TARGETING EFFICACY OF CHITOSAN NANOPARTICLES OF SN-38 FOR THE TREATMENT OF COLORECTAL CANCER

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Abstract- Colorectal cancer is the third most common cancer in United States. It starts in the colon and may spread to the rectum. In the present research polymeric nanoparticles of SN-38 was prepared by polyelectrolyte complexation method. Its physicochemical characterization was done by Fourier transform Infrared spectroscopy and Differential Scanning Calorimeter for any incompatibility, Malvern Zeta Sizer for particle size and zeta potential, TEM and SEM images for surface morphology. In vitro release studies were conducted in simulated gastrointestinal fluids for 48 hrs by dialysis membrane method. Particle size, drug entrapment and drug loading were taken as responses for optimization of nanoparticles. In vivo distribution studies were performed in rats to see the targeting potential of nanoparticles after oral administration of SN-38 loaded nanoparticles tagged with Nile red in rats. The optimized nanoparticles were found to be spherical, having positive zeta potential, and particle size between 190-250 nm. The entrapment efficiency was nearly 75% and drug loading was 11.1%. In vitro release in simulated gastric fluids showed maximum release in colonic fluid as compared to gastric and intestinal fluid. In vivo distribution studies show that Nile red tagged nanoparticles reached colorectal region and not other parts of the body after 4 hrs of oral administration. Thus they are not cytotoxic to other organs and these nanoparticles of SN-38 can be delivered orally for the treatment of colorectal cancer.

Keywords- SN-38, polymeric nanoparticles, colorectal cancer, polyelectrolyte complex

I. INTRODUCTION

Colorectal cancer is the third most common cancer among male and female in the United States [1]. Present treatment regimens mostly involve i.v. infusion due to poor bioavailability of chemotherapeutic drugs. Oral chemotherapy is still the need of cancer patients as it can be administered easily in conscious cancer sufferers. SN-38 (7-ethyl-10-hydroxycamptothecin) is 1000 times more cytotoxic than its prodrug CPT-11 (Irinotecan hydrochloride)(Atyabi et al., 2009) which is FDA approved chemotherapeutic agent, available with brand name Camptosar® (Pharmacia Corp., Peapack, NJ). SN-38 is in clinical trials by some companies' e.g. Insys, Enzon Pharma and Neopharm and is cytotoxic against cancers like lung cancer, cervical cancer, pancreatic cancer and colorectal cancer[3]. It does not require activation by the liver hence eliminating the interpatient variability [4]. Only 2-8% of CPT-11 is converted into SN-38 [5].Thus, promotion of SN-38 for cancer treatment may be beneficial for cancer treatment. SN-38 has been extensively studied for oral delivery in the form of prodrugs or nanomedicines [6]. For instance, SN-38 was encapsulated into lipid nanoparticles, which were stable in simulated gastrointestinal fluids and showed higher permeation across Caco-2 cell lines, but it has very less drug loading (0.04%). Similarly hydrophilic prodrug was synthesized by conjugating SN-38 to a macromolecular polyamidoamine (PAMAM) dendrimer. In another study, pH sensitive polymeric micelles (DDS-06E) of SN38 were developed which showed improved Caco-2 permeability [7]. After oral delivery of DDS-06E, tumour growth regression was similar to intravenous CPT-11 at an identical dose. Most of the studies were evaluated for permeability across Caco-2 cell line and if in vivo studies were conducted that were restricted to intravenous or subcutaneous route.

Here polymeric nanoparticles are developed using chitosan (cationic polymer) and Eudragit S100® (anionic copolymers based on methacrylic acid and methyl methacrylate) by polyelectrolyte complexation method. The nanoparticles formed are having positive surface charge due to chitosan coating. These NPs will stay for longer period of time inside gut as mucus is negatively charged. Both hydrophilic and lipophilic drugs can be included in polymeric nanoparticles (NPs). They have the potential to release the drug at a specific site, improves oral delivery of drugs with poor chemical, enzymatic or metabolic stability and permeability[8]. As compared to other methods, polyelectrolyte complexation method has a potential for oral controlled release [9].

II. MATERIAL AND METHODS

Materials: SN-38 was obtained as a gift sample from Avra Labs (Hyderabad, India). Eudragit ®S100 was obtained as a gift sample from Evonik Industries (Mumbai, India). Chitosan (deacetylation degree≥75%), Pluronic F 68, Dialysis bag (cut-off mol. wt. 8000 Dalton), Nile Red, HPLC grade acetonitrile and acetone were purchased from Hi Media Labs (Mumbai, India). All other chemicals and solvents were of analytical grade.

Methods

2.1 Preparation of Chitosan nanoparticles (CENP)

It was prepared by modified ionic gelation method as described by Prasad and Dangi [10]. Briefly, SN-38

and chitosan were stirred in 1% glacial acetic acid. Pluronic F 68 was added to this solution in different concentration. ES 100 solution in acetone was added slowly with a syringe in the above mixture. This mixture was stirred for 6 hrs. It was bath sonicated, centrifuged and pellets washed with distilled water, lyophilized and stored at 2-4^{o}C till further use. For tagging with Nile red, 0.05% Nile red was added to the ES 100 solution in acetone [11].

2.2 Optimization of CENP by Design expert®software

Optimization of CENP, its physicochemical characterization and in vitro release studies were published earlier [10]. In brief, optimization of CENP was performed by Design Expert software 9® (Stat-Ease Inc., Minneapolis, MN, USA). A 3-level experimental response surface methodology using the Box-Behnken design[12] was used to study the effect of different variables on formulation properties like mean particle size, entrapment efficiency and drug loading of CENPs. Independent variables are polymer (Eudragit® S100) concentration, surfactant concentration and sonication time. Total 17 formulations were prepared. As per the desirability obtained from the experimental design a new formulation of CENP was developed and characterized.

2.3 Physicochemical characterization and in vitro release studies in simulated gastrointestinal fluids

Physicochemical characterization was done with Fourier transform infra red spectroscopy (FTIR), Differential scanning calorimeter (DSC) and X-ray diffraction (XRD) are shown in fig 1. TEM images (TEM; FEI Philips Morgagni 268D, 100 kV, and Magnification: up to 2,80,000 X) were taken and shown in figure 1.Entrapment efficiency, drug loading, particle size, zeta potential and polydispersity index are given in table 1 of the optimized CENP. In vitro drug release in simulated gastrointestinal fluids were conducted by dialysis membrane method [13][14]with modification method for 48 hrs and it is graphically represented in fig1.

2.4 In vivo distribution of CENP after oral delivery

Study protocol was approved by the Institutional Ethical Committee, Institute of Pharmaceutical Sciences, 994/a/GO/06/CPCSEA, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India. 3 female Sprague-Dawley rats were taken and fasted overnight and water was given ad libitum.

10 mg of Nile red tagged CENP was given orally and were observed for any adverse symptoms. They were sacrificed after 4 hrs. Different organs (Brain, spleen, liver, colon, heart) were isolated and fixed by keeping inside 10% formalin solution. Tissue slides were prepared by using haematoxylin as a staining agent. The slides were observed under Digital Photomicroscope (Zeiss, Primostar, Germany) and the photomicrographs of colon tissue slides are shown in Figure 2 and photomicrographs of other organs are given in Figure 3.

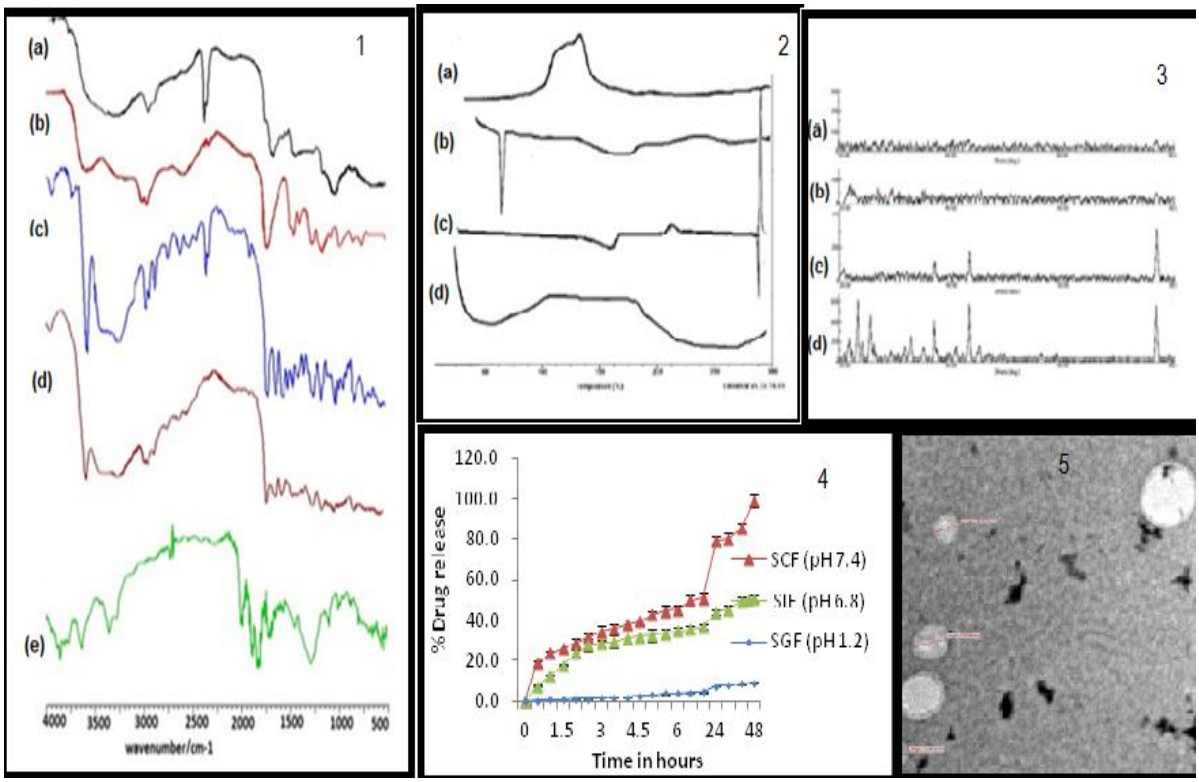


Figure 1: 1) FTIR of Chitosan (a), ES-100 (b), SN-38 (c), Physical mixture (d), CENP (e). 2) DSC of Chitosan (a), ES100 (b), SN-38 (c), and CENP (d). 3) XRD of CENP (a), Chitosan (b), ES-100 (c), SN-38 (d). 4) Drug release study in simulated fluids. 5) TEM image of CENP.

Table1. Evaluation parameters of CENP and blank CENP

S.No	Formulation	Predicted values (Experimental design)			Observed values			PdI	Zeta potential (mV)
		Particle size	Drug entrapment	Drug loading	Particle size (nm)	Drug entrapment (%)	Drug loading (%)		
1	Blank CENP	-----	-------	------	137.5± 2.5	-------	------	0.341 ± 0.04	25.4± 1.3
2	CENP	211.65	74.52	11.45	212.5± 1.61	75.3± 1.01	11.1± 0.6	0.318 ± 0.062	25.6 ± 0.47

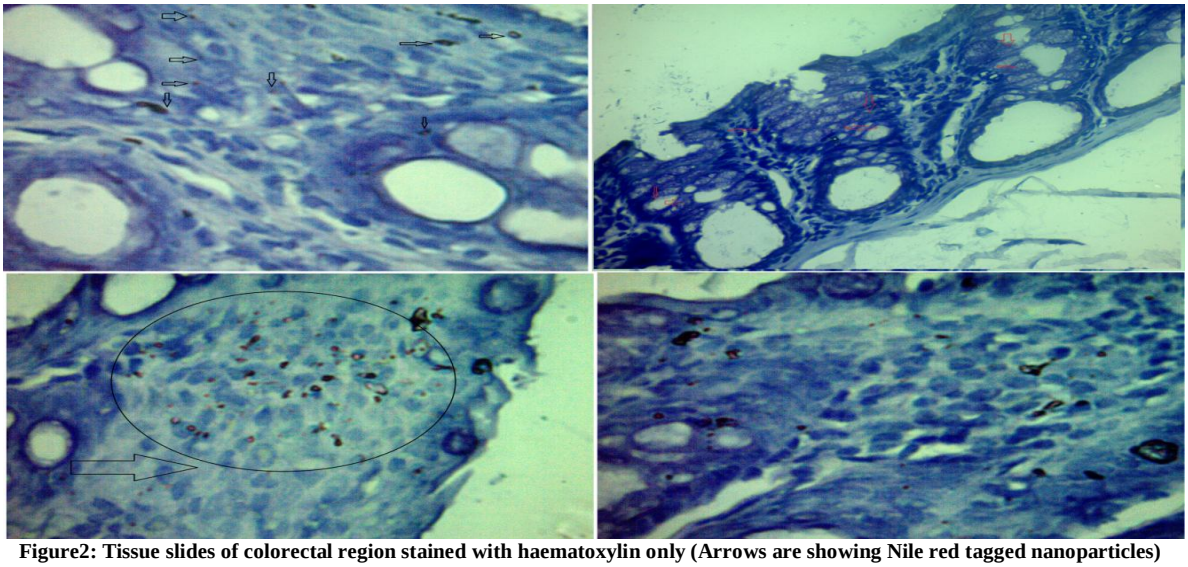


Figure2: Tissue slides of colorectal region stained with haematoxylin only (Arrows are showing Nile red tagged nanoparticles)

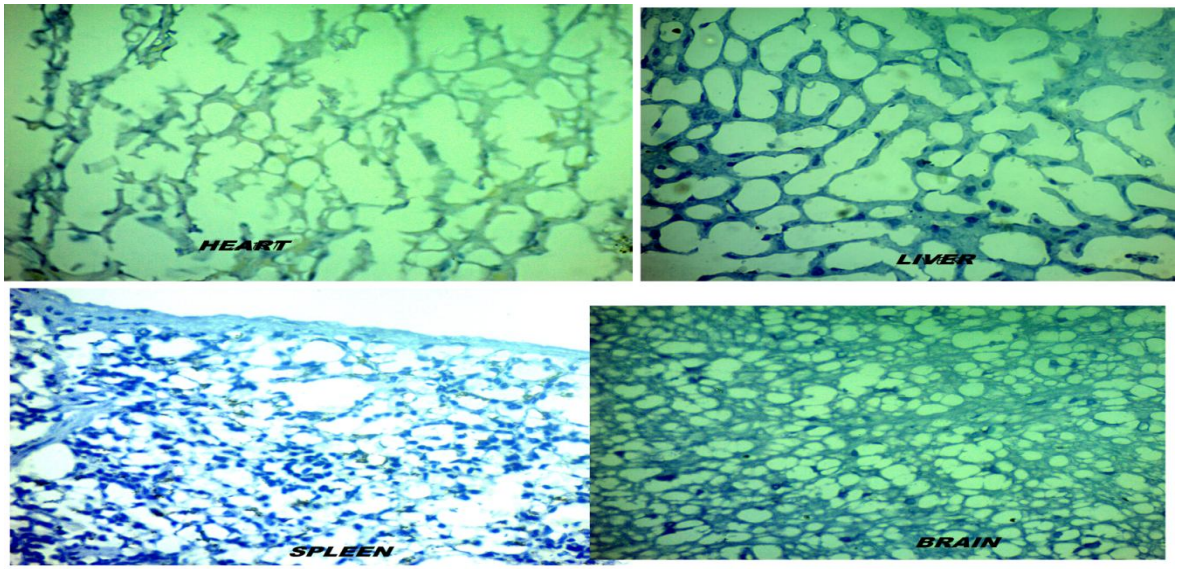


Figure3: Tissue slides of other organs (Heart, liver, spleen and brain) stained with haematoxylin only.

III. RESULTS AND DISCUSSION

3.1 Preparation and optimization of CENP

CENP was prepared by polyelectrolyte complexation method using ES-100 which is often used for coating of pellets [15][16] for colon targeting as it has pH dependent solubility above pH 7. In the present design its ionic properties as well as its pH sensitive properties were used for the formation of pH sensitive nanoparticles. Box Beckhen design with response surface models is used for getting optimized formulation with reducing the number of experimental runs, thus preventing wastage of time. The desirability value obtained from Design-Expert software is 0.743. So, a new batch of CENP was prepared with the predicted levels of formulation factors to confirm the validity of the optimization procedure. The particle size, entrapment efficiency, drug loading represented good agreement with the predicted values (Table 1).

High zeta potential indicates stability of nanoparticles dispersion as particles having high charge cause less aggregation and thus lesser PdI. Positive zeta potential is good for mucoadhesion as mucus is negatively charged.

3.2 Physicochemical characterization of CENP

FTIR, DSC and XRD peaks depicted crystalline structure of SN-38 and no incompatible reaction between any components of CENP. TEM images clearly shows spherical structure with narrow size distribution without any aggregation in the range of 135-205 nm (Fig 1). In vitro drug release studies showed release of SN-38 in simulated gastric fluid was negligible. In Simulated intestinal fluid at pH 6.8 it released almost 54% drug and in simulated colonic fluid the release almost 90% during 48 hours. This type of drug release pattern favoured the objective to target the drug to colon given by oral route.

3.3 In vivo distribution of CENP after oral delivery

In vivo distribution studies were conducted in rats after oral delivery to see the targeting potential of CENP for colon delivery. After oral administration the rats do not show any type of adverse symptoms. The microscopic images of colon (Fig.2) and other organs (Fig.3) shows that Nile red tagged CENP reaches to the colon area and does not reaches and affects the other organs like brain, heart, liver and spleen. It also not reached to lungs and kidney (images not shown). This shows that CENP has the potential to bypass first pass metabolism as it is not reaching the liver. Other part of this result is that CENP are able to reach colon in its intact form for which it is targeted thus not being cytotoxic to other organs.

CONCLUSION

This research reveals the potential of chitosan nanoparticles formed by complexation with Eudragit S100 (anionic copolymer). These particles are stable and have the properties of both the polymers. They can be prepared easily and are able to reach the colon without being degraded by the enzymatic activity and different pH values of different parts of gastrointestinal tract. Their antitumor efficacy for colorectal cancer has also shown positive results. So, they can be further tried for clinical trials.

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