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GALACTOSYLATED ALBUMIN NANOPARTICLES BEARING CIMETIDINE

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Abstract

The galacotsylated albumin nanoparticles were prepared for the selective delivery of Cimetidine to the asialoglycoprotein receptor (ASGP-R) which is particularly presents on mammalian 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 galactose coated) was found to be in range of 200-250 nm, and maximum drug payload was found to be 19.08% ± 1.10 .The maximum drug content was found to be 30.80% ± 0.3 and 27.09% ± 0.5 respectively in plain and galactose coated nanoparticles while the maximum entrapment efficiency was found to be 90.68% ± 0.5 and 91.75% ± 0.59 in plain and 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.

Keywords: Hepatotoxicity, galactose, cimetidine, targeting, asialoglycoprotein receptor.

1. 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].

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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 mammalian hepatocytes can be utilize 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.[5]

Nanoparticles can be defined as the colloidal particles having size ranging from 10 to 1000 nm. The advantages of nanotechnology is to provide the safe and the effective medicine (nanomedicine). A large number of drugs can be delivered using a nanoparticulate carriers via a large number of routes. These includes 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.[7,8,9]

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 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 maintain the required concentration of drug at the desired site. Moreover, as nanoparticles have high carrier capacity (i.e drug molecules can be incorporated in the polymer matrix), helps to provides the sustain action and thus reduces the dose frequency and increases the patient compliance. In the current work, we had prepared galactosylated albumin nanoparticles using desolvation method. The coating of nanoparticles with galactose helps to gets binds the nanocarriers with the asialoglycoprotein receptor presents in hepatocytes and improves the immunotherapeutic effects. The developed formulations overcome the drawbacks and limitations of the conventional drug delivery systems.

2. Materials and methods

2.1 Materials

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

2.2 Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as the investigation of physical and chemical properties if drug substance alone or in combination with excipients. The overall objective of preformulation studies is to generate information useful to formulator in developing stable and bioavailable dosage forms which can be mass produced**.**

2.2.1. Identification tests

2.2.1.1. Identification of drug by FTIR

Infrared spectrum of Cimetidine was determined by using Fourier Transform Infrared Spectrophotometer using KBr disks method. The sample (0.5 to 1.0 mg) is finely grounded and intimately mixed with approximately 100 mg of dry potassium bromide powder. Grinding and mixing can be done with mortar and pestle. The mixture is then pressed into a transparent disk in an evacuable die at sufficiently high pressure. Suitable KBr disks or pellets can often be made using a simpler device such as a hydraulic press. The base line correction was done using dried potassium bromide. Then ,the spectrum of dried mixture of drug and potassium bromide was scanned from 4000 cm^{-1} to 400 cm^{-1}.[10]

2.2.1.2. Organoleptic characteristics

The colour, odour and taste of the drug were characterized and recorded.

2.2.1.3. Solubility

The solubility of the drug were checked in different solvents like distilled water, buffers, ethanol and organic solvents and recorded. This might be helpful in selection of a suitable solvent to dissolve drug as well as excipients used in formulations.

2.2.1.4. Melting Point determination:

The sample was loaded into a sealed capillary (melting point capillary) which was then placed in the melting point apparatus. The sample is then heated & as the temperature increases the sample was observed to detect the phase change from solid to liquid phase. The temperature at which this phase change occurs gives the melting point.

2.2.1.5. Particle size analysis

Determination of Particle Size by optical microscopy

The size of drug particle was determined with the help of optical microscopy. Firstly, the least count of microscope was determined by adjusting stage and ocular micrometer. Then, a drop of drug suspension was placed in a slide and spread into a thin film with coverslip and observed under microscope at 45X with ocular to count the particle size. A total of 100 particles were counted and their size was determined. The average particle size in micrometers was determined and recorded.

2.2.2. Spectral Studies

2.2.2.1. Drug - excipient Compatibility Study

Drug and polymer was mixed in the equal ratio and finally grounded and intimately mixed with approximately 100 mg of dry potassium bromide powder. Grinding and mixing was done with mortar and pestle. The mixture is then pressed into a transparent disk in an evacuable die at sufficiently high pressure. Suitable KBr disks or pellets have been prepared using a simpler device such as a hydraulic press. The base line correction was done using dried potassium bromide. Then, the spectrum of dried mixture of drug and potassium bromide was scanned from 4000 cm^{-1} to 400 cm^{-1}.

2.2.2.2. Preparation of Calibration Curve of Cimetidine

a. Preparation Of Phosphate Buffer pH 7.4

Accurately weighed 6.804gm of potassium dihydrogen phosphate was dissolved in 250ml of distilled water; 2.00 gm of sodium hydroxide was dissolved separately in 250 ml of distilled water. Then, 62.5 ml of potassium dihydrogen phosphate was taken in a separate 200 ml volumetric flask, added 48.87 ml of sodium hydroxide and then add water to volume. The pH was determined by pH meter.

b. Determination of λ_{max} of Cimetidine

Phosphate Buffer pH 7.4 was investigated to develop a suitable UV-spectrophotometric method for the analysis of Cimetidine in formulations. For selection of media the criteria employed were

sensitivity, ease of sample preparations, solubility of drug, cost of solvents and applicability of method to various purposes. An UV spectroscopic scanning run (200-400 nm) was carried out to select the best UV wavelength for detection of Cimetidine in buffer. The analysis was carried out using phosphate buffer pH 7.4 as blank. Absorbance of Cimetidine was determined.

c. Calibration Curve of Cimetidine

A stock solution of 100 mcg/ml of Cimetidine was prepared in phosphate buffer pH 7.4 by dissolving 10 mg of drug in 100ml of the media. For preparations of different concentrations, aliquots of stock solution were transferred into a series of 10 ml standard flasks and volumes were made with respective media. The different concentrations were prepared in the range of 0.2-4 mcg/ml of Cimetidine in phosphate buffer pH 7.4 for standard curve.

2.3. Formulation of Nanoparticles

2.3.1. Preparation of Master Formula

Table 1.1: 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

2.3.2. 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.

2.3.3. 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 10mM 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.

2.3.4. 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 10mM 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^{o} C.

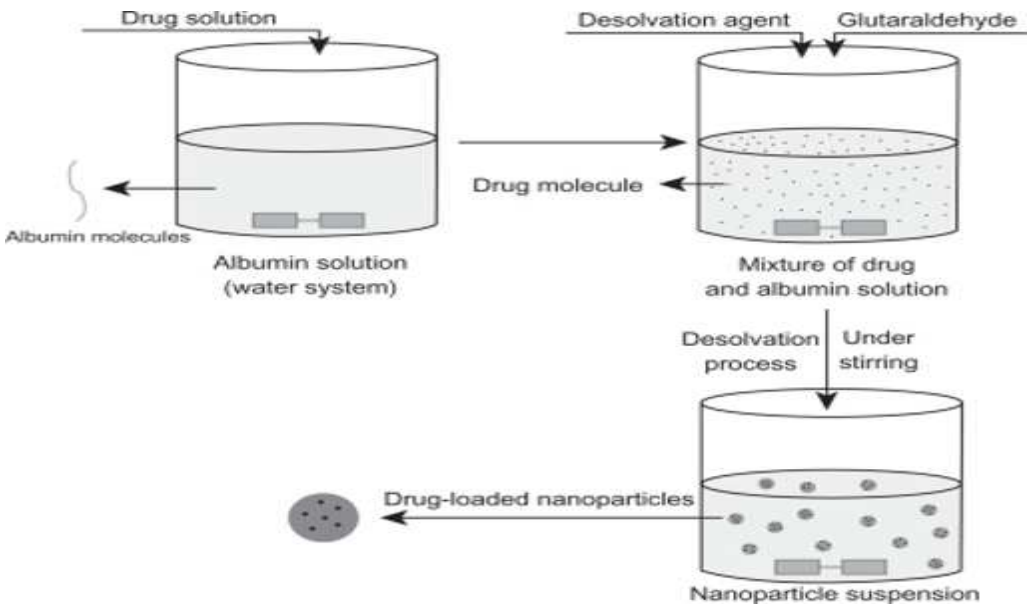


Fig.1.1: Schematic Representation of Desolvation Method

2.4. 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.

2.4.1. Shape and Size

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

2.4.2. Drug content uniformity

500 mg of nanoparticles were weighed and crushed in mortar and pestle. A powder equivalent to 10 mg 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 234 nm.

2.4.3. Entrapment efficiency and Loading efficiency

500 mg of nanoparticles were weighed and crushed in mortar and pestle. A powder equivalent to 10 mg 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 234 nm.

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

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

$$Drug\ loading\ efficiency\ (\%) = \frac{Amount\ of\ drug\ in\ nanoparticles}{Amount\ of\ drug\ loaded\ nanoparticles} \times 100$$

2.4.4. 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:

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

2.4.5. *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 (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. 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 was analyzed by UV spectrometer at 234 nm.

2.4.6. 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.

3. Results and discussions

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, *in-vitro* drug release study.

3.1. Preformulation Studies

3.1.1. Identification of drug by FTIR

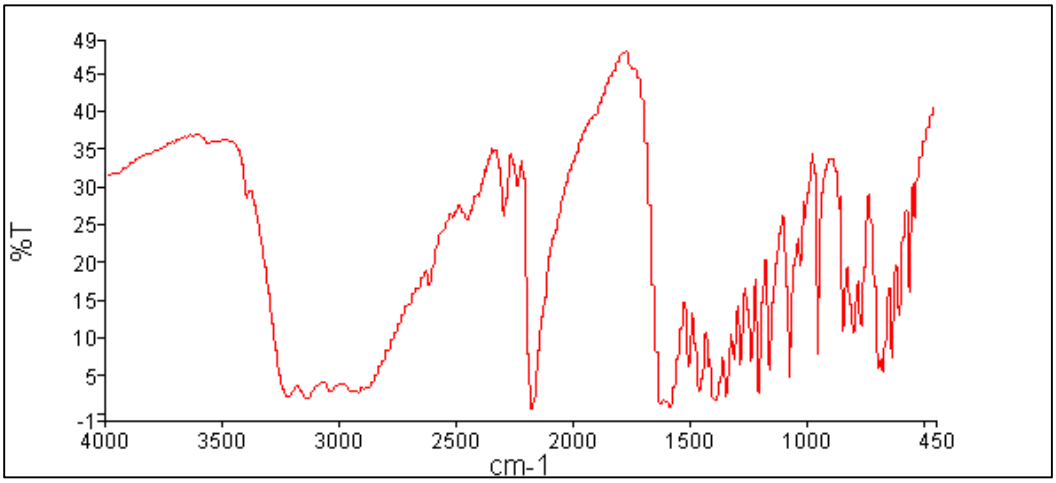


Fig 2.1: FTIR spectrum of Cimetidine

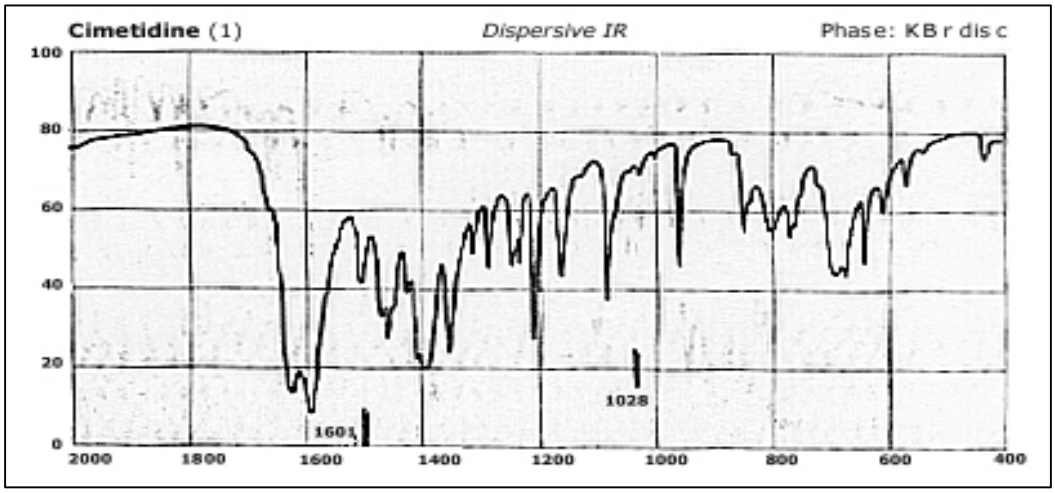


Fig 2.2: FTIR spectrum of Cimetidine (with reference from I.P.).

Table 2.1: Characteristics peaks of Cimetidine (with reference from I.P.)

S.No.	Peaks (cm^{-1})	Functional Group	Stretching/Bending
1.	1601	C=N	Stetching
2.	1300	C-N	Stetching
3.	1400	CH_2	Bending
4.	1580	C= C	Bending
5.	1375	CH_3	Bending

Table 2.2: Characteristics peaks of Cimetidine

S.No.	Peaks (cm^{-1})	Functional Group	Stretching/Bending
1.	3400.67	N-H	Stetching
2.	2943.76	C-H	Stetching
3.	2455.93	S-H	Stetching
4.	1346.49	C-N	Stetching
5.	1465.87	CH_2	Bending
6.	1386.79	CH_3	Bending
7.	1622.80	C=N	Stetching
8	1587.03	C =C	Bending

The comparison between the peaks of two graphs shows that the characteristics peaks of Cimetidine (taken from I.P.) was found similar to the given drug sample, which shows that the drug is Cimetidine.

3.1.2. Organoleptic characteristics

The colour, odour and taste of the drug were characterized and recorded using descriptive terminology, the results are shown in Table No: 2.3

Table 2.3: Results of Organoleptic properties.

Properties	Results
Description	Crystalline solid
Colour	Almost white powder
Odour	Characteristics
Taste	Bitter

3.1.3. Solubility

Cimetidine is very soluble in mineral acids, soluble in water, soluble in alcohol and freely soluble in phosphate buffer pH 7.4 as shown in Table No: 2.4

Table 2.4: Results of Solubility studies

S.No.	Solvent	Solubility	Solubility (mg/ml)
1.	Water	Soluble	20
2.	Phosphate Buffer pH 7.4	Freely Soluble	10
3.	Alcohol	Soluble	30
4.	Mineral acids	Very Soluble	1

3.1.4. Melting Point determination

The melting point of Cimetidine was found to be 140-145º C. This value is same as that of the literature citation of 139-144º C.

Table 2.5: Results of Melting Point determination

Reported melting point	139-144 °C
Observed melting point	140-145 °C

3.1.5. Particle size analysis:

The average particle size of the Cimetidine was found to be 1.505

3.2. Spectral Studies

3.2.1. Drug - excipient Compatibility Study

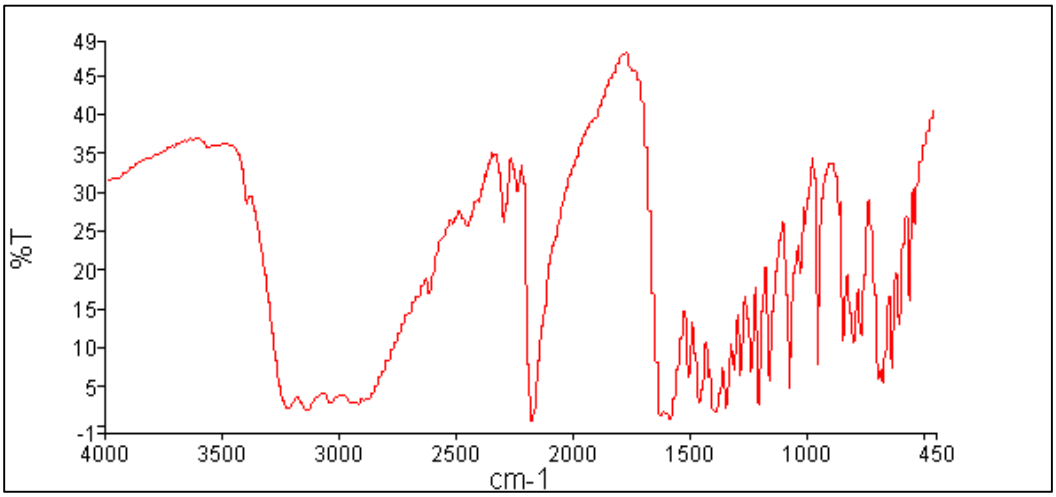


Fig 2.3: FTIR spectrum of Cimetidine

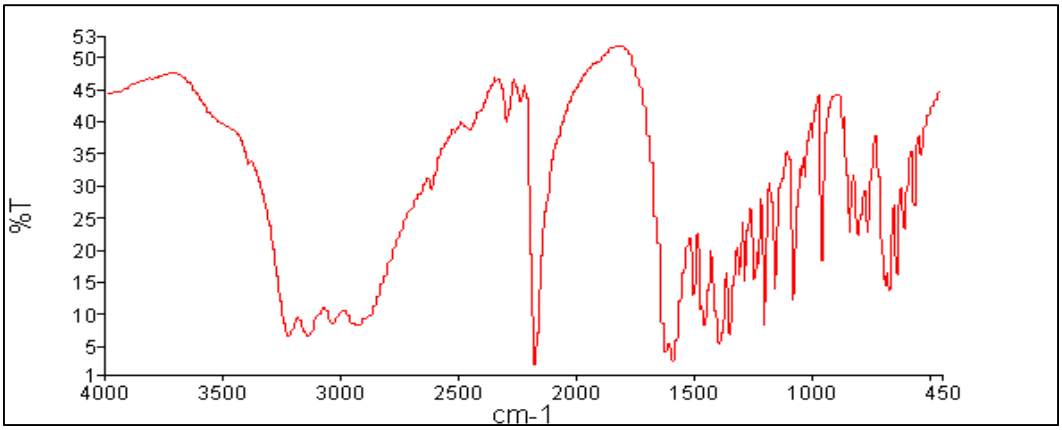


Fig 2.4. : FTIR spectrum of Cimetidine + Bovine Serum Albumin (polymer)

Table 2.6: Characteristics peaks of Cimetidine

S.No.	Peaks (cm^{-1})	Functional Group	Stretching/Bending
1.	3400.67	N-H	Stetching
2.	2943.76	C-H	Stetching
3.	2455.93	S-H	Stetching
4.	1346.49	C-N	Stetching
5.	1465.87	CH_2	Bending
6.	1386.79	CH_3	Bending
7.	1622.80	C=N	Stetching
8	1587.03	C =C	Bending

Table 2.7: Characteristics peaks of Cimetidine + Bovine Serum Albumin physical mixture

S.No.	Peaks (cm^{-1})	Functional Group	Stretching/Bending
1.	3435.94	N-H	Stetching
2.	2943.76	C-H	Stetching
3.	2456.23	S-H	Stetching
4.	1346.8	C-N	Stetching
5.	1587.22	C=C	Stetching
6.	1386.79	CH_3	Bending

The drug-polymer interactions shows that there was no major shifts in the absorption bands(peaks) of Cimetidine in presence of polymer and it was observed that all the characteristics peaks of drug is present in the combination of drug and polymer spectra indicating the compatibility of drug with the polymer used.

3.2.2. Preparation of Calibration Curve of Cimetidine

Determination of λ_{max} of Cimetidine

UV absorption spectrum showed λ_{max} to be 234nm.The graph of absorbance Vs concentration for Cimetidine was found to be linear in the concentration range of 0.2-4 mcg/ml at 234 nm. Hence, the drug obeys Lambert-beer's law in this range. Fig.2.5 shows UV spectrum of Cimetidine and Fig. 2.6. Shows the calibration curve of Cimetidine in phosphate buffer pH 7.4.

The calibration curve was prepared and results was shown in Table 6.8.

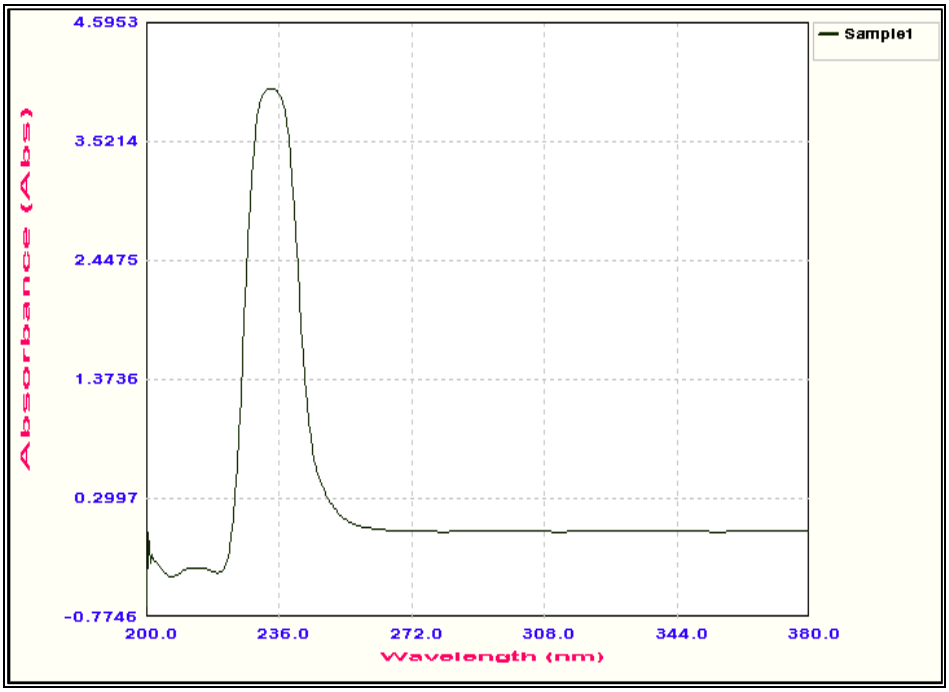


Fig 2.5.: UV Spectrum of Cimetidine

Wavelength of maximum absorption (λ_{max}) in Phosphte buffer pH 7.4 was found to be 234nm.

Table 2.8: Data for Calibration Curve of Cimetidine in Phosphate buffer pH 7.4

S.No.	Conc.(mcg/ml)	Absorbance (λ=234Bnm)
1.	0	0
2.	0.4	0.1475
3.	0.8	0.2269

S.No.	Conc.(mcg/ml)	Absorbance (λ=234Bnm)
4.	1.2	0.3047
5.	1.6	0.3792
6.	2.0	0.4127
7.	2.4	0.4862
8.	2.6	0.5731
9.	3.2	0.6413
10.	3.6	0.7126
11.	4.0	0.8749
12.	4.4	0.9523

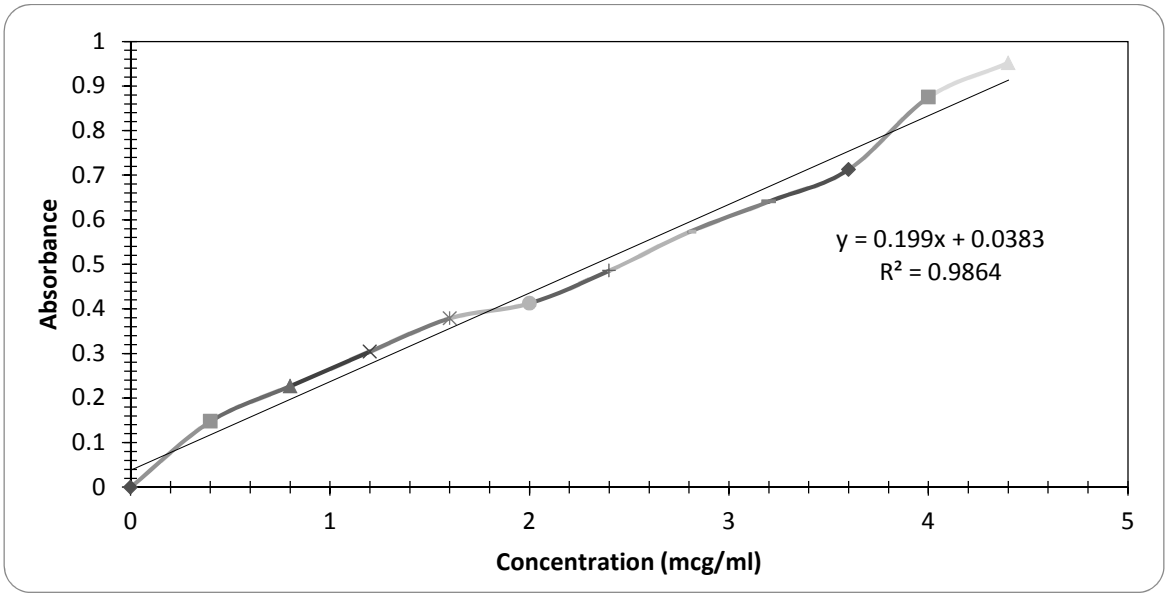


Fig. 2.6.: Calibration Curve of Cimetidine in Phosphate buffer pH 7.4

Line of Equation: y = 0.199x+ 0.038

Beer's Range: 0.2-4 mcg/ml

R^2 Value: 0.986

λ_{max}: 234nm

Fig.2.6 shows the calibration curve with slope 0.199 and regression value 0.986

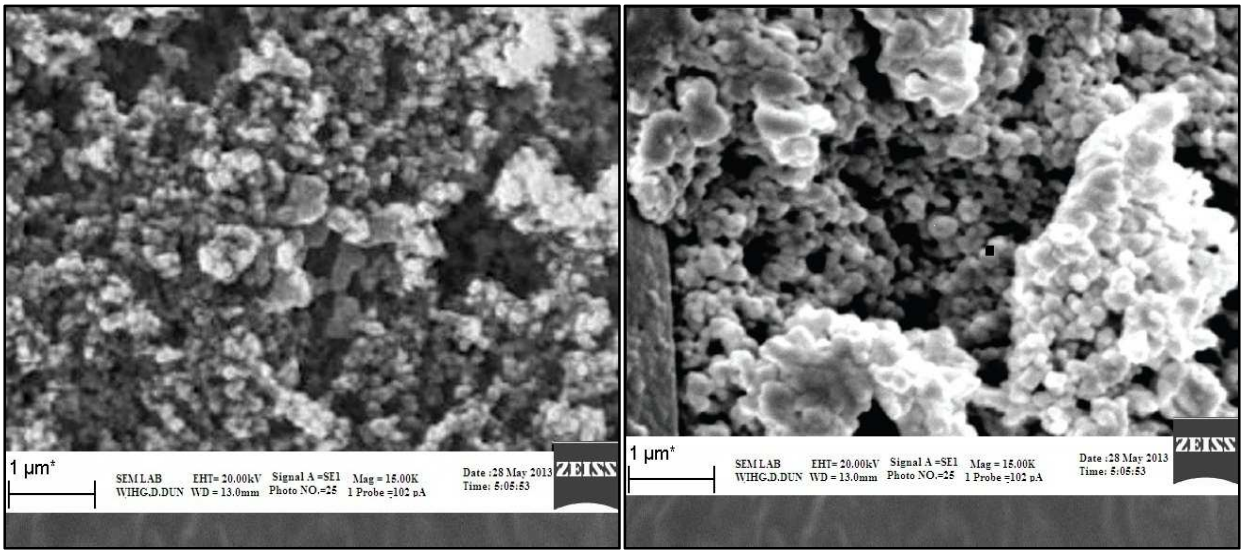
3.3. Characterization of Nanoparticles

3.3.1. 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.2.7 (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.



A **B**

Figure 2.7. (A) Scanning electron microscopy (SEM) photomicrograph of Albumin-NPs; (B) SEM photomicrograph of Galactose coated NPs.

3.3.2. 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 2.9. Comparison of drug content for formulations F1 to F4 is shown in Fig.2.8

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 2.9: Drug Content of Plain and galactose coated Cimetidine nanoparticles (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

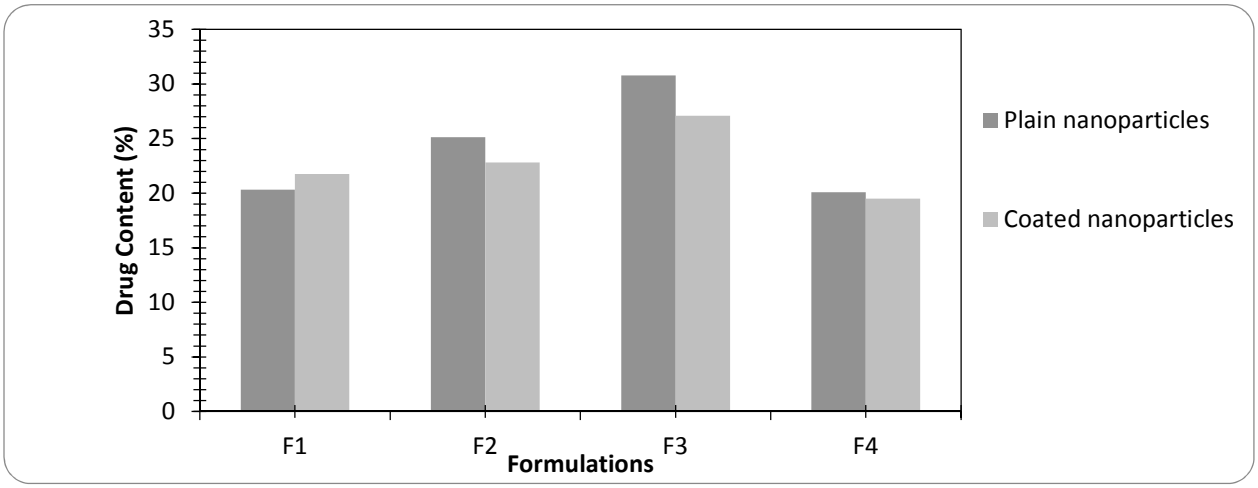


Fig 2.8: Drug Content Comparison of different formulations

3.3.3. Entrapment efficiency and Drug loading efficiency:

The entrapment efficiencies of all four formulations were given in the Table 2.10 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. Comparison of entrapment efficiency for formulations F1 to F4 is shown in Fig. 2.9.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 entrapment efficiency increases with increasing polymer concentration upto 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 2.10: Entrapment efficiency of Plain and galactose coated Cimetidine nanoparticles (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

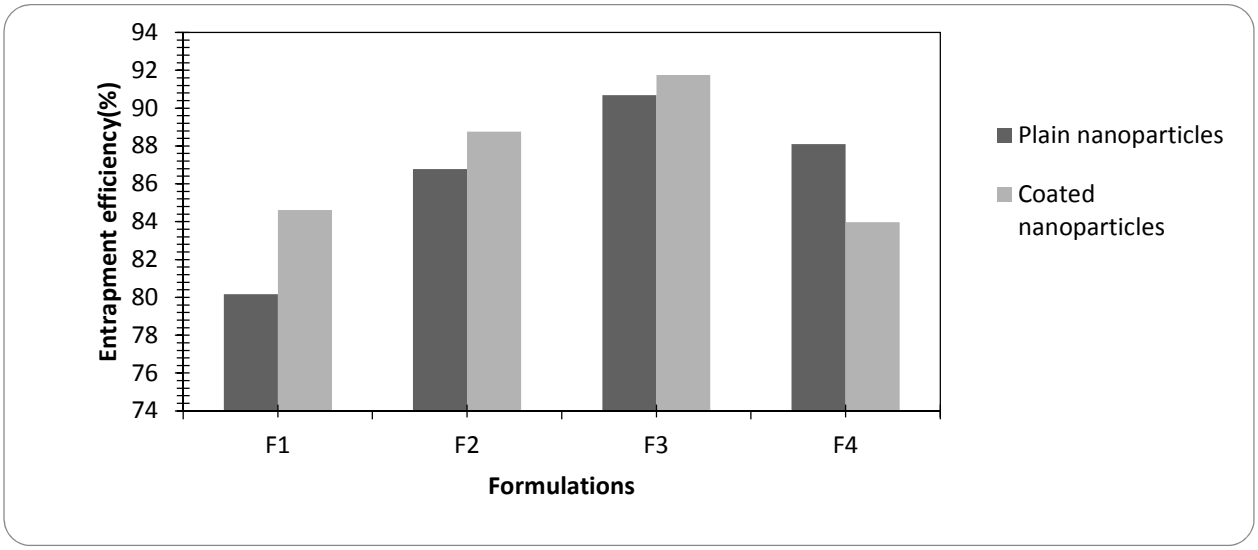


Fig 2.9: Entrapment efficiency Comparison of different formulations

Drug loading efficiency

The drug loading efficiency of all four formulations were given in the Table 2.11 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. Comparison of entrapment efficiency for formulations F1 to F4 is shown in Fig.2.10.

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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 diffuses 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.

Table 2.11 : Drug Loading efficiency of Plain and galactose coated Cimetidine nanoparticles (n =3)

Formulation Code	Drug Loading efficiency (%)± S.D.	
	Plain Nanoparticles	Coated Nanoparticles
F1	3.45 ± 0.8	3.80 ± 0.45
F2	7.09 ± 0.45	7.67 ± 0.79
F3	16.98 ± 0.78	18.09 ± 0.9
F4	18.98 ± 0.98	19.08 ± 1.10

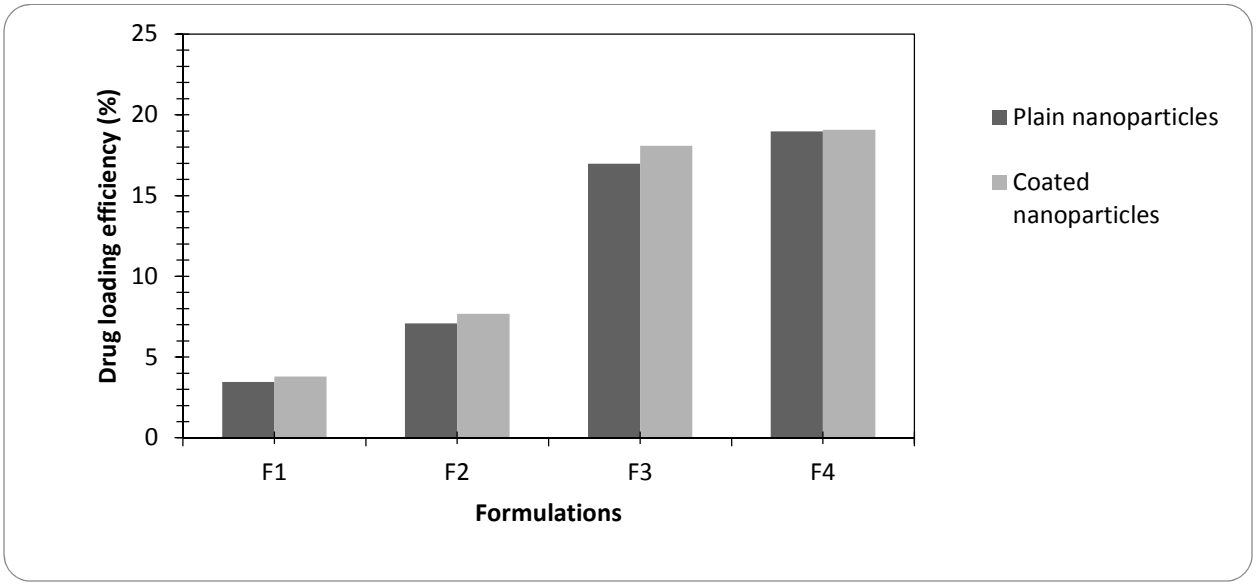

Fig 2.10: Drug loading efficiency comparison of different formulations

3.3.4. 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 2.12.Comparion of percentage yield for formulations F1 to F4 is shown in Fig.2.11.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 occur due to the loss of nanoparticles during the coating process.

Table 2.12: Percentage Yield of Plain and galactose coated Cimetidine nanoparticles

Formulation Code	Total amount of ingredients (mg)		Percentage Yield (%)	
	Plain Nanoparticles	Coated Nanoparticles	Plain Nanoparticles	Coated Nanoparticles
F1	850	870	32.14	25.98
F2	950	970	45.43	37.09
F3	1050	1070	55.71	46.72
F4	1150	1170	70.24	62.32

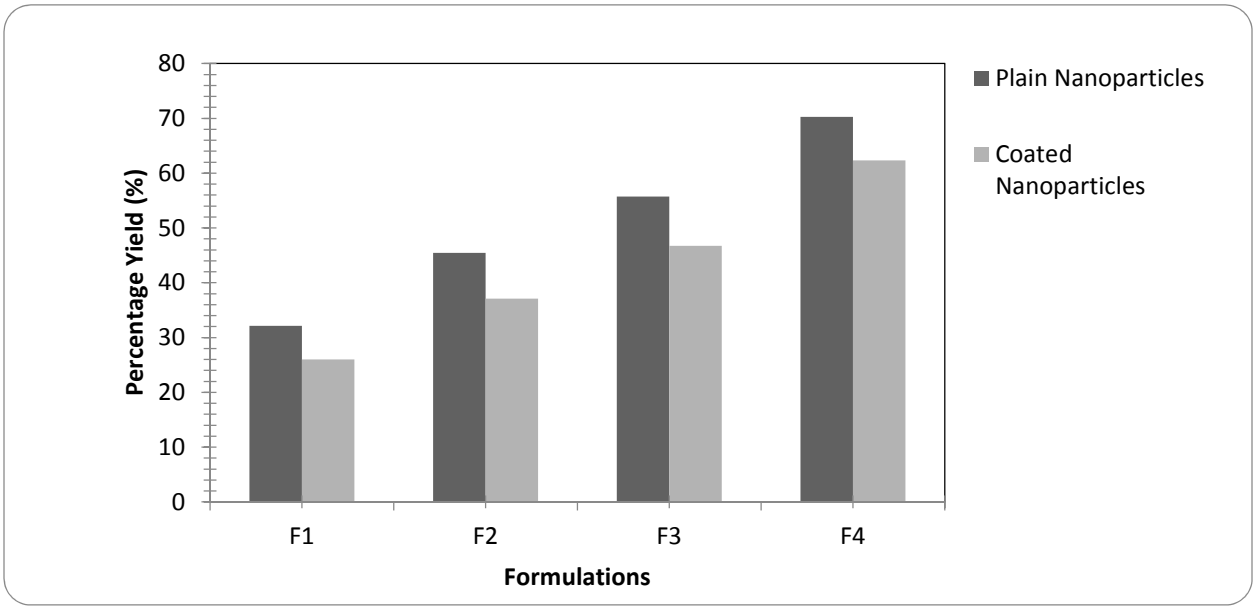

Fig 2.11: Percentage Yield Comparison of different formulations

3.3.5. *In vitro* drug release

The dissolution study on all four formulations of Cimetidine were carried out in phosphate buffer pH 7.4 using egg membrane and modified apparatus. The *in-vitro* drug release of all four formulations F1 to F4 are shown in Table 2.13.The cumulative percent drug release after 10 hrs was found to be between 35.01 % to 51.78% for formulations F1 to 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.

Table 2.13: *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

Table 2.14 : *In-vitro* release profile of Cimeitidine from Formulations F1

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
			Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated
0	0	-	0	0	100	100	-	-	2	2

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
1	1	0	16.48	15.23	83.52	84.77	1.21	1.18	1.92	1.92
2	1.414	0.3	18.05	17.32	81.95	82.68	1.25	1.23	1.91	1.91
3	1.732	0.47	19.9	18.67	80.1	81.33	1.29	1.27	1.9	1.91
4	2	0.6	21.57	20.09	78.43	79.91	1.33	1.3	1.89	1.9
5	2.236	0.69	25.76	26.8	74.24	73.2	1.41	1.42	1.87	1.86
6	2.449	0.77	32.67	34.56	67.33	65.44	1.51	1.53	1.82	1.81
7	2.645	0.84	40.89	38.98	59.11	61.02	1.61	1.59	1.77	1.78
8	2.828	0.9	46.67	42.87	53.33	57.13	1.66	1.63	1.72	1.75
9	3	0.95	48.98	44.98	51.02	51.02	1.69	1.65	1.7	1.74
10	3.162	1	51.78	47.75	48.22	52.25	1.71	1.67	1.68	1.7

Table 2.15: *In-vitro* release profile of Cimeitidine from Formulations F2

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
			Plain	**Coated**	**Plain**	**Coated**	**Plain**	**Coated**	**Plain**	**Coated**
0	0	-	0	0	100	100	-	-	2	2
1	1	0	14.54	13.98	85.46	86.02	1.16	1.14	1.93	1.93
2	1.414	0.3	16.78	15.89	83.22	84.11	1.22	1.2	1.92	1.92
3	1.732	0.47	18.45	17.69	81.55	82.31	1.26	1.24	1.91	1.91
4	2	0.6	19.89	18.98	80.11	81.02	1.29	1.29	1.9	1.9
5	2.236	0.69	23.78	24.31	76.22	75.69	1.37	1.38	1.88	1.87
6	2.449	0.77	31.98	32.98	68.02	67.02	1.5	1.51	1.83	1.82
7	2.645	0.84	36.98	35.87	63.02	64.13	1.56	1.55	1.79	1.8

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
8	2.828	0.9	42.14	40.09	57.86	59.91	1.62	1.6	1.76	1.77
9	3	0.95	46.78	44.81	53.22	55.19	1.67	1.65	1.72	1.74
10	3.162	1	49.73	48.91	50.27	51.09	1.69	1.68	1.7	1.7

Table 2.16: *In-vitro* release profile of Cimeitidine from Formulations F3

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
			Plain	**Coated**	**Plain**	**Coated**	**Plain**	**Coated**	**Plain**	**Coated**
0	0	-	0	0	100	100	-	-	2	2
1	1	0	12.56	10.98	87.44	89.02	1.09	1.04	1.94	1.94
2	1.414	0.3	14.89	12.87	85.11	87.13	1.17	1.10	1.92	1.94
3	1.732	0.47	15.76	13.32	84.24	86.68	1.19	1.12	1.925	1.93
4	2	0.6	18.78	15.78	81.22	84.22	1.27	1.19	1.9	1.92
5	2.236	0.69	21.46	22.83	78.54	77.17	1.33	1.35	1.89	1.88
6	2.449	0.77	30.98	29.75	69.02	70.25	1.49	1.47	1.83	1.84
7	2.645	0.84	33.56	33.1	66.44	66.9	1.52	1.51	1.82	1.83
8	2.828	0.9	36.86	38.09	63.14	61.91	1.56	1.58	1.8	1.79
9	3	0.95	39.02	38.90	60.98	61.1	1.59	1.58	1.78	1.77
10	3.162	1	43.15	42.09	56.85	57.91	1.63	1.62	1.75	1.73

Table 2.17: *In-vitro* release profile of Cimeitidine from Formulations F4

Time (hrs)	√T	Log T	% CDR		%Cumulative drug retained		Log % CDR		Log %Cumulative drug retained	
			Plain	Coated	Plain	Coated	Plain	Coated	Plain	Coated
0	0	-	0	0	100	100	-	-	2	2
1	1	0	10.97	9.99	89.03	90.01	1.04	0.99	1.94	1.95
2	1.414	0.3	12.45	11.67	87.55	88.33	1.09	1.06	1.94	1.94
3	1.732	0.47	13.89	12.67	86.11	87.33	1.14	1.10	1.93	1.94
4	2	0.6	15.76	14.98	84.24	85.02	1.19	1.17	1.92	1.92
5	2.236	0.69	19.87	20.01	80.13	78.99	1.29	1.32	1.9	1.89
6	2.449	0.77	27.13	25.96	72.87	74.04	1.43	1.41	1.86	1.86
7	2.645	0.84	29.61	27.12	70.39	72.88	1.43	1.47	1.84	1.84
8	2.828	0.9	33.14	30.98	66.86	69.02	1.49	1.56	1.82	1.79
9	3	0.95	36.98	34.98	63.02	65.02	1.54	1.61	1.79	1.77
10	3.162	1	37.13	35.01	62.87	64.99	1.54	1.62	1.79	1.76

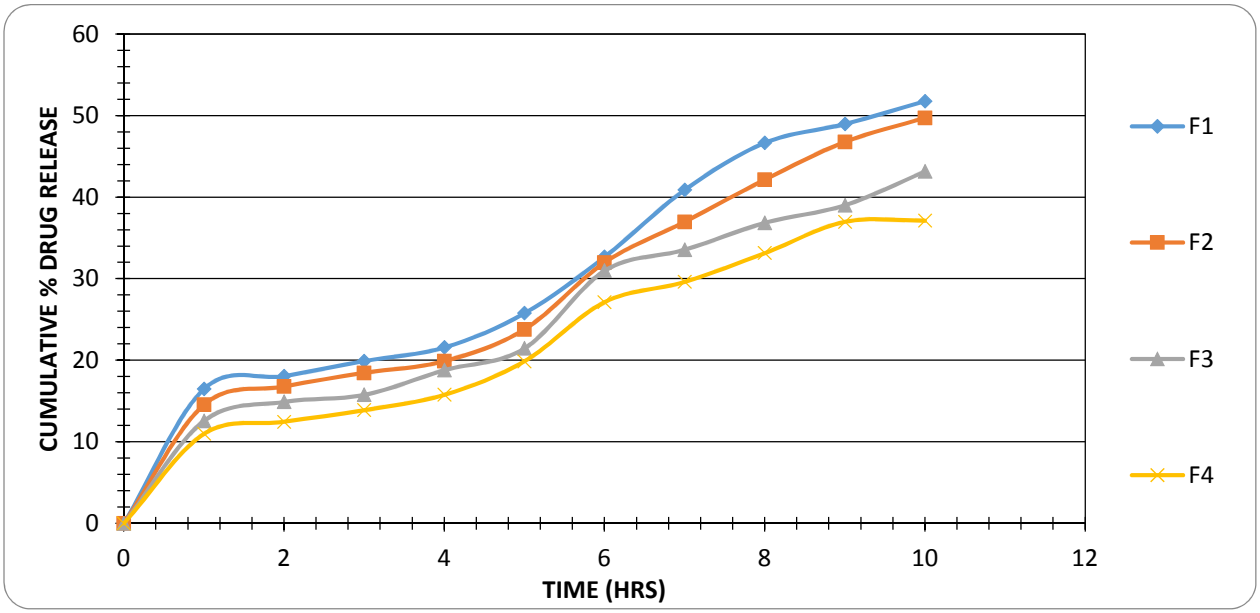

Fig 2.12: Zero order release Plot of Cimetidine plain nanoparticles

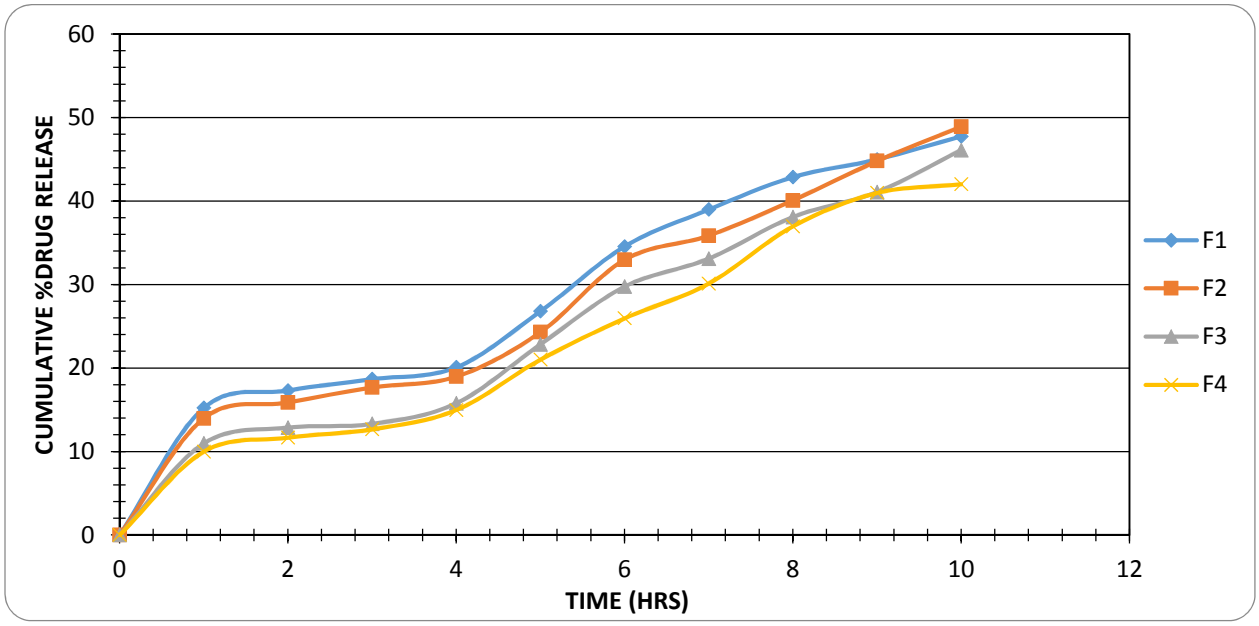

Fig 2.13: Zero order release Plot of Cimetidine galactose coated nanoparticles

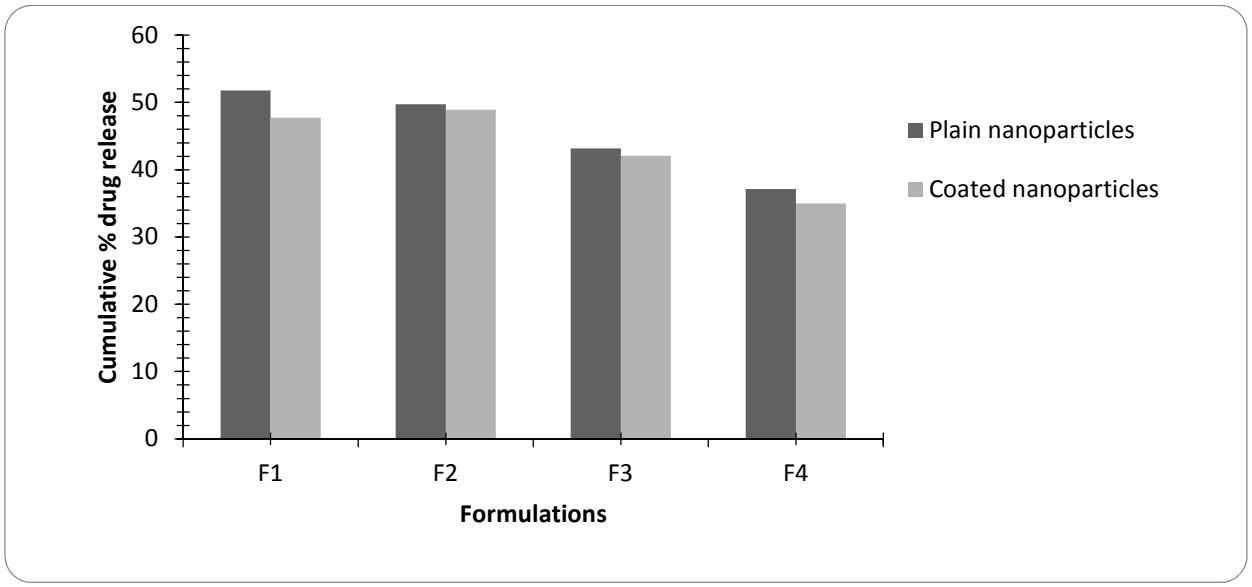

Fig 2.14: Cumulative % drug release Comparison of different formulations

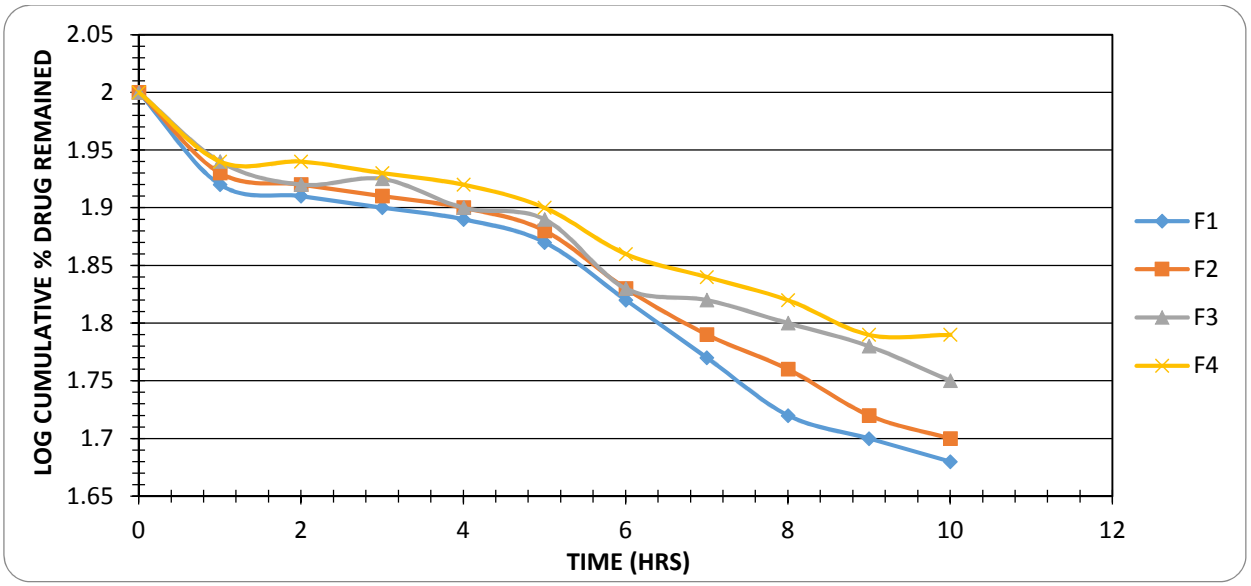

Fig. 2.15: First order release Plot of Cimetidine plain nanoparticles

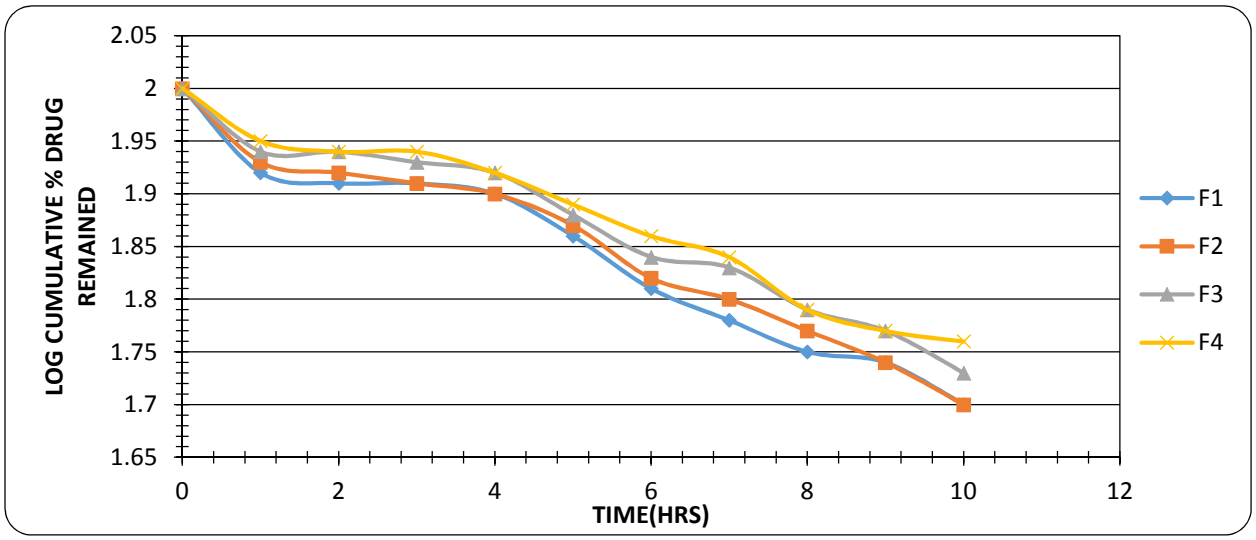

Fig. 2.16: First order release Plot of Cimetidine galactose coated nanoparticles

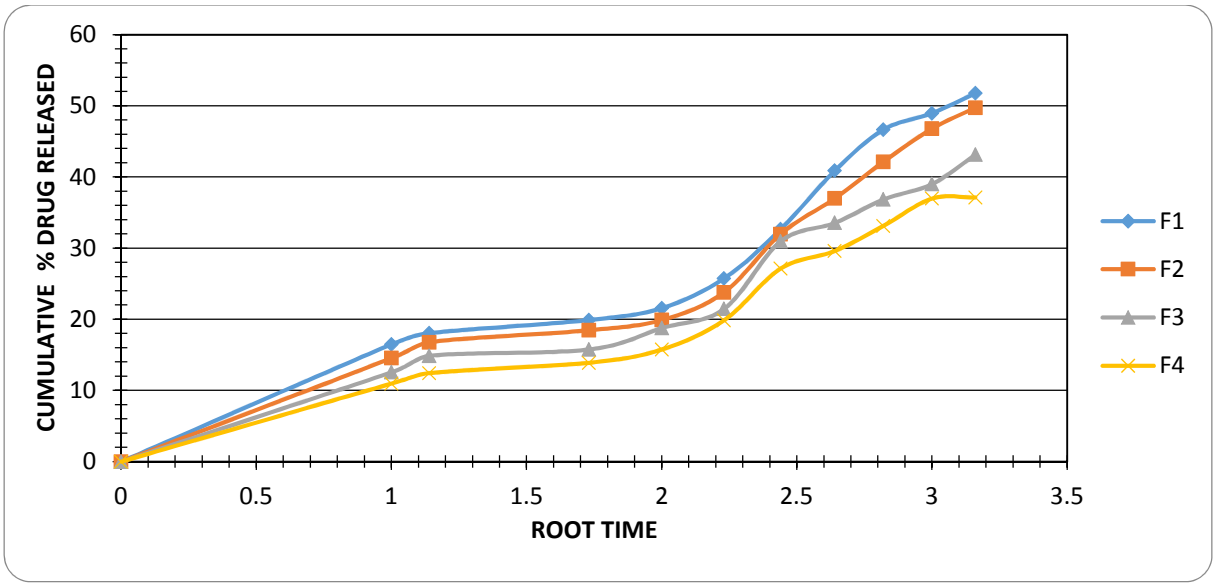

Fig.2.17: Higuchi Plot of Cimetidine plain nanoparticles

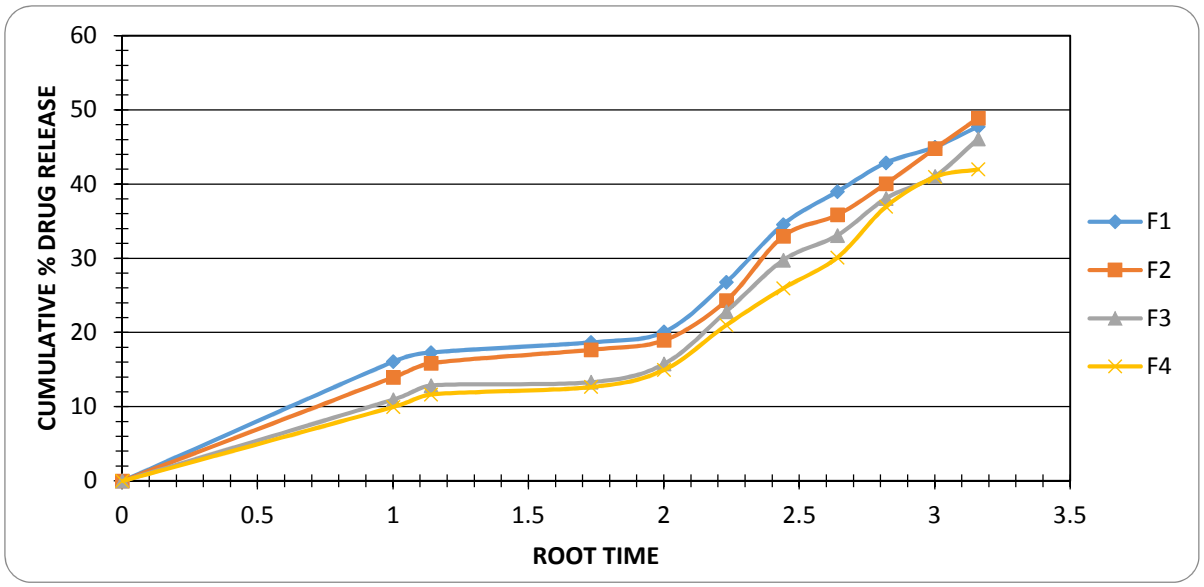

Fig.2.18: Higuchi Plot of Cimetidine galactose coated nanoparticles

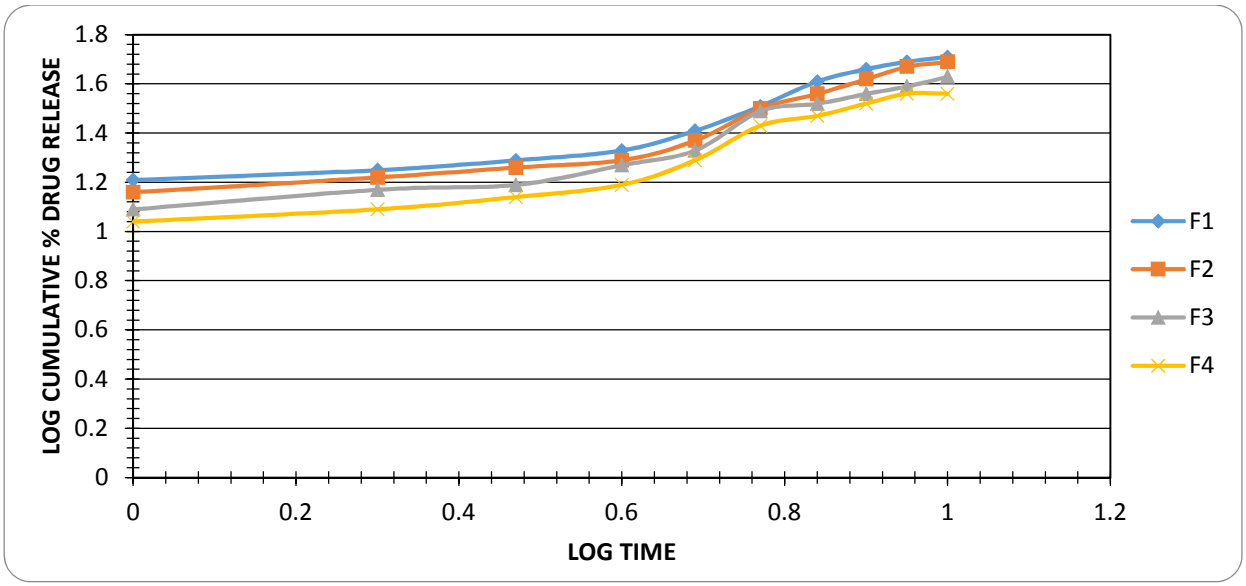

Fig.2.19: Korsmeyer Peppa's Plot of Cimetidine plain nanoparticles

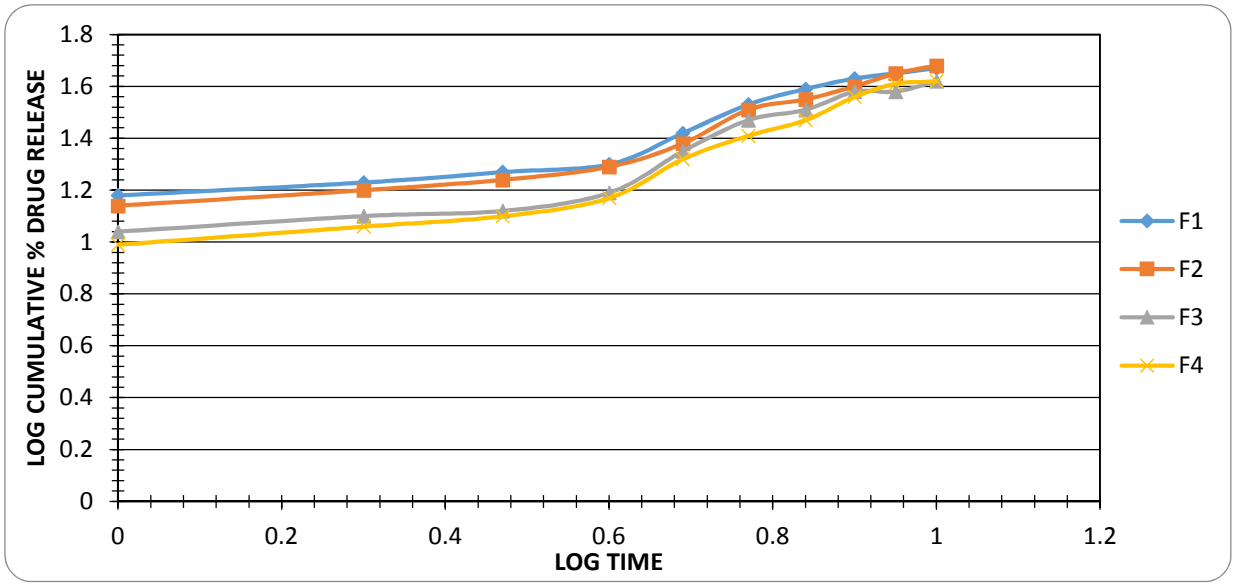

Fig.2.20 : Korsmeyer Peppa's Plot of Cimetidine galactose coated nannoparticles

3.3.6. 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.

The regression coefficients of the all formulations F1 to F4 are shown in Table 2.18.

It was found that all the formulations follows 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 tramsport mechanism.

Table 2.18: 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

4. Conclusion

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.
- 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 size 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.

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