PREPARATION AND EVALUATION OF DIACEREIN MICROSPHERE BY MULTIPLE EMULSION METHOD

THESIS SUBMITTED TO FACULTY OF ENGINEERING AND TECHNOLOGY, JADAVPURUNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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By

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CERTIFICATE OF APPROVAL

This is to certify that Miss Monalisha Sengupta had carried out the work entitled "Preparation and Evaluation of Diacerein microsphere by Multiple Emulsion Method" under my supervision in the Division of pharmacology and I appreciate her endeavour to do the works with proper care and attention. Her project work being submitted at the Department of Pharmaceutical Technology, Jadavpur University for the partial fulfillment for the completion of "**Master of Pharmacy"** has reached my entire satisfaction.

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DECLARATION OF ORIGINALITY

AND

COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that the thesis contains literature and original research work by the undersigned candidate, entitled "Preparation and Evaluation of Diacerein Microsphere by Multiple Emulsion Method" studies.

All information in this document have been obtained and presented in accordance with academic rule and ethical conduct.

I also declare that as require by these rules and conduct, I have fully cited and referenced all materials that are not original to this work

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Monalisha Sengupta

DEDICATED TO MY RESPECTABLE AND LOVING PARENTS AND ALMIGHTY WHO SHOWS LIGHT OF EARTH, AND TEACH HARD WORK, HONESTY AND LOVE.

Contents

CHAPTER 1

INTRODUCTION

INTRODUCTION

1.1 A SHORT VIEW ON SUSTAINED/CONTROLLED RELEASE DRUG DELIVERY SYSTEM:

The terms used to identify drug delivery system that are design to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose of drug are sustained release, sustained action, prolonged action, controlled released, depot release. The controlled release/sustained release dosage forms get a great fem in modern therapeutics amongst these dosage form.

"Sustained release" is a well-known term from many decades, in the medical and pharmaceutical literature, for its retarding release of therapeutic agent such that delaying its appearance in the systemic circulation and its plasma profile is sustained in duration. Nowadays these dosage forms are designed to release a drug at a predetermined rate by maintaining a constant drug level for a specific period of time with minimum side effects[10].Matrix system, generally prolongs and controls the release of the therapeutic drug, which is dissolved or dispersed in later. Sustained release constitutes any dosage form that provides medication over an extended time or denotes that the system is able to provide some actual therapeutic control whether this is of a temporal nature, spatial nature or both. Sustained release system generally do not attain zero order type release and usually try to mimic zero order release by providing drug in a slow first order [21].

A sustained release dosage form will provide a therapeutic concentration of the drug in the blood

i.e maintained throughout the dosing interval with a reduction in a peak concentration ratio[23],[24].For most drugs, conventional method of drug administration is effective, but some drugs are shorter half-life, unstable or toxic and have narrow therapeutic ranges. Some drugs also posses' solubility problems. To overcome these problems, controlled drug delivery systems were introduced.

1.1.1. THERE ARE CERTAIN CONSIDERATIONS FOR THE FORMATION OF SUSTAINED RELEASE FORMULATION

- The compound is sustained on its own when it has a long half-life (over 6 hours).
- The development of a time release product may be problematic when the absorption of the active compounds involves an active transport.
- A broad therapeutic windows is necessary to avoid toxicity when the active compound has short half-life as it would require a large amount to maintain a prolonged effective dose; otherwise, the risk is unwanted and another mode of administration would be recommended [26,25].
- The time releasing has no purpose when the pharmacological activity of the active compound is not related to its blood levels [25].

1.1.2. REASONS FOR DEVELOPING SUSTAINED RELEASE DOSAGE FORM [10]

- To extend the duration of action of the drug
- To reduce the frequency of dosing
- To minimize the fluctuations in plasma level
- To improve drug utilization

- To reduce adverse effects

1.1.3. ADVANTAGES OF SUSTAINED/CONTROLLED RELEASE DRUG DELIVERY SYSTEM [10]

- The reduction of frequency of drug administration.
- For better patient compliance.
- For convenient drug administration.
- For reduction of characteristic blood level variations due to multiple dosing of conventional dosage forms.
- The total amount of drug administered can be reduced, thus:
    1. Maximizing availability with minimum dose.
    2. Minimizing the local side effects
    3. Eliminating systemic side effects.
    4. Minimize drug accumulation with chronic dosing.

1.1. DIFFUSION CONTROLLED SYSTEMS POLYMERIC MICROCAPSULES

For the drugs which are require frequent administration to maintain therapeutic levels in the body, diffusion control of drug from dosage form is a logical approach. However major limitations are imposed on maintaining controlled drug delivery by the normal physiological process of the body and how much drug can be enclosed conveniently in a single dosage form. Drugs which are undergo high first-pass metabolism also poor candidates for the development of

controlled-release dosage form.

Polymers can be used to control the drugs by-

i) Controlling how rapidly the drug molecules moves through the polymer molecular matrix or
ii) Controlling the movement of dissolve drug molecules through the channels or pores in a matrix permeated by the dissolution medium.

In the first case, the rate of diffusion depends on solubility of the drug in the polymer matrix, size of the drug molecules and the hindrance of movement by the matrix structure.

In the second case, rate of diffusion depends on the availability, length and tortuosity of the pores; solubility of the drug in the penetrating medium and the viscosity of the medium.

In a microcapsule both the mechanism may contribute to the movement of the drug molecules into the dissolution environment. The extent of contribution by each mechanism will depend on the nature of the drug, polymer and parameters of the microcapsules and also the dissolution environment. Control of drug release with a polymer also can be accomplished by erosion of the polymer to release the drug or by a combination of drug diffusion and polymer erosion.

CHAPTER 2

AIM AND OBJECTIVES

AIM AND OBJECTIVES

The aim of the study, is to formulate and evaluate *in-vitro* and *in-vivo*, microsphere a sustained release preparations ofpoorly water soluble drug Diacerein. It is done by double emulsion (W/O/W) technique, where sodium alginate is used as matrix and ethyl cellulose used as a coating polymer.

The main objectives of the present study are:-

- To develop a microsphere of poorly water soluble drug of Diacerein.
- To study the *in-vitro* release of Diacerein when concentration of matrix polymer is increased.
- To evaluate the toxicity study of drug Diacerein.
- To study the pharamacological activity (*in-vivo*) of the prepared microsphere.

CHAPTER 3

MICROSPHERE

MICROSPHERE

3.1. MICROSPHERE [7] [8].

Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. It has a particle size of (1-1000nm).

3.2.TYPES OF MICROSPHERES [29]

a) Floating Microspheres:

Floating microspheres generally are gastro-retentive, low-density systems thathave sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period without affecting gastric emptying rate. The drug is released slowly at the desired rate.

b) Magnetic Microspheres:

Magnetic microspheres are supra-molecular particles that are smallenough to circulate through capillaries without producing embolic occlusion (<4μm)but are sufficiently susceptible (ferromagnetic)to be captured in micro-vessels and dragged into the adjacent tissues by magnetic field of 0.5-0.8 tesla

c) Bioadhesive Microspheres:

These kinds of microspheres exhibit a prolonged residence timeat the site of application and causes intimate contact with the absorption site and produces better therapeutic action.

d) Radioactive Microspheres:

Radioactive microspheres deliver high radiation dose to thetargeted areas without damaging the normal surrounding tissues. They are injected to the arteries that lead to tumour of interest. The different kinds of radioactive microspheres are α emitters, βemitters and γ emitters.

e) Polymeric Microspheres:

Biodegradable polymeric microspheres are those which containinbiodegradable polymers which prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. Synthetic polymeric microspheres are those which are made up of synthetic polymers and are used as used as bulking agent, fillers, embolic particles, drug delivery vehicles etc.

3.3.ADVANTAGESOF MICROSPHERE [11]

1. Reduces the dosing frequency and thereby improve the patient compliance.
2. Microspheres provide constant and prolonged therapeutic effect.
3. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
4. Microsphere morphology allows a controllable variability in drug release and

degradation.

5. They could be injected into the body due to the spherical shape and smaller size.

3.4. DISADVANTAGES OF MICROSPHERE [11]

1. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit through gut.
2. Differences in the release rate from one dose to another.
3. Dosage forms of this kind should not be crushed or chewed.
4. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.

3.5. CRITERIA MAINTAINED AT PREPARATION OF MICROSPHERES

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersibility in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability.
6. Susceptibility to chemical modification.

3.6. DRUG RELEASE MECHANISM[1]

The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug as release of the active constituent is an important consideration in case of microspheres. Structure or micro-morphology of the carrier and the properties of the polymer determine the release of drug from both biodegradable as well

as non-biodegradable microspheres. The drug release from the microspheres can be done by any of the three methods:

- First is the osmotically driven burst mechanism,
- Second by pore diffusion mechanism, and
- Third by erosion or the degradation of the polymer.

The burst effect is mainly controlled by three factors

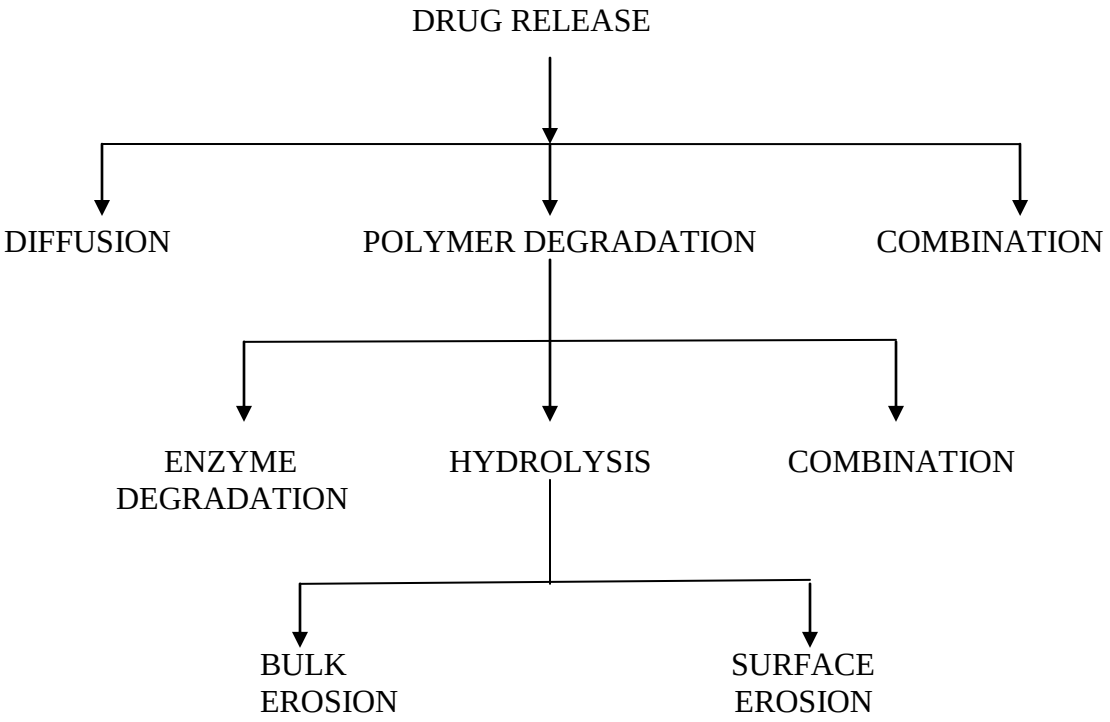
- the macromolecule/polymer ratio,
- particle size of the dispersed macromolecule and
- the particle size of the microspheres.

The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix.

Drug release from the non-biodegradable type of polymers can be understood by considering the geometry of the carriergoverns overall release profile of the drug or active ingredients. The geometry of the carrier:

- whether it is reservoir type where the drug is present as core, or
- matrix type in which drug is dispersed throughout the carrier.

Fig.1: Possible drug release mechanism for polymeric drug delivery



3.7. POLYMERS USED TO PREPARE MICROSPHERE

They are generally two types

a) Natural polymers

Proteins, carbohydrates and chemically modified carbohydratesare different sources of natural polymers.

Proteins: Albumin, Gelatin, And Collagen,

Carbohydrates: Agarose, Carrageenan, Chitosan, Starch,

Chemically Modified Carbohydrates: Poly (acryl) dextran, Poly (acryl) starch.

b) Synthetic polymers

Nonbiodegradable polymers:

Polymethyl- methacrylate (PMMA), Acrolein, Glycidylmethacrylate, Epoxy polymers

Biodegradable polymers:

Lactides,theirglycolides and their copolymers, Poly-alkylCyanoAcrylate, Poly-anhydrides.

CHAPTER 4

DRUGS &POLYMER PROFILE

DRUG & POLYMER PROFILE

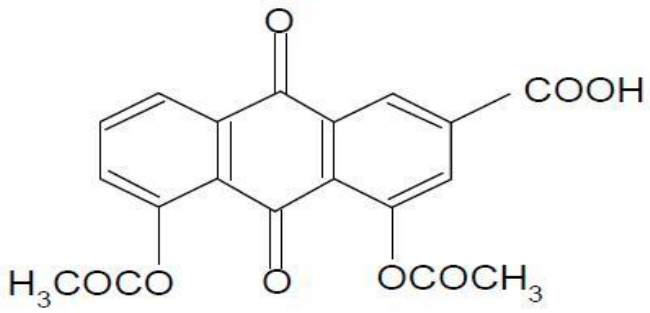
4.1.DRUG PROFILE:

DIACERIN [37]

Diacerein has anti-inflammatory effects through inhibition of Interleukin-1B after converting to its active metabolite "Rhein". It is also the drug to be proved as disease modifying agent. It decreases the fibrinolytic synovial Fibroblasts. Diacerein [4, 5-bis [acetyloxy]-9, 10-dioxo-dioxo-2–anthracene] is an Anthracene derivative.

4.1.1. MONOGRAPH OF DIACERINE [38]

Structure:



Molecular formula: $C_{19}H_{12}O_8$

Molecular weight: 368.3

Chemical Name: 9, 10 – dihydro-4, 5-dihydroxy-9, 10-dioxo-2-anthranoic acid diacetate

Standards: Diacerein contain not less than 98.0 % and not more than 101.0 % of $C_{19}H_{12}O_8$, calculated on the anhydrous basis.

Category: Antirheumatic

Dose: 50 to 100mg

Description: A fine yellow powder

Solubility: methanol, dimethyl formamide, dimethyl sulfoxide.

Identification:

Test A may be omitted if test B and C are carried out. Test B and C may beomitted if test A is carried out.

A) Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with Diacerein RS or with the reference spectrum of Diacerein.

B) Determine by thin-layer chromatography (2.4.17) coating the plate with silica gel GF254.

Mobile phase: A mixture of 60 volumes of 2-propanol, 30 volumes of ethyl acetate and 2 volumes of water.

Test solution: Dissolve about 10 mg of the substance under examination in 100 ml of acetone.

Reference solution: A 0.01 % w/v solution of Diacerein RS in acetone.

C) In the assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests:

pH (2.4.24): 4.5 to 5.5, determined in a 1% w/v suspension.

Related substances: Determine by liquid chromatography (2.4.14)

Aloe-emodin content: Determine by liquid chromatography (2.4.14)

Heavy metals (2.3.13): 2.0 gm. complies with the limit test for heavy metals method B, (10 ppm).

Sulphated ash (2.3.18): Not more than 0.2%

Chromium: Not more than 5 ppm.

Water (2.3.43): not more than 0.5%, determined on 0.5 gm

Assay: Determined by liquid chromatography (2.4.14)

Storage: Storeprotected from light.

4.1.2. MECHANISM OF ACTION [39]

It directly inhibits IL-1 synthesis and release in vitro and downmodulates IL-1 induced activities and have been shown to possess disease modifying effect in experimental models of osteoarthritis and in human subjects with finger joint and knee osteoarthritis. IL-1 plays a fundamental role in osteoarthritis Pathophysiology and cartilage destruction. IL-1 also promotes expression of inducible nitric oxide synthase, increase release of prostaglandin E2, IL-6, IL-8 in human osteoarthritis chondrocytes, which promote joint degradation. Hence, by inhibiting IL-1 Diacerein retards all pathological prepossess initiated in OA. Diacerein also inhibits IL-1 induced expression of cartilage degrading enzymes. It also enhances expression of TGF BETA-1 and TGF BETA 2 thus favoring matrix synthesis and turnover in articular chondrocytes, thereby accounting for disease modifying property of Diacerein. It also inhibits superoxide production, chemo-taxis and phagocytic activity of neutrophils in addition to effect on macrophage migration and phagocytosis. In contrast to NSAIDS Diacerein does not inhibit synthesis of prostaglandins; hence no gastroduodenal toxicity has been observed with Diacerein. It is also demonstrated to be involved in prevention of loss of hydroxiproline and proteoglycans in the joint cartilage, an effect not observed with conventional NSAIDS or COX-2 inhibitors.

4.1.3. PHARMACOLOGY [39]

Diacerein has efficacy on functional manifestations of osteoarthritis and onstructural component. It exerts its pharmacologic action through its active metabolite rhein. Diacerein is entirely converted to rhein before reaching systemic circulation and rhein later gets eliminated byrenal route (20%) or conjugated in liver to rhein glucronide (60%) and rhein sulphate (20%),these metabolites are

mainly eliminated by renal route. The pharmacokinetics after a single oraldose are linear in normal therapeutic doses with equal efficacy in normal young and elderly volunteers. The absorption in systemic circulation is delayed with standard meal but isassociated with 25% increase in amount absorbed. In contrast to other NSAIDS the interactions are minimal as highly binding of rhein to plasma proteins is not saturable. It does not alter renal or platelet COX activity and can be tolerated easily by patients with prostaglandin dependent renal function. Though dose modification is required in mild to severe renal insufficiency [50% dose reduction in severe renal failure], no reduction in initial dose is proposed in liver cirrhosis.

4.1.4. SAFETY PROFILE [39]

Drug watch data and clinical trials have confirmed the safety and tolerabilityofDiacerein. So there is no limitation on the duration of its use. The optimal daily dose which relief symptoms in osteoarthritis knee calculated from effect on VAS assessment criteria of pain on movement was found to be 100mg/day. Diacerein is well tolerated, the predominant adverse effect include transient change in bowel habits. It seems neither responsible for gastrointestinal bleeding nor for renal, liver nor hematological toxicities. Non-significant discoloration of urine occurs during treatment because of urinary elimination of metabolites of Diacerein. No allergic cutaneous reactions were reported in knee osteoarthritis trial. In 3 year hip osteoarthritis trial, rash or purities' was noted in 3% patients on placebo and in 7% patients on Diacerein 100mg daily. No severe allergic reaction has been reported till date.

4.2. POLYMER PROFILE [2]:

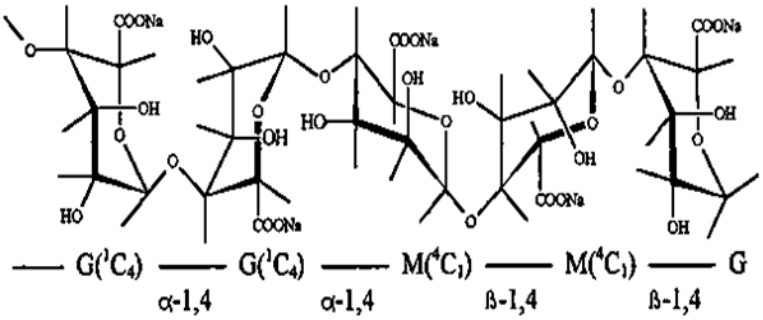
4.2.1. SODIUM ALGINATE

Sodium alginate is a linear unbranched, amorphous copolymer composed of β-D-mannuronic acid (*M*) and α-L-guluronic acid (*G*) linked by $1 \rightarrow 4$ glycosidic bonds. The *M* and *G* units in the alginates may be randomly or non- randomly organized as heterogeneous or homogeneous sequences. Commercially available sodium alginate is usually extracted from various seaweeds. The chemical composition and sequence distribution of sodiumalginate depends on the species and parts of the seaweed employed for extraction [31]. Sodium alginate is a natural polymer consists mainly of the sodium salt of alginic acid, which is a mixture of polyuronic acids $[C_6H_8O_6)_n]$ composed of residues of D-mannuronic acid and L-guluronic acid, and is obtained mainly from algae belonging to the *Phaeophyceae.* This natural polymer is used as a matrix for the drug in microsphere preparation. Increased alginate concentration decreased the percent drug release. Thus, alginate microspheres showed extended in vitro drug release thereby offering sustained release profile along with improved drug delivery. Higher alginate concentration improves the gelling properties and lower concentrations of sodium alginate sustain the drug release for an extended period.

4.2.1.1. MONOGRAPH OFSODIUM ALGINATE

Structure:

Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The water molecules associated with the alginate molecule are not shown in the above structural formula.

Chemical formula: $(C_6H_7NaO_6)_n$

Characters: A white or pale yellowish-brown powder.

Solubility: Slowly soluble in water forming a viscous, colloidal solution, practically insoluble in alcohol.

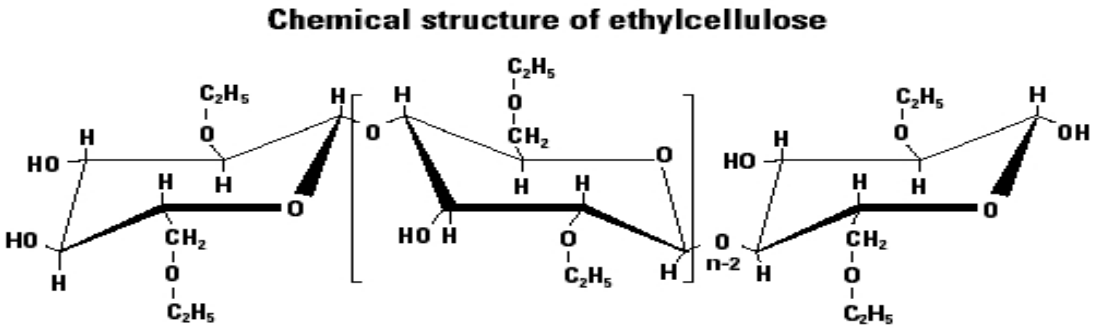
Melting point: Greater than 300^oC

4.2.2. ETHYL CELLULOSE

Ethyl-cellulose is the widely used polymer for oral dosage form. It has beenused as a controlled release excipient for several decades. In most applications, ethylcellulose has been solubilized in an organic solvent(s) and used as a film coating for tablets, beads, and particles to impart a controlled release or taste-masking effect. Ethyl cellulose is partly O- ethylated cellulose. It contain s not less than 44.0 per cent and not more than 51.0 per cent of ethoxy ($-OC_2H_5$) groups, calculated with reference to the dried substance.

4.2.2.1. MONOGRAPH OF ETHYL CELLULOSE

Structure:



Approved name: Ethyl cellulose [IP 1985, Addendum (II)], (32)

Empirical formula: $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_{n-2}C_{12}H_{23}O_5$

Appearance: White or yellowish-white powder or granular powder, odorless or almost odorless.

Typical properties:

Specific gravity: 1.14

Solubility: practically insoluble in water, soluble in methylene chloride and in a mixture of 20 g of alcohol and 80 g of toluene, slightly soluble in ethyl acetate and in methanol, practically insoluble in glycerol (85 per cent) and in propylene glycol. The solutions may show a slight opalescence.

CHAPTER 5

LITERATURE REVIEW

LITERATURE REVIEW

5.1. REVIEWS ON SODIUM-ALGINATE USED IN SUSTAINED/CONTROLLED RELEASE FORMULATION

Preparation of spherical microspheres of theophylline using sodium alginate by Soni *et al.* to prolong the release rate of drug concluded from the results that alginate concentration and percentage of drug release is inversely proportional that means increased alginate concentration decreased the percent drug release. Thus, alginate microspheres showed extended in vitro drug release thereby offering sustained release profile along with improved drug delivery [15]. Kesavan *et al.*also studied the release kinetics of Gatifloxacin using sodium alginate as a polymer which showed the release of the drug was fast and the system got completely depleted of drug within 2 h. A little amount like 0.4 % to 2 % w/v concentration of sodium alginate can modulate drug release significantly. Higher alginate concentration improves the gelling proper ties and lowerconcentrations of sodium alginate sustain the drug release for an extended period[16] period of time.

Study on the influence of micro-environmental pH of alginate facilitated ethyl-cellulose microspheres on entrapment efficiency and release characteristics of fluconazole was successfully done by Sabyasachi Maiti *et al*. The use of hydrophilic polymer at a concentration of 2% (w/v), increased the viscosity of internal aqueous phase and held the active principlefirmly from partitioning out into the external water phase were anticipated by them. Above this concentration, the viscosity of alginate solution became higher, and it was difficult to formulate microspheres. Hence, the concentration of sodium alginate was kept constant at 2% (w/v). To

investigate the effect of the nature of aqueous alginate phase on the properties of the microspheres, the alginate solution was adjusted to different pH values: 4, 6 and 8, while keeping all other formulation and processing parameters fixed. The percentage yield of the microspheres was above 90% for all the formulations. It was observed that the variations in pH of alginate solution significantly affected drug entrapment efficiency ($p<0.05$). The drug entrapment efficiencies decreased at pH above or below 6 and as high as 72.79% fluconazole entrapment was noted at pH 6. As sodium alginate solution is pH sensitive and tends to become insoluble at a pH value below 5, the homogenous distribution of Fluconazole in the aqueous alginate solution was difficult and therefore, the drug diffused to the external medium quite easily leading to lower entrapment efficiency at pH 4. Even, the adjustment of alginate solution to pH 8 led to lower drug entrapment efficiency of the microspheres. The study revealed that the viscosity of alginate solution having pH 8 was lower (872.2 cp) than that of the solution having pH 6 (1087 cp). Hence, it could be suggested that such lowering of viscosity of alginate solution having pH 8 caused easier diffusion of the incorporated drug from polymer/solvent droplets to the external processing medium and resulted in lowerentrapment efficiency at pH 8 [37].

Malay K. Das and Prakash C. Senapati prepared Furosemide-loaded Alginate microspheres by Ionotropic External Gelation Technique. They study the effect of sodium alginate concentration on furosemide release; they used sodium alginate at four different concentrations and observed the more sustained effect with an increase in the concentration of sodium alginate. The steady state release was achieved after an initial lag time and it was directly proportionalto theconcentration of sodium alginate [18].

5.2. REVIEWS ON ETHYL-CELLULOSE USED AS A RETARDANT MATERIAL INSUSTAINED/CONTROLLED RELEASE FORMULATION

Ethyl-cellulose (EC) based microencapsulated drug delivery systems are being extensively studied throughout the world for achieving extended drug release and protecting the core substance from degradation. The *in vitro* evaluation of EC microcapsules have elucidated that their particle characteristics are very useful to control drug release behavior, since these enable drugs to be released at a certain controlled release rate based on the characteristics of drug-EC linkage.

Ethyl-cellulose has good film forming properties and wide compatibility with different type of drugs, and hence it is the most commonly used polymer for sustained/controlled release formulation. It provides a membrane around the dosage form and remain intact throughout the g.i.t. However it does permit water to permeate the film, but dissolve the drug and diffuse out again.

Mingna Song et al. [IL Farmaco, 60(2005)] showed the effects of polymer viscosity and concentration on the properties of amoxicillin microcapsules prepared by emulsion solvent evaporation method. They studied the effect of pore-inducer and emulsifier on release of the drug. Influence of Polymer viscosity on the surface properties of the microcapsules. Both encapsulation increase and smooth surface microcapsules are obtained when viscosity is increased. But meanwhile rate and extent of drug release is decrease with increase in viscosity[19].

M. Kar, P.K. Choudhury [Govi-veriag, 62(2) 2007] prepared microsphere by double emulsion solvent diffusion method to study the entrapment efficiency and extend of drug release of Metformin HCl by using Ethyl-cellulose as retardant material. They used mixed solvent system

consisting of acetonitrile and dichloromethane in 1:1 ratio and light liquid paraffin chosen as the primary and secondary oil phases respectively. Span 80 was used as the surfactant for stabilizing secondary oil phase. The in-vitro release studied was performed in a series of buffer solutions with variables pH. Drug loaded microspheres showed 55-85% of entrapment and the release were extended for up to 12 hrs. SEM study revealed that the microspheres were spherical andporous in nature. Drug release was found to be diffusion controlled [40].Saravanam et al. (2003) prepared ethyl-cellulose microspheres with or without polystyrene (0-25%) by emulsion solvent evaporation technique. It was found that EC/polystyrene shows prolonged drug release and less burst release in comparison to microsphere prepared with ethyl-

cellulose alone and high co-relation was obtained in Higuchi and Korsmeyer-Peppas model [41]. Narong Sariusuta *et al.* [*DDIP,25(3), 1999*] the effect of surfactant on the release characteristics of Coniine HCl from ethyl-cellulose film microsphere. They found that release increase with increase in surfactant concentration and the release found to conform to the solution matrix model[42].

K.R.Rao *et al.* [J. of Microencapsulation 2005] prepared and developed a controlled release micro particle of Zidovudine by w/o/o double emulsion method. They used a mixed solvent system of acetonitrile: dichloromethane (1:1) and light liquid paraffin used as a secondary oil phase. To stabilize the secondary oil phase they were used span80. They showed the effects of variation in drug-polymer ratio on particle size, entrapment efficiency, and release behavior of Zidovudine**.** In vitro release of the drug was performed in phosphate buffer pH 7.4. The drug loaded microsphere showed 41-55% entrapment efficiency and release was extended up to 18-20 hr. SEM studies of the microsphere showed that the microspheres are spherical and porous in nature. From IR spectra, DSC, and DTA thermograms they have been found that there was

absence of drug-polymer interaction and stable character of Zidovudine loaded microsphere [43]. Bhupender Sing and R.Agarwal, [Ind. J. of pharm. Sciences, 64 (4), 2002] studied the development and optimization of controlled release microcapsules of Diltiazem Hydrochloride by emulsion solvent evaporation method. The microcapsules were prepared by factorial design taking rate control polymer, ethyl-cellulose and span80 as an emulgent as the factor at three levels each. The formulations were tested for drug release, size, entrapment efficiency, surface tropography using SEM. They were constructed a polynomial equation, for each response variable and the resultant data were critically analyzed to locate the composition of the optimum formulations and thoroughly evaluated. From SEM study they observed that the microcapsules were topographically regular with over 90% encapsulation efficiency. Release profile for optimized formulations was found to be regulated for controlled release purposes with little dosedumping and release up to 16 hr≈ 99% [44].

Yi-Yan Yang et al. describes the influence of preparation temperature on the variouscharacteristics and release profiles of poly(DL-lactide-co-glycolide) (PLGA) microspheres of bovine serum albumin (BSA)-loaded microspheres using the water-in-oil-in-water (w/o/w) technique with poly(vinyl alcohol) as surfactant in the external aqueous phase. Temperature variation shows the effect on microsphere characteristics such as the microsphere shrinking rate during formation, particle size, density, surface and internal morphology, BSA encapsulation efficiency, BSA initial release, microsphere degradation and BSA in vitro release behavior. They reported that a low preparation temperature of 5^0C gives the fastest initial but the slowest overall shrinking rate, but at high temperatures of 38^0C and 42^0C there is lowest initial yet the highest overall shrinking rate. Subsequently, microsphere means size increases and the

particlesize distribution widens with increase in the preparation temperature. Although all the microspheres have a porous surface as well as internal structure, microspheres fabricated at high temperatures have a uniform internal pore distribution and a very thin dense skin layer, while microspheres fabricated at lower temperatures have a thicker but porous skin layer and biggerpores in the middle of the sphere. Microspheres formed at 33^0C are found to give the highest initial burst release. In terms of in vitro release, microspheres fabricated at low temperatures (5^0C, 15^0C and 22^oC) exhibit similar, steady rates. Microspheres formed at higher temperatures however give very low release rates after their initial release. The results obtained suggest that preparation temperature significantly affects microsphere formation, resulting in their structural and protein release profile differences. These differences ultimately work together to affect the initial release and overall release patterns of the microspheres.

Harri Heiskanen et al. reported the effect of stirring speed on the size of the microsphere and as expected, the size of the microspheres decreased with increasing stirring speed. At low surfactant concentrations the size of the microspheres was independent of the surfactant concentration. However, the size of the microspheres decreased as the surfactant concentration was further increased. The size of the microspheres was not only affected by the surfactant concentration but also by the volume ratio of the dispersed phase to the continuous phase. At a low volume ratio of the phases the effect of the surfactant on the size of the microspheres was larger than the effect of the increased volume ratio of the phases. At high volume ratios of the phases the effect of the volume ratio of phases on the size of the microspheres became more significant than the effect of the surfactant. A slow solidification increased the formation of non-spherical microspheres.

CHAPTER 6

RHEUMATOID ARTHRITIS

RHEUMATOID ARTHRITIS

6.1. VIEWS ON RHEUMATOID ARTHRITIS: [12, 13, 14]

RA is the most common autoimmune disease, and the second most common form of arthritis compared to osteoarthritis (OA); Rheumatoid arthritis (RA) is a chronic, inflammatory disease in which the immune system destroys synovial joints and accessory structures resulting in localized erosion to the joint and its accessory structures; RA affects approximately 1% of adults all over the world, Individuals are usually diagnosed between the third and fifth decade of life and women are 2 to 3 times more likely to be diagnosed than men. Therefore, individuals with RA may experience a lower quality of life and amass a large amount of direct and indirect costs due to the management of the disease, hospitalizations, and physician visits.Due to the progressive nature of the disease, extra-articular complications will occur in multiple organsystems.

6.2. ETIOLOGY

The etiology of RA is not fully understood, but environmental and genetic factors havebeen proposed as potential theories. Genetic predisposition results in the destructive nature of this autoimmune disease. The inheritance of certain genes in the major histocompatibility complex can increase the susceptibility of developing RA. For example, an individual with expression of a human leukocyte antigen (HLA) DR-4 antigen will be 3.5 times more likely to develop RA compared to someone with other HLA-DR antigens. Other potential risk factors include female

gender, use of oral contraceptives, tobacco use, and infectious agents.

Over the past decade, the management of RA has evolved with disease-modifying anti-rheumatic agents with biologic activity targeting specific components of the immune system. With advanced therapy, management includes halting further progression of the disease and maintaining quality of life. The American College of Rheumatology has provided updated guidelines regarding the use of biologic therapies as monotherapy or in combination with non-biologic therapy such as Methotrexate. Unmet needs remain, however, for the management of RA due to the complexity of the disease. Innovative agents are needed to create additional strategies and achieve desired goals for its management. (Formulary. 2011; 46:532–545.)

6.3. PATHOPHYSIOLOGY

Synovial joints, such as the knee, have the most flexibility due to unity ofbones by connective tissue of an articular capsule and accessory ligaments. In RA, the immune response will be activated in an early stage of life. This immune response could be triggered by genetic and environmental factors. Once the immune system is unbalanced, subclinical inflammation will occur due to activation of T cells from an antigen-presenting cell. Once T cells are proliferated, a cascade of events occurs in the immune system: activation of B cells and macrophages, as well as other pro-inflammatory mediators such as tumor necrosis factor (TNF) and interleukin (IL). As the immune system remains unchecked, symptoms associated with RA will occur and the criteria for the disease will be fulfilled. Once the diagnosis is confirmed, the pathologic inflammatory response can continue, resulting in joint destruction and extra-articular complications. Within a synovial joint, bone and cartilage erosion will occur, causing a swollen

joint capsule and inflamed joint synovium. The extra-articular complications can occur over time and include infections, lymphomas, cardiovascular disease, and osteoporosis.

6.4. CLASSIFICATION OF ANTI-RHEUMATIC DRUGS [15, 16]

- **Disease-modifying anti-rheumatic drugs (DMARDs):**

  The most commonDMARDs are-Methotrexate, Sulfasalazine, Hydroxychloroquine, and Leflunomide. Less frequently used medications include Gold salts, Azathioprine, and Cyclosporine.

- **Biologic agents / Biologic DMARDs:**

  Another class of medications used in persons withrheumatoid arthritis and related inflammatory rheumatic conditions is biologic agents, sometimes called biologic DMARDs, including Etanercept, Adalimumab, Infliximab, Certolizumabpegol, and Golimumab, which are all part of a class of drugs called tumor necrosis factor (TNF) inhibitors, and a variety of other agents with different targets, including Anakinra, Abatacept , Rituximab, Tocilizumab, and Tofacitinib. These medications are often combined with Methotrexate or other DMARDs to improve efficacy.

CHAPTER 7

MICROENCAPSULATION

MICRO ENCAPSULATION

7.1. MICRO ENCAPSULATION

Microencapsulation is one of the most interesting fields in the area of pharmaceutical technology by which very tiny droplets or particles of liquid or solid material are surrounded or coated witha continuous film of polymeric material [22, 3].

Microencapsulation is a process by which solid, liquid or even gaseous particles are coated with a continuous film of polymeric material, having a diameter in range of 1 to 1000 µm and are widely used as drug carriers. Usually the drug substances are encapsulated in a polymer-forming particle with a diameter in range of 1-1000 µm and are widely used as a drug carrier[29].

Product smaller than 1 µm are referred to as nanocapsule. Microencapsulated pharmaceutical products have an active agent known as the core material surrounded by a shell known as the coating material or embedded into a matrix structure. Commercially available microcapsules contained 10-90 % w/w core [5]. Microencapsulation technology allows protection of the drug from the environment, stabilization of sensitive drug substances, elimination of incompatibilities, or masking of unpleasant taste, conversion of liquid drugs in a free flowing powders, preventionof vaporization of many volatile drugs, *etc*. [22, 4]. Microcapsules provide constant drug concentration in blood thereby increasing patient compliance, decreasing dosing frequency and toxicity. Thus, microencapsulation technology continues to be of much interest in controlled release-based partly on relative ease of design and formulation and partly on the advantages of micro-particulate delivery systems. The latter include sustained release from each individualmicrocapsule and offer greater uniformity and reproducibility [6]. Microcapsules may be isolated as free flowing products, collected as dry aggregates or suspended directly into

vehicles for administration. The various shapes of microcapsules are- globular, spherical, kidney like rice grains, flocculants and massive etc. drug absorption from microcapsules may be improve due to the minute size of the particles and hence wide distribution of the drug throughout the G.I.T.

7.2.MORPHOLOGY OF MICROCAPSULES [27]

The morphology of microcapsules depends mainly on the core material and the deposition process of the shell.

- **Mononuclear** (core-shell) microcapsules contain the shell around the core.
- **Polynuclear** capsules have many cores enclosed within the shell.
- **Matrix encapsulation** in which the core material is distributed homogeneously into the shellmaterial.
- In addition to these three basic morphologies, microcapsules can also be mononuclear with multiple shells, or they may form clusters of microcapsules.

Two general micro-morphologies of micro-particles can be distinguished-

“Micro-capsules” are the particles in which drug containing core is completely surrounded by a polymer shell. The core can be solid, liquid or gas; the shell is a continuous porous or non-porous polymeric layer. On the other hand “Microspheres” are defined as micro-particles in which the drug substance is either homogenously dissolved or dispersed in a polymeric matrix.

7.3. DIFFERENT TECHNIQUES USED IN MICROENCAPSULATION PROCESS

A) Physico- Chemical Process:

-Phase separation from aqueous solution

-Phase separation from organic solution

-Emulsion solvent evaporation techniques

B) Chemical process:

-interfacial polymerization

-in-*situ* polymerization

-rapid insolubilization (orifice method)

-surface neutralization method

C) Mechanical process

-Air suspension (Wurster)

-Spray drying

-vacuum coating

-Electrostatic aerosol method

-Centrifugal multi-orifice method

Mechanism and Kinetics of Drug Release

Major mechanisms of drug release from microcapsules include diffusion, dissolution, osmosis and erosion [28] (Brazel and Peppas 2000).

Diffusion is the most commonly involved mechanism wherein the dissolution fluid penetrates the shell, dissolves the core and leak out through the interstitial channels or pores [9] (Korsmeyer *et al*. 1983). Thus, the overall release depends on-

(i) The rate at which dissolution fluid penetrates the wall of microcapsules,

(ii) The rate at which drug dissolves in the dissolution fluid, and

(iii) The rate at which the dissolved drug leak out and disperse from the surface [30] (Gunder, Lippold and Lippold 1995). The kinetics of such drug release obeys Higuchi's equation (Higuchi 1963) as below:

$$\mathbf{Q = [D/J\ (2A - \varepsilon C_S)\ C_S\ t]^{1/2}}$$

Where Q is the amount of drug released per unit area of exposed surface in time t; D is the diffusion coefficient of the solute in the solution; A is the total amount of drug per unit volume; C_S is the solubility of drug in permeating dissolution fluid; ε is the porosity of the wall ofmicrocapsule; J is the tortuosity of the capillary system in the wall. The above equation can be simplified to **Q = vt**

where, **v** is the apparent release rate.

Dissolution: Dissolution rate of polymer coat determines the release rate of drug from themicrocapsule when the coat is soluble in the dissolution fluid [29] (Korsmeyer *et al*. 1983). Thickness of coat and its solubility in the dissolution fluid influence the release rate [22] (Costa and Lobo 2001).

Osmosis: The polymer coat of microcapsule acts as semi permeable membrane and allowsthe creation of an osmotic pressure difference between the inside and the outside of the microcapsule and drives drug solution out of the microcapsule through small pores in the coat.

Erosion: Erosion of coat due to pH and/or enzymatic hydrolysis causes drug release [32] (Sachacht and Van Bos 1987) with certain coat materials like glyceryl-monostearate, bee's wax

and stearyl alcohol.

Attempts to model drug release from microcapsules have become complicated due to great diversity in physical forms of microcapsules with regard to size, shape and arrangement of the core and coat materials [33] (Nokhodchi *et al*. 2002; Haznedar and Dortue 2004). The physiochemical properties of core materials such as solubility, diffusibility and partition coefficient, and of coating materials such as variable thickness, porosity, and inertness also makes modeling of drug release difficult. However, based on various studies concerning the release characteristics, the following generalizations can be made:

i) Drug release rate from microcapsules conforming to reservoir type is of zero order.

ii) Microcapsules of monolithic type and containing dissolved drug have release rates that are $t_{1/2}$dependent for the first half of the total drug release and thereafter decline exponentially.

iii) However, if a monolithic microcapsule containing large excess of dissolved drug, the release rate is essentially $t_{1/2}$dependent throughout almost the entire drug release.

In monolithic capsules the path traveled by drug is not constant; the drug at the center travels a large distance than the drug at the surface. Therefore, the release rate generally decreases with time.

7.4. VIEWS ON EMULSION SOLVENT EVAPORATION METHOD [34]

The emulsion solvent evaporation technique was fully developed at the end of 1970s and has been successfully in the microspheres made from several biocompatible polymers. Solvent evaporation techniques are carried out in a liquid manufacturing vehicle (emulsion), which is prepared by agitation of two immiscible liquids. The process involves dissolving microcapsule

coating (polymer) in a volatile solvent, which is immiscible with the liquid manufacturing vehicle phase. Several methods can be used to achieve dispersion of the oil phase in the continuous phase. These are as follows:

i) Oil/water emulsion followed by solvent evaporation:

ii) Water-oil-water multiple emulsion followed by solvent evaporation system:

iii) Water/oil/oil or water/oil/oil/oil multiple emulsion followed by solvent evaporation system

iv) Modified water-in-oil-in-water ($W_1/O/W_2$) double emulsion followed by solvent evaporation.

Factors influencing microencapsulation by solvent evaporation technique include but not are limited to,

Effect of polymer,

Drug to polymer ratio,

Effect of organic solvents,

Effect of drug solubility in continuous phase,

Rate of solvent removal, Effect of preparation temperature,

Effect of interaction between drug and polymer,

Effect of buffer or added salt,

Effect of internal aqueous phase volume on loading capacity,

Effect of external aqueous phase volume on loading capacity,

Effect of stirring speed,

Important factor that must be considered during the preparation of microcapsules by solvent evaporation technique include the choice of vehicle phase and solvent for the polymer coating because they greatly influence microcapsule properties as well as the choice of solvent recovery

techniques.

This technique is applicable to produce wide variety of micro-capsules of liquid and solid core materials. The core material may be either water soluble or water insoluble but the polymer used must be water insoluble.

The technique is simple, economic and easy to perform depending on the stability of the active agent in the polymer solution; the product can be either homogenous or heterogeneous microsphere.

CHAPTER 8

INFLAMMATION

INFLAMMATION

8.1. VIEWS ON INFLAMMATION [47]

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair.

Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

8.2. SIGNS AND SYMPTOMS [47]

Signs of acute inflammation are

- Pain
- Heat
- Redness
- Swelling
- Loss of function

Signs of chronic inflammation are

- Hay fever
- Periodontitis
- Atherosclerosis
- Rheumatoid arthritis
- Cancer (e.g., gallbladder carcinoma)

8.3. ANTI-INFLAMMATORY [47]

Anti-inflammatory or anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system.

Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by counteracting the cyclooxygenase (COX) enzyme. On its own, COX enzyme synthesizes prostaglandins, creating inflammation. In whole, the NSAIDs prevent the prostaglandins from ever being synthesized,

reducing or eliminating the pain.

Some common examples of NSAIDs are aspirin, ibuprofen, and naproxen. The newer specific COX-inhibitors are not classified together with the traditional NSAIDs even though they presumably share the same mode of action.

On the other hand, there are analgesics that are commonly associated with anti-inflammatory drugs but that have no anti-inflammatory effects. An example is paracetamol (known as acetaminophen in the U.S). As opposed to NSAIDs, which reduce pain and inflammation by inhibiting COX enzymes, paracetamol has as early as 2006 been shown to block the reuptake of endo-cannabinoids, which only reduces pain, likely explaining why it has minimal effect on inflammation.

CHAPTER 9

MATERIALS & INSTRUMENTS

MATERIALS AND INSTRUMENTS

Details of materials and instruments have been discussed in this chapter. Details of drug, polymer and different instruments used in the experiment are given bellow:

9.1. MATERIALS

Table 1: list of chemical or materials usedin experimental work

SL.No.	Materials / Chemicals Used	Manufacturer
1	Diacerine	Virdev Intermediates Pvt. Ltd
2	Ethyl cellulose	Sigma Aldrich
3	Sodium Alginate	Star chemicals
4	Disodium hydrogen phosphate	MERCK
5	Potassium hydrogen phosphate	MERCK
6	Dichloromethane	MERCK
7	Span 80	LOBA CHEMICALS
8	Tween 20	MERCK
9	Carrageenan	HIMEDIA
10	Diclofenac Sodium (standard drug for in-vivo study)	NOVARTIS

9.2. INSTRUMENTS

Table 2: list of various instruments used in experimental work

SL.No.	Instrument	Model No.	Manufacturer
1	Homogenizer	RQ-127A	REMI MOTOR
2	Mechanical stirrer	BL-433	REMI MOTOR
3	Magnetic stirrer	MS2012	ELTEK LABORATORY
4	Melting Point Apparatus	—	MP-I
5	UV Spectrometer	M5	SPECTRAMAX
6	Digital Electronics Balance	PB 303 Delta range	METLER TOLEDO
7	pH Meter	CYBER SCAN 500	EUTEK INSTRUMENTS
8	Dissolution Apparatus	(USP) TdL08L	Electro lab
9	SEM	JSM6360	JEOL MAKE
10	FTIR	IR-Prestige-21	SHIMATZU MAKE (JAPAN)
11	Plethysmometer	—	—

CHAPTER 10

METHODOLOGY

METHODOLOGY

10.1. PREPARATION OF MICROSPHERE BY MULTIPLE EMULSION TECHNIQUE

Multiple emulsion (W/O/W) technique is the main protocol which is followed in case of preparation of microsphere. The process is described briefly by the schematic diagram is as follows:

Water + Natural polymer from plant gum exudates

Stirring for 30minutes at 30-35^{0}c

↓

Suspension (W_1)

Polymer (ethyl cellulose) + Dichloromethane + Span80 {oil phase O_1}

Stirring few minutes and W_1 added drop by drop with Homogenization

↓

Primary Emulsion (W_1/O_1)

Distilled water + Tween 80 (W_2)

(W_1/O_1) is taken in a 12 no. syringe added drop by drop by continuous mechanical stirring at 600-700 r.p.m for 5-6 hrs

↓

Microsphere formation in solution by evaporation of the solvent oil

↓

Separate Microsphere by filtration & Washout, Collect & Dry

10.2.FORMULATION PARAMETERS

Table 3: Categorization and amount of items present in formulation:

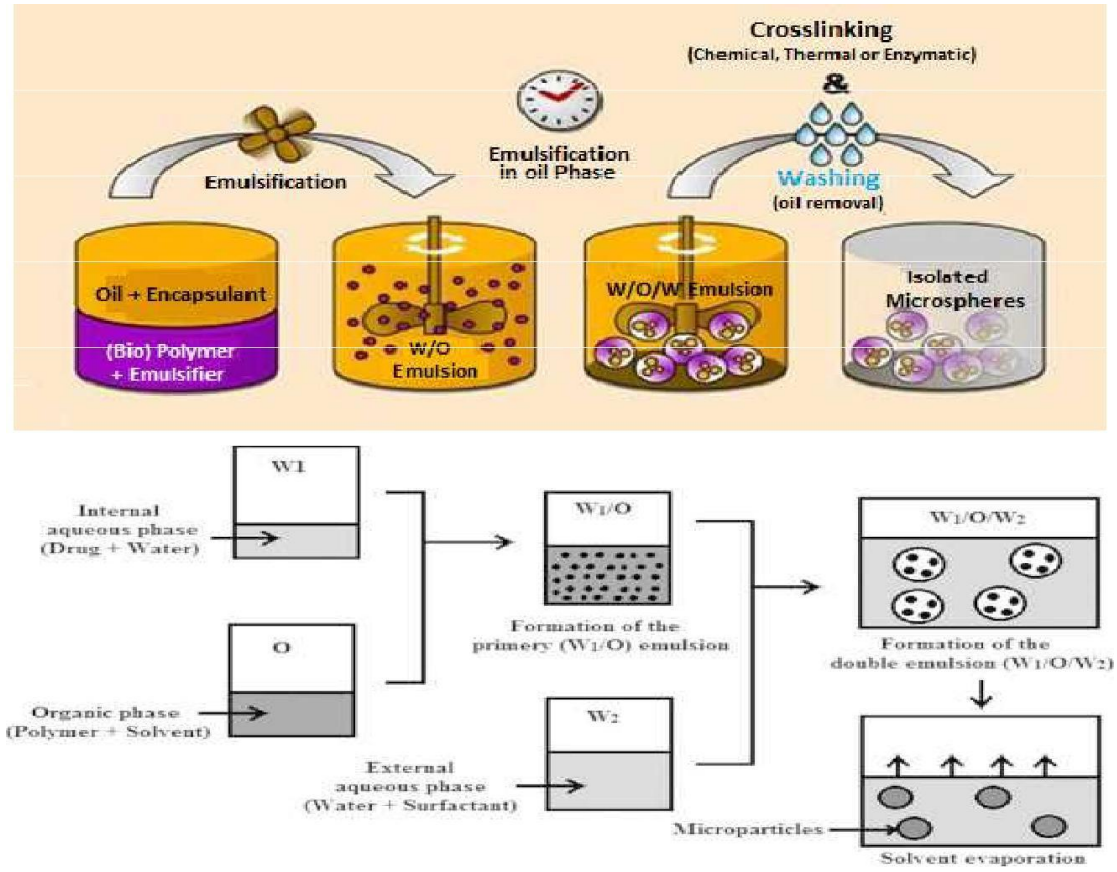
CATEGORY	NAME	AMOUNT
Drug	Diacerein	25mg
Polymer	Ethyl cellulose	600 mg
Gum	Sodium alginate	25-150 mg
Oil phase	Chloroform or Dichloromethane	20 ml
Internal aqueous phase	Water or phosphate buffer pH range 6-8	8-10 ml
External aqueous phase	Water	100 ml
Surfactant	Tween 80 & Span 80	0.2 ml

10.3. PREPARATION OF DIACEREIN LOADED MICROSPHEREBY MULTIPLE EMULSION TECHNIQUE

In the multiple emulsion technique, we prepared Diacerein microsphere by water in oil in water (w/o/w) format, as per drug's demand where the internal and external aqueous phases are separated by an oil layer. For their formation and stability, at least Two surfactants, one having a low HLB value(Span 80) and the other with higher HLB value (Tween 80) are used to form the

primary water in oil (w/o) emulsion and to achieve secondary emulsifications respectively are required for formation and for getting better stability of prepared microsphere. In the preparation of microsphere, an aqueous solution or suspension of gum with drug (internal aqueous phase) was emulsified in a solution of polymer in organic solvent containing 0.2 ml surfactant(low HLB value) using a homogenizer at 5000 rpm for 5 min. The resulting water-in-oil (w/o) emulsion was then transferred into 100 ml water containing 0.2 ml surfactant (high HLB value) with continuous mechanical stirring with the r.p.m 1000 at room temperature to form W/O/W types multiple emulsion. The stirring was continued for a period of 3 hrs to allow complete evaporation of the organic solvent. Upon solvent evaporation, the polymer precipitated and the core of the microspheres solidified. The microspheres were then filtered off with the help of muslin cloth, washed with cold doubled distilled water and dried at room temperature for 24 hr. The same procedure was adopted for the preparation of blank microspheres (without drug), and drug-loaded microspheres (without gum).

***Fig.2**: Schematic representation of microspheres-preparation by double emulsion technique*



CHAPTER 11

EVALUATION

EVALUATION

11.1. *IN-VITRO* EVALUATION

There are some parameters which ensure us about the originality and purity of the active drug. This study also includes the release of prepared formulation, drug loading, inter-relationship between the active drug and other excipients.

11.1.1. CHARACTERIZATION OF THE DRUG

The drug was identified by physical observation, melting point, UV-spectroscopy and IR-spectroscopy.

11.1.1.1. Physical observation:

The color and the type of forms (crystalline, amorphous, powder etc) of the drug should observed and identified as per the Indian Pharmacopoeia (7^{th} edition 2014) reported by WHO expert committee.

11.1.1.2. Determination of melting point:

The melting point of Diacerein should be determined by and compared with the reference sample cited in Indian pharmacopoeia.

11.1.1.3. Ultra-violet spectroscopy:

The spectrophotometry analysis of the drug was carried out in phosphate buffer pH 6.8 in the

range of 200-400 nm and the spectra was analyzed to find out the suitable λ-max which was used for quantitative analysis.

11.1.1.4.Development of Calibration Curve:

i) Preparation of phosphate buffer pH 6.8

Stated amount of disodium hydrogen phosphate andpotassium hydrogen phosphate in IP is taken in a beaker to prepare 1000 ml buffer of pH 6.8which should be adjusted with ortho-phosphoric acid by using pH meter.

ii) Estimation of absorption maxima (λ max)

By dissolving 10 mg of Diacerein in 100 ml of phosphate buffer pH 6.8 to obtain a stock solution of 100 µg /ml. Then a solution of 10 µg/ml is prepared from the stock solution in pH 6.8. The resulting solution was scanned between 200 nm to 400 nm in a double beam UV-Visible spectrophotometer.

iii) Preparation of calibration curve in phosphate buffer pH 6.8

10 mg of Diacerein was accurately weighed and transferred to 100 ml of volumetric flask, then dissolved and made up the volume upto 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 100 µg/ml (stock solution I). One ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 10 ml to obtain a solution of 10 µg/ml (stock solution II). From stock solution II aliquots of 2, 4, 6, 8, 10 ml were transferred to a series of 10 ml volumetric flasks and the volume made up with the same fluid to give 2, 4, 6 & 8, 10 µg/ml of concentration. The absorbance's of these solutions was measured at 260 nm against blank.

11.1.1.5. Infrared Spectroscopy:

IR spectrum of Diacerein was recorded using IR Spectrophotometer between the ranges of 400and 4000 cm^{-1}. It was then compared with the IR-reference spectra of Diacerein.

11.1.2.DRUG-EXCIPIENT INTER-RELATIONSHIP STUDY

Fourier transform infrared spectroscopy:

FTIR spectra of pure drug, formulation and blank formulation (without drug) were recorded by KBr disc method using FTIR spectrophotometer. FTIR spectra of physical mixture were compared with FTIR spectra of pure drug.

11.1.3. CHARACTERIZATION OF MICROSPHERES

11.1.3.1. Percentage Yield of Microspheres:

After the preparation of microsphere, they are accurately weighed and the percentage yield was calculated by using the following formula:

Percent Yield = (Amount of microspheres obtained in mg ÷theoretical amount) ×100

11.1.3.2. Drug entrapment efficiency

A predetermined amount of drug loaded microspheres were crushed into powder which diluted to 30 ml phosphate buffer by stirring for 5h using the magnetic stirrer. The stock solution was collected by filtered through whatmann filter paper which was diluted using phosphate buffer and analyzed spectrophotometrically for Diacerein content at 260 nm. The drug entrapment efficiency was determined using the following relationship:

Drug entrapment efficiency = (experimental drug content ÷ theoretical drug content) × 100

Results were based on triplicate determination.

11.1.3.3. Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope which is capable of producing high-resolution images of a sample surface. Due to the manner in which the image is created, SEM images have a characteristic three dimensional appearance and are useful for judging the surface structure of the sample. Scanning Electron Microscope was used to characterize the shape and surface topography of the microspheres. Photo-microgram of the microspheres before and after the release of the drug was taken. Prior to examination, samples were gold sputter coated at 17kV to render them electrically conductive.

11.1.3.4. *In-Vitro* Drug Release Study

In-vitro drug release study of drug loaded microsphere was carried out by using USP paddle typedissolution apparatus at 37^{o}C and 50 r.p.m in change pH. In change pH initially the dissolution process started with acidic buffer pH 1.2 (500ml) for first 2h and after that it is continued with phosphate buffer pH 6.8 (750 ml) for rest of the study. An accurate amount of microsphere (30 mg) were added to dissolution medium and at preset interval 5 ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. Aliquotsfollowing suitable dilution, were analyzed spectrophotometrically at 260 nm. This study was performed for the period of 12 hrs. The concentration of Diacerein in the test samples were corrected for sampling effect using following formula-

$$\mathbf{C_n = M_n\,(V_T/V_T\text{-}VS) \times (C_{n-1}/M_{n-1})}$$

Where, C_n is the corrected concentration of the n^{th} sample,

M_n is the original concentration of the n^{th} sample,

V_T is the volume of dissolution medium,

V_s is the volume of the sample,

C_{n-1} is the corrected concentration of the $(n-1)^{th}$ sample,

M_{n-1} is the original concentration of the $(n-1)^{th}$ sample.

Result was based on triplicate determination.

11.2. *IN-VIVO* EVALUATION

This type of evaluation shows different types of pharmacological activity and toxicity of the active drug as well as the prepared formulations which performed on suitable animal model stated on the standard officials.

11.2.1. ACUTE TOXICITY STUDY

Acute oral toxicity study was performed as per OECD (Organization of Economic Cooperation and Development) 423 guideline (Acute toxic class method).Healthy female (should be nulliparous and non-pregnant) albino Wister rats were randomly divided into 6 groups with 6 animals in each group. The animals were kept fasting overnight providing only water, after which the solution of active Diacerein was administered orally with increasing doses (50, 100, 500, 1000 and 2000 mg/kg) by intra-gastric tube to determine the safe doses. The animals were noticed continuously for 1 h, then frequently for 4 h and later at the end of 24 h for general behavioral, neurological and autonomic profile. Further, one group was administered high dose of Diacerein orally once daily for 15 days and observed for any lethality.

11.2.2. ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced rat hind paw edema

The anti-inflammatory activity of the prepared Diacerein microsphere was assessed by the carrageenan-induced right hind paw edema method in Male Wister rats. Acute inflammation was produced by sub-planar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats 1 h after the oral administration of test materials. The paw volume was measured by plethysmometer at 0h, 1hr, 1.5hr, 2hrand 3hr after the carrageenan injection .The formulation of two different batches were administered at 25 and 50 mg/kg body weight orally. Diclofenac Sodium at a dose of 40 mg/kg body weight was used as standard anti-inflammatory agent. The negative control group received 0.1% Na-CMC in saline solution. The anti-inflammatory effect of the drug was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%) inhibition} = \frac{Pc - Pt}{Pc} \times 100$$

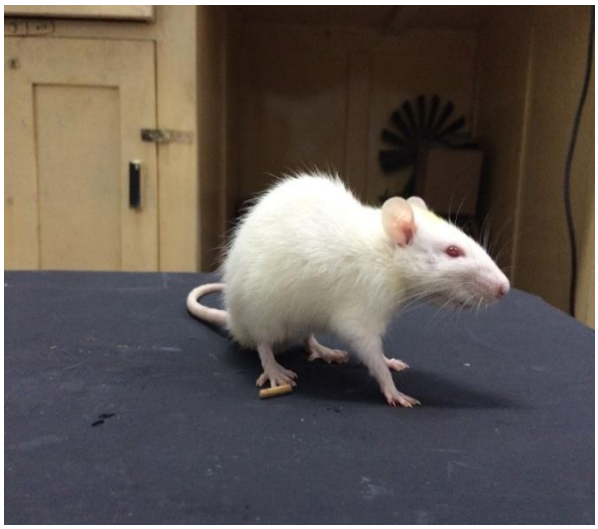
Where,

P_c= Increase in paw thickness of the control group

P_t = Increase in paw thickness of the control group

Some photos of the anti-inflammatory study which performed in the lab are given below.

Fig: 3 Wister albino rat used for the anti-inflammatory study



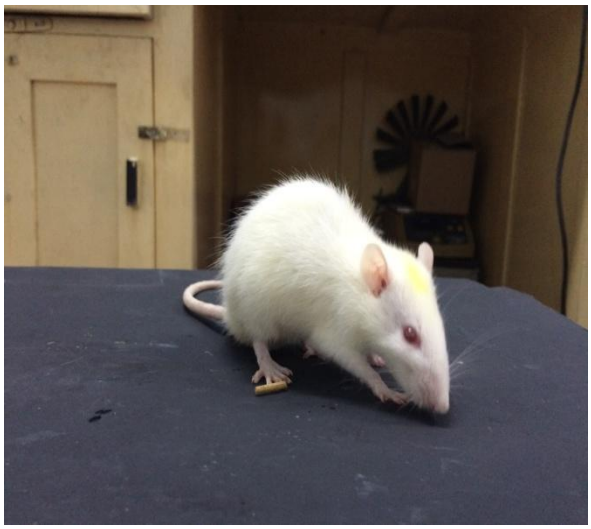


Fig: 4 Feeding of Diacerein formulation to the Wister albino rat

Fig:5 Inducing paw edema on Wister albino rat by injecting 1% carrageenan solution

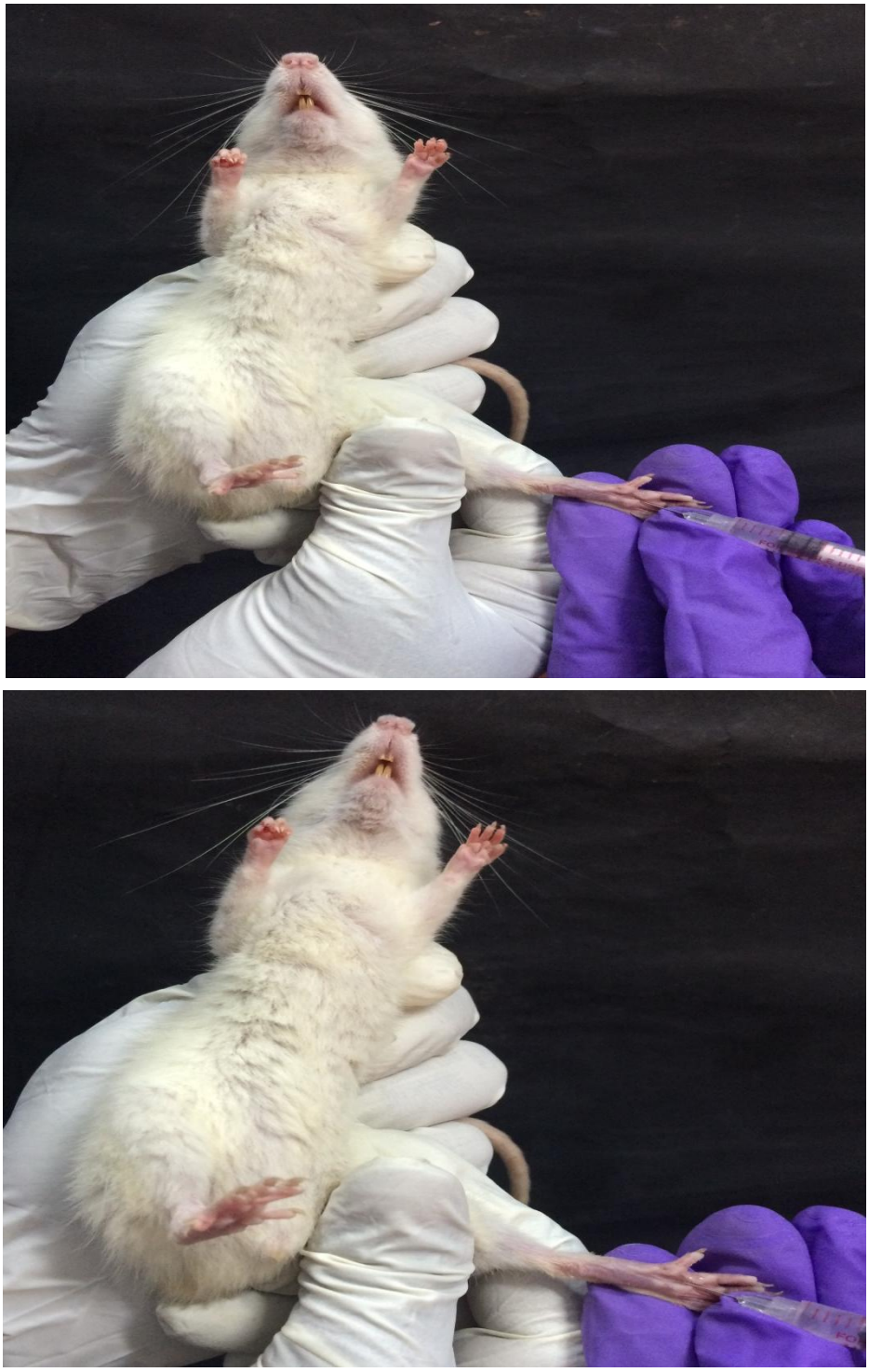
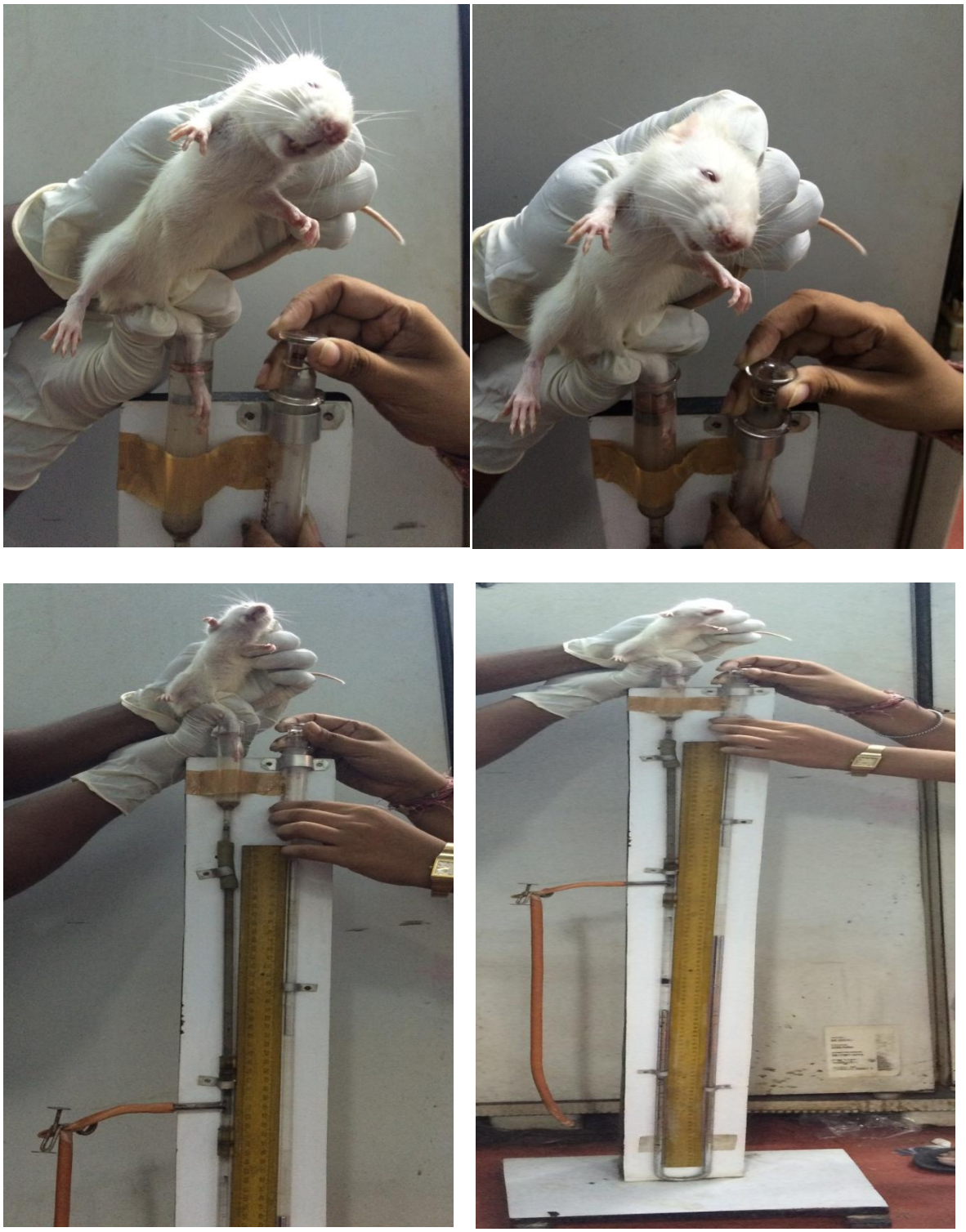


Fig:6 Checking Paw edema of Wister albino rat by using plethysmometer



CHAPTER 12

RESULTS & DISCUSSION

RESULT AND DISCUSSION

In this chapter result of different analyses were described of pure drug as well as of formulation too. From these results we can assure about the originality, purity of the core drug. Not only that we know also about the amount of presence, entrapment, release of drug from the prepared formulation and effects of drug on animal model. Here the results are discussed to draw a suitable conclusion.

12.1. IN-VITRO RESULT

12.1.1. RESULTS OF CHARACTERIZATION OF THE DRUG

12.1.1.1. Physical observation:

The color of supplied Diacerein is yellow and the type is fine crystalline powder.

12.1.1.2. Melting point determination:

At 217^{o} melting point of Diacerein is found which matches the official literatures.

12.1.1.3. Ultra-violet (UV) absorption spectra:

An absorbance maximum (λ_{max}) of drug Diacerein was determined by spectrophotometer at the range of 200-400nm wavelength was 260nmwhich was used later for quantitative analysis.

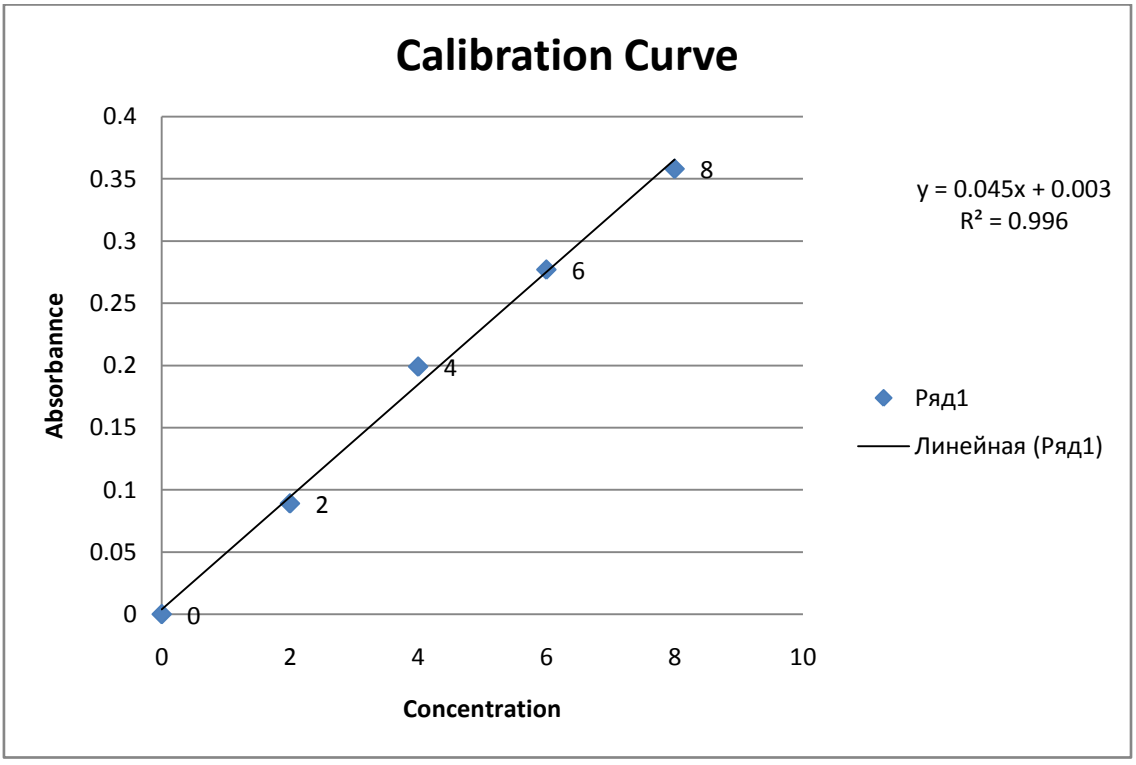
12.1.1.4. Calibration curve of Diacerein:

Calibration curve of Diacerein was prepared in phosphate buffer pH 6.8 to calculate and utilized Regression equation for quantitative estimation of the drug. The correlation coefficient (R^2) was found to be 0.9963.

Table 4: Data of calibration curve of Diacerein (mean ± SD, n=3)

Concentration (mcg/ml)	Absorbance (nm)
0	0.00
2	0.089
4	0.199
6	0.277
8	0.358

Fig 7: Calibration curve of Diacerein in phosphate buffer 6.8



12.1.2. RESULTS OF DRUG-EXCIPIENT INTER-RELATIONSHIP STUDY

Fourier transform infrared spectroscopy:

The FTIR patterns of pure Diacerein, formulations and blank (without drug) are shown in (Figure 8, 9, 10). The FT-IR spectra of pure Diacerein showed sharp peak. All the peaks are also obtained in the microspheres, confirming the compatibility. This result suggests drug stability during encapsulation process.

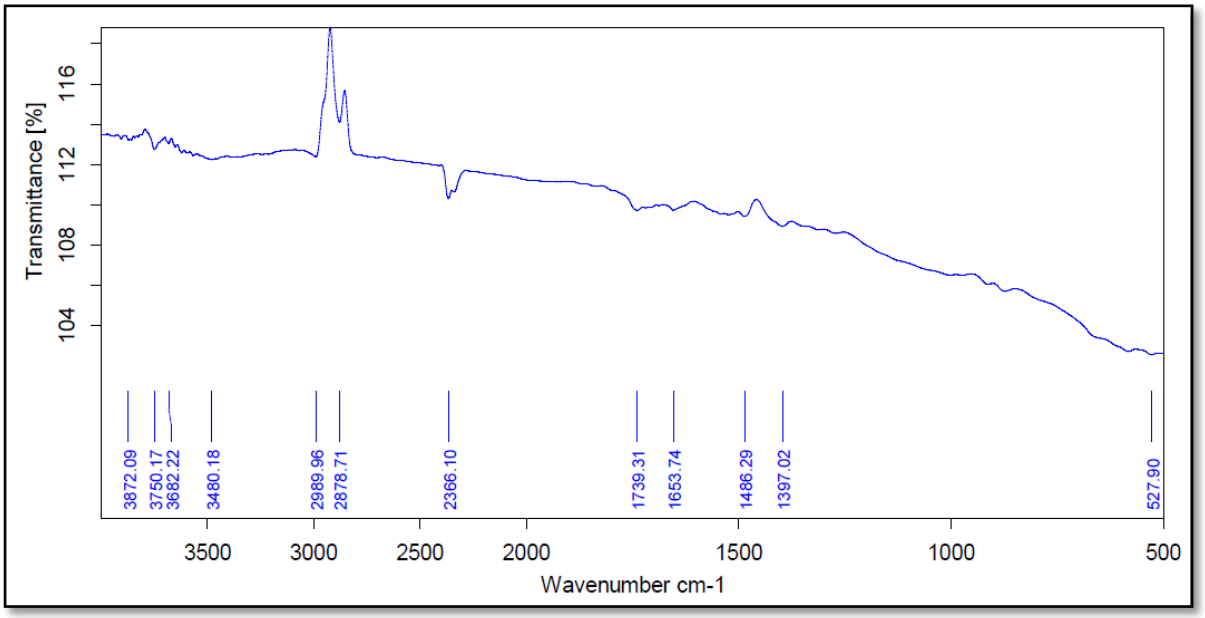


Fig:8 FT-IR Spectrum of Blank formulation (without drug)

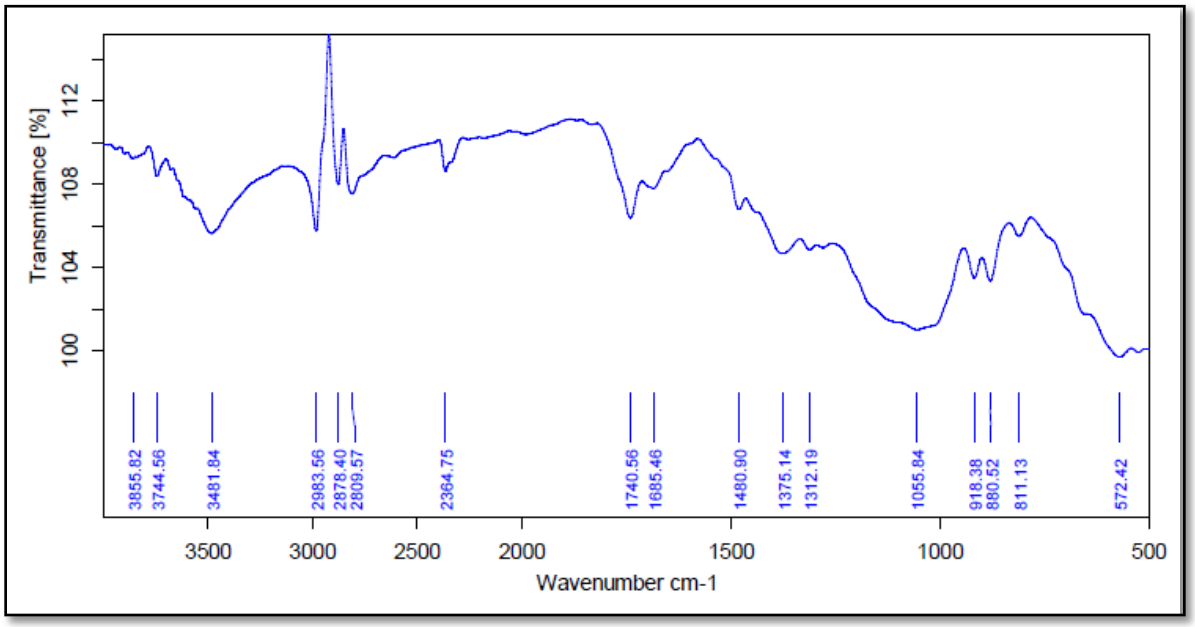


Fig: 9 FT-IR Spectrum of Blank formulation (with drug)

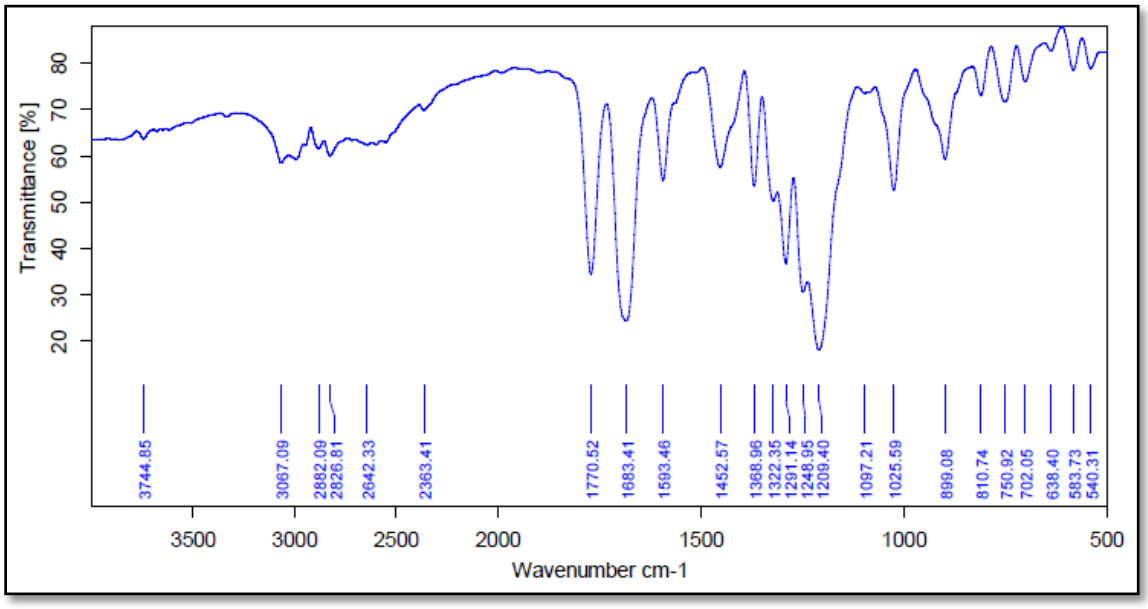


Fig: 10 FT-IR Spectrum of pure drug Diacerein

12.1.3. RESULTS OF CHARACTERIZATION OF MICROSPHERES

12.1.3.1. Percentage Yield of Microspheres:

Several formulations of microspheres were prepared with different drug to polymer ratio. Here the results are given who give the satisfactory result. Yields were found of different batches range from the71-84%.

Table 5: percentage yield of Diacerein microsphere

Batch No. and Drug: sodium-alginate: ethyl - cellulose ratio	% yield (mean, n=3)
B06(1:2:24)	84.15
B08(1:1:24)	76.31
B11(1:3:15)	71.43
B12(1:5:12)	82.29

12.1.3.2. Drug Entrapment Efficiency:

Satisfactory results of percentage of drug entrapment efficiency of different batches are given in the following chart.

Table 6: Percentage of drug entrapment efficiency of Diacerein microsphere

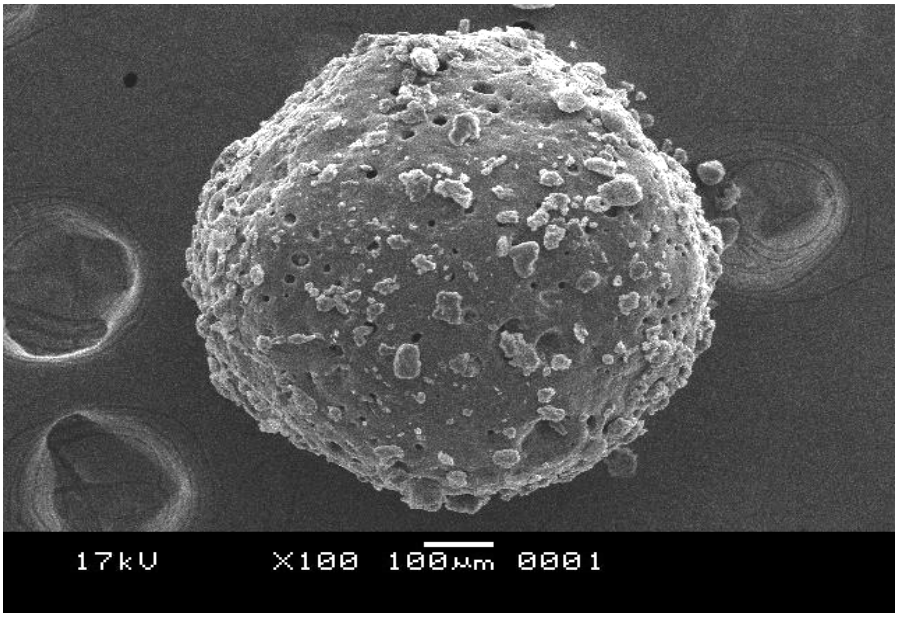
Batch No. and Drug: sodium-alginate: ethyl - cellulose ratio	Entrapment efficiency (%) (mean, n=3)
B06(1:2:24)	77.23
B08(1:1:24)	62.96
B11(1:3:15)	59.33
B12(1:5:12)	73.79

12.1.3.3. Scanning Electron Microscopy (SEM):

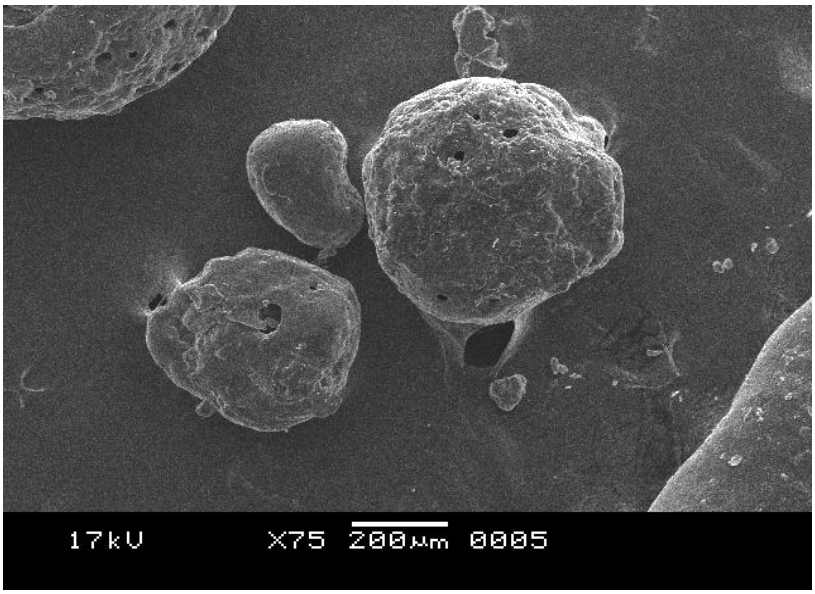
The scanning electron microscopy (SEM) of drug loaded microsphere of sodium-alginate and ethyl-cellulose reveals that the microspheres possess spherical, smooth surface, non-aggregated and porous in nature(Fig 11 –a, b, c). By SEM study it has been seen that the size of the optimized Diacerein loaded microspheres was 100 to 300 µm. The particle size of the microsphere increases with decrease in revolution speed and duration. More porosity on the surface of the formulation, results from low concentration of polymer which followed by the low entrapment of drug and lower release profile. It happens may be due to the solvent penetration through more pores, resulting the swelling of the internal matrix which release the drug either by burst release or through diffusion. So this may be the reason for fast release of drug during dissolution.

Fig 11: SEM photograph (a, b, c) of different batches of Diacerein loaded microsphere

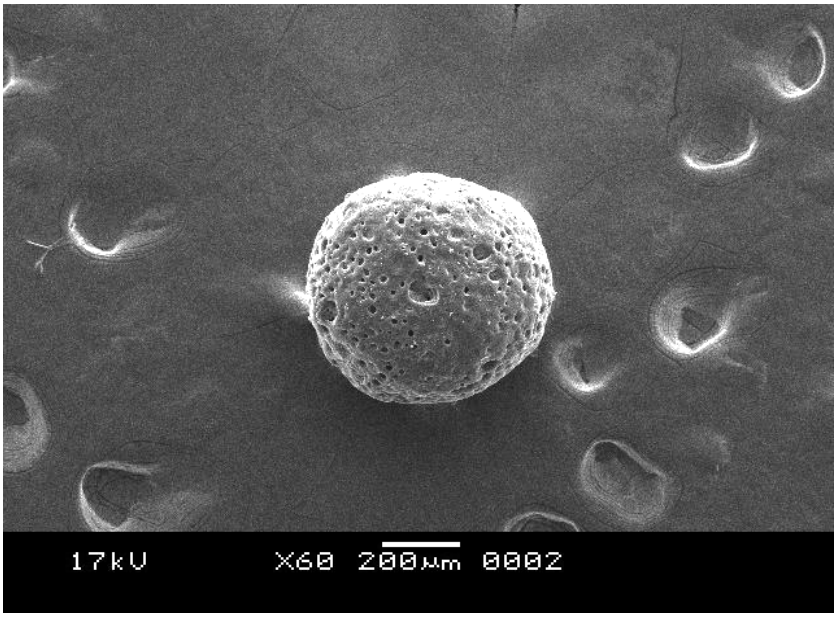
(a)



(b)



(c)



12.1.3.4. In-Vitro Drug Release Study:

In-vitro drug release studies were performed for all the formulations by dissolution study employing USP type II (paddle)dissolution apparatus in change pH (first 2h in pH 1.2 and rest of the 10h in pH 6.8) dissolution medium for 12 hr. The samples withdrawn samples were analyzed by using UV spectrophotometer. For the different formulations, it has been observed that after 2hthe drug releases initially through burst effect in pH 6.8which was followed by a slow and continuous manner. As it is sustained release dosage form which is one type of controlled release dosage form, an excipient should be used to retard or controlled the release of drug. For that reason a polymer should be used. A polymer's ability to retard the drug release rate is related to its viscosity. Higher the viscosity rate of alginate slowed down the drug release rate. The results showed that sodium alginate matrices can sustain drug release for 12 hr. Release details of some formulations which gave the satisfactory results in comparison to others are given below.

Table 7: Percentage of drug release and AUC of formulation B06

Time(hr)	% release, (Mean, n=3)	AUC
0	0	0
0.5	0.64176	9.6264
1	2.09485	31.42275
2	3.50529	105.1587
3	8.503703	255.11109

4	10.737505	322.12515
5	18.5468	556.404
6	26.718988	801.56964
7	31.71492	951.4476
8	46.43663	1393.0989
9	50.18968	1505.6904
10	53.11126	1593.3378
11	56.36097	1690.8291
12	56.7904	1703.712

Fig 12: Release curve of formulation B06in change pH

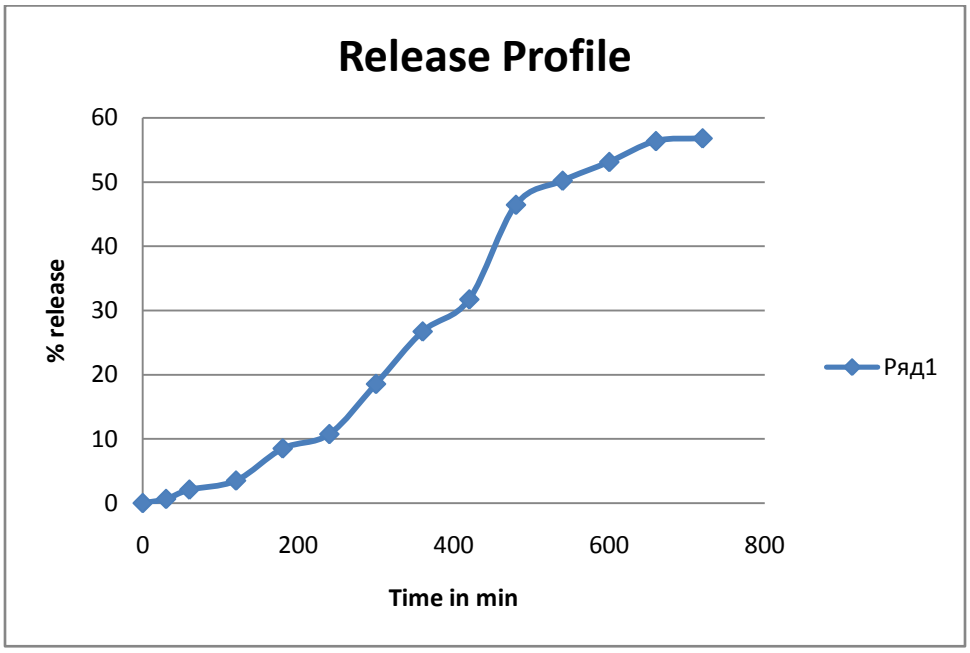


Table 8: Percentage of drug release and AUC of formulation B08

Time(hr)	% release, (Mean, n=3)	AUC
0	0	0
0.5	0.2688	4.032
1	1.8857	28.2855
2	3.0012	90.036
3	6.9973	209.919
4	9.8769	296.307
5	20.3254	609.762
6	26.019925	780.59775
7	30.23897	907.1691
8	48.00132	1440.0396
9	51.3987	1541.961
10	53.0967	1592.901
11	55.30087	1659.0261
12	56.2364	1687.092

Fig 13: Release curve of formulation B08 in change pH

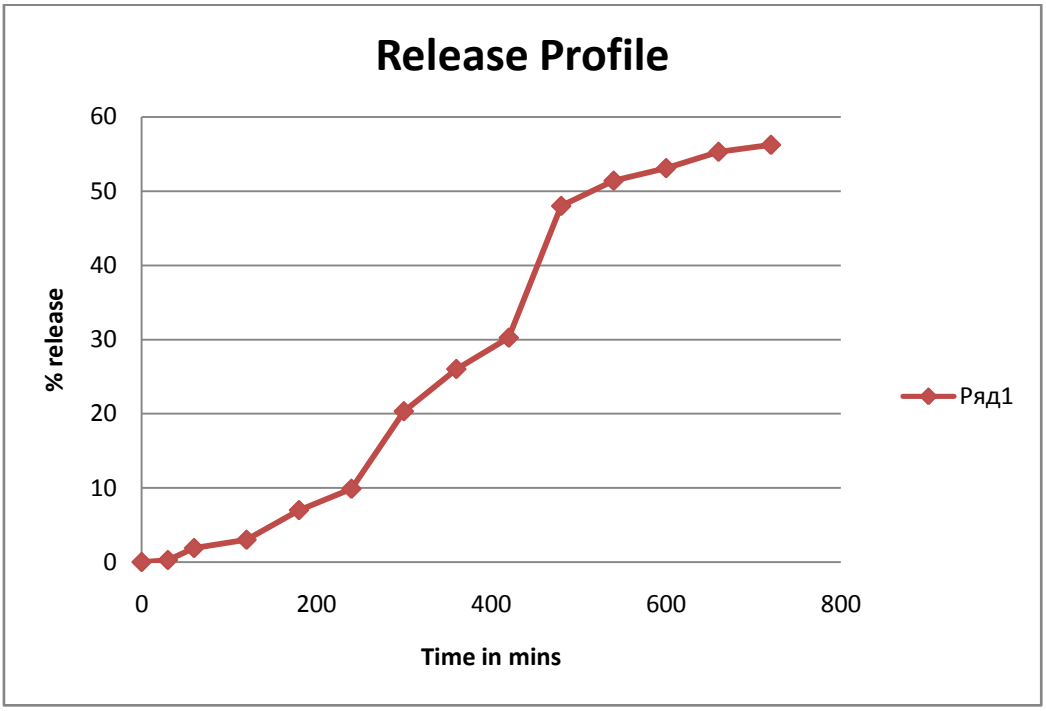


Table 9: Percentage of drug release and AUCof formulation B11

Time(hr)	% release, (Mean, n=3)	AUC
0	0	0
0.5	1.0023	15.0345
1	2.10508	31.5762
2	2.99895	89.9685
3	6.2547	187.641
4	10.8976	326.928
5	15.8092	474.276
6	22.547	676.41

7	25.8879	776.637
8	36.2654	1087.962
9	44.08867	1322.6601
10	49.2295	1476.885
11	52.8688	1586.064
12	53.6502	1609.506

Fig 14: Release curve of formulation B11 in change pH

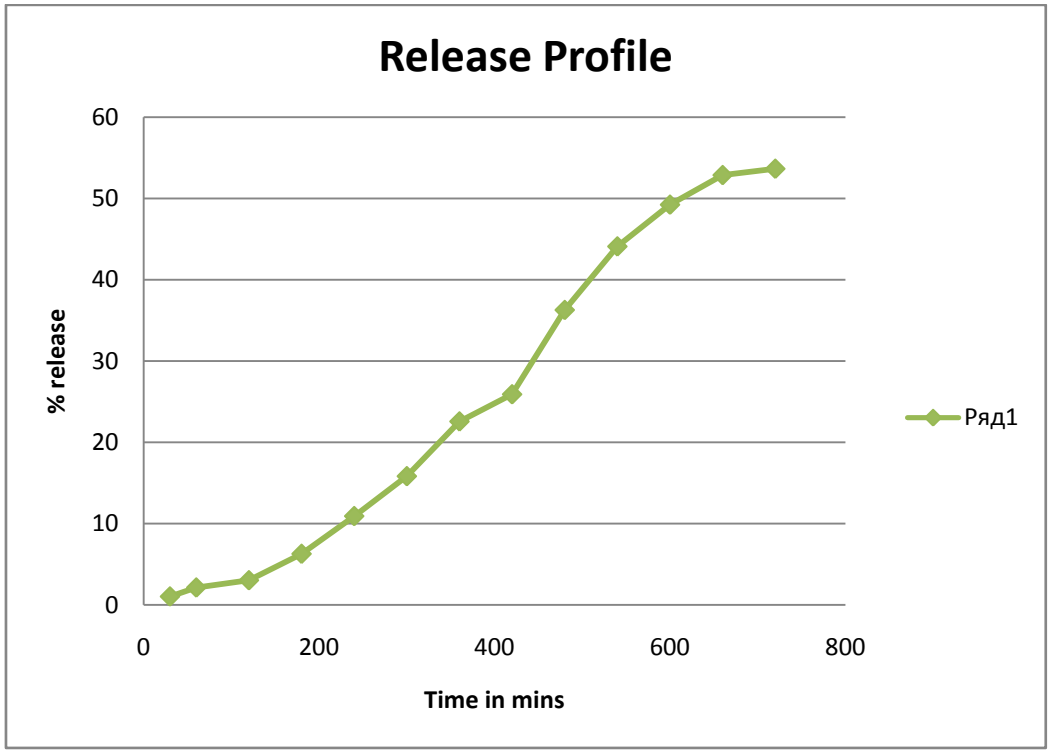


Table 10: Percentage of drug release and AUC of formulation B12

Time(hr)	% release, (Mean, n=3)	AUC
0	0	0
0.5	0.8978	13.467
1	1.9095	28.6425
2	4.08022	122.4066
3	8.0099	240.297
4	14.08022	422.4066
5	21.9942	659.826
6	28.03325	840.9975
7	33.8927	1016.781
8	45.80732	1374.2196
9	48.90987	1467.2961
10	51.29867	1538.9601
11	52.30077	1569.0231
12	52.90394	1587.1182

Fig 15: Release curve of formulation B12 in change pH

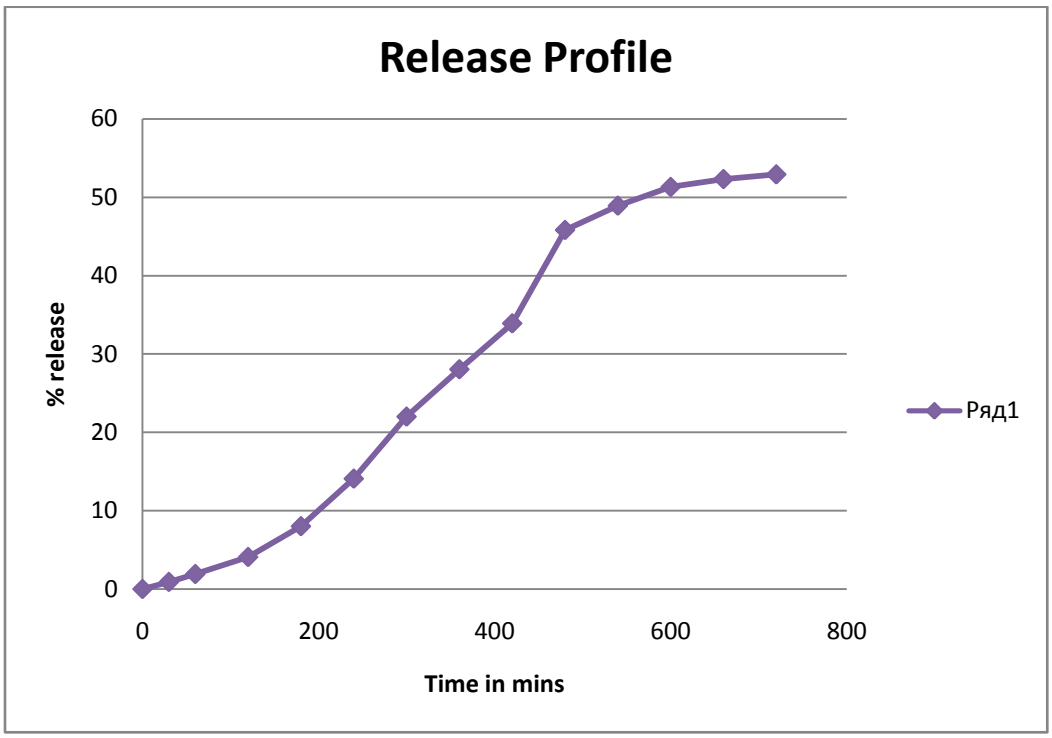
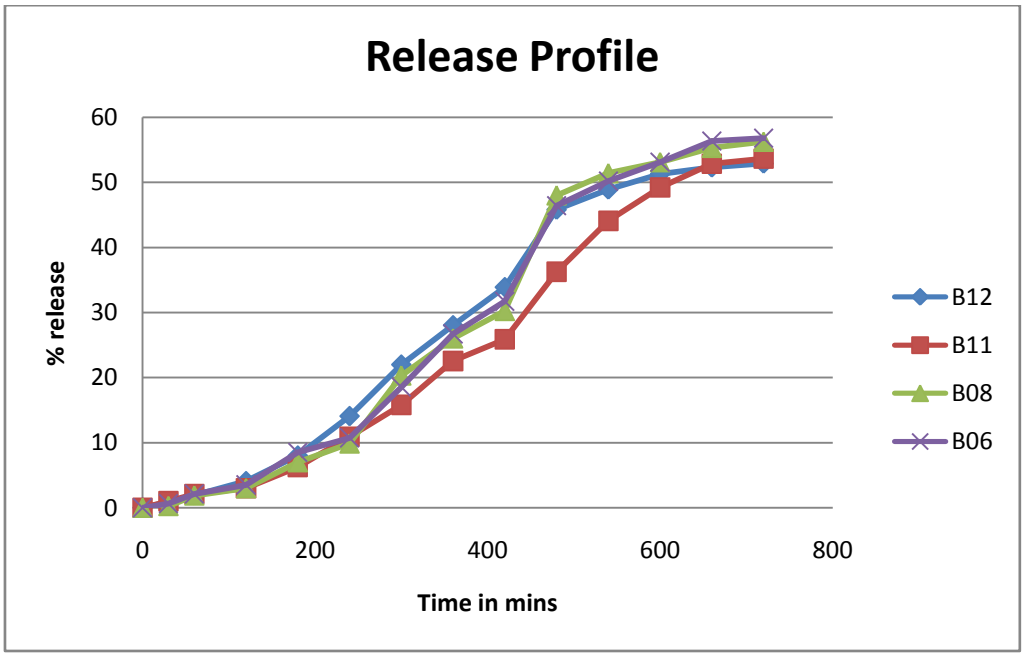


Fig 16: Comparison of Release curve of different formulation in change pH



The release of drug in pH 1.2 is very low for first 2h of about 5% to maintain initial burst effect and controlled the rate of release at pH 6.8 for rest of the hours. This contributed to get the desired effect which ensures initial therapeutic plasma concentrations of drug. The drug release was controlled with high viscosity of the polymer as described above. It is understood that higher polymer concentration results in a longer diffusion path length, so drug release is extended.

12.2. RESULTS OF IN-VIVO STUDY

12.2.1. Toxicity study

The drug Diacerein showed no toxicity at the dose of 2000mg/Kg body weight and also no lethality at higher dose .So the drug Diacerein is a safe drug.

12.2.2. Anti-inflammatory study

The anti-inflammatory activity of the Diacerein was measured at a dose of 25 and 50 mg/kg body weight of prepared formulation against acute paw edema induced by carrageenan. An inhibition of the paw edema was observed with the different doses of the Diacerein and with Diclofenac Sodium (standard drug) at a dose of 40mg/kg body weight. The two doses tested (25 and 50 mg/kg) produced significant ($p<0.05$) anti-inflammatory activity and reduced the paw volume by 58.26% and 59.055%respectively of B08 and 59.05% and 59.84% of B12, whereas Diclofenac Sodium caused 50.35% reduction when used as a standard drug. Result of anti-inflammatory activity is written on table-11 & 12and plotted in figure-17&18.

Table 11: Anti-inflammatory effect of Diacerein formulation B08 against carrageenan induced paw edema in rats

(a)Table of volume inhibition

Treatment	Dose mg/kg	Initial paw thickness	Paw thickness after 1 h	Paw thickness after 2 h	Paw thickness after 3 h	Paw thickness after 4 h	Paw thickness after 5 h	Paw thickness after 6 h
Control(CMC)	1ml (1%)	0.67 ±0.1	1.06 ±0.1	1.35 ±0.1	1.75 ±0.1	1.71 ±0.1	1.59 ±0.1	1.03 ±0.3
Diclofenac. Sod	50mg/kg	0.63 ±0.2	0.75 ±0.3	0.82 ±0.2	0.87 ±0.2	1.18 ±0.2	1.16 ±0.2	0.89 ±0.2
Diacerein formulation	25mg/kg	0.64158 ±0.2	0.87 ±0.2	1.02 ±0.4	1.07 ±0.1	0.84 ±0.3	0.65 ±0.1	0.66 ±0.5
Diacerein formulation	50mg/kg	0.65416 ±0.1	0.89 ±0.4	0.93 ±0.1	0.99 ±0.3	0.87 ±0.1	0.67 ±0.3	0.68 ±0.1

(b)Table of % of inhibition

Treatment	Dose mg/kg	Initial paw thickness	% of inhibition after 1 h	% of inhibition after 2 h	% of inhibition after 3 h	% of inhibition after 4 h	% of inhibition after 5 h	% of inhibition after 6 h
Control(CMC)	1ml (1%)	0.66 ±0.1	-----	-----	-----	-----	-----	-----
Diclofenac. Sod	50mg/kg	0.63 ±0.1	28.57	39.252	50.36	30.88	27.56	13.41
Diacerein formulation	25mg/kg	0.64 ±0.2	15.48	24.3	38.85	49.26	58.27	35.37
Diacerein formulation	50mg/kg	0.65 ±0.1	17.86	30.84	43.17	50.74	59.06	36.59

*P < 0.05 when all treated groups are compared with CMC group and the value indicate paw volume in rats.

Values are expressed as mean ±SEM for six independent observations (n=6). Statistical difference determined by ANOVA followed by Dunnett's test.

Fig 17: Changes of % of inhibition of paw thickness in consecutive hours by bar graph

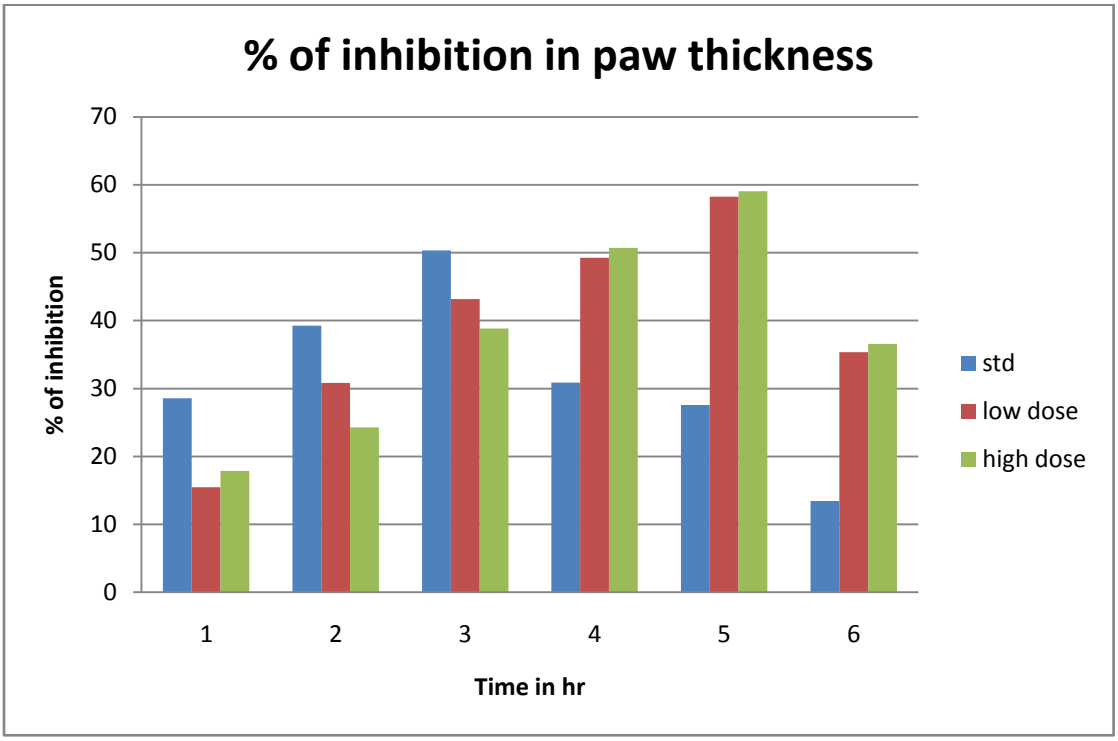


Table 12: Anti-inflammatory effect of Diacereinformulation B12 against carrageenan induced paw edema in rats

(a)Table of volume inhibition

Treatment	Dose mg/kg	Initial paw thickness	Paw thickness after 1 h	Paw thickness after 2 h	Paw thickness after 3 h	Paw thickness after 4 h	Paw thickness after 5 h	Paw thickness after 6 h
Control(CMC)	1ml (1%)	0.67 ±0.1	1.06 ±0.1	1.35 ±0.1	1.75 ±0.1	1.71 ±0.1	1.6 ±0.1	1.03 ±0.3

Diclofenac. Sod	50mg/kg	0.63 ±0.2	0.78 ±0.3	0.8 ±0.2	0.89 ±0.2	1.19 ±0.2	1.15 ±0.2	0.9 ±0.2
Diacerein formulation	25mg/kg	0.64 ±0.2	0.84 ±0.2	1.0 ±0.4	1.05 ±0.1	0.82 ±0.3	0.63 ±0.1	0.68 ±0.5
Diacerein formulation	50mg/kg	0.65 ±0.1	0.88 ±0.2	0.94 ±0.3	0.97 ±0.4	0.85 ±0.1	0.69 ±0.3	0.65 ±0.1

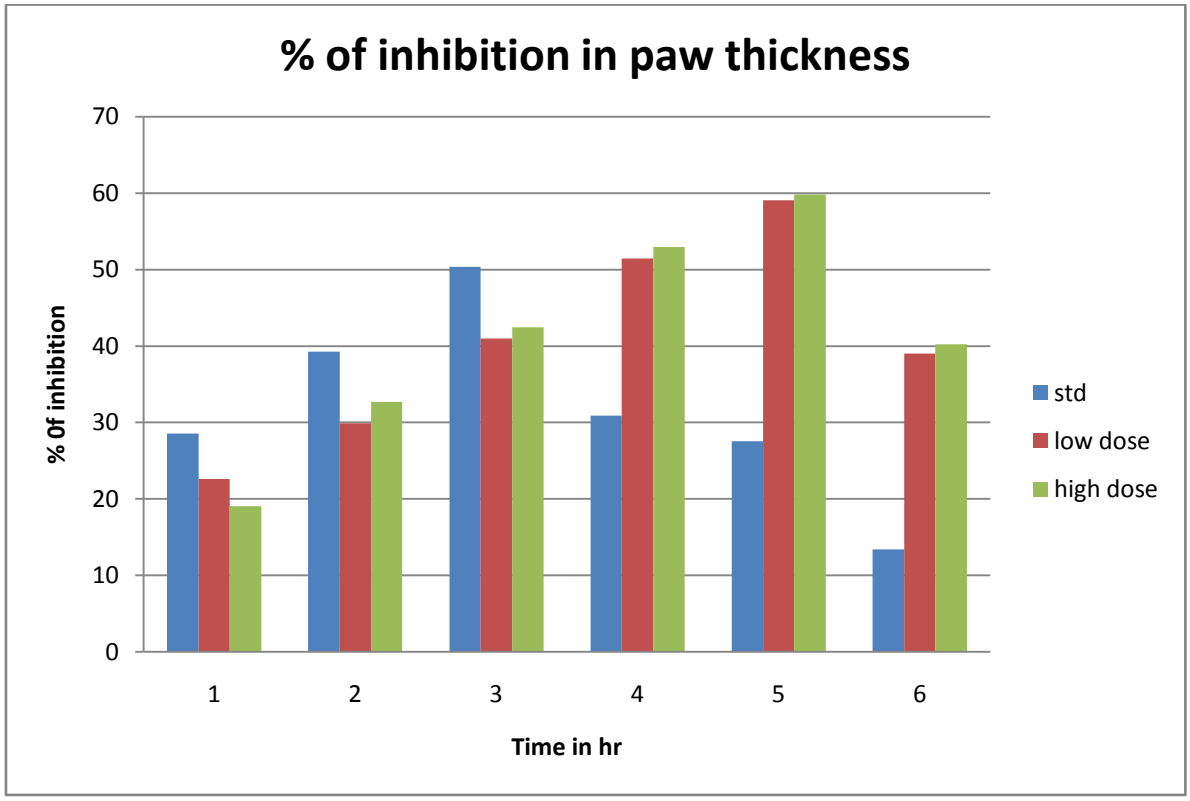
(b)Table of % of inhibition

Treatment	**Dose mg/kg**	**Initial paw thickness**	**% of inhibition after 1 h**	**% of inhibition after 2 h**	**% of inhibition after 3 h**	**% of inhibition after 4 h**	**% of inhibition after 5 h**	**% of inhibition after 6 h**
Control(CMC)	1ml (1%)	0.67 ±0.1	-----	-----	-----	-----	-----	-----
Diclofenac. Sod	50mg/kg	0.63 ±0.1	28.57	39.25	50.36	30.88	27.56	13.41
Diacerein formulation	25mg/kg	0.62 ±0.2	22.62	29.91	41.01	51.47	59.06	39.02
Diacerein formulation	50mg/kg	0.60 ±0.1	19.05	32.71	42.45	52.94	59.84	40.24

*P < 0.05 when all treated groups are compared with CMC group and the value indicate paw volume in rats.

Values are expressed as mean ±SEM for six independent observations (n=6). Statistical difference determined by ANOVA followed by Dunnett's test.

Fig 18: Changes of % of inhibition of paw thickness in consecutive hours by bar graph



CHAPTER 13

CONCLUSION & FUTURE SCOPE

CONCLUSION AND FUTURE SCOPE

Diacerein is a popular marketed drug, used for mainly osteoarthritis. In market it is already established as conventional dosage form but no such controlled release dosage form is available. Arthritis is a chronic pain, which require at least three dose/day to manage the severity of pain because the $t_{1/2}$of the maximum drug is maximum up to 3-4 hrs. This condition is not demandable for patient health. In general, people already avoid taking medicine frequently which creates a gap between the dosage regimens.But in case of this type of chronic pain, if people forget to take require dose then it is harmful for them.To avoid these types of consequences controlled released dosage forms are preferable.

On the other hand, conventional dosage forms are present in the systemic circulation in large amount due to sudden burst in the system, which cause damaging of mucous when it is used for the treatment of peripheral organ. But in case of controlled release dosage form, the drug release from the dosage slowly and in controlled manner, which cause no damage of mucous membrane. At the same time, Diacerein has not reported any of side effects on GIT like other NSAIDs, as it is not under NSAIDs. So by preparing Diacerein loaded microsphere, we want to overcome the above said problem as it is a controlled release dosage form.

The result of FTIR study proves that microsphere of drug Diacerein can be prepared by using sodium alginate and ethyl cellulose as they have no interference with each other. Results of percentage yield and entrapment efficiency of formulations prove that Diacerein can entrap into

the microsphere. Result of SEM study shows that Diacerin loaded microsphere are spherical, smooth surfaced and porous in nature which is demandable. In-vitro drug release profile supports that Diacerein release from microsphere can be controlled by following the mentioned formula. Finally all the research work is also strengthen by the satisfactory result of in-vivo study.

Therefore it can be concluded from above study and their result that Diacerein can be formulated as controlled release dosage form. By changing the polymer or gum or the ratio of any of them better release can be achieved and in future it can be marketed for better patient compliance.

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