3: FTIR of Pravastatin + Chitosan

Particle Size and zeta potential

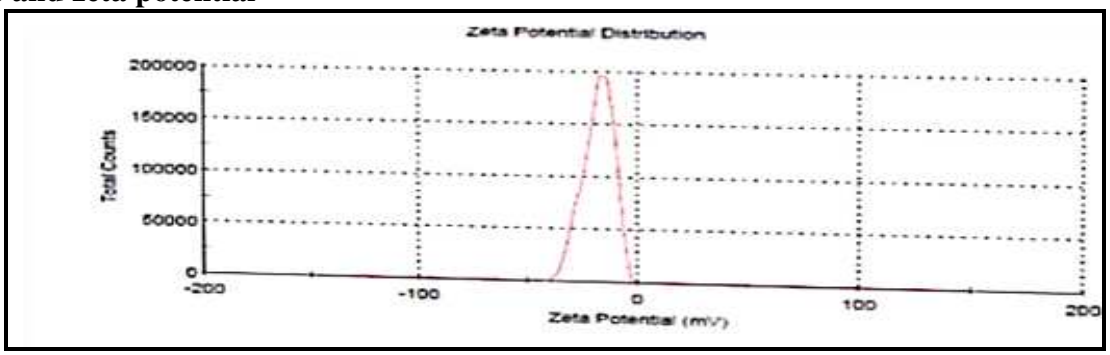
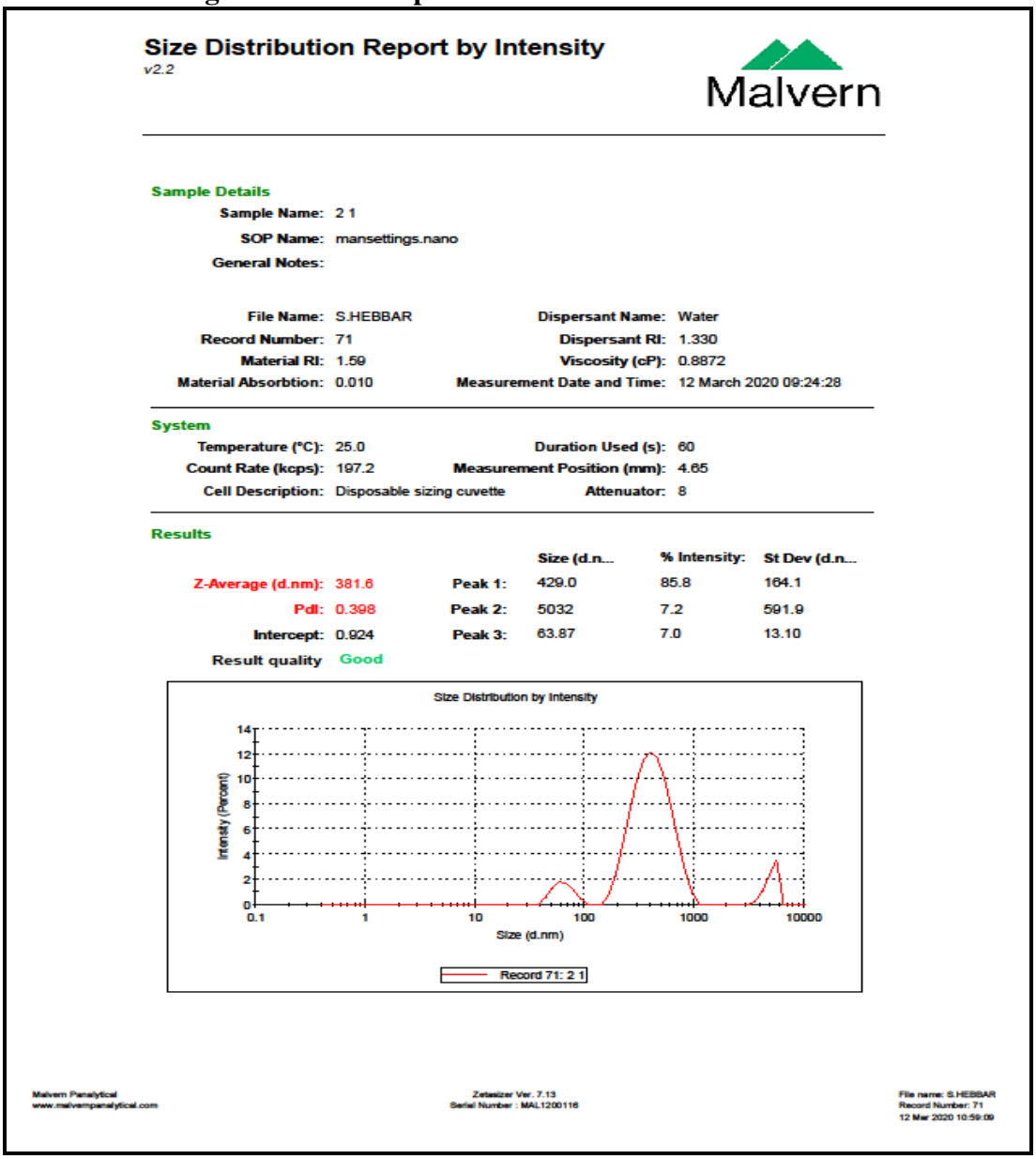


Figure No.4: Zeta potential studies of F3 formulation



Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	2 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	S.HEBBAR	**Dispersant Name:**	Water
Record Number:	71	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	12 March 2020 09:24:28

System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	197.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	381.6	**Peak 1:**	429.0	85.8	164.1
PdI:	0.398	**Peak 2:**	5032	7.2	591.9
Intercept:	0.924	**Peak 3:**	63.87	7.0	13.10
Result quality	Good				

Malvern Panalytical
www.malvernpanalytical.com

Zetasizer Ver. 7.13
Serial Number : MAL1200116

File name: S.HEBBAR
Record Number: 71
12 Mar 2020 10:59:09

Shape and Surface morphology

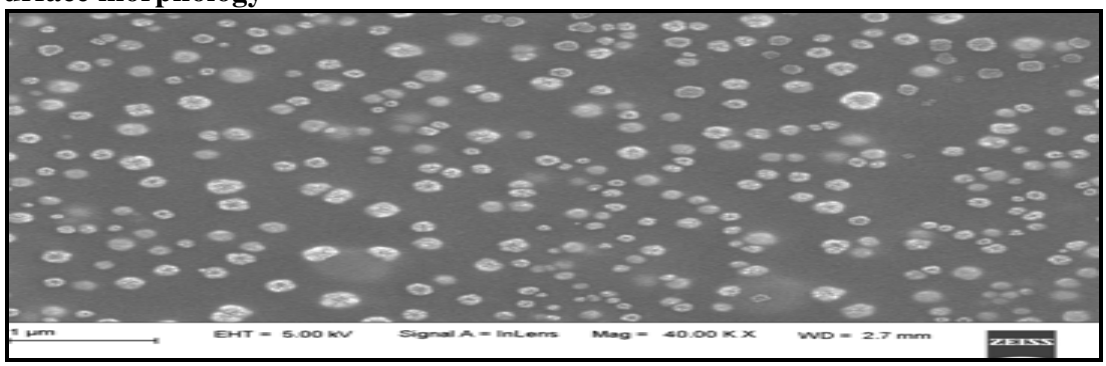


Figure No.5: Particle size analysis of F3 formulation

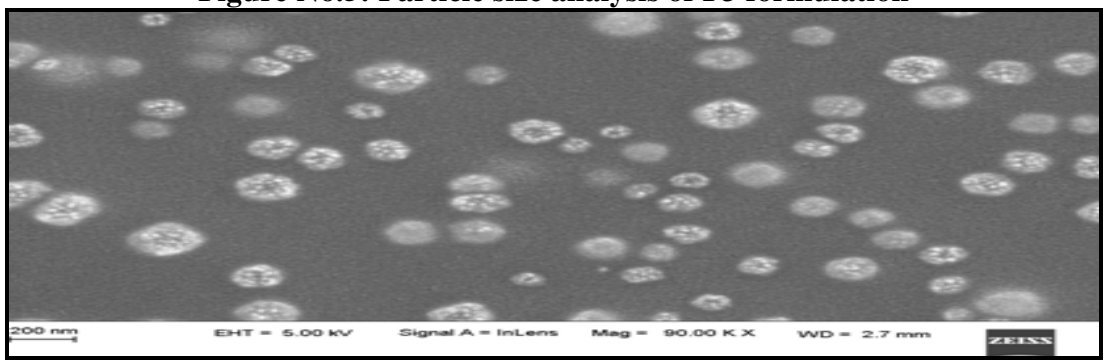


Figure No.6: SEM of F3 formulation

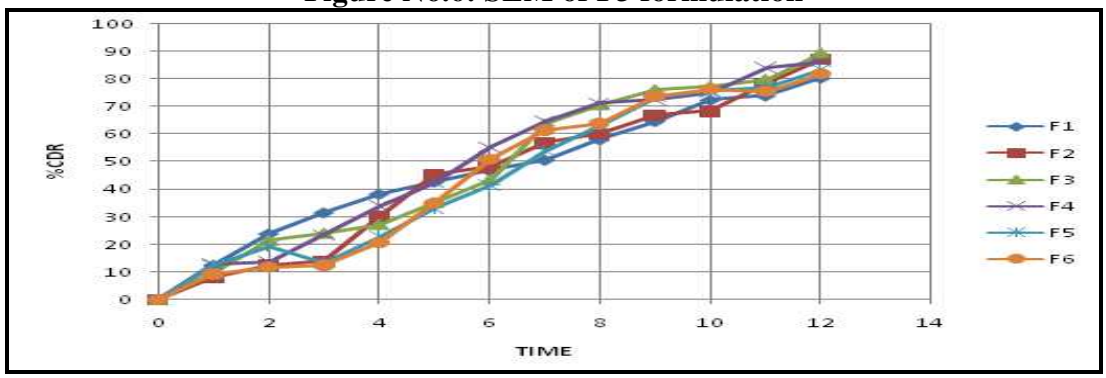


Figure No.7: In-vitro release profile of Pravastatin nanoparticles (F1-F6)

***In-Vitro* Drug release kinetics**

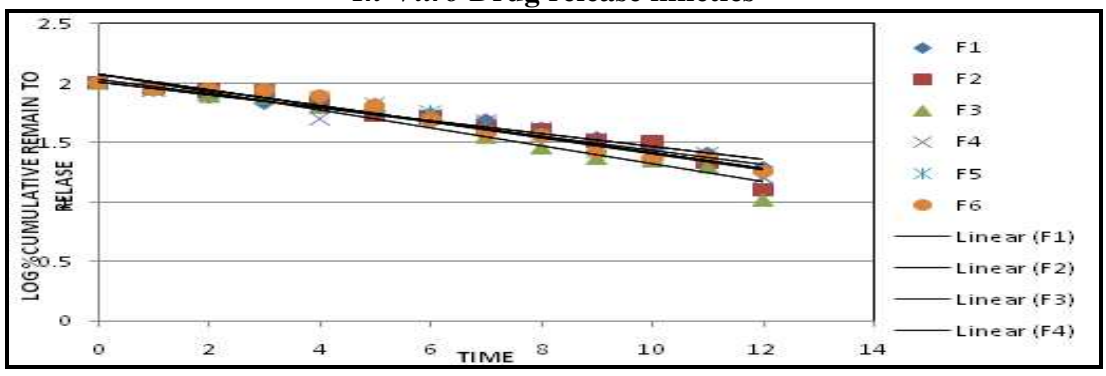


Figure No.8: First order

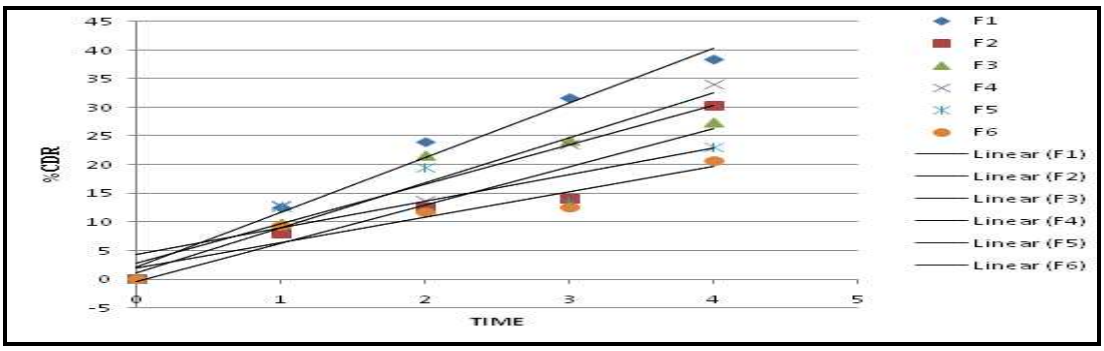


Figure No.9: Zero order

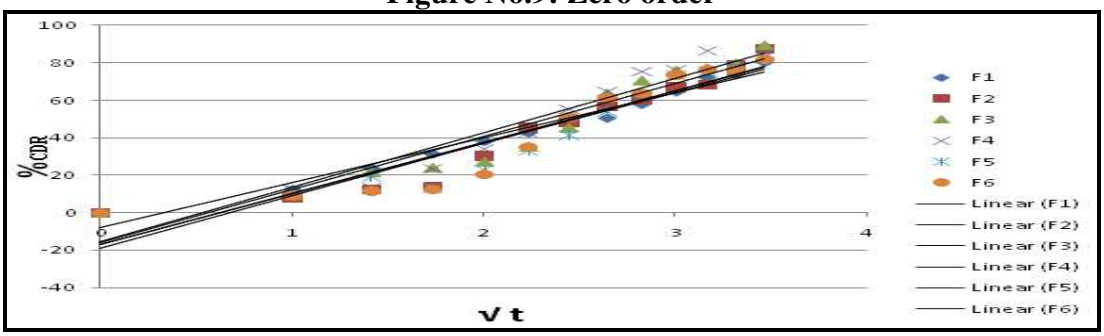


Figure No.10: Higuchi

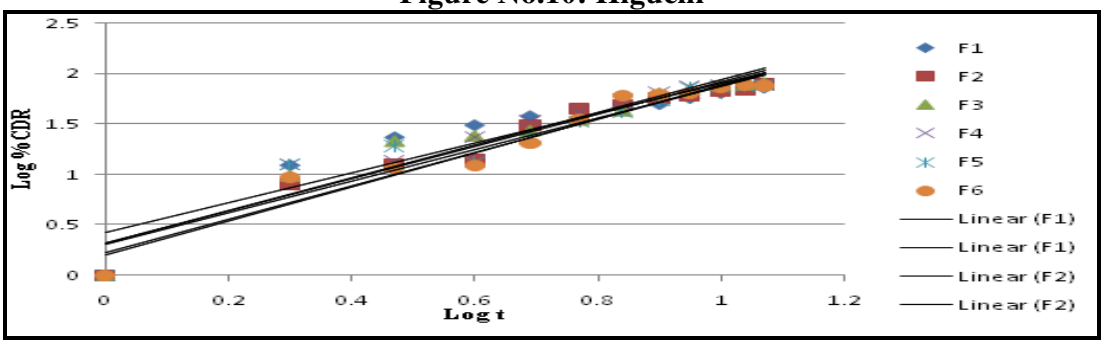


Figure No.11: Peppas

CONCLUSION

Nanoparticles of Pravastatin were prepared successfully by ionotropic gelation method using different concentration of chitosan, Sodium tripolyphosphate, and acetic acid.

The following conclusions were drawn from the present investigation

- Pre-formulation studies like melting point, solubility and UV analysis were carried out and they comply with the standards.
- The FTIR spectral data indicates that there was no interaction between drug and the utilized polymers. All the polymers were compatible with the drug.
- Six different formulations were prepared by ionotropic gelation method and were evaluated. The prepared nanoparticles were subjected to various parameters like entrapment efficiency, *in-vitro* drug release study, particle size analysis and SEM and it shows satisfactory results.
- Entrapment efficiency and drug content also increased with an increase in the polymer concentration. From the results it can be inferred that there was a proper distribution of Pravastatin in the nanoparticle and deviation was within acceptable limits.

- From the *In-vitro* release studies it could be concluded that F3 formulation gives maximum drug release at the end of 12 hours.
- Short-term stability studies of the formulations indicated that there are no significant changes in the appearance, drug content and dissolution parameter values after 60 days of storage at 25±2°C with 65±5% RH and 40±2°C with 75±5% RH.
- It can be concluded that the developed formulation can be effective formulation with improved efficacy, promised release and patient compliance.

ACKNOWLEDGEMENT

I would like to thank Mangalore University, Konaje for allowing to perform Scanning Electron Microscopy (SEM) and particle size stidies. Thanks to VGST for the grants provided to establish the laboratory setup.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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