LIPID POLYMER BASED NANO PARTICLES FOR THE THEROPY OF ULCERATIVE COLITIS TO IMPROVE THE THERAPEUTIC EFFICIENCY

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Abstract: Conventional treatment of inflammatory bowel disease is based on the daily administration of high doses of immune-suppressant or anti-inflammatory drugs, is very complicated by serious adverse effects. A carrier system that delivers the drug specifically to the inflamed intestinal regions and shows prolonged drug release would be desirable. It may enter any portion of the gastrointestinal tract though it is mostly found in the terminal ileum often with extension into the cecum and sometime into the ascending colon. Half of the cases, multiple areas of both small and large intestine are involved in segmental fashion i.e. lengths of normal intestine separate areas of disease called skip area. Crohn's disease granulomatous colitis. Lesions of the small and large bowels are associated with anal lesions it is the first manifestation of the disease Inflammatory bowel disease is mostly common in western countries and in some areas of northern latitude. Polymeric nanoparticles can increase the permeability of an ulcerative colitis through biological barriers, such as the mucosal barriers, skin barriers and cell membrane barrier. The conventional treatments are mostly restricted to control inflammations. However, the main Aim of clinical ulcerative colitis therapy is to not only to control inflammation, but also to achieve mucosal healing. Therefore, the novel therapeutic strategies are urgently needed for addressing the limitations of existing treatments will Increase the stability of any of the volatile pharmaceutical agents, easily and also can be cheaply fabricated in large quantities by a multitude of methods. Delivers a higher concentration of pharmaceutical agent to a desired location.

***Key words*: Dissociation of the drug from the polymer and from its de-adsorption (or) release from the swelled nanoparticles**

I. INTRODUCTION

Ulcerative colitis begins in the rectum or sigmoid and progresses to involve part or all of the colon .It can be diagnosed by protoscopic 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 infection. The disease is

systemic in nature and is often associated with arthritis, uveitis, venous thrombosis, liver disease and various skin lesions; especially pyoderma gangrenosum.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, malabasorption, 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-threating. 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.

Crohn's disease

It may involve any portion of the gastrointestinal tract though it is most commonly found in the terminal ileum often with extension into the cecum and sometime into the ascending colon. Half of the cases, multiple areas of both small and large intestine are involved in segmental fashion i.e. lengths of normal intestine separate areas of disease called skip area. Crohn's disease granulomatous colitis. Lesions of the small and large bowels are associated with anal lesions it is the first manifestation of the disease.

Statistical Data for Ulcerative Colitis

Inflammatory bowel disease is most prevalent in western countries and in 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 a prevalence of 20-40 per 100,000 people ([12, 13]. The incidence of Crohn's disease varies considerably among studies, but has clearly 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. The incidence of ulcerative colitis has remained relatively constant over many years. Although most epidemiologic studies combined ulcerative proclitic with ulcerative colitis, 17% to 49% of cases are classified as proclitic. Both sexes are affected equally with inflammatory bowel disease, although some studies show slightly greater numbers of women's with Crohn's disease and males with ulcerative colitis. Ulcerative and Crohn's disease have bimodal distributions in 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

Need of Nanoparticles Drug Delivery

- Conventional drug therapy in tumor 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 Nano particulate to reduce the systemic side effects associated with the present anti-cancer drugs.
- Nanoparticles of pulmonary surfactant 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

Advantages of nanotechnology techniques

- Nanotechnology-based delivery systems can also protect drugs from degradation.
  - Longer shelf-stability
  - Ability to incorporate hydrophilic and hydrophobic drug molecules
  - Can be administered via different routes
  - Longer clearance time
  - Ability to sustain the release of drug
  - Can be utilized for imaging studies

- Targeted delivery of drugs at cellular and nuclear level
- Development of new medicines which are safer
- Prevent the multi-drug resistance mediated efflux of chemotherapeutic agents

- Improved products may be available with a change in the physical properties when their sizes are shrunk.
- Make treatment a better experience and reduce treatment expenses.
- Nano-based systems allow delivery of insoluble drugs.
- Drug targeting can be achieved by taking advantage of the distinct pathophysiological features of diseased tissues.
- An ideal targeting system should have long circulating time; it should be present at appropriate concentrations at the target site.
- It should not lose its activity or therapeutic efficacy while in circulation.
- Tumors allow an enhanced permeability and retention effect.
- Passive targeting of drugs to the macrophages present in the liver and spleen.
- Improve the oral bioavailability of the agents that are not effectively used orally

Disadvantages of Nanoparticles

- Involves higher manufacturing costs which may in turn lead to increase in the cost of formulation.
- Involves use of harsh toxic solvents in the preparation process
- May trigger immune response and allergic reactions
- Extensive use of poly (vinyl alcohol) as stabilizer may have toxicity issues

Applications

- Cancer therapy
- Intracellular targeting
- Prolonged systemic circulation
- Vaccine adjuvant
- Perioral absorption
- Ocular delivery

Classification of Nanoparticles

i) Polymeric Nanoparticles

- Polymeric nanoparticles are nanoparticles which are prepared from polymers.
- The drug is dissolved, entrapped, encapsulated or attached to a nanoparticles and depending upon the method of preparation, nanoparticles, Nano spheres or Nano capsules can be obtained.
- Nano capsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymer membrane, while Nano spheres are matrix systems in which the drug is physically and uniformly dispersed. As name only suggest polymeric nanoparticles are nanoparticles which are prepared from polymers. The drug is dissolved, entrapped, encapsulated or attached to nanoparticles and depending upon the method of preparation. Polymeric Nanoparticles are mainly two types:
- Nano capsule is a vesicular systems where the drug is confined in to a cavity surrounded by a polymer membrane.
- Nano spheres are matrix systems where the drug is physically and uniformly dispersed.

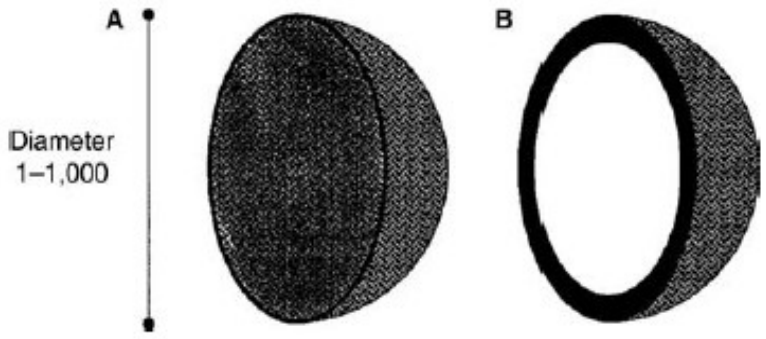


Fig.1 Schematic Representation of Nanospheres (A) And Nano capsules (B).

In Nano spheres, the whole particle consists of a continuous polymer network. Nano capsules present a core-shell structure with a liquid core surrounded by a polymer shell. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in drug targeting to Particular organs/tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through a per oral route of administration

Need of Polymeric Nanoparticles for Ulcerative Colitis:

Polymeric nanoparticles can increase the permeability of an 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:

The polymeric nanoparticles (PNPS) are prepared from 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, Nano spheres or Nano capsules can be obtained. Nano capsules are systemic in which the drug is confined to a cavity surrounded by a unique polymer membrane, while Nano spheres are matric system in which the drug is physically and uniformly dispersed. 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 object of delivering the drugs to specific target; such an advantage improves the drug safety. Polymer- based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their Nano meter-size promotes effective permeation through cell membranes and stability in the blood stream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticles construct with many potential medical applications.

Advantages of Polymeric Nanoparticles:

- Increase the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous method of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modified drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.

Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

MATERIALS AND METHODS:

- Tacrolimus
- PLEA-
- Pluronic F-127
- Ethyl Acetate
- Distilled Water

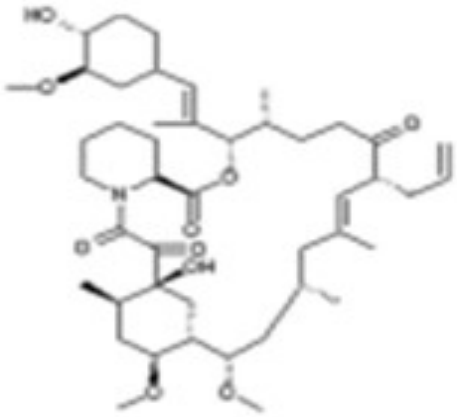
POLYMER PROFILE

PLGA in drug delivery systems

- Devices based on polymers of lactic and glycolic acids are widely used in a number of biomedical and pharmaceutical applications.
- Co-polymers of lactide and glycoside, referred to as PLGA, have generated tremendous interest because of their excellent biocompatibility, biodegradability and mechanical strength.

Methods for the preparation of PLGA-based nanoparticle

- Emulsification solvent diffusion/evaporation,
- Salting out and
- Nano precipitation



DRUG PROFILE:

Tacrolimus

Tacrolimus is a medicate used to treat ulcerative colitis.

- It is an immunosuppressant macrolide anti biotic obtained from Streptomyces tsukubaensis.

Molecular formula: C44H69NO12.H2O
Melting point: 126^0c
Metabolism: Liver
Bioavailabity: 11.2-19.1%.
Biological Half Life: 12hr

Mechanism of Drug Release

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 site of delivery, there by releasing the drug from the entrapped inner core. Dissociation of the drug from the polymer and it's de-adsorption / release from the swelled nanoparticles.

Preparation of Nanoparticles

Conventionally, Nano Particles are prepared mainly by two methods:

i) Dispersion of the preformed polymers
ii) Polymerization of monomers.

Dispersion of Preformed Polymers

Many methods have suggested to prepare biodegradable Nano Particles from PLA, PLG, PLGA by dispersing the preformed polymers.

Emulsification Diffusion Method

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

Solvent Evaporation Method

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 emulsifying agent like gelatin poly(vinyl alcohol) poly sorbet 80 poloximer 188 etc. 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 Nanoparticles was discussed earlier .The W/O/W method has been also used to prepare the water soluble drug –loaded Nanoparticles. In the above methods a high-speed homogenization or sonication is used. but for a large –scale pilot production, alternative methods using low –energy emulsification are required.

Spontaneous Emulsification/Solvent Diffusion Method

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

Salting Out /Emulsification –Diffusion Method

These methods discussed about the use of organic solvents, which are hazardous to the environment as well as to the physiological system. In order to meet these requirements, Aleman and co-workers have developed two methods preparing Nanoparticles.

The first one is a salting –out method.

The second one is the emulsification –solvent diffusion technique.

Characterization Studies Drug Loaded Nanoparticles:

Determination of Particle Size

The mean particle size and size distribution analysis of nanoparticles were performed by the dynamic light scattering (DLS) method at 25^0c with a 90^0c scattering angle for optimum detection. Ten mille grams of drug drug loaded nanoparticles was dispersed in 10 ml of deionized water. The dispersion was sonicated for 1 min and size immediately. The results shown (mean± standard deviation (SD)) are representatives of three independent.

Determination of Drug Entrapment Efficiency

Drug concentrations were determined by HPLC-MS. Ten mille grams of nanoparticles containing drug were dissolved in 10 ml of methanol. The solution was stirred for 1 hrs and sonicated for 5 min. afterwards the solution was diluted with a specific volume of the mobile phase and injected into HPLC.EE was calculated with following equation: EE (%, w/w) = weight of drug in nanoparticle/weight of drug used in preparation of nanoparticles in 100.

Measurement of Zeta Potential:

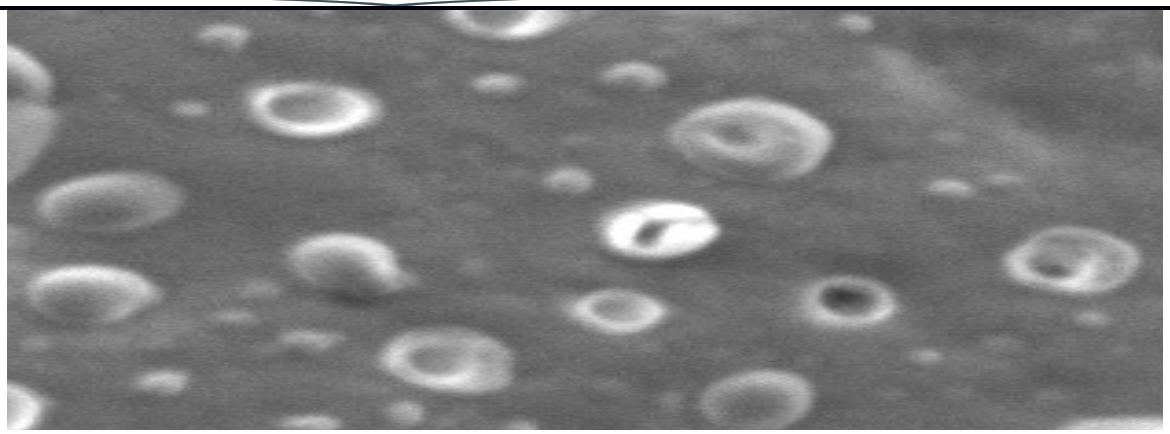
The zeta potential of prepared nanoparticles was measured by the ELS-8000 zeta potential analyze was triplicate among different batches to assess the surface charge and stability of nanoparticle. Zeta potential of Nano particles was measured in aqueous dispersion. The measurements were carried out using the medium of water. The results (mean ± SD) are representatives of three independent experiment.

RESULTS AND DISCUSSION:

SEM Analysis

SEM Analysis of the TPNF 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 TP was loaded as shown

SEM Analysis of TPNF 2



***Invitro* Drug Release Studies (IDRS):**

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

Time (Hr)	TPNF 1	TPNF 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

In vitro drug release of TP loaded PLGA nanoparticles

Compatibility Studies:

Differential scanning calorimetric:

DSC is an important technique to analyses the polymer -drug interactions and also it has previously been used to show the disparity 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 , desolation, recrystallization and solid phase transformations indicated by endothermic or exothermic peaks at thermo gram. DSC thermo gram showed 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 thermo gram of TP, PLGA and their combinations.

Fourier Transform Infra-Red Spectroscopy (FTIR) Studies:

- The Fourier transform infra-red analysis was conducted for the structure characterization.
- FTIR spectra of the pure abacavir sulphate, 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.

Wave number for Tacrolimus and PLGA&

Sl.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

Various Formulations of Tacrolimus Loaded PLGA Nanoformulations

S.No	Ingredients	TPNF 1	TPNF 2
1	Tacrolimus	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

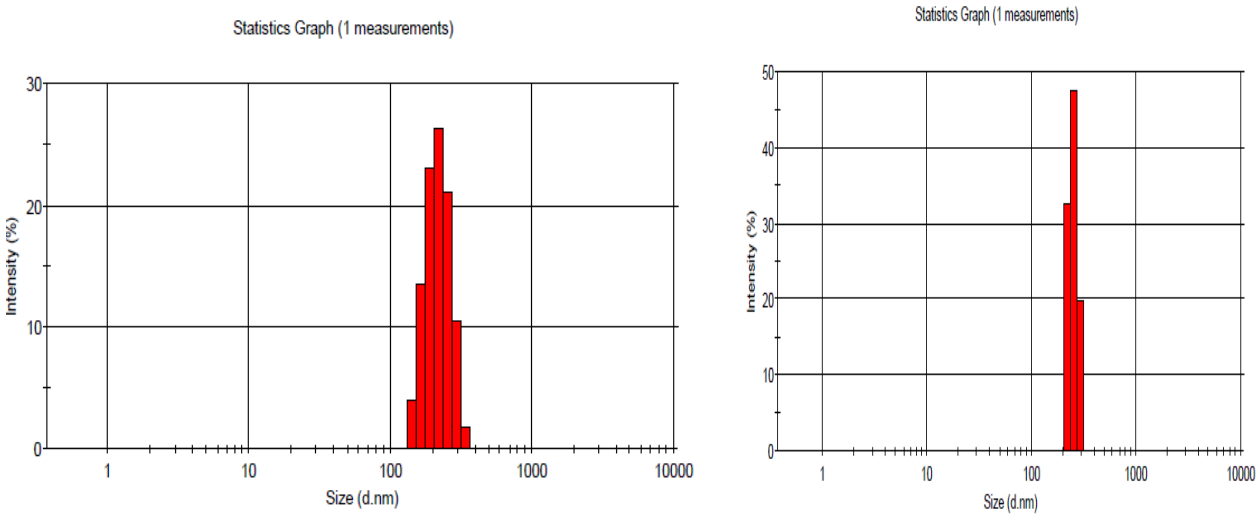
The above table showed that FTIR Spectrum values of Tacrolimus and PLGA combination.The FTIR spectra acquired were taken from physical mixer of Tacrolimus and PLGA and interaction study between drug and polymer evaluated the characteristic peaks owing amino and hydroxyl group which confirm the stability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

Particle Size Analysis

Particle size is well known that sizes of particles are highly dependent on the preparation method and condition employed. Differential scanning thermo gram of (A) TP (B) PLGA (C) physical mixture of TP, PLGA also they can influence the drug loading, drug release and stability of drug in side 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.

Particle size and zetapotential of TPNF1, TPNF2

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



TPNF-1 **TPNF-2**

Particle size and distribution of TPNF-1, TPNF-2

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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.

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10	41.72 ± 2.2	55.21 ± 1.1			
12	47.52 ± 2.3	60.11 ± 1.2			

SUMMARY

PLGA nanoparticles containing tacrolimus were developed for sustain release on i.v administration, which may help to improve the patient compliance and to reduce the adverse effects. The selected polymer was found to possess a good compatibility with tacrolimus without any mutual interactions as shown in FTIR TPNF2 were prepared by modified emulsification diffusion method using PLGA as a polymeric nanoparticles are capable of delivering the loaded drugs on to the respective site in a sustained fashion .PLGA is a biodegradable polymer which can be comfortably used for the preparation of nanoparticles.

The increased proportion of the polymer caused an increase in particle size of the nanoparticles. Pluronic F-127 as 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 tacrolimus from formulation TPNF2 is consistent and with a better release and permeation than other formulations at the end of 24hrs.

Among the two nanoparticles formulation of tacrolimus, prepared with PLGA (TPNF1, TPNF2). The formulation TPNF2 exhibit higher drug content, entrapment efficiency, *invitro* drug release for 24hrs**.**

CONCLUSION

- Tacrolimus –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 drug is in sustained fashion, maximum drug was encapsulated in the Nano formulation.
- Their entrapment efficiency is about 84% at the optimal condition. And there is a possibility that the tacrolimus release from PLGA is pH dependent. Generally most of nanoparticles accumulate to the target site during continuous systemic circulation due to physicochemical characterization such as particle size, zeta potential etc. .
- Based on the results of our study, we could conclude that this mechanism was related to drug delivery system of tacrolimus loaded nanoparticles. Therefore, it was suggested that the prepared tacrolimus loaded nanoparticles can be good for the treatment of ulcerative colitis.

Hence, it can be concluded that tacrolimus 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.

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