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An Ex-Post Facto on Formulation and Charecterization of Camptothecin and its Derivatives in Nanoparticle Formulation

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ABSTRACT

This effective retrospective research analysis will provide you basic research evidence in designing a nanoparticulate formulation based on camptothecin derivatives of Aromatase inhibitors. This paper provides us the novel information on formulation and characterization of camptothecin and its derivatives in nanoparticle formulation. The potential physical and chemical interactionsbetween the drug and excipients can affect the chemical nature, stability, bioavailability of drugs and subsequently, affects their therapeutic efficacyand safety. Various types of single and diblock polymers were used in formulation of nanoparticles under different method. The particle size characterization was differentiated based on the methods adopted for the formulation and types of polymer used. The comparative In-vitro study of all the formulations provides better understanding in dosing the formulations. The behavior of the camptothecin based nanoparticles was clearly reviewed by making comparative study along with a graphical representation.

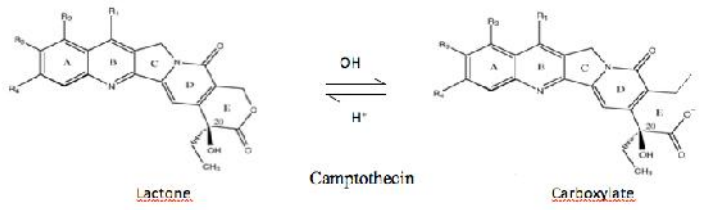
Key words: *Camptothecin, Nanoparticles, Aromatase Inhibitors, In-vitro study, Diblock polymers.*

INTRODUCTION

Nanotechnology is a revolutionary field of micro manufacturing involving physical and chemical changes to produce nano-sized materials. The word "nano" is a Latin word meaning "dwarf". Mathematically a nanometer is equal to one thousand millionth of a meter. Camptothecin (CPT) is a quinoline alkaloid derived from the bark, wood and fruit of the Asian tree Camptotheca acuminata. It wasfirst discovered in 1966 by Drs. Wall and Wani and developed at National Cancer Institute (NCI) todemonstrate potential antitumor activity. Initial preclinical testing in mouse L1210leukemia and rat Walker carcinosarcoma models showed promising results in terms of tumor inhibition. This was followed by early clinical trials carriedout in mid 1970s that demonstrated partial success, butwhich were subsequently discontinued due to serioustoxicity concerns. The water insolubility of the activeform was one of the major issues in further clinicaldevelopment at that time [1].

Camptothecin has a five ring heterocyclic structure with Camptothecin has a five ring heterocyclic structure with α-hydroxylactone within its E-ring which is essential for its anti-tumor activity.

Figures



Compound	R1	R2	R3	R4
Camptothecin	H	H	H	H
Irinotecan	C_2H_5	H		H
Topotecan	H	$CH_2N(CH_3)_2$	OH	H
SN38	C_2H_5	H	OH	H
9-aminocamptothecin	H	NH_2	H	H
9-nitrocamptothecin	H	NO_2	H	H
10-hdroxycamptothecin	H	H	OH	H

* Irinotecan and topotecan are the hydrochloride of the structures shown above

Fig. 1: Structure and chemistry of Camptothecin and analogs

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Targeted delivery of a drug molecule to organ or special sites is one of the most challenging research areas in pharmaceutical sciences. By developing colloidal delivery systems such as liposomes, micelles and nanoparticles a few frontiers was opened for improving drug delivery. Nanoparticles with their special

characteristics small particle size, large surface area and the capability of changing their surface properties have numerous advantages compared with other delivery systems. Nanoemulsions are nanometric-sized emulsion, typically exhibiting diameters of upto 500 nm [2].

Nanoparticles are solid colloidal particles ranging from 10 to 1000 nm (1.0 μm), in which the active principles (drug or biologically active material) are dissolved, entrapped, and/or to which the active principle is adsorbed or attached. The advantages of nanoparticles as drug delivery systems are that they are biodegradable, non-toxic, and capable of being stored for longer periods [2].

Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to tradition colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles. Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system. SLN are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals[3].

In a separate study, Camptothecin-loaded Solid Lipid Nanoparticels(SLNs) were prepared by High Pressure homogenization (HPH) method. The prepared SLNshad an average diameter 196.8 nm, zeta potential of −69.3 mV and drug encapsulation efficiency of 99.6%. The specificchanges in body distribution of Camptothecin were investigated following oral delivery of SLN and solution formulations of Camptothecin in mice. In tested organs, the area under curve (AUC) and mean residence time (MRT) of SLN formulationincreased significantly as compared with solution formulation.The increase of brain AUC was the highest among all testedorgans. The study suggested that SLNs could be a promisingsustained release and targeting system for Camptothecin or other lipophilic antitumor drugs after oral administration [4].

1. Camptothecin Delivery Challenges:

The discovery of the anti-tumor potential of CPTand subsequently that of taxol were two major breakthroughs in the field of cancer chemotherapeutics.Various CPT derivatives have been evaluated in severalpre-clinical and clinical trials since then. However, inspite of possessing potent anti-tumor activity, a fullrealization of this potential has not yet been realized inthe clinic. One of the major obstacles in achieving thisgoal as mentioned previously is that of severe toxicities or side effects associated with these compounds. Acritical second issue is the challenge in terms ofdelivering the optimum concentration of the requiredform of the drug to the tumor site and ultimately insidethe tumor cells, a challenge associated with both theinsolubility of the parent compound in addition to rapidclearance. To combat the insolubility, early clinical trialswere conducted using sodium salt of CPT.

The successful generation of several modificationsof CPT with the aim of delivering more soluble form ofCPT to tumors led to renewed interest and ultimately intwo clinically approved forms of CPT, Irinotecan and Topotecan currently in clinical use for the treatment ofvarious cancers. The structurally labile nature of the E-ringat physiological pH poses separate and distinctchallenge for all CPT analogs in terms of delivering anactive form of the drug to the tumor site in that pHdependent opening of the lactone ring renderscarboxylate form that is inactive as a TOP1 inhibitor.Although reversible, re-lactonization of ring open format physiological pH is seldom possible. At neutral pHhalf-life of this conversion is few minutes irrespective ofthe type of CPT, hence, shortly after administration verysmall quantities of CPT and its analogs are available inthe active lactone form. This plasma inactivation of CPTis further exacerbated by preferential albumin binding ofcarboxylate form which is observed to be speciesdependent. Thus,inherent physico-chemical characteristics, unstable lactone ring and toxicities due to non-tumor specifications pose a daunting task of delivering sufficientamounts of active form of CPT to the site of action.

Synthesizing a stable analog that possesses adequatebiological activity till it reaches the site of action withoutpossessing significant toxicity is a difficult task. Hence,designing a delivery vehicle that can be use to achievethis goal can be a more practical approach.

2. Preparation of Nanoparticles:

2.1. Preparation of Camptothecin nanoparticles:

The Nano precipitation method was employed for the formation of drug-encapsulated Poly (d,1-lactide-co-glycolide)- Poly (ethylene glycol)(PLGA-PEG) nanoparticles. The polymers of optimized PLGA-PEG) was dissolved in acetone, then the copolymer solution containing exact amount of drug was addeddrop-wise into Poly vinyl alcohol (PVA) aqueous solution (pH was adjusted to 3 by 0.1 N HCl) and stirred magnetically at room temperature until complete evaporation of the organic solvent.

Next, the nanoparticle suspension was centrifuged by ultracentrifuge. The separated nanoparticles were redispersed and centrifuged three times in distilled water (pH 3) in order to removefree drug and excess surfactant completely. The acidity of the medium used in this process was because of stabilizing the lactone form of 9-nitrocamptothecin. Finally, nanoparticles were dried via desiccator at room temperaturefor 24 hours, and then were characterized[5].

2.2. Preparation of CPT-loaded amphiphilic β-cyclodextrin nanoparticles:

Nanoparticles were prepared according to the Nano precipitationmethod introduced by Fessi*et al*. and further modified to prepare the nanoparticles directly from preformed inclusion complexes of CPT and cyclodextrins (CD). The organic phase (0.8 ml) consisting of 1 mg of CPT:amphiphilicβ-CD inclusion complex dissolved in absolute alcohol was prepared, and 0.2 mlof CPT solution in absolute alcohol was added to this organic phase.

This solution was added at room temperature under constant stirring to 2 ml of the aqueous phase consisting only of ultrapure water. After stirring for 30 min at room temperature, the organic solvent was evaporated under vacuum, and the nanoparticle dispersion was concentrated to the desired volume (2 ml). This technique was called high-loading method. Highly loaded nanosphereswere prepared directly from preformed drug:cyclodextrin inclusion complexes and by further dissolving an additional amount of drug in the organic phase during preparation [6].

2.3. Preparation of Camptothecin nanoparticle (CPT- TMC):

Camptothecin encapsulated with N-trimethyl chitosan (CPT-TMC) was prepared by combination of microprecipitationand sonication as follows: Firstly, 6 mg/ml of Camptothecine was prepared by dissolving 30 mg Camptothecineinto 5 ml dimethyl sulfoxide (DMSO) solution. Then N-trimethyl chitosan (TMC) was dissolved in water at the concentration of 5 mg/ml. Subsequently, 0.1 ml of Camptothecine solution was added drop wisely into 2 ml of TMC solution at 4°C.

The obtained colloid solution was ultrasonicated for 10 min also at 4°C. Finally, the colloid solution was dialyzed against water using a membrane with a molecular weight cutoff of 8,000-14,000 (Solarbio, China) for 3 days, then the solution was centrifuged at 10,000 × g for 10 min toremove insoluble CPT. The encapsulation rate of CPT to N-trimethyl chitosan (TMC) was about 10% in this paper. The prepared CPT nanoparticles are well-dispersed and physical stable at 5 mg/ml TMC solution. The morphology of resulting CPT nanoparticles was investigated by transmission electron microscopy (TEM) observation. We could find that the needle-liked CPT nanoparticles were successfully prepared.

The size of nanoparticles was only about 30-50 nm and vertical size of nanoparticles was about 500 nm. The zeta potential of resulting CPT nanoparticles was about +15 mv. Camptothecin encapsulated with N-trimethyl chitosan (CPT-TMC), CPT and TMC were dissolved in 0.9% NaCl solution (NS) for vitro and vivo studies [7].

2.4. Preparation of lipid nanoparticles:

The lipid and aqueous phases were separately prepared in glass vials. The lipid phase consisted of solid or liquid lipids, a lipophilic emulsifier (Myverol), and Camptothecin, while the aqueous phase consisted of double-distilled water and a hydrophilic emulsifier (PF68). The 2 phases were separately heated to 85 °C for 15 min. The aqueous phase was added to the lipid phase and then mixed using a high-shear homogenizer (Pro 250; Pro Scientific, Monroe, CT, USA) for 5 min. The mixture was further treated using a probesonicator (VCX600; Sonics and Materials, Newtown, CT, USA) for 10 min at 25-35 W. The whole systems consisted of the water phase, the lipid phase, and the lipid/ water interphone [8].

2.5. Production of solid lipid nanoparticles:

SLN were prepared following a recently published protocol (Martins et al., 2011). Briefly, lipid and drug were heated at approximately 5–10 ◦C above the melting point of the lipid followed bythe addition of an aqueous surfactant solution at the same temperature. A hot emulsion was formed using an ultra-turrax T25 and then subjected to homogenisation in MicronLab 40 high pressurehomogeniser. The resultant hot oil-in-water (o/w) nano emulsionwas cooled down to room temperature forming SLN [9].

2.6. Complexation of SN-38 with PAMAM Dendrimers:

Complexes are designated as G*x*S*y*where *x* represents the PAMAM dendrimer generation and *y* represents the number of SN-38 molecules complexed. A typical procedure for complexation, as described for G4S5 was as follows: SN-38 (0.005 g, 14.1 μmol) was added into the solution of G4-PAMAM dendrimer (0.025 g, 1.76 μmol) in dimethyl sulfoxide (DMSO; 10 ml) and the solution was stirred for 48 h at room temperature. DMSO was evaporated in vacuum to obtain crude G4S5 complex. The crude complex was re-dissolved in water and purified by extensive dialysis against distilled water using dialysis membrane of 1000 MWCO (Spectrum Laboratories, Inc., Rancho Dominguez, CA, and USA). The product was further purified by size exclusion chromatography using PD-10 column. Purified PAMAM dendrimer was then freeze dried and solid product was stored at 4°C. G4S11 and G4S26 were prepared following a similar procedure with 16 and 32 M equivalents of SN-38 respectively [10].

2.7. Preparation and identification of HCPT-loaded nanoparticles:

HCPT-loadedpoly(γ-benzyl L-glutamate) andpoly (ethylene oxide) (PEG-PBLG) nanoparticles were prepared by dialysis, as described previously. Briefly, PEG-PBLGdiblock copolymer and HCPT (1:1 W/W) were dissolved in N,Ndimethyl formamide (DMF), then dialyzed using adialysis bag (molecular cut-off 3500 g/mol; Spectrum Medical Industries, Inc., Houston, TX) against double-distilled water for 24 h. The solution inside the dialysis bag was centrifuged and supernatant (nanoparticles) was filteredthrough a 0.45 μm filter. A 640 UV spectrophotometer (Beckman) was used to identify the HCPT-loaded PEG-PBLG nanoparticles at the wavelengths, 200–400 nm. The morphology of nanoparticles was observed using a scanning electron microscope (SEM, HITACHI-600; Japan)[12].

2.8. Synthesis of CPT-PGA encapsulated SNPs (CPT-PGA3SNPs):

In 200-ml solution of (Adamantanamine-Poly ethylene Glycol) Ad-PEG (10 mg, 50 mg/ml), 10-ml DMSO solution of Ad-PAMAM with various concentrations (22 and 44 mg/ml) was slowly injected under vigorous stirring. Followed by addition of 120-mlCyclodextrin-grafted branched Poly ethylenimine(CD-PEI) (10.44 mg, 87 mg/ml) into the mixture and incubating for 20 min. After the incubation, the mixture was slowly added into 700-mlCamptothecinPoly (L-glutamic acid) CPT-PGA (10 mg/ml) solution and heated to 50 _C for an additional 20 min. Two different sizes of CPT-PGA-SNPs (37--nm and 104-nm CPT-PGA-SNPs) were obtained after the solution cooled down.

3. Characterization:

3.1. 9-nitro Camptothecin loaded Nanoparticle characterization:

The morphology of the optimized Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol)(PLGA-PEG) nanoparticles examined by Scanning electron microscopy (SEM) was spherical. Average size of nanoparticles was found to be around 148.5 ± 30 nm with a polydispersity of 0.07%. It is concluded that the steric barrier of PEG shell could be the main cause to avoid aggregation of internal phase droplets in preparation pathway; therefore PLGA-PEG nanoparticles have a smaller size than Poly (d,1-lactide-co-glycolide) (PLGA) ones. The zeta potential of the nanoparticles was about +1.84 mV over what is suitable for stable colloidal dispersion of nanoparticles.

The DSC curve of 9-NC showed a single melting peak which started to degrade as it melted. The PLGA-PEG thermo gram displayed an endothermic peak investigative of polymer transition temperature (Tg). No 9-NC melting peak was visible in the case of drug-loaded nanoparticles. This might be due to the amorphous state of the drug when dispersed in the nanoparticles. Since the curve of copolymer has not any shift in Tg, it is concluded that there is no occurrence of prominent interaction between the drug and copolymer.

XRD patterns curves of 9-NC exhibit a sharp peak at about 2θ scattered angle 27 indicating the crystalline nature of 9-NC. This kind of trait was absent in pattern of nanoparticles investigating that 9-NC was not in crystalline form in nanoparticles[5].

3.2. CPT-loaded amphiphilic β-cyclodextrin nanoparticles characterization:

3.2.1. Zeta potential measurement:

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) in triplicate to determine the surface charge and the potential physical stability of the nano system. Zeta potential of nanoparticles was measured in aqueous dispersion. Measurements were realized in triplicate at a 120 ^{0}angle at 25 ^{0}C.

3.2.2. Scanning electron microscope analysis:

A scanning electron microscope (SEM) (Jeol-SEM ASID-10 Device in 80 KV, Japan) was used to evaluate surface characteristics of the nanoparticles. Nanoparticles were mounted on the metal stubs with conductive silver paint and then coated with a 150 Å thick layer of gold in a Bio-Rad sputter apparatus. SEM images of the samples were obtained at different magnifications.

3.2.3. Entrapment efficiency:

Loaded drug quantity was determined according to the following procedure: unbound drug was separated by centrifugation (Hermle Z-323 K, Germany) at 5000 rpm for 15 min. Supernatant was then collected and lyophilized and the resulting powder containing the loaded nanoparticles was dissolved in dimethylsulfoxide to obtain a clear solution and analyzed by HPLC (Agilent 1100, Germany). Drug-loading values were expressed in terms of entrapped drug quantity, entrapment efficiency and associated drug percentage.

3.3. Camptothecinlipid nanoparticles characterization:

3.3.1. Determination of the mean diameter and surface charge:

The mean particle size (z-average) and zeta potentialof the SLN, NLC, and LE were measured by photon correlation spectroscopy (Nano ZS90; Malvern, Worcestershire,UK) using a helium-neon laser with a wavelengthof 633 nm. Photon correlations of spectroscopic measurements were carried out at a scattering angle of 90°. A1:100 dilutions of the formulations were made using doubledistilled water before the measurement. The stability of thedrug delivery systems was determined by monitoring the size and zeta potential at 37 °C for 28 days.

SLN made of Precirol (SLN-P) or Compritol (SLN-C) as the core material were stabilized with PF68 and Myverol. Precirol is a glycerol palmitostearate with a melting point of 58 °C. Compritol is a glycerol behenate consisting of mono-, di-, and triglycerides. Squalene was used as the liquid matrix in the NLC and LE formulations.

Squalene is an all-trans isoprenoid containing 6 isoprene units, which has been used without evidence of safety concerns according to the World Health Organization Weekly Epidemiological Record (14 July 2006). The lipid nanoparticle systems were developed by hot homogenization followed by ultrasonication. In the Camptothecin loaded mixtures, we observed no distinct, undissolved crystals. Even though the exact solubility of Camptothecin in the inner phase could not be measured, it appeared that most of input drug was solubilized. After production, the mean diameters of the particles were in the range of 190~310 nm, depending on the lipid loading.

The characterization of the particle size revealed that the average diameter of the SLN-P was considerably smaller (P<0.05) than that of the SLN-C. The average diameter of the LE was comparable (P>0.05) to that of the SLN-P.

NLC exhibited the smallest size (P<0.05) among the formulations tested, the zeta potentials of the lipid nanoparticles were negative. There was no significant difference (P>0.05) among the zeta potentials of the SLN and NLC formulations. The LE showed a surface charge of –12.6 mV, which was lower (P<0.05) than those of the systems with solid lipids (approximately -35 mV)[8].

3.4. Solid lipid nanoparticles characterization:

3.4.1.Assessment of particle size and size distribution:

The average hydrodynamic diameter in volume and polydispersity index (PI) of submicron SLN were analyzed by PCS (Nicomp model 370, PSS Nicomp, Sta Barbara, CA) as described previously in Martins et al. (2011). Supplementary optical single particle sizing (OSPS, Accusizer 780, PSS-Nicomp, Sta Barbara, CA)

was used to detect any particles in the micrometre range or aggregates of SLN as described in Martins et al. (2011). The number-weighted distribution of particles in the micro range was evaluated; number of microparticles >1 µm/ml and presence of microparticles>5 µm [9].

3.4.2. Zeta potential:

The electrophoretic mobility, zeta potential (ZP), was measured using a Zetasizer Nano ZS (Malvern, Worcestershire, UK). The samples were diluted with Milli Q-water having a conductivity adjusted to 50 µS/cm by drop wise addition of 0.9% (m/v) NaCl solution.

3.4.3. Differential scanning calorimetry analysis:

Differential scanning calorimetry (DSC) analysis was done, DSC analyses were performed on bulk lipids and unloaded and Camptothecin-loaded SLN on the day of production and one year after the production.

3.5. HCPT-loaded nanoparticles Characterization:

3.5.1.Drug-loading capacity and drug encapsulation:

HCPT-loaded PEG-PBLG nanoparticles were added into the dialysis bag, which was placed in DMF. The solution outside the dialysis bag was stirred at 37°C for 3 h and then the drug concentration was measured using a UV spectrophotometer at 326 nm. Absorbency of the solution (A) was used to calculate the drug-loading capacity anddrug encapsulation according to the following formulae: drug loading capacity = MHCPT/MHCPT/PEG-PBLG and drug encapsulation = MHCPT/M drug devoted, where MHCPT was the drug content of the detected solution (MHCPT = DHCPT × V, DHCPT = Asample/Astandard) × Dstandard, D: concentration, V: volume), MHCPT/PEG-PBLG was the quantity of the detectedsolution of HCPT/PEG-PBLG nanoparticles, and Mdrug devoted was the initial quantity of HCPT [12].

3.6.CPT-PGA encapsulated SNPs (CPT-PGA3SNPs) Characterization:

3.6.1.Dynamic light scattering (DLS):

DLS experiments were performed with a Zetasizer Nano instrument (Malvern Instruments Ltd., United Kingdom) equipped with a 10-mW helium-neon laser (l ¼ 632.8 nm) and thermoelectric temperature controller. Measurements were taken at a 90 ^{0}scattering angle.

3.6.2.Transmission electron microscope (TEM):

The morphology and sizes of CPT-PGA3SNPs were examined on a Philips CM 120 transmission electron microscope (TEM), operating at an acceleration voltage of 120 kV. The TEM samples were prepared by drop-coating 2-ml of CPT-PGA3SNPs solutions onto carbon-coated copper grids. Excess amounts of droplets wereremoved with filter paper after 45 s. Subsequently, the surface-deposited CPTPGA3SNPs were negatively stained with 2% uranyl acetate for 45 s before TEM studies.

3.6.3. Zeta potential (z) measurements:

Zeta potentials of CPT-PGA3SNPs were determined by photon correlation spectroscopy using a Zetasizer Nano instrument (Malvern Instruments, Malvern, Worcestershire, UK). The measurements were performed at 25^{0}C with a detection angle of 90^0, and the raw data were subsequently correlated to Z average mean sizeusing a cumulative analysis by the Zetasizer software package.

3.6.4. Drug encapsulation efficiency:

Free CPT was removed from CPT-PGA3SNPs by centrifugation of CPTPGA3SNPs solution at 1300 rpm for 30 min using centrifugal filter devices (3000 NMWL). After recovering the filtrate containing free CPT, CPT concentration was analyzed by ultraviolet absorption at a wavelength of 370 nm. The measurements were performed in triplicate. The amount of the CPT encapsulated in the SNPs was then calculated by the total loading amount of CPT subtracts the free CPT in thefiltrate.

Table No. 1: Characterization of Camptothecin and its derivatives in Nanoparticles Formulation using Various Polymers.

S.No	Drug	Polymer	TEM	SEM (nm)	Drug loading	DSC	EE	Zeta potential	*In-vitro* drug release
1	Camptothecin	Poly(L-glutamic acid) (PGA)	37±8nm	37 nm	--	--	- 90±3 %	-11±0.7Mv	20 % after 144 h
2	9- nitro Camptothecin	PLGA-PEG-NPs	---	148.5 ± 30	More than 45%	Mixture of drug and polymer no Interaction	45.3±2.9	1.84Mv	Initial Rapid release - 5h 4%; Prolonged release - 120 h 75%
3	Camptothecin	β cyclodextrin derivative β –CDC6	--	142 ± 3		Mixture of drug and polymer no Interaction	28±2	-22±0.7mV	30% release in 2h; Complete release after 144 h
		6-0-capro-β-CD		271 ± 15			48±4	-13±0.9mV	30% release in 5h ;Complete release after 288 h
		PLGA		187 ± 9			13±0.1	-0.06±1mV	30% release in 30 min ; Complete release after 48 h
		PCL		274 ± 0.8			7.2±0.5	-19±0.2mV	30% release in 1h; Complete release after 48 h
4	Camptothecin	mPEG		185 nm	2.56±0.32	Mixture of drug and polymer no Interaction	79%	-12.38±0.45 mV	25 % of Initial burst release in 24 h After 25 days 100%
5	Camptothecin (HCPT)	PEG-PBLG	200nm, 30 nm	--	7.5%	--	56.8%	--	Initial release after 2h (0.7 %) Sustained release after 96 h(30 %)
6	Camptothecin	Precirol	35+/-7nm	247.7 ±7.5		--	--	-36.1±3.6mV	Sustained release 30 h (45 %)
		Campritol		309.9 ±2.1				-34.7±6.9mV	Sustained release 30 h (45 %)
		NLC		192.3 ±10.2				-36.8±2.8 mV	Rapid release release 30 h (65 %)
		LE		252.7				-12.6±3.4	Rapid release

				±0.9				mV	release 30 h (75 %)
7	Camptothecin	Eudragit S 100	--	--		263.77°c	--	--	--
		β cyclodextrin				262.12°c			
		poloxamer				260.44°c			
8	Camptothecin	Cetylpalmitate	--	< 200 nm	-	Mixture of drug and polymer no Interaction	--	--	90% release after 8 h
9	Camptothecin	PLGA-PEG	--	148.5 ±30	More than 45%	Mixture of drug and polymer no Interaction	45.3±2.9%	+1.84mV	Rapid release for 5h 2.5% and prolonged release for 120h (60%)

TEM: Transmission Electron Microscopy; **SEM:** Scanning Electron Microscopy; **DSC:** Differential Scanning Calorimetry; **EE:** Entrapment Efficiency; **HCPT:** 10-Hydroxycamptothecin; **PGA:** Poly (L-glutamic acid); **PLGA-PEG:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol); **PLGA:** poly(lactide-co-glycolide); **PCL:** poly-E-caprollactone; **mPEG:**Methoxypolyethylene glycol succinate *N*-hydroxysuccinimide ester; **PEG-PBLG:** poly(ethylene glycol)-poly(γ-benzyl-L-glutamate); **NLC:** Nanostructured Lipid Carriers (Precirol+squalene)**; LE:** Lipid Emulsion (Squalene); **PLGA-PEG:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol);

4. *In-vitro*drug release:

4.1. *In-vitro* drug release of Camptothecin Nanoparticles.

The 9-NC release profiles from optimum PLGA-PEG nanoparticles. The procedure was performed using the dialysis technique using a dialysis membrane having a molecular weight cutoff of 12,000 Da (Sigma, St. Louis, MO) which was fixed on Franz diffusion cell and donor and acceptor medium was PBS (pH 7.4). In the release curve, two parts are seen. The first part shows the initial phase of release which has a rapid trend during the first 5 hours, followed by a distinct prolonged release for more than 120 hours. The rapid initial release can be due to a part of drug on the surface of nanoparticles and the delayed part is thought to be because of diffusion of dissolved drug through polymeric matrix and its exit to the dissolution medium, where as in our previous study about 20% of the drug was released over a period of 20 hours, followed by an extended release period of more than 160 hours.

This difference of release profile could be explained by presence of the hydrophilic PEG shell around the nanoparticles, causing increased diffusion of water to the core of particles. On the other hand, compared to plain PLGA nanoparticles, the hydrophilic surface decreases adsorption of lipophilic drugs on the surface of nanoparticles and decreases initial burst drug release. In general, *in vitro* release profiles shows that these nanoparticles have the capacity to successfully release 9-NC using a sustained rate.

4.2.*In-vitro* drug release of CPT-loaded amphiphilic β-cyclodextrin nanoparticles.

Release profiles of CPT from nanoparticle formulations were determined in 100 ml of isotonic PBS (pH7.4) containing 0.1% Tween 80 providing sink conditions in a thermo stated shaker bath system at 37 ^{0}C with the dialysis technique (Spectra/Por Cellulose Ester Membrane MWCO:100,000 Da, Spectrum Labs, Rancho Dominguez, CA). At predetermined time intervals, 1 ml samples were withdrawn from the system and replaced with equal volume of fresh release medium maintained at the same temperature. The released amount of CPT was assayed for lactone and carboxylate forms by HPLC as described above.

4.3. Camptothecin release from lipid nanoparticles

Camptothecin release from the drug carrier systems was measured using a Franz diffusion cell. A cellulose membrane was mounted between the donor and receptor compartments.The donor medium consisted of 1 ml vehicle containing Camptothecin. The receptor medium consisted of 10 ml of 30% ethanol in pH 7.4 buffer in order to maintain sink conditions during the experiments. The available diffusion area between cells was 1.767 cm^2. The stirring rate and temperature were kept at 600 r/min and 37 °C, respectively. At appropriate intervals, 300 µl aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh buffer. The amount of drug released was determined by HPLC. Camptothecin in solution was used as the control by dissolving Camptothecin (3 mg) in a 10 ml mixture of polyethylene glycol 400, propylene glycol, and Tween 80 (40:58:2.)

A key issue investigated in this study was the feasibility of using lipid nanoparticles to deliver Camptothecin. The ability of nanoparticles to deliver Camptothecin was examined by determining the drug release.

The amount of Camptothecin released from each formulation was plotted as a function of time. The free control showed a quick release of Camptothecin. The inclusion of the drug in lipid nanoparticles significantly reduced the release. The release kinetics from nanoparticles could be fitted with a zero-order model. It was found that the release rate of the drug greatly depended on the inner phases in the lipid nanoparticles. Both SLN systems showed the most sustained release (P<0.05), with ~45% of total drug amount released within 30 h. A more rapid release of Camptothecin from the NLC and LE was observed, with ~65% and ~75% of Camptothecin being released within 30 h, respectively[8].

4.4.*In-vitro* drug release of Solid lipid Nanoparticles.

In vitro drug release from a controlled release formulation frequently displays a biphasic release pattern and this pattern was observed for all Camptothecin-loaded formulations developed. The initial release phase represents a burst release followed by a sustained release of Camptothecin.

Such burst release has been frequently reported for SLN formulations because SLN could not efficiently avoid drug from the particle surface diffusing into the water phase. A drug-enriched

Shell is frequently formed on the SLN surface, due to a large surface area and drug deposition on the particles surface. To minimize such burst release, a lower amount ofsurfactant is recommended.

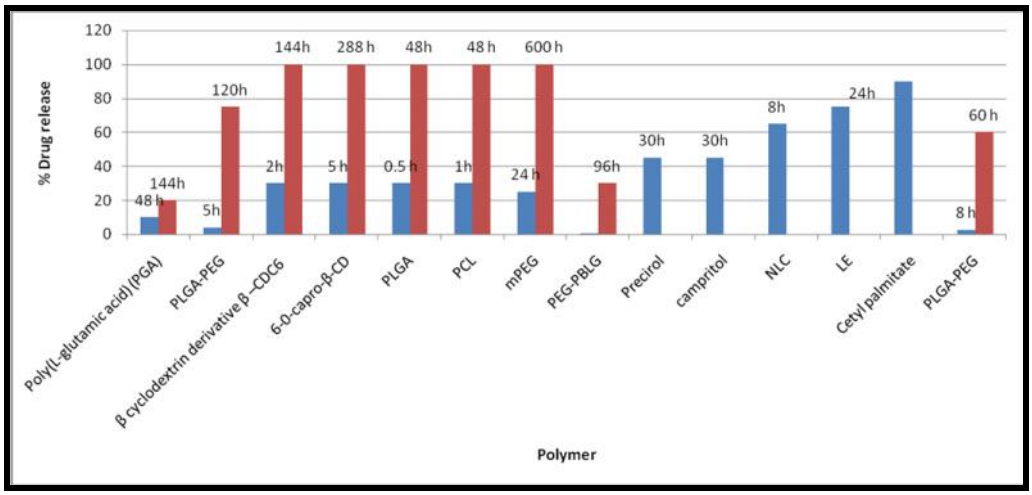
Camptothecin *in-vitro* release was generally faster in human plasma than in PBS, which is explained by the better solubility of Camptothecin in plasma due to Camptothecin-binding to albumin (Fleury et al., 1997). The different matrices showed similar biphasic shape profiles, but cetylpalmitate-based SLN released almost all the drug within 72 h in both mediums and Witepsol E85-based SLN released in the same period only 62% (PBS) or 74% (plasma).

For cetylpalmitate based-SLN 90% of the drug was released within 8 h. For the same time Dynasan-based SLN released around 70% (PBS) and 90% (plasma) and for Witepsol-based SLN 51% (PBS) and 67% (plasma). Therefore, faster release was achieved with cetylpalmitate-based SLN and slower release with Witepsol E85. This is in contradiction to the fact that only cetylpalmitate-based SLN is in solid state (DSC) which should rend the release of the drug from carrier more controlled than for the other two lipids. Cetylpalmitate is, according to literature a wax with a better in vitro degradation and an associated faster release. Knowing that brain maximum drug concentration is achieved generally in the first 30 min and cetylpalmitate-based SLN are able to release 90% of the camptothecin in the first 8 h, the release of the drug from cetylpalmitate –based SLN seems to be the most appropriate [9].

Table No. 2: Comparative study on *In-vitro* drug release for Camptothecin derivatives in Nanoparticle Formulation

Various polymers combination with Camptothecin and its derivatives	% Drug release		Dissolution medium	pH of Buffer
	Phase I	Phase II		
A	10 % (48 h)	20 % (144 h)	Phosphoric acid (85%, 100 µL)	pH 2
B	4 % (5 h)	75 % (120 h)	Phosphate buffer (0.1M)	pH7.4
C	30 % (2 h)	100 % (144 h)	Phosphate buffer	pH 7.4
D	30 % (5 h)	100 % (288 h)	Phosphate buffer	pH 7.4
E	30 % (30 min)	100 % (48 h)	Phosphate buffer	pH 7.4
F	30 % (1 h)	100 % (48 h)	Phosphate buffer	pH 7.4
G	25 % (1 day)	100 % (600 h)	Phosphate buffer	pH 7.4
H	0.7 % (2 h)	30 % (96 h)	Phosphate buffer	pH 6.86 & 9.18
I	45 % (1 day 6 h)	--	Phosphate buffer	pH 7.4
J	45 % (1 day 6 h)	--	Phosphate buffer	pH 7.4
K	65 % (1 day 6 h)	--	Phosphate buffer	pH 7.4
L	75 % (1 day 6 h)	--	Phosphate buffer	pH 7.4
M	90 % (8 h)	--	Phosphate buffer	pH 7.4
N	2.5 % (5 h)	60 % (120 h)	Phosphate buffer	pH7.4

A: Poly (L-glutamic acid); **B:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol); **C:** β cyclodextrin derivative β –CDC6; **D:** 6-0-capro-β-CD; **E:** poly(lactide-co-glycolide); **F:** poly-E-caprollactone; **G:**Methoxypolyethylene glycol succinate *N*-hydroxysuccinimide ester; **H:** poly(ethylene glycol)-poly(γ-benzyl-L-glutamate); **I:**Precirol; **J:**campritol; **K:**Precirol+squalene (nanostructured lipid carriers); **L:**Squalene (a lipid emulsion); **M:**Cetylpalmitate ; **N:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol);



Graph1: *In-vitro* drug release for Camptothecin derivatives in Nanoparticle Formulation.

h: Hour; **PGA:** Poly (L-glutamic acid); **PLGA-PEG:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol); **PLGA:** poly(lactide-co-glycolide); **PCL:** poly-E-caprollactone; **mPEG:**Methoxypolyethylene glycol succinate *N*-hydroxysuccinimide ester; **PEG-PBLG:** poly(ethylene glycol)-poly(γ-benzyl-L-glutamate); **NLC:** Nanostructured Lipid Carriers (Precirol+squalene); **LE:** Lipid Emulsion (Squalene) ; **PLGA-PEG:** Poly(d,1-lactide-co-glycolide)- Poly (ethylene glycol);

4.5. Drug Release of SN-38 with PAMAM Dendrimers

To determine *in-vitro* release characteristics, complexes (G4S5 and G4S11, 2 mg/ml) were dissolved in neutral (pH 7.4) and acidic (pH 5.0) buffers and stirred continuously at 37°C. At various time points 10 µl of the solution was used to separate free drug from the polymer complex. Separation was achieved using PD-10 column and the fractions collected were used to quantify, by fluorimetry (Ex/Em 375/550 nm) the amount of drug retained in the complex and the amount of drug released.

Due to the various physiological environments encountered by the drug–dendrimer complexesduring their transport from the gastrointestinal tract to the systemic circulation.we determinedthe stability of these complexes at a range of pH values. Initial studies suggested that G4S5 isrelatively stable at pH 7.4 thus retaining >80% of the drug in the first 2 h at pH 7.4.

The amount of drug retained by G4S5 reduced to 60% after 4 h and 46% after 24 h. G4S11 showed similar trend with little improvement in stability. For G4S11, 65% of the drug was still retained on the polymer after 24 h. Initial stability studies in acidic environment (pH 1–2) suggested that the drug is released rapidly from the complex (data not shown). Further studies at milder acidic conditions were conducted to observe the release of the drug at simulated endocytic environment (pH 5.0). Both the complexes retained <15% and <10% of the drug after 30 min and 24 h respectively at these milder acidic conditions. It is important to realize that equilibrium exists between neutral phenolic OH and deprotonated phenolic OH in SN-38. It can be postulated that at acidic pH the equilibrium will shift towards neutral form of the phenolic OH thus eliminating the ionic interactions between deprotonated phenolic OH and dendrimer, thereby releasing the free drug. Similar observations were reported previously where binding of polarity responsive probe 5-(di-methylamino)-1-napthalene sulfonic acid (DNS) with amine terminated PAMAM dendrimer was studied at several pH levels.

Optimal binding was observed when both DNS and PAMAM dendrimers were in ionic forms.At lower pH when DNS was present in the protonated form, no binding occurred. Various other investigators have studied release of free drug after incubation of dendrimers containing drug in buffered solutions. Patri*etal*have reported more than 70% release of free methotrexate within first 2.5 h from non-covalent methotrexate-dendrimer inclusion complex. Complete release of efavirenz within first 24 h from drug containing polypropylenimine (PPI) dendrimers while significantly slower release from *t*-Boc glycine conjugated and mannose conjugated PPI dendrimers was reported. Drug release was also found to be controlled by molecular architecture of dendrimer and introducing poly (ethylene oxide) chains on the periphery of the dendrimer.

These observations suggest that further studies need to be conducted to avoid the premature release as well as control the

release of SN-38 from the complexes. Encapsulation of these complexes can also be considered as an alternative to avoid premature release of SN-38 from the complexes in harsh GI environment.

4.6. *In-vitro* drug releaseHCPT-loaded nanoparticles.

HCPT-loaded nanoparticles were added to a dialysis bag and then introduced into a vial with PBS at different pHs (6.86 and 9.18). The medium was stirred at 94 ± 4 revolutions/ min at 37°C. At the indicated time intervals (observed until 96 h), the medium was removed and replaced with fresh PBS. The absorbency of samples of these replaced media was detected by an UV spectrophotometer at 326 nm. The released HCPT in these replaced media at different time intervals was calculated from the standard curve, which was set up in the same way. Then, the release curve of HCPT-loaded nanoparticles was described[12].

4.7.Drug release profile

CPT-PGA (0.249 mg/ml) or 37-nm CPT-PGA3SNPs (0.977 mg/ml) was dispersed in 50% human serum (human serum:1 × PBS ¼ 1:1, v/v) and equally distributed to 20 vials with 1 mlsolution per vial, and then incubated at 37 ^{0}C. At selected time intervals, one selected vial of each group was taken out of the incubator. The solution was mixed with an equal volume of methanol (1 ml) and centrifuged at 15,000 rpm for 10 min. The supernatant (1 ml) was transferred to an eppendorf tube without disturbing the precipitates and brought to pH 2 with phosphoric acid (85%, 100 ml). The resulting solutionwas directly injected into an HPLC equipped with an analytical C18 column. A mixture of acetonitrile and water (containing 0.1% TFA) at a volume ratio of 1:3 was used as the mobile phase. The flow rate was set at 1 ml/min. The area of the HPLC peak of the released CPT (labs ¼ 370 nm) was intergraded for the quantification of CPT as compared to a standard curve of free CPT prepared separately[11].

Similar to the self-assembly preparation of DNA encapsulated SNPs,which used the coulombic interactions between the negatively charged DNA plasmid with the positively charged SNP vector, 5 KD anionic poly(L-glutamic acid) (PGA) was employed as a carrier to covalently link with CPT molecules, enabling encapsulation into SNP vectors. Approximately five CPT molecules were conjugated to each PGA polymer chain (via ester bond formation) to give CPT-grafted PGA, denoted as CPT-PGA.

It is noteworthy that the connecting ester bonds can be degraded via esterase-mediated hydrolysis, which allows controlled release of CPT under physiological conditions. The encapsulation of CPTPGA into SNP vectors to generate CPT-PGA encapsulated SNPs (CPT-PGA3SNPs) can be accomplished (Scheme 1) by simply mixing the drug conjugated polymer, CPT-PGA (Scheme 2), with the other two SNP building blocks (CD-PEI: CD-grafted branched polyethylenimine and Ad-PAMAM: Ad-grafted polyamido amine dendrimer), as well as a solvation ligand (Ad-PEG: Ad-grafted poly(ethylene glycol)) drug encapsulation efficiency (The drug encapsulation efficiency for 37-nm and 104-nm CPTPGA3SNPs are 90 ± 3% and 95 ± 2%, respectively drug release kinetics (The accumulative release of free CPT from CPT-PGA3SNPs was quantified by HPLC. The data point out that CPT-PGA3SNPs release 20% of CPT after 6 days without any associated burst release.

CONCLUSION

Nanoparticle research is currently an area of intense scientific research due to a wide variety of potential applications in the field of madecine. Nanomedicine has shown best therapeutic potentials to treat cancer in clinical applications. Abnormalities in tumor, such as growth induced solid stress, abnormal blood vessel networks, elevated interstitial fluid pressure, and dense interstitial structure contribute to resistance to anticancer therapy. An effective formulation can be done by appropriate polymer selection and method of preparation, which will improve the patient compliance.

REFERENCE:

1. NileshPatankar, Dawn Waterhouse. Nano-particulate Drug Delivery Systems for Camptothecins, Cancer Therapy, **2012**; 8: 90-104.
2. BiswajitBasu, Kevin Garala, Ravi Bhalodia, Bhavik Joshi, Kuldeep Mehta. Solid lipid nanoparticles :A promising tool for drug delivery system, Journal of Pharmacy Research, **2010**; 3(1): 84-92.
3. RoohiKesharwani, Suresh Kumar Nair, Dilip Patel. Solid Lipid Nanoparticle (SLN): A Modern Approach for Drug Delivery. Journal of Pharma Research, **2013**; 2(11): 13-21.
4. Surajit Das,AnumitaChaudhury. Recent Advances in Lipid Nanoparticle Formulations with Solid Matrix for Oral Drug Delivery, AAPS Pharm. Sci. Tech., **2011;** 12(1): 62-76.
5. Katayoun Derakhshandeh, MarziehSoheili, Simin Dadashzadeh, Reza Saghiri.Preparation and *in vitro* characterization of 9-nitrocamptothecin-loaded long circulating nanoparticles for delivery in cancer patients, International Journal of Nanomedicine, **2010**; 5: 463-471.
6. YaseminCirpanli, EremBilensoy, A. Lale Dogan, Sema Calis. Comparative evaluation of polymeric and amphiphiliccyclodextrin nanoparticles for effective camptothecin delivery, European Journal of Pharmaceutics and Biopharmaceutics, **2009**; 73: 82-89.
7. Xian-ping Liu, Sheng-tao Zhou, Xing-yi Li, Xian-cheng Chen, Xia Zhao, Zhi-yongQian, Li-na Zhou, Zhiyong Li, Yu-mei Wang, QianZhong, Tao Yi, Zheng-yu Li, Xiang He and Yu-quan Wei. Anti-tumor activity of N-trimethylchitosan-encapsulated camptothecin in a mouse melanoma model, Journal of Experimental & Clinical Cancer Research, **2010**; 29: 76.
8. Zih-rou HUANG, Shu-chiou HUA, Yueh-lung YANG, Jia-you FANG. Development and evaluation of lipid nanoparticles for camptothecin delivery: a comparison of solid lipid nanoparticles, nanostructured lipid carriers, and lipid emulsion, Acta Pharmacol. Sin., **2008**; 29(9): 1094-1102.
9. Susana Martinsa, IngunnTho, IsoldeReimold, GertFricker, Eliana Soutoe, DomingosFerreiraa, Martin Brandlb, Brain delivery of camptothecin by means of solid lipid nanoparticles: Formulation design, in vitro and in vivo studies, International Journal of Pharmaceutics, **2012**; 439: 49-62.
10. Rohit B. Kolhatkar, Peter Swaan, and Hamidreza Ghandehari, Potential Oral Delivery of 7-Ethyl-10-Hydroxy-Camptothecin (SN-38) using Poly (amidoamine) Dendrimers, Pharm. Res., **2008**; 25(7): 1723-1729.
11. Kuan-Ju Chen, Li Tang, Mitch André Garcia, Hao Wang, Hua Lu, Wei-Yu Lin, Shuang Hou, Qian Yin, Clifton K.-F. Shen, Jianjun Cheng, Hsian-Rong Tseng.The therapeutic efficacy of camptothecin-encapsulated supramolecular nanoparticles Biomaterials, **2012**; 33: 1162-1169.
12. AnxunWang, Su Li. Hydroxycamptothecin-loaded nanoparticles enhance target Drugdelivery and anticancer effect, BMC Biotechnology, **2008**; 8: 46.

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