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Formulation, Development and Evaluation of Atorvastatin Calcium loaded Nanosponges (ATRC-Nsgs) by Using Factorial Design and It's In Vivo Study

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ABSTRACT

Introduction: Atorvastatin calcium is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of HMG-CoA reductase, the rate- limiting enzyme that converts 3-hydroxy-3-methyl-glutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol.

Methodology: The Determination of Calibration curve by UV visible spectrophotometer and Analytical method validation by UV visible spectrophotometer. The Analytical Techniques Used to Detect Drug-Excipient Compatibility of drug.

Aim: The aim of this study was to design, formulate, and characterize the atorvastatin calcium loaded nanosponges (NSGs) by using a suitable factorial design.

Results and Discussion: The in vitro release profile of ATRC, Marketed tablet, lyophilized ATRC-NSGs in phosphate buffer pH 6.8 at 37°C shown in Fig IA. 71-72. The % drug release was not identical for all the batches. Initially the NSGs showed immediate release of ATRC, this may be due to release of drug from the surface of NSGs, followed by slow and controlled release of drug which was encapsulated in nanosponges.

Conclusion: Formulated nanosponges formulation was found to be stable at accelerated stability conditions as per ICH guidelines up to 6 months. Comparative in-vivo pharmacodynamic study (which was done as per approved protocol by CPCSEA committee) in male Wistar rats at fasted and fed conditions reveled that, formulated nanosponges successfully evade variation in optimized freeze dried ATRC-NSGs which showed better lessening in prominent level of TG, TC, LDL as well as VLDL and better improvement in HDL level.

Keywords: Atorvastatin calcium, nanosponges, ICH guidelines, in-vivo pharmacodynamic study, TG, TC, LDL.

Introduction

Solubility is the trait of a solid, liquid, or gaseous chemical matter called solute to break up in a solid, liquid, or gaseous solvent to obtain a homogeneous solution of the solute in the solvent. The solubility of a material vitally depends on the solvent employed in adding up to on pressure and temperature. The degree of the solubility of a material in a definite solvent is calculated as the saturation concentration where adding up additional solute does not amplify its concentration in the solution. The solvent is usually a liquid, which can be a pure material or a mixture of two liquids. One may also articulate of solid solution, but seldom of

solution in a gas. The degree of solubility ranges broadly, from infinitely soluble (fully miscible) such as ethanol in water to poorly soluble, such as silver chloride in water. The term insoluble is over and over again useful to poorly or incredibly poorly soluble compounds. Solubility transpires beneath dynamic equilibrium, which does mean that solubility outcome from the simultaneous and opposing methods of dissolution and phase joining (e.g., precipitation of solids)[1-4]. Solubility equilibrium arises when the two techniques keep on at a constant rate. Under definite circumstances, equilibrium solubility may be exceeded to endow with a so-called supersaturated solution, which is metastable. Solubility is not to be perplexed with the capability to dissolve or soften a material, since these techniques may take place not only because of dissolution but also because of a compound interaction. The Biopharmaceutics Classification System (BCS) is a channel for envisage the intestinal drug absorption made available by the U.S. Food and Drug Administration. This arrangement restricts the prophecy by means of the parameters of intestinal permeability and solubility. Solubility is based on the elevated-dose strength of an instant release product. A drug is considered highly soluble when the uppermost dose strength is soluble in 250 mL or fewer of aqueous media over the pH series of 1 to 7.5. The volume approximation of 250 mL is obtained from typical bioequivalence study protocol that lay down administration of a drug product to fasting human volunteers with a glass of water.

Nanosponges are poised of hypercrosslinked cyclodextrin linked in a 3-D network. Nanosponges form spongy NPs with sizes less than 500 nm, so they effortlessly travel in the systemic circulation. As ‘sponges’, they can soak up toxins, fragments and secretions obtained by cancerous cells themselves. Their globular shape and negative surface charge give them a good capacity for embedding small molecules, ions, gases and macromolecules within their structure. Therefore, NSGs have been intended to enhance chemotherapeutic effectiveness by targeting drug-resistant cells[5-9]. The erythrocyte membrane can be employed as a cloak consisting of >3,000 nanosponges. Once they are entirely embedded with toxins, nanosponges are safely inclined of by the liver with lesser toxicity.

Benefits of Nanosponges: [10-11]

- Biodegradable, Predictable release and targeted site-specific drug delivery.
- These formulations are compatible with most vehicles and ingredients.
- Can be used to mask unlikable flavors and to alter liquid substances to solids.
- Fewer harmful side effects (since minor quantities of the drug make contact with healthy tissue).
- Nanosponge particles are water soluble, so the hydrophobic drugs can be embeded within the core of polymer, succeeding mixing with a chemical called an adjuvant reagent.
- The main limitation of these nanosponges is their capacity to embrace only smallmolecules.
- Nanosponge depends upon loading capacities.
- Dose dumping may occur at times.

Characteristic Features of Nanosponges:

- Nanosponges demonstrate a range of dimensions (1 µm or less) with tunable polarity of the pockets which can be formulated by different cross linker to polymer share.
- They are of para-crystalline or in crystalline appearance, depending on the methodcondition, crystal structure of nanosponges plays a very vital role in their complexation with actives.
- The drug loading capability of nanosponges mostly depends on the degree of crystallization. Para-crystalline nanosponges have revealed diverse drug loading competency.
- They are non-hazardous, permeable particles insoluble in most of organic solvents.

Atorvastatin Calcium

Atorvastatin calcium is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of HMG-CoA reductase, the rate- limiting enzyme that converts 3-hydroxy-3-methyl-glutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Triglycerides (TG) and cholesterol in the liver are incorporated into very low density lipoprotein (VLDL) and released into the plasma for delivery to peripheral tissues. Low density lipoprotein (LDL) is formed from VLDL and

is catabolised primarily through the high affinity LDL receptor.

Methodology

Analytical studies:

- Determination of Calibration curve by UV visible spectrophotometer
- Analytical method validation by UV visible spectrophotometer
- Determination of melting point
- Drug stability study
- Saturation Solubility study
- Preformulation Studies
- Analytical Techniques Used to Detect Drug-Excipient Compatibility
- Thermal Technique
- Differential Scanning Calorimetry (DSC)
- Non thermal Techniques or Spectroscopic techniques:
- FT-IR Spectroscopy (Vibrational spectroscopy)
- Powder X-ray diffraction (PXRD

Table 1: Design matrix of formulation

Batches Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Weight of drug (mg)	40	40	40	40	40	40	40	40	40
PVA: EC (%w/w)	1:1			1:2			1:3		
WPI :EC (%w/w)		1:2			1:3			1:1	
SPI:EC (%w/w)			1:3			1:1			1:2
DCM (ml)	20	20	20						
GTAD (ml)				20	20	20			
DMC (ml)							20	20	20
DW (ml)	150	150	150	150	150	150	150	150	150

Table 2: Design matrix of formulation

Step I: Synthesis of β-CD Nanosponges					
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
β-CD:DMC (mmol or gm)	1:1	1:2	1:3	1:4	1:5
β-CD (gm)	17.42	17.42	17.42	17.42	17.42
β-CD (mmol)	15.34	15.34	15.34	15.34	15.34
DMC (gm)	9.96	19.92	29.88	39.84	49.8
DMC (mmol)	61.42	122.84	184.26	245.68	307.1
Ethanol (ml)	100	100	100	100	100
Step II: Preparation of ATRC-Loaded NS					
ATRC: β-CD (w/w)	1:1	1:2	1:3	1:4	1:5
Drug	40	40	40	40	40
β-CD NSGs	40	80	120	160	200
Dist. water (ml)	20	20	20	20	20
Centrifugation (RPM)	2000	2000	2000	2000	2000

Table 3: Design matrix of formulation

Step I: Synthesis of β-CD Nanosponges			
Ingredients	**F1**	**F2**	**F3**
DMF	100	100	100
β-CD:DMC (mmol or gm)	1:2	1:4	1:8
β-CD (gm)	17.42	17.42	17.42
β-CD (mmol)	15.34	15.34	15.34
DMC (gm)	19.92	39.84	79.68
DMC (mmol)	30.68	61.36	122.72
Ethanol (ml)	100	100	100
Dist. water (ml)	100	100	100
Step II: Preparation of ATRC-Loaded NS			
ATRC: β-CD (w/w)	1:5	1:10	1:15
Drug	40	40	40
β-CD NSGs	200	400	600
Dist. water (ml)	20	20	20
Centrifugation (RPM)	2000	2000	2000

Table 4: Design matrix of formulation

Formul-ations	Weight of drug(mg)	Dimethyl sulfoxide(ml)	β-CD (mg)	Ethyl cellulose(mg)	Whey Protein isolate(mg)	Dimethyl carbonate(ml)	Distilled water(ml)	Ethanol (ml)
F1	100	10	500	500	100	23.15	150	100
F2	100	10	500	500	100	46.3	150	100
F3	100	10	500	500	100	69.45	150	100
F4	100	10	1000	1000	100	23.15	150	100
F5	100	10	1000	1000	100	46.3	150	100
F6	100	10	1000	1000	100	69.45	150	100
F7	100	10	1500	1500	100	23.15	150	100
F8	100	10	1500	1500	100	46.3	150	100
F9	100	10	1500	1500	100	69.45	150	100

Table 5: Formulation of NSGs using factorial design

Ingredients Batches	ATRC (mg)	EC mg (X_1)	Amountof DMSO(ml)	Amount of DMCml (X_2)	Amountof WPI(mg)	Amountof DW	Amountof ethanol (ml)
F1	100	1500	10	69.45	100	150	150
F2	100	1000	10	69.45	100	150	150
F3	100	1000	10	23.15	100	150	150
F4	100	1500	10	46.3	100	150	150
F5	100	500	10	23.15	100	150	150
F6	100	500	10	69.45	100	150	150
F7	100	500	10	46.3	100	150	150
F8	100	1000	10	46.3	100	150	150
F9	100	1500	10	23.15	100	150	150

Aim

- The aim of this study was to design, formulate, and characterize the atorvastatin calcium loaded nanosponges (NSGs) by using a suitable factorial design.

Objectives

- To fabricate the nanoporous drug delivery system of atorvastatin calcium for enhancement of solubility, better encapsulation, dissolution rate and oral therapeutic effectiveness.
- To explore its application in the improvement of bioavailability,
- sustained release/ controlled release preparations as well as stability enhancement.
- To investigate the influence of various experimental parameters on nanoparticles like type, concentration and molecular weight of cross linkers, drug concentration and process parameters of ultrasonication.
- To investigate the oral bioavailability of developed formulations by in-vivo pharmacodynamic study in animals.

Results and Discussion

Table 6: FTIR spectra observed frequencies of Atorvastatin calcium

Number	Functionalgroups	Reported frequency(cm^{-1})	Observedfrequency(cm^{-1})
3	C-H Bending	755	746.317
5	C=C bending	840-790	841.776
10	C-O stretching strong	1210-1163	1159.01
12	C–N stretching	1250-1020	1241.93
13	C–F stretching	1400-1000	1317.14
14	O-H bending	1440-1395	1435.74
15	N-O stretching strong	1550-1500	1509.03
16	N-O stretching strong	1550-1500	1551.45
17	C=C stretching strong	1650-1566	1578.45
18	N-H bending medium	1650-1580	1650.77
20	C–H stretching	3000-2840	2970.8
21	N-H stretching	3400-3300	3364.21
22	O-H stretching Free	3700-3584	3668.91

Table 7: Absorbance data for ATRC in phosphate buffer 6.8

Sr. No.	Concentration (µg/ml)	Absorbance ± Standard Deviation (SD)	% CV
1.	0	0	0
2.	2	0.0843±0.0015	1.8113
3.	4	0.1760±0.0010	0.5682
4.	6	0.2843±0.0006	0.2031
5.	8	0.3753±0.0067	1.7740
6.	10	0.5260±0.0020	0.3802
7.	12	0.6150±0.0010	0.1636
8.	14	0.7013±0.0006	0.0823
9.	16	0.8240±0.0026	0.3211
10.	18	0.933±0.0036	0.3864
11.	20	1.0440±0.0036	0.3454

Parameters	Values
Correlation coefficient (r^2)	0.998
Slope	0.051
Intercept	-0.023
Equation	y = 0.051x - 0.023

Table 8: Recovery studies

Sr. No	Level of % Recovery	Initial amount present(µg/ml)	Amountof Std.Added (µg/ml)	Total Amount present (µg/ml)	Total amount Recover ed (µg/ml)	%Recovery	% Mean Recovery
1	80	10	8	18	18.0078	100.04	99.98±0.079
	80	10	8	18	17.998	99.89	
	80	10	8	18	18.001	100.01	
2	100	10	10	20	20	100	99.94±0.076
	100	10	10	20	19.97	99.86	
	100	10	10	20	19.99	99.98	
3	120	10	12	22	22.005	100.02	99.94±0.110
	120	10	12	22	21.960	99.82	
	120	10	12	22	22	100	

Table 9: Statistical validation for recovery studies.

Level of % Recovery	% Mean Recovery	Standard Deviation	Coefficient of variance	Standard mean error	Lower 95% Confidence limit	Upper95% Confidence limit
80	99.98±0.079	0.0794	0.0794	0.0459	99.890	100.070
100	99.94±0.076	0.0758	0.0758	0.0438	99.860	100.032
120	99.94±0.110	0.1102	0.1102	0.0636	99.822	100.072

Table 10: Results of robustness

	Drug in standardsolution (µg/ml)	Measured conc.(µg/ml)	% Recovery
0.1 N NaOH with2% Methanol	14	13.918	99.41
	14	13.959	99.707
	14	14.020	100.14
	MEAN (n=3±SD)	13.97±0.052	99.76±0.367
0.1 N NaOH	14	13.857	98.979
	14	13.877	99.125
	14	13.79	98.54
	MEAN (n=3±SD)	13.84±0.046	98.88±0.305

Table 11: Robustness data for system suitability

Conc (µg/ml)	Sample	Different Wavelength		
	(% Assay)	245 nm	246 nm	247 nm
10 PPM	1	0.498	0.507	0.507
10 PPM	2	0.491	0.499	0.505
10 PPM	3	0.501	0.504	0.491
MEAN (n=3±SD)		0.497± 0.0051	0.503±.004	0.501±0.0087
± 2% from mean assayvalue of method Precision		0.008	0.002	0.004

Table 12: Stability of standard and sample preparation.

Time (Hrs)	Drug in standardsolution (µg/ml)	Absorbance*n=3,±SD	RSD	% RSD
0	12	0.5577±0.0045	0.0081	0.8086
After 1	12	0.5544±0.0031	0.0055	0.5511
After 3	12	0.555±0.0036	0.0065	0.6496
After 6	12	0.557±0.0050	0.0090	0.8977
After 12	12	0.5614±0.0025	0.0045	0.4483
After 24	12	0.5627±0.0045	0.0080	0.8014
Limit at (1hr)	0.5978			
Limit at (3hrs)	0.4781			
Limit at (6hrs)	0.1196			
Limit at (12hrs)	-0.6575			
Limit at (24hrs)	-0.8966			

Table 13: Multimedia solubility studies of ATRC a

Media	Solubility (µg/mL)
Dist. water	12.56±0.235
pH 1.2	1.60±0.119
pH 6.8	18.55±0.565
pH 7.4	14.25±0.114

Table 14: Reported and observed IR frequencies of ATRC

Number	Functionalgroups	Reported frequency (cm^{-1})	Observedfrequency (cm^{-1})
3	C-H Bending	755	746.317
5	C=C bending	840-790	841.776
10	C-O stretching strong	1210-1163	1159.01
12	C–N stretching	1250-1020	1241.93
13	C–F stretching	1400-1000	1317.14
14	O-H bending	1440-1395	1435.74
15	N-O stretching strong	1550-1500	1509.03
16	N-O stretching strong	1550-1500	1551.45
17	C=C stretching strong	1650-1566	1578.45
18	N-H bending medium	1650-1580	1650.77
20	C–H stretching	3000-2840	2970.8
21	N-H stretching	3400-3300	3364.21
22	O-H stretching Free	3700-3584	3668.91

Table 15: Percent drug encapsulation and drug loading of NSGs[a]

Run	ATRC: EC (% w/w)	Percent drug encapsulation (%)	Unentraped drug (%)	Drug Loading (%)	Solubility (µg/ml)
F1	1: 15	80.56±0.25	19.43±0.25	14.39±0.427	75.42±0.111
F2	1: 10	79.06±0.25	20.94±0.25	21.96±0.786	89.63±0.652
F3	1: 10	84.36±0.78	15.64±0.78	23.43±0.471	107.57±0.412
F4	1: 15	82.87±0.56	17.13±0.56	14.80±0.893	133.32±2.328
F5	1: 5	86.60±0.38	13.40±0.38	39.36±0.361	163.46±2.394
F6	**1: 5**	**93.89±0.26**	**6.11±0.26**	**42.68±0.711**	**313.86±3.093**
F7	1: 5	89.62±0.25	10.38±0.25	40.74±0.917	184.69±0.903
F8	1: 10	81.53±0.34	18.47±0.34	22.65±1.082	226.96±1.749
F9	1: 15	79.49±0.44	20.51±0.44	14.20±0.878	139.73±0.867

Table 16: ^{1}H-NMR chemical shifts of ATRC-NSGs

δ values	Integration	Proton Type	Number
1.238 Multiplet	4.13	C-H	1
1.375, 1.372 Doublet	21.33	2XCH3	6
1.361, 1.359 Doublet			
1.610, 1.689 Multiplet	3.18	CH2	2
1.946, 1.930 Doublet	3.22		
2.047,2.039 Doublet	3.09, 3.06	H-C-H (CH2)	2
3.228 Multiplet	3.49	C-H	1
3.646 Multiplet	3.81	CH2	1
3.744, 3.736 Multiplet	6.15	CH2 (N-CH2)	2
3.948 Multiplet	3.20	C-H	1
6.980, 7.522 Multiplet	6.06, 11.89, 18.37, 6.00	2 (H), 4H, 6H, 2H Aromatic C-H	14
9.838 Singlet	3.03	N-H	1

Table 17: 1) Stability study of ATRC-NSGs

Time (Month/s)	Physical Appearance	Particle size(nm)	EntrapmentEfficiency (%)	% CDR	Solubility (µg/ml)
25±2ºC/65± 5% RH					
0	white crystalline powder	108.39±1.99	93.13±0.13	93.81	311.26±2.17
3	white crystalline powder	109.61±0.94	93.01±0.12	93.44	310.03±0.23
6	white crystalline	110.43±1.37	93.18±0.1	93.27	309.39±0.82
	powder				
30±2ºC/70±5% RH					
0	white crystalline powder	109.50±2.28	93.34±0.19	93.45	311.15±1.62
3	white crystalline powder	109.67±1.24	93.22±0.19	93.26	310.82±1.23
6	white crystalline powder	111.16±0.90	93.09±0.18	92.91	309.90±0.14
40±2ºC/ 75±5% RH					
0	white crystalline powder	110.29±2.26	93.18±0.4	93.27	312.78±0.94
3	white crystalline powder	111.03±1.52	93.09±0.37	92.73	311.26±0.22
6	white crystalline powder	111.56±1.45	92.95±0.4	92.55	311.01±0.21

Table 18: Hyperlipidemic efficiency of Std-M (group III), Pure-ATRC (groupIV) and ATRC–NSGs (group V) compared to negative control (group I) and positive control (group II) animals (n=3; mean± SD)

Parameters	Group I	Group II	Group III	Group IV	Group V
TC	55.98 ±5.29	124.53±3.72 55.22% increase	68.80±1.41 44.75% reduction	73.47±2.48 41% reduction	65.84±2.97 47.13% reduction
TG	70.14±3.77	109.53±1.33 36.24% increase	93.08±1.78 14.41% reduction	87.02±1.98 21.61% reduction	75.28±3.01 32.19% reduction
HDL-C	41.97±1.97	31.48±2.02 35.39% reduction	33.54±3.12 6.54% increase	35.40±2.67 12.46% increase	54.22±3.18 72.22% increase
LDL-C	47.61±1.81	120.15±3.14	84.66±2.59	95.25±3.77	61.47±1.55
		60.33% increase	28.49% reduction	20.72% reduction	48.84% reduction
VLDL	14.41±1.55	73.26±1.80 80.26% increase	23.52±3.25 39.94% reduction	24.23±2.40 66.93% reduction	19.34±2.26 73.60% reduction

Discussion

The atorvastatin calcium was procured as a gift sample obtained from Vitalife Laboratories, Haryana, India and its standardization was done as per USP specifications. Atorvastatin calcium was tested for the following.

Appearance: Color and morphology of drug was observed visually.

Solubility: Solubility was checked in various solvents like methanol, ethanol, dist. water, acetonitrile, phosphate buffer of different pH and aqueous solution of pH 4 etc.

Identification tests: Infrared spectrum of drugs was investigated using FTIR Infrared Spectrophotometer using potassium disk method. Spectrum was scanned over the wave number range 4000-400 cm^{-1}.

Loss on drying: Drug (1gm) was weighed and dried in an oven at 100°C- 105°C to constant weight for 4 hours. The weight was again recorded.

Melting point: Melting point of ATRC was found in the range 159-161°C which is in close agreement with the literature.

UV spectrum: Ultraviolet spectrum of drug was taken using (Shimadzu, UV- 1800) Ultraviolet Spectrophotometer in dist. water and methanol, as solvents. Spectrum was scanned over wavelength range of 400- 200 nm.

Assay: Percent drug content was considered as mentioned in Certificate of Analysis of drug obtained from the supplier and confirmed by the analytical method described in later section.

The in-vitro drug release profile of the optimized freeze dried ATRC-NSGs (F6) formulation is depicted in Fig IA. 76-78. It was observed that, formulation (F6) showed analogous to that of initial cumulative drug release profile which was done before stability. Thus, no significant difference has been revealed in the formulation when exposed at different temperature and humidity conditions like 25ºC ± 2ºC and RH 60% ±5%, 30ºC ± 2ºC and RH 65% ± 5% and 40ºC ± 2ºC and RH 75% ± 5% as per the stability guidelines. The % CDR study indicated that there was no significant difference observed as compared to the initial cumulative drug release profile of optimized freeze dried ATRC-NSGs. Thus, the FT-IR spectrum of physical mixture revealed that the functional groups of ATRC, EC, WPI and other excipients were seen in the spectrum. Hence it is concluded that, all excipients in the mixture were found to be compatible with each other and does not showed any interaction with each other[12-15]. ATRC API exhibited a series of high peaks intensity and showed sharp characteristic diffraction patterns at 2θ angle of 9.07, 9.41, 10.18, 10.46, 11.76, 12.10, and 16.96°, indicating crystalline nature of ATRC. EC showed a two characteristics broad hen peak diffraction pattern at 2θ angle of 13.41, 13.71, 14.92, 20.53, 21.733, 21.84, 22.03° indicates the amorphous nature of EC. FESEM analysis of ATRC API, EC, WPI and Physical mixture (Fig IA.26-29) showed combination of sharp, prismatic, slender and flattened crystal habits with an average size range of > 20 microns confirm the crystalline nature of ATRC whereas, FESEM image of EC exhibits aggregate and paracrystalline in nature.

The particles were often located near to each other and particle size was found to be in almost micron in size. Moreover, surface was found to be irregular in shape and few porous cavities was also seen. This method was found to be tiresome and non consistent for easy scale up. Hence, this method was considered as discontinued for further scale up.

The in vitro release profile of ATRC, Marketed tablet, lyophilized ATRC-NSGs in phosphate buffer pH 6.8 at 37°C shown in Fig IA. 71-72. The % drug release was not identical for all the batches. Initially the NSGs showed immediate release of ATRC, this may be due to release of drug from the surface of NSGs, followed by slow and controlled release of drug which was encapsulated in nanosponges. It was evident that, marketed tablet showed significant improvement in dissolution rate with respect to ATRC alone. Furthermore, the lyophilized ATRC-NSGs (F6) exhibit faster dissolution as compared to plain ATRC as well as marketed formulation and it was found to be 93.98%, 37.81 % and 59.56% respectively. The concentration of polymer and cross-linker affects the release rate of ATRC from nanoparticles. Conversely, as the polymer

concentration increases, decrease in the rate and extent of drug release was observed[16-17].

This may be due to increase in the density of the polymer matrix and also an increase in the diffusion path length that the drug molecules have to travel. The results were evaluated for the statistically significant difference using the one-way ANOVA (P <0.05) when all test groups were compared with control group. Triglyceideslevel was found to be decreased in Gr-5 when compared with the control group (Gr-2). LDL cholesterol level was found to be reduced in Gr-5 when compared with the control group (Gr-2). HDL cholesterol level in Gr-5 was enhanced when compared with the control group (Gr-2). VLDL level was found to be decreased in Gr-5 when compared with the control group (Gr-2) and thus exhibited significant antihyperlipidemic activity (P<0.05) whereas plain ATRC was found to be insignificant (P > 0.05). Pharmacodynamic study substantiates enhancement of solubility of ATRC because of nanosponges drug delivery system as compared to plain ATRC. Optimized freeze dried ATRC-NSGs showed better lessening in elevated level of TG, TC, LDL and VLDL and better improvement in HDL level, as these nanosize formulations has the potential to lessen the inconsistency in absorption and in addition to it, first pass metabolism ofATRC also avoided.

Conclusion

- Atorvastatin calcium is a synthetic lipid-lowering agent. Nanonization techniques confirmed to be a superior alternative in formulation development of poorly soluble drugs.
- There was no interference of different components observed with respect to time. The technique was productively validated as per ICH principles. UV spectroscopy method was developed for the investigation of dissolution samples. A series of such standard curves were constructed and the linearity range was determined in dist. water and different dissolution media (pH 1.2, 6.8 and 7.4).
- A preliminary nanonization protocol was set up for screening of formulation components. Range of stabilizers from the category of polymers (Polyvinyl alcohol, Whey protein isolate, Soya protein isolate and Kollidon K30), cross-linkers (Dichloromethane, Dimethyl carbonate, glutaraldehyde) and their rational combinations were systematically screened for development of stable NSGs of ATRC.
- Various proportion of polymer, cross-linker and stabilizer modulation has been done by considering the negligible effect on particle size after freeze drying. Finally formulated NSGs were subjected to freeze drying and resultant freeze dried powder was transformed into capsule using routine excipients.[18-19]
- The optimized batch of NSGs was in depth characterized at three key stages viz. nanosuspension (formulation) stage, freeze dried powder stage and capsule stage.
- Formulated nanosponges formulation was found to be stable at accelerated stability conditions as per ICH guidelines up to 6 months. Comparative in-vivo pharmacodynamic study (which was done as per approved protocol by CPCSEA committee) in male Wistar rats at fasted and fed conditions reveled that, formulated nanosponges successfully evade variation in optimized freeze dried ATRC-NSGs which showed better lessening in prominent level of TG, TC, LDL as well as VLDL and better improvement in HDL level, as these nanosize formulations has the potential to lessen the unpredictability in absorption and in addition to it, it was also evade first pass metabolism of ATRC.

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