TO PREPARE AND EVALUATE THE EFFECT OF POLYMERIC NANOPARTICLES FOR ANTI-ARTHRITIS ACTIVITY

A Thesis Submitted
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF PHARMACY

In

Pharmaceutics

by

Harshita Chaturvedi

Enrollment No

2102270566007

Under the Supervision of

Mrs. Roshan Zehra

Associate Professor

Innovative College of Pharmacy, Greater Noida

&

Co-Supervisor

Mrs. Sandhya Sharma

Assistant Professor

Innovative College of Pharmacy, Greater Noida

to the

FACULTY OF PHARMACY

DR. A.P.J. ABDUL KALAM TECHNICAL UNIVERSITY

(Formerly Uttar Pradesh Technical University) LUCKNOW

JUNE, 2023

DECLARATION

I hereby declare that the work presented in this report **entitled "TO PREPARE AND EVALUATE THE EFFECT OF POLYMERIC NANOPARTICLES FOR ANTI-ARTHRITIS ACTIVITY"**, was carried out by me. I have not submitted the matter embodied in this report for the award of any other degree or diploma of any other University or Institute.

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Name : Harshita Chaturvedi

Roll No. : 2102270566007

Branch : M Pharmacy (Pharmaceutics)

(Candidate Signature)

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Certified that Harshita Chaturvedi (enrollment no. **2102270566007**) has carried out the research work presented in this thesis entitled **"TO PREPARE AND EVALUATE THE EFFECT OF POLYMERIC NANOPARTICLES FOR ANTI-ARTHRITIS ACTIVITY"** for the award of Master of Pharmacy from Dr. A.P.J. Abdul Kalam Technical University, Lucknow under my/our supervision. The thesis embodies results of original work, and studies are carried out by the student himself/herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Signature

Mrs Roshan Zehra

Associate Professor

Innovative College of Pharmacy,

Greater Noida.

Signature

Mrs Sandhya Sharma

Assistant Professor

Innovative College of Pharmacy,

Greater Noida.

Date:

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Enrollment No. 2102270566007

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(Formerly Uttar Pradesh Technical University, Lucknow)

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2. Enrollment No.: 2102270566007
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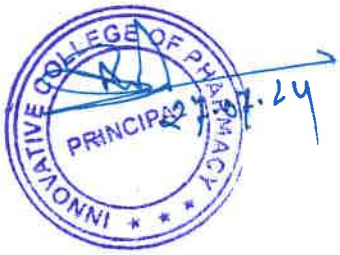
ABSTRACT

The purpose of the prepare and test the effect of polymeric nanoparticles for anti-arthritis activities. Nanoparticles can be created with rutin medication and tamarind seed gum. Preparation is done using a solvent antisolvent technique. In the present work, the nanoparticles are generated by employing the natural polymer and used in the therapy of arthritis. Due to their non-toxicity, safety, lack of immunogenicity, and good biocompatibility, natural polymers have been used in drug delivery. Nanoparticles are created to boost therapeutic efficacy and bioavailability. It has been hypothesized that nanostructures can keep drugs from deteriorating in the digestive tract. Furthermore, this technique permits the targeted administration of medications to specific parts of the body. With this new strategy, medications that aren't highly water soluble can find a way to escape being metabolised by the liver totally. In the present work nanoparticles are generated by utilising single emulsion solvent evaporation method. Nanoparticles manufactured will be studied further to evaluate the formulation's impact on arthritis.



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Harshita Chaturvedi

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CHAPTER 1

INTRODUCTION

1.1 NATURAL POLYMER:

The German physicist Herman Staudinger, Nobel Prize laureate invented the term "Macromolecules" to reference the polymer in 1953. The 'Polymer' word derived from Greek word, which consists of 'Poly' and 'meros' word. 'Poly' denotes 'many' and 'meros' denotes 'high molecular mass parts or units'. The polymer molecule is composed of repeated monomer units bonded with covalent bonds. Polymer is a macromolecule and monomer is a single molecule. Chemical process known as polymerization, which helps in the joining of two or more monomers together to form polymers [1]. Polymers play a significant part in every dosage form as excipients. They affect the release of drugs and must be safe, nontoxic, economic and stable. They have a large range of uses so selection of the polymer is the main step in formulating any type of novel form of dosage. Recently, the manufacturers are reluctant to use natural polymers due to certain issues associated with drug release and side effects [2].

The natural polymer is defined as materials that widely occur in nature or extracted from the animals and plants. Natural polymers can also be derived from microorganisms. As a living organism, we rely heavily on natural polymers, which are essential to our own structure. Some of the examples of natural polymers are nucleic acid and proteins that occur in the human body, other example includes natural rubber, cellulose, wool and silk [3]. Drugs, drug delivery systems, scaffolds for tissue regeneration, and imaging agents are just a few of the ways in which natural polymers contribute to medical practise. In the medical field of wound care, these serve as templates for new tissue to develop on and as long-term dressings for wounds of all types, including the acute and chronic varieties. [4].

Natural polymers have become a vast area of research because it is utilized in the delivery of drug. Synthetic excipients are not always necessary for drug delivery, and natural gums that have been changed can be just as efficient. Gums can be easily and affordably obtained from plants. When a plant is exposed to stress, such as dehydration or the breakdown of the plant's cell wall, it creates extracellular material, including pathologic products like gums, which are discharged upon cutting open the plant (gummosis) (gummosis). Guargum, acacia, tragacanth, and many more gums are

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examples of those that are easily accessible. Sugars including glucose, fructose, galactose, arabinose, and mannose are hydrolyzed to their component sugar units. The plants-based polymer has been used as film coating agents, microsphere, nanoparticles, buccal films, matrix-controlled systems, ophthalmic solutions, suspensions and implants [5,6].

1.1.1 Advantages of natural polymer:

- It is easily available, biodegradable and very cheap to fabricate.
- It is compatible with the environment and inert.
- Must be less toxic.
- It does not have any action related to pharmacology [7,8].
- For drug delivery polymer must have high stability, good biodegradability, hydrophilicity and good biocompatibility [9].

1.1.2 Disadvantages of natural polymer:

- There is a decrease in viscosity with time when gums are stored dry; viscosity rises when water is added to the mixture. It was discovered that after storage, viscosity decreases due to the presence of gums, which is a complex substance in and of itself. [10].
- Variations in time, creatures, geography, and climate all influence the relative abundance of a material's chemical components, hence the pace at which it absorbs water is unpredictable. [10].
- Variation in batch to batch- Manufacturing related to synthetic material is a regulated process with fix ingredients quantities, whereas gum processing depends on seasonal and environmental factors [11].
- Contamination by microbes- Content of moisture in the gums is typically 10% or more. They are carbohydrates according to structure and disclosed to the external environment throughout processing. Contamination risk by microbes can be barred through proper handling and by using the preservatives [11].

1.1.3 Applications of natural polymer:

Natural polymers with combination of other synthetic or natural polymers is used for pharmaceutical applications in the form of a hydrogel. Natural polymer used in the transdermal drug delivery system. Chitosan mucoadhesive property made it topically usable and used as gels which applied topically for localized and efficacious delivery.

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Gelatin is another natural polymer used in the preparation of hydrogels for delivery of drug. Hydrogel prepared by using polyvinyl alcohol and gelatin used in the Cisplatin delivery for anticancer activity [12].

Almost all polymers from natural origin are used in non-food industries applications. The main consumers of the natural polymer is paper and textile industries, they use the polymer as agents for coating and sizing. While numerous synthetic polymers are used in the food industry, many natural polymers are also put to use. Carrageenan is widely used in the food processing and savoury product industries, whereas pectin is commonly used in baking, and xanthan gum is widely used in the beverage and dairy industries. [13].

Xanthan gum is used as a thickener, stabiliser and emulsifier in cosmetic, culinary, pharmaceutical sector and for dairy products. Pseudoplastic properties of gum xanthan used in the formulation of ointment and toothpaste because it has a shape which makes it easier to spread readily. Acacia is used as an emulsifying, suspending agent and as a tablet binder. It has demulcent properties that are employed in different diarrhoea, cough and throat preparations. It has been used as a binding agent in the manufacturing of cough pastilles and other medical preparations or as a coating for pills. Agar is used in confectionery items, jellies preparation, microbiology and studies of tissue culture [14].

Natural polymers are used by industry related to food as a thickening agent for snacks, fruit juices and meat products. These are also used for making disposable products such as fast food utensils and containers. These polymers were widely used in solid oral dosage formulations from a pharmaceutical perspective, where they were utilized as binder, disintegrants, diluent and matrix agent. Natural biopolymers such as chitosan, starch and gelatin have been used in industries such as textiles, food, cosmetics, adhesives, plastics, paper and pharmaceuticals. Starch is used mainly in paper, adhesive and clothing industry. The starch is applied in food thickeners, cosmetic creams and paper production. Uses for chitin include film and gel production, polyoxysalt creation, and metal ion chelation. Chitosan is widely used in the delivery of transdermal drugs delivery. Hyaluronic acid helps in proliferation of cell and tissue development. Hyaluronic acid with combination of alginate used in the surgical application for the healing of wounds. Alginic acid has numerous biological functions and as stabilizing material, viscosifier, carrier of drug and binding material have various industrial applications. In addition to its use as a food additive, silk fibroin has been put to good use

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in transdermal medicine administration, cosmetics, scaffolds, and fibres due to its excellent chemical and mechanical capabilities. The silk fibroin is extremely flexible and can be formulated in different ways like as gels, fiber, powder and films [15].

1.1.4 Tamarind seed gum:

The pharmaceutical industry shows interest in Tamarindus indica L. (Tamarind), also known as Imli. Tamarind seed gum is rich in polysaccharides, specifically glucose, xylose, and galactose, in a ratio of 3:2:1. Extracting and characterizing tamarind seed gum is a simple and inexpensive process. Its high molecular weight causes it to produce a very thick viscosity when mixed with water. Leguminosae in origin, it is found all throughout South and Southeast Asia in places like India, Myanmar, Bangladesh, Malaysia, Thailand, and Sri Lanka. [16].

1.1.5 Application of tamarind seed gum:

Tamarind gum is biocompatible, biodegradable, non carcinogenic and non irritant. Gum is soluble in warm water and has gel forming ability with a wide pH range with adhesivity. It is stable in acidic pH. It has film forming property, high drug holding capacity and high thermal stability. It is used as bioadhesive and hasmucomimetic property [17].

1.2 GRAFT COPOLYMER:

When a mixture of oligomers is "polymerized," the resulting surface adheres to the substrate via physical forces, whereas "grafting" describes the process of covalently joining or altering monomers to the polymer chain. Curing fills in the surface troughs, resulting in a smoother appearance. Natural polymers based on graft copolymers such as cellulose are seeing expanded use for metal ions removal from water systems with low-cost technologies. Grafting is a method used to add functional groups for the metal ions sorption on the polymer's backbone [18].

1.2.1 Acrylamide grafting:

Grafting of the polymer by using acrylamide is the most promising method that deals with the involvement of microwave irradiation. Acrylamide grafting is the technique used for the modification of polymer. The resulting enhanced product offers numerous benefits. It includes, increased swelling index, higher yield and various formulation

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related advantages. This is used in the metal ions removal from the wastewater released from the industry. Acrylamide grafted polymer is used in the formulation of nanoparticles which is cost effective and may be used for the prolonged time frame (both as controlled and sustained release) [19].

1.2.2 Polymer grafting and their advantages:

The interest in graft copolymers stems in part from the protection provided by the grafts on the backbone and this particular feature has led to a variety of applications in polymer blends such as emulsifiers, coating materials, surface-modifying agents and compatibilizers. Amphiphilic graft copolymers, in which grafts and backbone solubility activity are very different, play a significant role as an emulsifier [20].

Polymers are incompatible, as compared to metals, they can not be easily combined. They do not combine but shape separate materials with brittle microphase. Binding the polymers together by covalent bonds overcome chemical repulsion and produce variety of new things. The preparation of branches of grafted polymer are fastened by covalent bonds. Radiation is especially appropriate for polymer activation as polymer free radicals and involved in initiation of polymerization. It is the big advantage of copolymerization with the radiation initiated grafting [21].

1.2.3 Microwave-assisted acrylamide grafting of polymer:

The capability of the aqueous reaction mixture to transform microwave energy into heat is improved by the addition of exogenous redox initiators, which produce ions in processes associated with grafting. Microwave dielectric heating triggers the initiators to release free radicals, which speeds up the grafting reaction. Unlike conventional grafting reactions, those triggered by microwaves require no outside initiators. It was hypothesised that grafting happened via a process of free radical grafting since a small amount of a radical inhibitor, like hydroquinone, is enough to halt grafting events while subjected to microwave irradiation. [22].

A significant source of energy and effective method for copolymerization of graft is microwave irradiation which have an advantage of fast energy transfer into the mixture of reaction resulting in specific reagent interactions. The use of microwave irradiation further limits the utilization of harmful solvents and the time of reaction. Polysaccharide

copolymers synthesized by microwave have good industrial processing assets compared with conventionally synthesized copolymers [23].

Electromagnetic radiation is microwave radiation. Polar bonds selective excitation occurs in the microwave radiation presence. These outcomes in cleavage or rupture at exposure points and contributes in creation of sites of free radicals. The polymer backbone "C – C" is nonpolar, the microwave radiation stays uninfluenced. Area of copolymer grafting synthesis was revolutionized by the utilization of microwave radiation to create sites of free radical on the backbone of polymer. Unlike traditional synthesis methods, microwave-based methods do not require an inert atmospheric state. These methods of grafting are simple, operation is easy, effective, reproducible [24].

Due to more effective transfer of energy than conventional heating, in various polymerization techniques microwave-assisted heating is used, like step-growth polymerization, radical polymerization and ring opening polymerization. In method of microwave-assisted polymerization, microwave reactors enable accurate and fast monitoring of various parameters like pressure and temperature, for production of material with high purity in various forms. Such materials have vast use in field of science, technology, wastewater treatment and controlled delivery of drug [25]. Figure 1 shows the microwave irradiation method of polymer grafting.

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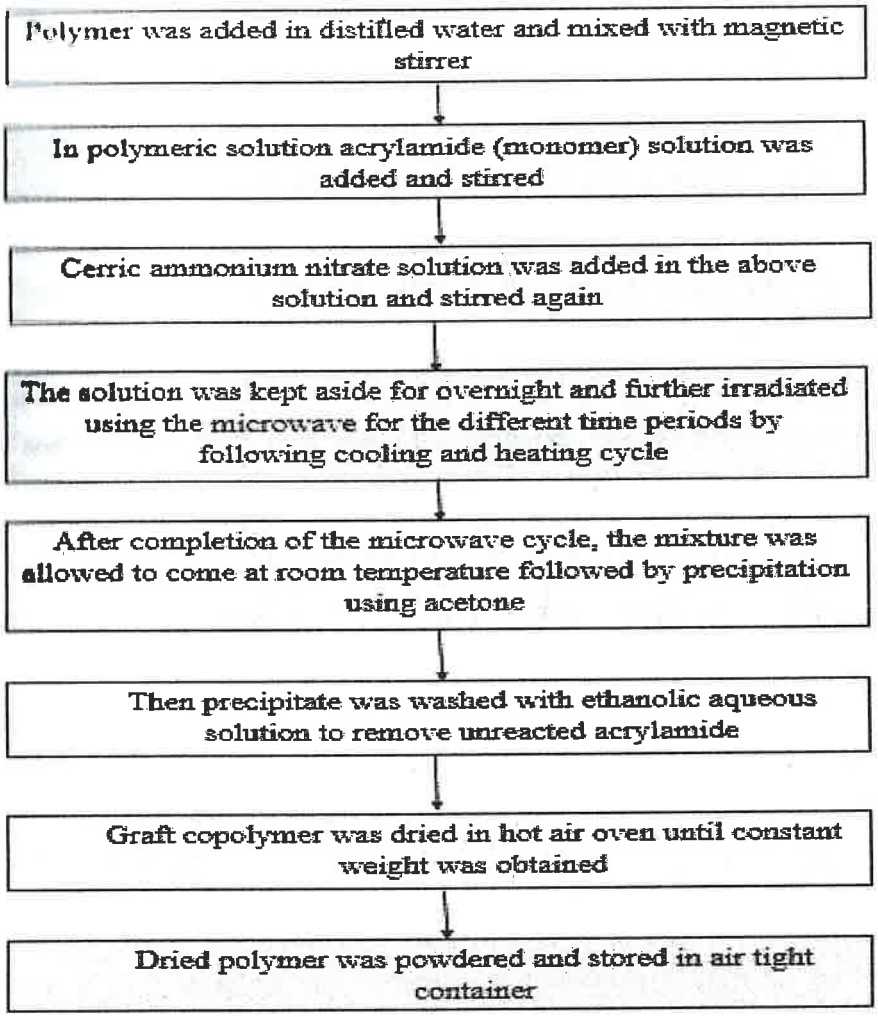

Figure 1.1. Schematic diagram to show the microwave irradiation method for polymer grafting

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NANOPARTICLES:

Nanoparticles are one of the most widely studied substances of this century, resulting in the development of a new 'nanotechnology' branch of science. Particles with at least one dimension between 1 and 100 nm can differ from the bulk substance from which they were isolated in terms of their physicochemical qualities. Nanoparticles, with their distinctive properties and wide range of possible applications, have already begun to permeate many areas of modern life and the energy sector. The chemical composition, size, and/or shape of a bulk material determines the properties of the resulting nanoparticles. [26].

The various types of nanoparticles need a particular process of purification, which also influences the quality and quantity of nanoparticles synthesized. Also, the action of synthesized nanoparticles is significantly impacted by experimental conditions. Previous studies have shown that the reaction conditions are determined by stability, reactivity, and physicochemical properties. In a study, it was found that the crystalline structure of zinc sulfide nanoparticles immediately changed when their atmosphere changed from a wet to a dry state [27].

Nanoparticles have numerous significant implications, especially in the fields of engineering of biomedical, agriculture and techniques related to environmental remediation. Because of their special properties Nanoparticles are commonly used in many industries. The nanoparticles toxic effect can be decreased by monitoring different parameters like surface area, diameter and a suitable range of routes [28].

Nanoparticles central objective is to monitor and biomacromolecular structures manipulation and assemblies of supramolecules that are vital to living cells to enhance the human health quality. By nature, these structures are of nanoscale and contain entities like DNA or RNA, drugs, viruses, proteins, cellular lipid bilayers, cellular receptor sites and immunologically antibody for various regions and also in nano-scale events. The advent of these nanotherapeutics or diagnostics would help to understand the longevity of human and illnesses like cardiovascular disease, cancer and genetic disorders [29].

1.3.1 Advantage:

- Bioavailability increased by improving solubility in aqueous medium.
- Raising the resistance period in body.
- Medication targeting to different locations throughout body (site of action).
- Reduces in dosage toxicity and drug required quantity.
- Toxic therapeutic drugs delivered safely and protection of cells and tissues which is non targeted from side effects [30].
- Stability is high.
- Carrier efficiency is high.
- Applicability of incorporation of both hydrophobic and hydrophilic drugs.
- Applicability of various administration route of drug, such as inhalation, oral application.
- Used as drug carriers.
- Engineered to permit controlled and sustained release of drugs from the matrix [31].

1.3.2 Disadvantage:

- Inorganic nanoparticles are not sufficiently assessed for their long-term toxicity and clearance.
- Magnetic nanoparticles are causes burst drug release and have poor stability.
- The drug delivery assisted by nanoparticles has limitations because the clearance of the drug is occurring by the reticuloendothelial system (RES) by opsonization.
- The large surface area and small size can result in aggregation of particle, making the nanoparticles physical handling in dry and liquid forms difficult [32,33].

1.3.3 Applications:

Nanoparticles are used in the biomedical field including, imaging, biosensing, delivery of drug and antimicrobial application. Nanoparticles have been studied in the environmental sector for applications in diverse contaminant bioremediation, water treatment and renewable energy production [34].

Nanomaterials are utilized in gene and drug delivery, fluorescent biological labels, pathogens biodetection, DNA structure probing, protein detection, engineering of tissue, destruction of tumour by heating, MRI contrast enhancement, purification and separation

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of biological molecules, cells and in studies of phagokinetic [35]. It is used in the oncology, bioseparation, hyperthermia, phototherapy, chemotherapy [36].

1.3.4 Classification of nanoparticles:

1.3.4.1 Carbon nanoparticles:

The most widely used carbon-based nanoparticles are carbon nanotubes and fullerenes. Their tremendous power, electrical conductivity, electron affinity, structure, and adaptability have garnered a lot of attention from the commercial world. Such materials have hexagonal and pentagonal carbon units arranged while every carbon is hybridized sp^2. Such materials not only used in pure form because of their unique chemical, mechanical and physical characteristics and also in nanocomposites for various industrial uses like for environmental remediation as efficient gas adsorbents, fillers and means of support for various organic and inorganic catalysts [37,38].

1.3.4.2 Metallic nanoparticles:

Nanoparticles that have a metallic chemistry are made solely from metal precursors. Because of their limited surface plasmon resonance properties, metallic nanoparticles can be employed as photodetectors. The visible area of the sun spectrum is a broad absorption band for nanoparticles of alkaline and precious metals like copper, silver, and gold. The size of synthesised metal nanoparticles may be precisely controlled. [39].

1.3.4.3 Ceramics nanoparticles:

Inorganic solids that aren't metals are created by heating and then cooling. It can be thick, amorphous, polycrystalline, hollow, or brittle, among other possible textures. Nanoparticles like this have attracted a lot of interest from scientists because of their potential use in fields including imaging, catalysis, photodegradation of dyes, and photocatalysis. [40].

1.3.4.4 Semiconductor nanoparticles:

This possess properties related to metals and nonmetals and therefore numerous applicability are described in the literature. Those with wide bandgaps experienced drastic changes to their characteristics. Thus, photonics, photocatalysis, and electronics are fundamental components. Nanoparticles of various semiconductors, thanks to their

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perfect bandgaps and banding locations, have been demonstrated to be extremely successful in water-splitting applications. [41,42].

1.3.4.5 Lipid nanoparticles:

It includes lipid motility and utilized successfully in various applications of biomedicals. It is typically spherical and have diameter between 10 and 1000 nm. The external center of nanoparticles was stabilized by emulsifiers or surfactants. It helps in the designing and lipid nanoparticles synthesis for different applications like carriers of drug, cancer therapy and release of RNA [43,44].

1.3.4.6 Polymeric nanoparticles:

Organic nanoparticles are frequently cited as their source in the scientific literature. Nanoscale structures of this type often take the shape of spheres or capsules. The matrix particles are typically stiff, whereas the other molecules adsorb on the surface's periphery. The second type features a particle that completely encloses the solid mass. [45,46].

Specific methods of preparing polymeric nanoparticles are used, including spontaneous emulsification, solvent evaporation, polymerization and solvent diffusion. Different parameters which influences the size, polymeric nanoparticles stability, release profile are solubility of drug, molecular weight, polymer composition, pH, solvent, mixing time and speed of homogenization [47].

If the polymers that make up the structure of Nanoparticles are biodegradable or non-toxic [48]. Polymeric nanoparticles play significant role in delivery of drugs because of their biocompatibility. They encapsulate drug molecules that are hydrophobic for effective pharmacological formulation [49].

Polymeric nanoparticles can incorporate both therapy and imaging for controlled drug delivery . which provides optimized drug delivery and drug safety, which enables enhanced therapeutic indexes [50].

Polymeric nanoparticles which are biodegradable are chosen. They are compatible with cells, tissues and prepared by polymers like chitosan, poly(lactic acid), poly(lactic-co-glycolic acid) and poly-ɜ-caprolactone. It have subcellular sizes and are non inflammatory, non toxic and non immunogenic [51,52].

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1.3.5 Polymeric nanoparticles applications:

Polymeric nanoparticles have many potential uses in the biomedical industry, including as carriers for gene therapy and other medications, and as release control systems for other drugs that must be delivered to specific organs and tissues. [48].

Polymeric nanoparticles are an excellent candidate for topical distribution and they commonly use natural polymers such as gelatin, chitosan, alginate and albumen. The following compounds have been used successfully to distribute topically: retinol (vitamin A), acyclovir (antiviral), quercetin (antioxidant), plasmid DNA and antisense oligonucleotides [53].

Recent developments in the delivery of drugs are the biodegradable amphiphilic nanoparticles for diagnosis and treatment of cancer. Synthetic polyesters like polycyanoacrylate and associated polymers poly(lactide-co-glycolide) or poly(lactic acid) have been utilized among the polymeric nanoparticles in drug delivery at target, silencing of gene and delivery of siRNA [54].

Polymeric nanoparticles improve the cutaneous drug delivery through skin by enhancing the concentration gradient and side effects decrement, such as irritation of skin from topical drugs [55]. Table 1 shows the patent list related to the polymeric nanoparticles.

Table 1.1. Patent list of polymeric nanoparticles.

S.No.	Patent No.	Workdone
1.	2012138013A1	The paclitaxel-loaded polymeric nanoparticles are those with paclitaxel surrounded by a copolymer, have water solubility and biocompatibility, and release and transport the drug, paclitaxel, to multi-drug - resistant cancer cells (MDR), thereby delivering an anti-cancer effect several thousand times higher than the effect of the initial paclitaxel [56].
2.	EP2741775A1	Biodegradable polymeric nanoparticles are prepared, containing an inner core composed of a photodynamic agent that can be triggered to produce cytotoxic singlet oxygen. Such nanoparticles have anti-cell proliferation activity and are effective for treating both cancerous and non-cancerous conditions like actinic keratosis, psoriasis, and acne vulgaris [57].
3.	US20150283254A1	In some examples the polymers include a first block consisting of hydrophilic monomers, the first block forming substantially an outer shell of the nanoparticles, and a second block consisting of cationic monomers and hydrophobic monomers, while the second block forming substantially a nucleus of the nanoparticles [58].
4.	WO2006052285A3	Nanoparticles are crosslinked using radiation (g-radiation) as the mechanism for free radical polymerization and not through harmful chemical means. For hydrophobic, hydrophilic, ionic groups or moieties or with enzymes, the nanoparticles and nanogels can be modified without restriction [59].
5.	US8420123B2	The present disclosure usually relates to methods

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		of producing nanoparticles having about 0.2 to about 35 percent weight of a therapeutic agent; and about 10 to about 99 percent weight of biocompatible polymer like a poly(lactic) acid-poly(ethylene)glycol diblock [60].
6.	US20160151298A1	The present disclosure primarily concerns the suspensions and compositions of polymeric nanoparticles like docetaxel and methods for the treatment of different cancers, like drug-resistant cancers and refractory cancer [61].
7.	WO2013160773A2	The present innovation relates in particular to the field of nanotechnology, the development of biodegradable polymeric nanoparticles. The present invention offers a biodegradable polymer nanoparticle consisting of a copolymer block and a method to manufacture the same. The nanoparticles are produced without any emulsifier and have a size of between 30-120 nm [62].
8.	WO2017223398A1	The invention provides polymers and polymeric nanogels in which nucleic acid molecules can be stably trapped or encapsulated and distributed and released controllably when the nano-structures are weakened in response to different micro-environmental triggers, as well as formulations and preparation methods and their use [63].
9.	WO2017120098A1	Embodiments of this disclosure provide for compositions like polymer particles, composition producing methods, composition processing methods, stimulus-responsive agent delivery methods and the like [64].
10.	US7594949B2	Stable polymeric materials are given internally cross-linked, each particle consisting essentially of a single macromolecule in the form of

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		significantly spherical particles. These have the unusual property of being soluble or dispersible in a liquid medium without greatly raising the mediums viscosity, making them useful in imaging applications like inkjet printers [65].
11.	US20150232642A1	Disclosed herein are compositions of latex emulsion consisting of water, turmeric, at least one surfactant and at least one organic monomer whereby the composition of the latex emulsion has an average particle size of less than 250 nm. Usage of latex emulsion compositions containing turmeric such as paints, toners, inks,cosmetics, beauty aids, foodstuffs, health aids and coatings [66].
12.	WO2013127004A1	Synthesis and characterization of pH-responsive starch based nanoparticles are identified for controlled delivery of drug [67].
13.	WO2017081606A1	The present disclosure typically concerns methods of evaluating the structural characteristics that lead to the rate of release of a therapeutic agent in a therapeutic nanoparticle. The nanoparticles contain a therapeutic agent [68].

CHAPTER 2

LITERATURE REVIEW

Prasad *et al.* 2019 The Latin name for the plant Ruta graveolens is where the word "rutin" is thought to have originated. Naturally occurring flavonoid found in plant foods. Buckwheat seeds, tea, apricots, grapes, apricots, grapefruits, plums, oranges, and onions are just some of the plant-based foods that contain this safe compound. Multiple biological effects of rutin (also called rutoside or vitamin P) have been the subject of substantial study. Antioxidant, anticancer, antidiabetic, antiinflammatory, antibacterial, antifungal, neuroprotective, and cardioprotective are just some of the many pharmacological activities attributed to rutin. Some of rutin's cancer-fighting properties include the ability to induce apoptosis and block cell cycle progression in cancer cell lines. The anticancer effects of rutin are complemented by evidence that it can also serve as a chemopreventive agent and make cancer cells more sensitive to chemotherapy. Antioxidant enzyme levels including glutathione, superoxide dismutase, and catalase are raised when rutin is taken as a supplement. [69].

The author described that it has been documented that about 4000 types of flavonoids are found in plants. Rutin is a glycoside of citrus flavonols known as quercetin-3-rutinoside, rutoside, and sophorin. Rutin was also observed as neutraceutical actions and it hasa nutritional component of foodstuff. Chemically rutin is a glycoside consists of quercetin with flavonolicaglycone along with disaccharide rutinose. It is also used in the prevention of neuroinflammation, promotion of neural crest cell survival, sedative activity, anticonvulsant, antialzheimer, antidepressant, analgesic, antiarthritic, antiplatelet, antiulcer, antiasthmatic, antiosteoporotic, antiosteopenic, anticataract, antimalarial, antiretroviral, antiviral, antifatigue activity [70].

Gullon *et al.* Rutin 2017 can be taken safely for up to six months at a time, as long as the recommended daily dosage of 500 to 2000 milligrammes is adhered to. There are exactly 610.518 g/mol of medication in the bottle. It melts at 195 degrees Celsius and has a crystalline yellow powder form. The drug dissolves at a rate of 0.13 g/L in water, 37.3 g/L in pyridine, 55 g/L in methanol, and 5.5 g/L in ethanol. According to the permeability coefficient, the speed of sound is 14.7 m/s. [71].

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The author pointed out that although rutin is a BCS Class II medicine, its limited value is due to its low absorption and poor solubility. The bioavailability of a medicine can be improved by increasing its solubility. Some methods for increasing drug solubility include decreasing particle size, dispersing the drug within the carrier, altering the crystal habit, complexing the drug with cyclodextrin, and employing surfactants. [72].

Phan *et al.* 2017 has prepared rutinnanosuspension using sodium tripolyphosphatecrosslinker and chitosan. Both reagents are used as an encapsulating agent and stabilizer in the preparation of nanosuspension. Based on these results, the average particle size of a rutin nanosuspension was 198 nm, and the sedimentation rate was 93%. After keeping an eye on particle size for six months at room temperature, we didn't see any major shifts. When rutin powder was redistributed into solutions of varied pH levels, it was found that the powder remained crystalline and did not form discernible agglomerates. [73].

In the study, Kizibey*et al.* 2017 have been prepared PLGA nanoparticles loaded with PLGA by applying single emulsion solvent evaporation method. Scientists have been studying the effects of dissolving rutin in propylene glycol to augment the drug's low bioavailability and examine the resulting nanoparticles. The reaction yield of the nanoparticles was 47%, while their encapsulation efficiency was 81%. The nanoparticle size distribution was measured to be 252.6 2.854 nm, and the Poly Dispersity Index value was found to be 0.209 0.008. The nanoparticles all looked to be the same size and shape. These findings illustrate the value of PLGA encapsulation as a strategy to improve the bioavailability of poorly soluble medicines. [74].

Cosco*et al.* 2019 have been prepared rutin loaded chitosan microspheres. Rutin was microencapsulated using the spray-drying technique in a chitosan matrix and studied the resulting system. High quantities of rutin were effectively trapped within polymeric microspheres and these microparticles were characterized by a smooth surface and provided the active compound with controlled release. The prepared microsphere has a spherical shape. These rutin loaded chitosan microspheres have been shows anti-inflammatory activity [75].

The author created the nanoparticles by applying neem gum, carboxymethylated neem gum and acrylamide grafted neem gum. Formulation used an ultrasonic solvent-antisolvent strategy. The nanoparticles stabilised with carboxymethylated neem gum

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demonstrate a reduced size and enhances the solubility and dissolving rate. The prepared nanoparticles do not show any changes in size in the presence of solvent after 45 days [76].

Sana *et al.* 2019 have been prepared the starch nanoparticles by using spinning disc reactor with solvent-antisolvent precipitation method. It was investigated that on increasing the antisolvent to solvent ratio induces a decraese in particle size was produced by increased antisolvents. The size of particle was not greatly affected by disk texture, the grooved disk surface obtained higher yields. Therefore, the grooved disk provides the potential for more efficiency in the starch precipitation with improved product consistency in the solvent-antisolvent [77].

Kumar *et al.* 2016 have been prepared the size controlled nanoparticles of griseofulvin utilizing polymeric stabilizers with evaporation assisted solvent-antisolvent interaction method. This process was used for the improvement of solubility. The solvent acetone was used and for antisolvent purpose vapour was utilized. Particle size was found to be regulated by varying the concentration of griseofulvin in acetone. The prepared nanoparticles were found to be less than 40nm. The reducing particle size and the development of lyophilic polymers have resulted in significant improvement in solubility and the rate of release of drugs [78].

In the study Han *et al.*, 2018 have been investigated the activity of rutin on *Candida albicans* causes septic arthritis. During the study, it was observed that rutin produces the anti-arthritis activity which can stops the production of nitric oxide from proliferation of T-cells and macrophages. It can inhibit the development of *Candida albicans* yeast. This describes anti-fungal and anti-arthritic effects produced by rutin [79].

Chua et al. 2019 share the findings of their study on the pharmacological activity of rutin. Articular elastase activity was used to evaluate rutin's efficacy as a therapy for arthritis because of its link with joint inflammation. The level of cellular activity at the injury site is reflected by the number of polymorphonuclear leukocytes (PMNs) secreted by hard-at-work granulocytes. In addition to the enzymatic analysis, the detection of nitric oxide, a crucial signalling molecule involved in the inflammatory response of activated macrophages and T-cells, is available. [80].

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Mediros *et al.* 2003 have been prepared rutin loaded microparticles which increases the analgesic effect. The microparticles loaded with rutin were prepared with pectin and casein using the compound physicochemical coacervation technique, ensuing in 4.903 μm ± 4.421 average particle size, irregular surfaces and round shape, crystals of rutin can be detected for adsorption on the microparticles outer surface. Microparticles loaded with rutin, *in vivo* study demonstrated the greater inhibition of mechanical hyperalgesia caused by carrageenan 64% than nonmicroencapsulatedrutin 28%. Rutin was distributed in the microparticles in an amorphous matrix tended to offer increased absorption, resulted in analgesic efficacy improvement related to rutin which is non-microencapsulated [81].

In the study Selloum *et al.2016* have been found that rutin shows at paw oedema of rat anti inflammatory effect. The rutin had an important effect ($p<0.05$) on paw oedema of rat. Rutin showed a important ($p<0.05$) activity of inhibition (58.3 ± 6.4%) that was determined at 5 hours after induction of oedema with 1% λ carrageenan. These results showed that rutin has anti-inflammatory properties [82].

The author prepared a complex of rutin-phospholipid, a phyto formulation and determined for the activity of anti diabetic in diabetic model, induced with streptozocin. Scanning electron microscope data shows that complex of rutin-phospholipid has phosphatidylcholine composed of vesicles with irregular size. Using DSC, researchers found that the rutin-phospholipid complex had a single endothermic peak at 147.68°C. Similar to how the OH group in rutin's phospholipid is located at a lower frequency, we see this in FTIR spectra of phytosomes. When compared to rutin alone, the rutin-phospholipid complex (50 and 100 mg/kg b.w. p.o.) significantly reduced blood glucose levels and rectified the parameters of altered lipid. Histopathology analyses confirmed the preservation of both the quantity and quality of beta cells in the pancreatic islets. Treatment of the rutin phospholipid group complex, together with careful monitoring and comparison to rutin, led to an increased concentration of rutin in the serum (13.2 g/ml). The elevated rutin concentration in rat serum was maintained for an extended period of time by a phospholipid rutin molecule. [83].

Zhang *et al.2018* have been prepared novel zein-sodium caseinate loaded rutin nanoparticles that were formulated in an aqueous medium with antioxidant activity. The mass ratio zein to sodium caseinate, ethanol volume fractions and rutin dosage

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considerably affects the characteristics of zein nanoparticles. The zein-sodium caseinate loaded rutin nanoparticles were round in shape, with more particle yields upto 86.6% and encapsulation efficiency upto 71.6%. The best results were 52.7% and 71.2% respectively from the 2,2-diphenyl1-1-pricrylhydrazyl and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays and total antioxidant capacity was found to be 0.40nmol g^{-1}. This result shows that zein-sodium caseinate loaded rutin nanoparticles have activity of antioxidant [84].

Rejaie *et al.* 2013 have been examined the hypercholesterolemia effects of rutin on hepatotoxicity induced rats. Rutin in combination with high cholesterol diet induces a protecting effect against hepatotoxicity by decreasing the level of plasma of alanine transaminase, aspartate aminotransferase, triglyceride, low density lipoprotein and total cholesterol. High cholesterol diet has shown a reduction in glutathione reductase, glutathione peroxidase and enhancement in the expression of glutathione Sα, glutamate-cysteine ligase, sulfiredoxin and paraoxonase-1 genes. The study showed that the levels of expression of certain genes involves the pathway of oxidative stress which were altered by high cholesterol diet feedings, resulting in damage of DNA and hepatotoxicity. Rutin has a hepatoprotective function by increasing the effect of antioxidant and oxidative stress genes amelioration [85].

Gera *et al.* 2020 After analysing numerous stabilisers and stabiliser combinations at varying concentrations, a rutin nanosuspension was created applying the antisolvent precipitation approach. With an increased rutin nanosuspension solubility of 87.632.2 percent compared to 39.772.86 percent pure medication, an optimised nanosuspension revealed a minimal particle size of 122.855.02 nm. Particle size, solubility, and surface area were optimised, which increased gastrointestinal absorption and perceived permeability. The *in vitro* assays with rutin nanosuspensions showed increased cell proliferation, the activity of antioxidants and the production of osteocalcin in osteoblast cells of MG-63. The protective effect of rutinnanosuspensions was demonstrated by the enhanced biochemical *in vivo* biomarkers and strength of bone. The results provided support for rutin nanosuspensions as a possible therapy to maintain health of bone [86].

Pedrozo *et al.2020* Nanoparticles of rutin were synthesised from bovine serum albumin using a nanospray drying technique. Under optimal conditions, spray drying produces

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spherical particles with an average size of 316 nm, zeta potential of 32 mV, and encapsulation effectiveness of roughly 32%. [87].

Sharma *et al.* 2020 have been prepared rutin nanoemulsion loaded with Tocopheryl polyethylene glycol 1000 succinate and oxidative stress induced in rats by ameliorate free radical. It was discovered that nanoemulsions provided a greater relative bioavailability of rutin than did pure drug suspension. When compared to rutin suspension, rutinnanoemulsion had an AUC and Cmax that were 1.8 and 1.9 times higher, respectively. Positive pharmacodynamics outcomes have been observed in rutinnanoemulsion clinical trials. Stress was reduced after administration of rutin nanoemulsion and rutin suspension, which increased SOD and GSH levels and decreased brain MDA. At 1 g/ml, anticancer activity was dose-dependent. In a rutin suspension, the IC50 was 36.7%, while in a rutin nanoemulsion, it was 25.4%. An effective method for lowering oxidative stress, rutin nanoemulsions have been demonstrated. [88].

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CHAPTER 3

SCOPE, AIM, OBJECTIVE AND PLAN OF WORK

3.1 SCOPE:

Polymeric nanoparticles of the rutin drug were prepared and further evaluated. Rutin is glycoside which combines disaccharide rutinose and flavonol quercetin. Rutin is drug which has mainly anti-inflammatory and antioxidant effect. It is used in the treatment of arms or leg swelling caused by damage to the lymph system and osteoarthritis. The rutin drug is having low bioavailability due to poor absorption, it also has a high metabolism and rapid excretion. To overcome this problem rutin nanoparticle is prepared. Tamarind seed gum is used in the preparation of nanoparticles to evade the chemical agents uses which having the stabilizing and capping property. The polymer itself acts as a stabilizing agent so the stability of the prepared nanoparticle is getting increased. It is also less toxic and helps in drug targeting. The prepared polymeric nanoparticle shows good anti-inflammation and anti-arthritic properties and will be used as a good therapeutic agent in the treatment of arthritis.

3.2 AIM OF STUDY:

To prepare and evaluate the effect of polymeric nanoparticles for anti-arthritis activity.

3.3 OBJECTIVE OF STUDY:

3.3.1 To prepare polymeric nanoparticle

3.3.2 To evaluate prepared nanoparticles

3.3.3 To evaluate anti-arthritis activity of optimized nanoparticles

3.4 PLAN OF WORK:

3.4.1 Literature review

3.4.2 Selection of drug

3.4.2.1 Rutin drug

3.4.3 Formulation development

3.4.3.1 Nanoparticles formulation

3.4.4 Nanoparticles characterization

3.4.4.1 Physical appearance

3.4.4.2 Particle size and zeta potential

3.4.4.3 Efficiency entrapment and morphology

3.4.4.4 Study of *in vitro* drug release

3.4.4.5 Anti-arthritic activity of optimized formulation

CHAPTER 4

DRUG PROFILE

4.1 Synonyms: Rutin

Quercetin-3-rutinoside, rutoside, eldrin, and sophorin [89].

Molecular Formula: $C_{27}H_{30}O_{16}$ [90]
Molar mass: 610.52 g/mol [91]
Melting point: 190 ^{0}C [90]
Colour: Yellowish colour [92]
Solubility: For instance, acetic acid and pyridine solutions, as well as inorganic base solutions, are soluble, while ether, benzene, toluene, and petroleuxi-ether are not. The Ethylene Dichloride, Carbon Tetrachloride, Chloroform, Chlorobenzene, and 1-Nitropropyl Components [93].

4.2Introduction:

Rutin was initially discovered and investigated in 1842. It's also known as rutinum, vitamin P, sophorin, and quercetin-3-O-rutinoside. This compound is mainly found in many parts of the plant like fruit skins, leaves, flowers and roots. Rutin has a structure that possesses significant chelating, antioxidant and antimicrobial properties [94,95]. Rutin has more antioxidant property among other available antioxidants. It has to scavenge properties on oxidizing species like superoxide radicals, OH radicals and peroxyl radicals [96]. Rutin has limited utility because of its low bioavailability and poor water solubility. Water insolubility prevents rutin from being taken orally as a therapy. [97].

4.3 Sources:

Tobacco leaves were commonly used as a source of rutin. Tobacco's low rutin content and high cost prompted numerous attempts to find appropriate replacements. Buckwheat Phytolacca *(Fagopyrumesculentum)*, *Fagopyrumtartaricum*, flower buds *ofSophora japonica*, and leaves of a few species of *Eucalyptus* have been used for the production of rutin [98].

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4.4 Uses:

This powerful antibacterial, antifungal, and antiallergic medication is effective in treating a wide range of chronic diseases [99], treating Alzheimer's disease while also having antimicrobial, antiprotozoal, antitumor, anti-inflammatory, antiviral, cytoprotective, vasoactive, antiplatelet, antispasmodic, and antihypertensive properties [100]. [101] In the therapy of rheumatoid arthritis[102].

CHAPTER 5

METHODOLOGY

1. RUTIN STANDARDS AND PREPARATION CURVES

a. Calibration medium preparation

0.1N Hydrochloric Acid with 0.1 percent w/v SLS

Add sodium lauryl sulphate (1 gramme) to an 8.5 ml hydrochloric acid in distilled water solution until the volume reads 1000 ml. (IndianPharmacopoeia., 2010).

Phosphate Buffer pH 6.5 with 0.1 percent w/v SLS

A solution of 100 ml, pH 6.5, and 0.1% sodium lauryl sulphate was prepared by adding 41.2 ml of 1M monobasic sodium phosphate monohydrate, 39.2 ml of 0.5M dibasic sodium phosphate anhydrous, and 1 gm of sodium lauryl sulphate to a 1000 ml volumetric flask. First add 900 ml of distilled water, then gradually dilute 1M sodium hydroxide with more distilled water until the desired concentration is reached. (IndianPharmacopoeia., 2010 & U S Pharmacopoeia., 2007).

Phosphate-buffered saline (PBS) with 0.1% sodium lauryl sulphate is used for an incubation period at 7.4 degrees.

Distilled water is mixed with measured amounts of sodium chloride (8 g), potassium dihydrogen phosphate (0.19 g), and disodium hydrogen phosphate (2.38 g) to create a solution of a given concentration (to make up to 1000 ml).

b. Absorption Maximum Estimation (λmax)

The main stock solution (100 g/ml) is made by dissolving ten milligrammes of Rutin into one hundred millilitres of methanol. To achieve a concentration of 10 g/ml, the stock solution is diluted with either 0.1N Hydrochloric acid containing 0.1 percent w/v SLS or

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pH 6.5 phosphate buffer containing 0.1 percent w/v SLS. Following generation, the solutions are scanned in the UV Spectrophotometer's 200-400nm band for maximum absorption.

c. Preparation of standard curves

It is possible to generate a series of solutions with concentrations ranging from 5 to 25g/ml by diluting the original stock solution with 0.1N Hydrochloric acid containing 0.1 percent w/v SLS. The maximum absorbance of the solutions can be found at 362 nm (max) by using a UV- spectrophotometer (max). A standard curve is generated by plotting the absorbance (y-axis) against concentration (X-axis) (X-axis). A standard curve is also presented using a pH 6.5 SLS buffer solution containing 0.1 percent w/v SLS. (Sandeep Kumar D et al., 2011, Patel V P et al2011 & Hariprasanna R C et al., 2010).

2. Drug-poymer interaction studies

Compatibility studies

In order to ensure that a medicine and polymer are compatible, scientists utilise Fourier transform infrared spectroscopy (FT-IR).

a. Application of the Fourier Transform to Infrared Spectroscopy (FT-IR)

Through the use of the KBr disc technique and an FT-IR Spectrophotometer (Shimadzu, Japan), we were able to collect Fourier Transform Infrared spectra of a variety of materials, including a pharmaceutical (Rutin), polymers (Eudragit L & S 100), and a surfactant (Triclosan) (Pluronic F 68 & Polyvinyl Alcohol). This is crucial to determining if the polymer and drug will react with one another. A hydraulic press is used to compress samples of a known weight with KBr powder at 150 bar for 30 seconds, resulting in discs with a diameter of 10 mm. By comparing the spectra, we can track how the functional peaks shift over time and whether any new peaks emerge. Over a scanning range of 400 to 4000cm-1, the resolution is 4cm-1. (Mishra B et al., 2010).

3. FORMULATION OF RUTIN LOADED POLYMER NANOPARTICLES

The Rutin-loaded polymeric nanoparticles are prepared via solvent displacement/nanoprecipitation. (Bivash Mandal et al., 2010, Suganeswari M et al., 2011 & Srinivas P et al., 2012).

Different quantities of the polymers eudragit L 100 and eudragit S 100 create an organic phase when added to a solution containing 10 mg of Rutin in 10 ml of methanol (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, and 1:70). Aqueous phase contains water stabilized with 1% pluronic F 68 and 1% polyvinyl alcohol, and it occupies 20% of the total volume. While the aqueous mixture is still being stirred, the organic component is added slowly. When a polymer solution dissolved in an organic solvent is combined with a miscible non-solvent of the polymer (usually water), a solid is formed by the process known as nanoprecipitation. When the composition is within a limited range, the polymer precipitates out almost instantly, and the organic solvent evaporates over the period of three to four hours of steady stirring to create nanoparticles. Drug-free nanoparticles undergo the same processing processes as their drug-containing counterparts. (Nepolean R et al., 2012).

4. CHARACTERIZATION OF RUTIN POLYMERIC NANOPARTICLES

Drug concentration, entrapment efficiency, particle size and polydispersity index, zeta potential measurement, in vitro drug release testing, and kinetics of release are all evaluated for the Rutin polymeric nanoparticles generated.

a. Drug analysis for potency

The concentration of the pharmaceutical ingredient is measured by ultraviolet (UV) spectrophotometric analysis. One milligramme of Rutin in polymeric nanosuspension is dissolved in one millilitre of methanol, and then ten times that quantity is diluted into one

hundred millilitres of buffer solution. The absorbance of the aforementioned solution can be determined by observing its spectrum in a UV-visible spectrometer set to 362 nm. The following formula can be used to calculate the total dosage from a standard curve:

b. Determination of Entrapment efficiency

Ultracentrifugation (Eppendorf Centrifuge, 5417R, Germany) at 14,000 rpm for 90 min at 4 C is used to remove the aqueous medium and calculate the amount of Rutin bound to the polymeric nanoparticles (entrapped drug). An absorbance reading at 362 nm made using a UV- spectrophotometer can be used to calculate the concentration of free medication after a sample of the supernatant liquid has been collected, diluted with the buffer solution, and then analysed. Subtracting the total drug content from the quantity of drug that remains in the aqueous medium after entrapment yields the amount of Rutin entrapped in polymeric nanoparticle formulation. For instance, consider the following illustration of the formula for determining entrapment efficiency (EE):

c. Size and Zeta-potential of Individual Particles

Measurements of the size, polydispersity index (PI), and zeta potential of a suspension of polymeric nanoparticles loaded with Rutin using a Zetasizer 3000 and the dynamic light scattering method (Malvern Instruments, UK). In addition to the average particle size and standard deviation, the width of the particle size distribution is also quantified (polydispersity index,PI). Dilution with sterile water and uniform 90 degree spreading of the nanoparticle suspension precedes analysis. When it comes to measuring the physical stability of colloidal dispersions, the zeta potential provides an extremely accurate way. The zeta potential of a particle is a measure of its surface charge. (Rezaei Mokarram A et al., 2010 & Srinivas P et al., 2012).

d. *in vitro* drug release studies

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Here, utilizing an in vitro drug release setup consisting of a dialysis bag, we assess the diffusion mechanism of a polymeric nanosuspension containing Rutin. For this method, a dialysis bag is filled with a nanoparticle suspension containing 1 mg of Rutin and sealed at both ends. During dialysis, both the donor solution in the dialysis bag and the receiver solution in the beaker are kept at 37 C2 C and circulated at a slow rate.

Dialysis bags are soaked for two hours in 0.1N hydrochloric acid with 0.1 percent w/v SLS acid buffer, and then the acid buffer is replaced with a pH 6.5 solution containing phosphate buffer. Aliquots of the diffusion medium, usually 5 mL, are removed at regular intervals and replaced with the same volume of buffer solution to keep the sink constant. Samples are taken every 15 minutes for the first two hours, and then every 30 minutes for the remaining ten hours. Next, an absorbance reading at 362 nm is taken with a UV-spectrophotometer to get an idea of how much dissolved Rutin there is. Aside from that, the experiment is replicated three times with no crossover between the duplicates. (Mishra B et al., 2010 & Umar faruksha A et al., 2013).

c. Research into the drug-release kinetics

In vitro drug release studies of nanoparticles can shed light on the kinetics and mechanism of drug release by fitting the data to several kinetic equations, such as the first-order rate equation.

1. Zero order (cumulative %release vs time).
2. First order (log % drug remaining vs time).
3. Higuchi's model (cumulative %drug release vs square root of time).
4. Hixon-Crowell cube root law.
5. Korsmeyer-papas model.

1. The zero-order equation describes a system where changes in drug concentration do not alter the rate of drug release.

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$$C = k_0 t$$

The drug concentration at time t is denoted by C, while the release rate constant at time t = 0 is denoted by k0.

2. A first order equation is used to characterise the release when the rate of release changes as a function of concentration, as it does in this system.

$$\log C = \log C_0 - kt / 2.303$$

In this equation, C represents the current drug concentration at time t, C0 represents the initial drug concentration, and k represents the first-order release rate constant.

3. Higuchi postulated that drug release from a porous, insoluble matrix would be a Fickian diffusion-based, square-root-of-time dependent process, and he provided the diagram below to illustrate his theory.

$$Q = kt^{1/2}$$

In which Q is the dose of medication released at time t.

4. The Hixson-Crowell cube root law characterises the release when there is a change in the particle's surface area or size.

$$W_0^{1/3} - W_t^{1/3} = \kappa t$$

To calculate the remaining drug load at time t, we use the following formula: where W0 is the initial drug load, Wt is the drug load at time t, and is the constant that includes the surface-volume relationship.

5. Korsmeyer – According to Peppas's model, the rate of drug release exponentially rises over time.

$$M_t/M_\infty = Kt^n$$

This equation shows the release exponent (n), the release rate constant (k), and the fraction of medicine released at time t (Mt/M). (Abdul Hasan Sathali A and Gopinath M etal., 2013 & Bivash Mandal et al., 2010).

f. Best-formulation selection and assessment

Analyzing particle size, entrapment efficiency, in vitro drug release studies, and drug release dynamics helps researchers determine the most effective formulations.

i. Making Nanosuspensions from Lyophilized Powders

Lyophilizing Rutin nanosuspensions in a freeze drier enhances their chemical stability (Lyodel- Delvac Pumps Pvt. Ltd, USA). The newly made nanosuspensions are lyophilized using a cryoprotectant (mannitol). To sum up, Rutin nanosuspensions are rapidly cooled to -500C for 2 hours, followed by primary drying at I.03 mbar and secondary drying at 0.001 mbar.

Then, the entrapment effectiveness and In vitro drug release of the lyophilized polymeric nanoparticles (F7 EL 100 with 1% pluronic F68) are compared to those of the pure drug (Rutin). (Rainer H Muller et.al., 2008).

ii. Analysis by means of infrared spectroscopy

Following the technique outlined in drug polymer interaction studies 2(a), an infrared (IR) spectrum analysis is performed on the chosen optimal formulation in order to determine the interactions between the drug and the excipients used 2(a).

iii. Researches on the Measuring of Solubility

Purified Rutin's solubility and the best formulations of Rutin loaded polymeric nanoparticulate suspension in distilled water and phosphate buffer pH 6.5 with 0.1 percent w/v SLS are evaluated using mechanical shakers. To a 250 ml stoppered conical flask

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holding 10 ml of their respective solvents, 1 mg of pure Rutin and nanosuspension are added. The hermetically sealed flasks are shaken for twenty-four hours by a mechanical system. After filtering an aliquot, the filtrate is diluted appropriately and put through its paces on a UV-spectrophotometer. Scanning electron microscopy morphology of polymeric nanoparticles

Scanning electron microscopy revealed the asymmetrical shape of polymeric nanoparticles in a Rutin dispersion (S-4800, Hitachi Technologies Corporation, Japan). Samples are sprayed with a thin layer of gold in a vacuum before being mounted on metal stubs for analysis. An acceleration voltage of 1.5kv is usual for SEMs.

CHAPTER 6

RESULTS

i. RUTIN STANDARDS AND PREPARATION CURVES

ii. Calibration medium preparation

Distilled water, 0.1N hydrochloric acid, phosphate buffer pH6.5, and phosphate buffer pH7.4 were all produced with 0.1 percent w/v SLS for use as calibration media in accordance with the Indian pharmacopoeia.

b. Absorption Maximum Estimation

We calculated the max absorption of Rutin by scanning a 10 g/ml solution in the UV- region (200- 400nm) (200- 400nm). Maximum observed spectral intensity was recorded at 362.nm in distilled water containing 0.1% w/v SLS, in 0.1N hydrochloric acid containing 0.1% w/v SLS, in phosphate buffer at pH 6.5, and in phosphate buffer at pH7.4 (20a-20d).

c. Rutin standard curves are being prepared for use.

Distilled water, 0.1N hydrochloric acid with 0.1 percent w/v SLS, phosphate buffer pH 6.5 with 0.1 percent w/v SLS, and these were used to make Rutin standard curves. The UV-Spectrophotometer was used to determine the absorbance of the solutions between 5 and 25 g/ml. The obtained linear correlation coefficient for 0.1N hydrochloric acid with 0.1 percent w/v SLS was found to be the same as that for phosphate buffer pH6.5 with 0.1 percent w/v SLS, at = 0.999. Within the concentration range of (5-25g/ml), Rutin follows the Beer's law. A plot of Rutin's standard curves was presented in.

(Tables 1A-1D) and (Figure 1) (20e-20h).(Sandeep Kumar D et al., 2011, Patel V P et al.,2011, Hariprasanna R C et al., 2010, Raghavendra Rao N G et al., 2010).

2. INTERACTION STUDIES BETWEEN DRUGS AND POLYMERS

a. Analysis by means of infrared spectroscopy

We used infrared spectroscopy to confirm that the nanoparticles and the polymers used to make them were completely safe for human consumption. Both pure pharmaceuticals and polymers, as well as a composite of the two, were tested using infrared spectroscopy. This diagram presented the entire spectrum, from 400 to 4000 nanometers (Figure 21a-21i).

There was no detectable functional peak shifting when comparing the IR spectrum of pure drug to spectra of physical mixes of drug and the polymers.

S.NO	CHARACTERISTICS	WAVE NUMBER (cm^{-1})
1	N-H stretching	3095.85 cm^{-1}
2	C-H stretching in CH_2	2963.93 cm^{-1}
3	C-H stretching	2902.96 cm-1
4	C=O stretching	1696.45 cm^{-1}
5	C=C stretching	1662.69 cm-1
6	C-N stretching	1363.72 cm^{-1}
7	C-C stretching	1172.76 cm-1
8	C-OCH_3 stretching	1058.96 cm-1
9	C-Cl	705.01 cm^{-1}

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All the primary peaks seen in the pure drug spectra were present, with only modest modifications, in the spectra of the drug combos. Research showed that there were no negative interactions between the drug and the excipients.

3. FORMULATION OF RUTIN LOADED POLYMERIC NANOPARTICLES

Polymeric nanoparticles of eudragit L100 and eudragit S100 were prepared using nanoprecipitation. Fessi et al.,1992 devised a straightforward and replicable method. Using this method, a polymer is deposited at the solution/air interface after a semi-polar solvent miscible with water is removed from a lipophilic solution.(Bivash Mandal et al., 2010, Ugo Bilati et al., 2005 &Snjezana Stolnik et al.,1999).

Polymers (Eudragit L100 and Eudragit S100) and stabilisers (Pluronic F68 and Polyvinyl alcohol, both at 1 percent) were mixed in varying proportions to create Formulations F1 through F28 (1:10,1:20,1:30,1:40,1:50,1:60 & 1:70). The outcomes of varying polymer to surfactant concentrations and vice versa are shown in Table 1. (2A-AD).(Swarnali Das et al.,2010).

After dissolving the polymers (Eudragit L100 and Eudragit S100) in an organic solvent (methanol), with or without Rutin, a colloidal suspension was prepared by slowly adding the organic phase into the stirred surfactant aqueous phase containing a concentration of 1% pluronic F68 and 1% polyvinyl alcohol. Polymer deposition at the interface between the organic phase and water instantly resulted in the formation of a colloidal suspension after the introduction of a relatively water-miscible organic solvent.

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Each intermediate particle dissipated into the surrounding water quite quickly due to methanol diffusion.

4. CHARACTERIZATION OF RUTIN LOADED POLYMERIC NANOPARTICLES

a. Drug analysis for potency

Table 1 demonstrates that the drug content of each final formulation (F1-F28) was between 91.08 and 94.82 percent (3A-3D). All of the nanoparticle formulations showed signs of equal medication distribution.

b. Measuring the effectiveness of trapping

For polymeric nanoparticle formulations, entrapment efficiency was considerably affected by polymer and stabiliser content. The ranges of entrapment efficiency of formulations F1-F28 were 29.72 percent to 63.95 percent as reported in Table(4A-4D) and Figure(22).

F7 eudragit L100 with 1% pluronic F68 was determined to have the highest entrapment efficiency (as a percentage) among the formulations tested (F1-F28). It's possible that this is due to the higher polymer content (1:70) in the nanoparticle formulation. Maximum entrapment of Rutin was achieved by manipulating fabrication parameters including drug-polymer ratios and the amounts of stabilising agents.

i. Drug-polymer ratio effects

Varied drug-polymer ratios resulted in drastically different entrapment efficiencies for eudragit L100 and eudragit S100 nanoparticles loaded with felopidipine. The results are shown in Tables 4A-4D and Figures 2A-2D. (22)

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The entrapment efficiencies ranged from 29.72% to 63.95% across seven distinct formulations (F1–F7) made with varied polymer (Eudragit L100):stabilizer ratios (1:10,1:20,1:30,1:30,1:40,1:50,1:60,1:70) containing 1% pluronic F68. The formulations F8-F14 showed entrapment efficiencies of 30.81 percent, 34.9 percent, 43.3 percent, 51.0 percent, and 59.38 percent, respectively, using various ratios of polymer (Eudragit L100) (1:10,1:20,1:30,1:40,1:50,1:60&1:70) with 1 percent polyvinyl alcohol as stabiliser. Entrapment efficiencies ranged from 31.55% in formulation F15 to 58.025% in formulation F21, which used different polymer (Eudragit S100):stabilizer (1:10,1:20,1:30,1:40,1:50,1:60,1:70) ratios. Efficiency of entrapment is best at F15, followed by F16, F17, F18, F19, F20, and finally F21.

Depending on the polymer (Eudragit S100) ratio utilised, the entrapment efficiency of the formulations F22-F28, which all contained 1% polyvinyl alcohol as a stabiliser, ranged from 30.45 to 34.9%. (1:10,1:20,1:30,1:40,1:50,1:60, &1:70). There was a rise in entrapment effectiveness from filter sizes F22 to F23 to F24 to F25 to F26 to F27 to F28.

Research showed that a 1:70 ratio of eudragit L100 to Rutin in nanoparticle formulations resulted in the best entrapment efficiency (1:10,1:20,1:30,1:40,1:50, &1:60). It was hypothesized that Rutin's high entrapment effectiveness was due to a combination of circumstances, including the drug's strong affinity in methanol and the polymer's high solubility in water. By increasing the viscosity of the organic phase, which in turn increases the diffusional barrier to drug molecules from the organic phase to the aqueous phase, one can increase the amount of drug trapped in the polymeric nanoparticles by increasing the polymer concentration in the organic phase.

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ii. Influence of stabiliser concentration

The effect of stabilisers (Pluronic F68 and Polyvinyl alcohol) on the entrapment efficiency of Rutin-loaded eudragit L100 and eudragit S100 nanoparticles was also studied (Pluronic F68 and Polyvinyl alcohol). The outcomes are shown in Tables 4A–4D and Figure 4. (22). (22). To better encapsulate Rutin, pluronic F68 polymeric nanoparticles were created and compared to polyvinyl alcohol.

It was shown that pluronic F68 polymeric nanoparticles entrapped Rutin more effectively than polyvinyl alcohol nanoparticles. (Anilkumar J Shinde et al., 2011).

c. Polydispersity index, zeta potential, and particle size determination

Through the use of dynamic light scattering microscopy, the nanoparticle diameter (Z-AVE), polydispersity index (PI), and zeta potential were investigated. (Annick Ludwig et al.,2006).

Particles smaller than 400 nm were favoured in the development of pharmaceutical procedures. High entrapment efficiency was achieved with mean particle sizes of 192.4nm, 238.7nm, 210.3nm, and 298.4nm for Formulations F7, F14, F21, and F28, respectively (Table(5A) & (Figure23B)).

Variations in drug/polymer ratios, surfactant concentration, and external phase volume led to large differences in particle size across formulations (23d).(Jawahar N et al.,2009).

i). Particle size and the drug-polymer ratio:

It was investigated how the size of Rutin-loaded eudragit L100 and eudragit S100 nanoparticles changed when the drug-polymer ratio changed from 1:10 to

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1:70. The results were laid out in a table for easy viewing. (5A&5B)&(Figure23a-23d).

The F7 and F14 formulations were both created with polymer (Eudragit L 100) (1:70) that included 1% pluronic F68 & 1% polyvinyl alcohol as a stabiliser, and their particle sizes were measured to be 192.4 nm and 238.7 nm, respectively. There was a 7–14-fold increase in the typical particle size.

The F21 and F28 formulations, both of which used polymer (Eudragit S 100) (1:70) containing 1% pluronic F68 and 1% polyvinyl alcohol as a stabiliser, had particle sizes of 210.3 nm and 298.4 nm, respectively. The average particle size increased from the F21 to F28 range.

Nanoparticles were shown to increase in size with increasing polymer content. In other words, the size of the Rutin-loaded polymeric nanoparticles increased as their concentration of (Eudragit L100 & Eudragit S100) increased.

This is so because a higher concentration of polymer leads to a thicker organic phase. In this way, the strong viscous resistance to the shear forces limits the creation of nanoparticles. Mass transfer is slowed down, polymer-solvent phase diffusion into the surrounding aqueous phase is reduced, and larger nanoparticles are formed when the organic phase is thicker. (Annick Ludwig et al.,2006).

ii). Particle Size and Stabilizer Effects

At a concentration of 1%, Pluronic F68 and Polyvinyl alcohol were used to create Rutin polymeric nanoparticles. (5A&5B)&(Figure23a-23d).

The average particle size of Formula F7, which included plutonium F68 at a concentration of 1%, was 192.4nm.

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The average particle size of Formula F14, which was produced using 1% polyvinyl alcohol, was measured to be 238.7nm.

The mean particle size of Formula F21, which is 1% pluronic F68, was determined to be 210.3nm.

The mean particle size of Formula F28 with 1% polyvinyl alcohol was calculated to be 298.4nm.

Therefore, it was concluded that a higher concentration of stabilisers could be oriented at the organic solvent/water interface to efficiently reduce the interfacial tension, thereby significantly increasing the net shear stress at a constant energy density during emulsification and encouraging the formation of smaller emulsion droplets (Anilkumar J Shinde et al., 2010).

Binding to the surface of nanoparticles is essential for stabilising them in a polymeric nanosuspension. It has been demonstrated that the hydrophobic polyoxypropylene centre block of Pluronic F68 strongly adsorbed to the surface of hydrophobic nanoparticles, stabilising them.

Formulations containing pluronic F68 were found to have a particle size range of 192.4nm-210.3nm (F7, EL 100 and F21 ES 100). For the polyvinyl alcohol-based formulations, particle size was measured at 238.7nm and 298.4nm (F14 EL 100 and F28 ES 100, respectively). The formulation was stabilised using pluronic F68 and polyvinyl alcohol. Average particle sizes were found to be lower for pluronic F68 than for polyvinyl alcohol. (Annick Ludwig et al.,2006 & Jawahar N et al.,2009).

Measure of Polydispersity

The polydispersity index (PI) is a key factor in defining the nanosuspension's physical stability, so it's ideal to keep it as low as possible. Tables

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(5A, 5B) showcased four different formulations (F7, F14, F21, and F28) with a high polydispersity index for effective entrapping (Figure23a-23d). The degree of uniformity in a distribution can be measured on a scale from 0 to 1. The closer to zero the value, the more uniform the distribution, and the higher the value, the more dispersed the data.

Formulations (F7&F14) made up of EL 100 and ES 100 at 1 percent pluronic F68 and 1 percent polyvinyl alcohol had polydispersity indices between 0.132 and 0.165.

When using 1% pluronic F68 and 1% polyvinyl alcohol, formulations (F21&F28) were made using EL 100 and ES 100 with a polydispersity index of 0.159-0.172.

Every one of their PI values was lower than 0.3, indicating that they were all rather close together. (Kristl B et al., 2002).

Zeta Potential

Colloidal stability of the final formulations was evaluated with the Zeta potential. The effect of various polymers and stabilisers on the surface charge of nanoparticles was investigated by measuring their zeta potential in solutions of Rutin-loaded polymeric nanoparticles. The results can be seen in Tables 6A and 6B, as well as in Figure 6. (24a-24d).

Formulations F7 and F14 made with EL 100 showed negative zeta potential (-19.4mV and -12.1mV, respectively) (-19.4mV& -12.1mV).

Incorporating ES 100 resulted in a negative zeta potential in formulations F21 and F28 (-16.2mV & -10.9mV).

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Because of the polymer carboxyl groups attached to the nanoparticles' surfaces, they were discovered to exhibit a negative zeta potential (Eudragit L100 & Eudragit S100). It is preferable to achieve high potential values because doing so guarantees a robust energy barrier and favours a good stability. According to Muller, nanoparticles are most effectively stabilised at a zeta potential of roughly -25mV, where repulsions prevent age-related aggregation. A nanosuspension needs a zeta potential of at least 30mV to be electrostatically stable, and a zeta potential of at least 20mV is needed for steric and electrostatic stabilisation. (Dianrui Zhang et al., 2012 & Philippe Maincent et al.,1998).

d. Invitro release studies of Rutin loaded polymeric nanoparticle using dialysis membrane.

The dialysis bag diffusion method was utilised to examine the in vitro release of Rutin from the polymeric nanoparticles to get insight into this phenomenon. Research into the first two hours of the release was done in 0.1N hydrochloric acid with 0.1 percent w/v of SLS.

Since the nanoparticles were meant to be ingested by the subjects in this investigation, they were kept at Phosphate buffer pH6.5 with 0.1 percent w/v of SLS for the following 10 hours to simulate the in vivo state. Nanoparticles were retained by the dialysis bag and the diffusion of the medication to the cell's receptor site without any delays.

Different concentrations of Eudragit L100 with 1% pluronic F68 (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, and 1:70) resulted in initial burst releases of 19.67%, 18.03%, 18.91%, 18.42%, 18.50%, and 18.66%, respectively, in formulations F1 through F7.

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At 2 hours, the rate was 15.71 percent; at 72 hours, it was 72.69 percent; at 48 hours, it was 69.30 percent; at 48 hours, it was 67.40 percent; at 48 minutes, it was 63.01 percent; at 48 minutes, it was 59.69 percent; at 48 minutes, it was 15.71 percent.

At 12 hours, the percentage was 56.12 percent. It was found that the cumulative percentage of drug release decreased from F1 > F2 > F2 > F5 > F6 > F7.

Initial burst releases of 21.40 percent, 20.33 percent, 18.92 percent, 18.46 percent, 17.25 percent were observed in formulations F8 through F14 that were created using varied concentrations (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, and 1:70) of Eudragit L100 with 1% Polyvinyl alcohol.

16 pct at 2 hours; 15 pct at 2 hrs; 17 pct at 4 hrs; 20 pct at 6 hrs; 25 pct at 12 hrs; 20 pct at 24 hrs; 15 pct at 48 hrs; 20 pct at 48 hrs;

12 hours later, that number rose to 57.36 percent. Reduced cumulative drug release occurred from F8 > F9 > F10 > F11 > F12 > F13 > F14.

The initial burst release of Eudragit S100 in the formulations F15–F21 created at various concentrations (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, and 1:70) with 1% Pluronic F68 ranged from 21.74 percent to 18.73 percent.

Results: 15.96%, 16.15% at 2 hours; 75.60%, 73.12%, 70.382%, 66.292%, 65.202%, 60.022%,

A corresponding 58.21 percent at 12 hours.There was a reduction in the overall percentage of drug release, and it was on the order of. When ranked in order of increasing speed, F15 > F16 > F17 > F18 > F19 > F20 > F21.

The initial burst release of Eudragit S100 in the formulations F22–F28 created with varied concentrations of the compound (1:10, 1:20, 1:30, 1:40, 1:50, 1:60, and 1:70) was 21.56–21.22 percent, 20.06–18.91 percent, 18.10–18.10 percent, and 18.10–18.10 percent, respectively.

There were 16.00 percent, 15.26% at 2 hours, 75.62%, 71.80%, 67.52%, 67.56%, 66.46%, 61.85%, and 61.84%, respectively.

At 12 hours, the percentage was 58.55. It was in the range of a reduction of that the total percentage of medication release was slowed down. To rank them in order: F22 > F23 > F24 > F25 > F26 > F27 > F28.

Table. (7A-7D) & Figure. presented the cumulative percent drug release for all the produced formulations (F1-F28).

(25a-25d). It is possible that the increased nanoparticle surface, leading to a larger drug fraction exposed to the dissolution medium, and the higher amount of drug loading explain why nanoparticles prepared with a smaller size and lower amount of Eudragit L100 & Eudragit S100 showed a higher drug release rate. Drug release rates were slower from nanoparticles of bigger size that had been synthesised using a greater concentration of Eudragit L100 and Eudragit S100. This may have been because the smaller nanoparticle surface area exposed less of the drug to the dissolution media. As reported by (Jawahar N et al., 2009).

Table.(7A- 7D) & Figure.show that the formulations with a lower polymer ratio(1:20) showed a greater burst release within the first two hours (25a-25d). Because the medication was entrapped in the polymer matrix of the nanoparticles, its release was prolonged by increasing the polymeric concentration up to a (1:70) ratio, which resulted in a sustained release lasting for 12 hours.

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In comparison to the other formulations (F1-F7), F2 has the lowest and highest burst release.

There was a decreasing trend in burst release from formulation F8 through F14, with F8 being the least effective, followed by F9, F10, F11, F12, and F13.

If you compare formulas F15–F21, you'll notice that the burst release decreases from lowest to highest as you move from F15 to F21.

The burst release was reduced from F22 to F28, with F22 being the least, followed by F23, F24, F25, F26, F27, and finally F28.

Drug adsorption in the polymeric surface causes the burst phase, but subsequent hydration of the nano-matrix increases the diffusion channel length of the molecules, slowing the diffusion rate. Therefore, it is reasonable to believe that the relative hydration rate of the polymer and the integrity of the hydrated matrix are critical to the acquisition and maintenance of a regulated release profile. Therefore, the efficacy of entrapment, the avoidance of burst release, the attainment of a regulated release profile, and the maintenance of that profile over time can be used to determine which formulation is preferable. F7 Eudragit L100, 1% Pluronic F68 has a lower burst release profile (15.71%) and higher entrapment efficiency (63.95%) than the other formulations. (Mishra B et al., 2010 & Bivash Mandal et al., 2010).

e. Drug release kinetics

The results acquired from the in vitro release tests were attempted to fit into various mathematical models as follows:

a) Calculating the rate of cumulative drug release over a given period of time (zero order rate kinetics)

b) Total amount of medicine remaining as a log plotted against time (first order rate kinetics)

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c) Annualized medication release % against time squared (Higuchi classical diffusion model)

d) Relationship between the cube root of the amount of medication left and time (Hixon Crowell erosion equation).

e) Cumulative drug release as a logarithmic function of time (Korsmeyer Peppas exponential equation)

Figure displayed a number of different plots, including those of zero order, first order, Higuchi matrix, Korsmeyer-Peppas, and Hixon-Crowell (26-30). Table displayed the r2 values and the n counts for the regression analysis (8A-8D).

To explain how drugs are released, Higuchi uses Fick's law, which states that the rate of diffusion is proportional to the square root of the time it takes for the medication to reach the patient. Drug release from polymeric nanoparticle formulations containing Rutin (F1-F28) was found to increase linearly with the square root of time. Therefore, the higuchi diffusion model (r2=0.976 to 0.988) might be used to describe the drug release rate. All formulations had high correlation coefficients for first order drug release kinetics (r2 = 0.975–0.991).

The literature provides a range of n values appropriate for cylindrical, spherical, and slab geometries. Fickian diffusion, anomalous transport, case II transport, and super case II transport are all associated with values of n between 0.5 and 1.0 for spherical geometries. The tabular results showed that the drug release mechanism followed non- fickian diffusion, with exponent n values within 0.5n.(Abdul Hasan Sathali A and Priyanka et al.,2012, Annick Ludwig et al., 2006 & Prasanthi. B et al., 2012).

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f. Best-formulation selection and assessment

Characterization of F7 EL 100 with 1% Pluronic F68 Based on the Above Data was chosen because it provided the clearest demonstration of,

Particle size	: 192.4
nm.Entrapment efficiency	: 63.95%
In vitro drug release	: 56.12% in 12 hours
Release kinetics	: Closest linearity to first order kinetics.

i. Preparation of Lyophilized Nanosuspensions

Lyophilization of nanosuspensions to obtain the polymeric\s\nanoparticles.

Examining the trapping efficiency and in vitro release

The entrapment efficiency the formulation (F7 Eudragit L 100 with 1 percent Pluronic F68) including pluronic F68 as stabiliser demonstrated greater entrapment efficiency. The entrapment efficiency of lyophilized polymeric nanoparticles is estimated to be 62.82 percent. Due to significant trapping, drug release from polymeric nanoparticles was sustained. This drug's release profile, if accurate, would mean that 55.37 percent would be absorbed after 12 hours. According to Table. (7E) & Figure., it was discovered that the particle size reduction used in the F7 EL 100-formulation resulted in a higher cumulative percentage of drug release compared to the pure drug (25e).

ii. Infra-red spectrum analysis

The optimal formulation (F7 EL 100 with 1% Pluronic F68) was recorded and its IR spectra is displayed in Figure (31). Spectra of the pure medication (Rutin) showed peaks at 3095.85, 2963.93, 2902.96, 1696.45, 1662.69, 1363.72, 1172.76,

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1058.96, 705.01 cm-1. When the resulting IR spectrum of pure medicine was compared to the spectra of the best formulations, it was found that the functional peaks had not moved. Without any noticeable shifts, the best formulations retained all the prominent peaks seen in the spectrum of the pure medication. Based on the data, it was determined that the medicine and formulation excipients did not interact with one another.(Abdul Hasan Sathali A and Priyanka K et al.,2012).

iii. Solubility measurement studies

The optimal formulation (F7 EL 100 with 1% Pluronic F68) and the pure medication's solubility in distilled water and phosphate buffer pH 6.5 with 0.1 percent SLS are shown in Table(9) and Figure(9), respectively (32a&32b). For formulation F7 EL 100 containing 1% pluronic F68, the solubility in distilled water was 117.23 g/ml, while that of pure medicine was 29.91 g/ml. Formulation F7 EL 100 with 1% pluronic F68 was found to be 260.58 g/ml soluble and pure medication was found to be 43.89 g/ml soluble in a phosphate buffer at pH6.5 containing 0.1 percent SLS. Table(9) and Figure(9) show that the most effective formulations had the highest solubility in both distilled water and phosphate buffer pH6.5 with 0.1 percent SLS, in comparison to the pure medicine (32a&32b) (Dianrui Zhang et al., 2012, Arunkumar N et al., 2009 & Abdul Hasan Sathali A and Gopinath M et al.,2013).

iv. Morphology of polymeric nanoparticles by SEM examination

In order to evaluate the shape of nanoparticle formulations SEM examination was used. In Figure, you can see a scanning electron micrograph (SEM) of one of the winning formulations, F7 (Rutin+Eudragit L100+1 percent Pluronic F68) (33). As can be seen from the data, the nanoparticles have a spherical

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form and are significantly smaller than 1 m. (Julijana Kristl et al.,2009 &.Abdul Hasan Sathali A and Priyanka K et al.,2012).

v. ***Intestinal permeability testing outside of the living organism***

Figure and Table (10A-10C) displayed the obtained data (34a - 34c).

After 2 hours, the duodenum of the intestine showed a cumulative permeability of around 0.22 mg for pure drug solution and about 0.85 mg (F7) for the polymeric nanoparticle formulation.

Similarly in the jejunum region likewise, the cumulative amount of drug permeated for pure drug solution was 0.25 mg and for the polymeric nanoparticle formulations had the permeability of 0.89 mg (F7) at the end of 2 hrs. Similar findings were made in the ileum of rat intestines. After 2 hours, the total drug permeability through the pure drug solution was 0.23 mg, while the permeability through the solid lipid nanoparticle formulations was 0.87 mg (F7). F7 EL 100 with 1% Pluronic F68 > Pure medication was found to have increased intestinal permeability.

According to the findings, nanoparticle formulations have increased drug permeability compared to the pure drug solution.

WATER WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.080±0.0015
2	10	0.181±0.0015
3	15	0.265±0.0020
4	20	0.361±0.0035
5	25	0.448±0.0026

n =3*

γ = 0.999564

TABLE 1B: CALIBRATION CURVE OF RUTIN USING 0.1N HYDROCHLORIC ACID WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.087±0.0015
2	10	0.186±0.0020
3	15	0.270±0.0025
4	20	0.363±0.0045
5	25	0.456±0.0041

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BUFFER pH 6.5 WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.086±0.0017
2	10	0.187±0.0015
3	15	0.270±0.0025
4	20	0.364±0.0015
5	25	0.447±0.0026

n =3*

γ = 0.999459

TABLE 1D: CALIBRATION CURVE OF RUTIN USING PHOSPHATE BUFFER pH 7.4 WITH 0.1% SLS

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE± SD
1	5	0.079±0.0015
2	10	0.176±0.0015
3	15	0.269±0.0025
4	20	0.368±0.0026
5	25	0.450±0.0025

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TABLE 2A: COMPOSITION OF RUTIN LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING1% PLURONIC F68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT L 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F1	1:10	10mg	100mg	1%	-
F2	1:20	10mg	200mg	1%	-
F3	1:30	10mg	300mg	1%	-
F4	1:40	10mg	400mg	1%	-
F5	1:50	10mg	500mg	1%	-
F6	1:60	10mg	600mg	1%	-
F7	1:70	10mg	700mg	1%	-

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TABLE 2B: COMPOSITION OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT L 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F8	1:10	10mg	100mg	-	1%
F9	1:20	10mg	200mg	-	1%
F10	1:30	10mg	300mg	-	1%
F11	1:40	10mg	400mg	-	1%
F12	1:50	10mg	500mg	-	1%
F13	1:60	10mg	600mg	-	1%
F14	1:70	10mg	700mg	-	1%

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TABLE 2C: COMPOSITION OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT S 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F15	1:10	10mg	100mg	1%	-
F16	1:20	10mg	200mg	1%	-
F17	1:30	10mg	300mg	1%	-
F18	1:40	10mg	400mg	1%	-
F19	1:50	10mg	500mg	1%	-
F20	1:60	10mg	600mg	1%	-
F21	1:70	10mg	700mg	1%	-

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TABLE 2D: COMPOSITION OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING1% PLURONIC F 68 AND 1% POLYVINYL ALCOHOL AS STABILIZER.

FORMULATION CODE	DRUG:POLYMER RATIO	WEIGHT OF DRUG	EUDRAGIT S 100	CONCENTRATION OF PLURONIC F68	CONCENTRATION OF POLYVINYL ALCOHOL
F22	1:10	10mg	100mg	-	1%
F23	1:20	10mg	200mg	-	1%
F24	1:30	10mg	300mg	-	1%
F25	1:40	10mg	400mg	-	1%
F26	1:50	10mg	500mg	-	1%
F27	1:60	10mg	600mg	-	1%
F28	1:70	10mg	700mg	-	1%

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TABLE 3A: DRUG CONTENT OF RUTIN LOADED EUDRAGIT L100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F1	93.58%±2.14
2	F2	91.08%±0.81
3	F3	92.68%±1.11
4	F4	93.75%±1.63
5	F5	92.50%±1.60
6	F6	93.04%±2.44
7	F7	93.04%±1.60

n=3*

TABLE 3B: DRUG CONTENT OF RUTIN LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F8	93.04%±1.92
2	F9	92.33%±2.16
3	F10	93.22%±2.16
4	F11	91.26%±1.34
5	F12	91.08%±2.94
6	F13	91.79%±1.63
7	F14	93.04%±2.14

INNOVATIVE COLLEGE OF PHARMACY
27.07
PRINCIPAL

TABLE 3C: DRUG CONTENT OF RUTIN LOADED EUDRAGIT S100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F15	94.82% ±1.11
2	F16	92.86% ±3.47
3	F17	94.82% ±2.69
4	F18	92.15% ±2.22
5	F19	93.22% ±2.41
6	F20	93.04% ±2.44
7	F21	91.26% ±1.11

n=3*

TABLE 3D: DRUG CONTENT OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	DRUG CONTENT (%) ± SD*
1	F22	94.82% ±2.16
2	F23	94.29% ±3.48
3	F24	93.93% ±1.72
4	F25	93.58% ±2.14
5	F26	92.51% ±2.14
6	F27	92.15% ±1.71

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

EUDRAGIT L 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY (%) ± SD*
1	F1	30.05±3.53
2	F2	29.72±3.27
3	F3	31.90±3.20
4	F4	33.65±3.60
5	F5	45.66±1.05
6	F6	57.86±3.07
7	F7	63.95±3.50

n=3*

TABLE 4B: ENTRAPMENT EFFICIENCY OF RUTIN LOADED EUDRAGIT L 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY (%) ± SD*
1	F8	30.81±2.88
2	F9	31.46±0.58
3	F10	34.99±0.63
4	F11	39.42±3.31
5	F12	43.37±3.82
6	F13	51.07±3.33
7	F14	59.38±1.34

INNOVATIVE COLLEGE OF PHARMACY PRINCIPAL

TABLE 4C: ENTRAPMENT EFFICIENCY OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% PLURONIC F 68

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY (%) ± SD*
1	F15	31.55±3.63
2	F16	32.02±0.82
3	F17	33.05±1.38
4	F18	35.75±2.27
5	F19	44.77±2.07
6	F20	50.22±2.22
7	F21	58.02±2.24

n=3*

TABLE 4D: ENTRAPMENT EFFICIENCY OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES USING 1% POLYVINYL ALCOHOL

S.NO	FORMULATION CODE	ENTRAPMENT EFFICIFICENCY (%) ± SD*
1	F22	30.45±0.15
2	F23	31.90±1.72
3	F24	34.12±2.89
4	F25	34.28±2.48
5	F26	40.48±1.51
6	F27	46.02±2.26
7	F28	54.47±1.24

TABLE 5A: PARTICLE SIZE OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	PARTICLE SIZE (nm)	POLYDISPERSITY INDEX
1.	F7	192.4	0.132
2.	F14	238.7	0.165

TABLE 5B: PARTICLE SIZE OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	PARTICLE SIZE (nm)	POLYDISPERSITY INDEX
1.	F21	210.3	0.159
2.	F28	298.4	0.172

TABLE 6A: ZETA POTENTIAL OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F68 & 1% POLYVINYL ALCOHOL AS STABILIZER

S.NO	FORMULATION CODE	ZETA POTENTIAL (mV)
1.	F7	-19.4
2.	F14	-12.1

TABLE 7A: COMPARISON OF *IN VITRO* RELEASE OF RUTIN LOADED EUDRAGITL 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F1 EL 100	F2 EL 100	F3 EL 100	F4 EL 100	F5 EL 100	F6 EL 100	F7 EL 100
1.2	0.25	5.17±0.83	5.17±0.83	4.99±0.54	4.99±0.54	4.99±0.54	4.99±0.54	4.62±0.31
	0.50	6.52±0.81	7.61±0.86	6.69±0.85	6.51±1.15	6.51±0.86	5.97±0.86	6.31±0.56
	0.75	9.20±1.16	9.44±1.16	8.84±1.69	9.74±1.19	9.19±1.44	7.71±1.51	8.80±1.13
	1.00	12.73±0.91	11.88±0.94	12.35±1.51	13.11±1.55	12.17±1.38	9.89±1.51	10.49±1.47
	1.50	15.68±1.47	15.34±1.15	15.83±0.51	15.54±2.37	14.92±0.99	12.71±1.03	12.42±1.25
	2.00	19.67±0.81	18.03±1.12	18.91±0.82	18.42±1.60	18.50±0.98	16.01±0.42	15.71±0.49
6.5	2.50	25.56±0.70	23.83±0.87	25.11±1.70	24.27±1.56	24.45±1.59	21.72±0.94	21.09±1.10
	3.00	27.36±1.18	25.45±0.54	26.74±1.09	25.70±1.87	25.70±1.61	22.80±0.91	21.97±0.91
	3.50	29.43±1.01	27.70±1.14	28.45±0.45	27.20±1.77	28.13±0.98	24.67±1.73	23.44±1.25
	4.00	31.59±1.85	29.86±1.22	30.42±0.62	29.13±1.24	29.55±1.51	26.62±1.24	24.97±1.14
	4.50	33.48±2.52	31.75±1.01	32.66±0.88	31.15±0.90	31.03±0.99	28.47±1.40	26.94±1.18
	5.00	35.63±2.19	34.64±1.53	34.99±1.38	33.43±1.51	32.56±0.99	30.02±1.14	28.44±0.89
	5.50	37.85±1.91	36.35±1.55	36.86±1.19	35.44±1.45	34.89±1.14	31.81±1.24	30.36±1.22
	6.00	39.98±1.43	38.30±1.57	39.16±1.24	37.71±1.46	37.13±0.67	33.49±1.27	31.81±1.23
	6.50	43.48±1.44	40.87±2.20	41.36±1.55	39.69±1.17	39.27±1.09	35.03±1.52	33.48±1.27
	7.00	46.01±2.27	43.53±2.57	43.08±1.65	41.36±1.12	41.48±1.40	36.62±1.26	35.58±1.06
	7.50	48.80±2.04	45.74±2.42	45.78±2.02	43.82±1.46	43.02±1.15	38.81±1.00	37.38±1.36
	8.00	51.31±2.37	48.56±2.79	48.00±2.41	45.99±1.26	44.98±1.18	41.08±1.04	39.61±1.11
	8.50	54.28±2.14	51.47±2.67	50.11±2.50	48.23±1.10	46.98±1.22	42.86±1.17	41.36±1.19
	9.00	56.77±1.90	53.37±3.06	51.91±1.15	50.72±1.32	49.04±1.25	45.44±1.19	43.15±1.45
	9.50	59.34±1.65	56.23±2.74	54.48±1.72	53.10±1.33	51.35±1.59	47.53±0.78	44.81±1.25
	10.00	61.96±1.42	58.80±2.58	56.94±1.80	55.17±1.64	53.53±1.65	49.88±1.63	47.24±1.26
	10.50	64.47±1.42	61.25±2.64	59.09±1.63	57.47±1.62	55.95±1.50	52.28±1.97	49.38±1.30
	11.00	67.03±1.42	63.20±3.15	61.85±2.16	59.83±1.67	58.07±1.75	54.56±2.03	51.57±1.31
	11.50	69.65±1.12	66.66±2.88	64.68±2.22	62.43±1.86	60.42±1.81	57.28±1.78	53.82±1.35
	12.00	72.69±1.41	69.30±2.94	67.40±2.57	65.65±1.16	63.01±1.77	59.69±1.82	56.12±1.40

n=3*

TABLE 7B: COMPARISON OF *IN VITRO* RELEASE OF RUTIN LOADED EUDRAGIT L100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F8 EL 100	F9 EL 100	F10 EL 100	F11 EL 100	F12 EL 100	F13 EL 100	F14 EL 100
1.2	0.25	5.90±0.31	5.53±0.54	5.53±0.54	5.17±0.31	4.62±0.31	4.62±0.31	4.62±0.31
	0.50	8.38±0.84	8.00±0.57	8.00±1.12	7.43±1.09	6.31±0.56	6.31±0.54	6.31±0.56
	0.75	11.52±0.89	10.75±0.88	10.39±0.91	9.98±0.60	8.62±1.17	8.44±0.85	8.44±0.86
	1.00	15.15±1.23	14.17±0.92	13.24±0.62	12.81±0.38	11.03±1.47	10.84±0.58	10.84±0.98
	1.50	18.22±0.96	17.30±0.85	15.85±0.95	15.40±0.59	13.72±1.42	13.34±0.68	12.97±0.65
	2.00	21.40±0.70	20.33±0.78	18.92±0.69	18.46±1.13	17.25±1.34	16.66±0.79	15.73±0.68
6.5	2.50	28.36±1.21	27.27±1.20	25.30±1.12	24.90±1.08	22.60±1.92	22.14±1.11	21.15±0.91
	3.00	29.82±1.37	28.56±1.22	26.75±0.86	26.17±1.08	23.84±1.94	23.76±1.45	22.40±0.92
	3.50	32.27±1.19	30.08±1.22	28.45±1.13	27.51±1.08	25.69±1.96	25.26±1.65	24.07±1.10
	4.00	34.28±1.39	32.60±1.79	30.41±1.15	29.63±0.78	27.62±2.49	27.21±1.20	25.63±0.94
	4.50	36.55±1.61	35.23±1.83	32.45±1.16	31.67±0.77	29.27±2.33	28.49±1.11	27.26±1.27
	5.00	38.55±1.45	37.22±1.61	34.77±0.91	34.34±1.31	31.16±2.05	30.19±0.82	28.95±1.15
	5.50	40.25±1.47	39.29±1.85	36.62±0.70	36.01±1.33	33.32±2.06	32.14±1.12	30.89±1.31
	6.00	42.38±1.82	41.07±1.96	38.90±1.21	37.93±1.09	35.19±1.97	34.17±1.11	32.54±1.83
	6.50	44.95±2.02	43.27±1.79	40.90±2.09	40.09±1.22	37.12±2.29	36.27±1.40	34.24±1.38
	7.00	47.43±2.07	46.29±0.90	43.70±1.87	42.33±1.42	39.30±2.00	38.26±1.16	36.37±1.58
	7.50	49.81±1.96	48.86±1.22	45.86±1.64	44.64±1.67	41.37±2.27	39.94±0.78	38.38±1.43
	8.00	52.26±1.84	51.33±0.94	48.46±1.73	46.66±1.71	43.32±1.83	42.41±0.80	40.09±1.64
	8.50	55.34±1.71	53.87±0.96	50.96±1.89	49.10±1.98	45.70±1.97	44.59±0.63	42.59±1.67
	9.00	57.97±2.09	56.49±1.45	53.35±1.84	51.25±2.03	47.78±2.04	46.65±1.13	44.61±1.71
	9.50	60.85±2.09	59.00±0.97	55.80±2.20	53.63±2.37	50.10±1.99	49.33±1.17	46.88±1.56
	10.00	63.45±1.81	61.57±1.21	58.32±2.05	56.45±2.10	52.49±1.99	51.53±1.64	48.84±1.77
	10.50	66.12±1.52	64.20±1.28	61.08±1.97	58.99±1.88	55.31±2.00	53.97±1.54	51.03±1.81
	11.00	69.22±1.04	66.91±1.42	63.18±2.31	61.40±1.91	57.85±1.73	56.11±1.26	53.10±2.08
	11.50	72.03±0.71	69.67±1.62	65.87±1.81	64.06±1.69	60.45±1.77	58.47±1.47	55.20±2.21
	12.00	74.54±0.54	72.50±1.34	68.81±1.61	66.59±1.71	63.29±2.09	60.53±1.78	57.36±1.95

n=3

COLLEGE OF PHARMACY
PRINCIPAL
27.07.24

TABLE 7C: COMPARISON OF *IN VITRO* RELEASE OF RUTIN LOADED EUDRAGIT S100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F15 ES 100	F16 ES 100	F17 ES 100	F18 ES 100	F19 ES 100	F20 ES 100	F21 ES 100
1.2	0.25	5.90±0.31	5.72±0.31	5.53±0.54	5.53±0.54	5.72±0.31	4.81±0.31	4.62±0.31
	0.50	8.38±0.82	8.55±1.09	8.18±0.85	7.81±0.84	7.28±0.64	6.32±0.56	6.31±0.54
	0.75	11.33±0.84	11.15±1.14	10.40±0.35	9.83±0.85	9.63±1.30	8.45±0.67	8.62±0.64
	1.00	14.96±0.96	13.50±0.73	13.25±0.56	12.11±1.14	12.45±1.24	11.21±0.89	11.21±0.36
	1.50	18.20±0.62	16.66±0.65	15.49±1.13	15.21±0.82	14.84±1.24	13.36±0.93	13.54±0.58
	2.00	21.74±0.25	19.59±0.54	18.73±0.68	18.07±0.31	17.50±0.84	15.96±0.78	16.15±0.91
6.5	2.50	29.26±0.53	26.48±1.12	25.09±0.24	24.48±0.43	23.67±1.41	21.43±1.28	21.64±0.84
	3.00	31.12±1.03	28.32±1.14	26.72±0.41	25.76±0.46	25.30±1.59	22.68±1.29	22.89±0.42
	3.50	33.06±1.54	30.98±1.48	28.99±0.63	27.83±0.58	27.57±1.77	24.54±1.31	24.38±0.71
	4.00	35.46±1.45	32.84±1.44	30.80±1.00	30.18±1.09	29.57±1.59	26.29±1.19	25.93±1.02
	4.50	37.58±1.51	35.51±0.72	33.06±1.31	31.70±1.13	31.46±1.80	28.68±1.98	27.92±1.03
	5.00	39.80±1.18	37.37±0.64	35.22±1.13	33.65±1.33	33.62±1.85	30.43±1.70	29.43±1.21
	5.50	41.90±1.16	39.48±0.40	36.92±1.19	36.05±1.56	36.23±1.40	32.24±1.41	31.37±1.52
	6.00	43.90±0.69	42.03±0.62	39.40±1.57	38.17±1.45	38.01±1.29	33.93±1.93	33.39±1.74
	6.50	46.33±0.70	43.94±1.05	41.43±1.43	40.17±1.69	40.04±1.00	36.05±2.29	35.48±1.65
	7.00	49.04±1.40	46.09±1.26	43.52±1.82	42.43±1.57	41.57±1.18	37.87±1.94	37.09±1.60
	7.50	51.27±1.35	48.48±1.64	46.23±2.03	44.58±1.81	44.07±1.40	40.12±2.29	39.30±1.62
	8.00	54.13±1.35	50.95±2.02	48.85±2.44	46.97±1.69	46.46±1.46	41.88±2.12	41.40±1.66
	8.50	56.53±1.68	53.87±1.78	51.36±2.25	49.44±2.06	48.56±1.47	43.88±2.16	43.38±1.69
	9.00	59.36±1.19	56.69±2.28	53.95±2.40	51.80±2.41	50.53±1.73	46.30±2.52	45.23±1.47
	9.50	61.90±1.21	59.59±2.48	56.25±2.16	54.22±2.19	52.73±1.76	48.25±2.90	47.68±1.27
	10.00	64.51±1.51	62.21±2.27	58.78±2.53	56.71±2.34	54.99±1.78	50.23±2.34	49.83±1.28
	10.50	67.19±1.29	64.89±1.88	61.94±2.37	58.89±2.20	57.49±2.51	52.45±2.38	51.86±1.14
	11.00	69.92±1.63	67.45±1.93	64.44±1.90	61.12±2.16	60.24±2.42	54.73±2.42	53.93±1.46
	11.50	72.91±1.37	70.07±1.99	67.38±2.21	63.58±2.22	62.87±2.23	57.43±2.38	56.05±1.30
	12.00	75.60±1.40	73.12±1.96	70.38±2.33	66.29±1.96	65.20±1.97	60.02±2.67	58.21±1.18

n=3*

TABLE 7D: COMPARISON OF *IN VITRO* RELEASE OF RUTIN LOADED EUDRAGIT S100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE						
		F22 ES 100	F23 ES 100	F24 ES 100	F25 ES 100	F26 ES 100	F27 ES 100	F28 ES 100
1.2	0.25	5.90±0.31	5.90±0.31	5.35±0.31	5.35±0.31	5.35±0.31	4.81±0.31	4.62±0.31
	0.50	8.56±1.09	8.74±0.81	7.44±0.56	7.44±0.53	7.44±0.54	6.87±0.54	6.13±0.32
	0.75	11.52±0.85	11.35±0.86	10.71±0.85	10.53±0.54	10.35±0.66	9.20±1.16	7.88±0.33
	1.00	14.98±1.47	14.98±1.06	13.40±0.70	13.40±0.82	12.66±0.60	11.09±0.92	10.07±0.32
	1.50	17.85±1.21	18.40±0.50	16.93±0.67	16.01±1.13	15.24±0.35	13.05±0.67	12.17±0.87
	2.00	21.56±0.94	21.22±0.27	20.06±0.57	18.91±0.64	18.10±0.37	16.00±0.86	15.26±0.69
6.5	2.50	28.51±1.12	28.74±0.57	26.72±0.81	25.28±0.50	24.35±0.73	21.50±0.72	20.36±1.02
	3.00	30.35±1.32	30.23±0.77	28.37±1.46	26.92±0.75	25.80±1.05	23.12±1.20	21.78±1.12
	3.50	33.37±1.82	32.71±0.80	30.46±1.84	28.81±1.64	27.87±0.88	25.37±1.36	23.46±0.98
	4.00	35.05±2.02	34.56±0.81	32.45±1.90	30.79±1.69	29.67±0.58	27.16±1.77	25.21±1.31
	4.50	37.17±1.59	36.85±1.39	35.09±1.93	33.04±2.01	31.53±1.23	28.84±2.07	27.03±1.16
	5.00	39.56±1.81	39.05±1.84	37.28±2.46	34.64±2.19	33.85±1.15	30.96±1.58	28.55±1.18
	5.50	41.85±1.48	41.15±1.82	39.19±2.40	36.48±2.04	35.88±1.44	32.79±1.89	30.32±0.99
	6.00	43.86±1.54	43.33±2.20	41.16±1.84	38.57±2.21	38.18±1.50	34.69±1.43	32.33±1.29
	6.50	46.49±1.67	45.57±2.23	43.75±2.20	40.92±1.86	40.37±2.14	36.65±1.55	33.87±1.61
	7.00	48.84±2.12	48.08±2.14	46.24±1.87	42.79±1.91	42.63±1.93	38.67±1.79	35.63±1.65
	7.50	51.44±1.62	50.49±1.81	48.46±1.67	45.28±2.36	44.79±1.75	40.76±1.93	38.20±1.95
	8.00	53.58±1.59	53.15±1.68	51.10±1.69	47.67±2.12	47.01±1.20	42.92±1.65	40.30±2.06
	8.50	55.77±1.61	56.08±1.65	53.64±1.96	50.31±2.17	49.48±1.83	45.14±1.55	42.09±1.82
	9.00	58.76±1.84	58.55±1.47	56.08±1.76	52.49±1.85	51.47±1.58	47.42±1.61	44.30±1.82
	9.50	60.92±1.66	60.90±1.51	58.58±2.10	54.90±1.64	53.69±1.62	49.77±1.34	46.58±1.61
	10.00	63.67±2.14	63.49±1.46	61.14±2.07	57.38±1.43	56.33±1.73	52.00±1.35	48.92±1.42
	10.50	66.32±1.90	65.96±1.49	63.58±2.11	59.74±1.64	58.68±1.77	54.29±1.72	51.32±1.77
	11.00	69.40±1.72	68.49±1.92	66.26±2.45	61.97±1.41	61.27±1.74	56.63±1.75	53.79±1.70
	11.50	72.38±1.46	71.07±2.12	68.64±2.21	64.80±1.20	63.93±1.54	59.21±1.47	56.14±1.74
	12.00	75.62±1.48	73.52±1.89	71.80±1.70	67.52±1.43	66.46±1.57	61.85±1.59	58.55±1.78

n=3*

TABLE 7E: COMPARISON OF *IN VITRO* DRUG RELEASE OF PURE DRUG (RUTIN) WITH BEST FORMULATION (F7 EL 100 with 0.1% SLS)

pH	TIME IN HOURS	CUMULATIVE % DRUG RELEASE	
		PURE DRUG	F7 EL 100 + 1% PLURONIC F 68
1.2	0.25	0	0
	0.5	0	0
	0.75	0	0
	1.00	0	0
	1.50	0	0
	2.00	0	0
6.5	2.50	1.42±0.32	11.42±0.64
	3.00	2.60±0.31	14.22±0.28
	3.50	4.40±0.32	17.12±0.86
	4.00	5.72±0.32	20.32±0.36
	4.50	6.54±0.33	22.72±0.25
	5.00	7.58±0.67	25.19±0.05
	5.50	8.83±0.38	27.36±0.53
	6.00	9.76±0.40	29.96±0.81
	6.50	-	32.08±1.17
	7.00	-	34.06±1.35
	7.50	-	36.26±1.29
	8.00	-	38.32±1.01
	8.50	-	40.61±0.87
	9.00	-	42.38±0.81
	9.50	-	44.73±0.61
	10.00	-	46.94±0.30
	10.50	-	49.00±0.37
	11.00	-	51.28±0.08
	11.50	-	53.22±0.27
	12.00	-	55.37±0.55

n=3*

INNOVATIVE COLLEGE OF PHARMACY PRINCIPAL 07.24

TABLE 8A: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F1	0.988	5.582	0.981	-0.042	0.981	22.77	0.994	0.701	0.991	-0.122
F2	0.990	5.275	0.983	-0.038	0.980	21.46	0.996	0.676	0.993	-0.111
F3	0.982	5.074	0.987	-0.035	0.987	20.72	0.995	0.680	0.992	-0.107
F4	0.984	4.889	0.986	-0.033	0.984	19.90	0.994	0.663	0.992	-0.101
F5	0.979	4.713	0.989	-0.031	0.988	19.23	0.995	0.661	0.992	-0.096
F6	0.985	4.475	0.986	-0.029	0.980	18.23	0.991	0.673	0.990	-0.089
F7	0.984	4.182	0.990	-0.026	0.982	17.00	0.995	0.647	0.993	-0.081

INNOVATIVE COLLEGE OF PHARMACY PRINCIPAL

TABLE 8B: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F8	0.982	5.558	0.975	-0.043	0.982	22.59	0.995	0.646	0.987	-0.124
F9	0.984	5.439	0.980	-0.041	0.983	22.13	0.996	0.658	0.990	-0.119
F10	0.986	5.157	0.983	-0.037	0.981	20.95	0.995	0.649	0.991	-0.110
F11	0.985	4.988	0.984	-0.035	0.982	20.29	0.996	0.656	0.991	-0.104
F12	0.986	4.738	0.984	-0.031	0.980	19.29	0.995	0.679	0.990	-0.096
F13	0.986	4.588	0.987	-0.030	0.981	18.68	0.995	0.673	0.992	-0.092
F14	0.986	4.327	0.990	-0.027	0.981	17.59	0.995	0.657	0.993	-0.085

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

TABLE 8C: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING PLURONIC F 68 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F15	0.980	5.659	0.978	-0.044	0.987	23.06	0.996	0.655	0.989	-0.127
F16	0.985	5.493	0.979	-0.041	0.982	22.34	0.996	0.655	0.990	-0.121
F17	0.987	5.258	0.978	-0.038	0.979	21.35	0.995	0.653	0.989	-0.113
F18	0.986	4.999	0.988	-0.035	0.983	20.33	0.995	0.650	0.993	-0.104
F19	0.985	4.903	0.988	-0.033	0.985	19.95	0.994	0.649	0.994	-0.102
F20	0.987	4.504	0.990	-0.029	0.983	18.33	0.995	0.664	0.994	-0.090
F21	0.985	4.397	0.991	-0.028	0.983	17.89	0.996	0.658	0.994	-0.087

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TABLE 8C: KINETICS RELEASE STUDIES OF FELODIPINE LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING POLYVINYL ALCOHOL 1% AS STABILIZER

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORS-MEYER & PEPPAS MODEL		HIXON – CROWELL MODEL	
	R^2	K_0 h^{-1}	R^2	K_1 h^{-1}	R^2	K_H $h^{1/2}$	R^2	n	R^2	K_{HC} $h^{1/3}$
F22	0.980	5.612	0.978	-0.044	0.987	22.86	0.997	0.651	0.989	-0.126
F23	0.980	5.528	0.984	-0.042	0.988	22.51	0.996	0.645	0.992	-0.123
F24	0.983	5.408	0.984	-0.040	0.986	22.05	0.996	0.669	0.992	-0.118
F25	0.983	5.039	0.985	-0.035	0.984	20.49	0.996	0.650	0.991	-0.106
F26	0.986	4.993	0.986	-0.035	0.983	20.30	0.996	0.652	0.993	-0.104
F27	0.988	4.658	0.988	-0.030	0.980	18.94	0.995	0.664	0.993	-0.094
F28	0.989	4.411	0.986	-0.028	0.976	17.93	0.993	0.672	0.991	-0.087

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

TABLE 9: COMPARISON OF SOLUBILITY OF BEST FORMULATION (F7 EL 100 with 1% PLURONIC F68) WITH PURE DRUG USING DISTILLED WATER WITH 0.1% SLS AND PHOSPHATE BUFFER pH 6.5 WITH 0.1% SLS

TIME (hrs.)	SOLVENT USED	SOLUBILITY (µg/ml)	
		PURE DRUG	F7 EL 100 + PLURONIC F 68
24 hrs.	Distilled water	29.91µg/ml±3.218	117.23µg/ml±3.215
	Phosphate buffer pH 6.5 with 0.1% SLS	43.89µg/ml±3.207	260.58µg/ml±3.210

n=3*

TABLE 10A: COMPARISON OF CUMULATIVE AMOUNT OF DRUGPERMEATED ACROSS DUODENUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED (mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1036±0.008	0.2105±0.013
0.50	0.1373±0.008	0.3655±0.042
1.00	0.1708±0.016	0.5617±0.045
1.50	0.1991±0.016	0.7298±0.021
2.00	0.2219±0.011	0.8572±0.011

n=3*

TABLE 10B: COMPARISON OF CUMULATIVE AMOUNT OF DRUGPERMEATED ACROSS JEJUNUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED (mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1125±0.010	0.2266±0.026
0.50	0.1484±0.013	0.4287±0.038
1.00	0.1874±0.019	0.5916±0.041
1.50	0.2231±0.011	0.7882±0.055
2.00	0.2533±0.008	0.8975±0.016

n=3*

TABLE 10C: COMPARISON OF CUMULATIVE AMOUNT OF DRUGPERMEATED ACROSS ILEUM SEGMENT

TIME(HRS)	CUMULATIVE AMOUNT OF DRUG PERMEATED (mg)± SD	
	PURE DRUG SOLUTION	F7 EL 100 + 1% PLURONIC F 68
0.25	0.1071±0.010	0.2194±0.016
0.50	0.1446±0.014	0.4158±0.050
1.00	0.1765±0.013	0.5731±0.059
1.50	0.2065±0.011	0.7517±0.032
2.00	0.2329±0.011	0.8743±0.013

n=3*

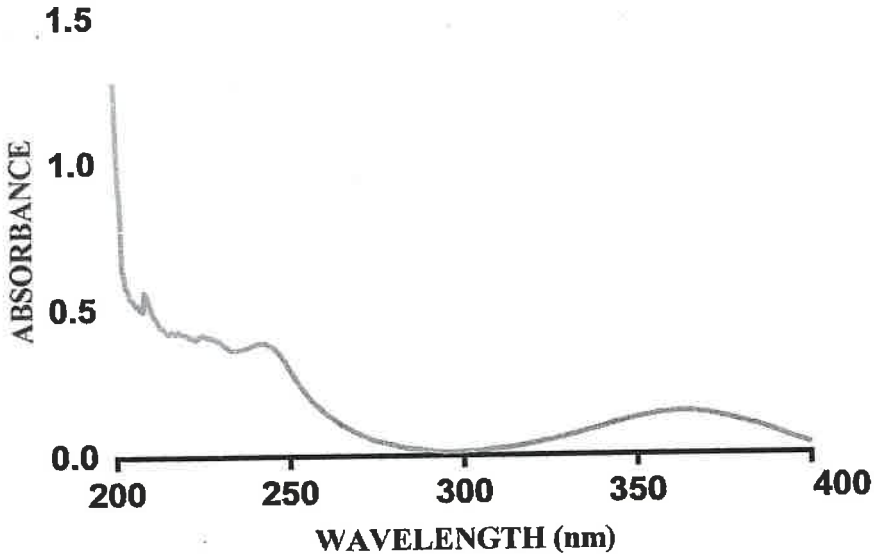

FIGURE 20a: λMAX OF RUTIN USING DISTILLED WATER WITH0.1% SLS

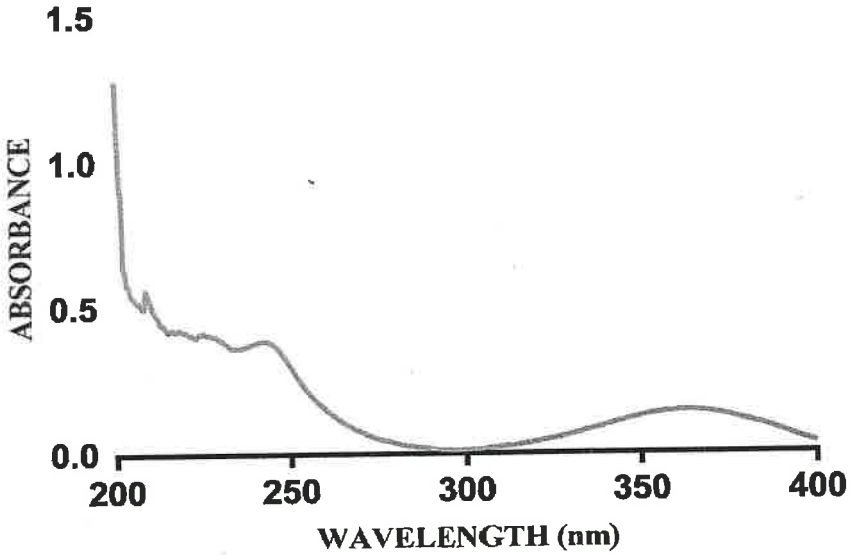

FIGURE 20b: λMAX OF RUTIN USING 0.1N HYDROCHLORIC ACIDWITH 0.1% SLS

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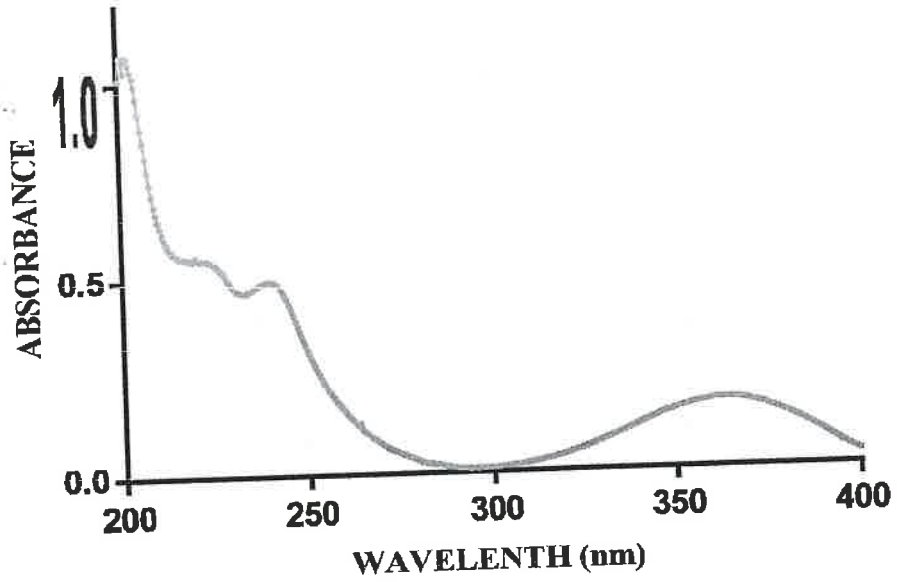

FIGURE 20c: λMAX OF RUTIN USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

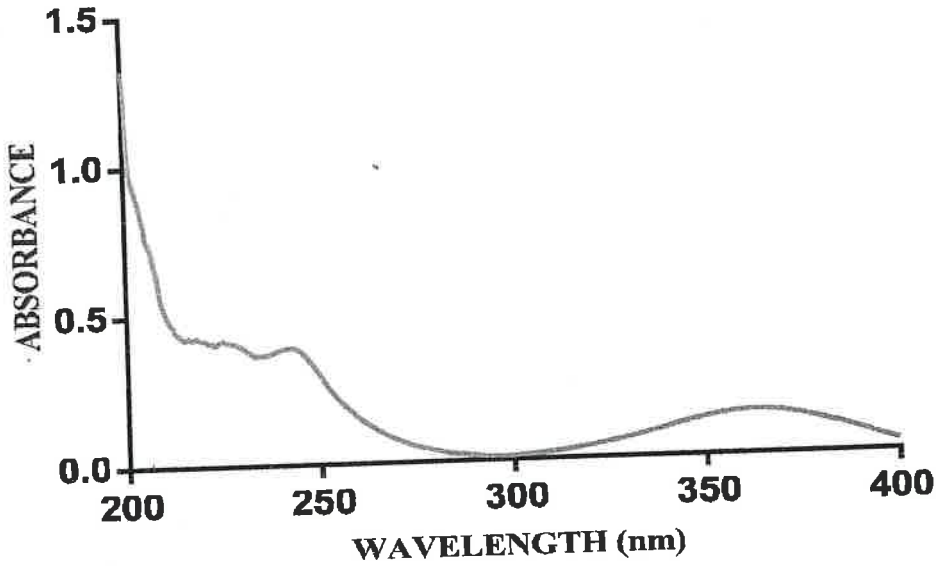

FIGURE 20d: λMAX OF RUTIN USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

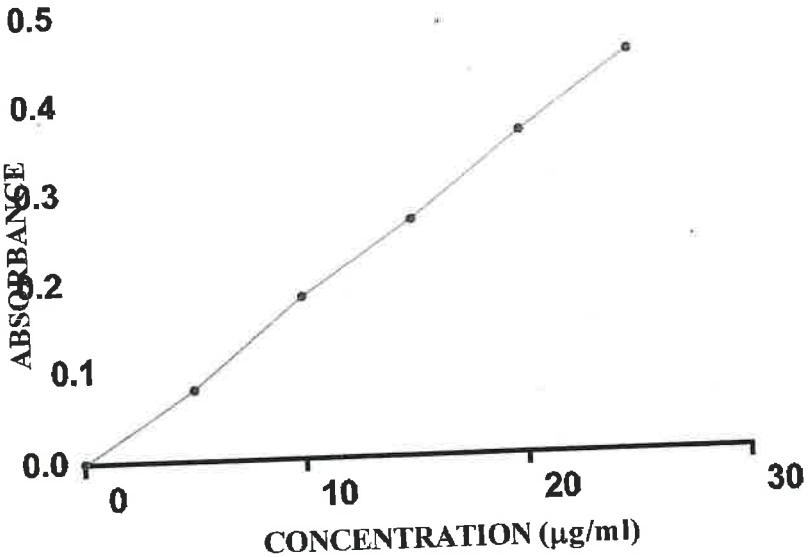

FIGURE 20e: CALIBRATION OF RUTIN USING DISTILLED WATERWITH 0.1% SLS

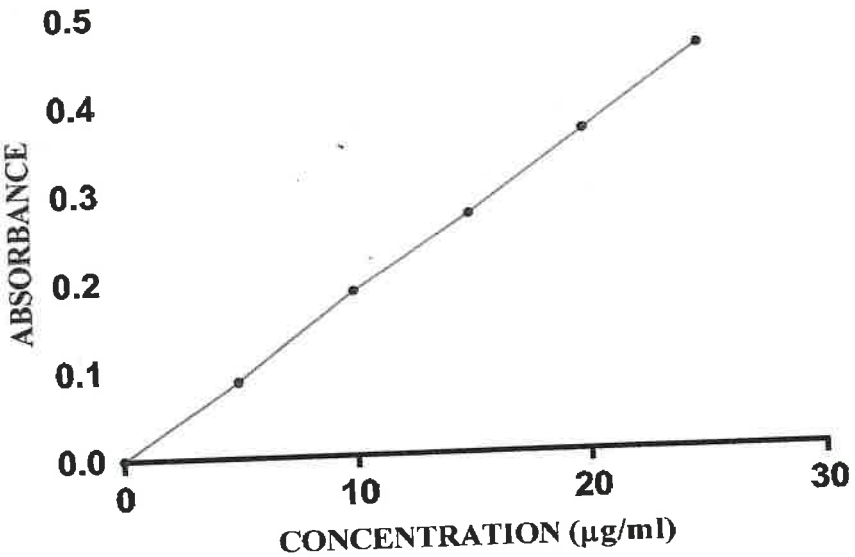

FIGURE 20f: CALIBRATION OF RUTIN USING 0.1N HYDROCHLORIC ACID WITH 0.1% SLS

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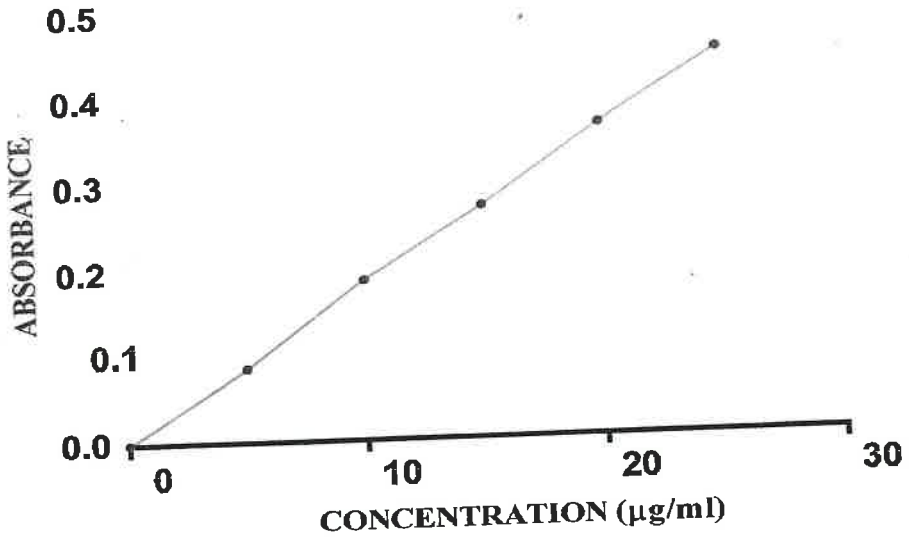

FIGURE 20g: CALIBRATION OF RUTIN USING PHOSPHATE BUFFER PH 6.5 WITH 0.1% SLS

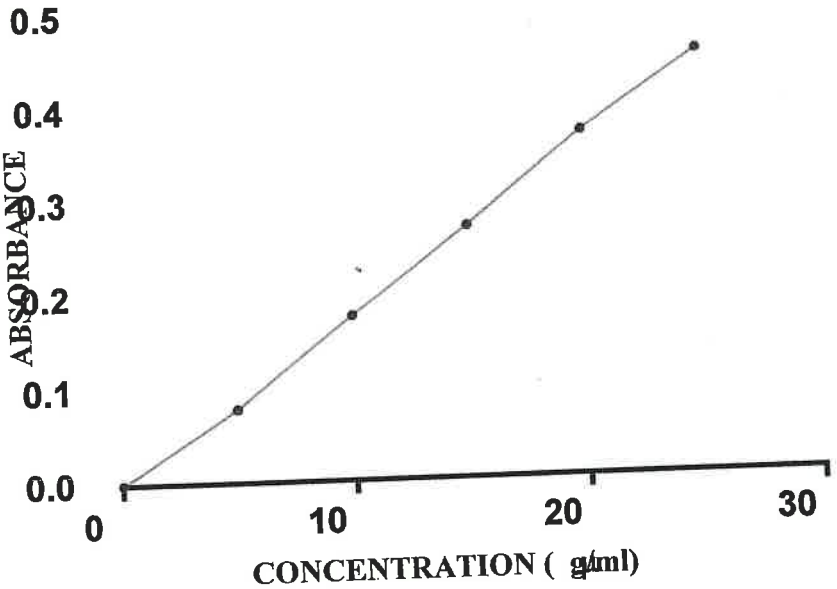

FIGURE 20h: CALIBRATION OF RUTIN USING PHOSPHATE BUFFER PH 7.4 WITH 0.1% SLS

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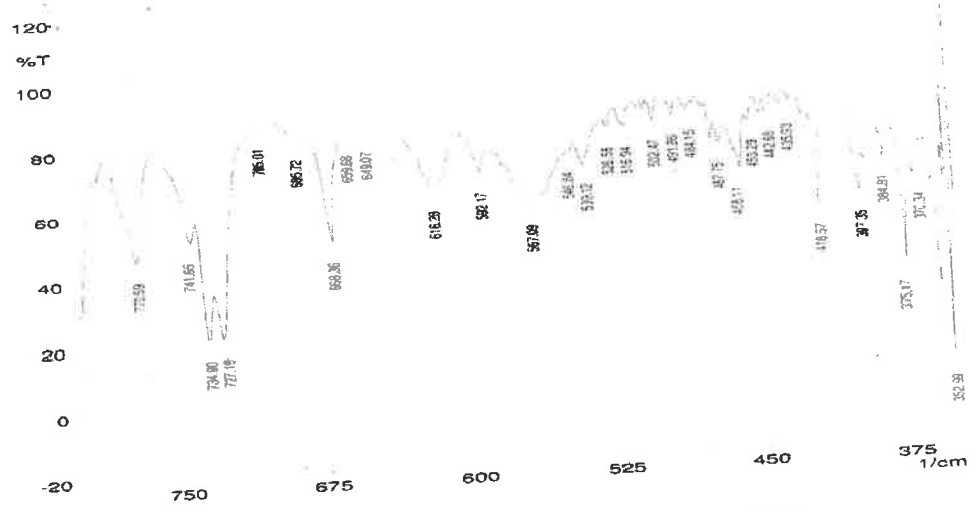


FIGURE 21a: INFRARED SPECTRAM OF RUTIN

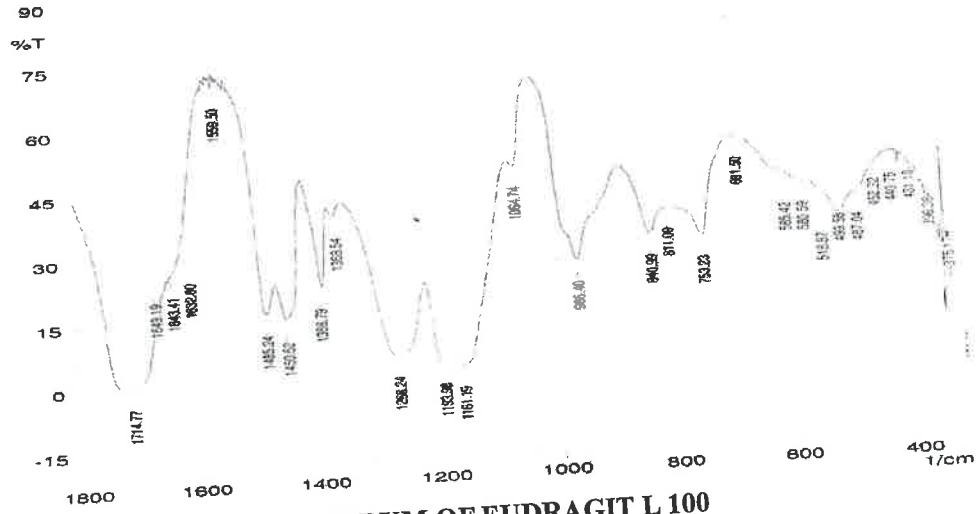


FIGURE 21b: INFRARED SPCTRUM OF EUDRAGIT L 100

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

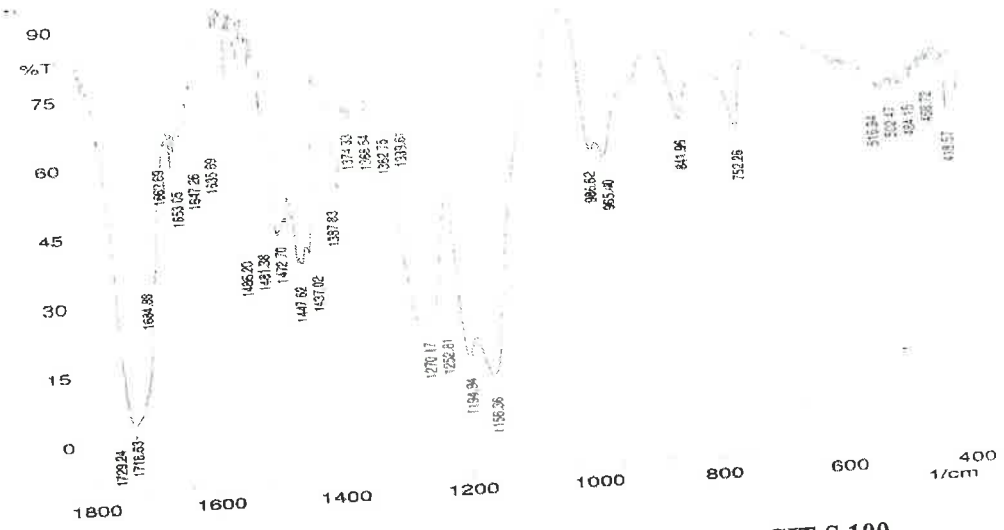


FIGURE 21c: INFRARED SPECTRAM O EUDRAGIT S 100

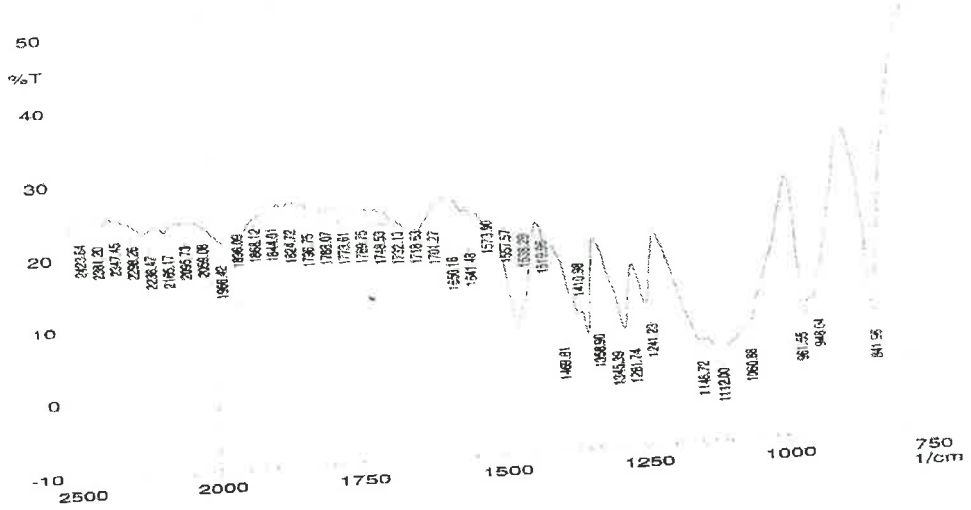


FIGURE 21d: INFRARED SPECTRAM OF PLURONIC F 68

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

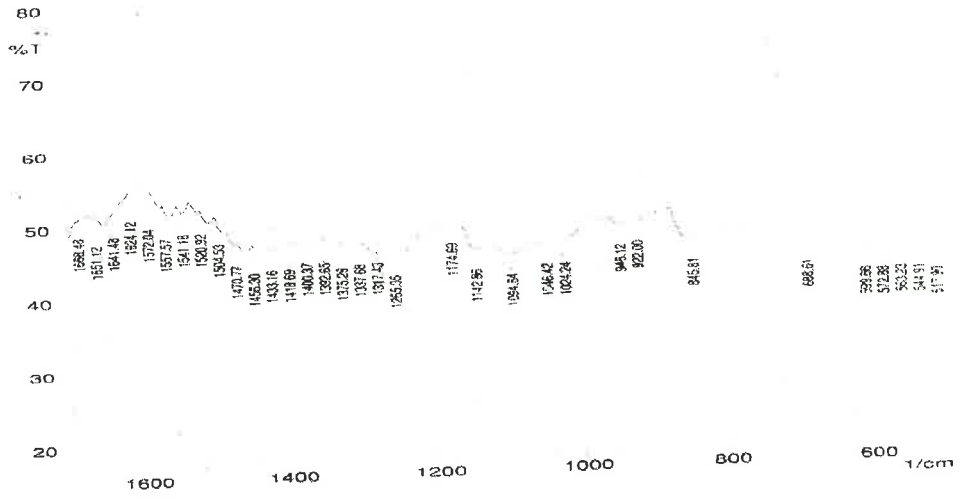
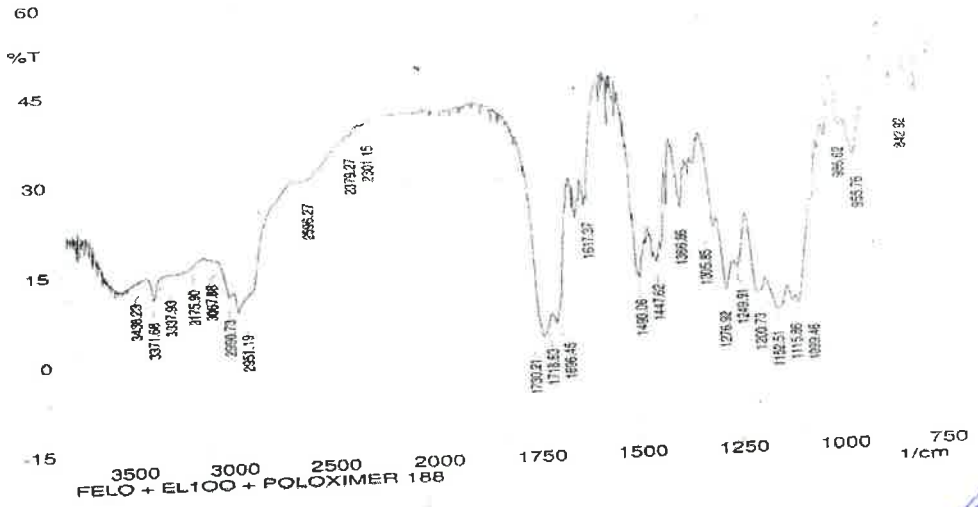


FIGURE 21e: INFRARED SPECTAM OF POLYVINYL ALCOHOL

FIGURE 21f: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT L 100+ POLOXAMER 188



INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

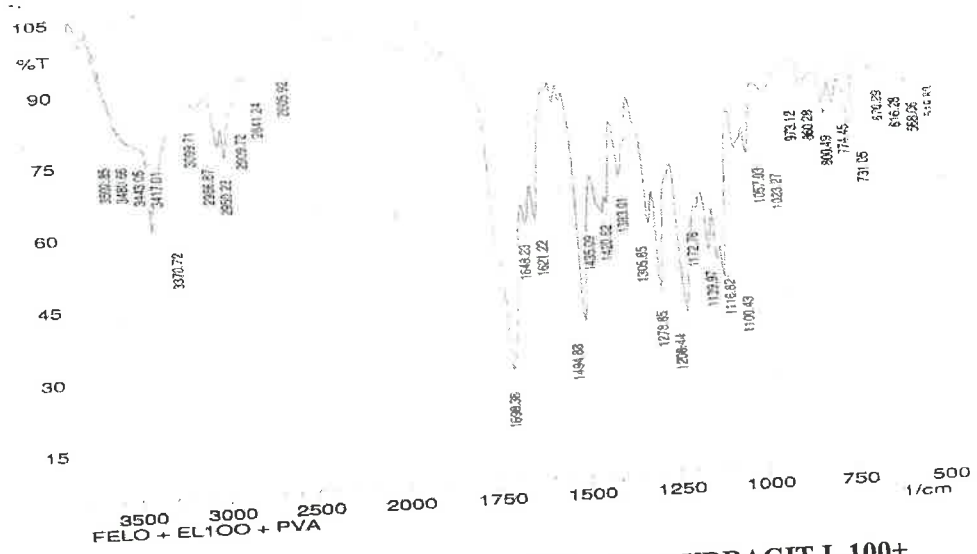


FIGURE 21g: INFRARED SPECTAM OF RUTIN + EUDRAGIT L 100+ POLYVINYL ALCOHOL

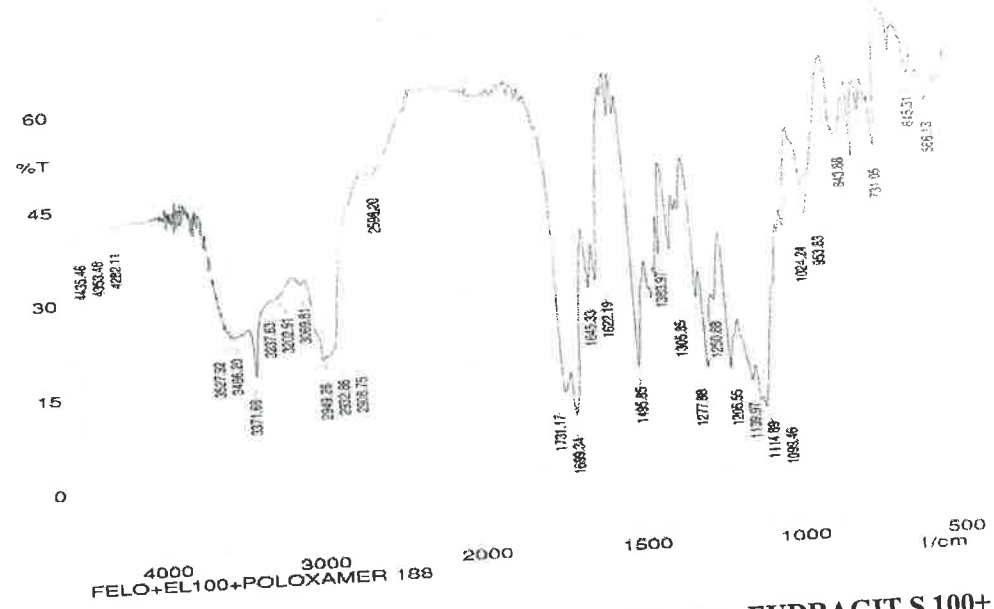


FIGURE 21h: INFRARED SPECTAM OF FELODIPINE + EUDRAGIT S 100+ POLOXAMER 188

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

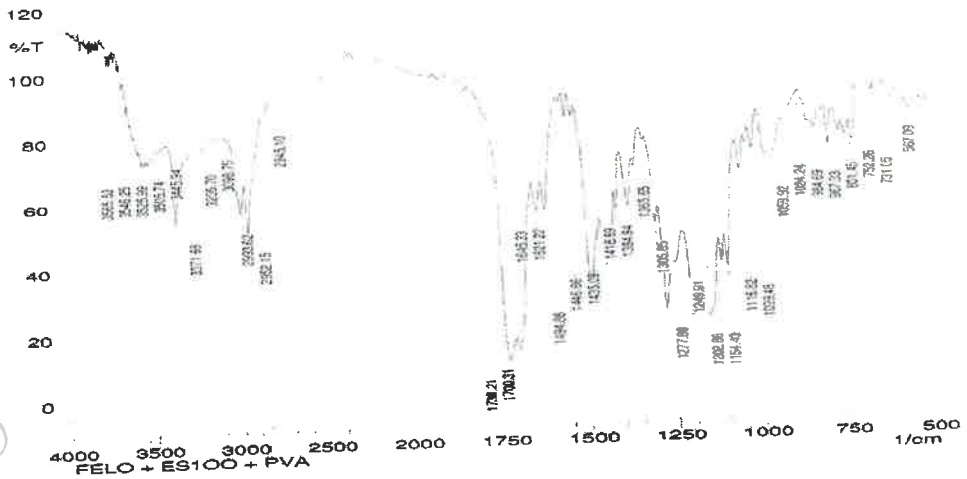


FIGURE 21i: INFRARED SPECTAM OF RUTIN + EUDRAGIT S 100+ POLYVINYL ALCOHOL

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

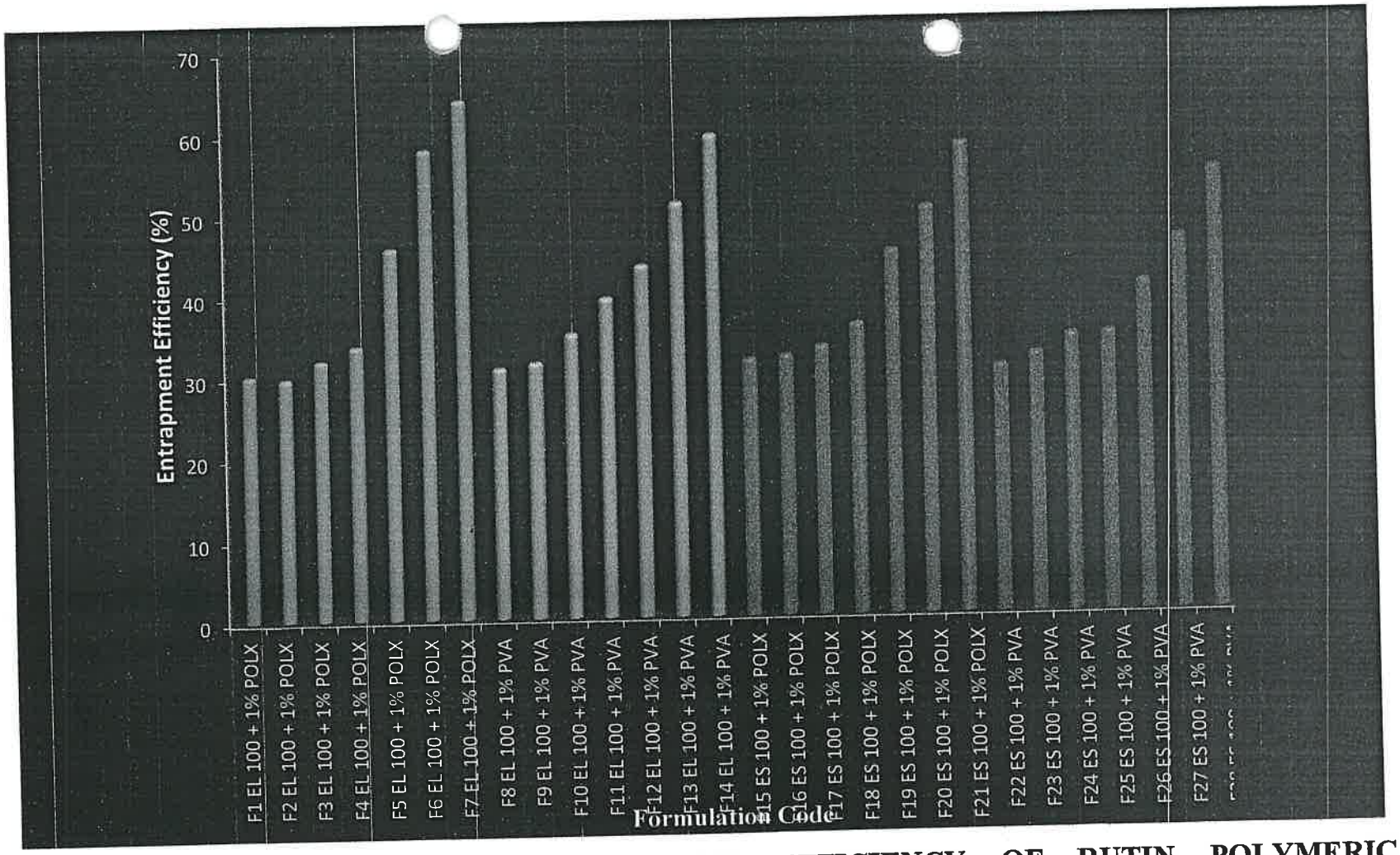


FIGURE 22: PERCENTAGE ENTRAPMENT EFFICIENCY OF RUTIN POLYMERIC NANOPARTICLES

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

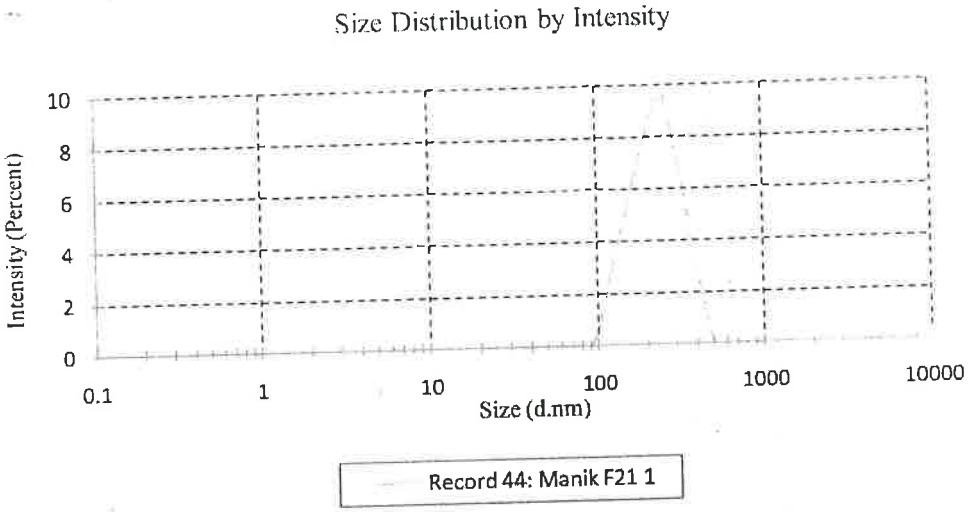


FIGURE 23c: PARTICLE SIZE DISTRIBUTION CURVE OF F21 ES 100 + 1% PLURONIC F 68

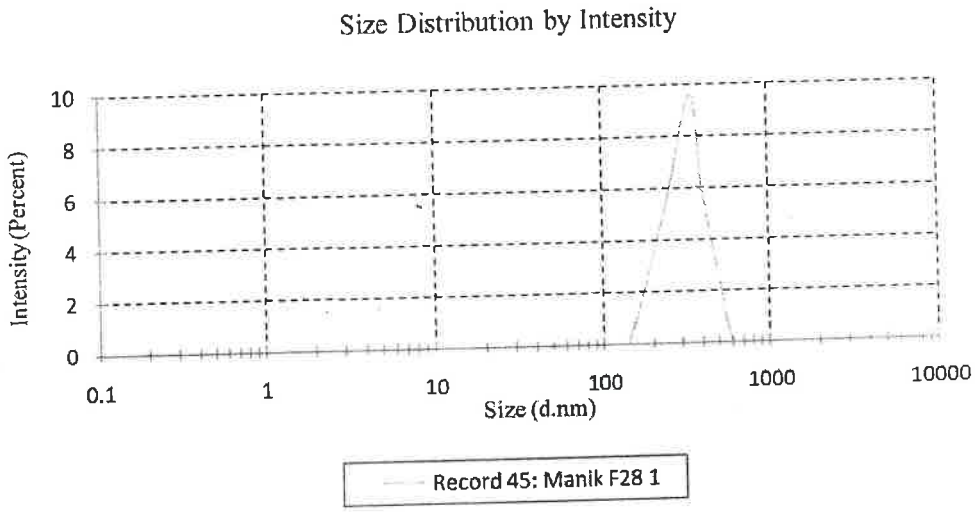


FIGURE 23d: PARTICLE SIZE DISTRIBUTION CURVE OF F28 ES 100 + 1% POLYVINYL ALCOHOL

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

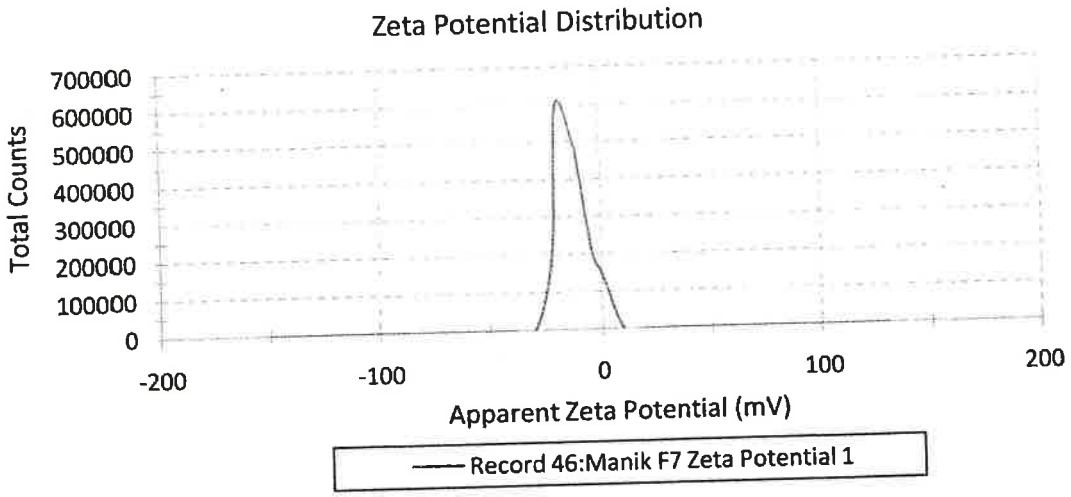


FIGURE 24a: ZETA POTENTIAL OF FORMULATION F7 EL 100 + 1% PLURONIC F 68

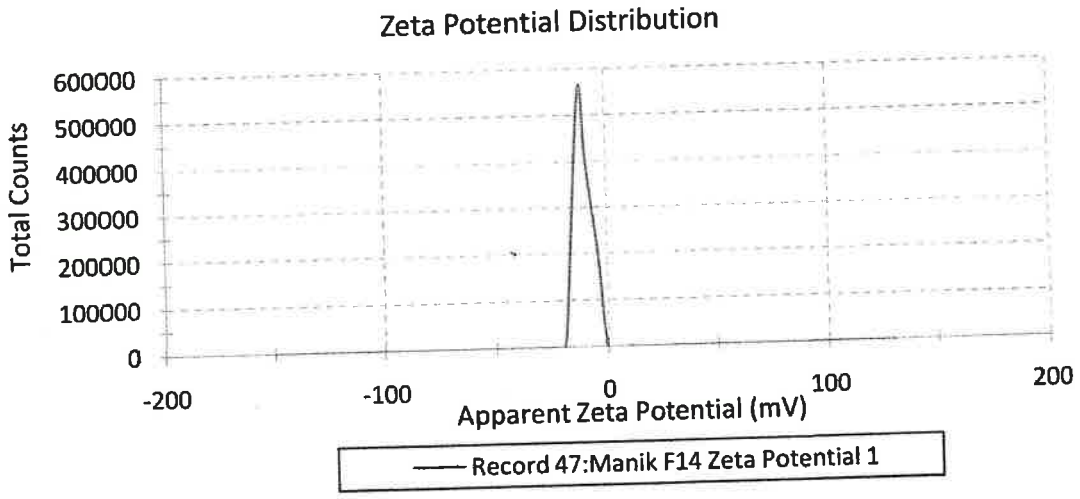


FIGURE 24b: ZETA POTENTIAL OF FORM LATION F14 EL 100 + 1% POLYVINYL ALCOHOL

INNOVATIVE COLLEGE OF PHARMACY
PRINCIPAL

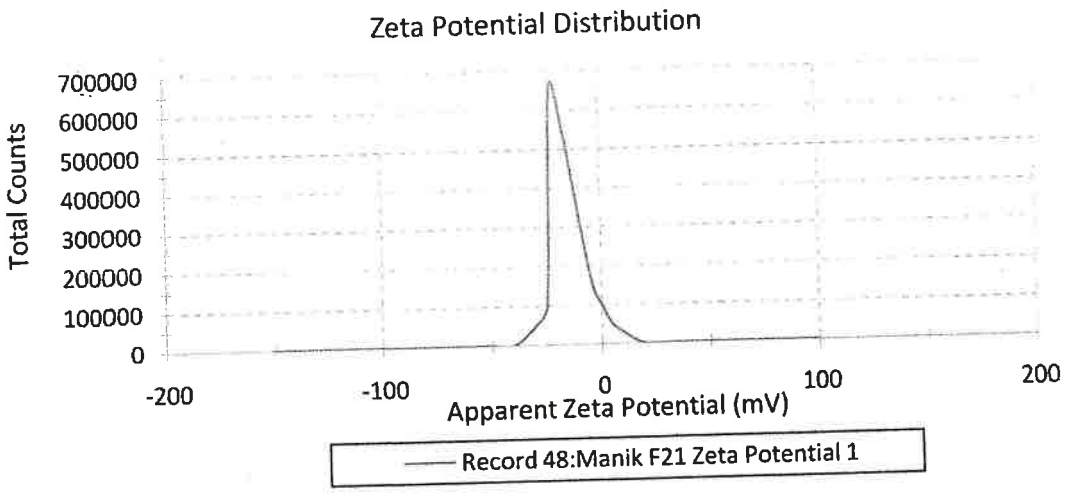


FIGURE 24c: ZETA POTENTIAL OF FORM ULATION F21 ES 100 + 1% PLURONIC F 68

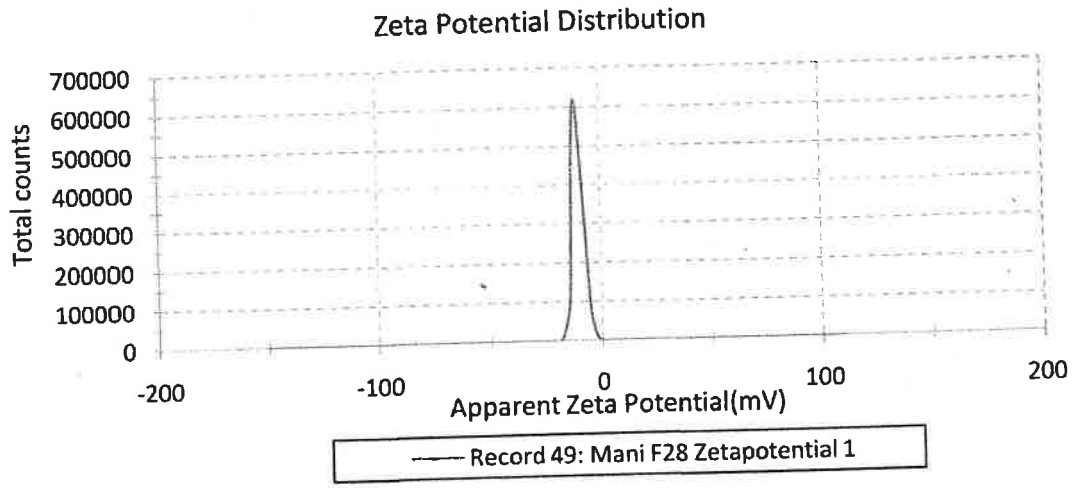


FIGURE 24d: ZETA POTENTIAL OF FORMU LATION F28 EL 100 + 1% POLYVINYL ALCOHOL

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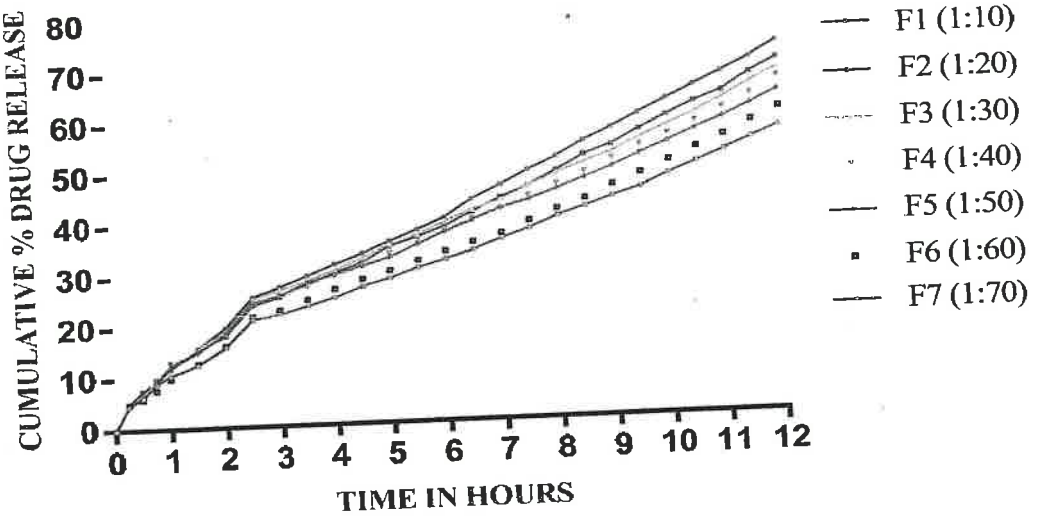

FIGURE 25 a: INVITRO RELEASE STUDIES OF RUTIN LOADED EUDRAGIT L 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68 AS STABILIZER

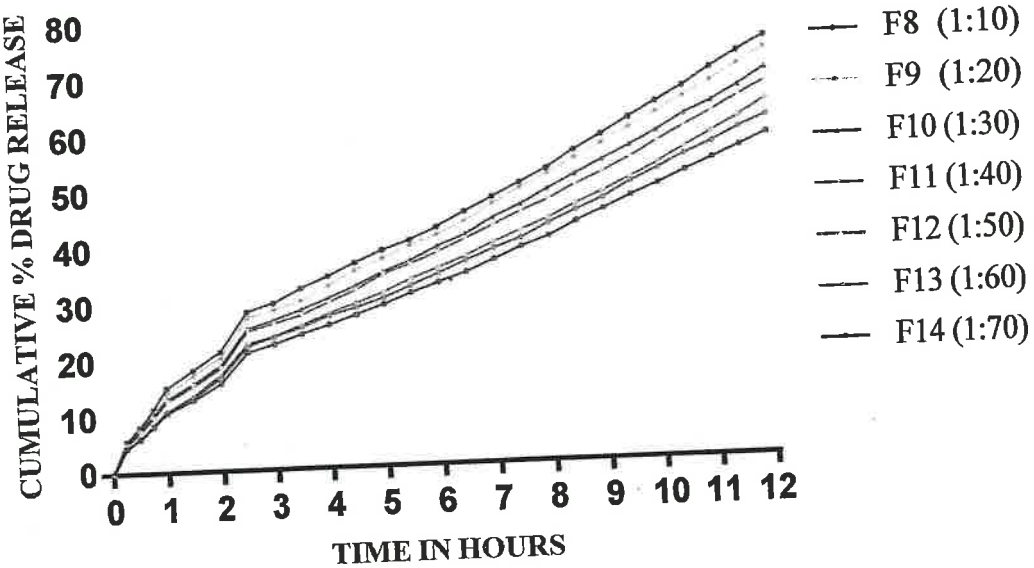

FIGURE 25b: INVITRO RELEASE STUDIES OF RUTIN LOADEDEUDRAGIT L100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

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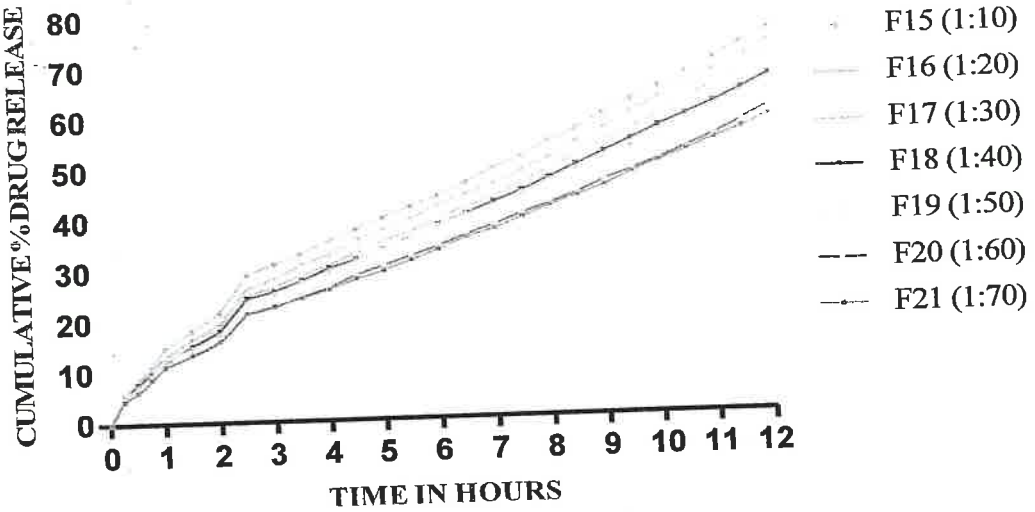

FIGURE 25c: INVITRO RELEASE STUDIES OF RUTIN LOADED EUDRAGIT S 100 NANOPARTICLES CONTAINING 1% PLURONIC F 68AS STABILIZER

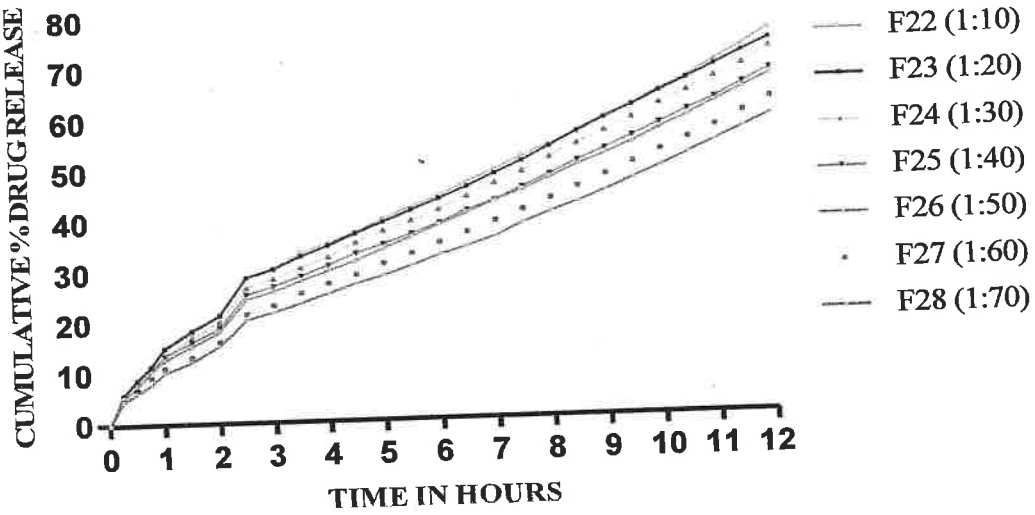

FIGURE 25d: INVITRO RELEASE STUDIES OF RUTIN LOADED EUDRAGIT S100 NANOPARTICLES CONTAINING 1% POLYVINYL ALCOHOL AS STABILIZER

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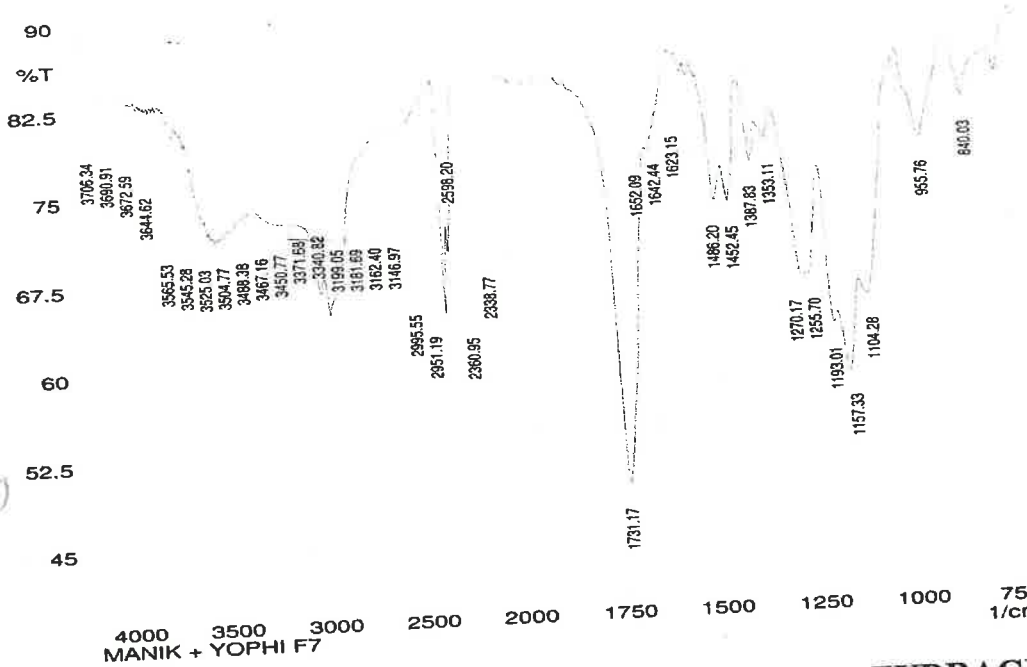

FIGURE 31: INFRARED SPECTAM OF RUTIN + EUDRAGIT L 100+ 1%PLURONIC F68

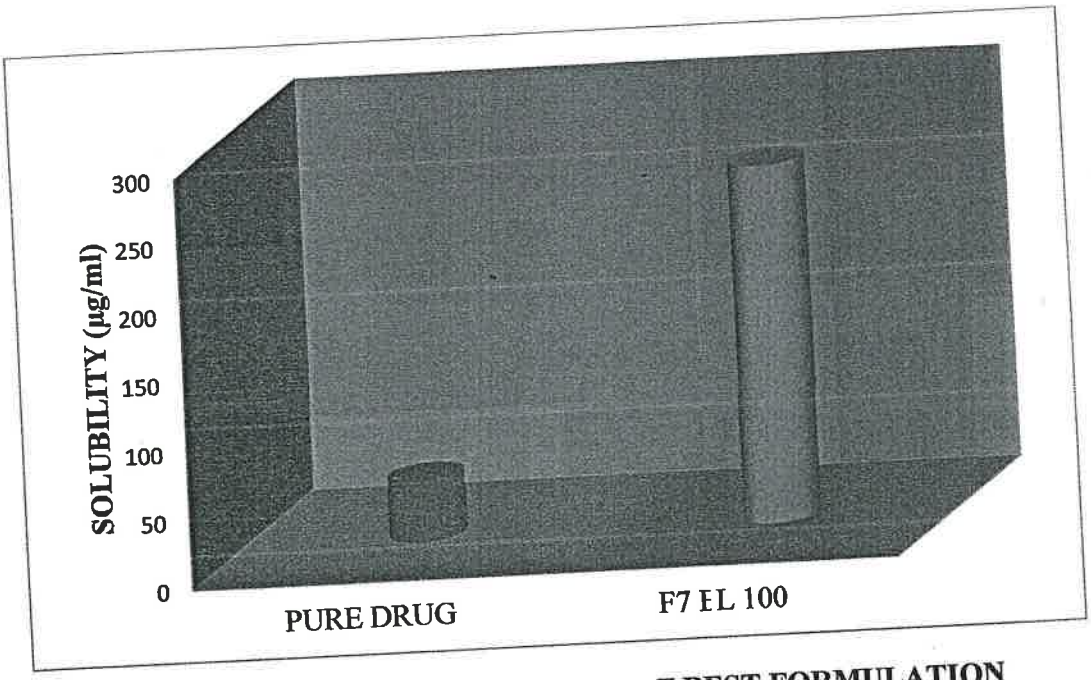

FIGURE 32a: COMPARISON OF SOLUBILITY F BEST FORMULATION WITH PURE DRUG USING PHOSPHATE BUFFER pH 6.5 with 0.1% SLS

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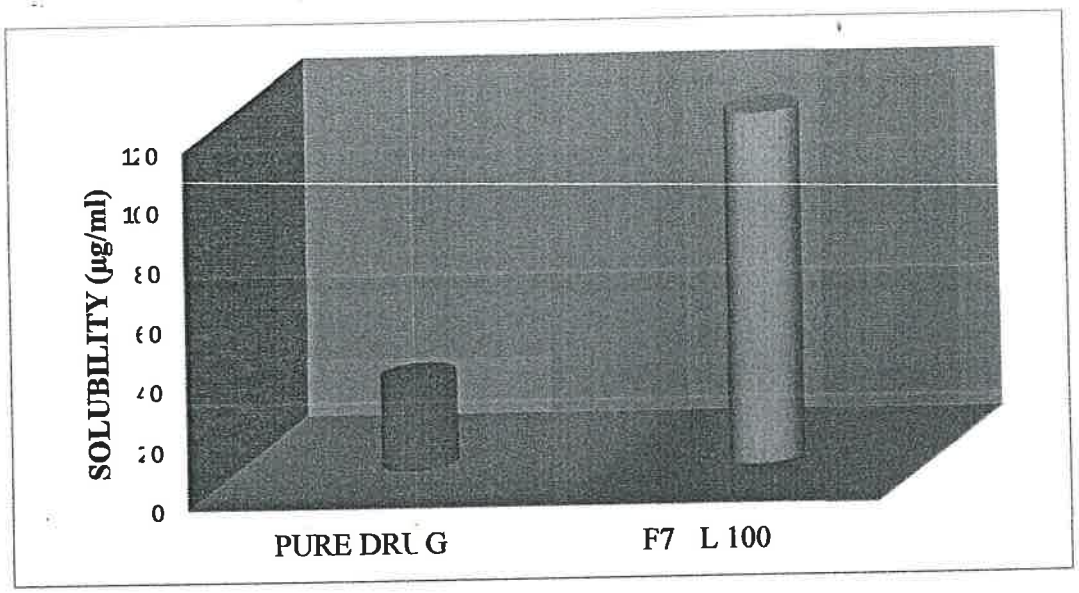

FIGURE 32b: COMPARISON OF SOLUBILITY OF BEST FORMULATIONWITH PURE DRUG USING DISTILLED WATER

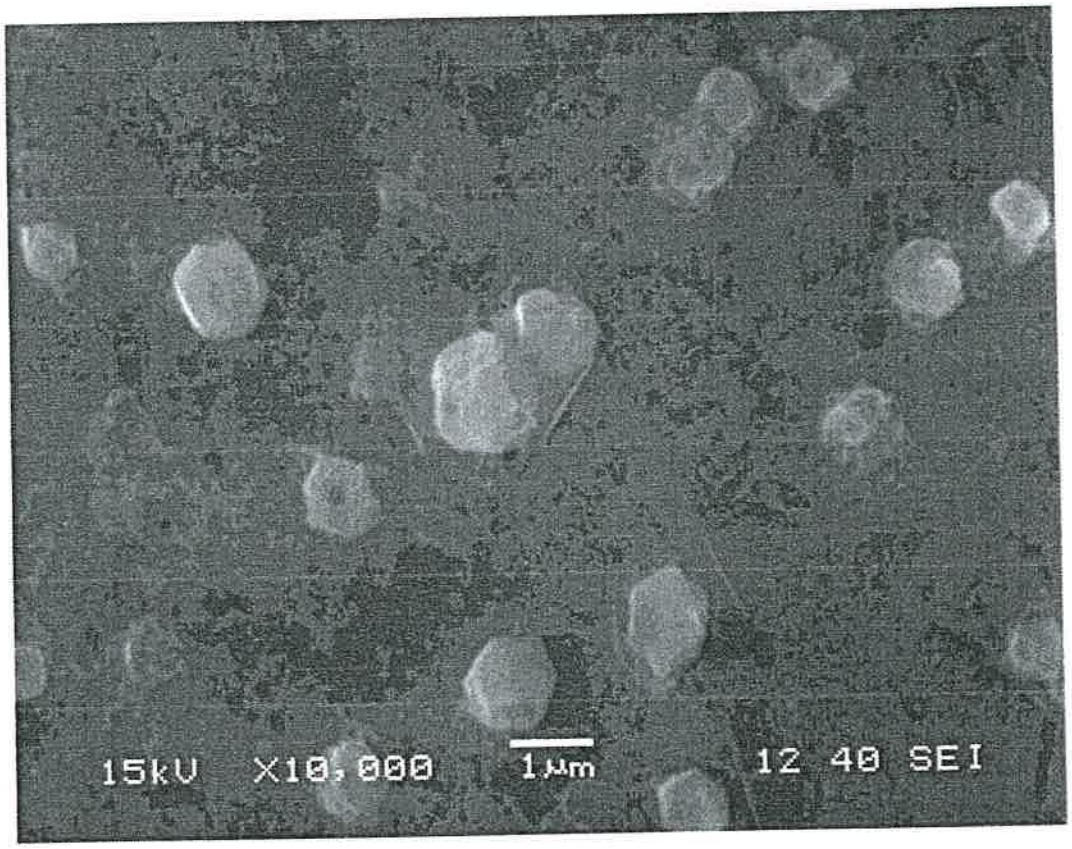

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FIGURE 33: SEM IMAGE OF F7 EL 100 + 1% PLURONIC F 68

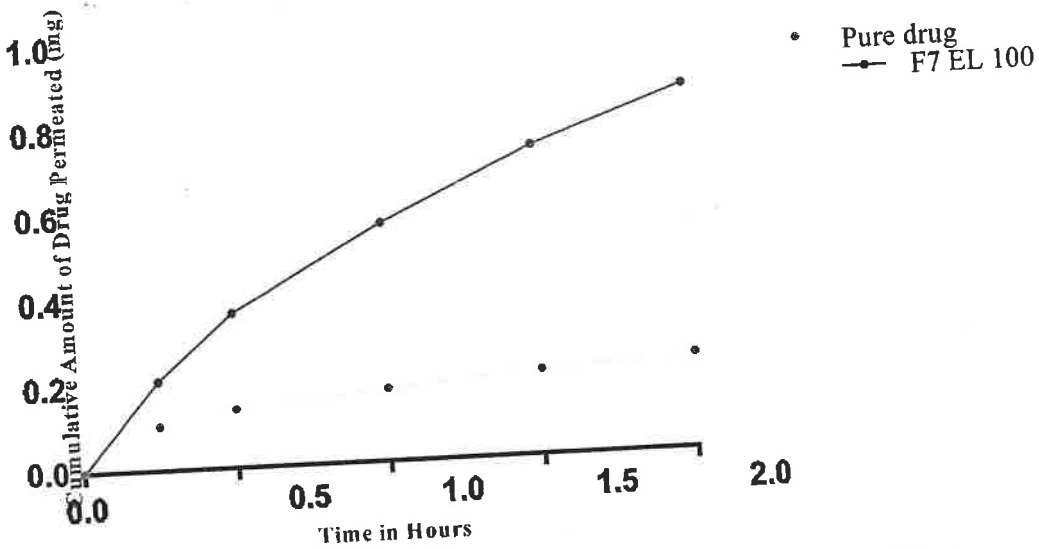

FIGURE 34a: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS DUODENUM SEGMENT

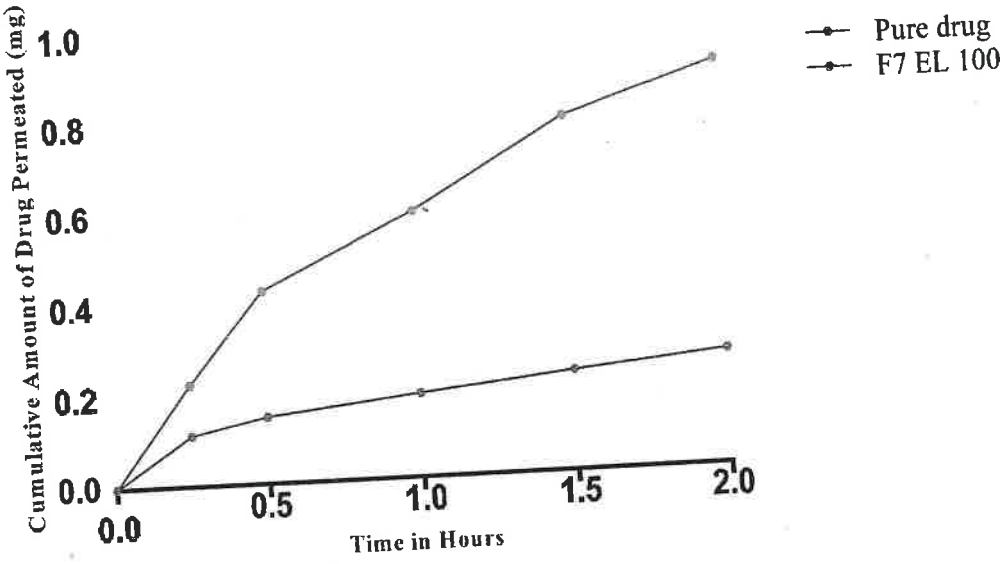

FIGURE 34b: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS JEJUNUM SEGMENT

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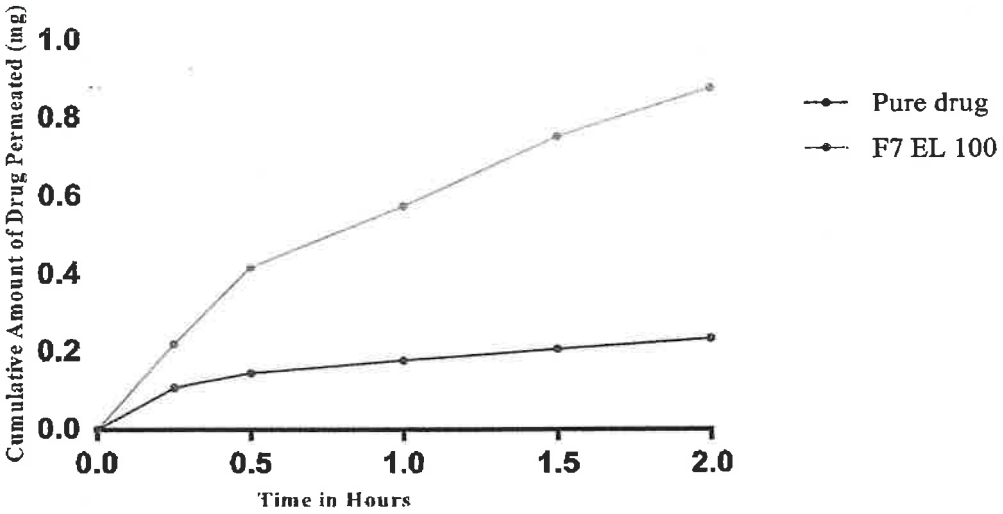

FIGURE34c: COMPARISON OF CUMULATIVE AMOUNT OF DRUG PERMEATED ACROSS ILEUM SEGME

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CHAPTER 7

SUMMARY AND CONCLUSION

This study intended to improve the solubility and dissolution rate of Rutin at the nanoscale while maintaining its therapeutic effectiveness by encapsulating it in Eudragit L and Eudragit S 100.Second, research using infrared spectroscopy found no evidence of drug-polymer interaction. Nànoprecipitation using Eudragit L100 and Eudragit S100 as polymers in the presence of stabilisers resulted in the effective preparation of Rutin-loaded polymeric nanoparticles (Pluronic F 68 and Polyvinyl alcohol).The study of the drug's composition indicated little fluctuations, which is consistent with a widely dispersed medication. Five, increasing polymer concentration improved entrapment efficiency throughout the board, whereas boosting stabiliser concentration had the opposite effect.Rutin-loaded polymeric nanoparticles were shown to have an optimal particle size of 192.4 nm -302.4 nm, according to a particle size analyzer. Eight, the formulation of polymeric nanoparticles had a polydispersity index of less than 0.3, indicating a rather homogeneous dispersion. Rutin-loaded polymeric nanoparticles with terminal carboxylic groups exhibited a negative zeta potential, indicating a negatively charged surface.It was found in in vitro drug release trials that the medication was released in two phases: an initial, rapid release within two hours, followed by a longer, more gradual release over the course of 12 hours. The in vitro drug release kinetics demonstrated a non fickian diffusion mechanism and a prolonged release profile. As can be seen from the release data and kinetic analyses, F7 has an excellent sustained release profile and high entrapment efficiency. Thirteen, compared to a pure drug solution, the solubility of Rutin-loaded polymeric nanoparticles rose by a factor of 10. Scanning electron microscopy investigation confirmed the spherical nature of the nanoparticles produced from the polymer.

Conclusion

Thus, it was determined that nanoprecipitation of polymeric nanoparticles is one of the beneficial strategies for the successful inclusion of Rutin with high entrapment efficiency. Results from solubility and ex vivo intestinal permeability tests showed that Rutin bioavailability might be increased with the use of nanoparticle formulations. In addition, it is reasonable to assume that bioavailability may be improved if particles in the nanometer range were obtained. As a result, we may infer that polymeric nanoparticles served as an effective drug delivery mechanism for Rutin, a weakly water-soluble lipophilic medication.

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List of Publications

1. Harshita Chaturvedi, Roshan Zehra, Sandhya Sharma, Amarjeet Singh European Chemical Bulletin, Issue 8, 2023.

Diagnosis of Neurodegenerative Diseases (Arthritis) towards Adequate Treatment in Nanomedicine

Harshita Chaturvedi[1], Mrs. Roshan Zehra[2], Mrs. Sandhya Sharma[3], Dr. Amarjeet Singh[4]

M.Pharm student[1], Associate Professor[2], Assistant Professor[3], Principal & Professor[4]

Innovative College of Pharmacy, Greater Noida, Uttar Pradesh-201306, India[1,2,3,4]

Abstract

Medical Laboratory Science is an autonomous profession that entails the examination of human, animal, and environmental samples for accurate diagnosis and illness treatment that is efficient and effective. has been overlooked in neurodegenerative illnesses in the past (NDDs). NDDs are progressive neurodegenerative illnesses that primarily affect the central nervous system, particularly the neurons of the brain. NDDs are most often represented by asynucleinopathies, Huntington's disease (HD), amyloidoses, Alzheimer's disease (AD), tauopathies, Parkinson's disease, amyotrophic lateral sclerosis (ALS), prion disease, and TDP-43 proteinopathies. Currently, cerebrospinal fluid (CSF) and blood are the most common diagnostic samples for neurodegenerative diseases (NDDs) based on the related biomarkers and nanoparticles. Although different forms of diagnosis and symptoms are utilised to diagnose NDDs, each NDD has a unique and particular Medical Laboratory diagnostic that is used to identify the many neurodegenerative diseases of public health significance. An efficient use of Medical Laboratory diagnostics in Nanomedicine for neurodegenerative illnesses would be a significant advancement in the field.

Keywords: Medical Laboratory diagnosis, Neurodegenerative diseases, NDDs, Nanomedicine

Introduction

Neurons are the brain cell type, and in most cases they cannot multiply or replace themselves. Neurodegenerative diseases (NDDs) are chronic conditions that deteriorate nerve cells in the brain and spinal cord over time (primarily neurons in the brain). The incidence rises as people become older. The most prevalent of them include a-synucleinopathies, HD, amyloidoses, AD, tauopathies, PD, ALS, prion disease, and TDP-43 proteinopathies. The illnesses, which are fatal and cause cognitive decline and dyskinesia, are defined by the slow and progressive death and

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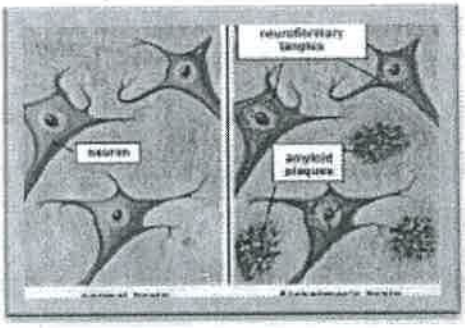
extrapyramidal and pyramidal movement disorders. While some affected individuals have what could be termed "pure syndromes", majority have combined clinical features **[2]**. They are mainly caused by genetic mutations. Other causes may include apoptosis, protein misfolding, mitochondrial dysfunction, DNA damage, and necrosis. Neurodegeneration may be brought on by a number of different things, including genetic mutations, the accumulation of harmful proteins in the brain, and the creation of neurotoxic chemicals brought on by the loss of mitochondrial activities.**[5]**. There may be more than one neurodegenerative disease process in one person and the protein misfoldings and pathophysiological processes that characterize NDDs may be present long time prior to the appearance of clinical features **[2,3]**. The progressive death and loss of neurons cause problems with ataxias and dementias. Early diagnosis is particularly important for timely intervention and treatment **[4]**.

Neurodegenerative diseases provides evidence of neuronal loss of structures or neuronal death as seen in Alzehemer's disease (figure 1) and figure 2 due to tangles of neurones.



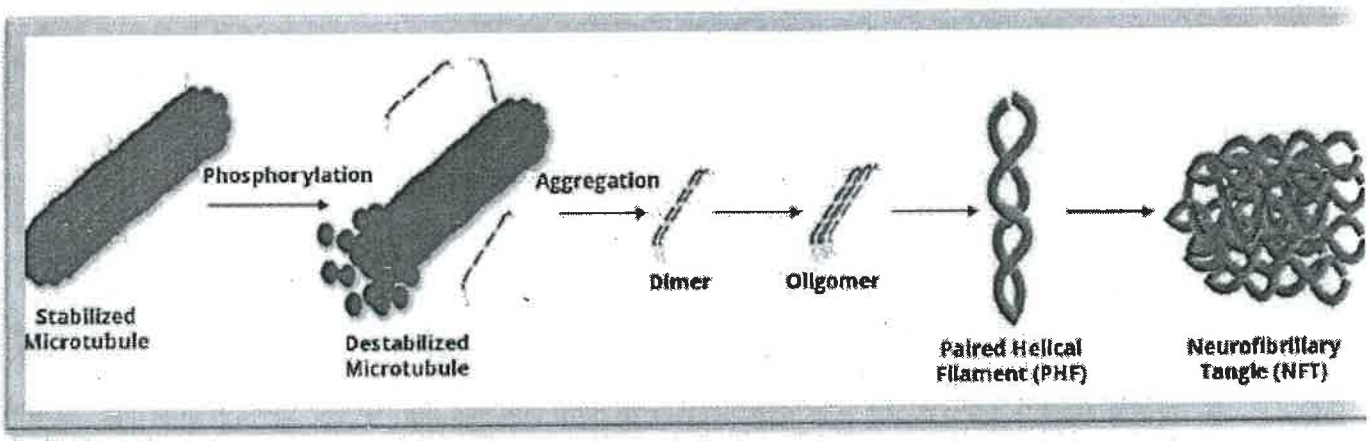


Figure 1. Neurodegenerative (Alzheimer's) disease when compared with normal neurones

Figure 2. Stages of Neuronal Disease Development

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Medical Laboratory Testing and Diagnostics contribute immensely to the clinical and medical cases available towards detection of diseases and monitoring of treatments. Studies have shown that medical laboratories contributes up to 70% of decisions taken on any clinical case. It also contributes up to 7o% of the income generated to any healthcare establishment **[6,7]**. Though, medical laboratory science may differ in nomenclature in various countries [8], their services remain same across the globe.

The contribution of medical laboratory science in disease management and treatment gives impetus to this chapter to provide a literature towards contribution of medical laboratory in the diagnosis and treatment of neurodegenerative diseases in general medical practice including nanomedicine.

This chapter provides a short review, summary, and discussion of ND disorders and their laboratory diagnostics for successful treatment in nanotechnology-based nanomedicine. Nanobiotechnology is a contemporary scientific discipline that has the potential to enhance medical practise and may play a significant role in the study and treatment of neurodegenerative disorders (NDDs). It may be used in neurodegenerative disease diagnosis, medicine delivery, and treatment.**[1,9]**.

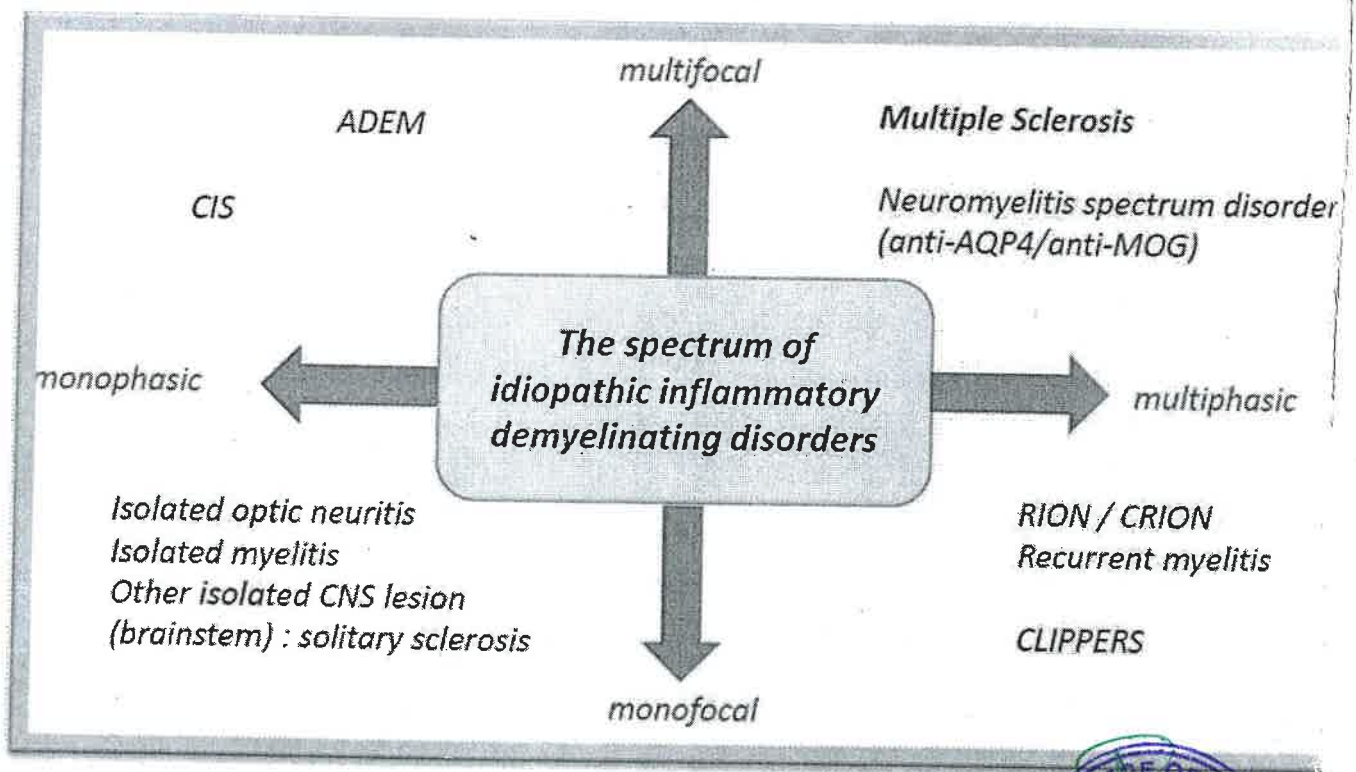

Figure 3. Nueroinflammatory or Neurodegenerative Disorders

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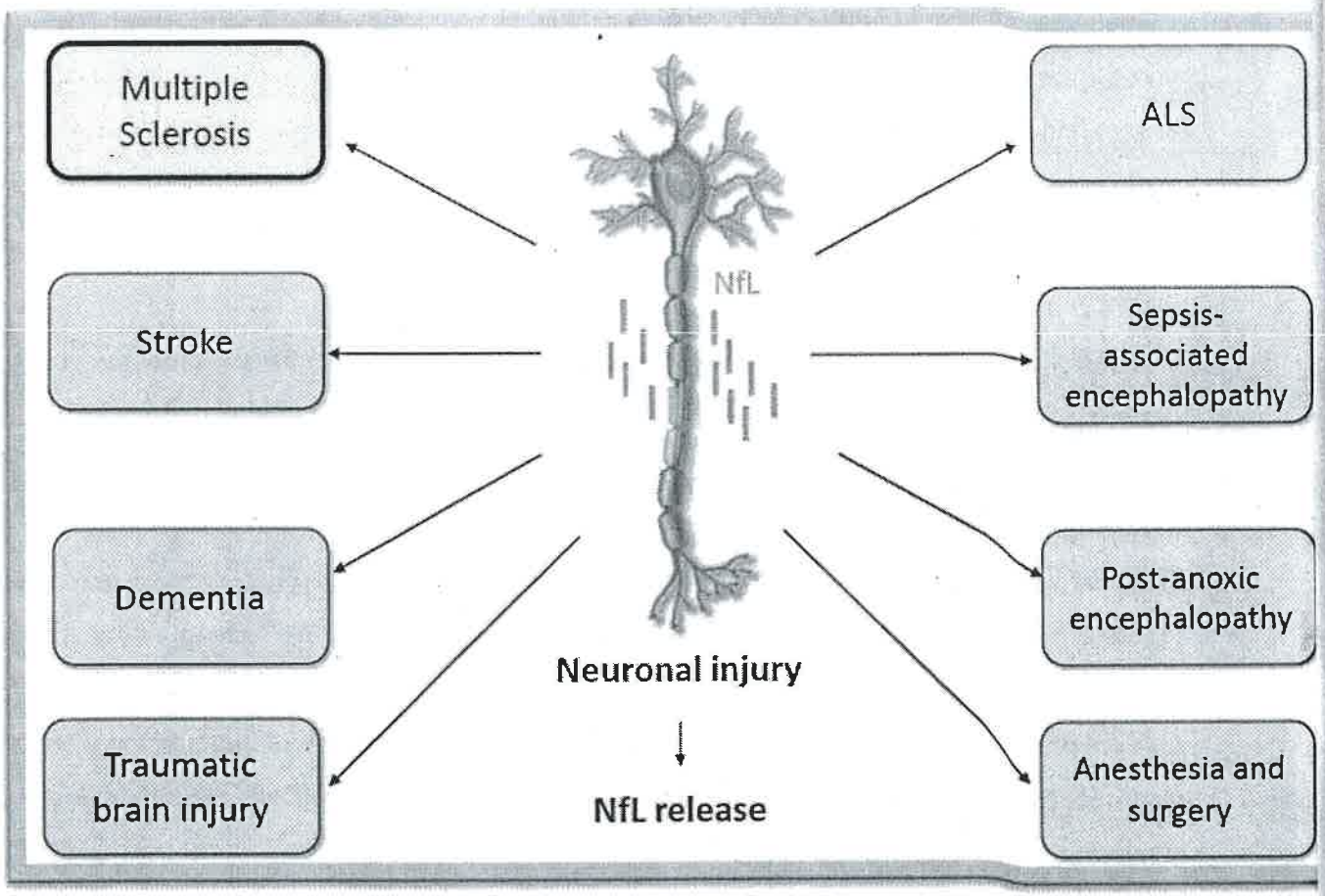

Figure 4. various Neuronal damages leading to Neurofilament release with various biomarkers

Medical Diagnosis of NDDs-neurodegenerative diseases

There has been detailed information gathered from successful thorough examination of neurodegenerative disease (NDDs) especially in histology, there are still limitations on the early diagnoses and treatment of the NDDs from routine medical laboratory perspective. This is attributable to the fact that majority of drugs and agents that effectively treat the disorders cannot pass through the blood–brain barrier (BBB) **[1,9]**.

Autopsy and examination of neuropathologic findings after a patient's death have remained the gold standard for diagnosing neurodegenerative illness at this time. There is a paucity of information on the available diagnostic biomarkers for the illnesses, except in rare instances when a genetic mutation is established to be the cause of the disease. **[2,10]**. There is a significant societal cost associated with diagnosing and treating NDDs; as a result, advances in the medical laboratory are desperately needed. To a large extent, a differential diagnosis of NDDs is based on observation of clinical symptoms. However, neurodegenerative illnesses

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cannot be diagnosed just by clinical physical examination. Hence, economical, specific, and sensitive biomarkers are recommended **[4]** and this chapter shall provide an insight.

Diagnosis of NDDs may involve stepwise processes (Figure 5) like taking the patient's medical history, neuropsy testing, neuroimaging techniques, and estimation of blood and/or CSF biomarkers (CSF Aβ42, CSF Tau,) **[11]**.

Figure 5. Steps for Clinical use of Neurofilaments in Neurological disease management

Biomarkers of Neurodegenerative Diseases

Biomarkers are "biological molecules found in blood and other body fluids or tissues that indicate sign of normal or abnormal process in normal or diseased conditions". A biomarker can be referred to as nanomolecule or nanoparticle and has helped in detecting how the normal or diseased body responds to various normal or disease conditions [12].

Nanoparticles with sizes of about 1–100 nm can provide innovative approach towards solving issues associated with ND diseases. The small sizes of nanoparticles make them able to cross the BBB and can interact with biological and biochemical systems at cellular levels. Thus, their biochemical and physical properties can be exploited for diagnosis, therapy, tissue regeneration, and drug administration **[1]**.

Biomarkers are used in nanomedicine and general medical practice for diseases management and not an exception in neurodegenerative diseases. Such biomarkers are mostly used in Medical Laboratory Science, imaging technologies and advanced medical and diagnostic techniques. The biomarkers has a relation with proteinopathies /proteopathies especially when it involves neurodegenerative diseases [13,14] no wonder the biomarkers of neurodegenerative diseases are mostly proteins (figure 6). The tables 1 & 2 presents biomarkers based on sample or types of neurodegenerative diseases involved.