Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Galactosylated Albumin Nanoparticles of Simvastatin

KUMAR GANESH, DHYANI ARCHANA* , KOTHIYAL PREETI

Sri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun

ARTICLE DETAILS

Article history:
Received on 11 August 2013
Modified on 15 September 2013
Accepted on 20 September 2013

Keywords:
Hepatotoxicity,
Galactose,
Targeting,
Asialoglycoprotein receptor.

ABSTRACT

In the present study, an attempt was made to develop galactosylated albumin nanoparticles of Simvastatin for treatment of hypercholesterolemia. By developing the galactosylated nanoparticulated delivery the reqiured action of drug at the target site i.e at liver can be provided. The advantage of targeting helps to reduce the systemic side effects which may be occur due to the distribution of the drug to the other organs and thus helps in maintaing the required concentration of drug at the desired site. The galacotsylated albumin nanoparticles were prepared for the selective delivery of an, Simvastatin to the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) the rate limiting enzyme in the pathway of cholesterol biosynthesis which is particularly presents on liver. The albumin nanoparticles (NPs) were prepared by using desolvation method and efficiently conjugated with galactose. Various parameters such as particle size, % entrapment efficiency and drug loading efficiency, percentage yield, *in vitro* drug release, were determined. The size of nanoparticles (both plain and coated NPs) was found to be 200 and 250 nm.The maximum drug content was found to be 79.98 and 79.8 % respectively in plain and galactose coated nanoparticles while the maximum entrapment efficiency was found to be 70.10% and 71.03% in plain and coated nanoparticles. It was also found that coating of nanoparticles increases the size of nanoparticles.

© KESS All rights reserved

INTRODUCTION

The liver is the primary organ for regulation of total body cholesterol homeostasis in mammalian systems. Hepatic coordination of cholesterol biosynthesis with assembly, secretion, and uptake of plasma lipoproteins depends in part on cellular mechanisms coupling the activities of the key enzymes of sterol synthesis with the receptors governing lipoprotein clearance [1]. Thus an important target for pharmacological regulation of plasma low density lipoptotein cholesterol is liver 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate limiting enzyme in the pathway of cholesterol biosynthesis.

Drug low solubility and stability in physiological environment constitutes a main hurdle in attaining the appropriate bioavailability. Several polymer-based nanotechnologies are being intended in order to optimize the technological (e.g., solubility, stability, bioavailability, etc.) aspects of drugs. Among them, polymeric nanoparticles, dendrimers, polymeric micelles and polymersomes appear as the most attractive and promising [2,3].

Dyslipidemia, including hypercholesterolemia, hypertriglyceridemia, or their combination, is a major risk factor for cardiovascular disease. Generally, dyslipidemia is characterized by increased fasting concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), in conjunction with decreased concentrations of high-density lipoprotein cholesterol (HDL-C). At present, these lipid imbalances are most routinely treated with pharmacological therapy.

Simvastatin is a poorly soluble lipid-lowering agent which is used for the treatment of primary hypercholesterolemia. When given orally, Simvastatin (a lactone) undergoes hydrolysis and is converted to the β, δ-dihydroxy acid form, a potent competitive inhibitor of 3-hydroxyglutaryl-CoA reductase the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis [4]. Water solubility of Simvastatin is very low, approximately 30 µg/ml [5]. It is

*Author for Correspondence:
Email: archana.dhyani89@gmail.com

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 [6]. Here, the solubility of Simvastatin is increased by the addition of surfactants and reduction of particle size.

Albumin is an attractive macromolecular carrier and widely used to prepare nanospheres and nanocapsules, due to its availability in pure form and its biodegradability, nontoxicity and nonimmmunogenicity. Both Bovine Serum Albumin or BSA and Human Serum Albumin or HSA have been used. As a major plasma protein, albumin has a distinct edge over other materials for nanoparticle preparation. On the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry reactive groups (thiol, amino, and carboxylic groups) on their surfaces that can be used for ligand binding and/or other surface modifications and also albumin nanoparticles offer the advantage that ligands can easily be attached by covalent linkage. Drugs entrapped in albumin nanoparticles can be digested by proteases and drug loading can be quantified. A number of studies have shown that albumin accumulates in solid tumors making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs [7].

Among the available potential colloidal drug carrier systems covering the size range described,protein-based nanoparticles play an important role. Basically three different methods for their preparation have been described, based on emulsion formation, desolvation, or coacervation. Most often serum albumin of different origin as well as gelatin were used as the starting material for the preparations.With respect to emulsion techniques applying human serum albumin (HSA), a complete and systematic study concerning the influence of protein concentration, emulsification time and power, stirring rate, heat stabilization temperature, and the type of the non-aqueous phase [8]. The disadvantage of the emulsion methods for particle preparation is the need for applying organic solvents, for the removal both of the oily residues of the preparation process and of surfactants required for emulsion stabilization. Therefore, as an alternative method for the preparation of nanoparticles a desolvation process derived from the coacervation method of microencapsulation was developed. In 1993, Lin et al. described the preparation of Human Serum Albumin nanoparticles of diameter around 100 nm using a surfactant-free pH-coacervation method [9]. The particles were prepared by the dropwise addition of acetone to an aqueous Human Serum Albumin solution at pH values between 7 and 9, followed by glutaraldehyde crosslinking and purification by gel permeation chromatography. It was found that with increasing pH value of the Human Serum Albumin solution particle size was reduced, apparently due to an increased ionization of the HSA (isoelectric point pI = 5.3) which leads to repulsion of the Human Serum Albumin molecules and aggregates during particle formation. Human Serum Albumin nanoparticles were obtained in a size range between 90 and 250 nm,by adjusting the pH and by controlling the amount of added acetone.

MATERIALS AND METHODS

Materials

Simvastatin was a gift sample from Ind. Swift Pharmaceutical Ltd, Chandigarh, sterile bovine serum albumin,sodium chloride,sodium lauryl sulphate, ethanol were obtained from Central Drug House Ltd, New Delhi. All the reagents and solvents used were of analytical grade satisfying Pharmacoepial standards.

Preparation of Bovine Serum Albumin nanoparticles

Bovine Serum Albumin nanoparticles were prepared by a desolvation[10]. In principle, between 50 and 1000 mg Bovine Serum Albumin in 2.0 ml of purified water or 10mM NaCl solution, respectively, both titrated to pH 7–10, the drug was also incorpoated and addition of few ml of 0.5% Sodium Lauryl Sulfate concentration were transformed into nanoparticles by the continuous addition of 8.0 ml of the desolvating agent ethanol under stirring (500 rpm) at room temperature.After the desolvation process, 8% glutaraldehyde in water was added to induce particle crosslinking (Table 1). The crosslinking process was performed under stirring of the suspension over a time period of 24 hrs.

Table 1: Composition of different Nanoparticle formulations

Ingredients	Formulations				
	F1	F2	F3	F4	F5
Drug(mg)	40	40	40	40	40
BSA(mg)	50	100	200	600	1000
Ethanol (ml)	8	8	8	8	8
Glutaraldehyde(%)	8	8	8	8	8
Galactose(mg)	20	20	20	20	20
Sodium Lauryl Sulfate (%)	0.5	0.5	0.5	0.5	0.5

Purification of BSA nanoparticles

The resulting nanoparticles were purified by three cycles of differential centrifugation (20,000 rpm, 8 min) and redispersion of the pellet to the original volume in water or 10mM NaCl at pH values of 7 and 9, respectively. Each redispersion step was performed in an ultrasonication bath over 5 min.The solvent was evaporated by rotary evaporated and the nanoparticles were stored at 2-8 °C.

Galactose coating of Nanoparticles

20 mg of galactose were added to 10 mg of BSA nanoparticles nanoparticles dispersed in 5 mL acidic PBS (pH 5.0), and the mixture was then stirred at room temperature over-night. The resulting nanoparticles were purified by three cycles of differential centrifugation (20,000 rpm, 8 min) and redispersion of the pellet to the original volume in water or 10mM NaCl at pH values of 7 and 9, respectively. Each redispersion step was performed in an ultrasonication bath over 5 min. The solvent was evaporated by rotary evaporated and the nanoparticles were stored at 2-8 °C.

Characterizations of Nanoparticles

Shape and Size

The morphology of plain and galactose-coated nanoparticles was determined by Scanning electron microscopy.

Drug content uniformity

10 mg of nanoparticle was taken and introduced in a 100ml volumetric flask. The nanoparticles were dissolved in phosphate buffer pH 7.4 and make up the volume upto 100ml. The above solution was analyzed by UV spectrometer at 238 nm.

Entrapment efficiency

10 mg of nanoparticle was taken and introduced in a 100ml volumetric flask. The nanoparticles were dissolved in phosphate buffer pH 7.4 and make up the volume upto 100 ml. The above solution was analyzed by UV spectrometer at 238 nm.The entrapment efficiency of the prepared nanoparticles was calculated by the formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Practical drug loading} - \text{Theoretical drug loading}}{\text{Theoretical drug loading}} \times 100$$

Percentage Yield

It is calculated to knowabout the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material. It can be calculated using following formula:

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

In vitro drug release

In vitro drug release study was carried out by Modified Diffusion Apparatus. The apparatus consists of a beaker containing 50 ml of phosphate buffer pH 7.4 maintained at 37□C under mild agitation using a magnetic stirrer acts as receptor compartment. An open ended tube acts as donor compartment and the egg membrane was tied into upper part of the donor compartment. The nanoparticles (plain and galactose coated) equivalent to 10 mg were placed into the donor compartment over the membrane which was dipped in the receptor compartment consisting buffer. Then, the samples were taken at different time intervals from the receptor compartment and were analyzed by UV spectrometer at 238 nm.

Mathematical modeling

The data obtained from *in vitro* release studies was treated by various conventional mathematical models (zero-order, first-order, Higuchi, Korsmeyer- Peppas) to determine the release mechanism from the designed nanoparticle formulations [10–12]. Selection of a suitable release model was based on the values of R (correlation coefficient), k (release constant) and n (diffusion exponent) obtained from the curve fitting of release data.

Receptor ligand Binding Study

After fasting overnight mice were killed by cervical dislocation, liver were excised, and homogenized with 0.1M phosphate buffer pH 7.4.The homogenate were homogenized in 0.25M sucrose containing EDTA (1mM). The homogenate was centrifuged at 30,000 rpm for 10 min. The resulting supernatant was centrifuged at 10,000 rpm for 10min. The supernatant was collected and suspended in the same buffer. 10mg of nanoparticles were added into the supernatent containing hepatocytes and homogenized at a high speed (20,000 rpm) for 20 min. Place 5 ml of this solution in donor compartment of Modified Diffusion Apparatus. Then, the samples were taken at definite time intervals from the receptor compartment and were analyzed by UV spectrometer at 234 nm.

RESULTS AND DISCUSSIONS

Five formulations of Simvastatin were formulated using different drug polymer ratios.The formulation is subjected to evaluation parameters like particle size, percentage yield, entrapment efficiency, zeta potential, drug content uniformity, invitro drug release.

Characterization of Nanoparticles

Particle Size

The particle size of all batches of plain nanoparticles was found to be in the size of 200 nm and that of galactose coated nanoparticles was found to be in the size range of 250 nm.

The SEM photomicrographs of nanoparticles are shown in Fig. 1 (a & b). It was observed from these photomicrographs that all samples of particles were smooth, sub-spherical in shape and aggregated to form small clusters.

The larger particle size of galactosylated nanoparticles as compared to plain nanoparticles could be due to the anchoring of galactose molecule at the surface of nanoparticles and hence an increment in size of nanoparticles was observed.

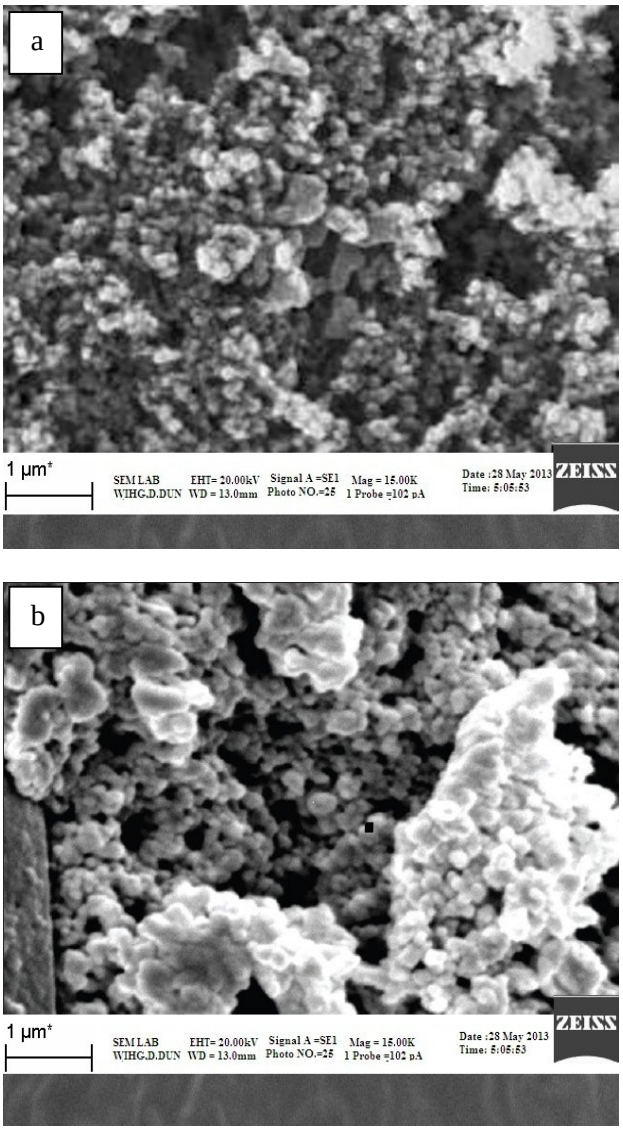

Figure 1: (a) Scanning electron microscopy (SEM) photomicrograph of Albumin-Nanoparticles; (b) SEM photomicrograph of Galactose coated Nanoparticles

Drug content uniformity

The drug content of different formulations F1 to F5 was calculated and the content was found to be in range of 45.09 to 93.80% for plain nanoparticles and 46.8 to 95.98% for coated nanoparticles. The maximum drug content was found to be 93.8% for plain and 95.98 % for coated nanoparticles for the formulation F3. The nanoparticles exhibited an increase in drug content with an increased in polymer ratio, up to particular concentration. A decrease in drug content was observed after that point due to saturation capacity of polymer. The results are shown in Table 2.

Table 2: Drug Content of Simvastatin nanoparticles

Formulation Code	Drug Content (%)	
	Plain Nanoparticles	Coated Nanoparticles
F1	45.09	46.8
F2	55.12	57.81
F3	93.80	95.98
F4	82.09	84.09
F5	76.98	79.8

Nanoparticulate yield

The percentage yields of different formulations F1 to F5 were calculated and the yield was found to be in the range of 32.14 to 83.24% for plain nanoparticles and 28.75 to 79.8 % for coated nanoparticles. Percentage Yield of all batches is shown in Table 3. Maximum particle yield was found in F5 (83.24 % and 79.8% for plain and coated nanoparticles) where the concentration of albumin is highest while the nanoparticle yield is lowest in F1 (32.14% and28.75% for plain and coated nanoparticles) where the concentration of albumin is low.

The reduction in percentage yield after coating of nanoparticles might be occurring due to the loss of nanopaticles during the coating process.

Entrapment efficiency

The encapsulation efficiencies of all four formulations were given in the Table 4 and the entrapment efficiency was found to be in range of 32.19 to 90.91% for plain nanoparticles and 38.09% to 93.27 % for coated nanoparticles. The maximum entrapment efficiency was found to be 90.91% and 93.27 % for the formulation F3.

Table 4: Entrapment efficiency of Simvastatin nanoparticles

Formulation Code	Entrapment efficiency (%)	
	Plain Nanoparticles	Coated Nanoparticles
F1	32.19	38.09
F2	48.67	50.98
F3	90.91	93.27
F4	78.09	81.29
F5	70.10	71.03

The relatively higher percent drug entrapment was obtained for coated nanoparticles as compared to the plain nanoparticles which could be due to minimum repulsion between drug and polymer.

In vitro release profile

The comparative plot of the percent release profile of Simvastatin loaded BSA nanoparticles is shown in Fig. 2 (a & b). The key results obtained by evaluation of the percent release values are summarized in Table 5. As observed in Table 5, the overall highest release was observed in the formulation F1, which contained lowest amount of BSA (92.6 % after 10 h).

It can be interpreted from this result that the formulation with the lowest polymer content showed the fastest release. In contrast, F5, which contained maximum BSA, showed minimum release (50.8 % after 10 h). Thus, it can be interpreted that the formulation with high polymer content showed the slowest release. It was also found that coating of nanoparticles with galactose also decreases the dug release F5 (48.71% after 10 h).

Mathematical modeling

Correct determination of the release mechanism depends greatly on the selection and application of a suitable model to the release data. Model fitting of 10 h reveals that all the batches follow the matrix or Higuchi and Korsmeyer-Peppas model. The R values in the case of all batches were higher for the Korsmeyer-Peppas model. The values of n suggest that all formulations followed Super case II transport release mechanism from the nanoparticles. The R values of model fitting data for 10 h show that Simvastatin release followed the zero-order and matrix/Higuchi model.

Receptor - ligand binding study

From the study, it was found that the amount of dug release from the formulation F3 after 10 hrs was only 5.67%, prior to that the release was 42.09%.So, the remaining 36.42% drug binds with receptor present in hepatocytes.

CONCLUSIONS

It can be concluded from the above studies that it was possible to prepare Simvastatin nanoparticles using bovine serum albumin as a macromolecular material with controlled release up to 10 h.The mathematical model fitting of the release data showed that the formulations followed case II transport mechanisms.

Table 3: Percentage Yield of Simvastatin nanoparticles

Formulation Code	Total amount of ingredients (mg)		Percentage Yield (%)	
	Plain Nanoparticles	Coated Nanoparticles	Plain Nanoparticles	Coated Nanoparticles
F1	90	110	32.14	28.75
F2	140	160	41.23	36.09
F3	240	260	55.74	51.29
F4	640	660	74.31	70.09
F5	1040	1060	83.24	79.8

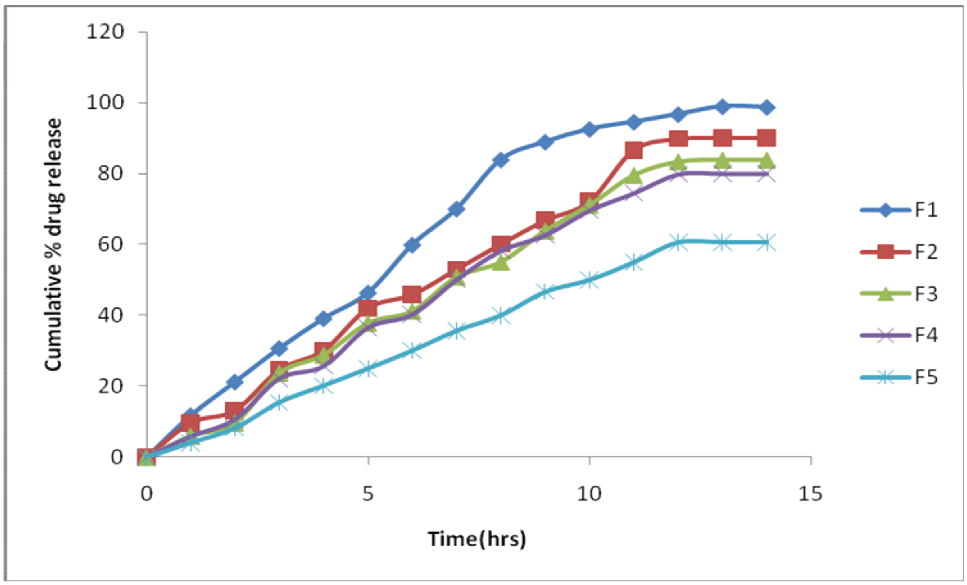

Figure 2(a): Zero order release Plot of Simvastatin plain nanoparticles

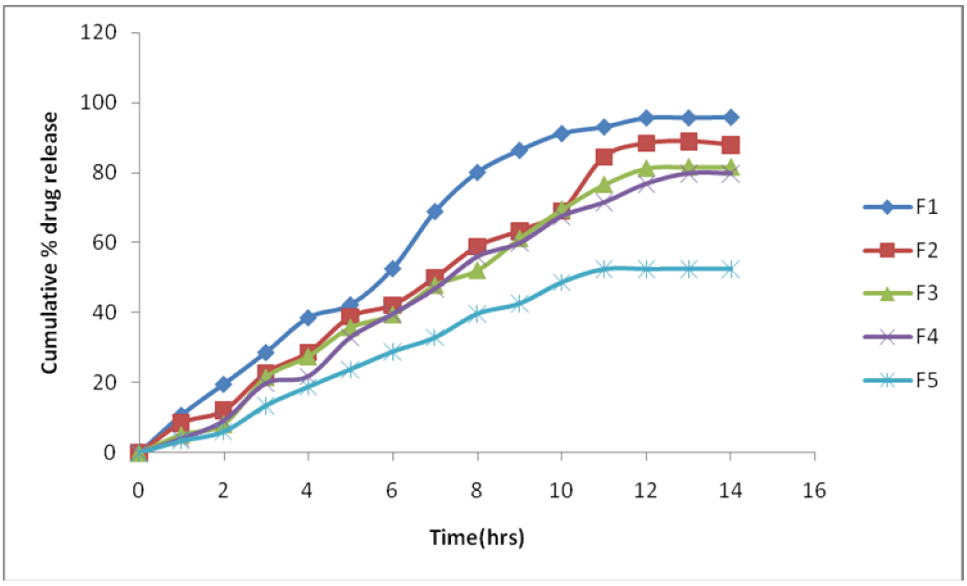

Figure 2(b): Zero order release Plot of Simvastatin galactose coated nanoparticles

Table 5: Cumulative % drug release of Plain and Galactose coated Nanoparticles

Time(hrs)	Cumulative % drug release									
	F1		F2		F3		F4		F5	
	Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated
1	11.84	10.84	9.76	8.54	6.06	5.21	5.92	4.12	4.16	3.45
2	21.31	19.65	13.26	12.12	9.78	8.25	10.76	9.23	8.29	6.08
3	30.78	28.73	24.7	22.87	23.79	21.65	22.26	19.87	15.59	13.52
4	39.19	38.63	30.18	28.72	28.9	27.56	25.78	21.87	20.29	18.9
5	46.42	42.34	42.08	38.9	37.91	35.87	36.59	32.98	25.12	23.87
6	59.96	52.67	45.87	42.24	41.29	39.5	40.31	39.64	30.19	28.92
7	70.1	68.97	52.98	50.07	50.89	47.89	50.1	46.82	35.79	32.96
8	83.96	80.14	60.1	58.98	55.12	52.15	58.26	55.97	40.16	39.71
9	88.98	86.43	66.78	63.45	63.91	61.2	62.64	59.87	46.76	42.65
10	92.6	91.23	72.12	69.08	71.18	69.53	69.63	67.5	50.13	48.71

Table 6: Kinetic Values Obtained from In-Vitro Release Profile of Nanoparticles (Zero order and First order)

Formulation	Zero Order Plot		First Order Plot	
	Regression Coefficient (r)		Regression Coefficient (r)	
	Plain Nanoparticles	Coated Nanoparticles	Plain Nanoparticles	Coated Nanoparticles
F1	0.992	0.993	0.917	0.921
F2	0.993	0.995	0.982	0.978
F3	0.993	0.993	0.969	0.965
F4	0.995	0.995	0.975	0.987
F5	0.998	0.993	0.990	0.986

Table 7: Kinetic Values Obtained from *In-Vitro* Release Profile of Nanoparticles (Higuchi, Korsmeyer Peppas models)

Formulation	Higuchi's				Korsmeyer Peppa's			
	Plain Nanoparticles		Coated Nanoparticles		Plain Nanoparticles		Coated Nanoparticles	
	Slope (n)	RegressionCoefficient (r)	Slope (n)	Regression Coefficient (r)	Slope (n)	Regression Coefficient (r)	Slope (n)	Regression Coefficient (r)
F1	32.28	0.921	30.97	0.945	0.898	0.985	0.897	0.984
F2	24.55	0.931	27.63	0.936	0.924	0.986	0.962	0.988
F3	26.72	0.946	26.98	0.956	0.987	0.983	0.978	0.980
F4	23.81	0.908	25.48	0.938	0.921	0.986	0.989	0.985
F5	19.22	0.943	17.4	0.943	0.921	0.997	0.923	0.978

The accumulation of galactose-coated albumin nanoparticles in liver is due their preferential macrophage uptake by RES organs.After administration, nanoparticles are selectively taken up by the macrophage rich organs by receptor-mediated endocytosis due to the presence of asialoglycoprotein receptor on the cell surface. After reaching inside the cell, these nanoparticles are degraded by lysosomes and entrapped Simvastatin is released, which is a potent competitive inhibitor of 3-hydroxyglutaryl-CoA reductase the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis. Based on these findings we can conclude that Simvastatin loaded albumin nanoparticles are promising agents for regional drug delivery in dyslipidemia, including hypercholesterolemia, hypertriglyceridemia.

REFERENCES

[1] Ma PT, Gil G, Sudhof TC, Bilheimer DW, Goldstein JL, Brown MS. Mevinolin an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. Proc Natl Acad Sci U S A. 1986: 83(21); 8370–8374.

[2] Allemann E, Leroux JC, Gurny R. Polymeric nano- and microparticles for the oral delivery of peptides and peptidomimetics. Adv Drug Deliv Rev. 1998; 34:171–18.

[3] Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci. 2002; 6: 319–327.

[4] Carlucci G, Mazzeo P, Biordi L, Bologna M, Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high performance liquid chromatography with UV detection. J Pharm Biomed Anal. 1992; 10: 693-697.

[5] O'Neil MJ. The Merck index—an encyclopedia of chemicals, drugs and biologicals 2006; 14: 1471-1472.

[6] Patel RP, Patel MM. Inclusion complexion-physico-chemical characterization and *in vitro* dissolution behavior of simvastatin-cyclodextrin inclusion compounds. Drug Deliv Technol 2007; 7: 50-56.

[7] Merodio M, Arnedo A, Renedo MJ, Irache JM, Ganciclovir loaded albumin nanoparticles: characterization and in vitro release properties, Eur J Pharm Sci. 2001; 12(3): 251-259.

[8] Gallo JM, Hung CT, Perrier DG, Analysis of albumin microsphere preparation. Int J Pharm. 1984; 22: 63–74.

[9] Lin W, Coombes AGA, Davies MC, Davis SS, Illum L, Preparation of sub-100 nm human serum albumin nanospheres using a pH-coacervation method. J Drug Target. 1993;1: 237–243.

[10] Chothy MF, Danenberg J, Lipophillic drug loaded nanospheres prepared by Nanoprecipitation technique: effect of formulation variables on size, drug delivery and release kinetics. J Control Release 2002; 83: 389-400.

[11] Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983; 15: 25–35.

[12] Ritger PL, Peppas NA. A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J Control Release 1987; 5: 37-42.