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Development and characterization of Irinotecan loaded colloidal drug delivery system

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Abstract

Irinotecan hydrochloride trihydrate is a drug of choice in the treatment of colon cancer. The most significant adverse effects of Irinotecan are severe diarrhea and extreme suppression of the immune systems. The present work deals with the preparation, characterization and optimization of irinotecan loaded nanoparticles for overall improvement in the efficacy, reduced toxicity and enhancement of therapeutic index of irinotecan. irinotecan nanoparticles were prepared by Emulsion droplet coalescence method with combination of Chitosan and Eudragit polymers.

The prepared nanoparticles were characterized for particle size and the surface morphology, results revealed that nanoparticles were found to be discrete and spherical with the mean size range of of 134.8 nm – 369.9 nm nm. Encapsulation efficiency was in the range of 49.6 to 60.5%. The drug content was uniform and reproducible in each batch of nanoparticles. FTIR and DSC studies discovered no interactions between drug and excipients used in the formulation. *In-vitro* drug release profiles results revealed that, irrespective of the polymer used, increase in concentration has drastically retarded the release of irinotecan. The mechanism of drug release was Non-Fickian diffusion controlled first order kinetics for optimized formulation F5. Stability studies indicated that, the prepared nanoparticles were more stable at room temperature.

Keywords: Colon, Nanoparticles, irinotecan, *In-vitro* release, Stability study.

INTRODUCTION

An ideal drug delivery system should improve therapeutic index of drug as well as it should improve patient compliance. Colloidal drug carriers have attracted increasing attention during recent years to achieve the objective of modern drug therapy. Investigated system includes solid lipid nanoparticles, nanoemulsion, liposomes and nanosuspension. The increasing interest gained by SLNs as a colloidal drug carrier is due to their properties like possible targeting by suitable modification, good protection of encapsulated drug, high encapsulation load, no biotoxicity of carrier and ease of production and scale up at low cost. Therefore solid lipid nanoparticles have been proposed as a drug delivery system for a number of drugs. Nanotechnology is one of the most promising approaches to deal with cancer, which is a life threatening ailment all over the world. [1].

Nanotechnology (NPs) is a rapidly emerging scientific field that is defined as the production of devices with atomic or molecular scale precision, but it also includes all devices with size less than 100 nm. One of the important areas of nanotechnology is nano medicine which refers to highly specific medical intervention at the molecular scale for diagnosis, prevention, and treatment of diseases. Nanoparticles (NPs) were proposed as drug carriers over 30 years ago and have received growing consideration since, mainly due to their stability related aspects, enhanced loading capabilities and control over physicochemical properties. In addition to systemic administration even localized drug release may be achieved using macroscopic drug depots close to the target site [2].

Chemically, Irinotecan is a synthetic analogue of the natural alkaloid camptothecin and is a chemotherapy agent that is a topoisomerase 1inhibitor. Irinotecan is mainly used as a component of first-line therapy in combination with fluorouracil and leucovorin for the treatment of metastatic carcinoma of the colon or rectum. It is also used as a single agent for treatment of metastatic carcinoma of the colon or rectum in patients whose disease has recurred or progressed following initial therapy with fluorouracil-based antineoplastic regimens. The most significant adverse effects of Irinotecan are severe diarrhea and extreme suppression of the immune systems. Irinotecan-associated diarrhea is severe and clinically significant, sometimes leading severe dehydration requiring hospitalization. Also the immune system is adversely impacted which is reflected in dramatically lowered white blood cell counts in blood. All these adverse effects limit the amount of drug to be given to the patients. To overcome these inherent drawbacks associated with parenteral drug delivery of Irinotecan an attempt will be made to provide an alternative drug delivery system for Irinotecan in the form of colloidal drug delivery system to reduce the adverse effects and to enhance therapeutic efficacy of the drug [3, 4].

MATERIALS AND METHODS

Matarials

Irinotecan was a gift sample from Cipla ltd. Bangalore, Chitosan was the gift sample from Primex company, Iceland, Eudragit was obtained from Evonik industries, Acetic acid, Sodium hydroxide, Tween 20, Liquid paraffin were procured from S.D Fine Chemicals, Mumbai.

Methods

Preparation of Nanoparticles by Emulsion droplet coalescence method

Chitosan was dissolved in 1% acetic acid to which 40mg of Irinotecan dissolved in phosphate buffered saline was added. This solution was further added to 10ml of liquid paraffin containing 5%v/v Tween 20 and the mixture was stirred using a high speed blender homogenizer (Remi Motors Ltd) for 3 minutes to form water in oil (w/o) emulsion. Similarly, Eudragit S 100 was dissolved in 3M sodium hydroxide solution. This solution was further added to 10ml of liquid paraffin containing 5%v/v Tween 20 and the mixture was stirred using a high speed blender homogenizer for 3 minutes to form water in oil (w/o) emulsion.

Then these two emulsions were mixed and stirred using homogenizer. As a result of coalescence of the droplets, chitosan in the system was solidified to produce nanoparticles. Eudragit S 100 producing second coating over chitosan nanoparticles. The resultant nanoparticles were centrifuged at 3000 rpm for 60 min (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hrs and stored in a dessicator [5].

Particle Size and Zeta Potential Determination

The particle size was determined by dynamic light scattering, using a Malvern systemwith vertically polarized light supplied by an argon-ion laser operated at 40 mw. Experiments were performed at a temperature of 25.0 ± 0.1° C at a measuring angle of 90° to the incident beam.

The zeta-potential of the nanoparticles was determined by laser doppler anemometry using a Malvern Zetasizer. Measurements

were performed at 25 ± 0.10º C. The nanoparticles were dispersed in 0.1 mM NaCl solution and were taken in clear disposable zeta cell and measured [6].

Scanning Electron Microscopy Analysis

The external and internal morphology of the Nanoparticles were studied using scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with platinum to a thickness of about 10 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The stub containing the coated samples was placed in the scanning electron microscope (JSM- 6360A; JEOL, Tokyo, Japan) chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 20 Kv [6].

Determination of Entrapment efficiency

The amount of Irinotecan entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non-entrapped drug remaining in the aqueous supernatant. The redispersed Irinotecan Nanoparticle suspensions were centrifuged at 6000 rpm in centrifuge at 15oC for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free Irinotecan in the supernatant was determined using UV-Visible spectrophotometer at 369 nm [7, 8].

Drug entrapment efficiency = Actual drug content/Theoretical drug content x 100

FTIR and DSC study

FTIR spectral analysis of pure Irinotecan and Irinotecan loaded nanoparticles was performed by means of the KBr method employing a FTIR spectrometer (Shimadzu Corporation 8600, Japan) [9].

The thermal behavior of the polymer, drug, and formulated nanoparticles were analyzed using a DSC (Shimadzu DSC-60) instrument. About 5 mg of polymer, drug (Irinotecan), nanoparticle formulation containing polymer drug mixture was weighed, crimped into an aluminum pan and analyzed at a scanning temperature range from 50 to 600˚C at the heating rate of 10˚C/m. Baseline optimization was performed before each run. Indium was used as the standard reference material to calibrate the temperature and energy scale of the apparatus [10].

***In vitro* release studies**

The *in-vitro* drug release studies were performed using the dialysis membrane diffusion technique. The dialysis membrane of 12000 mwco (Spectra, Sigma, USA) retaining NPs and allowing free drug into the release media was used. Briefly, drug loaded NPs equivalent to 10 mg Irinotecan were transferred to a glass cylinder having the length of 10 cm and diameter of 2.5 cm closed at one end with a membrane presoaked in distilled water. The cylinder was placed in a receiving compartment containing 50ml phosphate buffer saline pH 7.4. This volume provides complete sink conditions for the drug. The entire system was kept at 37 ± 0.5°C with continuous magnetic stirring at 100 rpm. At predetermined time intervals (1, 2, 3, 4, 5, 6, 8, 10, 12hrs); an aliquot of the release medium was withdrawn and analyzed using spectrophotometer at 369 nm. Withdrawn samples were replaced by fresh buffer. All the experiments were carried out in triplicate [11].

Determination of drug release kinetics

The mechanism of release from optimized formulation NPs were determined using the following mathematical models: zero-order kinetics, first- order kinetic, Higuchi kinetics, and the Korsmeyer-Peppas. The regression coefficient and slope values were determined [12, 13].

Stability studies

The stability study of the Nanoparticles was carried out in accordance with ICH guidelines at Refrigeration Temperature (4-8^{0}C), Room Temperature (25±2^{0}C) and oven Temperature (45±2^{0}C) for 30 days by storing the samples in stability chamber (Lab-Care, Mumbai).

RESULTS AND DISCUSSION

Irinotecan nanoparticles were prepared by emulsion droplet coalescence method with combination of chitosan and eudragit polymers [Table 1].

Table- 1. Formulation of Irinotecan NPs

Formulation	Chitosan 1%(w/v)	Eudragit S 100 (%w/v)	Amount of drug (Irinotecan) (mg)	Tween 20 (%w/v)
F1	0.5	1	40	0.5
F2	1	1	40	0.5
F3	1.5	1	40	0.5
F4	1	0.5	40	0.5
F5	1	1.5	40	0.5
F6	1	2	40	0.5

Scanning electron microscopy reveals that prepared Irinotecan nanoparticles had a homogeneous solid matrix structure. [Fig-1] The picture shows that the particles are spherical and nearly monodispersed.

The Particle size of different formulations is shown in [Table-2].

Table-2. Particle size of Irinotecan loaded nanoparticles

Formulation	Particle size(nm)
F1	361.2±0.5
F2	321.2±1.2
F3	288.2±0.3
F4	217.0±1.5
F5	369.9 ±1.8
F6	134.8±1.4

* All values are expressed as mean ± SD, +n=3

Particles size of Irinotecan NPs were found by dynamic light scattering, using a Malvern system the mean particle size of nanoparticles of drug Irinotecan was found to be in the range of 134.8 nm – 369.9 nm [Fig- 2].

Zeta potential of chitosan nanoparticles containing drug Irinotecan is shown in [Table-3].

Table-3. Zeta potential of Irinotecan nanoparticles

Formulation	Zeta potential(mV)
F1	-27.0±1.3
F2	-26.2±1.1
F3	-25.5±0.9
F4	-24.9±1.7
F5	-26.3±1.3
F6	-21.7±1.4

* All values are expressed as mean ± SD, +n=3

Zeta potential of Irinotecan nanoparticles is shown in [Fig-3]. The zeta potential is a measure of the charge of the particles, as such the larger the absolute value of the zeta potential the larger the amount of charge of the surface. Zeta potential is an important physicochemical parameter, which can influence factors like stability of a nano-drug carrier formulation. Nanoparticles with Zeta Potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Extremely positive or negative zeta potential values cause larger repulsive forces, while electrostatic repulsion between particles with the same electric

charge prevents aggregation of the particles. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions.

The amount of drug present in formulation was estimated using UV at 369 nm. The drug content in different formulations is shown in [Table-4].

Table-4. Drug Entrapment efficiency of Irinotecan loaded nanoparticles

Formulation	Drug Entrapment efficiency (%w/v)
F1	60.5±0.32
F2	56.3±0.2
F3	53.4±0.85
F4	49.6±0.62
F5	52.3±0.54
F6	56.2±0.46

* All values are expressed as mean ± SD, +n=3

The Entrapment efficiency in all the formulations was found to be between 49.6 to 60.5% w/v. In emulsion droplet coalescence (F1 to F3) as the chitosan concentration increases entrapment efficiency decreases this is due to increased viscosity of chitosan hindered the drug encapsulation efficiency, In (F4 to F6) as the eudragit concentration increases entrapment efficiency increases this is due to when highly concentrated, the polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary.. The results showed that, the entrapment efficiency was uniform and reproducible in each batch of different nanoparticle formulations.

It is clear from the observations and comparative study of the spectra of drug and formulation that there is no differences in the spectra and the positions of characteristics absorption of functional groups and bands present in drug molecule. Hence FTIR study revealed no interactions between drug and excipients [Fig- 4A and B].

The thermal behavior of the drug, and nanoparticles formulation were analyzed using a DSC (Shimadzu DSC-60) instrument. The DSC thermograms of the pure drug exhibited a broad endothermic peak at 254^0C corresponding to the melting point of the drug. The thermograms of the formulation F5 exhibited the endothermic peak at 253.5^0C which is almost equal to pure drug. From these observations it is quite obvious to conclude that the drug has not lost its properties and does not show any type of interactions with the carrier and excipients [Fig- 5A and B].

The *in-vitro* drug release studies were performed using the dialysis membrane diffusion technique. The drug release in different formulations is shown in [Table-5].

The drug release profiles from the nanoparticles were as shown in [Fig-6]. The formulations F5, F6, F1 and F2 showed good release from the polymers. The percentage cumulative drug release after 12 hours was 78.36, 74.26, 71.19, 69.20, 68.66 and 69.2% respectively. However about 15% initial burst release was found within first hour in all formulations. F5 released 78.36% of Irinotecan in 12 hrs with a initial burst nearly 14.25% of drug within first hour. Irrespective of the polymer used, increase in concentration has drastically retarded the release of Irinotecan.

Drug release kinetics was derived for best formulation from the in vitro profile. Result from Higuchi plot showed that Irinotecan NPs follows diffusion mechanism where the regression value was above 0.9. Slope value for formulation from Peppas plot was found to be more than 0.5 (Slope 0.711) for Irinotecan, which confirms that formulation follows non- Fickain's diffusion mechanism. Irinotecan formulation followed first order kinetics and the regression value was found to be (0.986).

The stability study of the Nanoparticles was carried out according to ICH guidelines of different formulations is shown in [Table-6]

Table-5. *In-vitro* release data of Irinotecan Nanoparticles

Time(hrs)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	6.746±1.9	8.99±0.21	4.65±0.25	4.69±0.14	9.138±0.25	7.33±0.87
1	13.31±0.25	14.30±0.45	10.39±0.54	9.44±0.54	14.25±0.98	13.96±0.59
2	16.58±0.24	17.99±0.85	14.17±0.88	14.75±0.68	23.43±0.88	17.10±0.89
3	22.56±0.89	23.62±0.46	18.35±0.41	18.67±0.24	28.59±0.97	22.69±0.49
4	28.46±0.45	27.46±0.65	23.53±0.65	26.3±0.78	34.93±0.74	26.76±0.69
5	37.39±0.74	34.10±0.31	30.42±0.25	34.15±0.98	42.00±0.59	32.31±0.21
6	44.40±0.26	42.18±0.61	36.96±0.02	43.00±0.89	48.50±0.89	39.61±0.25
7	52.11±0.24	48.40±0.87	44.68±0.25	50.18±0.85	54.91±0.66	47.45±0.44
8	58.36±0.85	56.43±0.99	51.36±0.74	57.87±0.52	62.52±0.96	58.61±0.75
10	64.50±0.01	62.37±0.27	57.6±0.48	64.29±0.36	70.59±0.35	66.56±0.61
12	71.19±0.21	69.20±0.87	62.70±0.55	68.66±0.69	78.36±0.87	74.26±0.82

* All values are expressed as mean ± SD, +n=3

Table 6 *In-vitro* release data of Irinotecan Nanoparticles after one month stability study

Formulation code	Before stability studies	After stability studies		
		At refrigeration temperature (4-8°C)	At room temperature (25±2°C)	At extreme temperature (45±2°C)
F1	71.19±0.21	70.98±0.15	70.12±0.59	70.67±5.89
F2	69.20±0.87	68.1±0.22	68.2±0.52	67.2±0.57
F3	62.70±0.55	62.1±0.22	61.4±0.47	61.0±0.12
F4	68.66±0.69	68.1±0.22	67.5±0.47	67.0±0.12
F5	78.36±0.87	77.4±0.52	77.1±0.57	76.5±0.51
F6	74.26±0.82	73.30±0.10	73.21±0.43	72.25±0.01

* All values are expressed as mean ± SD, +n=3

From stability studies minimal loss respect to erosion of lipid particles to some extent during storage, the results were in accordance with for instance any pharmacopoeia specifications. This indicated that that Irinotecan nanoparticle formulation remains fairly stable at refrigeration, room and oven temperature.

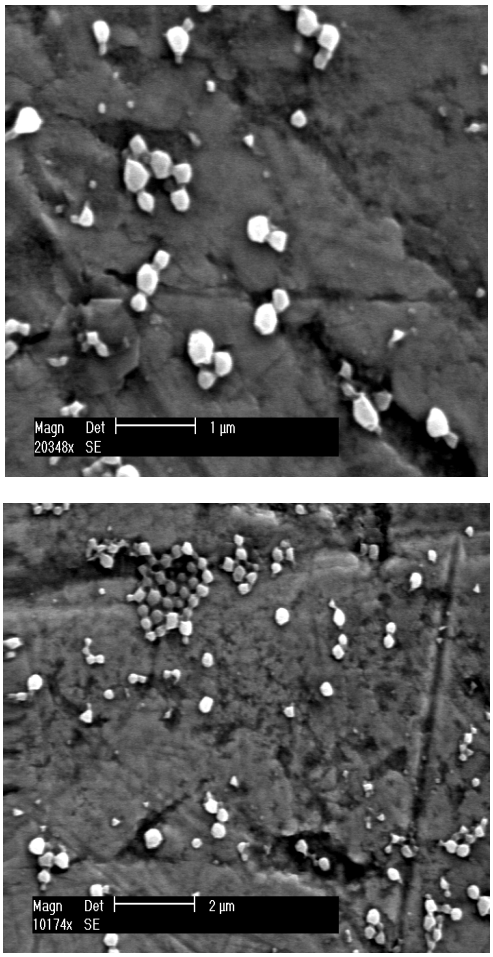

Fig 1.SEM Images Of Optimized Irinotecan Nanoparticles (F5) at Different Magnifications (philips xl30 feg sem).

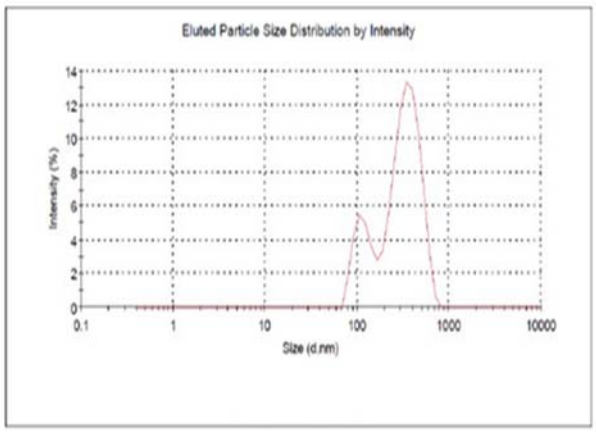

Fig 2. Particle size distribution of Irinotecan loaded chitosan nanoparticles

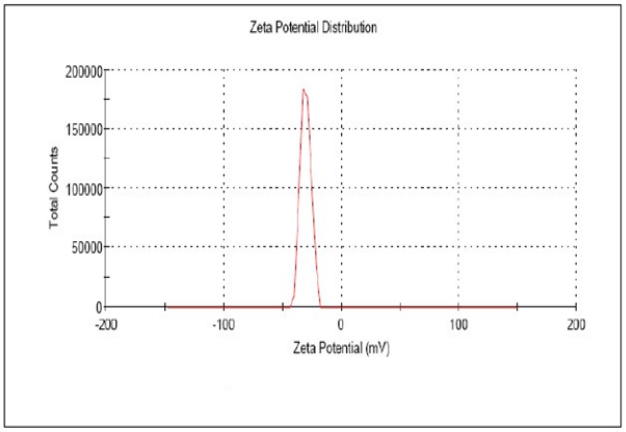

Fig 3. Zeta potential distributions of Irinotecan loaded chitosan nanoparticles (F5)

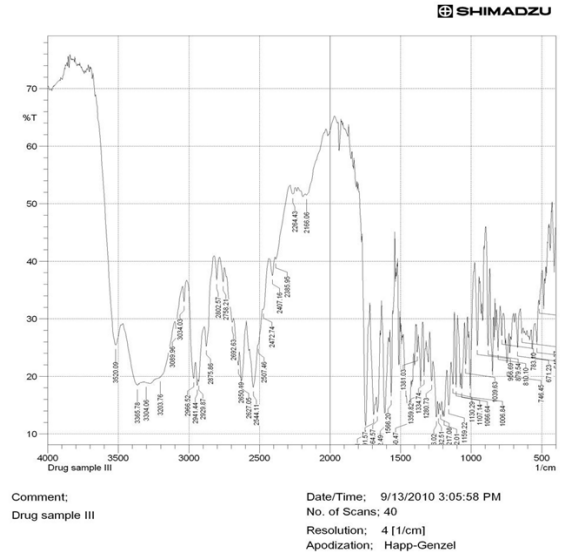

Fig 4 A. IR spectra of pure Irinotecan hydrochloride trihydrate

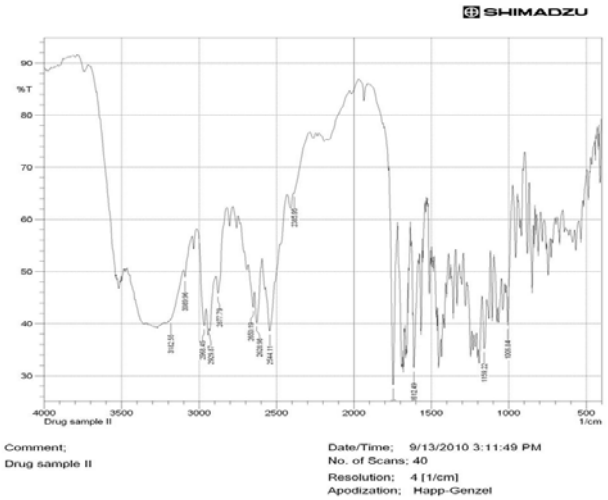

Fig 4 B. IR spectra of optimized formulation of Irinotecan hydrochloride trihydrate nanoparticles (F5)

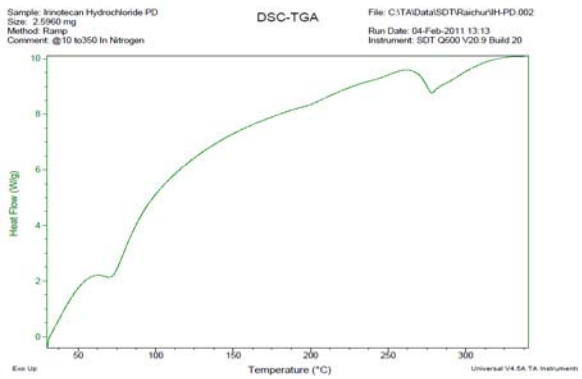

Fig 5 A. DSC Thermograms of pure Irinotecan hydrochloride trihydrate

Trihydrate Nanoparticles (F5)

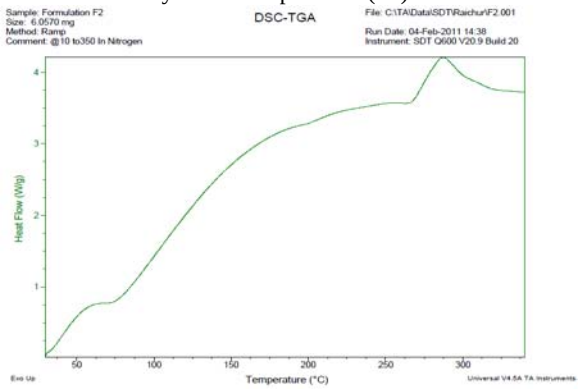

Fig 5 B. DSC Thermograms of optimized formulation of Irinotecan hydrochloride

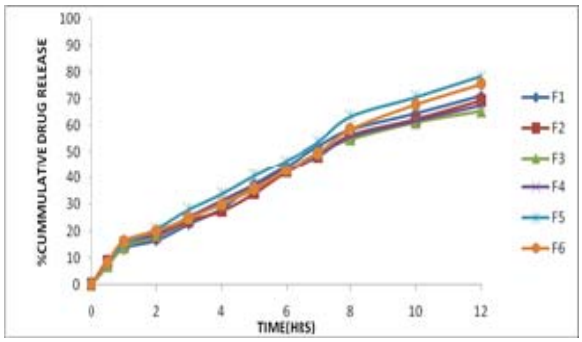

Fig 6. Comparative release profiles of Irinotecan loaded Nanoparticles

CONCLUSION

Irinotecan loaded nanoparticles were prepared with lower dose to improve the availability of the drug at the site and to minimize the drug related side effects. From the results we conclude that Irinotecan polymeric nanoparticles gives promising results with respect to particle size, drug content, in vitro release studies and stability studies.

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