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Original Article

BIODEGRADABLE PEG NANOPARTICLES FOR COLORECTAL CANCER USING IRINOTECAN AS ANTICANCER AGENT

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ABSTRACT

Objective: The idea behind this work was to formulate nanoparticles of irinotecan hydrochloride using poly ethylene glycol as carrier molecule and to evaluate the formulation.

Methods: Irinotecan loaded nanospheres were prepared by the emulsification solvent evaporation method.

Results: The preformulation studies of FT-IR and XRD shows better compatability between the drug and polymer. Evaluation of prepared Irinotecan hydrochloride nanoparticles was carried out for characteristics like SEM analysis and *In-Vitro* release kinetic studies.

Conclusion: The formulation shows better drug release performance of about 81.3 % in drug release kinetics. The in- vitro drug release profile shows the controlled drug release and better percentage of drug release in 24 hrs release study.

Keyword: Antineoplastic, Preformulation, Nanoparticle, Emulsification, Controlled drug release.

INTRODUCTION

Solid lipid nanoparticles are typically spherical with an average diameter between 1 and 1000 nm. It is an alternative carrier system to tradition colloidal carriers, such as, emulsions, liposomes, and polymeric micro and nanoparticles[1]. Controlled drug delivery systems offer numerous advantages over conventional dosages forms, including improved efficacy,reduce toxicity, and improved patient compliance, and can be utilized in the form of nanocarriers in drug delivery [2]. Release kinetics of biodegradable polymers are controlled by diffusion, erosion or a combination thereof and are depend on the polymer's properties like molecular weight, copolymer ratio, crystallinity, drug properties, preparation conditions, particle size, surface morphology, drug loading and the dissolution conditions. The sustained release nanoparticles can be prepared by an emulsion solvent extraction/evaporation technique. In the solvent evaporation method, the required amount of polymer and drug are dissolved in an organic phase which is emulsified under homogenization with surfactant to form an oil in water emulsion. Stirring is continued to evaporate the organic phase, the formed nanoparticles separate and dried [3]. In the present study Nanoparticlulate drug delivery system for Irinotecan Hcl was developed, that would overcome the therapeutic risks of conventional formulations and was evaluated with respect to particle size, drug content, in vitro release [4]. Irinotecan is a semisynthetic, water-soluble derivative of camptothecin, which is a cytotoxic, quinoline-based alkaloid extracted from plants such as *Camptotheca acuminata*, Irinotecan a prodrug, is converted to a biologically active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) by a carboxylesterase-converting enzyme, Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. It is thousand-fold more potent than its parent compound irinotecan[2,3] Irinotecan exhibits moderate plasma protein binding (30% to 68% bound). Irinotecan and SN-38 is predominantly binds with (approximately 95%) with albumin[2]. Irinotecan and its active metabolite, SN-38, inhibit the action of topoisomerase I, an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Irinotecan and SN-38 bind to the topoisomerase I-DNA complex and prevent religation of the DNA strand, resulting in double-strand DNA breakage and cell death. The precise contribution of SN-38 to the activity of irinotecan in humans is not known. Irinotecan is cell cycle phase-specific (S-phase)

MATERIALS AND METHODS

Materials

Irinotecan Hydrochloride was purchased from Shilpa medicare Ltd, (Raichur, India), PEG – 400 was obtained from Sigma aldrich pvt. Ltd, (Bangalore, India), Pluronic F68 (Poloxamer) was obtained from Signet corporation, (Mumbai, India), Dichloro methane was purchased from Thermo Fisher scientific India pvt. Ltd, (Mumbai, India).

Methods

Formulation of nanoparticles

Irinotecan loaded nanospheres were prepared by the emulsification solvent evaporation method. The hydrophobic drug Irinotecan (20 mg) and biodegradable polymer poly ethylene glycol were dissolved in 2.4 ml of organic solvent methylene chloride. This resultant solution was dispersed in an aqueous phase ie. DM water (117.6 ml) containing pluronic F68 (2mg) by using probe sonicator for 2 mins, there after the organic solvent was evaporated at 600 rpm for 2 hrs at room temperature using magnetic stirrer. Then the aqueous suspensions were concentrated in a low pressure system to final volume of 50 ml and filtered in a 0.8 µm Millipore membrane [5]. Different batches of the formulation(F1, F2 and F3) was carried out by keeping 20 mg of Irinotecan Hcl as constant and changing the concentration of polymer as 20 mg (F1), 40 mg (F2) and 60 mg (F3).

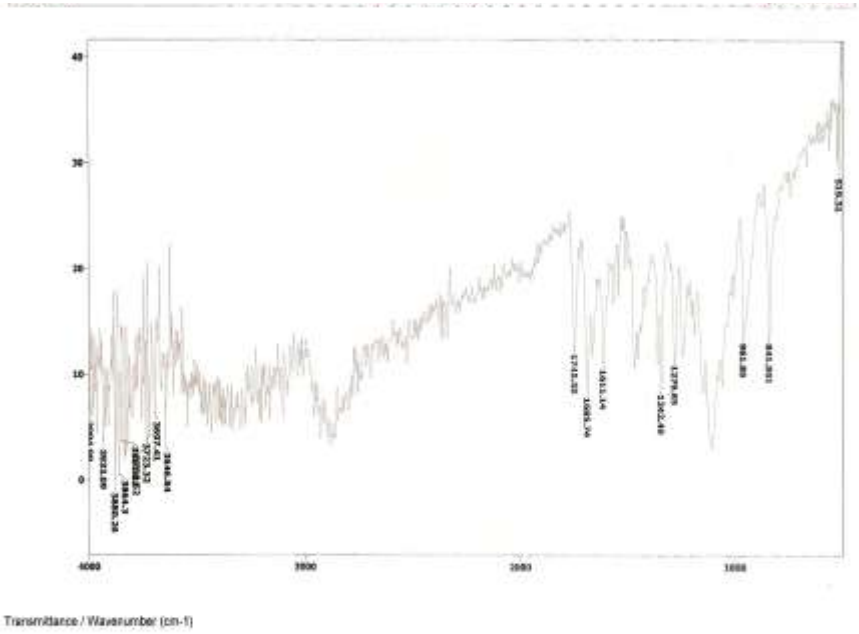
RESULTS

A. Fourier Transform Infrared Spectroscopy (FTIR).

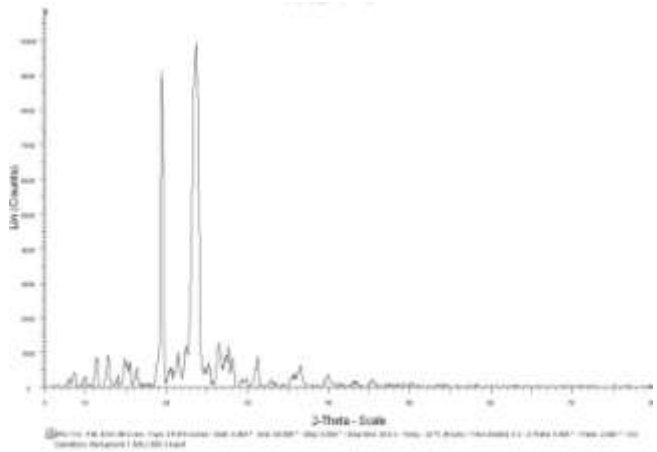
The IR spectroscopy studies were carried out for the standard Irinotecan Hydrochloride, poly ethylene glycol (PEG) and the combination of the drug Irinotecan with biodegradable polymer poly ethylene glycol using Perkin Elmer spectrum-1 FTIR spectrophotometer. The IR spectrum of the formulation was then analyzed in comparison with the spectrum of standard Irinotecan Hydrochloride to assess the compatibility of the excipients with the drug Irinotecan Hydrochloride [6].

Table 1: Composition of Irinotecan Hcl nanoparticle Formulations

Ingredients	Formulation		
	F1	F2	F3
Irinotecan Hydrochloride	20 mg	20 mg	20 mg
PEG – 400	20 mg	40 mg	60 mg
Pluronic F-68 (Poloxamer)	1.5 mg	1.5 mg	1.5 mg
Dichloromethane	2.4 ml	2.4 ml	2.4 ml
DM water	117.6 ml	117.6 ml	117.6 ml



Graph 1: FTIR peak for Irinotecan combined with polyethylene glycol.



Graph 2: XRD Peak for Polymer Polyethylene glycol with drug Irinotecan hydrochloride

B. X- ray diffraction (XRD)

The x – ray diffraction pattern of the Irinotecan loaded PEG nanoparticles demonstrated the amorphous state of the sample. The x – ray powder diffraction patterns of pure Irinotecan displayed crystallinity, whereas an amorphous pattern lacking crystalline peaks was observed for PEG. The diffractogram of the physical mixture consists of the superimposed figures of each of the pure components with the peaks of Irinotecan being attenuated due to dilution and particle size reduction during mixture. When compared to the diffraction patterns of pure Irinotecan and PEG, the diffractogram of the inclusion complex was superimposable with that of PEG, indicating the existence of molecular interactions between the two species [7].

C. Scanning Electron Microscopy (SEM)

Surface morphology of Irinotecan Hydrochloride nanoparticle formulations were studied by Scanning Electron Microscopy (SEM) operating at 5 keV.

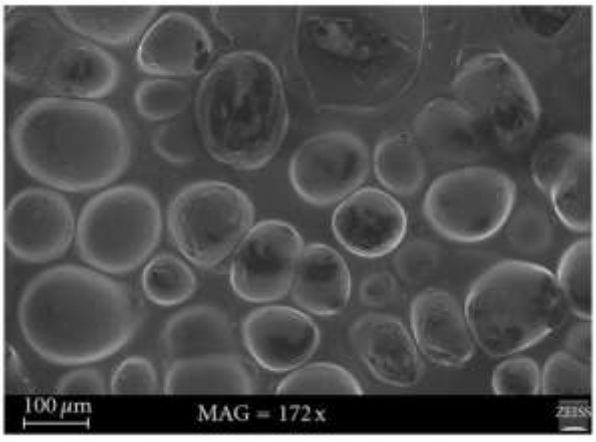


Fig. 1: SEM Photograph of Formulation F3

A thin layer of the formulations were dispersed on polycarbonate 0.05 µm filter membrane placed on the carbon adhesive tape in an aluminium stub. The samples were dried and then coated with platinum using autofine coater. Then the scanning electron microphotographs were taken by selecting the field, which consists of spot size setting of 1.0 mm and a working distance of 5 mm [8].

D. *In-vitro* release studies: The *in- vitro* release studies of the three batches of Irinotecan Hydrochloride formulations (F1, F2, and F3) were carried out using dialysis membrane in a beaker containing 100 ml of phosphate buffer saline (PBS) pH 7.4 maintained at constant room temperature and sink conditions, stirring was carried out at 50 rpm using a magnetic stirrer.

The samples were collected at regular intervals of 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 24 hrs and analyzed by using UV spectrophotometer for absorbance at and the results are tabulated in table and represented graphically.

Table 2: Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F1 – 1:1)

S. No.	Time (h)	Higuchi Plot	
		Square root of Time	Cumulative % of Drug release
1	0	0.000	0
2	1	1.000	9.35
3	2	1.414	12.25
4	4	2.000	18.6
5	6	2.449	18.75
6	8	2.828	19.5
7	10	3.162	21.3
8	12	3.464	23.2
9	14	3.742	23.92
10	16	4.000	24.9
11	20	4.472	26.0
12	24	4.899	38.9

Table 3: Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F2 – 1:2)

S. No.	Time (h)	Higuchi Plot	
		Square root of Time	Cumulative % of Drug release
1	0	0.000	0
2	1	1.000	31.65
3	2	1.414	33.0
4	4	2.000	34.4
5	6	2.449	39.45
6	8	2.828	43.1
7	10	3.162	44.1
8	12	3.464	49.15
9	14	3.742	52.26
10	16	4.000	53.55
11	20	4.472	58.9
12	24	4.899	60.75

Table 4: Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F3 – 1:3)

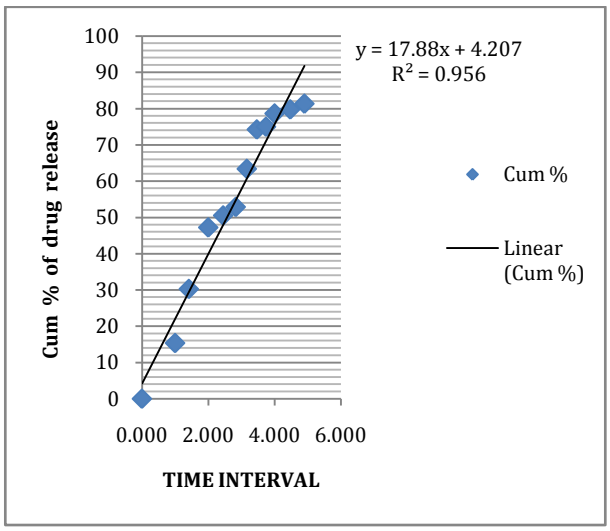
S. No.	Time (h)	Higuchi Plot	
		Square root of Time	Cumulative % of Drug release
1	0	0.000	0
2	1	1.000	15.35
3	2	1.414	30.25
4	4	2.000	47.2
5	6	2.449	50.5
6	8	2.828	52.85
7	10	3.162	63.35
8	12	3.464	74.2
9	14	3.742	74.95
10	16	4.000	78.6
11	20	4.472	79.72
12	24	4.899	81.3

DISCUSSION

The x – ray diffraction pattern of the Irinotecan hydrochloride of pure sample shows its crystallinity nature, whereas an amorphous pattern lacking crystalline peaks were observed for Poly ethylene glycol, The diffractogram of the physical mixture consisted of the superimposed figures of each of the pure components with the peaks of irinotecan Hcl When compared to the diffraction patterns of the pure irinotecan Hcl and Poly ethylene glycol. The diffractogram of the inclusion complex was superimposable with that of amorphous Poly ethylene glycol. The combination of drug with polymer complex have a Amorphous pattern, superimposed figure is due to dilution and particle reduction during the mixture. The comparative analysis of FT-IR spectrum of the standard drug and the polymer reveals that there is better compatibility between the drug Irinotecan and the biodegradable polymer Poly ethylene glycol. The Scanning Electron Microscopy photographs reveals the Anatomy of the particle along with their size of the formed nanoparticles.

Table 5: Comparative Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F1, F2, and F3 – 1:1, 1:2, and 1:3)

S. No.	Time (hrs)	Higuchi Plot			
		Square root of Time	Cumulative % of Drug release F-1	Cumulative % of Drug release F-2	Cumulative % of Drug release F-3
1	0	0.000	0	0	0
2	1	1.000	9.35	31.65	15.35
3	2	1.414	12.25	33.0	30.25
4	4	2.000	18.6	34.4	47.2
5	6	2.449	18.75	39.45	50.5
6	8	2.828	19.5	43.1	52.85
7	10	3.162	21.3	44.1	63.35
8	12	3.464	23.2	49.15	74.2
9	14	3.742	23.92	52.26	74.95
10	16	4.000	24.9	53.55	78.6
11	20	4.472	26.0	58.9	79.72
12	24	4.899	38.9	60.75	81.3



Graph 3: Release kinetics profile of PEG nanoparticle of Irinotecan Hydrochloride Formulation (F3 – 1:3)

Therefore it is suitable for nanoparticulate drug delivery system, achieves site specific action. Because of the smaller size it gets rapidly absorbed and it leads to increased bioavailability, this yields better therapeutic effect. The *in-vitro* release study reveals it is a bi-phasic pattern of drug release, in which 50% of the total loaded drug from the formulation get released within 5 hrs interval of time and the remaining amount of the drug is again extended for about 19 hrs in a 24 hrs release study. This reveals about the character of NP formulation in which it has a bi-phasic pattern of drug release. The *In-vitro* drug release is in the manner of prolonged action, this is due to its nanoparticle nature of the carrier molecule combining with the drug which is having a greater effect on drug delivery [9,10]. Finally the kinetics of drug release is considered as a controlled drug delivery in bi-phasic pattern of nanoparticle formulation. So it is clearly concluded that the *In-Vitro* release fits in the kinetic of Higucuchi equation.

CONCLUSION

In this work, I have proved that poly ethylene glycol can be combined with Irinotecan Hcl for the formulation to have better performance. It is clearly demonstrated that the solvent evaporation method is possible for poly ethylene glycol (PEG) nanoparticle preparation. Decreasing the concentration of Pluronic F- 68 leads to increase in size of the particle thereby decrease in polydispersity. This study has shown that poly ethylene glycol are not only well known carrier molecule, They constitute very powerful tool in drug targeting because they can increase dramatically the loading capacity of nanoparticles. For these reasons Irinotecan Hcl with poly ethylene glycol nanoparticles are likely to constitute a promising system for improving intravenous delivery of Irinotecan Hcl in the treatment in colorectal cancer. The formulation shows better drug release performance upto 24 hrs for about 80 %. The comparative *in-vitro* drug release profile of all the three batches showed the controlled drug release, the formulation F3 shows better percentage of drug release in 24 hrs release study. It is clearly concluded that formulation F3 is the best among the three batches while comparing with F1 and F2.

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ABBREVIATIONS

hrs – Hours

FTIR – Fourrier Transform Infrared Spectroscopy

XRD – X –Ray Diffraction

SEM – Scanning Electron Microscopy

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