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Galactosylated Albumin nanoparticles bearing Cimetidine for effective management of Acetaminophen induced hepatotoxicity

ABSTRACT

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In the present study, an attempt was made to develop galactosylated albumin nanoparticles of Cimetidine for treatment of Acetaminophen induced hepatotoxicity. By developing the galactosylated nanoparticulated delivery of Cimetidine the required 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 maintain the required concentration of drug at the desired site. The use of cimetidine to treat Acetaminophen induced hepatotoxicity was based on the observation that it would lead to the competitive inhibition of the enzyme CYP 450 2E1 and reduce the acetaminophen metabolism to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive, electrophilic molecule .Thus, it might be useful in treatment of Acetaminophen induced hepatotoxicity. The galactosylated albumin nanoparticles were prepared for the selective delivery of an, Cimetidine to the asialoglycoprotein receptor (ASGP-R) which is particularly presents on mammilla in hepatocytes. 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 in range of 200-250 nm, and maximum drug payload was found to be 19.08% $\pm$ 1.10 .The maximum drug content was found to be 30.80% $\pm$ 0.3 and 27.09% $\pm$ 0.5 respectively in plain and galactose coated nanoparticles while the maximum entrapment efficiency was found to be 90.68% $\pm$ 0.5 and 91.75% $\pm$ 0.59 in plain and coated nanoparticles. It was also found that coating of nanoparticles increases the size of nanoparticles.

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INTRODUCTION

Hepatotoxicity is a direct liver injury caused by the toxic metabolite of acetaminophen. When taken in therapeutic doses, greater than 90% of acetaminophen is metabolized to phenolic glucuronide and sulfate in the liver by glucuronyltransferases and sulfotransferases and subsequently excreted in the urine. Of the remaining acetaminophen, about 2% is excreted in the urine unchanged .Approximately 5% to 10% is metabolized by cytochrome P450, mainly the enzyme CYP2E1, to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive, electrophilic molecule that causes harm by formation of covalent bonds with other intracellular proteins. This reaction is prevented by conjugation with glutathione and subsequent reactions to generate a water-soluble product that is excreted into bile. With acetaminophen overdose, glucuronyltransferases and sulfotransferases are saturated, diverting the drug to be metabolized by cytochrome P450 and generating NAPQI in amounts that can deplete glutathione [1-4].

Targeted delivery of drugs and proteins to liver can be achieved via asialoglycoprotein receptor, which can recognize and combine the galactose- and N-acetygalatosamine terminated glycoproteins. Glycosyl is usually conjugated with drugs directly to fabricate prodrugs or with nanoparticles encapsulated drugs via forming covalent bonds, while the covalent bonds may lead to some shortages for drug release. Therefore, we can prepare nanoparticles for efficient targeting by glycosylation using galactosylated polymer as a carrier to entrap the model drugs in nanoparticles core physically rather than forming covalent drug conjugation. The means of incorporation of drug in nanoparticles may improve drug release to maintain its activity, raise its therapeutic index and diminish the adverse effect. Due to their nanometer-size and galactosyl, the nanoparticles may be a potential delivery system for passive and active targeting to liver parenchymal cells for therapy of hepatitis and liver injury [5].

The asialoglycoprotein receptor (ASGP-R) which is particularly presents on mammilla in hepatocytes can be utilized for active targeting by using its natural and synthetic ligands. By utilizing this receptors can provides a unique means for the development of liver-specific carriers, such as liposomes, recombinant lipoproteins, and polymers for drug or gene delivery to the liver, especially to hepatocytes. These receptors recognize the ligands with terminal galactose or N-acetylgalactosamine residues, and endocytose the ligands for an intracellular degradation process [6].

Nanoparticles can be defined as the colloidal particles having size ranging from 10 to 1000 nm. The advantages of nanotechnology are to provide the safe and the effective medicine (nanomedicine) is set to substantially influence the landscape of both pharmaceutical industries. A large number of drugs can be delivered using nanoparticulates carrier via a large number of routes. These include many hydrophilic drugs, hydrophobic drugs as well as for proteins, vaccines, biological macromolecules, etc. They can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation[6].The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

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 [6-9].

EXPERIMENTAL

Materials

Cimetidine was a gift sample from indlas Biotech Ltd, Dehradun, sterile bovine serum albumin, sodium chloride and ethanol were purchased from Central Drug House Ltd, New Delhi. All the reagents and solvents used were of analytical grade satisfying Pharmacoepial standards (Table 1).

Formulation of Nanoparticles

- **Preparation of Master Formula:**

Table 1. Formulation plan for Cimetidine nanoparticles

INGREDIENTS	FORMULATIONS			
	F1	F2	F3	F4
Drug(mg)	800	800	800	800
Bovine Serum Albumin (mg)	50	150	250	350
Acetone(ml)	8	8	8	8
Glutaraldehyde (%)	8	8	8	8
Galactose (coating agent) (mg)	20	20	20	20

- **Preparation of Bovine Serum Albumin nanoparticles from desolvation method**

Bovine Serum Albumin nanoparticles were prepared by a desolvation technique .The different amounts of bovine serum albumin (i.e 50, 150, 250, 350 mg) was dissolved in 2.0 ml of 10mM NaCl solution, respectively, titrated to pH 8. The specified amount of drug was then added into bovine serum albumin solutions followed by the continuous addition of 8.0 ml of the desolvating agent i.e. acetone under stirring (500 rpm) at room temperature. After the desolvation process, 8% glutaraldehyde in water was added to induce particle crosslinking. The crosslinking process was performed under stirring of the suspension over a time period of 24 h.

- **Purification of Bovine Serum Albumin nanoparticles**

The resulting nanoparticles were purified by three cycles of differential centrifugation (10,000 rpm for 10 min) and redispersion of the pellet to the original volume 10 mM NaCl at pH values of 8, respectively. Each redispersion step was performed in an ultrasonication bath over 5 min. The solvent was removed and the nanoparticles were collected and stored in a refrigerator.

- **Galactose coating of Nanoparticles**

20 mg of galactose were added to 10 mg of bovine serum albumin loaded nanoparticles which is dispersed in 5 mL acidic phosphate buffer saline (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 (10,000 rpm for 10 min) and followed by redispersion of the pellet to the original volume in 10 mM NaCl at pH 8, respectively. Each redispersion step was performed in an ultrasonication bath over 5 min. The solvent was evaporated and the nanoparticles were collected and stored at 2-8° C.

Characterization of Nanoparticles

The formulated nanoparticles were evaluated for particle size, shape, zeta potential, drug content uniformity, entrapment efficacy, drug loading and *in-vitro* drug release study.

- **Shape and Size**

The morphology and size of plain and galactose-coated nanoparticles was determined by Scanning electron microscopy (SEM). (Zeiss, Evo 40, India).

- **Drug content uniformity**

500 mg of nanoparticles were crushed in mortar and pestle.10 mg of powdered nanoparticles

were taken and introduced in a 100ml volumetric flask. The nanoparticles were dissolved in phosphate buffer pH 7.4 and make up the volume up to 100 ml. The above solution was analyzed by UV spectrometer at 234 nm [10].

- **Entrapment efficiency and Loading efficiency**

500 mg of nanoparticles were crushed in mortar and pestle. 10 mg of powdered nanoparticles was taken and introduced in a 100ml volumetric flask. The nanoparticles were dissolved in phosphate buffer pH 7.4 and make up the volume up to 100ml. The above solution was analyzed by UV spectrometer at 234 nm.

The entrapment efficiency and drug loading of the prepared nanoparticles was calculated by the formula:

Entrapment efficiency (%) =

$$= \frac{\text{Theoretical drug} - \text{practical drug}}{\text{Theoretical drug}} \times 100$$

Drug Loading efficiency (%) =

$$= \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of drug loaded nanoparticles}} \times 100$$

- **Percentage Yield**

It is calculated to know about 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℃ under mild agitation (50 rpm) 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.10 mg of nanoparticles (plain and galactose coated) 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 234 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 [11-13]. 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 10 min. The supernatant was collected and suspended in the same buffer.

10mg of nanoparticles were added into the supernatant 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 DISCUSSION

Four formulations of Cimetidine were formulated using different drug polymer ratios. The formulation is subjected to evaluation parameters like particle size, zeta potential, drug content uniformity, percentage yield, entrapment efficiency, drug loading efficiency and *in vitro* drug release study.

Particle Size

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 Figure 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.
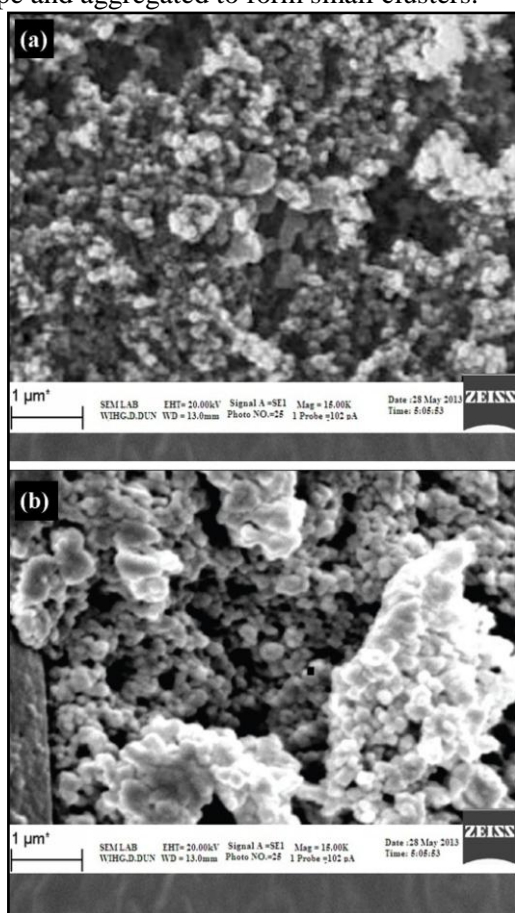

Fig. 1. (a) Scanning electron microscopy (SEM) photomicrograph of Albumin-NPs; (b) SEM photomicrograph of Galactose coated NPs.

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.

Drug content uniformity

The drug content of different formulations F1 to F4 was calculated and the content was found to be in range of 20.09 to 30.80 % to plain nanoparticles and 19.51 to 27.09 % for coated nanoparticles. The maximum drug content was found to be 30.80% for plain nanoparticles and 27.09% for coated nanoparticles in formulation F3.The results is shown in Table 2. The reason of low drug content was due to drug partitioning to the external aqueous phase during formulation, which also leads to the low drug loading efficiency.

Table 2. Drug Content of Plain and galactose coated Cimetidine nanoparticles
(For n =3)

Formulation Code	Drug Content (%) ± S.D.	
	Plain Nanoparticles	Coated Nanoparticles
F1	20.31 ± 0.5	21.76 ± 0.63
F2	25.12 ± 0.5	22.80 ± 0.71
F3	30.80 ± 0.3	27.09 ± 0.5
F4	20.09 ± 0.6	19.51 ± 0.62

Entrapment efficiency and Drug loading efficiency

The encapsulation efficiencies of all four formulations were given in the Table 3 and the entrapment efficiency was found to be in range of 80.17 to 97.68% for plain nanoparticles and 84.62 to 99.75 % for coated nanoparticles. The maximum entrapment efficiency was found to be 90.68% and 91.75 % for the formulation F3. The entrapment efficiencies of nanoparticles are larger than 80%, the drug can be effectively loaded inside the nanoparticles. The encapsulation efficiency increases with increasing polymer concentration up to a certain ratio.

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.

Table 3. Entrapment efficiency of plain and galactose coated Cimetidine nanoparticles
(for n =3)

Formulation Code	Entrapment efficiency (%) ± S.D.	
	Plain Nanoparticles	Coated Nanoparticles
F1	80.17 ± 0.84	84.62 ± 0.37
F2	86.78 ± 0.65	88.75 ± 0.46
F3	90.68 ± 0.5	91.75 ± 0.59
F4	88.09 ± 1.12	83.98 ± 1.10

Drug loading efficiency

The drug loading efficiency of all four formulations was given in the Table 4 and it was found to be in range of 3.45 to 18.98% for plain nanoparticles and 3.80 to 19.08 % for coated nanoparticles. Loading efficiency may be increased by increasing the polymer ratio, so that sufficient quantity of polymer will be able to entrap the drug present in solution.

The main reason for low drug loading efficiency was low drug-polymer binding. The drug has low protein binding therefore; most of the drug can easily diffuse through the matrix.

Further, the existing albumin-based drug delivery systems are often limited by their low drug loading capacity as well as noticeable drug leakage into the blood circulation.

Percentage Yield

The percentage yield of different formulations F1 to F4, were calculated and the yield was found to be in the range of 32.14 to 70.24% for plain nanoparticles and 25.98 to 62.32% for coated nanoparticles. Percentage yield of all batches is shown in Table 5. The maximum percentage yield was found to be 70.24% and

62.32% for plain and coated nanoparticles in formulation F4, where the concentration of albumin is highest while the nanoparticle yield is lowest in F1 i.e. 32.14% and 25.98 % where the concentration of albumin is lowest.

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

Table 4. Drug loading efficiency of Plain and galactose coated Cimetidine nanoparticles
(For n =3)

Formulation Code	Drug Loading efficiency (%)± S.D.	
	Plain Nanoparticles	Plain Nanoparticles
F1	3.45 ± 0.8	3.45 ± 0.8
F2	7.09 ± 0.45	7.09 ± 0.45
F3	16.98 ± 0.78	16.98 ± 0.78
F4	18.98 ± 0.98	18.98 ± 0.98

Table 5. Percentage Yield of Plain and galactose coated Cimetidine nanoparticles

| Formulation Code	Drug Loading efficiency (%)± S.D.		Drug Loading efficiency (%)± S.D.	
	Plain Nanoparticles	Plain Nanoparticles	Plain Nanoparticles	Plain Nanoparticles
F1	3.45 ± 0.8	3.45 ± 0.8	3.45 ± 0.8	3.45 ± 0.8
F2	7.09 ± 0.45	7.09 ± 0.45	7.09 ± 0.45	7.09 ± 0.45
F3	16.98 ± 0.78	16.98 ± 0.78	16.98 ± 0.78	16.98 ± 0.78
F4	18.98 ± 0.98	18.98 ± 0.98	18.98 ± 0.98	18.98 ± 0.98

- ***In vitro* drug release**

The dissolution studies on all four formulations of Cimetidine were carried out in phosphate buffer pH 7.4 buffer using egg membrane and modified apparatus. The *in-vitro* drug release of all four formulations F1 to F4 are shown in Table 6. The cumulative percent drug release after 10 hrs was found to be 35.01% to

51.78% for formulations of F1to F4, respectively. From the results, it was concluded that increase in polymer concentration, decreases the drug releases from the nanoparticles**.**

It was also found that coating of nanoparticles with galactose retard the rate of drug release as compared to plain nanoparticles(Figure 2 and 3).

Table 6. *In vitro* release profile of Formulations F1 to F4
(Plain and Galactose coated Nanoparticles)

Time(hrs)	Cumulative % drug release							
	F1		F2		F3		F4	
	Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated
0	0	0	0	0	0	0	0	0
1	16.48	15.23	14.54	13.98	12.56	10.98	10.97	9.99
2	18.05	17.32	16.78	15.89	14.89	12.87	12.45	11.67
3	19.9	18.67	18.45	17.69	15.76	13.32	13.89	12.67
4	21.57	20.09	19.89	18.98	18.78	15.78	15.76	14.98
5	25.76	26.8	23.78	24.31	21.46	22.83	19.87	21.01
6	32.67	34.56	31.98	32.98	30.98	29.75	27.13	25.96
7	40.89	38.98	36.98	35.87	33.56	33.1	29.61	27.12
8	46.67	42.87	42.14	40.09	36.86	38.09	33.14	30.98
9	48.98	44.98	46.78	44.81	39.02	38.9	36.98	34.98
10	51.78	47.75	49.73	48.91	43.15	42.09	37.13	35.01
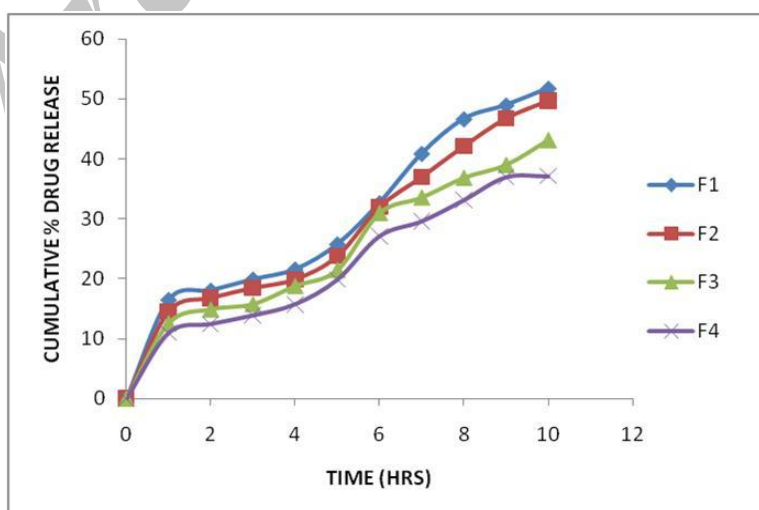
Fig. 2. Zero order release Plot of Cimetidine plain nanoparticles
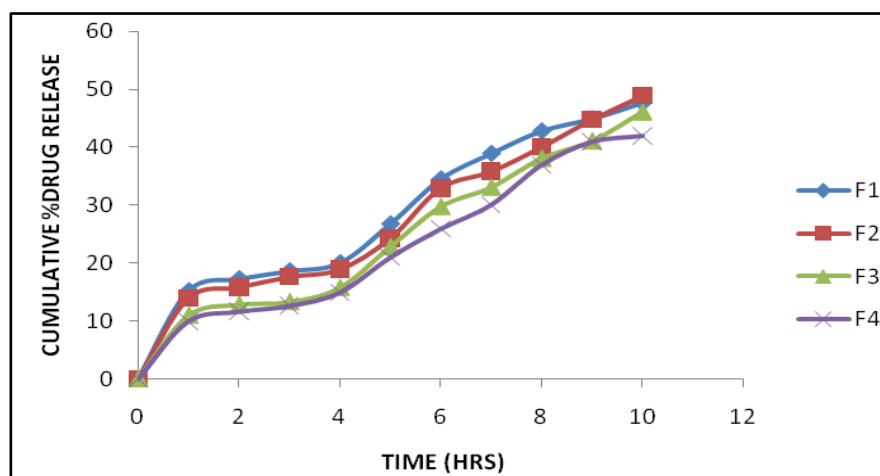
Fig. 3. Zero order release Plot of Cimetidine galactose coated nanoparticles

- **Mathematical modeling**

The data obtained from *in vitro* release studies was treated by various conventional mathematical models (zero-order, first-order, Higuchi and Korsmeyer- Peppa's) to determine the release mechanism from the designed nanoparticle formulations. 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.

In-vitro drug release data of all four formulations F1 to F4 are shown in Table 6. The regression coefficients of the all formulations F1 to F4 are shown in Table 7.

It was found that all the formulations follow the first order kinetics.

The regression coefficients for the formulations F1 to F4 of Higuchi plot was found to be almost linear. The linearity suggests that the release of Cimetidine nanoparticles was diffusion controlled.

Korsmeyer- Peppas release model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. The value of n could be used to characterize different release mechanism. The value of n for F1 to F4 was found to be respectively greater than 0.8. The formulations F1 and F2 indicates that the release approximates non-Fickian diffusion mechanism while the formulations F3 and F4 shows the Super Case-II transport mechanism.

Table 7. Model fitting release profile of Formulations F1 to F4

Formulation Code	Regression Coefficient (R^2)						Slope (n) value	
	Zero order		First order		Higuchi's		Korsmeyer- Peppas	
	Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated
F1	0.955	0.957	0.957	0.962	0.915	0.934	0.863	0.883
F2	0.964	0.968	0.968	0.972	0.915	0.923	0.872	0.893
F3	0.963	0.976	0.968	0.979	0.933	0.906	0.902	0.880
F4	0.965	0.975	0.966	0.970	0.932	0.899	0.892	0.898

- **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

In the present study , an attempt was made to develop galactosylated albumin nanoparticles of Cimetidine for treatment of Acetaminophen induced hepatotoxicity with a view to provide targeted action to the required site and helps to provides the sustain action and thus reduces the dose frequency and increases the patient compliance.

From the results, it can be concluded that:

- Nanoparticles were successfully prepared by desolvation method. The method was able to produce discrete, free-flowing nanoparticles.
- Bovine Serum Albumin is a biocompatible and biodegradable polymer for preparing targeted nanoparticles.
- FTIR studies were carried out to find out the possible interaction between the drug and the polymer. The study revealed that there was no interaction between the selected drug and polymer.
- The particle size analysis revealed that particle sizes were found 200nm for plain nanoparticles and 250 nm for coated nanoparticles. It was also found that coating of nanoparticles increases the size of nanoparticles.
- From the *in-vitro* studies, it was concluded that increase in polymer concentration, decreases the drug releases from the nanoparticles.
- From the percentage yield, it was concluded that the maximum percentage yield was found to be 70.24% and 62.32 % for plain and coated nanoparticles in formulation F4, where the concentration of albumin is highest while the nanoparticle yield is lowest in F1 i.e. 32.14% and 25.98 % for plain and coated nanoparticles where the concentration of albumin is lowest.
- The maximum entrapment efficiency was found to be 90.68% ± 0.5 and 91.75% ± 0.59 in plain and coated nanoparticles for the formulation F3. The entrapment efficiencies of nanoparticles are larger than 80%, the drug can be effectively loaded inside the nanoparticles. The encapsulation efficiency increases with increasing polymer concentration up to a certain ratio. Further, 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.
- The maximum drug loading efficiency was found to be 18.98% ± 0.98 in plain and 19.08% ± 1.10 in coated nanoparticles for formulation F4. Loading efficiency may be increased by increasing the polymer ratio, so that sufficient quantity of polymer will be able to entrap the drug in present in solution. The main reason for low drug loading efficiency was due to low drug-polymer binding. The drug has low protein binding therefore; most of the drug can easily diffuse through the matrix. However, the existing albumin-based drug delivery systems are often limited by their low drug loading capacity as well as noticeable drug leakage into the blood circulation.
- The maximum drug content was found to be 30.80% ± 0.3 and 27.09% ± 0.5 respectively in plain and galactose coated nanoparticles for the formulation F3.The reason of low values of drug content was low drug loading. The lower drug content was due to drug partitioning to the external aqueous phase during formulation
- The receptor ligand binding was determined by diffusion study. The amount of free drug was diffuses through egg membrane and its percentage in the media was very less (5.67%) only after 10 hrs. So, it can be concluded that the nanoparticles can easily bind with the asialoglycoprotein receptors present in hepatocytes.

REFERENCES

[1] Larson A. M., (2007), Acetaminophen hepatotoxicity. *Clin. Liver Dis.* 11: 525–548.

[2] Rumack B. H., (2002), A cetaminophen hepatotoxicity. *J. Toxicol. Clin. Toxicol.* 40: 3–20.

[3] Nelson S. D., (1990), Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Semin Liver Dis.* 10: 267–278.

[4] Jaeschke H, Bajt M. L., (2006), Intracellular signaling mechanisms of acetaminophen-induced liver cell death. *Toxicol. Sci.* 89: 31–41.

[5] Wu J.,Zern M. A., Hepatic S., (2000), A target for treatment of liver fibrosis. *J. Gastroenterol.* 35: 665-72.

[6] Takami A., Masanori B, Mitsuru A., (2007), Preparation of nanoparticles by self –organization of polymers consisting of hydrophobic and hydrophilic segments:Potential applications. *Polymer*. 48: 6729-6747.

[7] Merodio M,. Arnedo A, Renedo M. J. Irache J. M, (2001), Ganciclovirloaded albumin nanoparticles: Characterization and in vitro release properties. *European J. of Pharmceutical Sci.* 12: 251-258.

[8] Gallo J. M., Hung C. T., Perrier D. G., (1984), Analysis of albumin microsphere preparation. *Int. J. Pharm.,* 22: 63–74.

[9] Lin W., Coombes A. G. A., Davies M. C., Davis S. S., Illum L., (1993), Preparation of sub-100 nm human serum albumin nanospheres using a pH-coacervation method. *J. Drug Target.* 1: 237–243.

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

[11] Korsmeyer W, Gurny R., Doelker E., Buri P., Peppas N. A., (1983), Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* 15: 25–35.

[12] Ritger P. L., Peppas N. A., (1987), A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. *J. Control. Release*. 5: 37–42

[13] Philip A. K., Pathak K., (2006), Osmotic flow through asymmetric membrane: A means for controlled delivery of drugs with varying solubility. *AAPS Pharm. Sci. Tech*., **7**: E1–E11.