PREPARATION AND *IN VITRO* EVALUATION OF GRISEOFULVIN MICROPARTICLES USING CHITOSAN AND ETHYL CELLULOSE

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

in partial fulfillment for the award of degree of

MASTER OF PHARMACY

(PHARMACEUTICS)

MARCH 2008

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

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Certificate

This is to certify that the dissertation entitled "**PREPARATION AND *IN VITRO* EVALUATION OF GRISEOFULVIN MICROPARTICLES USING CHITOSAN AND ETHYL CELLULOSE"** was carried out by **ANISH JOHN** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct supervision and guidance to my fullest satisfaction.

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CONTENTS

INTRODUCTION AND OBJECTIVE OF THE INVESTIGATION

Novel drug delivery systems, are being employed both experimentally and therapeutically to alter the body distribution of drugs with a view to reduce the toxicity of existing drugs and delivering them more efficiently. One such area in which research is gaining much attention is “controlled drug delivery” because of their more precise, reduced toxicity and enhanced efficacy.

It has been reported that about 40% of the compounds being developed by the pharmaceutical industry are poorly water-soluble. A limiting factor to the *in vivo* performance of poorly water-soluble drugs after oral administration is their inadequate ability to be wetter and dissolved into the fluid in the gastrointestinal (GI) tract. Therefore increasing the dissolution rate of poorly soluble drugs is an important and significant challenge to pharmaceutical scientists, in order to maximize absorption [1].

Microspheres has more advantages over other soluble carriers like liposomes. Liposomes have low efficiency of drug entrapment, rapid leakage of water soluble drugs, especially in the presence of blood components, poor storage stability and method of preparation; which are poorly compatible with large scale production requirements. However microspheres can entrap various molecules in a stable and reproducible way.

Griseofulvin is an antifungal drug widely used for the treatment of the dermatophytoses. Because of the emergence of more effective antifungal drugs, Griseofulvin use reduced today. The physicochemical property of Griseofulvin as a lipophilic molecule which is practically insoluble in water makes formulation and delivery difficult.

However, this molecule still interests researchers. It was proven that Griseofulvin dissolution rate could be enhanced by micronization, complexation of Griseofulvn with cyclodextrin [2].

The main purpose of this piece of study was to compare the different character of Griseofulvin microsphere using two methods with different polymers Chitosan and Ethylcellulose. Chitosan is a natural polymer and the method adopted for preparing microsphere is chemical cross-linking method. Ethylcellulose is a natural, biodegradable polymer and the method adopted for preparing microsphere is solvent evaporation method.

The plan of work was desingned into different stages;-

- Preparation of Chitosan and Ethylcellulose microspheres using thermal cross linking and solvent evaporation methods respectively.
- Determination of size and shape of microspheres.
- Estimation of percentage yield of the microspheres and its drug encapsulation efficiency.
- Study of *in vitro* release from prepared microspheres.

1. INTRODUCTION [3,4,5]

Drugs were there, are there will be there till there is life on this planet. However over the years we have learnt that optimization of drug therapy is of paramount importance and in the process; patient safety, convenience, compliance, economic and industrial feasibility are also to be overlooked.

For decades an acute and chronic illness is being clinically treated through delivery of drug to patients in form of some conventional dosage forms. Moreover these conventional dosage forms need repetitive dosing with small dosing intervals thus causing inconvenience to patients.

In the recent years, researchers developed newer drug delivery systems having more precise, spatial and temporal placement of drug in the body through a controlled drug delivery.

Basically there are 3 basic modes of drug delivery that is targeted delivery, controlled, and modulated release. Targeted delivery refers to the systemic administration of drug carrier with the goal of delivering the drug to specific cell types, tissues, and organs. Controlled release refers to the use of delivery device with the objective of releasing the drug into the patient body at a predominant rate at a specific time or with specific release profiles. Modulated release implies of drug delivery device that release the drug at a variable rate controlled by environmental conditions, bio feed back, sensor input or an external control device.

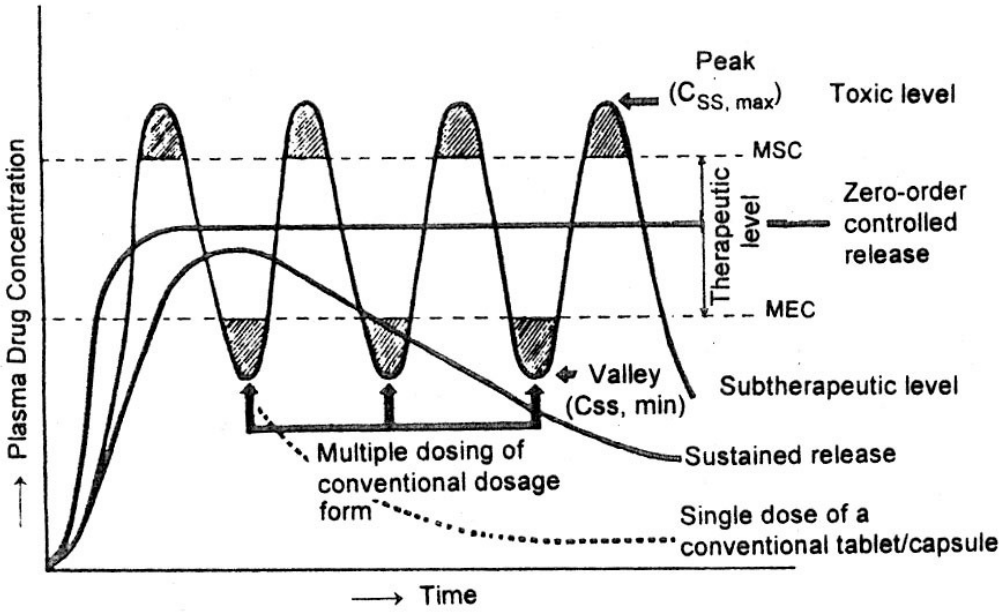
1.1. Definition

Controlled drug delivery system is one, which delivers the drug at a predetermined rate locally or systemically for a period of time.

Controlled release dosage forms have been referred to by a number of terminologies, such as delayed-action, extended-action, gradual-release, prolonged-release, depot, repeated-action, slow-release, sustained-release,

retard and timed-release dosage forms. Any of these terms, or similar labeling for designation of controlled release dosage forms.

Fig: 1



A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations

Objective of controlled delivery

- Get an extended duration of action, thus increase patient compliance.
- Localize drug action by spatial or controlled release system adjacent to or in the diseased tissue or organ.
- Targeted drug action by using carriers or chemical derivatization to deliver drugs to a particular target cell type.

1.2. Advantages

1) Decrease adverse effects and toxicity
2) Better drug utilization.
3) Controlled rate and site of release.
4) More uniform blood concentration.
5) Improved patient compliance.
6) Reduced dosing frequency.
7) More consistent and prolonged therapeutic effect.
8) A greater selectivity of pharmacological activity.
9) Avoidance of night time dosing.

1.3. Disadvantages

1) Increased variability among dosage units.
2) Stability problems.
3) Toxicity due to dose dumping.
4) Increase cost.
5) More rapid development of tolerance.
6) Need for additional patient education and counseling.
7) Poor *in vitro* and *in vivo* correlation.
8) Decreased systemic availability.
9) Retrieval of drug is difficult in case of toxicity of hypersensitivity reactions.

1.4. Factors governing the design of controlled release dosage form:-

Drug related

- Aqueous solubilityPartition coefficient
- Molecular size
- Drug stability
- Protein binding

Biological factors:

- Absorption.

- Cardiac rhythm.
- Distribution.
- Elimination.
- Duration of action.
- Margin of safety.
- Side effects.
- Disease state.

Physiological

- Prolonged drug absorption.
- Variability in GI emptying and motility.
- GI blood flow

Pharmacological

- Changes in drug effect upon multiple dosing.
- Sensitizing / tolerance.

Pharmacokinetic

- Dose dumping.
- First pass metabolism.
- Variability of urinary pH effect on elimination.
- Enzyme induction/ inhibition upon multiple dosing.

1.5. Classification of oral controlled release systems:

A) Continuous release systems

1. Dissolution controlled release systems
   a) Matrix type b) Reservoir type
2. Diffusion controlled release systems
   a) Matrix type b) Reservoir type

3. Dissolution and diffusion controlled release systems
4. Ion exchange resin drug complexes
5. Slow dissolving salts and complexes
6. Osmotic pressure controlled systems
7. pH dependent formulations
8. Hydrodynamic pressure controlled systems

B) Delayed transit and continuous release systems

1. Altered density systems
    a. High density
    b. Low density
    c. Floating
2. Mucoadhesive systems
3. Size based systems

C) Delayed release systems

1. Intestinal release systems
2. Colonic release systems

1.6. Future directions in controlled drug delivery

The most exciting opportunities in controlled drug delivery lies in the area of responsive delivery systems, with which it will be possible to deliver drugs through implantable devices in response to a measured blood level or to delivery a drug precisely to a targeted site. Much of the developments of novel materials in controlled drug delivery is focusing on the preparation and use of the responsive polymers with specifically defined macroscopic and microscopic structural and chemical features.

Such systems include,

- Copolymers with desirable hydrophilic / hydrophobic interactions
- Block or graft copolymers
- Complexation networks responding via hydrogen or ionic bonding
- Dendrimers or star polymers as nanoparticles for immobilization of enzymes, drugs, peptides or other biological agents.

1.7. MICROENCAPSULATION

Microencapsulation is one of the most intriguing fields in the area of drug delivery systems, moreover this method used to produce controlled release dosage forms. It is a technology devoted to entrapping solid, liquids or gels inside one or more polymeric coatings. Depending on the manufacturing process, various types of microcapsules structure can be obtained. The most common type is the mononuclear spherical. The microcapsulate drug delivery systems include mainly pellets, microcapsules, microspheres, liposomes, emulsions and multiple emulsions. The size range covered by the microparticle is between 1 to 1000 μm.

Classification of Microencapsulation methods:

Table:1

Process	Coating material	Solvent
Interfacial polymerization	Water soluble and insoluble monomer	Aqueous or organic solvent
Complex coacervation	Water-soluble polyelectrolyte	Organic solvent
Coacervation	Hydrophobic polymers	Organic solvent
Thermal denaturation	Proteins	Organic
Salting out	Water-soluble polymers	Water
Solvent evaporation	Hydrophilic or hydrophobic polymers	Organic or water
Hot melt	Hydrophilic or hydrophobic polymers	Aqueous or organic solvent
Solvent removal	Hydrophilic or hydrophobic polymers	Organic solvent
Spray drying	Hydrophilic or hydrophobic polymers	Air, nitrogen
Phase separation	Hydrophilic or hydrophobic polymers	Aqueous or organic

(i) Reasons for microencapsulation

- Taste and odor masking
- Conversion of oils and other liquids into solids for ease of handling
- Protection of drug against the environment
- Delay of volatilization
- Separation of flow of powders
- Safe handling of toxic substances
- Isolation from tissues
- Enteric coating
- Sustained and controlled release

- Detoxification

(ii) Properties of Drugs used for Microencapsulation

The core of microcapsules used in medicine contains one or more drugs either alone or in combinations with suitable additives to form a liquid or solid phase. Liquid core may be composed of polar or non polar substantial that comprises the active ingredient or that act as vehicle for dissolved or suspended drugs. The solvent properties of such liquids will critically influences the rate of drug release and the selection of coating materials. For drugs of low water solubility with known bioavailability problems associated with low rates of dissolution, decreases in particle size of suspended drugs may be important in enhanceing *in vivo* absorption. Smaller Microcapsules to have faster release rates because of their increased surface area per unit volume or weight of core material. Solid cores are used more frequently than liquid cores. Very small core particles tend to give aggregation problem during production, because of their surface attraction forces. Large particles can cause problems of their rapid sedimentation.

The shape of these cores also important, It is much easier to deposite uniform coating on regular spherical particles of narrow size range that are devoid of sharp edges. Accordingly, choice of particular polymorphs of drugs, reduction of irregular shaped crystalline material by grinding or use of spray drying forms. Density of cores very important in controlled the transit time in GI tract. Increasing density was the most factore promoteing, retention of pellets in stomach.

Ex: High density systems.

Decreaseing density is important in floating type dosage forms. Swelling of core with disruption of coating and leads to un controlled drug release.

(iii) Properties of Polymer used for Microencapsulation

The selection of the appropriate coating materials dictates, to a major degree the resultant physical and chemical properties of the Microcapsules. The coating material should be capable of forming a film that cohesive with the core material and the ideal coating material should be chemically compatible and non reactive with core material, and provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability.

Typical coating properties such as cohesiveness, permeability, moisture sorption, solubility, stability and clarity must be considered in the selection of proper coating material. The uniqueness of Microcapsules in their properties and use involves their characteristic smallness.

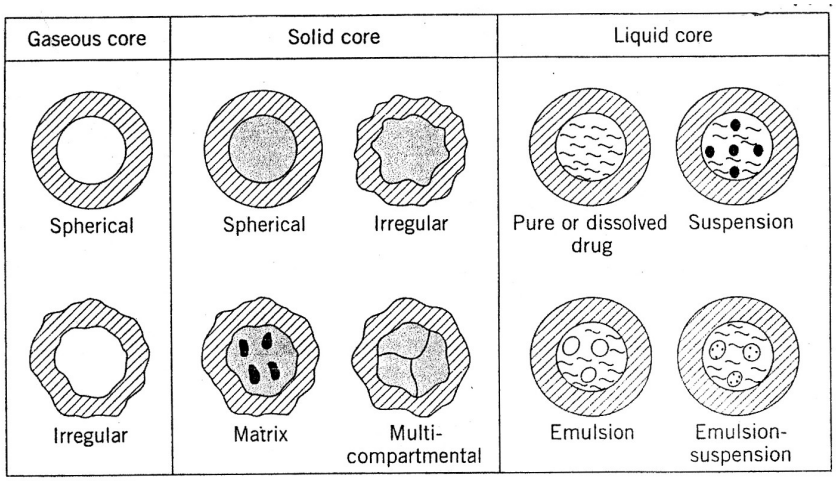
Consequently, the protective coating that are applied are quite thin. Although the active content of many Microcapsulated products can be varied from a few percent to over 99%, the effective coating thickness that can be realized, regardless of the method of application employed varies from tenths to few hundred microns, depending on the coating to core ratio and the particle size (surface area) of the core material.

1.8. MICROSPHERES AND MICROCAPSULES

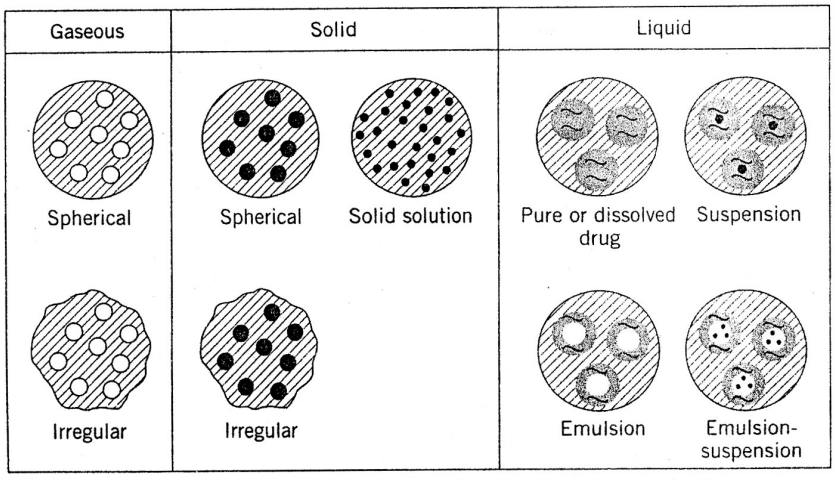
Microspheres can be defined as spherical empty particles ranging in size from 1 to 1000 micrometer. Microspheres are characteristically free flowing powder consisting of proteins or synthetic polymers, which are biodegradable in nature.

Microcapsules is system consists of a well-defined core and a well-defined envelope; the core can be solid, liquid or gas, the envelop is made of continuous process or nonporous polymeric phase.

It is also defined as a spherical particle with size varying from 50nm to 2mm containing a core substance.



(a)



(b)

Fig:2 various configurations of (a) microcapsules and (b) microspheres

Microspheres have been investigated for intravenous and intra arterial targeting and delivery systems. Microspheres and microcapsule have been injected in the vessels to ensure the passive targeting of drugs. The drug release is controlled by diffusion through the polymer matrix and/or by erosion of the polymer. The role of microspheres and microcapsules depend their size and site of injection.

Microparticles of diameter smaller than 2µm can be injected in an intravenous, intra arterial and intra peritoneal manner in order to target the

RES. Intravenous injection of microspheres of size from 3-12 micrometer is intended to block the capillaries of lungs, liver and spleen.

Vessels can be hyperselectively embolized with drug loaded particulate materials of more than 10 micrometer. Microspheres of 100-300 micrometer size are the most appropriate embolic agents. They reach the intra lesional precapillary arteries and cause reduction of blood flow.

Drugs can be incorporated in microsphere and microcapsule by two methods:

1. The polymer can either surround a core of drug (encapsulated type).

2. The drug can be dissolved or homogeneously dispersed in the polymer (matrix device – microsphere type).

(i) The advantages of microspheres

1. Taste and odor masking.
2. Conversion of oils and other liquids to solids for ease of handling.
3. Protection of drugs against the environment (moisture, light, heat, and/or oxidation) and vice versa (prevention of pain on injection).
4. Delay of volatilization.
5. Separation of incompatible materials (other drugs or excipients such as buffers).
6. Improvement of flow of powders.
7. Safe handling of toxic substances.
8. Aid in dispersion of water-insoluble substances in aqueous media.
9. Production of sustained-release, controlled-release, and targeted medications.

(ii) Route of administration:

Route of administration is selected depending on the drug properties, disease state being treated and the age and condition of the patient. Desirable properties of the microspheres to be used for the delivery will also change depending on the route of administration.

a) Oral delivery:

Oral delivery is the simplest way of drug administration. Constraints of the oral route are numerous on the whole it offers less potential danger than the parentral route. The relatively brief transit time of about 12hours trough the GI tract limits the duration of action that can be expected via the oral route. Recently, it has been reported that microsphers of less than 10μm in size are taken up by the Peyer's patches and may increase the retention time in the stomach. Also microsphere made from polymers with mucoadhesive propertied get attached to the stomach and prolong the residence time in the stomach. Bioavailability of drugs with limited solubility in the stomach or intestine and small absorption rate constant can be increasing by increasing the retention time in the stomach. Improved drug delivery was observed compared to the microspheres administered alone.

b) Parenteral delivery:

Most of the microspheres based controlled delivery systems are developed with the aim of using them for parenteral administration. Drug released is completely absorbed in this case. Microspheres used for parenteral delivery should be sterile and should be dispersible in a suitable vehicle for injection. Surfactants in small concentration are often necessary for reconstituting hydrophobic particles for injection in aqueous vehicles, which are reported to, came adverse tissue reactions and affect the release of the incorporated drug.

(iii) Polymers used:

Synthetic polymers

Non-biodegradale:

PMMA

Acrolein

Glycidyl methacrylate

Epoxy polymers

Biodegradable:

Lactides and Glycolides

Polyalkyl cyano acrylates

Polyanhydrides

Natural materials

Proteins:

Albumin

Gelatin

Collagen

Carbohydrates:

Starch

Agarose

Carrageenan

Chitosan

Chemically modified carbohydrates:

DEAE cellulose

Poly(acryl dextran)

Poly(acryl) starch

Prerequisites for an ideal microparticulate carriers

- Longer duration of action
- Control of content release
- Increase of therapeutic efficiency
- Protection of drug
- Reduction of toxicity
- Biocompatability

- Sterilizability
- Relative stability
- Water solubility or dispersability
- Bioresorbability
- Targetability
- Polyvalent

(iv) Methods of preparation:

The method of preparation and its choice are equicocally determined by some formulation and technology related factors as mentioned below:

1. The particle size requirement
2. The drug or the protein should not be adversely affected by the process
3. Reproducibility of the release profile and the method
4. No stability problem
5. There should be no toxic products associated with the final product

Synthetic polymers are now materials of choice for the controlled release as well as targeted micro particulate carriers. The various methods are described below.

Solvent evaporation

A technique is based on the evaporation of the initially phase of an emulsion by agitation. Initially, the polymeric supporting material is dissolved in a volatile organic solvent. The active principle to be encapsulated is then dispersed of dissolved in the organic solution to form a suspension, emulsion, or solution. In the following step the organic phase is emulsified under agitation in a dispersing phase consisting of a non solvent of the polymer, which is immiscible with the organic solvent, which contains an appropriate tension active additive. Once emulsion is stablilized, agitation is maintained and solvent is the creation of solid microspheres. On the completion of the solvent evaporation process, the microspheres held in

suspension in the continuous phase are recovered by filtration of centrifugation and are washed and dried.

1. **Thermal and Chemical cross linking:**

Micro spheres made from natural polymers are prepared by a cross linking process; the polymers include gelatin albumin, starch and dextran. A water oil emulsion is prepared, where the water phase is a solution of the polymer, which contains the drug to be incorporated. The oil phase is a suitable vegetable oil or oil-organic solvent mixture containing an oil-soluble emulsifier. Once the desired water-oil emulsion is formed, the water-soluble polymer is solidified by some kind of cross-linking process. This may involve thermal treatment or the addition of a chemical cross- linking agent like Glutaraldehyde. In the chemical and thermal cross linking both the amount of chemical and intensity of heating are critical in determining the release rates and swelling properties of the micro spheres. If the Glutaraldehyde is the cross linking agent, residual amounts can have toxic effects.

Precipitation

A variation on the evaporation method is the precipitation method. The emulsion consists of polar droplets dispersed in a no polar medium. Solvent may be removed from the droplets by the use of a co solvent. The resulting increase in the polymer-drug concentration causes a precipitation forming a suspension.

2. **Freeze drying**

This technique involves the freezing of the emulsion; the relative freezing points of the continuous and dispersed phases are important. The continuous-phase solvent is usually organic and is removed by sublimation at low temperature and pressure. Finally, the dispersed-phase solvent of the droplets is removed by sublimation, leaving polymer-drug particles.

3. Spray drying and spray congealing

Spray drying and spray congealing methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or the cooling of the solution, the two processes are named spray drying and the spray congealing respectively.

The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microsphers in a size range 1-100μm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vaccum drying.

Very rapid solvent evaporation, however leads to the formation of porous microparticles.

Advantages:

1. Feasibility of the operation under aseptic conditions.
2. The process is rapid.
3. Suitable for both batch and bulk manufacturing.
4. This technique is used to encapsulate a large number of drugs.

6. Phase separation coaservation techniques

Phase separation method is specially designed for preparing the reservoir type of the system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however, some of the preparations are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids. In matrix type device, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of the polymer

in the organic phase to affect the formation of the polymer rich phase called the coacervates. The coacervation can be brought about by addition of the third component to the system which results in the formation of the two phase, one rich in the polymer, while other one, i.e. supernatant, depleted of the polymer. This is achieved by the addition of salt, non-solvent addition, addition of the incompatible polymer or change in pH.

In this technique, the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution. Phase separation is then accomplished by changing the solution conditions by using any of the method mentioned above. The process is carried out under continuous stirring to control the size of the microparticles.

(v) Loading of drug:

The active components are loaded over the microspheres principally using two methods, i.e., during preparation of the microsphere or after the formation of the microspheres by incubation them with the drug/protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption.

Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives (eg: cross-linking agent, surfactants, stabilizers etc), heat of polymerization, agitation intensity etc. The loading is carried out in pre-formed microspheres by incubating them with high concentration of the drug in a suitable solvent. The drug in these microspheres is loaded via penetration of diffusion of the drug through the pores in the microspheres as well as adsorption on their surface. The solvent is then removed, leaving drug loaded microsphere.

The Freundlich model is applied to determine the adsorption of the drugs. The Frenudlich equation is,

$$\frac{X}{M} = KC^{P_{eq}}$$

Where,

K = Constant related to the capacity of the adsorbent for the adsorbate.

P = Constant related to the affinity of the adsorbent for the adsorbate.

(vi) Release of drug form the micro spheres

The rate of drug release form micro spheres dictates their therapeutic action. Drug release is governed by the molecular structure of the drug and polymer the resistance of the polymer to degradation and the surface area and porosity of the micro spheres.

Reservoir delivery systems extend the residence time of drug within the systemic circulation originally focused on zero-order dissolution kinetics.

In porous polymeric systems the rate of drug release is dictated by the device surface area, which is linked directly to its shape. The rate of release form spheres may result from polymer diffusion or erosion.

The internal structure may vary as a function of the microencapsulation process employed. Reservoir microcapsules have a core of drug coated with polymer. The drug is distributed homogeneously throughout the polymeric matrix in monolithic micro spheres.

The mechanism of drug release at a controlled rate from micro spheres include diffusion of drug through a polymeric excipient, diffusion of trapped drug as polymer erodes, and release of drug through pores in the polymeric excipient.

Increase the surface area, by reducing the particle size, results in an increased release rate. The path length travel by the drug in the matrix can be controlled by manipulating the micro sphere loading. Micro spheres with

high drug content release the outline ingredient more rapidly than those with a low load. Thicknesses of the polymer also influence the drug release rate.

Mechanism of drug release:

Factors affecting the release of the drug form the particulate system in relation to drug, microspheres and bioenvironment.

Drug:

Position in microspheres

Molecular weight

Concentration

Interaction with matrix

Microspheres:

Type and amount of the matrix polymer

Size and density of the microspheres

Extent of cross linking, denaturation or polymerization

Adjuvants

Environment:

PH

Polarity

Presence of Enzyme

The geometry of the carrier, ie, whether it is reservoir type where the drug is present as a core, or matrix type in which drug is dispersed throughout the carrier, governs overall release profile of the drug or active ingredients.

Reservoir type system

Release from the reservoir type system with rate controlling membrane proceeds by first penetration of the water through the membrane followed by dissolution of the drug in the penetrating dissolution fluid. The dissolved drug after partitioning through the membrane diffuses across the

stagnant diffusion layer. The release is essentially governed by the Fick's first law of diffusion as,

$$J = - D (dc/dx)$$

Where,

J = flux per unit area

D= diffusion coefficient

Diffusion across the membrane determines the effectiveness of the carrier system. The release rate from the carriers can be modified by changing both the composition and the thickness of the polymeric membrane.

Matrix type system

Release profile of the drug form this type of the device critically depends on the state of drug whether it is dissolved or dispersed in the polymer matrix.

In case of the drug dissolved in the polymeric matrix, amount of drug, and the nature of the polymer affect the release profile. If this is the case the amount of drug appearing in the receptor phase at 't' is approximated by two separate equations. The first equation determines the 60% of the drug release while the second shows the release profile at later stage.

$$\frac{dM_t}{dt} = 2\mathrm{M}_X \left(D/\pi l^2 t \right)^{1/2}$$

$$\frac{dM_t}{dt} = 8\, DM_X / l^2 \exp \pi^2 D_t / l^2$$

Where,

l = thickness of polymer slab

D = diffusion coefficient

Mx = total amount of drug present in the matrix

Mt = amount of drug released in time 't'

When the drug is dispersed throughout the polymer matrix then the release profile follows Higuchi's equation.

$$\frac{dM_t}{dt} = \frac{A}{2}(2DC_sC_o)$$

Where,

A = area of matrix

Cs = solubility of the drug in matrix

Co = total concentration in the matrix

(vii) Fate of microsphere in the body

Table:2 Proposed mechanism for uptake of microspheres

(viii) Applications of microsphers

- Microspheres in vaccine delivery
- Antigen release
- Immune system
- Targeting using micro particulate carriers (ocular, intranasal, oral)
- Magnetic microspheres
- Monoclonal antibodies mediated microspheres targeting immunomicrospheres
- Imaging
- Micro sponges: topical porous microspheres.

1.9. Chitosan Microspheres

Chitosan react with controlled amounts of multivalent anion result in cross inking between chitosan molecules. The cross linking may be achieved in acidic, neutral or basic environment depending on the method applied. This cross linking has been extensively used for the preparation of chitosan microspheres. The process that have been used for the preparation of chitosan microspheres are given below. Down outlines various methods which have been used for the preparation of chitosan microspheres.

Fig: 3 Schematic representation of various methods of preparation of chitosan microspheres.

(i) Thermal and chemical cross linking

Microspheres made from natural polymers are prepared by a cross linking process; the polymers include gelatin albumin, starch and dextran. A water oil emulsion is prepared, where the water phase is a solution of the polymer, which contains the drug to be incorporated. The oil phase is a suitable vegetable oil or oil-organic solvent mixture containing an oil-soluble emulsifier. Once the desired water-oil emulsion is formed, the water-soluble polymer is solidified by some kind of cross-linking process. This may involve thermal treatment or the addition of a chemical cross- linking agent like Glutaraldehyde. In the chemical and thermal cross linking both the amount of chemical and intensity of heating are critical in determining the release rates and swelling properties of the micro spheres. If the Glutaraldehyde is the cross linking agent, residual amounts can have toxic effects.

1.10. Ethyl cellulose Microspheres

Ethyl cellulose microsphere were prepared based on the principle of coacervation with certain modifications, the rational for selecting EC for the shell material was that this substance is commonly used as an additive to foods and drug because of its high inertness, and forms a stable, semipermeable capsular membrane.

Ethyl cellulose is synthetic polymer, synthetic polymers have the advantage that they can be easily and reproducibly prepared. This can be copolymerized with one another to alter their physical, chemical and mechanical properties and can be prepared as low or high molecular weight material by suitable reaction conditions. Chemical bonds which are susceptible to degradation include amides, esters, orthoesters, acetyls, glycosides and related groups. Biodegradability of the polymer depends on many factors such as polymer structure, molecular weight, physical form of the polymer and environment in which the polymer is placed since many Proteolytic enzymes specifically catalyzes the hydrolyses of peptide linkages

adjacent to substituted proteins, substituted polymers containing benzyl, hydroxyl, carboxy-methyl and phenyl groups have been prepared to improve biodegradability

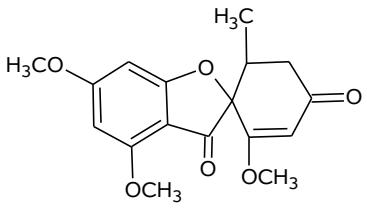
(i) Solvent evaporation process

Solvent evaporation technique was fully developed at the end of the 1990s since them numerous studies have been carried out on the basis of this method.

A technique is based on the evaporation of the initially phase of an emulsion by agitation. Initially, the polymeric supporting material is dissolved in a volatile organic solvent. The active principle to be encapsulated is then dispersed of dissolved in the organic solution to form a suspension, emulsion, or solution. In the following step the organic phase is emulsified under agitation a dispersing phase consisting of a non solvent of the polymer, which is immiscible with the organic solvent, which contains an appropriate tension active additive. Once emulsion is stabilized, agitation is maintained and solvent is the creation of solid microspheres. On the completion of the solvent evaporation process, the microspheres held in suspension in the continuous phase are recovered by filtration of centrifugation and are washed and dried.

1.11. Profiles of drug employed in the study [6,7,8,9,10]

GRISEOFULVIN



Chemistry

Griseofulvin is (2S,4R)-7-chloro-2,4,6-trimethoxy -4-methylspiro-[benzofuran-2(3H),3-cyclohexane]-3,6-dione, produced by the growth of certain strains of Penicillium griseofulvin or obtained by any other means. It is heterocyclic bezofuran).

Category

Antifungal

Description

White to yellowish white powder, the particles of which are generally up to 5 μm in maximum dimension, although larger particles which may occasionally exceed 30 μm may be present; almost odorless.

Molecular weight

352.77

Empirical formula:

$C_{17}H_{17}ClO_6$

Solubility

Freely soluble in dimethylformamide and In 1,1,2,2-tetrachloroethane ; soluble in acetone and in chloroform ; slightly soluble in ethanol(95%) and in methanol ; practically insoluble in water.

Storage

Stored in tightly closed containers.

Pharmacodynamics

Mechanism of action

Griseofulvin exerts its fungistatic action primarily by disrupting the cells miotic spindle structure, thus arresting the metaphase of cell division. Although the effect on mitosis is similar to that caused by colchicine, a different mechanism is probably involved. Another proposed mechanism of action is that griseofulvin causes production of defective DNA which is unable to replicate.

Pharmacokinetics

Absorption

Following oral administration griseofulvin is absorbed principally from the duodenum. Absorption of microsize griseofulvin is variable and unpredicatable and range from 25-70% of an oral dose. Absorption enhanced by administration of drug with fatty meals.

Ultramicrosize griseofulvin is almost completely absorbed following oral administration. A single 500mg dose of griseofulvin administered to fasting adults produces mean peak serum concentration of 0.5-2 mcg/ml 4 hours after oral administration.

Half life

10 – 30 hours

Distribution

Following absorption griseofulvin is concentrated in skin, nail, hair, liver, fat, and skeletal muscles. Griseofulvin is deposited in keratin precursor cells and has a high affinity for diseased, tissue; the drug is tightly bound to new keratin.

Within 4 hours after administration of a single 500mg dose of microsize griseofulvin, concentration of 1 mcg/gm of skin have been found ; after 8 hours of the dose 3 mcg/gm found in the skin.

Griseofulvin concentrations in skin are higher in warm climates than in cold.

Elimination

Griseofulvin has an elimination half life of 9-24 hours.

Griseofulvin is oxidatively demethylated and conjugated with glucuronic acid, principally in the liver. The major metabolites, 6-desmethylgriseofulvin, is microbiologically inactive which is excreted in urine. A large amount of dose of griseofulvin of reduced particle size appears unchanged in the faeces; less than 1% is excreted unchanged in the urine; some is excreted in the sweat.

Spectrum of activity

In vitro griseofulvin concentrations of 0.15-0.5 mcg/ml inhibit species of *Trichophyton*, *Microsporum* and *Epidermophyton* when they are exposed to the drug for 72 hours. Specifically, griseofulvin is active against *Microsporum andouinni, M.canis, M.gypseum* and *Epidermophyton*

floccosum, Trichophyton rubrum, T.eonsurans, T.mentagrophytes, T.megninii, T.gallinae and T.schoenleinni.

Antimicrobial action

Griseofulvin is fungistatic antibiotic which inhibits fungal cell division by disruption of the mitotic spindle structure. It may also interfere with DNA production. It is active against dermatophytes, including some species of Epidermophyton, Microsporum or Trichophyton.

Drug interaction

Phenobarbitone has been reported to decrease the gastrointestinal absorption of griseofulvin.

Griseofulvin may increase the rate of metabolism and effects of some drugs such as coumarin anticoagulants and oral contraceptives.

Griseofulvin has also been reported to reduce plasma concentration of salicyliate in a patient taking aspirin.

Griseofulvin may enhance the effect of alcohol.

Precautions

Griseofulvin is contra-indicated in patients with porphyria, liver failure, or systemic lupus erythematosus.

Griseofulvin is contra indicated in pregnancy.

Griseofulvin may reduce the effectiveness of oral contraceptives.

Griseofulvin may impair the ability to drive or operate machinery, and has been reported to enhance the effects of alcohol.

Griseofulvin has been associated with acute attack of porphyria and is considered cusate in patients with acute porphyria.

Adverse effects:

Side effects are usually mild and transient and consist of headache, skin rashes, dryness of the mouth, and altered sensation of taste, and gastro intestinal disturbances.

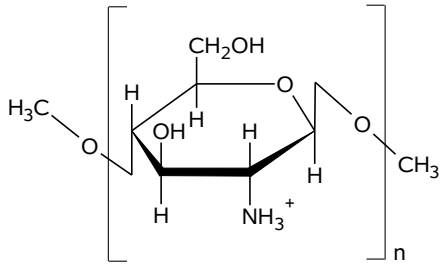
Depression, confusion, dizziness, insomnia and fatigue have also been reported.

There have been a few reports of hepatotoxicity attributed to griseofulvin.

1.12. Profiles of polymers employed in the study [11]

In the present study Chitosan and Ethyl cellulose were used in the preparation of micropartilces.

(i) CHITOSAN:



(ά – (1,4)2-amino-2-deoxy-β-D glucan) is a polysaccharide derived from chitin.

Source:

Chitin , a polysaccharide of animal origin is obtained from waste material of crustaceans such as crabs, lobsters, hrimps, prawns and crayfish. Also from the exo-skeleton and wings of insects crustacean shells.

Molecular Weight:

1-3 x10^5 Da

Physical Characters:

It occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look cotton like.

Chemical Characters:

Chitosan is a cationic polyamine with a high charge density at pH <6.5 so adheres to negatively charged surface and chelates metal ions. It is a linear polyelectrolyte with ractive hydeoxyl and amino groups so available for chemical reaction and salt formation.numerous studies have demonstrated that the salt form, molecular weight, and degreee of deacetylation as well as pH at which the chitosan is used all influence how this polymer is utilized in pharmaceutical application.

Solubility:

Chitin is insoluble in most of the solvent, acids and bases, chitosan is insoluble in neutral and alkaline pH values but dissolves in organic and inorganic acids (e.g. acetic acid, formic acid, glutamic acid, lactic acid. hydrochloric acid etc.) to produce viscous polyelectrolyte solution.

Preparation of chitosan from chitin:

Crustacean shells are the usual raw material of chitin. Shells are cleaned of all adhering flesh and washed with water. These are crushed into small pieces and dried and ground to fine powder. It is cleaned from proteins and lipids by treatment for 3 hours in 1N sodium hydroxide solution and for 12 hours in 1N hydrochloride acid solution at room temperature to remove calcium carbonate. The alkali and acid treatments are repeated twice, the chitin is decolourised by refluxing in acetone.

Toxicity:

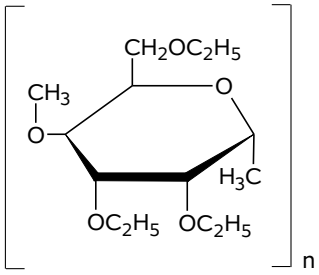
Chitosan caused concentration dependent toxicity towards cells in vitro and red blood cell lysis. It has similar structural characteristic as that of glycosamino glycans. It is tough, biodegradable and non-toxic. In vivo toxicity test indicated that chitosan is non-toxic, inert-LD 50 oral toxicity level of chitosan were established both in mice and rats. Lack of chitosan was noticed as evidenced by an oral LD 50.10 g/ Kg. in mice. Acute systemic toxicity tests in mice did not show any toxic effects of chitosan. Eye irritation in rabbits and skin irritation test in guinea pig did not reveal any undesirable toxic effects of chitosan. All the pharmacological test were undertaken as per USP.

Chitosan is cheap, widely available in Indian coastal areas giving wide opportunities to drug development professionally for better drug delivery system.

Applications

1. It is used for variety of biomedical applications including wound dressing and drug delivery system.
2. It is used for making absorbable and digestible surgical sutures.
3. Chitin and Chitosan are used as vehicle in compressed tablets.
4. It has been used as direct compression diluent.
5. It is an agent for the enhancement of the dissolution properties of some less soluble drugs.
6. Chitosan has acid-lowering effect of the stomach.
7. Chitosan is forming gel at low pH and has antacid and antiulcer activities that may prevent of reduce drug irritation in the stomach.

(ii) ETHYLCELLULOSE:



Synonyms:

Aquacoat ECD, aqualon E 462, ethocel, surelease.

Chemical name:

Cellulose ethyl ether

Description:

Ethyl cellulose is a tasteless, free flowing, white to light tan-coloured powder.

Density:

0.4 g/cm^3

Glass transition temperature:

129 – 133º C

Solubility:

Ethyl cellulose is practically insoluble in glycerin, propylene glycol, water.

Ethyl cellulose that contains less than 46.5% of ethoxy groups is freely soluble in chloroform. Methyl acetate, tetrahydrofuran, aromatic hydrocarbons and ethanol.

Freely soluble in ethanol, ethylacetate, methanol, toluene.

Specific gravity:

1.12 – 1.15 g/cm^3

Viscosity:

5 – 100m pascals (7 – 100cp)

Functional category

Coating agent, flavouring fixative, tablet binder, tablet filler, viscocity-increasing agent.

Method of manufacture

Ethyl cellulose is prepared by treating purified cellulose (sourced from chemical-grade cotton, linters and wood pulp) with an alkaline solution, followed by ethylation of the alkali. Cellulose with chloroethane as shown below, where R represents the cellulose radical.

The manner ethyl group is added to cellulose can be described by the degree of substitution(DS). The DS designates the average number of hydroxyl positions on the anhydroglucose unit that have been reacted with ethyl chloride. Since each anhydroglucose unit of the cellulose molecule has three hydroxyl groups, the maximum value for DS is 3.

Stability and storage conditions

Ethylcellulose is stable, slightly hygroscopic material. Chemically resistant to alkali, more sensitive to acidic materials. Ethyl cellulose is subjected to oxidative degradation in the presence of sunlight or UV light at elevated temperature. This may be prevented by use of antioxidant and chemical additives that absorb light in the 230-340nm range.

It should be stored at a temperature not exceeding 32ºC in a day area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

Safety

Ethyl cellulose widely used in oral and topical pharmaceutical formulations. It is also used in food products. Ethyl cellulose is not metabolized following oral consumption and is therefore a noncalorific substance. Because ethyl cellulose is not meatabolized it is not recommended for parenteral products; parenteral use may be harmful to the kidneys.

Ethyl cellulose generally regarded as a nontoxic nonallergenic and non irritating material. An ethyl cellulose is not considered to be a health hazard; the WHO has not specified an acceptable daily intake.

Incompatabilities:

With paraffin wax and microcrystalline wax.

Applications in pharmaceutical formulations:

1) hydrophobic coating agent for tablets and granules.
2) Modified the release of drug.
3) To mask the unpleasant taste of drug.
4) To improve the stability of formulation.
5) Thickness agent in creams, lotions, gels.
6) Used in cosmetics, food products.
7) Binder in tablets.

II. REVIEW OF LITERATURE

1. Akbuga et al.,[12] (1999) prepared Chitosan microspheres containing cis-platin using a w/o emulsion system. Variables important for microsphere properties were investigated: these include; chitosan, cis-platin and glutaraldehyde concentration, the types of chitosan and oil, cross linking process and stirring rate. Chitosan and glutaraldehyde concentrations, the type of oil and chitosan have a significant effect on cis-platin entrapment in chitosan microspheres. Cis-platin release from microspheres is characterized by an initial burst effect.

2. Denkbas et al.,[13](1999) prepared Chitosan microspheres by a suspension cross-linking technique. A petroleum ether/mineral oil mixture was used as the suspension medium which includes an emulsifier, e.g. Tween-80. Glutaraldehyde was used as the cross-linker.5-Fluorocuacil was incorporated in the matrix for the possible use of the microspheres in chemoembolization. Smaller microspheres with narrower size distributions were obtained when the chitosan/solvent ratio and drug/chitosan ratio were lower.

3. Tamilvanan et al.,[14](2000) prepared Ibuprofen-loaded polystyrene microparticles by the emulsion-solvent evaporation process from an aqueous system. The drug content in all the formulations was less than the theoretical drug loading. The lower drug content was due to drug partitioning to the external aqueous phase during formulation. The effect of size of the micropaticles in drug release was more important for the low drug-loaded microparticles than that for the high drug-loaded microparticles. Such release behavior from the microparticles was explained on the basis of the morphological structure of the microparticles.

4. Lin et al.,[15](2000) prepared water-soluble sodium diclofenac-loaded microspheres with three low-molecular weight polyesters, poly(L-lactic acid)(PLA), copoly(lactic acid/glycolic acid)(PLGA) and poly(δ-

valerolactone)(PV), using the oil-in-oil(o/o) emulsification-solvent evaporation method. The results indicate that high encapsulation efficiency and better monodispersity might be achieved by the o/o emulsification-solvent evaporation method, depending on the amount of drug loading used. The in vitro degradation rate of all the microspheres in pH 7.4 phosphate buffer solution showed first-order kinetics, and ranked in the order of PLGA>PLA>PV microspheres.

5. Fernandez et al.,[16] (2000) developed a controlled release formulations of alachlor in ethyl cellulose. Microencapsulted alachlor should have reduced potential for leaching in the soil while maintaining effective biological activity. Microspheres of alachlor were prepared using ethyl cellulose, according to the solvent evaporation method.

6. Michail Abainge et al.,[17](2000) studied the sustained release nature of ofloxacin microspheres-to eradicate bacterial biofilm associated with chronic infections from sensitive strains of bacteria-was determined both in vitro and in vivo. Ofloxacin microspheres were prepared by emilsion solvent evaporation procedure using poly(glycolic acid-co-dl-lactic acid)(PLGA) as the biodegradable polymer. The microspheres were characterized by scanning electron microscopy, in vitro release in an incubator, and in vivo release in the rat subcutaneous model. The microspheres were highly spherical with a very smooth surface.

7. Yang et al.,[18] (2000) studied microencapsulation of aspirin in ethyl cellulose was in a surfactant-free, water-in-oil type of emulsification/solvent evaporation process using non-toxic solvents. Ethanol was used as the dispersed phase and soybean oil as the continuous phase. The addition of a small amount of non-solvent (water) prior to the emulsification was found to have a significant impact on the microencapsulation process. The addition of non-solvent also markedly changes the microsphere characteristics, resulting in a coarser surface and an increased release rate.

8. Vandelli et al.,[19] (2001) proposed a new non-toxic cross linking agent, D.L-glyceraldehyde(GAL), to overcome the restriction in using crosslinked gelatin in the pharmaceutical field. Gelatin microspheres crosslinked with different concentrations of GAL (0.5, 1 or 2 % w/v) and for different time periods (1 or 24 h) were prepared. To evaluate the pharmaceutical properties, an antihypertensive drug, clonidine hydrochloride, was chosen as drug model and loaded into the microspheres.

9. Perumal[20] (2001) proposed a method to prepare modified release microsphre of ibuprofen by emulsion solvent diffusion. The technique was optimized for the following processing variables: the absence/presence of baffles in the reaction vessel, agitation rate and drying time. Thereafter, the influence of various formulation factors on the microencapsulation efficiency, in *vitro* drug release and micromeritic properties was examined. The variables included the methacrylic polymer, Eudragit RS 100, ibuprofen content and the volume of ethanol used during microencapsulation. The results obtained were then interpreted on a triangular phase diagram to map the region of microencapsulation, as well as those formulations that yielded suitable modified release ibuprofen microspheres.

10. Pérez-Martínez et.al.,[21] (2001) studied that the pesticide norfluazon has been microencapsulated using ethyl cellulose to develop controlled-release formulations that decrease its mobility through the soil and protect it from photodegradation. Ethyl cellulose microspheres loaded with norfluazon were prepared by the solvent-evaporation method. To obtain the microspheres, certain conditions (pesticide/polymer ratio, percentage of emulsifying agent and solvent) were varied. The shape and size of the microspheres obtained were studied by scanning electron microscopy. Other parameters, such as solids recovery, encapsulation efficiency and pesticide loading, were

also studied. The release rate of norfluazon from the different microspheres was slower than that of pure norfluazon. In particular, microspheres obtained with *o*-xylene, which provided the largest diameter, retarded the initial release of the pesticide relative to microspheres obtained with chloroform, or to pure norfluazon. Moreover, the studies showed that the pesticide/polymer ratio controlled the release of norfluazon, which was slower when this ratio was low. Release rates conformed to a generalized kinetic equation for a diffusion-controlled release mechanism, and the time taken for 50% of the active ingredient to be released into water, t_{50}, was calculated.

11. Sajeev et al.,[22] (2002) formulate and evaluate microencapsulated controlled release preparations of diclofenac sodium (DFS) using di. eren proportions of ethyl cellulose (EC) as the retardant material to extend the release. The formulated microcapsules were then compressed into tablets to obtain controlled release oral formulations. Phase separation-coacervation technique was employed to prepare microcapsules of DFS using di. erent proportions of EC in cyclohexane. Physical characteristics of microcapsules and their tablets, *in vitro* release pattern of the designed microcapsules and their tablets prepared from them were studied using USP dissolution apparatus (USP 2000) type 2 (paddle method) in triple distilled water. The prepared microcapsules were white, free ¯owing and spherical in shape, with the particle size varying from 49.94±52.72 mm. The duration of DFS release from microcapsules was found to be directly proportional to the proportion of EC and, thus, coat thickness. All tablets were of good quality with respect to appearance, drug content uniformity, hardness, weight variation, friability and thickness uniformity. *In vitro* release study of the tabletted microcapsules in triple distilled water showed zero order release kinetics and extended release beyond 24 h. A good correlation was obtained between drug

release (*t*60) and proportion of EC in the microcapsules. In the case of tabletted microcapsules, very good correlation could be established between *t*60, proportion of EC, weight of the tablets and between release rate constant (*K*) and proportion of EC. All the formulations were highly stable and possessed reproducible release kinetics across the batches.

12. Sinha et al.,[23] (2004) proposed that Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion. It has been shown that it not only improves the dissolution of poorly soluble drugs but also exerts a significant effect on fat metabolism in the body. Gel formation can be obtained by interactions of chitosans with low molecular counterions such as polyphosphates, sulphates and crosslinking with glutaraldehyde. This gelling property of chitosan allows a wide range of applications such as coating of pharmaceuticals and food products, gel entrapment of biochemicals, plant embryo, whole cells, microorganism and algae. This review is an insight into the exploitation of the various properties of chitosan to microencapsulate drugs. Various techniques used for preparing chitosan microspheres and evaluation of these microspheres has also been reviewed. This review also includes the factors that affect the entrapment efficiency and release kinetics of drugs from chitosan microspheres.

13. Jiahui Hu et al.,[1] (2004) Poor water solubility is an industry wide issue, especially for pharmaceutical scientists in drug discovery and drug development. In recent years, nanoparticle engineering processes have become promising approaches for the enhancement of dissolution rates of poorly water soluble drugs. Nanoparticle engineering enables manufacture of poorly water soluble drugs into nanoparticles alone, or incorporation with a combination of pharmaceutical excipients. The use of these processes has

dramatically improved in vitro dissolution rates and iv vivo bioavailability of many poorly water soluble drugs. This review highlights several commercially or potentially commercially available nanoparticle engineering processes recently reported in the literature for increasing the dissolution properties of poorly water soluble drugs.

14. Chowdary et al.,[24] (2004) prepared ethyl cellulose microspheres of glipizide were prepared by an industrially feasible emulsion-solvent evaporation technique and the microspheres were investigated. The microspheres are spherical, discrete and free-flowing. Encapsulation efficiency was in the range of 81-91 percent. Glipizide release from the microspheres was slow and diffusion controlled and extended over a period of 10 d and depended on core: coat ratio, wall thickness and size of the microspheres. Good linear relationships were observed between percent coat, wall thickness and release rate of the microspheres. In the in vivo, the microspheres produced a sustained hypoglycemic effect over 6 d in normal rabbits. These microspheres were found suitable for parenteral controlled release.

15. Yasunori Sato et al.,[25] (2004) formulated hollow microspheres (microballoons) floatable in JPX III No.1 solution as a dosage form characterized by excellent buoyant properties in the stomach. Microballoons were prepared by the emulsion solvent diffusion method utilizing enteric acrylic polymers codissolved with drug in a mixture of dichloromethane and ethanol. The release properties of five different drugs exhibiting distinct water solubility entrapped within microballoons were investigated. Buoyancy of the microballoons decreased with increasing drug release rate. In the case of aspirin, salicylic acid and ethoxybenzamide, the drug release profiles of microballoons proved a linear relationships by Higuchi plotting. However, indomethacin and riboflavin release pfofiles did not follow the Higuchi equation. When the loading amount of riboflavin was

higher than the solubility in the admixture of dichloromethane and ethanol, the drug release profiles of the microballoons displayed and initial burst release.Riboflavin crystals were released preferentially at the initial stage of the release test, which was attributable to the initial burst. In addition, by incorporating a polymer such as hydroxypropylmethylcellulose within the shell of microballoons, the release rate of riboflavin from the microballoons could be controlled while maintaining high buoyancy.

16. Martinac et al.,[26] (2005) Loratadine-loaded microspheres were prepared by spray-drying of dispersions, emulsions and suspensions differing in polymeric composition and solvents used. composed microspheres were obtained by spray-drying of two-phase systems composed of chitosan and ethylcellulose (EC). Microspheres differed in EC/CM weight ratio (0:1, 1:2 and 1:3) and in loratadine/polymers weight ratio (1:6 and 1:8). Tensile studies showed that both, EC/CM ratio and the type of spray-dried system influenced the bioadhesive properties of the microspheres in a way that the microspheres with higher chitosan content were more bioadhesive and microspheres prepared from suspensions were more bioadhesive than those prepared from emulsions, regardless of the same polymeric composition.

17. Zohra Zili et al.,[2] (2005) prepared poly-ε-caprolactone nanospheres and nanocapsules of Griseofulvin by nanoprecipitation and to characterize them. Nanoparticles of Griseofulvin were obtained with high encapsulation efficiency. The particle size was about 250-326 nm for nanospheres and 390-400 nm for nanocapsules. The dissolution rate of Griseofulvin nanoparticles was higher than that of micronized Griseofulvin therefore recourse to nanoencapsuation of Griseofulvin should enhance its bioavailability and possibly its efficiency for the treatment of dermatomycosis.

18. Jong et al.,[27] (2006) prepared hydrophobically modified glycol chitosan (HGC) nanopaticles as a carrier for paclitaxel. HGC conjugates were prepared by chemically linking 5β-cholanic acid to glycol chitosan chains using 1-ethyl-3-(3-dimethylaminopropyl)-carbodimide chemistry. Paclitaxel was efficiently loaded into HGC nanoparticles up to 10 wt% using dialysis method. The pacelitaxel-loaded HGC (PTX-HGC) nanoparticles were 400nm in diameter and were stable in PBS for 10 days. These PTX-HGC nanoparticles also showed sustained release of the incorporated of paclitaxel (80% of the loaded dose was released in 8 days at 37ºC in PBS). Finally, PTX was less toxic to the tumor-bearing mice when formulated in HGC nanopaticles than when formulated with Cremophor EL.

19. Kocbek et al.,[28] (2006) developed a method to enhance the dissolution of poorly soluble drugs by preparing nanosuspensions. The melt emulsification method traditionally used to prepare solid lipid nanoparticles was adapted to produce drug nanosuspensions. The method was evaluated in comparison with the well known solvent diffusion process for ibuprofen as a model drug. Control of the preparation variables (stabilizers, drug content, homogenization procedure and cooling conditions) allowed formation of nanosuspensions with diameters less than 100 nm. The major advantage of the melt emulsification method over the solvent diffusion method is the avoidance of organic solvents during production, although the mean particle size is slightly greater. The formulation of ibuprofen as a nanosuspension, either in the form of lyophilized powder of granules, was very successful in enhancing the dissolution rate when compared to the micronized drug. The increase *in vitro* dissolution rate may favorably affect bioavailability and improve safety for the patient by decreasing gastric irritancy.

20. Wong SM et al.,[29] (2006)The slow dissolution rate exhibited by poorly water-soluble drugs is a major challenge in the drug development process. Following oral administration, drugs with slow dissolution rates generally show erratic and incomplete absoption which may lead to therapeutic failure. The aim of this study was to improve the dissolution rate and subsequently the oral absoption and bioavailability of a model poorly water soluble drugs. Microparticles containing the model drug (Griseofulvin) were produced by spray drying the drug in the absence/presence of a hydrophilic surfactant. Poloxamer 407 was chosen as the hydrophilic surfactant to improve the particle wetting and hence the dissolution rate. The spray dried particles were characterized and in vitro dissolution studies and in vivo absorption studies were carried out. The results obtained showed that the dissolution rate and absolute oral bioavailability of the spray dried Griseofulvin/poloxamer 407 particles were significantly increased compared to the control. Although spray drying Griseofulvin alone increased the drugs in vitro dissolution rate, no significant improvement was seen in the absolute oral bioavailability when compared to the control. Therefore, it is believed that the better wetting characteristics conferred by the hydrophilic surfactant was responsible for the enhanced dissolution rate and absolute oral bioavailability of the model drug.

21. Gavini et al.,[30] (2006) studied the nasal administration of Carbamazepine (CBZ) has been studied using microspheres constituted by chitosan hydrochloride(CH) or chitosan glutamate(CG). The microspheres were produced using a spray-drying technique and characterized in terms of morphology (scanning electron microscopy(SEM), drug content, particle size(laser diffraction method) and thermal behavior (DSC). In vitro drug release studies were performed in phosphate buffer(pH 7.0). In vivo tests were carried out in

sheep using the microparticle containing chitosan glutamate, chosen on the basis of the results of in vitro studies.

III. EXPERIMENTAL

3.1 Materials used

- **Griseofulvin**
  Gift sample from CFL and Wallace pharmaceuticals
- **Chitosan**
  Fluca Biochemicals
- **Ethylcellulose**
  S d fine-chem Ltd.
- **Petroleum ether**
  Qualigens fine chemicals
- **Light liquid paraffin**
  Spectrum reagent and chemicals
- **Acetic acid glacial**
  S d fine-chem limited
- **Acetone**
  Qualigens fine chemicals
- **Tween- 80**
  Indian research products
- **Methanol**
  Qualigens fine chemicals
- **Polyvinyl alcohol**
  Loba chemie Pvt. Ltd.
- **Chloroform**
  Qualigens fine chemicals
- **Glutaraldehyde**
  Kemphasol

3.2. Instruments and equipments used

 # UV Visible spectrophotometer

Shimadzu 1400 double beam

 # Electronic balance

Denver instrument XS – 210

 # Dissolution test apparatus USP XXIV

M/s. Tab Machines, Mumbai

 # IR – hydraulic pellet press

Kimaya Engineers (Type – WT, SR No.330) India

 # Magnetic stirrer

Remi motors

3.3 Methods

Method I - Preparation of Chitosan microparticles by cross-linking method.

Method II- Preparation of Ethyl cellulose microarticles by solvent evaporation method.

METHOD- I

PREPARATION OF CHITOSAN MICROPARTICLES BY CROSS-LINKING METHOD [13]:

About 200 mg of chitosan was dissolved in 10 ml of 5 % acetic acid solution. Mix the various concentrations of drug (Griseofulvin) in the polymer solution and this whole was slowly added to 60 ml of the suspension medium containing mineral oil/ petroleum ether (60/40, v/v) mixture and 1 ml of Tween-80 as an emulsifier. The suspension medium was stirred at 2000 rpm for 10 minute and 1 ml of gltaraldehyde was added into the medium and stirring continued. After 1 and 2 hours add 0.5 ml of glutaraldehyde, respectively, were added into the suspension medium and stirring continued. At the end of 3 hour, stirring was stopped and the chitosan microspheres

were washed several times with petroleum ether, 5% sodium bisulphate solution and acetone, (consecutively) and then were dried in an oven at 65°C.

METHOD-II

PREPARATION OF ETHYLCELLULOSE MICROPARTICLES BY SOLVENT EVAPORATION METHOD [16]:

About 200 mg of ethyl cellulose was dissolved in 20 ml of dichloromehane (DCM) or chloroform. Different amounts of Drug (Griseofulvin) were dissolved in this polymer solution at 30°C. The drug/polymer solution was then added drop-wise to a dispersion phase (200 ml) consisting PVA solution, under stirring. After 2 hours of stirring, the products were filtered, washed with 1 liter of distilled water and air-dried at room temperature.

Table:3 Formulation Code For Chitosan Preparations

FORMULATION-CODE	DRUG:POLYMER RATIO	POLYMER NAME	GLUTARAL-DEHYDE (ML)
C1G1	10:200	CHITOSAN	1
C1G2			2
C1G3			3
C2G1	20:200	CHITOSAN	1
C2G2			2
C2G3			3
C3G1	30:200	CHITOSAN	1
C3G2			2
C3G3			3

Table: 4 Formulation Code For Ethylcellulose Preparations

FORMULATION-CODE	DRUG:POLYMER RATIO	POLYMER NAME	POLYVINYL ALCOHOL (%)
E1P1	10:200	ETHYL-CELLULOSE	0.15
E1P2			0.20
E1P3			0.25
E2P1	20:200	ETHYL-CELLULOSE	0.15
E2P2			0.20
E2P3			0.25
E3P1	30:200	ETHYL-CELLULOSE	0.15
E3P2			0.20
E3P3			0.25

Abbreviations:

G - Glutaraldehyde

PVA - Polyvinyl alcohol

IV. STUDIES ON DRUG DELIVERY USING GRISEOFULVIN WITH CHITOSAN AND ETHYLCELLULOSE POLYMERS

4.1. Preformulation studies

(i) Interaction study

Infrared (IR) absorption spectroscopy:

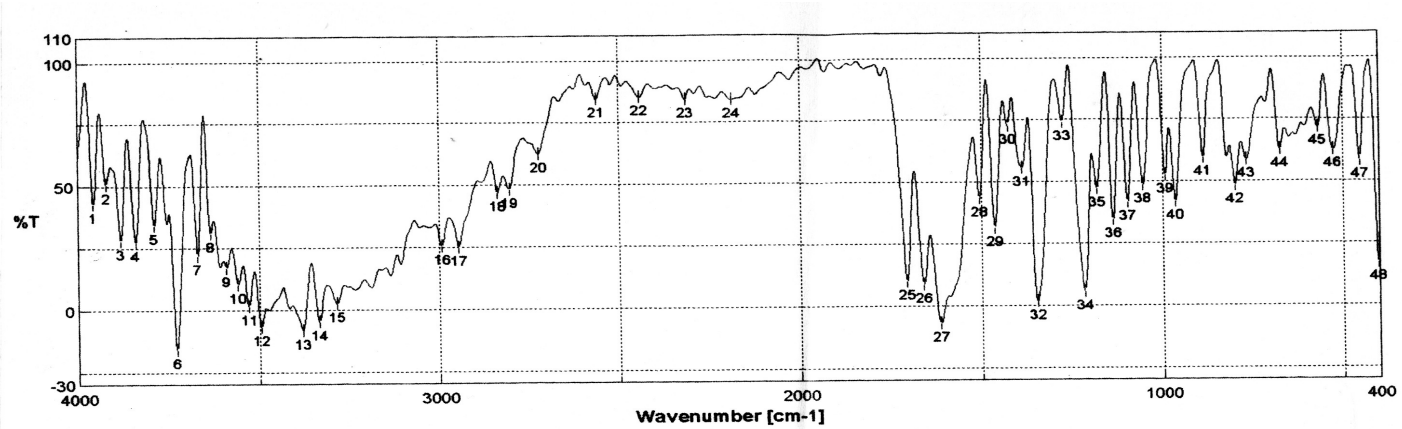
To investigate any possible interactions between the drug and the polymers, the IR spectra of pure drug Grieofulvin and its physical mixtures (1:1) with Chitosan and Ethylcellulose were carried out using Shimadzu IR-470 spectrophotometer (Tokyo, Japan). The samples were prepared as KBr disks compressed under a pressure of 6 ton/nm^2. The wave length selected ranged between 400- 4000 cm^{-1} in a Perkin Elmer FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymers and matching was done to detect any appearance or disappearance of peaks.

Procedure:

First a pinch of drug was added to the dried KBr, and triturated in mortar. The pellet was made by using pellet plate technique, and kept in IR chamber and scanned to get the spectra. The same procedure was followed for the combination of drug-polymer too.

By comparing the IR spectrum of drug and drug-polymer combinations, it was inferred that in both cases the characteristic peaks were obtained at same wave numbers. So from this it was concluded that no structural changes had occurred for the drug when combined with the polymers.

Fig :4 IR Spectra of Griseofulvin- pure sample



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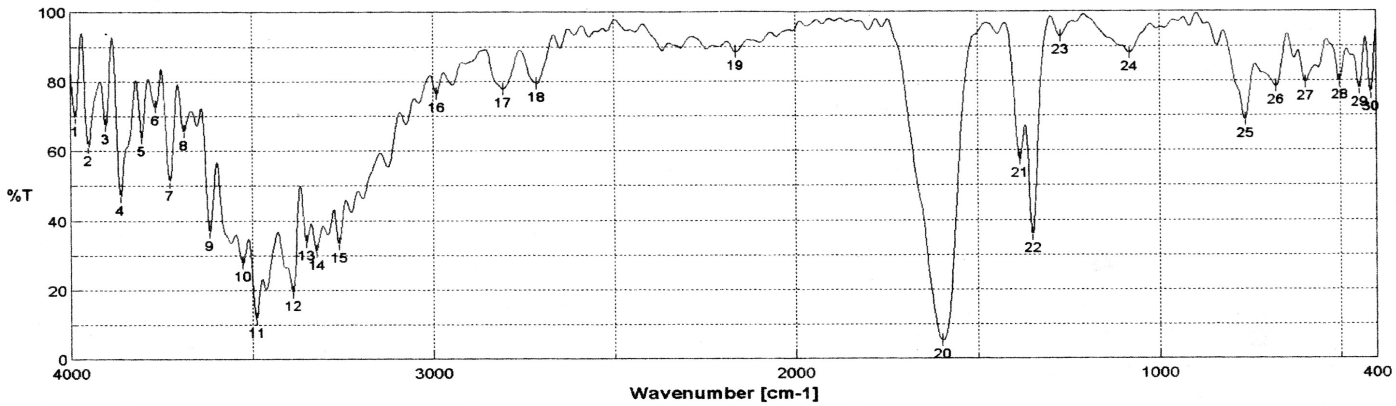
Zero Filling	ON	Apodization	Cosine
Gain	16	Scanning Speed	2 mm/sec
Date/Time	1/21/2008 10:35AM	Update	1/30/2008 4:57PM
Operator	C.Geetha		
File Name	Memory#7		
Sample Name	GF		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3957.21	42.8491	2	3922.5	50.8153	3	3882	28.2633	4	3840.54	27.665	5	3789.44	34.3986
6	3728.69	-15.7013	7	3669.87	22.0681	8	3633.23	31.026	9	3589.84	17.1953	10	3558.99	10.4501
11	3529.09	1.59401	12	3496.31	-6.75189	13	3380.6	-8.26115	14	3335.28	-4.73162	15	3286.11	2.59976
16	2993.94	25.6123	17	2947.66	25.2268	18	2838.7	46.9217	19	2806.88	48.2696	20	2724.92	62.2574
21	2562.93	84.5954	22	2442.4	85.3455	23	2318.02	84.4691	24	2189.77	84.1419	25	1706.69	10.0915
26	1659.45	8.8173	27	1613.16	-6.89219	28	1505.17	43.5332	29	1463.71	31.5304	30	1427.07	72.8406
31	1388.5	55.6184	32	1347.03	1.78592	33	1275.68	74.1377	34	1216.86	5.83288	35	1181.19	47.098
36	1135.97	34.5732	37	1096.33	41.7723	38	1053.91	48.5835	39	992.196	52.5616	40	963.269	41.9762
41	887.095	59.5671	42	797.421	48.4659	43	766.566	58.7401	44	674.963	62.6177	45	570.826	71.6626
46	527.436	62.6959	47	454.154	58.1896	48	404.978	17.168						

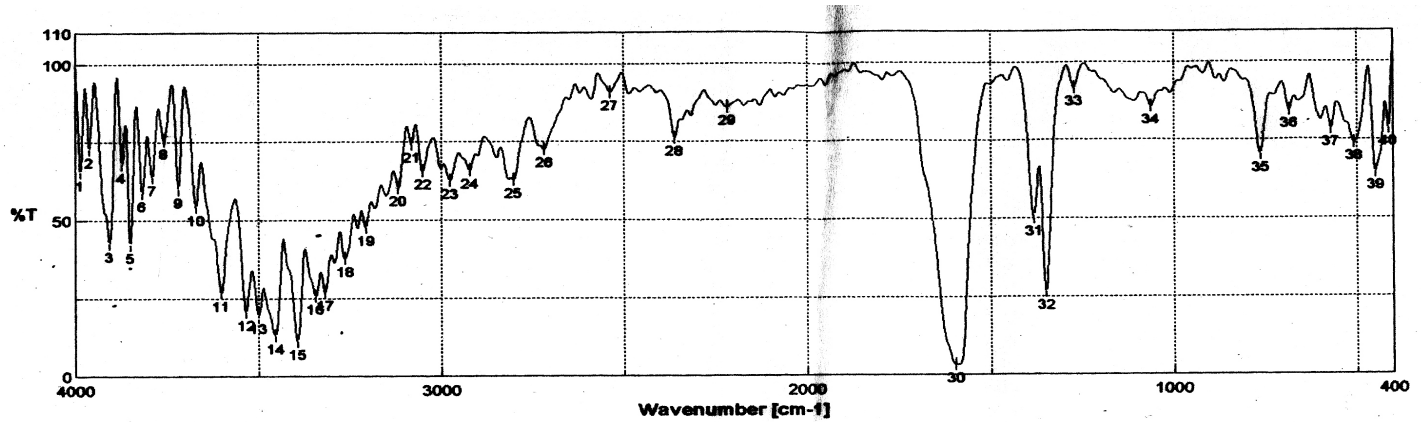
Flig:5 IR Spectra of Chitosan- pure sample



Accumulation	16	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	16	Scanning Speed	2 mm/sec
Date/Time	1/21/2008 10:42AM	Update	1/30/2008 4:51PM
Operator	C.Geetha		
File Name	CS -I		
Sample Name	CS -I		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3987.11	69.7154	2	3950.46	61.1747	3	3902.25	67.3768	4	3862.72	47.0343	5	3802.94	63.8932
6	3765.33	72.7921	7	3724.83	51.2464	8	3686.26	65.6972	9	3616.84	36.826	10	3526.2	27.9127
11	3489.56	11.6293	12	3390.24	19.3738	13	3351.68	34.0487	14	3323.71	31.2941	15	3261.04	33.3176
16	2991.05	76.5489	17	2807.85	77.8195	18	2714.32	79.4827	19	2166.63	88.4763	20	1597.73	5.40833
21	1383.68	57.3971	22	1348	35.785	23	1271.82	92.9373	24	1080.91	88.2441	25	763.673	68.7838
26	678.82	78.3657	27	596.861	79.51	28	502.366	79.8944	29	446.44	77.6585	30	416.549	76.2502

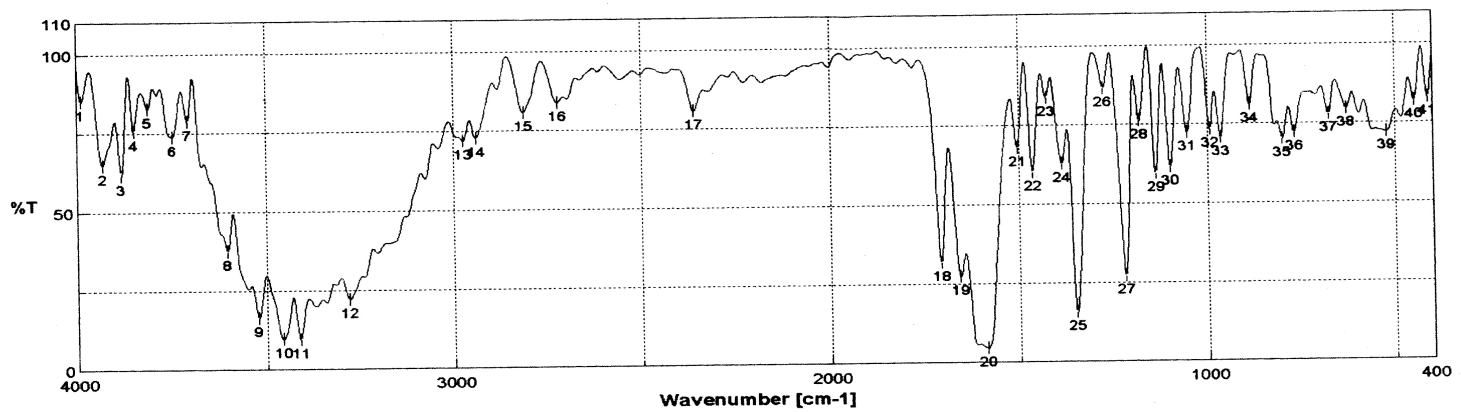
Fig: 6 IR Spectra of ethyl cellulose- pure sample



Accumulation	16	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	16	Scanning Speed	2 mm/sec
Date/Time	1/21/2008 11:04AM	Update	1/30/2008 4:53PM
Operator	C.Geetha		
File Name	Memory#5		
Sample Name	EC - II		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3997.11	65.4436	2	3962.04	72.886	3	3906.11	42.6027	4	3873.33	67.8983	5	3849.22	42.315
6	3818.36	58.7516	7	3790.4	63.8397	8	3758.58	75.5681	9	3719.05	60.0508	10	3670.84	54.2005
11	3601.41	26.8926	12	3533.92	20.7908	13	3500.17	19.7829	14	3454.85	13.2543	15	3395.07	11.4599
16	3344.93	25.8061	17	3318.89	26.944	18	3263.93	37.8584	19	3207.04	47.6987	20	3119.3	60.4217
21	3082.65	74.2083	22	3051.8	65.598	23	2977.55	62.635	24	2923.56	65.9582	25	2803.03	62.9238
26	2719.14	72.6124	27	2538.79	90.9602	28	2363.34	76.1985	29	2219.67	86.0024	30	1595.81	3.4078
31	1382.71	50.2079	32	1348.96	26.6507	33	1271.82	91.6787	34	1060.66	85.7691	35	763.673	70.4318
36	685.57	84.3439	37	571.79	78.5654	38	508.151	74.0227	39	448.369	64.6571	40	413.656	78.685

Fig :7 IR Spectra of Griseofulvin with Chitosan

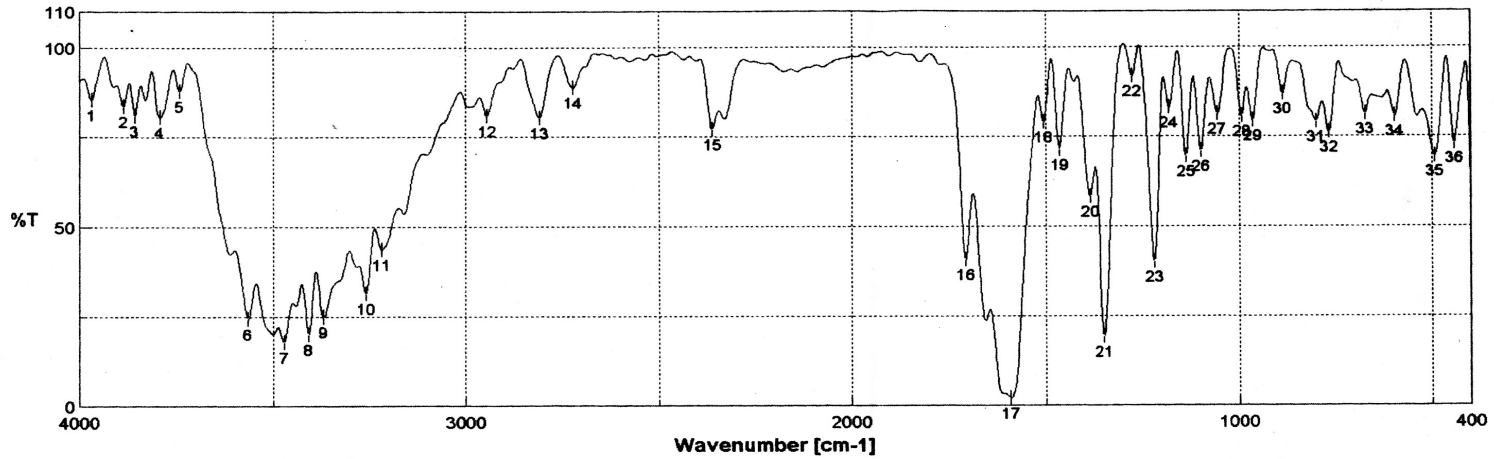


Accumulation	16
Zero Filling	ON
Gain	16
Date/Time	1/21/2008 11:06AM
Operator	C.Geetha
File Name	GF +CSI
Sample Name	GF +CSI
Comment	

Resolution	4 cm-1
Apodization	Cosine
Scanning Speed	2 mm/sec
Update	1/30/2008 4:59PM

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3988.07	85.1843	2	3934.07	64.7597	3	3883.93	61.5688	4	3850.18	75.6476	5	3813.54	82.7953
6	3747.98	73.7851	7	3708.44	78.6303	8	3603.34	37.6029	9	3521.38	16.4085	10	3458.71	9.72408
11	3412.42	9.60408	12	3280.32	22.1956	13	2975.62	71.9022	14	2941.88	72.7701	15	2815.56	80.7565
16	2723.96	83.7544	17	2361.41	80.6201	18	1707.66	32.0191	19	1657.52	27.348	20	1588.09	4.72589
21	1504.2	67.8607	22	1463.71	60.233	23	1427.07	83.7294	24	1385.6	62.8957	25	1348	15.7992
26	1274.72	86.6704	27	1218.79	27.3217	28	1180.22	76.1993	29	1136.83	59.5932	30	1098.26	61.5509
31	1054.87	72.2851	32	993.16	73.3369	33	964.233	70.6609	34	887.095	81.0286	35	799.35	70.0678
36	767.53	72.2202	37	676.892	77.9625	38	629.644	79.4718	39	524.543	72.0931	40	448.369	81.9387
41	413.656	82.9663												

Fig :8 IR Spectra of Griseofulvin with Ethyl cellulose



Accumulation	16	Resolution	4 cm-1
Zero Filling	ON	Apodization	Cosine
Gain	8	Scanning Speed	2 mm/sec
Date/Time	1/21/2008 11:12AM	Update	1/30/2008 5:02PM
Operator	C.Geetha		
File Name	GF +EC - II		
Sample Name	GF +EC - II		
Comment			

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3969.75	85.4147	2	3887.79	83.6234	3	3857.9	81.013	4	3792.33	80.6764	5	3741.23	87.836
6	3565.74	24.4778	7	3472.2	17.9935	8	3408.57	20.1131	9	3369.03	24.9217	10	3259.11	31.4708
11	3218.61	43.6866	12	2945.73	81.0077	13	2810.74	80.4093	14	2723.96	88.8875	15	2362.37	77.1526
16	1706.69	40.8294	17	1590.99	2.26766	18	1505.17	79.0166	19	1464.67	71.4404	20	1384.64	58.2957
21	1348	19.3276	22	1275.68	91.825	23	1218.79	40.1986	24	1180.22	83.0688	25	1136.83	69.5485
26	1098.26	71.0611	27	1055.84	81.3113	28	993.16	80.7817	29	964.233	79.4951	30	886.131	86.973
31	800.314	79.1742	32	767.53	76.2381	33	673.999	81.362	34	595.896	80.7457	35	492.723	69.4746
36	441.619	73.255												

4.2. Standard graph of Griseofulvin

Preparation of stock solution:

100 mg of Griseofulvin dissolved in 100 ml of pH 7.4 phosphate buffer to get a concentration of 1000 μg/ml.

From this take 10 ml and made up to 100 ml with pH 7.4 phosphate buffer to get a concentration of solution 100 μg/ml.

Preparation of various concentrations of drug solution

1. 0.05 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 5 μg/ml.
2. 1 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 10 μg/ml.
3. 1.5 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 15 μg/ml.
4. 2.0 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 20 μg/ml.
5. 2.5 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 25 μg/ml.
6. 3.5 ml of the stock solution was made up to 10 ml with pH 7.4 phosphate buffer to give 30 μg/ml.

Procedure:

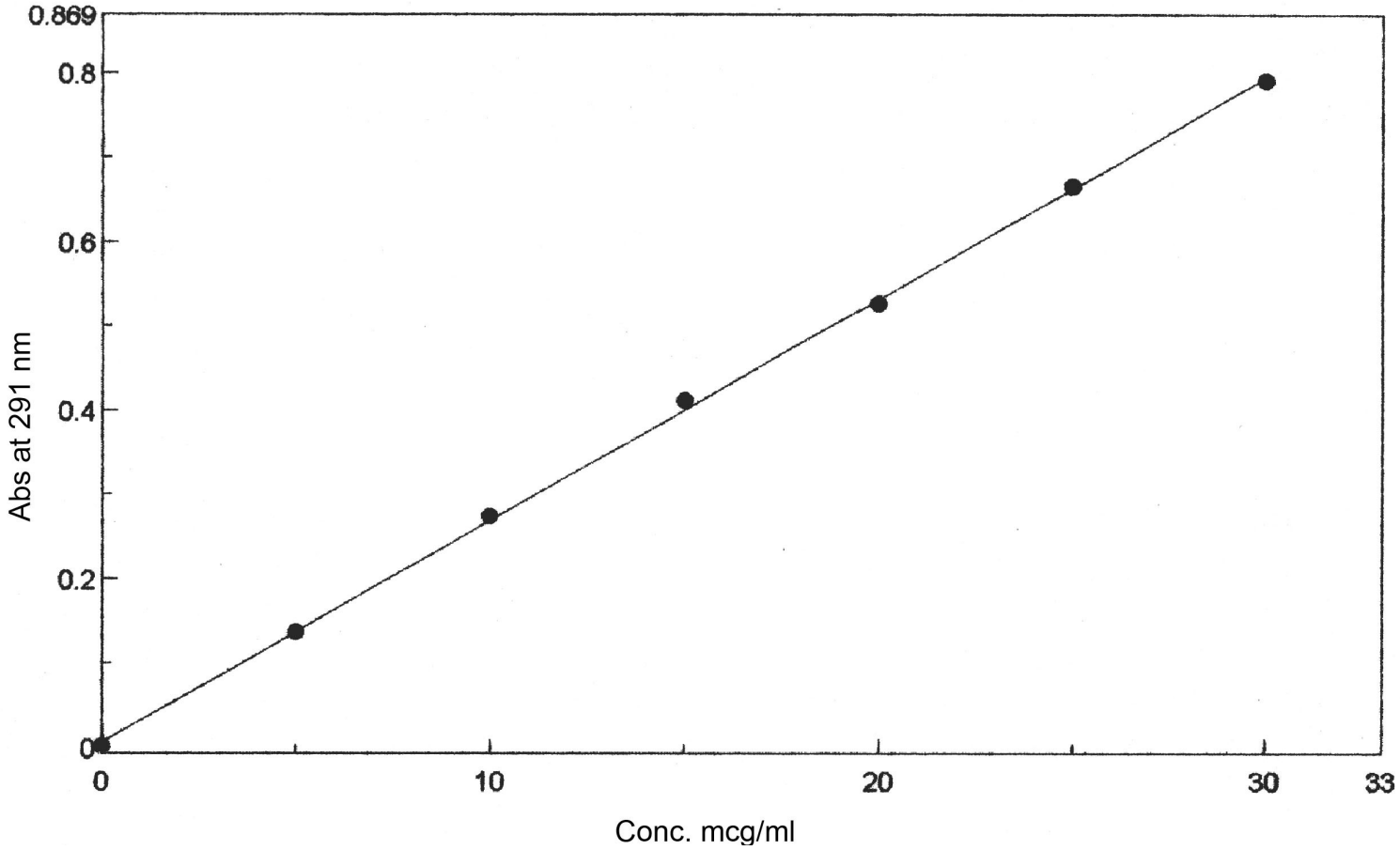
Various concentrations of Griseofulvin (5, 10, 15, 20, 25 and 30 μg/ml) were prepared as mentioned above. Absorbance of the solution was measured against reagent blank at 291 nm using UV Spectro photo meter. A standard graph between concentration Vs

absorbance was plotted. A straight line passing through origin was obtained.

Table: 5 Calibration Data for the Estimation of Griseofulvin (5-30 µg/ml)

S.No	Concentration in µg/ml	Absorbance at 291 nm
1	0	0.0000
2	5	0.1350
3	10	0.2720
4	15	0.4100
5	20	0.5250
6	25	0.6650
7	30	0.7900

Fig 9 Calibration Data Curve For the Estimation Of Griseofulvin (5-30 µg/ml)



4.3. Drug content analysis

UV spectrophotometric method was employed to verify the presence of drug in the micropartilces. Crush the microparticles and the drug was extracted with phosphate buffer 7.4 and absorbance was measured using UV spectrophotometer at 291nm. The amount of Griseofulvin in the micropartilces was estimated with the help of standard graph. A study was performed in the percentage yield and percentage encapsulation efficiency.

Table:6 Amount of Drug Loaded in Chitosan microparticles

DRUG: POLYMER (mg)	AMOUNT OF DRUG LOADED		
	1 ml G	2 ml G	3 ml G
10 : 200	5.464 mg	5.760 mg	5.869 mg
20 : 200	11.832 mg	12.48 mg	13.02 mg
30 : 200	24.973 mg	25.172 mg	26.88 mg

Fig:10

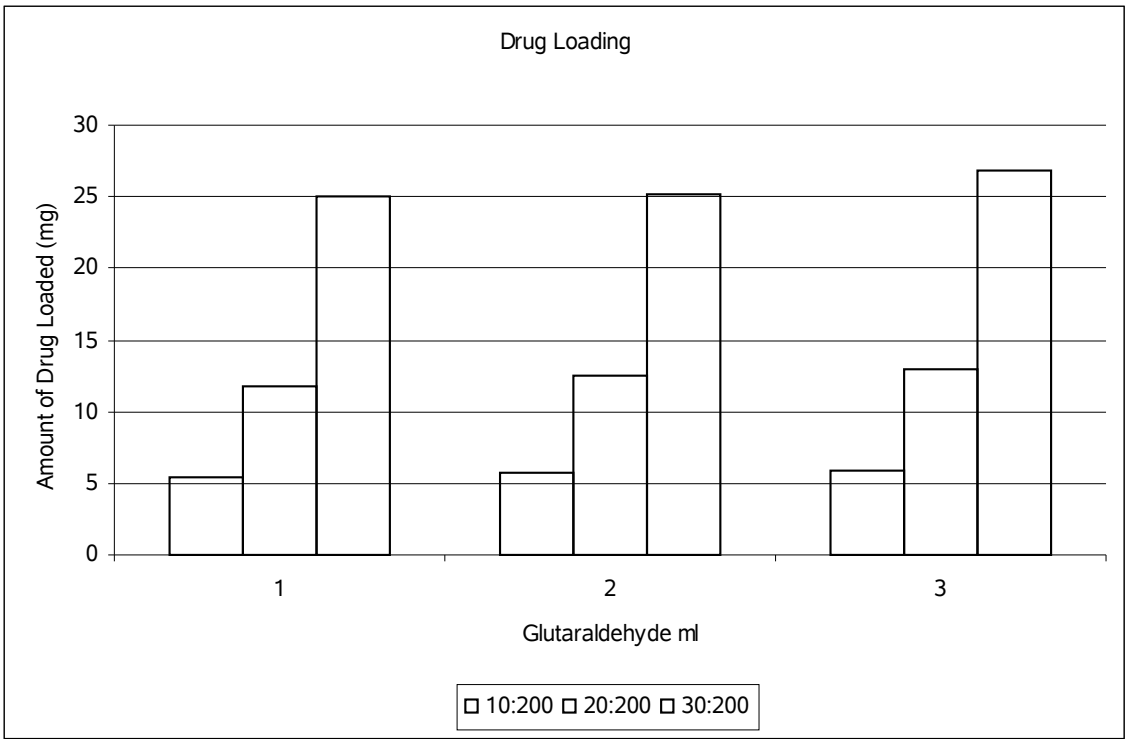


Table:7 Encapsulatin Efficiency Of Chitosan Microparticles

DRUG:POLYMER (mg)	PERCENTAGE DRUG LOADING		
	1 ml G	2 ml G	3 ml G
10 : 200	54.64 %	57.60 %	58.69 %
20 : 200	59.16 %	62.4 %	65.1 %
30 : 200	83.24 %	83.90 %	89.6 %

Fig:11

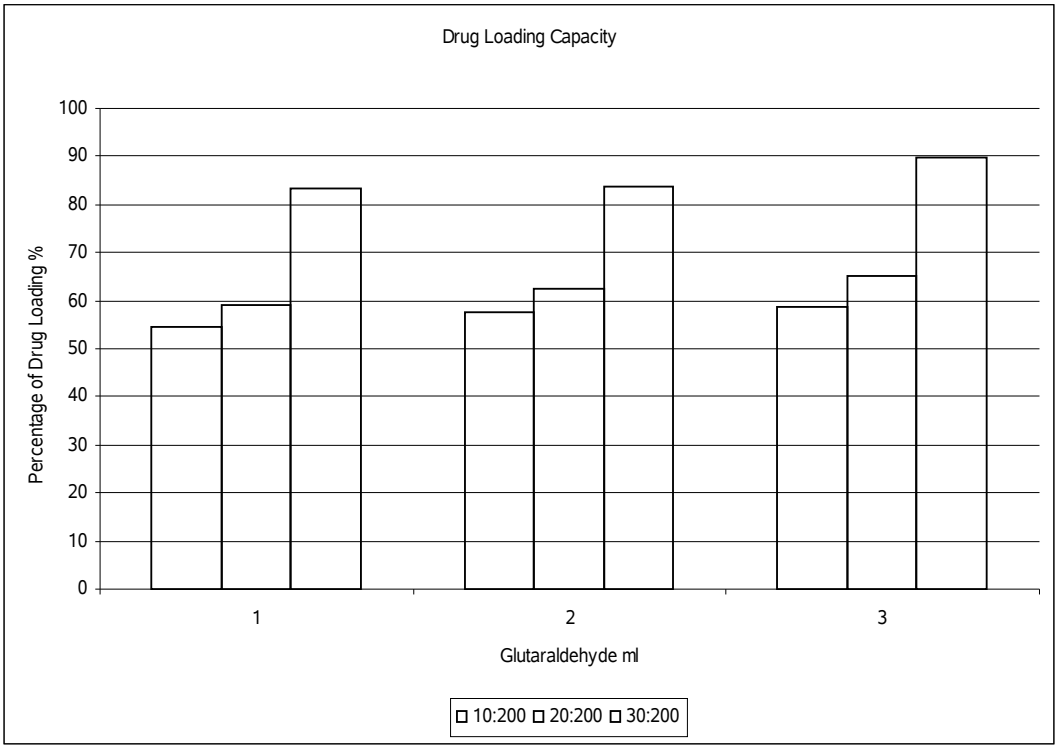


Table:8 Percentage Yield Of Chitosan Microparticles

DRUG:POLYMER (mg)	PERCENTAGE YIELD		
	1 ml G	2 ml G	3 ml G
10 : 200	94.28 %	95.24 %	95.71 %
20 : 200	92.72 %	94.54%	95.45 %
30 : 200	96.08 %	94.35 %	97.39 %

Fig:12

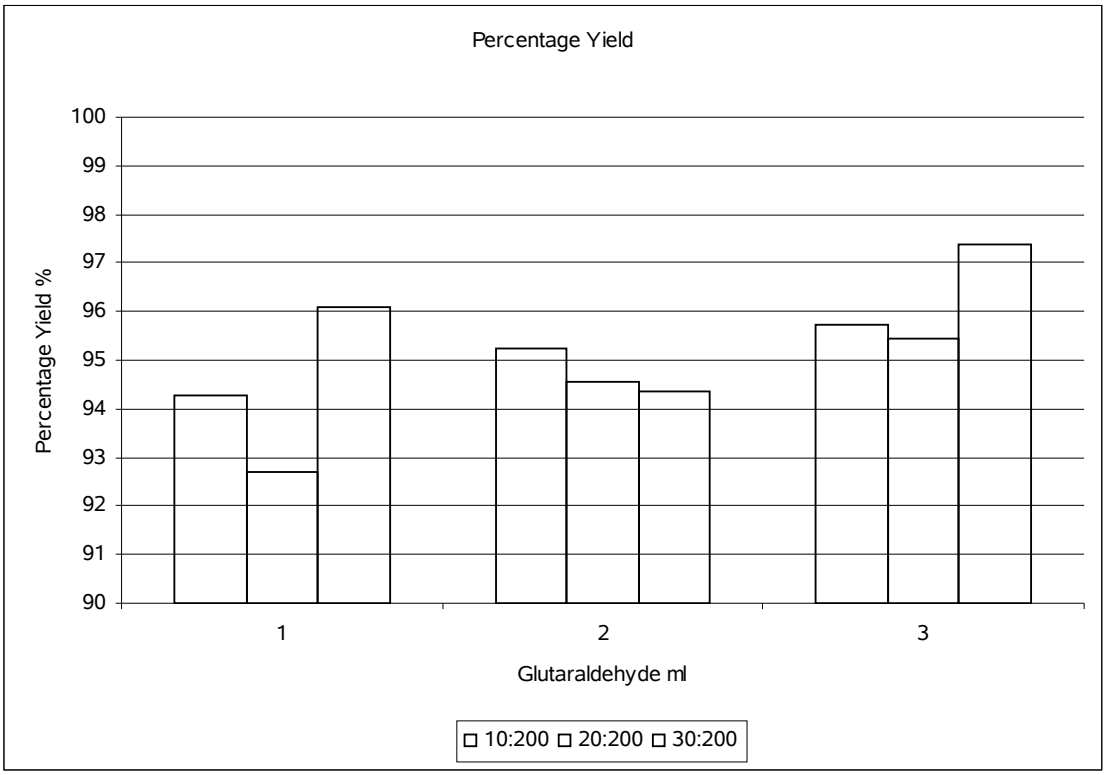


Table:9 Amount of Drug Loaded in Ethylcellulose microparticles

DRUG: POLYMER (mg)	AMOUNT OF DRUG LOADED		
	0.15 %PVA	0.20 % PVA	0.25 % PVA
10:200	4.961 mg	4.29 mg	3.48 mg
20:200	12.14 mg	10.08 mg	7.98 mg
30:200	19.79 mg	18.04 mg	15.09 mg

Fig:13

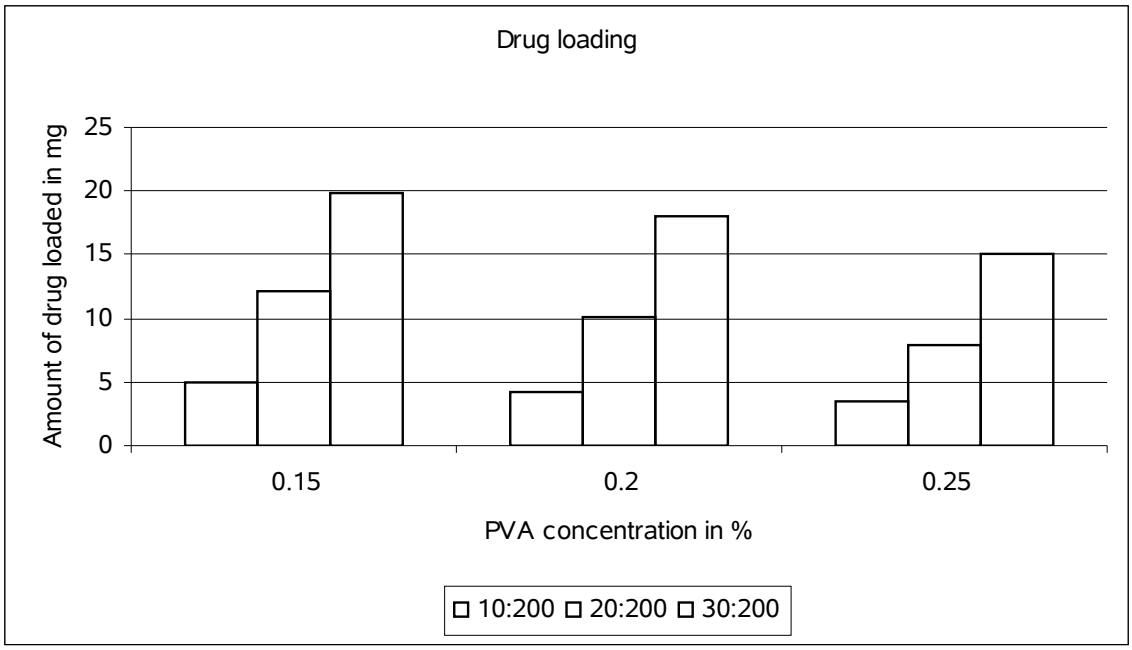


Table:10 Encapsulatin Efficiency Of Ethylcellulose Microparticles

DRUG: POLYMER (mg)	PERCENTAGE DRUG LOADING		
	0.15 % PVA	0.20 % PVA	0.25 % PVA
10:200	49.61 %	42.9 %	34.8 %
20:200	60.7 %	50.4 %	39.9 %
30:200	65.96 %	60.13 %	50.3 %

Fig:14

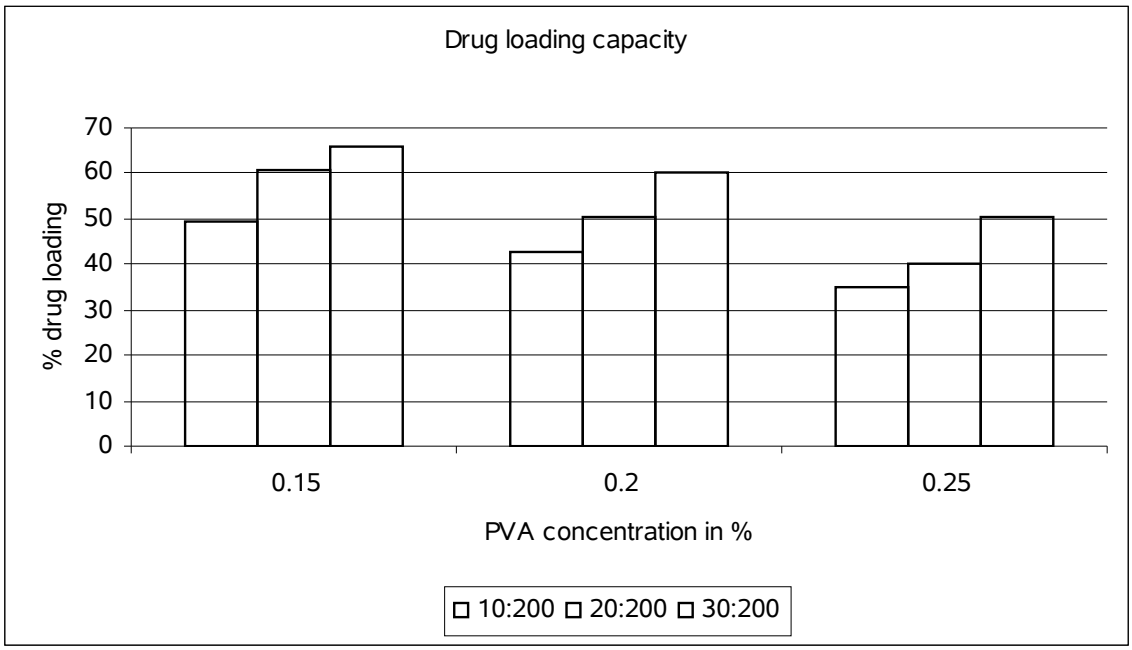
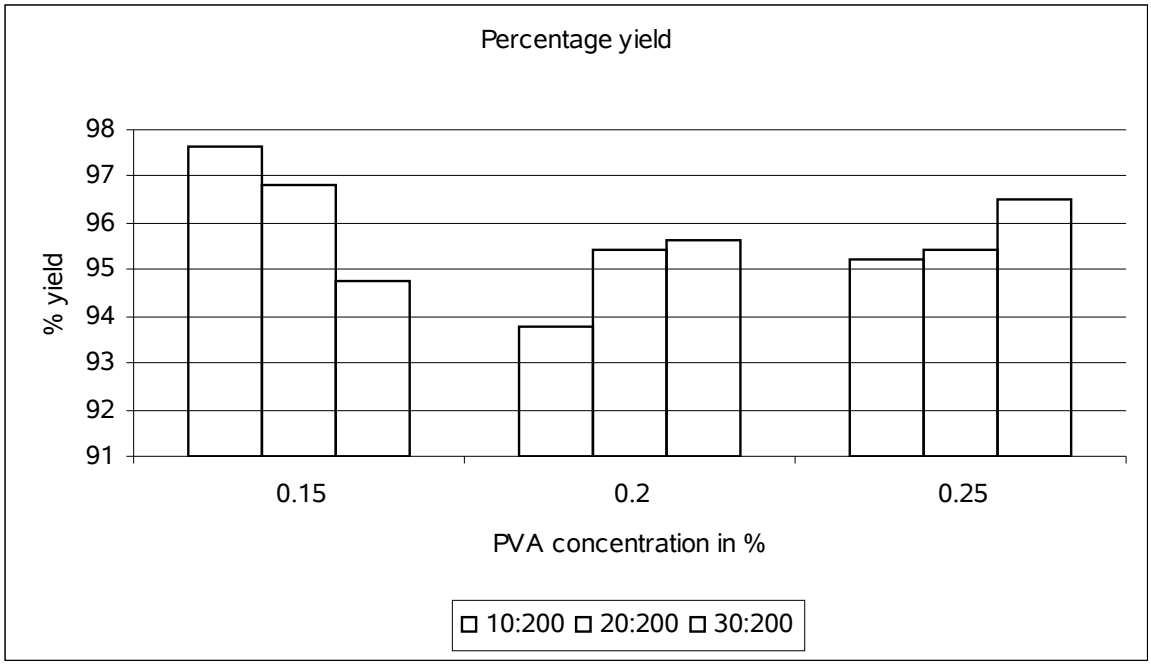


Table:11Percentage Yield Of Ethylcellulose Microparticles

DRUG:POLYMER (mg)	PERCENTAGE YIELD		
	0.15 % PVA	0.20 % PVA	0.25 % PVA
10: 200	97.62 %	93.80 %	95.23 %
20: 200	96.81 %	95.45 %	95.45 %
30: 200	94.78 %	95.65 %	96.52 %

Fig:15

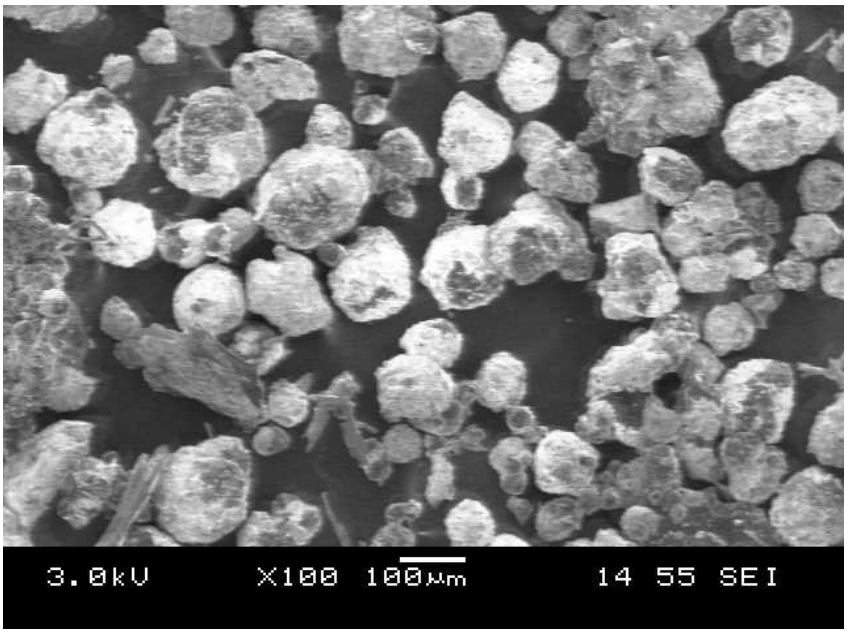


4.4 Determination of size and shape of the microparticles

(i) Determination of shape and size of microparticles by scanning electron microscope

The surface morphology and internal texture of griseofulvin mincrosparticles were observed by a scanning electron microscope (SEM). SEM photographs were taken on Jsm 6400 scanning electron microscope at 3K magnification at room temperature. Before scanning the microparticles were sputtered with gold to make the surface conductive. The figures shows that the particles are spherical enough having rough surface.

Fig:16 Scanning electron picture of chitosan micropartilce



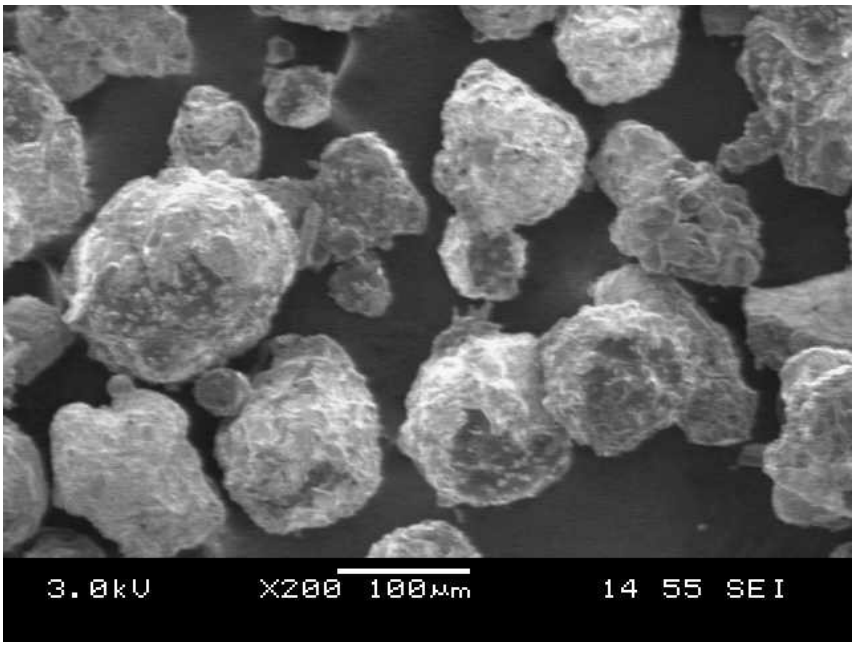
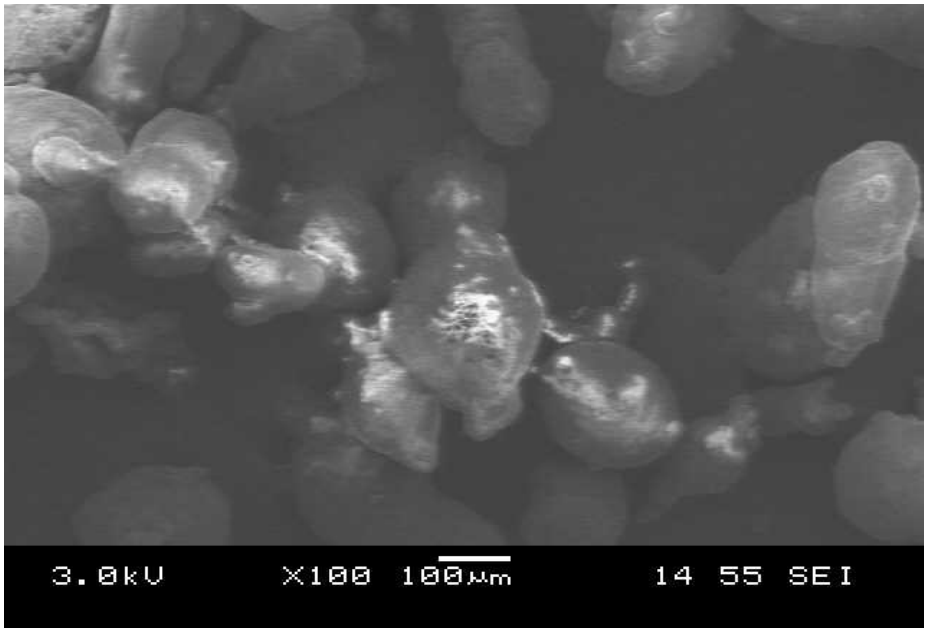
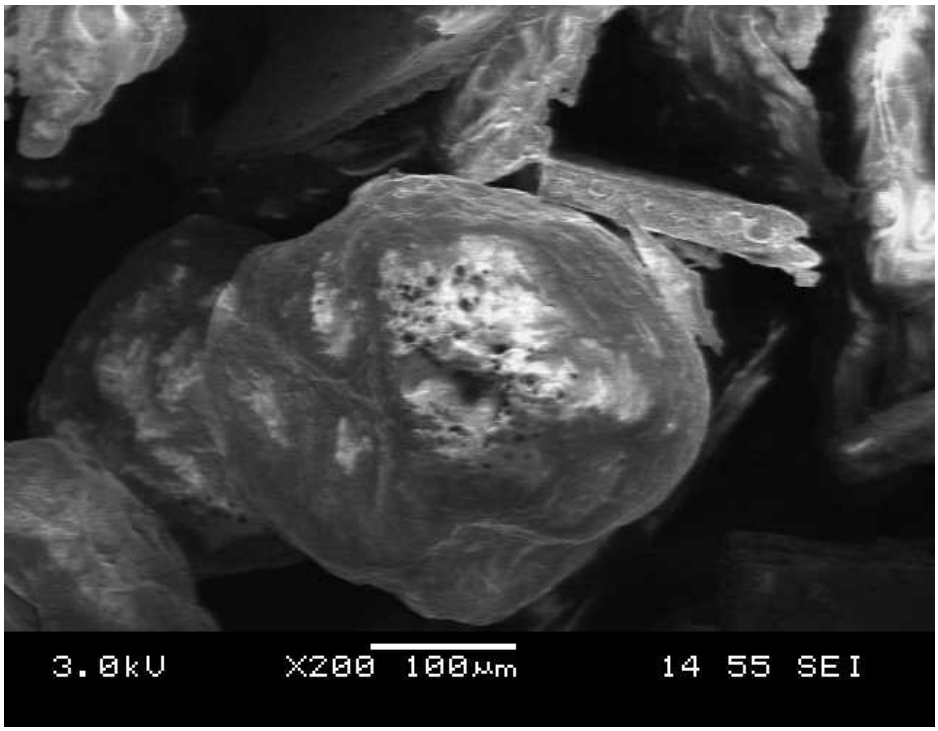


Fig:17 Scanning electron picture of ethyl cellulose microsparticle





(ii) Measurement of particle size determination of miroparticles by optical microscopic method:

First clean the microscope and place it in proper place where light is suitable for projection. Fix the eye-piece in microscope with micrometer. Calibrate the eye-piece micrometer with a standard stage micrometer. Mount the sample on the plain slide. Then measure the size of the particles with the help of eye-piece micrometer. Count accurately 200 particles.

Arithmetic mean = $\Sigma nd / \Sigma n$

Table:12 Calibration of Eye Piece Micrometer

STAGE MICROMETER	0	15	21	45	60
EYEPIECE MICROMETER	0	10	14	30	40

Particle Size Determination by Optical Microscopic Method

TABLE:13

The Arithmetic Mean of Particle Size – C1G1 = 165.37
C1G2 = 167.32
C1G3 = 169.12

TABLE:14

The Arithmetic Mean of Particle Size – C2G1 = 175.8
C2G2 = 178.5
C2G3 = 179.6

TABLE:15

The Arithmetic Mean of Particle Size – C3G1 = 189.75
C3G2 = 191.62
C3G3 = 200.62

TABLE:16

The Arithmetic Mean of Particle Size – E1P1 = 339
E1P2 = 340.5
E1P3 = 345

TABLE:17

The Arithmetic Mean of Particle Size – E2P1 = 348
E2P2 = 349.5
E2P3 = 352.5

TABLE:18

The Arithmetic Mean of Particle Size – E3P1 = 378
E3P2 = 384.75
E3P3 = 399

4.5 In vitro drug release studies:

Procedure:

Release of Griseofulvin from microspheres was studied in phosphate buffer 7.4 pH (100 ml) using a USP XXI/XXII dissolution rate apparatus, with a paddle rotating at a rate of 100 RPM and at 37±0.5°C, samples are with drawn through a syringe at different time interval and were assayed at 291 nm for Griseofulvin using UV visible double beam spectrophotometer. The drug release experiment was conducted in triplicate.

Calculation:

To calculate the actual percentage release, it is necessary to calculate the theoretical release of drug. It can be calculated by using the general formula,

Theoratical release = (Total amount of drug loaded in 1 mg of microparticles) × (Amount of microparticles taken for in vitro studies)

The exact percentage relese of drug can be obtained by the following general formula:

IN VITRO DISSOLUTION PROFILE IN PH 7.4

TABLE:19

TIME IN Hrs	PERCENTAGE RELEASE OF GRISEOFULVIN ON VARIOUS CONCENTRATIONS OF CHITOSAN (%)		
	C1G1	C1G2	C1G3
2	27.18	23.5	18.09
4	29.32	26.42	21.74
6	30.54	27.79	22.68
8	34.76	32.3	25.27
10	39.19	34.53	27.4
12	42.65	36.02	31.34
24	51.74	43.41	38.26

FIG:18

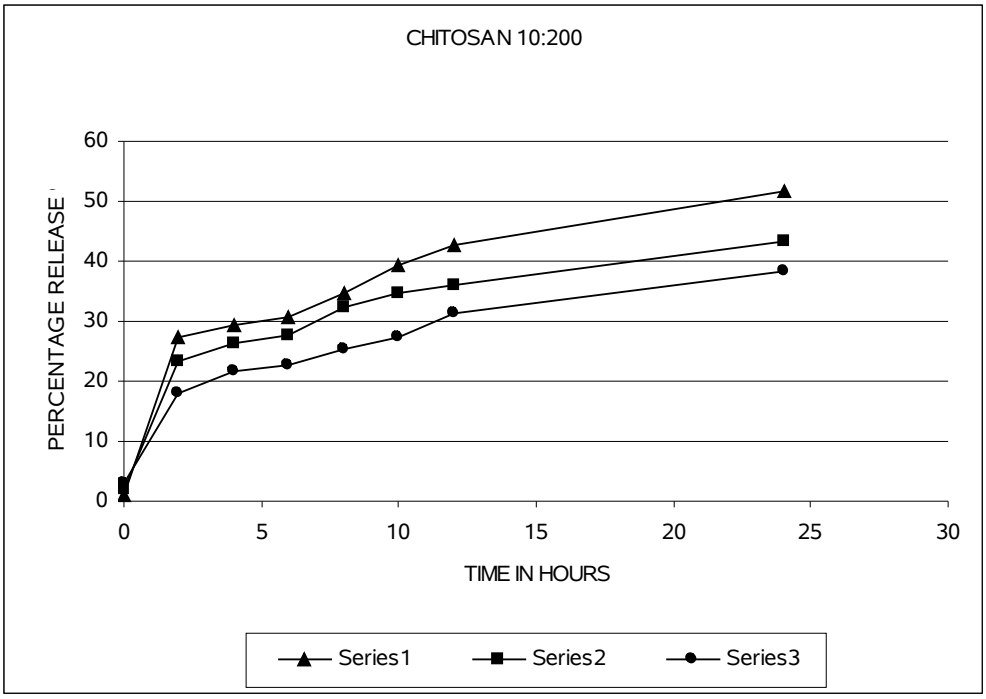


TABLE:20

TIME IN Hrs	PERCENTAGE RELEASE GRISEOFULVIN ON VARIOUS CONCENTRATIONS OF CHITOSAN (%)		
	C2G1	C2G2	C2G3
2	31.17	29.51	23.63
4	33.71	30.55	24.31
6	36.82	32.1	27.39
8	39.32	35.41	28.08
10	43.24	36.45	31.50
12	45.30	39.58	32.87
24	52.19	46.87	39.72

FIG:19

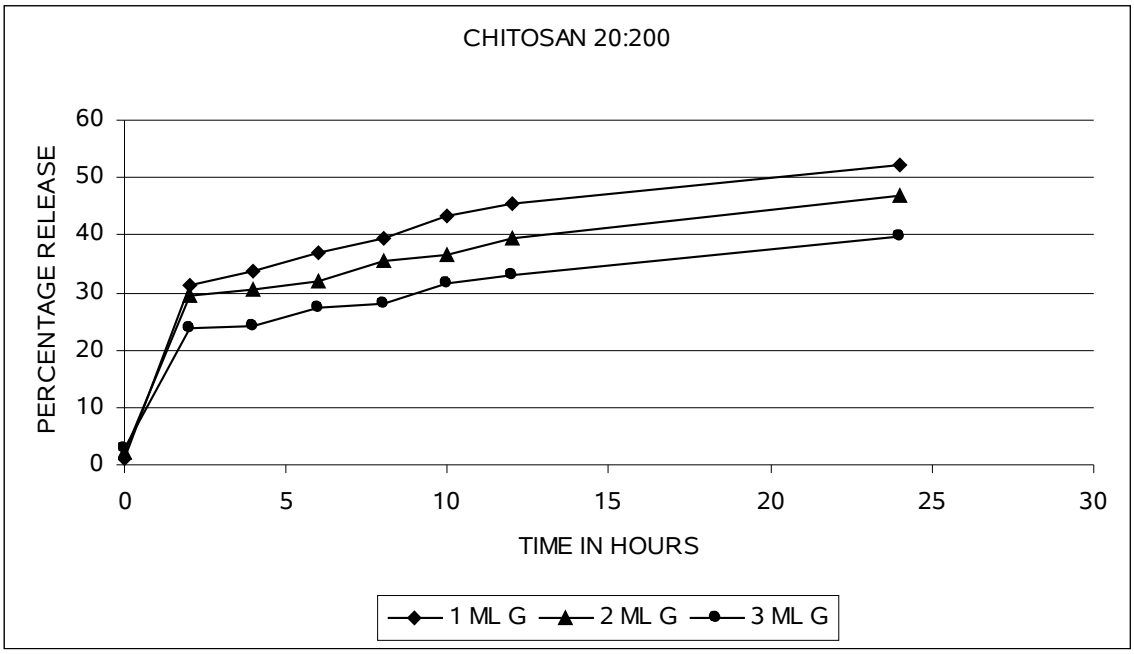


TABLE:21

FIG:20

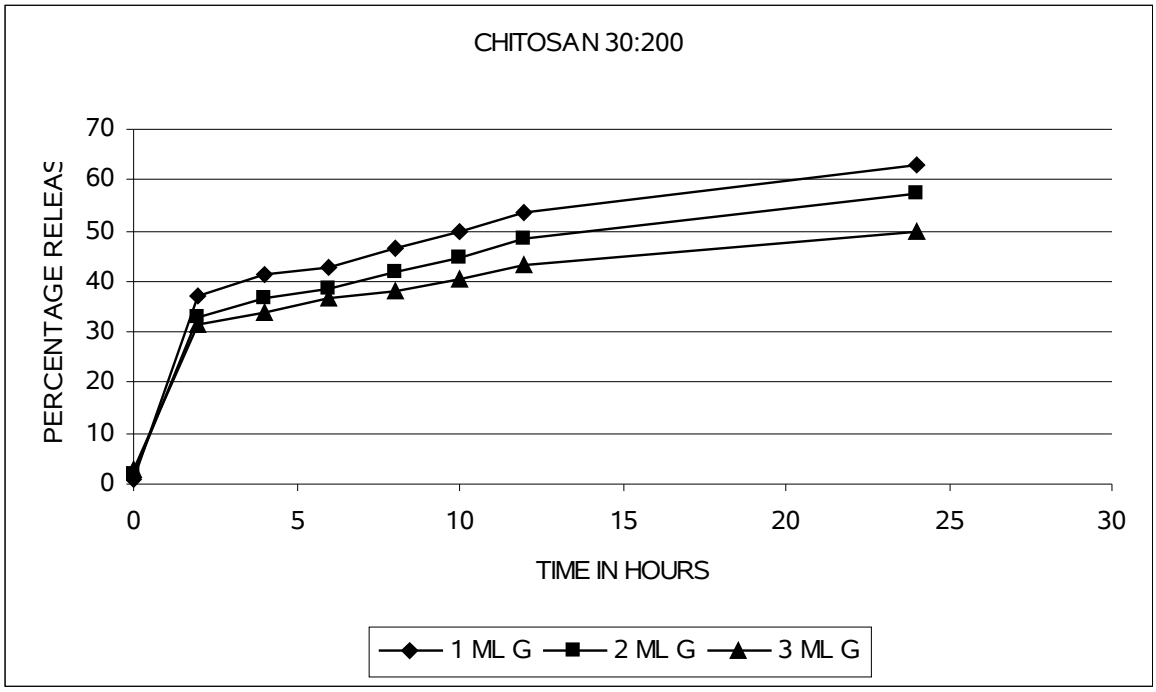


TABLE:22

TIME IN Hrs	PERCENTAGE RELEASE GRISEOFULVIN ON VARIOUS CONCENTRATIONS OF ETHYLCELLULOSE (%)		
	E1P1	E1P2	E1P3
2	8.2	6.31	2.5
4	9.71	9.74	4.17
6	13.18	12.56	5.82
8	15.4	16.18	9.31
10	18.94	17.87	12.65
12	20.37	19.69	13.08
24	27.17	25.27	18.17

FIG:21

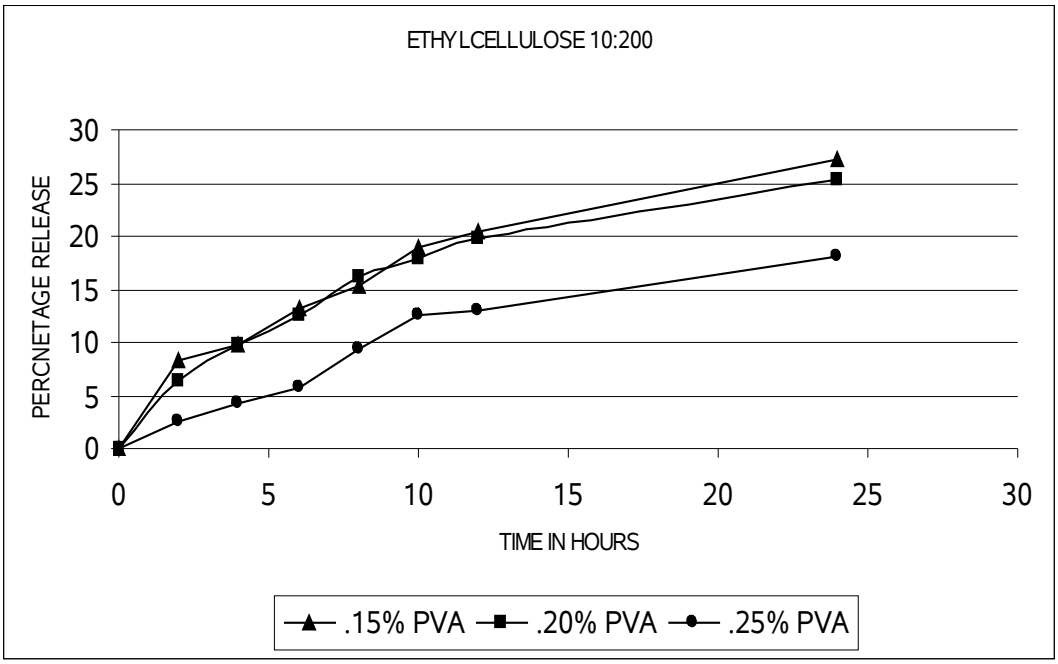


TABLE:23

TIME IN Hrs	PERCENTAGE RELEASE GRISEOFULVIN ON VARIOUS CONCENTRATIONS OF ETHYLCELLULOSE (%)		
	E2P1	E2P2	E2P3
2	9.17	7.63	3.63
4	13.08	8.31	5.11
6	17.03	11.96	6.24
8	18.81	14.40	10.6
10	21.4	18.07	12.82
12	24.62	21.61	13.41
24	33.91	29.54	23.05

FIG:22

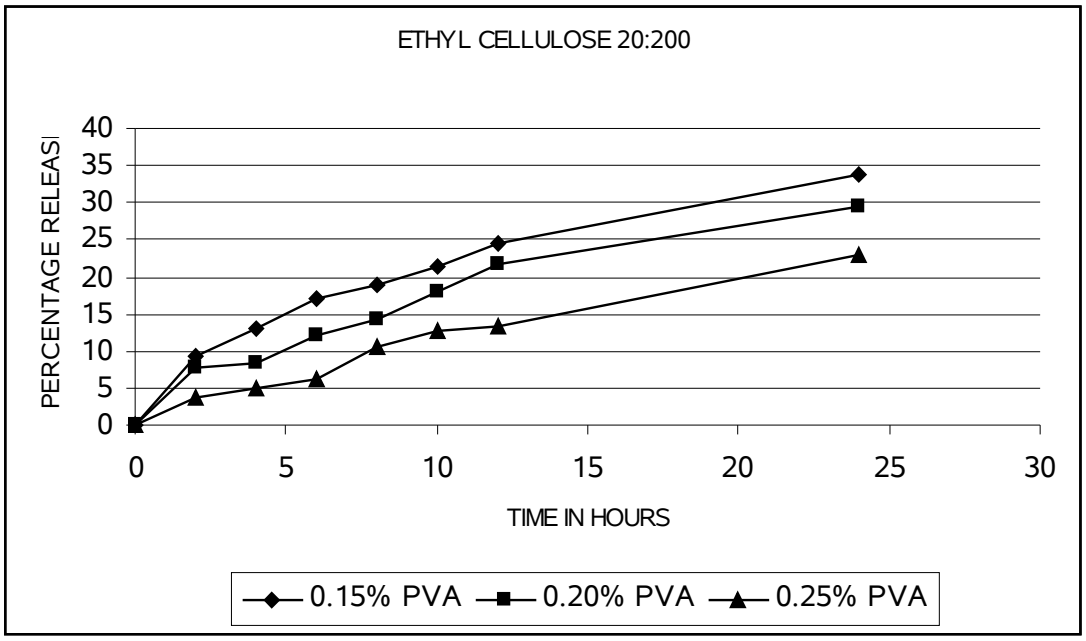
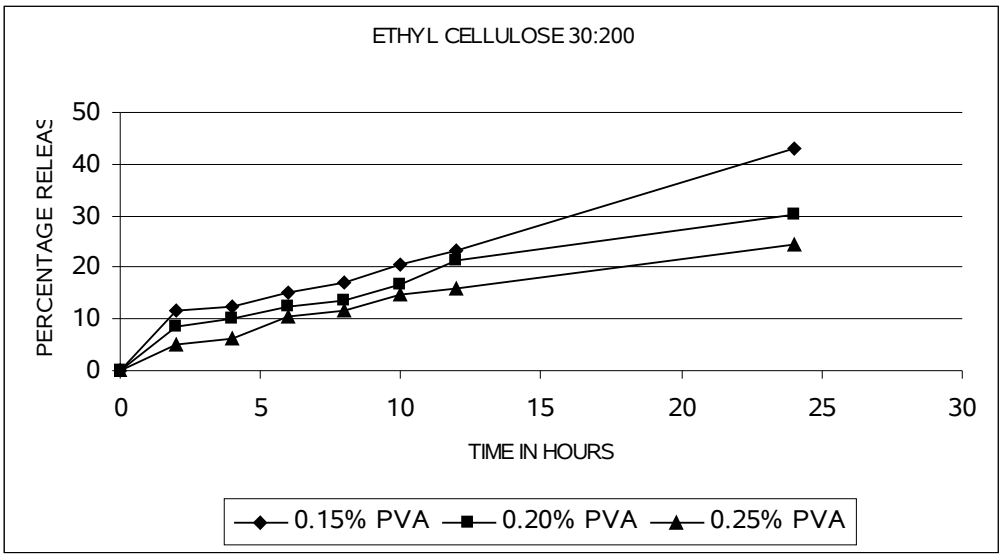

ETHYL CELLULOSE 20:200
PERCENTAGE RELEASI
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
TIME IN HOURS
0.15% PVA
0.20% PVA
0.25% PVA

TABLE:24

TIME IN Hrs	PERCENTAGE RELEASE GRISEOFULVIN ON VARIOUS CONCENTRATIONS OF ETHYLCELLULOSE (%)		
	E3P1	E3P2	E3P3
2	11.61	8.38	5.17
4	12.36	10.09	6.23
6	15.04	12.54	10.48
8	17.09	13.65	11.63
10	20.42	16.76	14.74
12	23.18	21.43	15.79
24	42.86	30.18	24.52

FIG:23



RESULTS AND DISCUSSIONS

CHITOSAN

1) IR spectra of chitosan microparticles:

An IR spectrum of the drug-polymer mixture was taken to study and check the drug-polymer interaction. The spectrum revealed no interaction.

2) Yield of the product:

An average product yield for the formulation 10:200 (drug: polymer) was 95.07%, 20:200 was 94.24% and 30:200 was 95.94%.

3) Preparation of chitosan microparticles:

Chitosan microparticles were prepared by the chemical cross-linking method. The batch of microparticles was subjected to SEM analysis for their size determination. The size range was found to be between 165.37µm – 200.62µm.

4) Study on the encapsulation efficiency of Griseofulvin into the microparticles:

To study the maximum amount of drug, which could be incorporated into the microparticles, different batches of microparticles were prepared containing 10mg, 20mg, and 30mg of the drug with various concentrations of the Gluteraldehyde amount. The amount of polymer used was kept as constant. The drug encapsulation efficiency of the microparticles containing 10mg, 20mg and 30mg of drug was found to be in average of 56.97%, 62.22% and 85.58% respectively. So 30 mg of drug Griseofulvin may be

considered as the ideal concentration to load into the prepared chitosan microparticles.

To estimate the amount of drug bound to microparticles, the chitosan microparticles containing 10mg, 20mg and 30mg of the drug was subjected to UV analysis and the calculation revealed that an average of 5.75mg, 12.44mg and 25.675mg of drug bound to the microparticles

5) **Study of in vitro release from prepared chitosan microparticles:**

The release studies were carried out for 24 hours. In the first two hours an average of 22.92% for 10:200 (drug: polymer), 28.10% for 20:200 and 33.87% for 30:200 were released. After the second hour onwards microparticles released the drug in a sustained manner.

ETHYLCELLULOSE

1) **IR spectra of ethylcellulose microparticles:**

IR spectra of the drug-polymer mixture were taken to study and check the drug-polymer interaction. The spectrum revealed no interaction.

2) **Yield of the product:**

An average product yield for the formulation 10:200 (drug: polymer) was 95.55%, 20:200 was 95.90% and 30:200 was 95.65%.

3) **Preparation of ethylcellulose microparticles:**

Ethylcellulose microparticles were prepared by the solvent evaporation method. The batch of microparticles was

subjected to SEM analysis for their size determination. The size range was found to be between 339µm – 399µm.

4) **Study on the encapsulation efficiency of Griseofulvin into the microparticles:**

To study the maximum amount of drug, which could be incorporated into the microparticles, different batches of microparticles were prepared containing 10mg, 20mg, and 30mg of the drug with various concentrations of the polyvinyl alcohol amount. The amount of polymer used was kept as constant. The drug encapsulation efficiency of the microparticles containing 10mg, 20mg and 30mg of drug was found to be in average of 42.44%, 50.3% and 58.79% respectively. So 30 mg of drug Griseofulvin may be considered as the ideal concentration to load into the prepared ethylcellulose microparticles.

To estimate the amount of drug bound to microparticles, the ethylcellulose microparticles containing 10mg, 20mg and 30mg of the drug was subjected to UV analysis and the calculation revealed that an average of 4.24mg, 10.06mg and 17.64mg of drug bound to the microparticles.

5) **Study of in vitro release from prepared ethylcellulose microparticles:**

The release study clearly shows that ethylcellulose releasing the drug in a sustained manner.

SUMMARY AND CONCLUSION

SUMMARY

The preparation and evaluation of microparticles containing Griseofulvlin using chitosan and ethyl cellulose were compared under the following headings.

1) **Estimation of the drug encapsulation efficiency of chitosan microparticles an ethyl cellulose microparticles:**

Different batches containing various concentrations of drug and same concentration of the polymers were prepared and estimated for the amount of drug loaded in each batch. A comparative result revealed that may be a maximum of 30 mg could be incorporated in both polymers.

A comparison of the drug binding capacity of the microparticles containing two polymers showed that the drug bound to chitosan was greater than ethyl cellulose.

2) In vitro release studies:

The release pattern of the chitosan microparticles revealed a faster release (28.29%) in the first two hours (because of burst release) but ethyl cellulose showed a sustained release through the period.

CONCLUSTION:

From the results obtained it is evident that microparticles containing Griseofulvin prepared using chitosan exhibited better yield, drug encapsulation and fast release when compared to ethyl cellulose microparticles. The fast release of chitosan microparticle may be due to the chemical cross-linking of the polymer glutaraldehyde.

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