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Formulation and Evaluation of Polymeric Nanoparticles of Sirolimus by Using Poloxamer-407

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ABSTRACT

To develop sirolimus loaded polymeric Nanoparticles and optimize the formulation parameters. To develop nanoparticles containing sirolimus with higher stability and better release profile. To reduce the overall dose of sirolimus require for ulcerative colitis treatment by loading in Nanocarriers. To study the physicochemical properties of sirolimus loaded polymeric Nanoparticles. To study the drug release profile of prepared Nanoparticles. Sirolimus also called Rapamycin. It is an immune suppressant macrocyclic antibiotic obtained from bacteria Streptomyces hygroscopic us and can be used in the prevention of organ transplant rejection and also to treat a rare lung disease called lymphangioleiomyomatosis. Based on the results of our study, we could conclude that this mechanism was related to the drug delivery system of sirolimus loaded nanoparticles. Therefore, it was suggested that the prepared sirolimus loaded nanoparticles can be good for the treatment of ulcerative colitis. Hence, it can be concluded that sirolimus loaded Poloxamer-407 Nano formulation can serve as a potential formulation for the treatment of ulcerative colitis. But more animal studies and extensive clinical studies are needed to check and confirm the efficacy of the prepared drug delivery system.

INTRODUCTION:

Ulcerative colitis begins in the rectum or sigmoid and progresses to involve part or all of the colon. It can be diagnosed by otoscopic examination which is not universally accepted. It is a mysterious, but common and serious disorder characterized by extensive ulcerations of the colon. The colon is the primary site of the attack. The disease is systemic and is often associated with arthritis, uveitis, venous thrombosis, liver disease, and various skin lesions; especially pyoderma gangrenous. [32]It is commonly associated with Crohn's disease. Typical symptoms of ulcerative colitis include bloody diarrhea (the most predominant symptoms) with mucus, abdominal pain with fever, and weight loss in severe cases. The typical appearance of a patient diagnosed with Crohn's disease is low BMI, malabsorption [1], weight loss, and growth retardation. Frank blood loss is more common in ulcerative colitis than Crohn's disease. The symptoms of ulcerative colitis are similar to Crohn's colitis with patients being tachycardia, anemic, febrile, fatigued, dehydrated, and thin. Approximately 50% of patients with ulcerative colitis have some form of relapse each year, and severe attacks can be life-threatening. Up until the 1960s, one-third of ulcerative colitis patient's diet from the condition; with advances in medical and surgical treatment death is now extremely rare.[2]

Statistical Data for Ulcerative Colitis [33]

Inflammatory bowel disease is most prevalent in western countries and areas of northern latitude. The reported rates of inflammatory bowel disease are highest in Scandinavia, Great Britain, and North America. Crohn's disease has an incidence of 3.6-8.8 per 100,000 persons in the United States and the prevalence of 20-40 per 100,000 people. The incidence of Crohn's disease varies considerably among studies but has increased dramatically over the last 3 or 4 decades. Ulcerative colitis incidence ranges from 3-15 cases per 100'000 persons per year among the while population with a prevalence of 80 to 120 per 100,000. [3]The incidence of ulcerative colitis has remained relatively constant over many years. Although most epidemiologic studies combined ulcerative proctitis with ulcerative colitis, 17% to 49% of cases are classified as proctitis. Both sexes are affected equally with inflammatory bowel disease, although some studies show slightly greater numbers of women with Crohn's disease and males with ulcerative colitis. Ulcerative and Crohn's disease have bimodal distributions in the age of initial presentations. The peak incidence occurs in the second or third decades of life, with a second peak occurring between 60&80 years of age.[4]

Classification of Drugs for Inflammatory Bowel Disease [5,6]

I. 5-amino salicylic acid (5-ASA) compounds

- Sulfasalazine(salicylazo sulfapyridine)

II. Corticosteroids

- Prednisolone
- Methylprednisolone
- Triamcinolone
- Hydrocortisone
- dexamethasone

III. Immunosuppressant

- Sirolimus,
- Azathioprine,
- Cyclosporine,
- prednisolone

IV. TNF α-Inhibitor

- Etanercept
- Infliximab
- Adalimunab

A Nanoparticle is a microscopic particle whose size is measured in nanometers (nm). It is defined as a particle with at least one dimension <200nm. or nanoparticles are solid colloidal particles ranging in size from 10nm to 1000nm. [30, 31]

Nanoparticles (NP) have been studied extensively as particulate carriers in several pharmaceutical and medical fields. Nanoparticles can be used to provide targeted (cellular/tissue) delivery of drugs, to sustain drug effect in a target tissue, to improve oral

bioavailability to solubilize drugs for intra-vascular delivery and to improve the stability of therapeutic agents against enzymatic degradation. [7]

Nanoparticles represent a very promising carrier system for the targeting of anti-cancer agents to tumors. Nanoparticles exhibit a significant tendency to accumulate in several tumors after i.v injection. Nanoparticles can also be used in Brain Drug Targeting. Poly (butyl cyanoacrylate) nanoparticles represent the only nanoparticles that were so far successfully used for in vivo delivery of drugs to the brain.

Need for Nanoparticles Drug Delivery [8, 9]

- Conventional drug therapy in tumors is associated with problems of drug intolerance and toxicity leading to permanent damage in certain organs.
- The long duration of therapy and the drug-related toxicity are common causes for non-compliance resulting in multi-drug resistant tuberculosis.
- Need to develop drug delivery forms that can be directly administered to the lungs as Nanoparticulate to reduce the systemic side effects associated with the present anti-cancer drugs.
- Nanoparticles of pulmonary surfactants can be employed as delivery agents for the anti-cancer drugs to the infected lung tissue.
- High stability.
- High carrier capacity.
- Feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration**.**

Need of Polymeric Nanoparticles for Ulcerative Colitis: [10]

Polymeric nanoparticles can increase the permeability of ulcerative colitis through biological barriers, such as the mucosal barriers, skin barriers, and cell membrane barrier.

- Biocompatible and biodegradable.
- Non-toxic
- Water-soluble

- They are easy to synthesize, inexpensive.

The conventional treatments are mainly restricted to control inflammation. However, the main goal of clinical ulcerative colitis therapy is to not only control inflammation but also achieve mucosal healing. Therefore, novel therapeutic strategies are urgently needed to address the limitations of existing treatments.

Polymeric Nanoparticles: [11]

The polymeric nanoparticles (PNPS) are prepared from the biocompatible and biodegradable polymer in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix. Depending upon the method of preparation nanoparticles, Nanospheres or Nanocapsules can be obtained. Nanocapsules are systemic in which the drug is confined to a cavity surrounded by a unique polymer membrane, while Nanospheres are matric system in which the drug is physically and uniformly dispersed. [29]The field of polymer nanoparticles (PNPS) is quickly expanding and playing an important role in a wide spectrum of areas ranging from electronics, photonics, conducting materials, sensors, medicine, biotechnology, pollution control, and environmental technology. PNPS are promising vehicles for drug delivery by easy manipulation to prepare carriers with the object of delivering the drugs to a specific target; such an advantage improves drug safety. Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometre-size promotes effective permeation through cell membranes and stability in the bloodstream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into a unique nanoparticles construct with many potential medical applications.

Mechanism of Drug Release [12]

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general Physico-chemical mechanisms.

1. By the swelling of the polymeric nanoparticles by hydration followed by release through diffusion.

2. By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at the site of delivery, thereby releasing the drug from the entrapped inner core.

Dissociation of the drug from the polymer and its de-adsorption / release from the swelled nanoparticles.

MATERIALS AND METHODS:

Preparation of Nanoparticles[13,14]

Conventionally, NPs have been prepared mainly by two methods:

i) Dispersion of the preformed polymers

ii) Polymerization of monomers

Dispersion of Preformed Polymers

Several methods have been suggested to prepare biodegradable NPs from PLA, PLG, PLGA, and poly (Є-caprolactone) by dispersing the preformed polymers.

Emulsification Diffusion Method [15]

The preparation of drug-loaded nanoparticles was prepared by the emulsification diffusion method. Polymer and drug were dissolved in ethyl acetate. add the above solution to aqueous phase containing surfactant and emulsified by homogenization using a homogeniser (20000rpm\10min) .add large volume of water to the emulsion and gentle stirring with a magnetic bar, allow the ethyl acetate to leave the droplets [51, 52]. The organic solvents and a part of water were removed by evaporation under reduced pressure for 3 hours to get purified and concentrated suspension.[28] The final product was obtained by centrifugation (12000rpm\20min), re-dispersion, and freeze-drying.

Solvent Evaporation Method [16]

In this method, the polymer is dissolved in an organic solvent like dichloromethane, chloroform, or ethyl acetate. The drug is dissolved or dispersed into the preformed polymer solution, and this mixture is then emulsified into an aqueous solution to make an oil (O) in water (W) i.e., O/W emulsion by using a surfactant for an emulsifying agent like gelatine poly(vinyl alcohol) poly sorbet 80 poloxamer 188, etc. [56,57]. After the formation of stable emulsion, the organic solvent is evaporated either by increasing the temperature/under pressure or continuous stirring the effect of process variables on the properties of NPs was discussed earlier [17]. The W/O/W method has been also used to prepare the water-soluble

drug-loaded NPs. Both methods use a high-speed homogenization or sonication. However, these procedures are good for a laboratory-scale operation, but for a large –scale pilot production, alternative methods using low –energy emulsification are required. In this pursuit, the following approaches have been attempted.

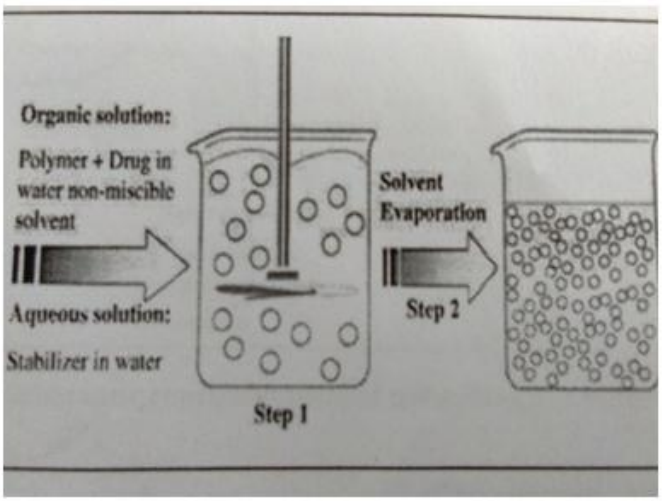


Figure No. 1: Schematic Representation of the Solvent-Evaporation Technique

Spontaneous Emulsification/Solvent Diffusion Method [18]

In a modified version of the solvent evaporation method. The water-soluble solvent like acetone or methanol along with the water-insoluble organic solvent like dichloride methane or chloroform was used as an oil phase due to the spontaneous diffusion of water-soluble solvent (acetone or methanol) interfacial turbulence is created between two phases leading to the formation of smaller particles. [27]As the concentration of water-soluble solvent (acetone) increases, considerable decreases in particle size can be achieved.

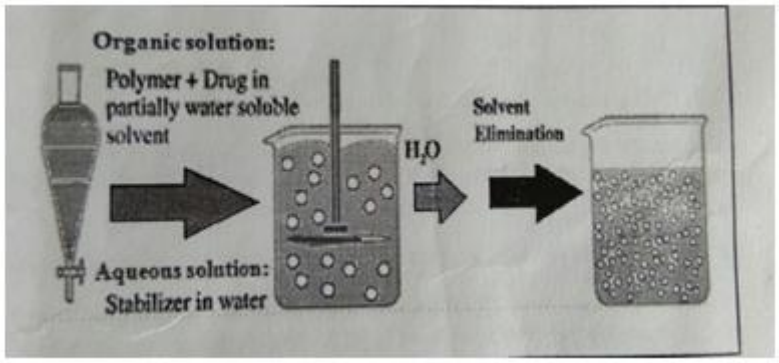


Figure No. 2: Schematic Representation of the Emulsification/Solvent Diffusion Technique

Salting Out /Emulsification –Diffusion Method [19, 20]

The methods discussed above require the use of organic solvents, which are hazardous to the environment as well as to the physiological system. The USFDA has specified the residual amount of organic solvents in the injectable colloidal system. To meet these requirements, Allemann and co-workers have developed two methods for preparing NPs. The first one is a salting-out method. While the second one is the emulsification –solvent diffusion technique (61-64).

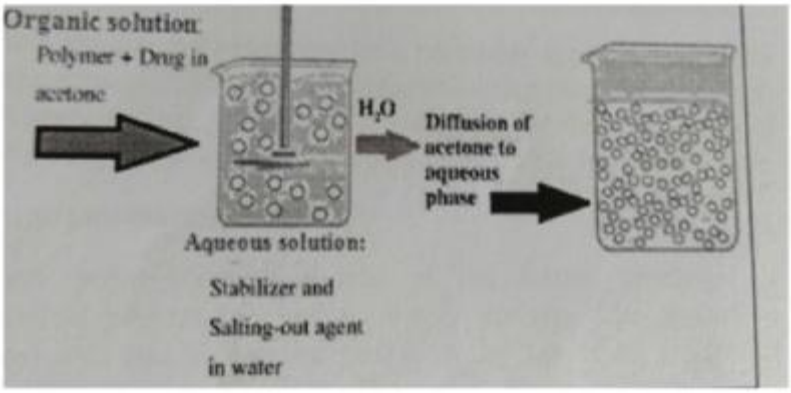


Figure No. 3: Schematic Representation of the Salting Out Technique

Production of Nps Using Supercritical Fluid Technology [21]

Production of NPs with the desired physicochemical properties to facilities the targeted drug delivery has been a topic of renewed interest in the pharmaceutical industry. Conventional methods like solvent evaporation, coacervation, and in situ polymerization often require the use of toxic solvent and /or surfactant. Therefore, research efforts have been directed to develop environmentally safer en-capsulation methods to produce the drug-loaded micron and sub-micron size particles. If solvent impurities remain in the drug-loaded NPs, then these become toxic and may degrade the pharmaceutical within the polymeric matrix.[26] Supercritical fluid has now become the attractive alternatives because these are environmentally friendly solvents and the method can be profitably used to process particles in high purity and without any trace amount of the organic solvent. The literature on the production of drug-loaded microparticles using supercritical fluids enormous. However comparatively much less has been investigated to produce NPs.It is beyond the scope of the present review to give an entire coverage supercritical fluid technology; we will discuss only two of the most commonly used methods of producing micro or nanoparticles.

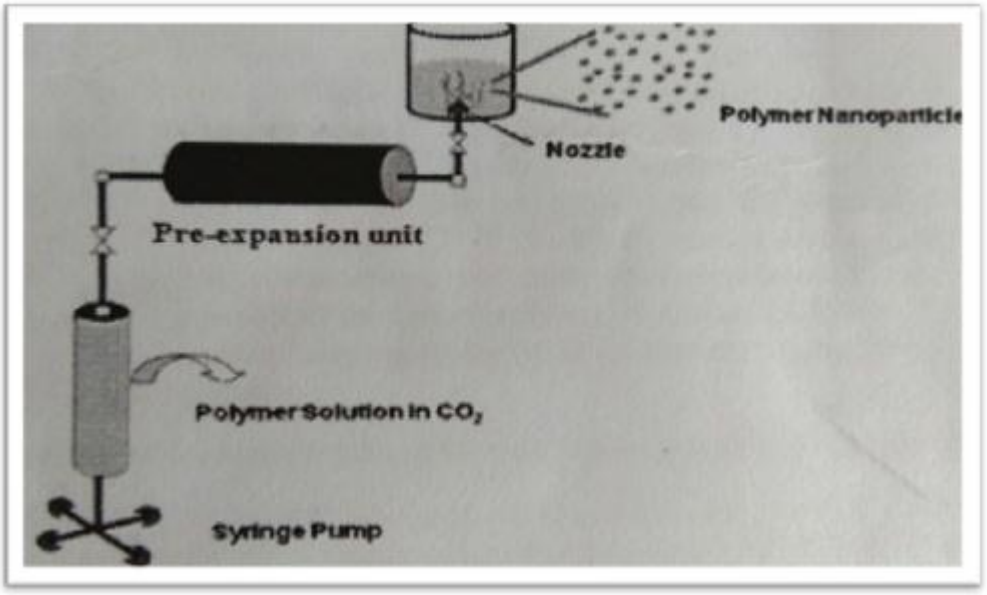


Figure No. 4: Experimental Set-Up for the Rapid Expansion of Supercritical Fluid Solution into Liquid Solvent Process

MATERIALS: [22, 23]

Table No. 1: Ingredients used

Sr. No.	Ingredients	Manufacture
1	Sirolimus	Biocon, Bangalore
2	PLEA	Sigma Aldrich
3	F127	Sigma Aldrich

Preparation of Sirolimus loaded nanoparticles: [24, 25]

Preparation of sirolimus loaded nanoparticles was prepared by the emulsification diffusion method .polymer (PLGA) and drug (sirolimus) was dissolved in ethyl acetate. add the above solution to aqueous phase containing surfactant (pluronic F-127) and emulsified by homogenization using a homogeniser (20000rpm\10min) .add large volume of water to the emulsion and gentle stirring with a magnetic bar, allow the ethyl acetate to leave the droplets.[34, 35] The organic solvents and a part of water were removed by evaporation under reduced pressure for 3 hours to get purified and concentrated suspension. The final product was obtained by centrifugation (12000rpm\20min), dispersion, and freeze-drying.

Table No. 2: Formulation of Sirolimus Loaded Nanoparticles

Sr. No.	Ingredients	Quantity
1	Sirolimus	10mg
2	PLEA	20mg
3	Ethyl acetate	10ml
4	2% Pluronic F 127	0.4mg\20ml
5	Purified water	90ml

RESULTS AND DISCUSSION

Organoleptic Properties:

The organoleptic characters of the drug-like color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

Table No. 3: Organoleptic Properties of Sirolimus

Sr. No.	Properties	Observation
1	Appearance	Fine powder
2	Colour	White
3	Odor	Odorless

Solubility Studies:

Table No. 4: Solubility Studies of Sirolimus

Sr. No.	SAMPLE	OBSERVATION
1	Water	Insoluble
2	Ethanol	Soluble
3	Methanol	Soluble
4	Acetone	Soluble
5	Chloroform	Insoluble

Melting Point:

Table No. 5: Melting Point of Sirolimus

Sr. No.	TRIALS	RESULTS
1	Trial-1	125.5˚C
2	Trial-2	126˚C
3	Trial-3	126˚C

Compatibility Studies:

Differential scanning calorimetry:

DSC is an important technique to analyze the polymer-drug interactions and also it has previously been used to show the dispersity of the molecules. Thermal analysis was used to evaluate the changes in thermodynamic properties that occur when the material supplied heat energy changes that can be observed in the process of melting, desolvation, recrystallization, and solid-phase transformations indicated by endothermic or exothermic peaks at thermogram. DSC thermogram showed a solid endothermic peak of TP at ℃. The polymer PLGA indicates endothermic peaks were observed and the surfactant Pluronic F127 indicated endothermic peak at 58.4℃ respectively. The endothermic peaks of TP, PLGA, and Pluronic F 127 observed at similar temperature ranges, which eliminate the possibility of any physical interaction. The DSC thermogram of TP, PLGA and their combinations are shown in figure:

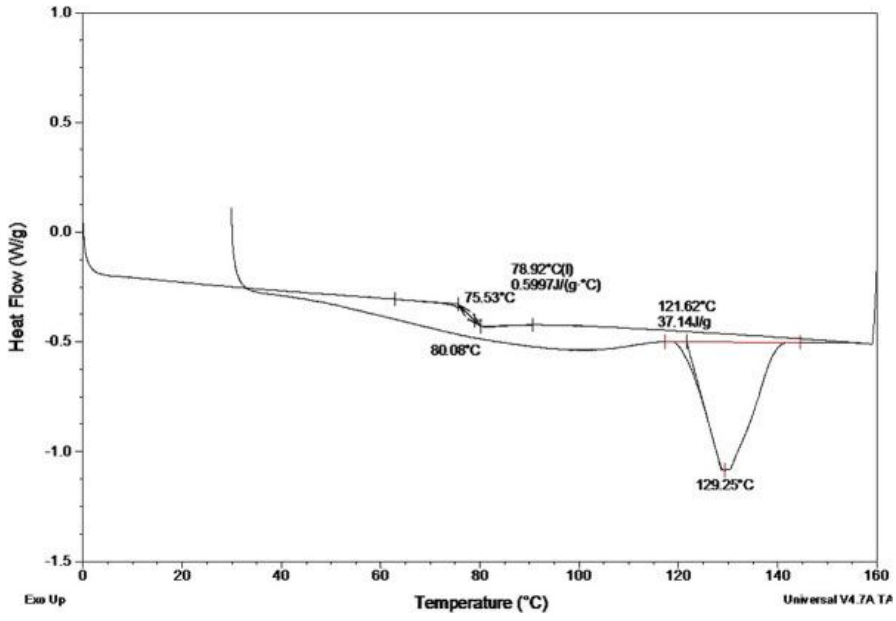


Figure No. 5: DSC Thermograms of Sirolimus

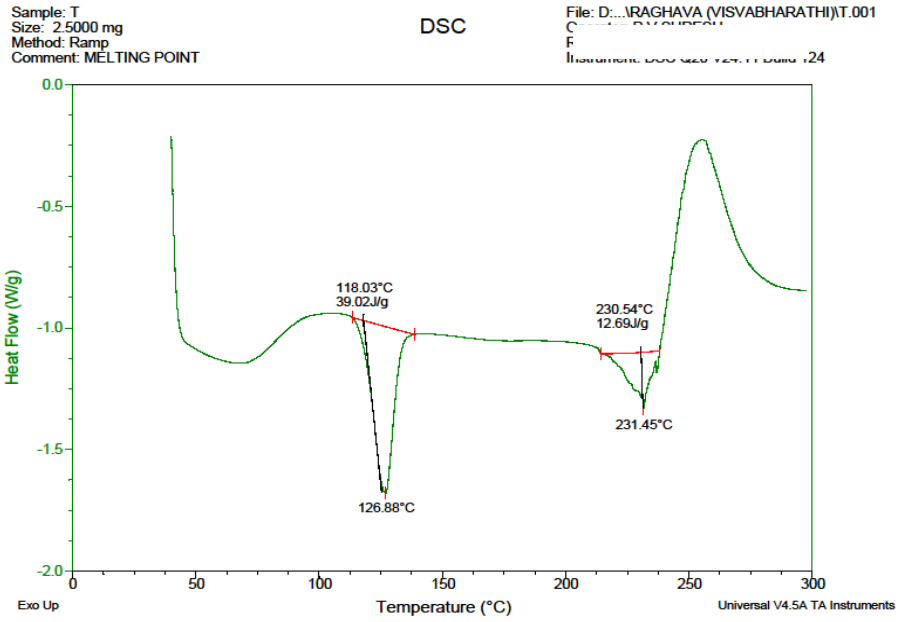


Figure No. 6: DSC Thermogram of SPNF

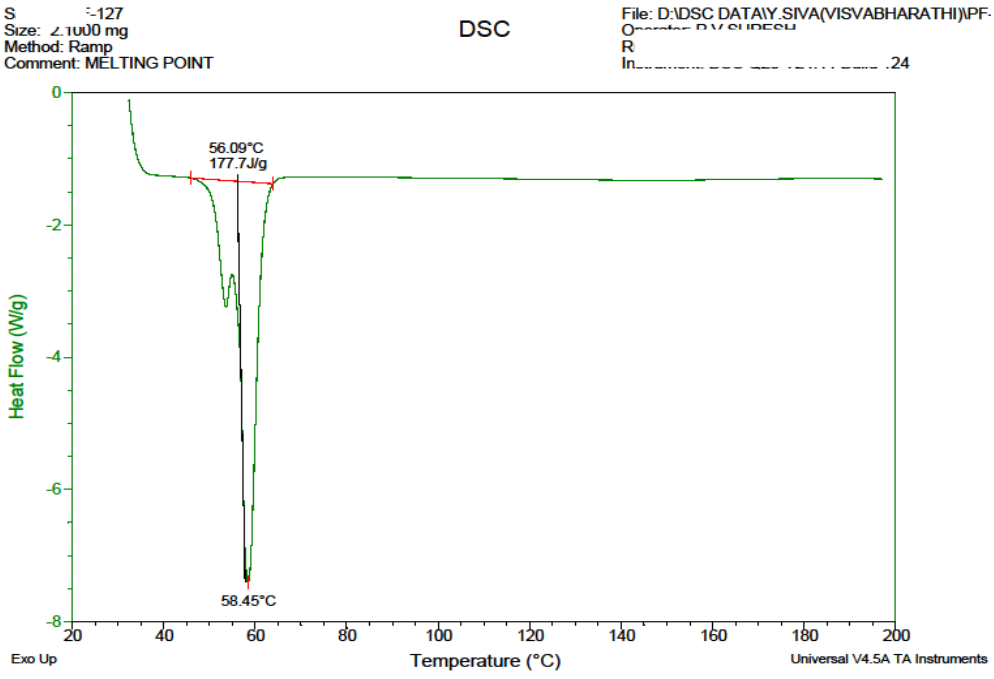


Figure No. 7: DSC Thermogram of Pluronic F-127

Fourier Transform Infra-Red Spectroscopy (FTIR) Studies:

- The Fourier transform infrared analysis was conducted for structural characterization.

- FTIR spectra of the pure abacavir sulfate, pure polymer, and formulated nanoparticle were recorded.
- FTIR spectra were recorded on Shimadzu Fourier transform infrared spectrophotometer.
- Test samples were mixed with KBr, pressed into a disk, and scanned from 400 to 4000cm.

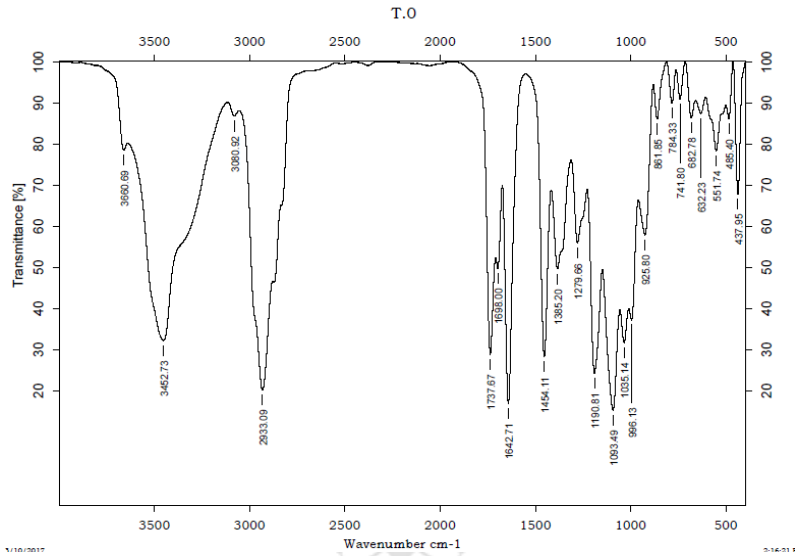


Figure No. 8: FTIR Spectrum of Sirolimus

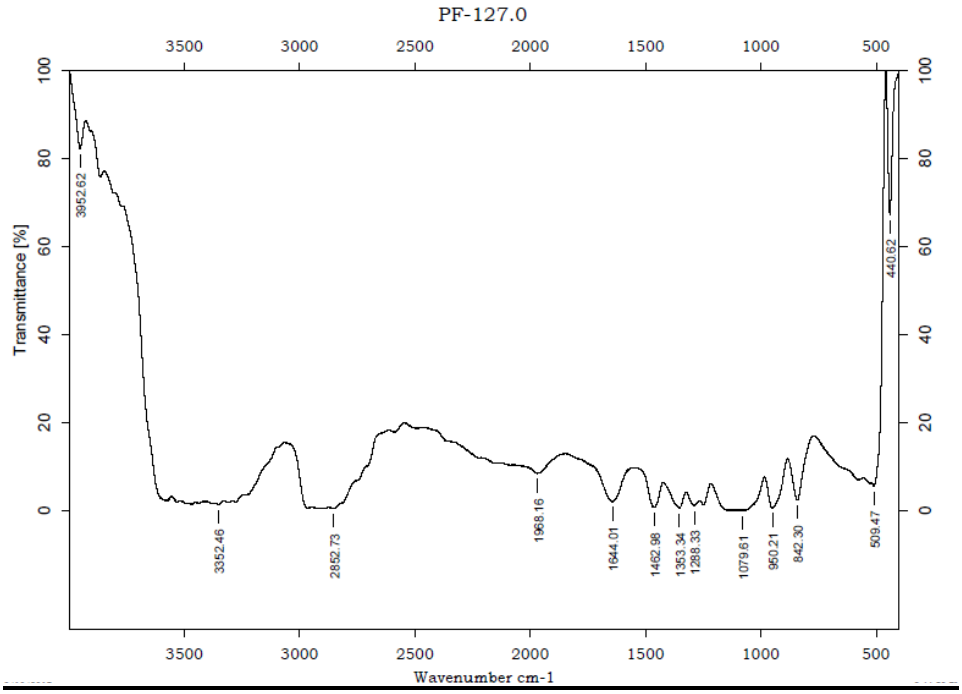


Figure No. 9: FTIR Spectrums of PLGA and Pluronic F-127

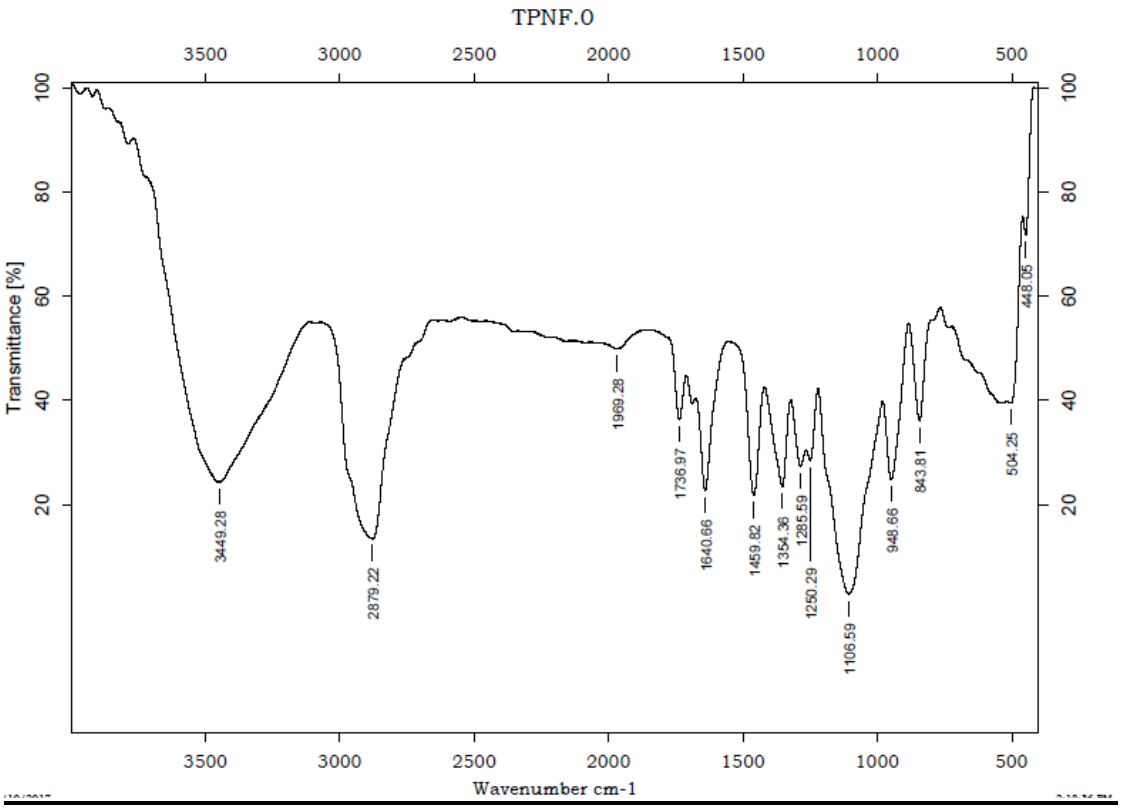


Figure No. 10: FTIR Spectrum of SPNF Solid Formation

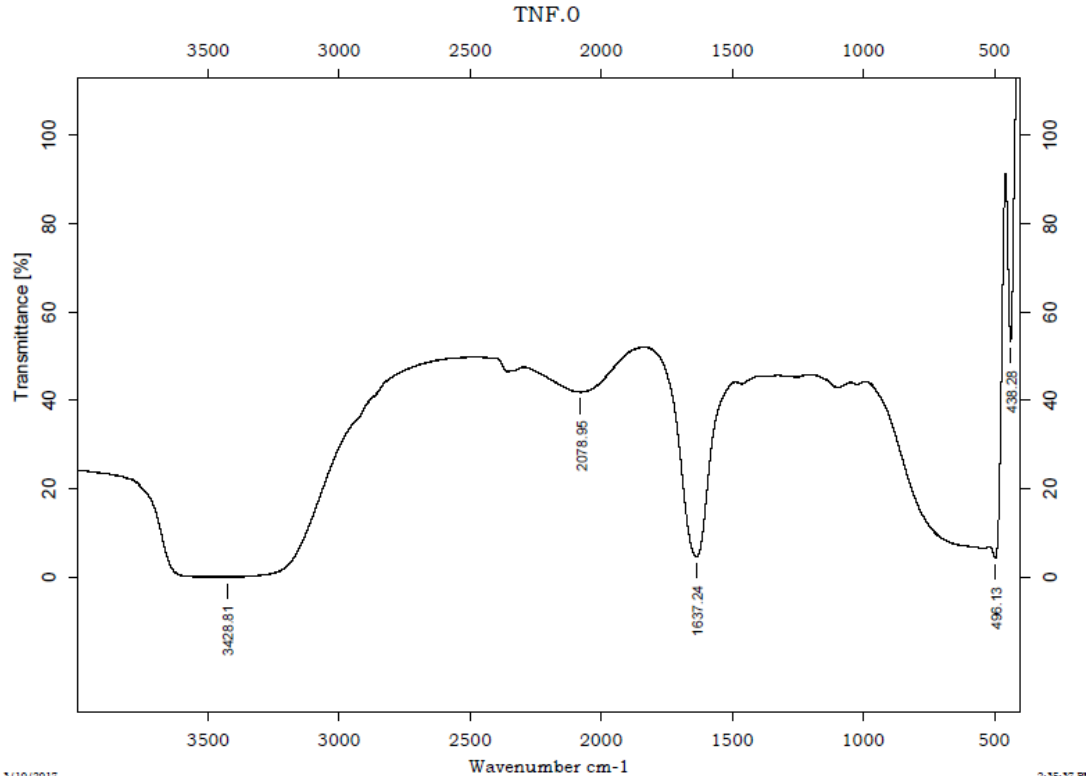


Figure No. 11: FTIR Spectrum of SNF Liquid Formation

Table No. 6: Wavenumber for Sirolimus and PLGA

Sr. No.	Vibrations	Wave Number cm^{-1}
1	N-H Stretching	3452.73
2	O-H Stretching	1737.67
3	CH_3 Stretching	1642.71
4	C=O Stretching	3560.69

The above table showed that FTIR Spectrum values of Sirolimus and PLGA combination. The FTIR spectra acquired were taken from physical mixer of Sirolimus and PLGA and interaction study between drug and polymer evaluated the characteristic peaks owing amino and hydroxyl group which confirm the stability of the formulation and by this study, it was confirmed that there was no significant change in the chemical integrity of the drug.

Table No. 7: Various Formulations of Sirolimus Loaded PLGA Nanoformulations

Sr. No.	Ingredients	SPNF 1	SPNF 2
1	Sirolimus	10 mg	10 mg
2	PLEA	20 mg	30 mg
3	1 % Pluronic F-127	10 ml	10 ml
4	Acetone	5 ml	5 ml

Particle Size Analysis

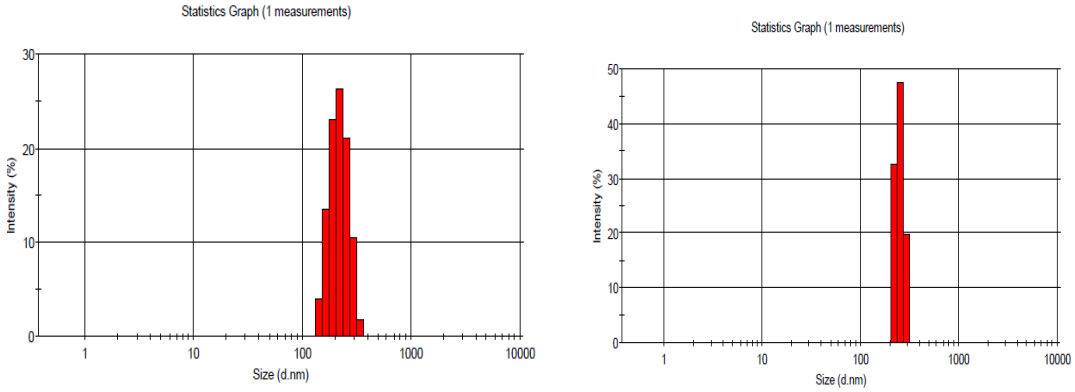
Particle size is well known that sizes of particles are highly dependent on the preparation method and condition employed. Differential scanning thermogram of (A) TP (B) PLGA (C) physical mixture of TP, PLGA also they can influence the drug loading, drug release, and stability of the drug inside nanoparticles. The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the particles. It has been demonstrated that the anionic surface of the drug delivery system could provide improved blood compatibility as compared to the cationic carrier. The zeta potential value of the plain nanoparticles and abacavir loaded nanoparticles were recorded.

Table No. 8: Particle size and zeta potential of SPNF1, SPNF2

Sr. No.	Code	Particle Size(nm)	Zeta potential(mV)
1	SPNF 1	244 ± 1.5	-26.3
2	SPNF 2	274 ± 1.4	-28.4

Graphs for Particle Size SNF-1, SNF-2

Values indicated are mean ±standard error mean of three trials.



SPNF-1 **SPNF-2**

Figure No. 12: Particle size and distribution of SPNF-1, SPNF-2

SEM Analysis

SEM Analysis of the SPNF 2 indicated that the particles are isolated, and it was clear that the particles were spherical in shape and hollow in structure, with a large central cavity in which SP was loaded as shown:

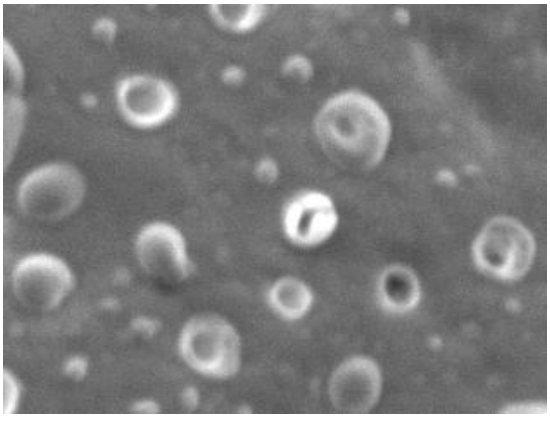


Figure No. 13: SEM Analysis of SPNF 2

***In-vitro* Drug Release Studies (IDRS):**

In-vitro release of SP loaded PLGA nanoparticles in buffer solution P^H 7.4. A typical to phase -release was observed, i.e., the burst release was observed from all the batches of SP loaded PLGA NPs in 1 hr, followed by a relatively slower and sustained release was observed up to 24 hrs. The SPNF shows the maximum drug released by the end of the 24th hour.

Table No. 9: *In-vitro* drug release of SPNF1, SPNF2

Time (Hr)	SPNF 1	SPNF 2
0	0	0
1	17.14 ± 1.3	23.89 ± 1.1
2	21.48 ± 1.2	35.53 ± 1.2
4	26.64 ± 1.2	42.67 ± 1.1
6	30.85 ± 1.1	48.42 ± 2.1
8	35.52 ± 2.3	52.48 ± 2.3
10	41.72 ± 2.2	55.21 ± 1.1
12	47.52 ± 2.3	60.11 ± 1.2
14	51.86 ± 2.1	67.92 ± 1.3
16	55.94 ± 1.3	73.51 ± 2.1
18	58.92 ± 1.1	76.77 ± 2.1
20	60.12 ± 1.3	79.84 ± 1.1
22	66.14 ± 1.2	83.72 ± 1.2
23	72.84 ± 1.3	86.58 ± 1.3

After 24 hours TP was released at a slower rate. The release rate of TP was also slower due to the presence of the external PLGA coating, which effectively delayed its release from the Nanoparticles. The sustained release of TP is highly beneficial in enhancing an immunosuppressant and improving drug accumulation at the targeted site.

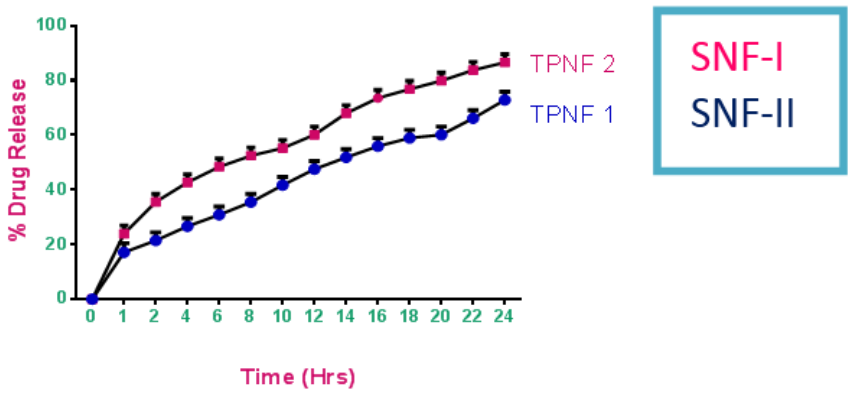


Figure No. 14: *In-vitro* drug release of S-loaded nanoparticles

SUMMARY:

PLGA nanoparticles containing sirolimus were developed to sustain release on i.v. administration, which may help to improve patient compliance and to reduce the adverse effects. The selected polymer was found to possess good compatibility with sirolimus without any mutual interactions as shown in FTIR S.

PNF2 was prepared by a modified emulsification diffusion method using PLGA as polymeric nanoparticles are capable of delivering the loaded drugs on to the respective site in a sustained fashion.PLGA is a biodegradable polymer that can be comfortably used for the preparation of nanoparticles.

The increased proportion of the polymer caused an increase in the particle size of the nanoparticles. Pluronic F-127 as a surfactant to enhance the activity which shows an increase in the drug content and entrapment efficiency were also observed. The surface morphology of PLGA nanoparticles was studied by SEM analysis. The Nano formulation indicates that the particles are discrete, smooth, and spherical. The release studies indicated that the release of sirolimus from formulation SPNF2 is consistent and with a better release and permeation than other formulations at the end of 24hrs.

Among the two nanoparticle formulation of sirolimus, prepared with PLGA (SPNF1, SPNF2). The formulation SPNF2 exhibit higher drug content, entrapment efficiency, *in-vitro* drug release for 24hrs.

CONCLUSION:

Sirolimus –loaded nanoparticles could be prepared easily and are reproducible by the emulsification-diffusion method. By drug release studies it was concluded that the release of drugs is in a sustained fashion, the maximum drug was encapsulated in the Nano formulation.

Their entrapment efficiency is about 84% of the optimal condition. And there is a possibility that the sirolimus release from PLGA is pH-dependent. Generally, most nanoparticles accumulate to the target site during continuous systemic circulation due to physicochemical characteristics such as particle size, zeta potential, etc.

Based on the results of our study, we could conclude that this mechanism was related to the drug delivery system of sirolimus loaded nanoparticles. Therefore, it was suggested that the prepared sirolimus loaded nanoparticles can be good for the treatment of ulcerative colitis.

Hence, it can be concluded that sirolimus loaded PLEA Nano formulation can serve as a potential formulation for the treatment of ulcerative colitis. But more animal studies and extensive clinical studies are needed to check and confirm the efficacy of the prepared drug delivery system.

REFERENCES:

1) Encyclopedia of pharmaceutical technology, second edition, volume-1.Edited by James Swarbrick, James c. Boylan, page no-21.
2) Encyclopedia of pharmaceutical technology, second edition, volume-2.Edited by James Swarbrick, James c. Boylan.-NP.
3) Nanotechnology and nanomaterials-applications and global market analysis Dr. Thomas Abraham.
4) Nanotechnology: advantages and drawbacks in the field of construction and building materials F. Pacheco. Torgal said, Jalali.
5) The advantages of nanotechnology in medical field Abdullah Alshahrani university of Bridgeport, electrical engineering department, Bridgeport. CTUS issue 4A, volume4.
6) International journal of pharmacy and pharmaceutical sciences -ISSN-0975-1491, VOL 3, SUPPL2, 2011.
7) International Journal of Pharmacy and Pharmaceutical Sciences, ISSN-0975-1491, VOL 3, ISSUE 4, 2011.
8) Essentials of medical pharmacology, 7th edition, KD Tripathi.-DG
9) 2016 Polysciences, Inc. Rev. #4 04.22.2016-PLM
10) European journal of pharmaceutics and Biopharmaceutics 74 (2010) pg.no:- 164 – 171
11) Robbins pathologic basis of disease-COTRAN, KUMAR, COLLINS, 6th edition, pg.no – 815-820
12) Pathologic basis of disease-STANLEYL. Robbins pg.no:-956-960
13) Anderson`s pathology - JOHN M.KISSANE, volume 2, 9th edition, pg.no-1161-1162
14) Clinical pharmacy and therapeutics –Roger walker, ate Whittlesea, 5th edition pg.no; - 185-200
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16) Langmead L, Rampton D, AG.P guide to inflammatory bowel disease practioner 2001; 245-229
17) Sandler RS Eisen GM. Epidemiology of IBD In; Krasner JB, ed. IBD-Philadelphia, PA, WB Saunders; 2000:89-112
18) Shanahan F, Bernstein CN .the evolving epidemiology of IBD. Current opinion gastroenterology 2009, 25:pg.no:301-305
19) Feldman M: Slazenger & Fordtrans, gastrointestinal & liver disease 7th edition, NewYork, NY Elsevier: 2002
20) Astete, C.M. (2006).synthesis and characterization of PLGA nanoparticles. Journal of biomaterials science-polymer. Edition 17(3):247-289
21) Uhrich, K.E., Cannizzaro, S.M., Langer, R.S., Shakesheff, K.M. Polymeric systems for controlled drug release. Chem. Rev.1999, 99, 3181-3198.
22) Wu, X.s., wamg, N.synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers. Part-II: Biodegradation, J.biomatter.sci.polym.Ed 2001, 12, 21-34.
23) Nair, L.S., Laurencin, C.T. Biodegradable polymers.as biomaterials. Prog.Polym.Sci.2007, 32,762-798.
24) Anderson, J.M., Shive, M.S., Biodegradation, and Biocompatibility of PLA and PLGA microspheres. Adv. Drug delivery.Rev.1997, 28, 5-24.
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28) Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparation of biodegradable nanoparticles of water-soluble and insoluble drugs with D, Lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. J. Control. Release 1993;25:89-98.
29) Yoo HS, Oh JE, Lee KH, Park TG. Biodegradable nanoparticles containing PLGA conjugate for sustained release. Pharm Res 1999; 16:1114-8.
30) Perez C, Sanchez A, Putnam D, Ting D, Langer R, Alonso MJ. Poly(lactic acid)-poly(ethylene glycol) nanoparticles a new carriers for the delivery of plasmid DNA.J Control Release 2001;75:211-24.
31) Lu W, Zhang Y, Tan Y-Z, Hu K-L, Jiang X-G, Fu S-K. Cationic albumin conjugated pegylated nanoparticles as novel drug carrier for brain delivery. J. Control release 2005; 107:428-48.
32) Saxene V, Sadoqi M, Shao J, Indocyanine green-loaded biodegradable nanoparticles: preparation, physicochemical characterization and in vitro release. Int J. Pharm 2004; 278:293-301
33) El-shabouri MH. Positively charged nanoparticle for improving the oral bioavailability of cyclosporin-A. Int J pharm 2002; 249:101-8
34) Vagras Began Debefve E, Konan-kouakou y, Lange N, Ballini JP.imroved photodynamic activity of porphyrin loaded into nanoparticles: an in vivo evaluation using chick embryos.Int j pharm 2004;286:131-45.
35) Konan yn, Gurny R, allemann E. state of the art in the delivery of photosensibilizers for photodynamic therapy. Photochem Photobiol B 2004; 66:89-106.