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Design and characterisation of antihyperlipidemic drug Rosuvastatin Calcium nanoparticles

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ABSTRACT

The objective of the present study was to formulate and evaluate polymeric nanoparticles of Rosuvastatinby Emulsification sonication method using Sodium alginate, Chitosanand Ethyl celluloseas a polymers.The nanoparticles were characterized for FTIR, particle size, poly-dispersity index, entrapment efficiency (EE), zeta potential, morphological study, DSC and*invitro* study. Infrared studies showed that there was no drug excipients interaction. Negative values of zetapotential indicated the good stabilization of the prepared nanoparticles.The entrapment efficiency was found in between 51.82% – 75.14%. The *in-vitro* drug release was extended maximum up to 48hrs with Chitosan. The curve fitting data shows that the drug release followed first order kinetics.SEM shows that nanoparticles were found spherical in structure without aggregation and uniform distribution of the drug within the nanoparticles.

Keywords: Rosuvastatin, Emulsification sonication method, odium alginate, Chitosan,Ethyl cellulose and Nanoparticles.

INTRODUCTION

Nanotechnology has gained huge attention over time. The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometres in size and are made up of carbon, metal, metal oxides or organic matter [1]. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, increased reactivity or stability in a chemical process, enhanced mechanical strength, etc. [2]. These properties of nanoparticles has led to its use various applications. The nanoparticles differs from various dimensions, to shapes and sizes apart from their material [3]. A nanoparticle can be either a zero dimensional where the length, breadth and height is fixed at a single point for example nano dots, one dimensional where it can possess only one parameter for example graphene, two dimensional where it has length and breadth for example carbon nanotubes or three dimensional where it has all the parameters such as length, breadth and height for example gold nanoparticles.

The nanoparticles are of different shape, size and structure. It be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular and differ from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated. Numerous synthesis methods are either being developed or improved to enhance the properties and reduce the production costs. Some methods are modified to achieve process specific nanoparticles to increase their optical, mechanical, physical and chemical properties. A vast development in the instrumentation has led to an improved nanoparticle characterisation and subsequent application. The nanoparticles are now used in every objects like from cooking vessel, electronics to renewable

energy and aerospace industry. Nanotechnology is the key for a clean and sustainable future.[4]

Advantages of nanoparticles

Nanoparticles offer numerous advantages in drug delivery system. These advantages include, but are not limited:

- Nanoparticles have many significant advantage over conventional and traditional drug delivery system.
- Nanoparticles are control and sustain release form at the site of localization, they alter organ distribution of drug compound. They enhance drug circulation in blood, bioavailability, therapeutic efficacy and reduce
- Nanoparticles can be administer by various routes including oral, nasal, parenteral, intra-ocular etc.
- In the tiny areas of body nanoparticles shows better drug delivery as compare to other dosage form and target to a particular cell type or receptor.
- Due to small particle size nanoparticles overcome resistance by physiological barriers in the body and easily penetrates to cell walls, blood vessels, stomach epithelium and blood–brain barrier.
- Nanoparticle enhance the aqueous solubility of poorly soluble drug, which improves bioavailability of drug.
- As a targeted drug carrier nanoparticles reduce drug toxicity and enhance efficient drug distribution.
- By using polymers drug release form nanoparticles can be modified which makes polymeric nanoparticle an ideal drug delivery system for cancer therapy, vaccines, contraceptives and antibiotics.
- Useful to diagnose various diseases
- Enhanced stability of ingredients
- Prolonged shelf life
- Used in dental surgery also as filling the tiny holes in teeth.
- Change the method of drug delivery to improve customer acceptance or reduce manufacturing costs.[5-8]

Limitations of Nanoparticles

a) Small size and large surface area can lead to particle particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

b) In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.[9,10]

Classification Of Nanoparticles

The nanoparticles are generally classified into the organic, inorganic and carbon based.

Organic nanoparticles

Dendrimers, micelles, liposomes and ferritin, etc. are commonly knows the organic nanoparticles or polymers. These nanoparticles are biodegradable, non-toxic, and some particles such as micelles and liposomes has a hollow core (Figure1), also known as nanocapsules and are sensitive to thermal and electromagnetic radiation such as heat and light. These unique characteristics makes them an ideal choice for drug delivery. The drug carrying capacity, its stability and delivery systems, either entrapped drug or adsorbed drug system determines their field of applications and their efficiency apart from their normal characteristics such as the size, composition, surface morphology, etc. The organic nanoparticles are most widely used in the biomedical field for example drug delivery system as they are efficient and also can be injected on specific parts of the body that is also known as targeted drug delivery.

Inorganic nanoparticles

Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide based nanoparticles are generally categorised as inorganic nanoparticles

*Metal based:*Nanoparticles that are synthesised from metals to nanometric sizes either by destructive or constructive methods are metal based nanoparticles. Almost all the metals can be synthesised into their nanoparticles. The commonly used metalsfor nanoparticle synthesis are aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver(Ag) and zinc (Zn). The nanoparticles have distinctive properties such sizes as low as 10 to 100nm, surface characteristics like high surface area to volume ratio, pore size,surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical andcolour, reactivity and sensitivity to environmental factors suchas air, moisture, heat and sunlight etc.

*Metal oxides based:*The metal oxide based nanoparticles are synthesised to modify the properties of their respective metal based nanoparticles, for example nanoparticles of iron (Fe) instantly oxidises to iron oxide (Fe_2O_3) in the presence of oxygen at room temperature that increases its reactivity compared to iron nanoparticles. Metal oxide nanoparticles are synthesised mainly due to their increased reactivity and efficiency. The commonly synthesised are Aluminium oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite(Fe_3O_4), Silicon dioxide (SiO_2), Titanium oxide (TiO_2), Zinc oxide (ZnO). These nanoparticles have possess an exceptional properties when compared to their metal counterparts.

*Carbon based:*The nanoparticles made completely of carbon are knows as carbon based. They can be classified into fullerenes, graphene, carbon nano tubes (CNT), carbonnanofibers and carbon black and sometimes activated carbon innano size.

*Fullerenes:*Fullerenes (C60) is a carbon molecule that is spherical in shape and made up of carbon atoms held together by sp2 hybridization. About 28 to 1500 carbon atoms forms thespherical structure with diameters up to 8.2 nm for a single layerand 4 to 36 nm for multi-layered fullerenes.

*Graphene:*Graphene is an allotrope of carbon. Graphene is a hexagonal network of honeycomb lattice made up of carbon atoms in a two dimensional planar surface. Generally the thickness of the graphene sheet is around 1 nm.

*Carbon Nano Tubes (CNT):*Carbon Nano Tubes (CNT), a graphenenanofoil with a honeycomb lattice of carbon atoms is wound into hollow cylinders to form nanotubes of diameters aslow as 0.7 nm for a single layered and 100 nm for multi-layeredCNT and length varying from a few micrometres to several

millimetres. The ends can either be hollow or closed by a half fullerene molecule.

Carbon Nanofiber: The same graphenenanofoils are used to produce carbon nanofiber as CNT but wound into a cone or cupshape instead of a regular cylindrical tubes.

*Carbon black:*An amorphous material made up of carbon, generally spherical in shape with diameters from 20 to 70 nm. The interaction between the particles is so high that they boundin aggregates and around 500 nm agglomerates are formed.[11-14]

MATERIALS

RosuvastatinProvided by SURA LABS, Dilsukhnagar, Hyderabad,Sodium alginate Procured from Gattefosse Pvt. Ltd., Mumbai, Chitosan Purchased from Merck Limited, Mumbai (India), Ethyl cellulosePurchased from Merck Limited, Mumbai (India), Tween 80 Purchasedfrom Merck Limited, Mumbai (India).

METHODOLOGY

Analytical Method Development Determination of absorption maxima

Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of Methanol(1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flaskand volume was made up with phosphate buffer (pH 6.8). Fromthis stock solution pipette out 1ml and dilute to 10 ml with

phosphate buffer and subject for UV scanning in the range of 200-400 nm using double beam UV spectrophotometer. The absorption maxima were obtained at 252 nm with a characteristic peak.

Preparation of calibration curve

It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Rosuvastatinwas prepared in Methanoland subsequent working standards (10, 20, 30, 40 and 50 µg/mL) were prepared by dilution with phosphate buffer of pH-6.8.

Table1: Composition of nanoparticles formulations (F1 to F9)

Excipients	R1	R2	R3	R4	R5	R6	R7	R8	R9

Rosuvastatin	10	10	10	10	10	10	10	10	10
Sodium alginate (%)	1	2	3	-	-	-	-	-	-
Chitosan (mg)	-	-	-	1	2	3	-	-	-
Ethyl cellulose	-	-	-	-	-	-	1	2	3
Tween 80 (mL)	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Dichloromethane (ml)	10	10	10	10	10	10	10	10	10
Methanol: Acetone Ratio	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3

Preparation of nanoparticles

Preparation of Rosuvastatin loaded nanoparticles Rosuvastatinloaded Nanoparticle was prepared by previously reported emulsification sonication method. These solutions were used for the estimation Rosuvastatin by UV method. The whole procedure was repeated three times and average peak area was calculated. Calibration plot was drawn between concentrations and peak area. Calibration equation and R^2 value are reported.

Rosuvastatinwas dissolved in organic solvent (10 ml, methanol). Polymers in different concentrations were dissolved in water. The organicphase was added drop wise into the polymericsolution foremulsification. Then the dispersion was sonicated (20 min)with the application of ultra-probe sonication (60 W/cm^3, Hielscher, Ultra-sonics, Germany). The formulation was stirred at 1500 rpm for 6 h using a magnetic stirrer to evaporate theorganic solvent.

The prepared NPs were centrifuged at15,000 rpm for 20 min at 4 °C (Remi, Mumbai, India). NPswere separated and lyophilized using cryoprotectant (Mannitol 0.2%) and stored for further evaluation Table and Fig. Equation for linearity curve and R^2 were calculated as Y=0.011X+0.011 and R^2 =0.997. Rosuvastatin showed maximum absorbance in phosphate buffer (pH 5.5) at 252 nm. The solution obeyed Beer-Lambert's law for concentration range of 10 to 50µg/mL with regression coefficient of 0.997. Standard curve of prepared Rosuvastatin in phosphate buffer pH 5.5 is shown below.

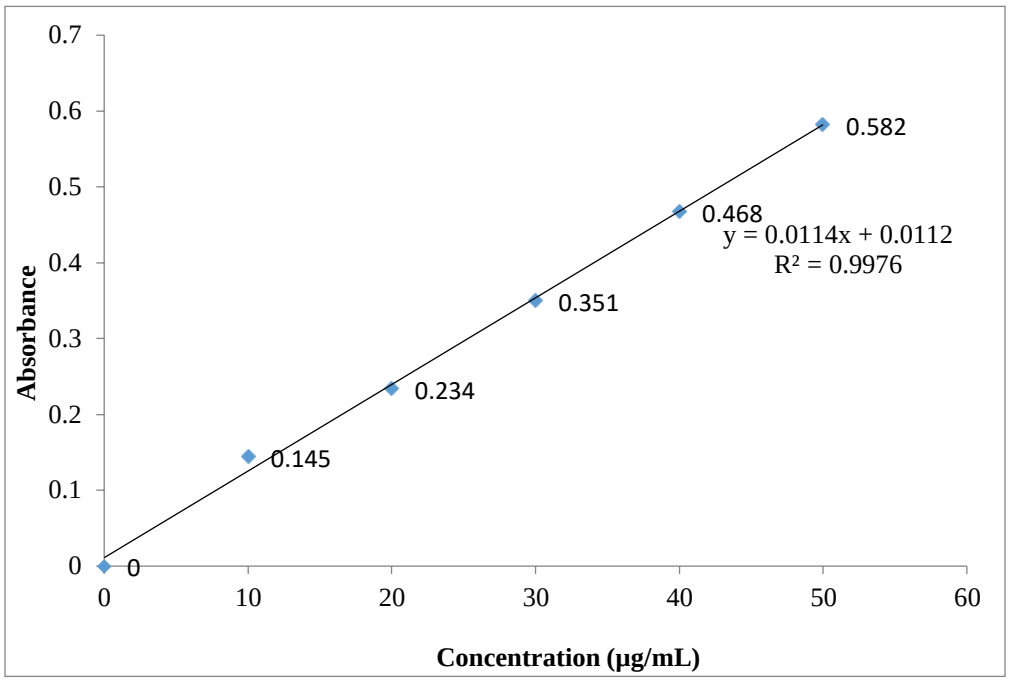

Fig 1:Calibration curve of Rosuvastatin in phosphate buffer pH 5.5

Characterization of nanoparticles

Table 2: Percentage yield, Drug Content, Entrapment Efficiency of all nanoparticles formulations

FORMULATION	Percentage yield	Drug Content	Entrapment Efficiency
R1	82.28	90.14	61.14
R2	89.34	93.04	71.25
R3	92.15	94.45	88.01
R4	91.54	95.03	51.82
R5	93.54	96.32	60.82
R6	**95.81**	**97.59**	**75.14**
R7	86.09	92.64	60.19
R8	90.47	94.82	69.25
R9	93.78	95.61	72.10

Table 3: Particle Sizes, PDI, Zeta Potential of all nanoparticles formulations

FORMULATION	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	175.9	0.095	-25.3
F2	162.2	0.092	-26.4
F3	156.2	0.162	-27.6
F4	168.5	0.185	-25.9
F5	151.9	0.109	-28.4
F6	**140.5**	**0.090**	**-29.5**
F7	173.9	0.159	-28.5
F8	152.2	0.132	-25.8
F9	148.3	0.123	-25.4

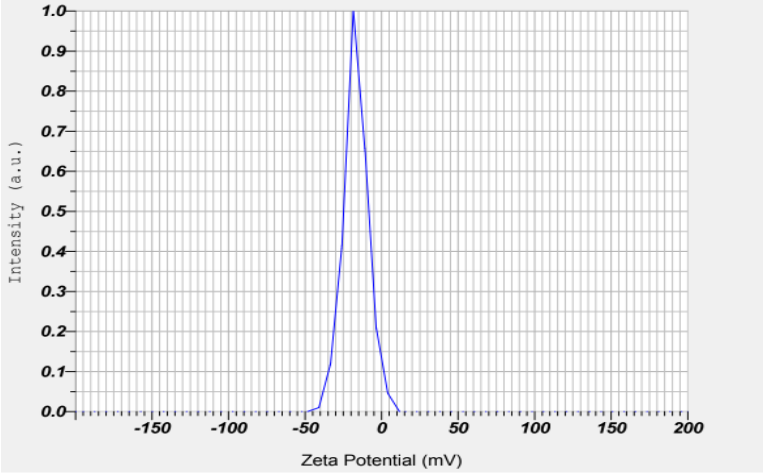

Fig 2: Zeta potential of R6 Formulation

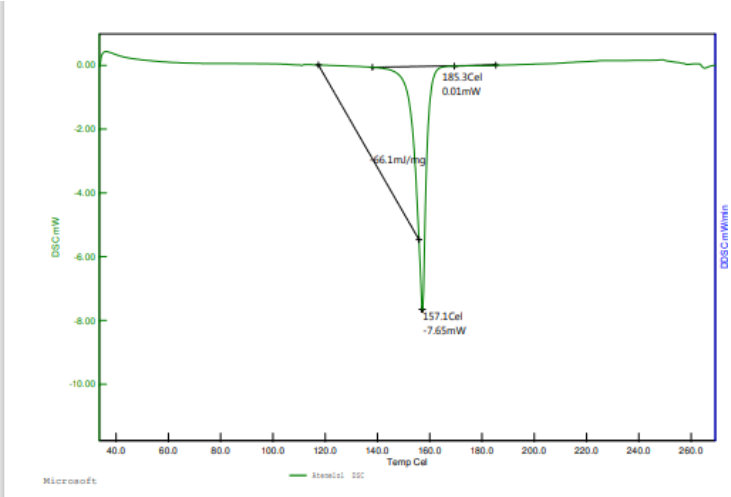

Fig 3: Rosuvastatin Pure

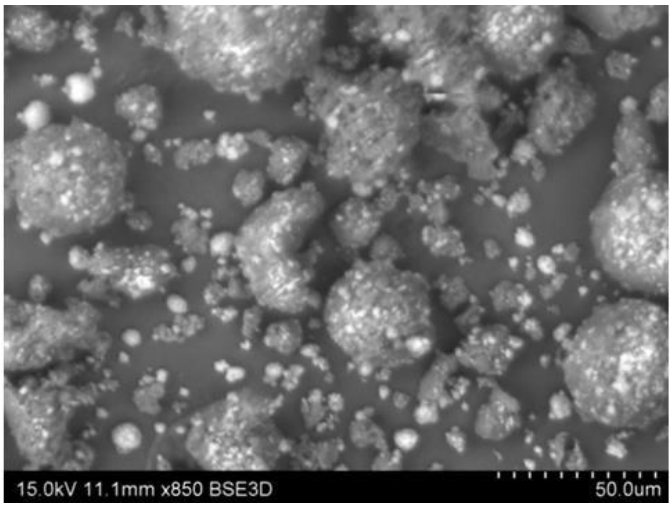

Fig4: RosuvastatinR6 optimisednanoparticles

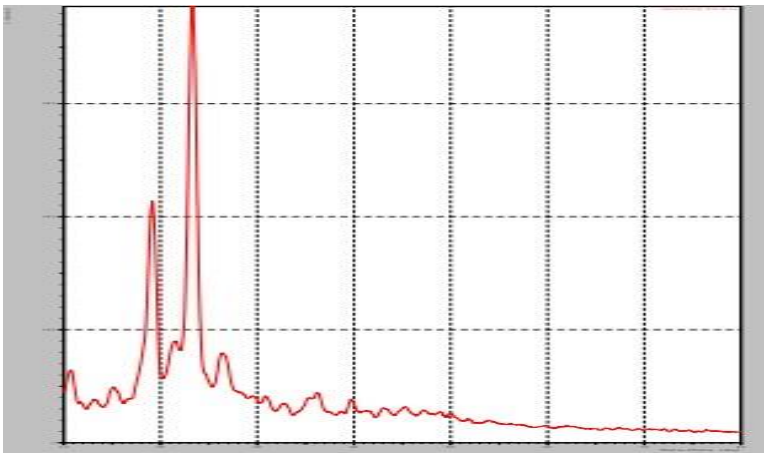

Fig5: XRDRosuvastatinR6nanoparticles

Table4:*In vitro* dissolution studies of R1-R9 nanoparticles formulations in percentage

Time (hour)	R1	R2	R3	R4	R5	R6	R7	R8	R9
0	0	0	0	0	0	0	0	0	0
1	12.52	8.39	7.19	10.96	14.62	10.58	13.72	10.41	12.38
2	17.37	16.17	19.72	14.83	19.68	15.64	18.14	16.34	18.29
4	27.48	25.35	23.93	21.78	25.64	27.11	25.76	21.92	23.71
6	42.26	36.17	29.54	27.41	31.48	38.97	35.10	28.76	32.92
8	54.18	48.86	35.41	35.79	36.95	45.65	46.28	33.63	38.49
10	58.71	56.61	39.76	41.86	48.72	52.74	55.19	45.21	46.58
12	66.33	69.14	56.19	67.31	69.39	64.22	64.98	49.34	58.26
18	75.85	75.59	64.72	73.22	78.14	75.94	69.75	57.27	69.15
24	83.95	83.61	67.29	81.89	87.58	90.19	74.15	68.34	76.87
48	86.78	85.34	72.34	92.15	94.11	**98.76**	89.37	83.27	80.62

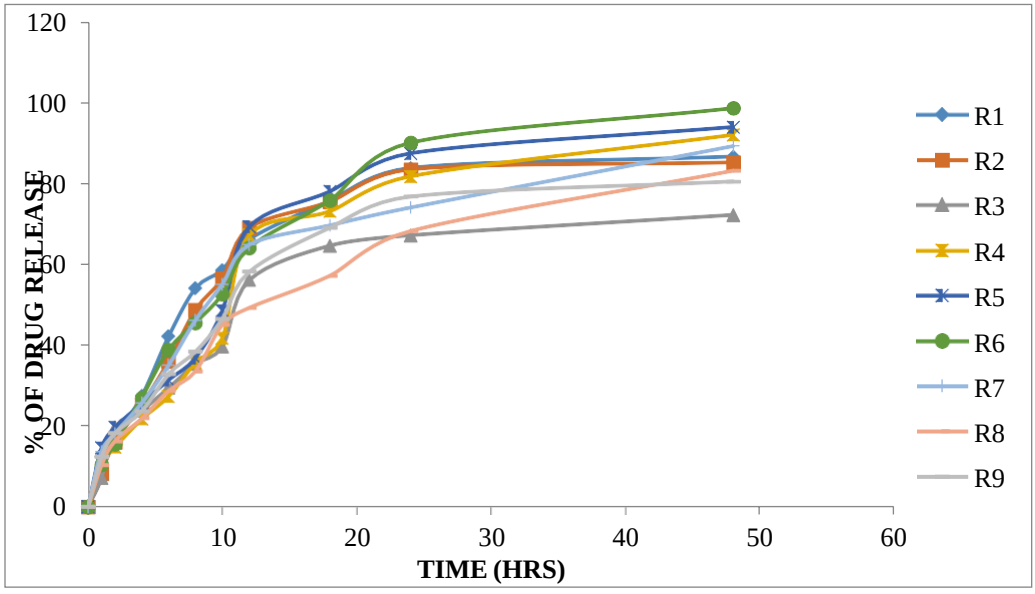

Fig 6: *In vitro* dissolution studies of R1-R9 nanoparticles formulations in percentage

Table 5: Release kinetics of optimised formulation

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE/t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
10.58	1	1.000	1.024	0.000	1.951	10.580	0.0945	-0.976	89.42	4.642	4.472	0.170
15.64	2	1.414	1.194	0.301	1.926	7.820	0.0639	-0.806	84.36	4.642	4.386	0.256
27.11	4	2.000	1.433	0.602	1.863	6.778	0.0369	-0.567	72.89	4.642	4.177	0.464
38.97	6	2.449	1.591	0.778	1.786	6.495	0.0257	-0.409	61.03	4.642	3.937	0.704
45.65	8	2.828	1.659	0.903	1.735	5.706	0.0219	-0.341	54.35	4.642	3.788	0.854
52.74	10	3.162	1.722	1.000	1.674	5.274	0.0190	-0.278	47.26	4.642	3.615	1.026
64.22	12	3.464	1.808	1.079	1.554	5.352	0.0156	-0.192	35.78	4.642	3.295	1.346
75.94	18	4.243	1.880	1.255	1.381	4.219	0.0132	-0.120	24.06	4.642	2.887	1.755
90.19	24	4.899	1.955	1.380	0.992	3.758	0.0111	-0.045	9.81	4.642	2.141	2.501
98.76	48	6.928	1.995	1.681	0.093	2.058	0.0101	-0.005	1.24	4.642	1.074	3.567

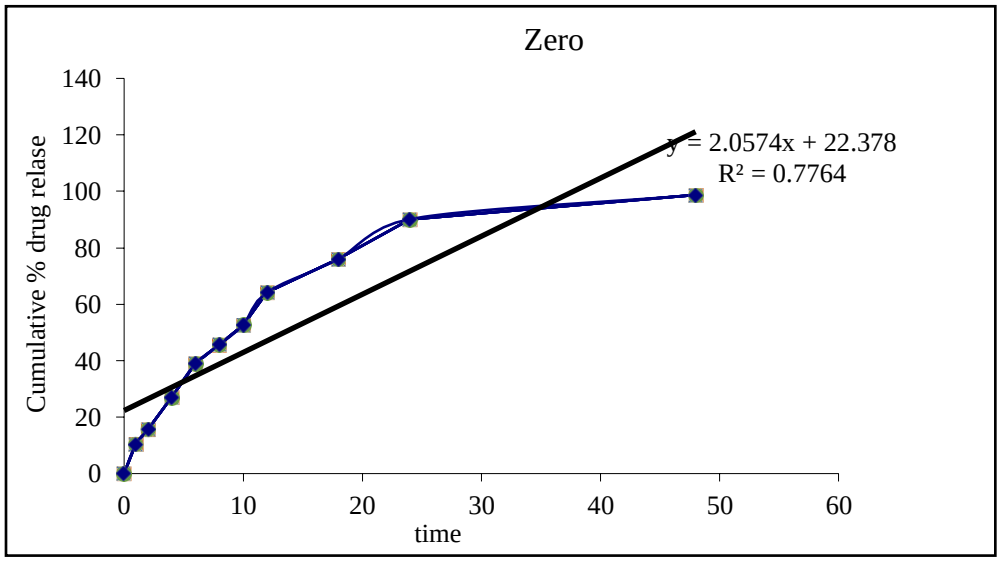

Fig 7: Zero order release kinetics graph

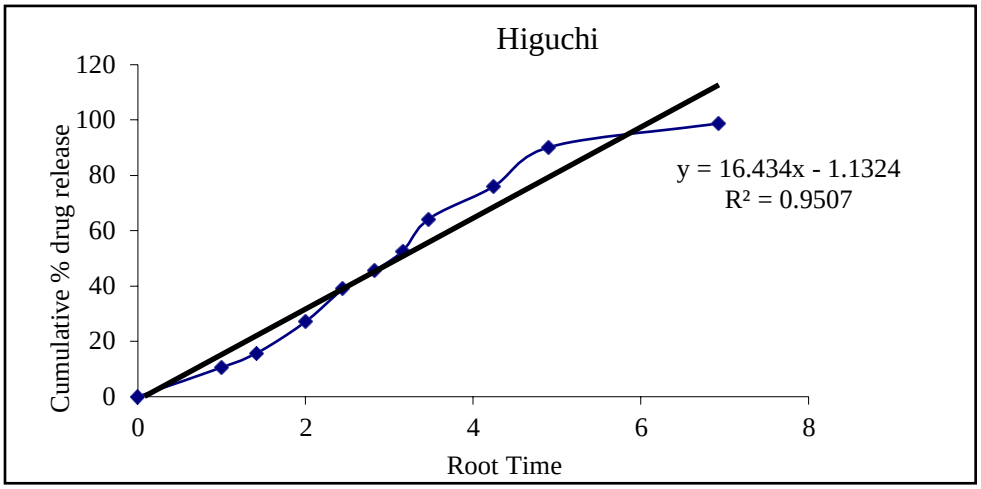

Fig8:Higuchi release kinetics graph

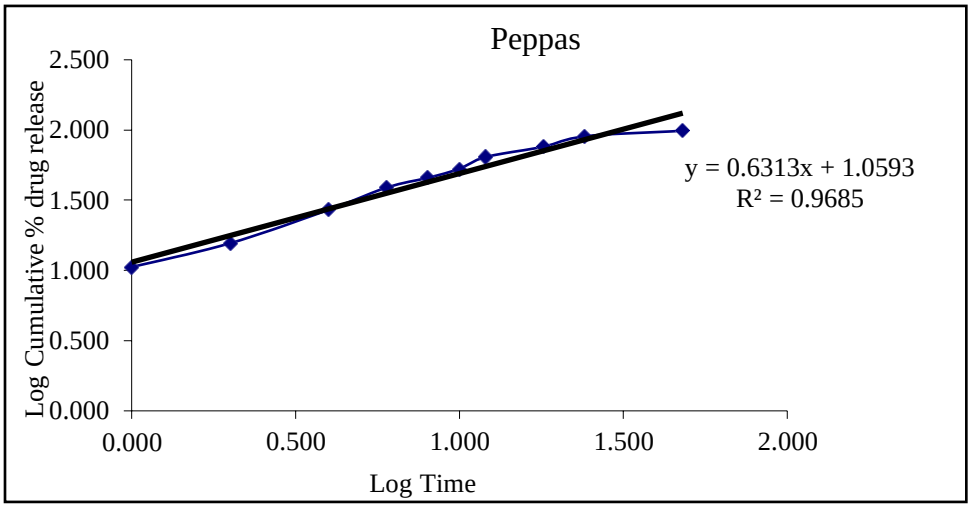

Fig9: Peppas release kinetics graph

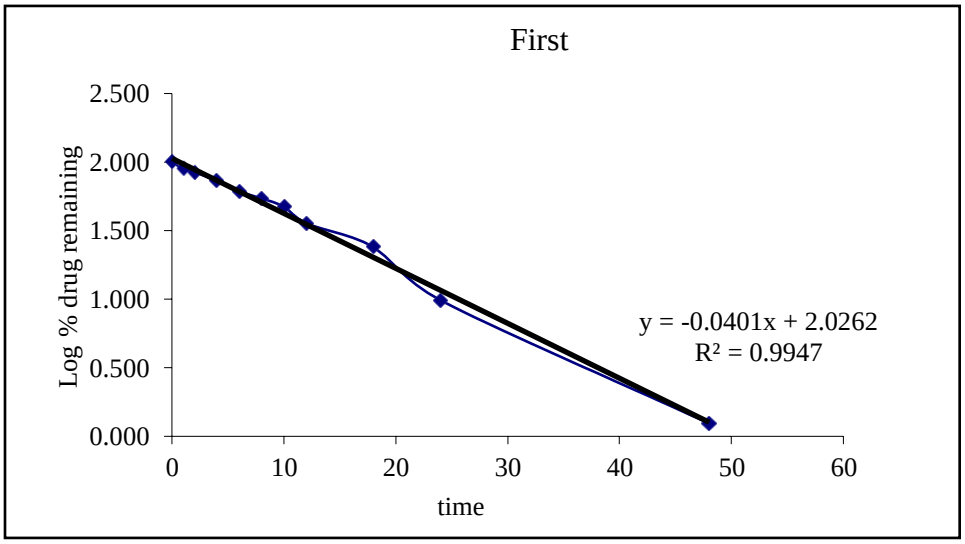

Fig10: First order release kinetics graph

Drug – Excipient compatibility studies

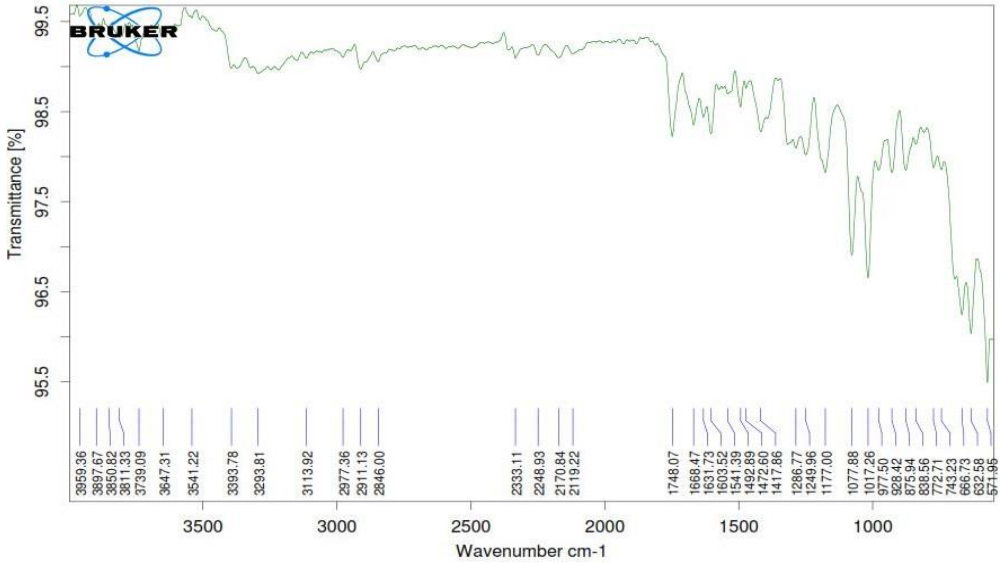

Fig 11: Rosuvastatin Pure drug FTIR

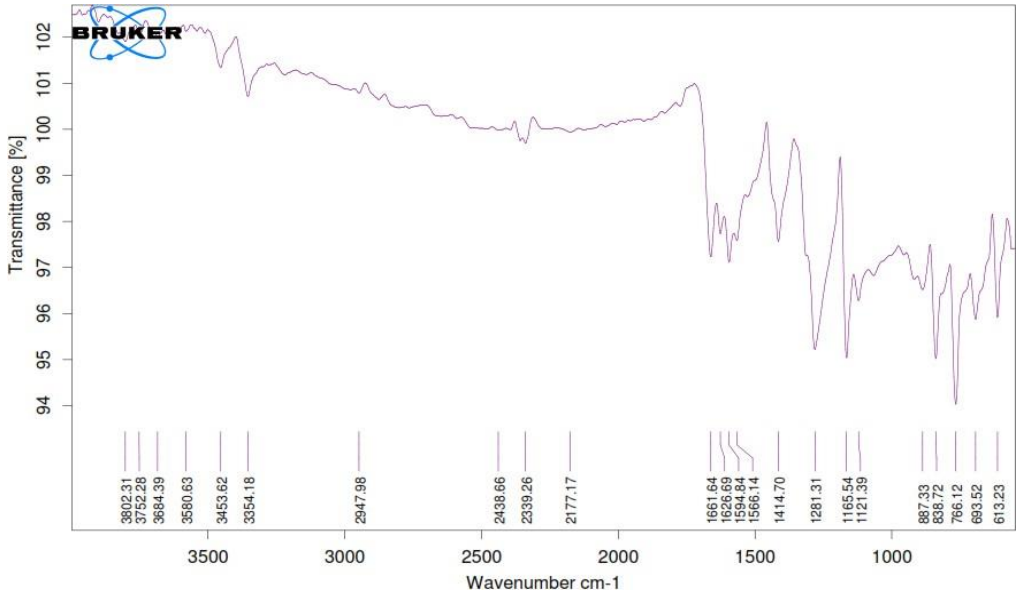

Fig 12: RosuvastatinR6 optimised

CONCLUSION

Rosuvastatin loaded polymeric nanoparticles were prepared by Emulsification sonication method. Drug polymer excipient comparability was confirmed by FT-IR and DSC investigationsconfirmed that there was no interaction between drugand polymers. The method resulted in consistent production of smaller size nanoparticles with narrow size distribution and good entrapment efficiency. From the results, formulation R6

containing Rosuvastatin nanoparticles using combination of polymers evolved as the optimized formulation and it releases more than 98.76% drug in 48 hrs. The optimized formulation R6 can be considered as a promising sustained drug delivery system of Rosuvastatin nanoparticles providing nearly first order drug release over a period of 48 hrs.These results may prove to be beneficial for the prolonged utilization of the formulation as an anti-Antihyperlipidemictherapy. Thus, the polymeric nanoparticles may provide an effective drug deliveryfor poorly water soluble lipophilic drugs.

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