FORMULATION AND EVALUATION OF CALCIUM DOBESILATE MICROSPHERES USING VARIOUS POLYMERS

A Dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI – 600 032

In partial fulfilment of the requirements for the degree of

MASTER OF PHARMACY

IN

BRANCH-I -> PHARMACEUTICS

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October 2017

Chennai

14.09.2017

CERTIFICATE

This is to certify that the dissertation **entitled "FORMULATION AND EVALUATION OF CALCIUM DOBESILATE MICROSPHERES USING VARIOUS POLYMERS"** submitted by **P.K.YUVARAJ (REGISTER NO:261411051)** in partial fulfilment of Degree of Master of Pharmacy in Pharmaceutics of the TamilNadu Dr.M.G.R Medical University, Chennai at Annai Veilankanni's Pharmacy College, Chennai 600 015 is the bonafide work carried out by him under my guidance and supervision during the academic year **2016-2017**. The dissertation or any part of this as not been submitted elsewhere for any other degree.

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DECLARATION

I hereby declare that the dissertation work **entitled "FORMULATION AND EVALUATION OF CALCIUM DOBESILATE MICROSPHERES USING VARIOUS POLYMERS"** is based on the original work carried out by me in Annai Veilankanni's Pharmacy College, Chennai under the guidance of **Dr.M.Senthil Kumar, Principal, and The Head, Department of Pharmaceutics** for submission to The Tamilnadu Dr.M.G.R Medical University in the partial fulfilment of the requirement for the award of Degree of Master of Pharmacy in Pharmaceutics. The work is Original and has not been submitted in part or full for any other Diploma or Degree of this or any other University. The information furnished in this dissertation is genuine to the best of my knowledge and belief.

Chennai

Date: 14.09.2017 261411051

ACKNOWLEDGEMENT

At the outset, I thank the God who brought this opportunity, gave me the abundance of requisite determination and strength to pursue and complete this course and dissertation successfully. It is my immense pleasure and privileges to acknowledge the untold contributions, thankfully received, the blessed inspiration and the unreserved support I have had from the individual and institutional sources with whom I have been in association during the course of my last two years of pursuit I hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

I am extremely grateful to **Dr.S.Devaraj, Chairman** and **Dr. D.Devanand, Secretary Annai Veilankanni's Pharmacy College, Saidapet, Chennai – 600 015** for providing me the opportunity to do my project.

It's a fact that every mission needs a spirit of hard work and dedication but it needs to be put on the right path to meet its destination and in my case this credit goes to my respected teacher and guide, **Dr.M.SenthilKumar, Principal, and The Head Department of Pharmaceutics, Annai Veilankanni's Pharmacy College**. I am very much thankful to him for his inspiration, kind co-operation, caring attitude, timely help, valuable guidance and constant encouragement during every phase of this dissertation. His patience way of sharing knowledge, numerous discussions and support has always propelled and boosted me to perform better.

My sincere and heartful thanks to my guide my teachers **Mrs.S.Valarmathi** and **Mr.R.Sathish** for this help and co-operation.

I would like to extend my sincere thanks to the **entire staffs of the Annai Veilankanni's Pharmacy College,** Saidapet, Chennai.

I would also like to thank my friends Vinoth, Mohan, Jabastein, Nagarajan, Rahul for their co-operation and help in carrying out my project work.

I thank everyone who helped me directly or indirectly in the successful completion of this dissertation.

At last but not least my heartiest and dearest gratitude to my lovable friends for their love, faith, care and support.

I would like to express my deep sense of love and affection to my beloved family members especially to My father **Mr.P.D.Kesavan** and My mother **Late Mrs. K.Pushpalatha** for their strong piety and pantheism enable me to face the world without fear and with pedantic strength.

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LIST OF ABBREVATIONS

HPMCK 100	Hydroxy Propyl Methyl Cellulose K 100
EC	Ethyl Cellulose
Min	Minute
DC	Diffusion Coefficient
Nm	Nanometer
Rpm	Revoluation per minute
SA	Sodium Alginate
CR	Controlled Release
BP	British Pharmacopoeia
UV	Ultra Violet
w/v	Weight by Volume
µg	Microgram
%	Percentage
FTIR	Fourier Transformed Infrared Spectroscopy

1.INTRODUCTION

CHRONIC VENOUS DISEASE [1,2,3,4,5]:-

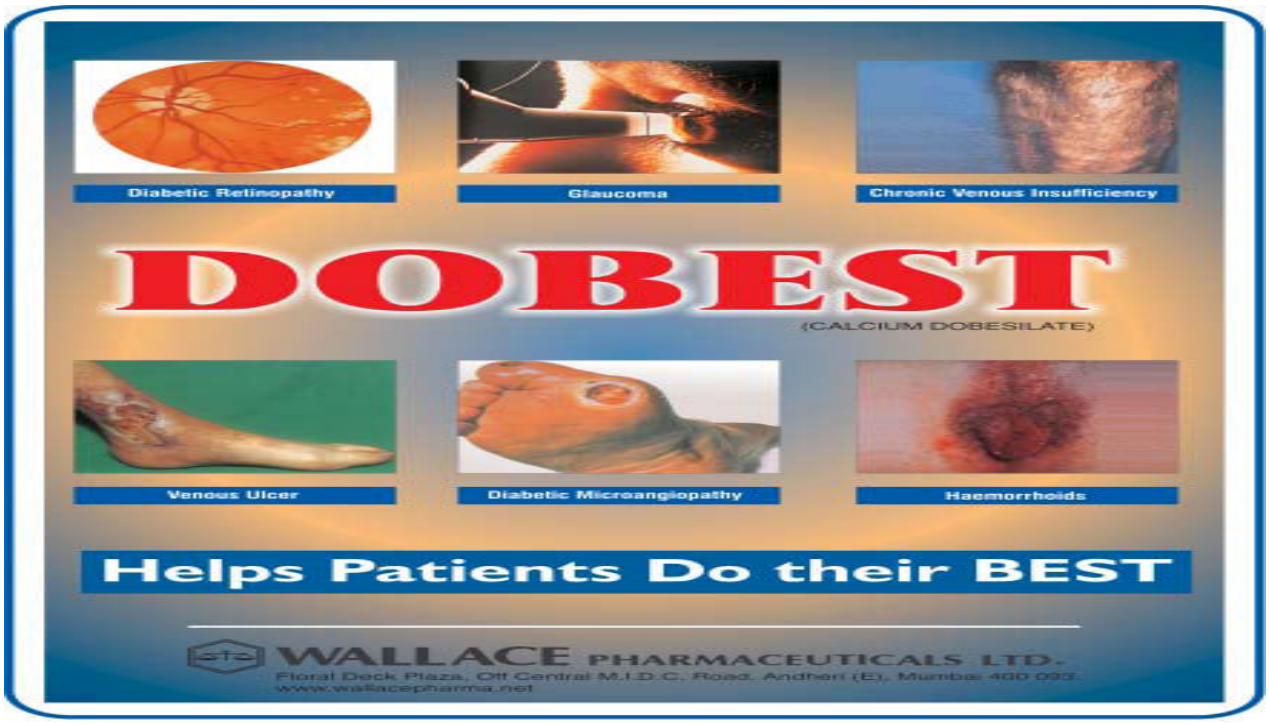
Chronic venous insufficiency (CVI) is a condition that occurs when the venous wall and/or valves in the leg veins are not working effectively, making it difficult for blood to return to the heart from the legs. CVI causes blood to "pool" or collect in these veins, and this pooling is called stasis.

Causes Chronic Venous Insufficiency:

- Veins return blood to the heart from all the body's organs. To reach the heart, the blood needs to flow upward from the veins in the legs. Calf muscles and the muscles in the feet need to contract with each step to squeeze the veins and push the blood upward. To keep the blood flowing up, and not back down, the veins contain one-way valves.
- Chronic venous insufficiency occurs when these valves become damaged, allowing the blood to leak backward. Valve damage may occur as the result of aging, extended sitting or standing or a combination of aging and reduced mobility. When the veins and valves are weakened to the point where it is difficult for the blood to flow up to the heart, blood pressure in the veins stays elevated for long periods of time, leading to CVI.
- CVI most commonly occurs as the result of a blood clot in the deep veins of the legs, a disease known as deep vein thrombosis (DVT). CVI also results from pelvic tumors and vascular malformations, and sometimes occurs for unknown reasons. Failure of the valves in leg veins to hold blood against gravity leads to sluggish movement of blood out of the veins, resulting in swollen legs.

- Chronic venous insufficiency that develops as a result of DVT is also known as post-thrombotic syndrome. As many as 30 percent of people with DVT will develop this problem within 10 years after diagnosis.

Fig. No.1: Symptoms of Chronic Venous Insufficiency[6,7]:



Symptoms of Chronic Venous Insufficiency[8,9,10]:

- The seriousness of CVI, along with the complexities of treatment, increase as the disease progresses. The problem will not go, and the earlier it is diagnosed and treated, the better chances of preventing serious complications.

Symptoms include:

- Swelling in the lower legs and ankles, especially after extended periods of standing

- Aching or tiredness in the legs
- New varicose veins
- Leathery-looking skin on the legs
- Flaking or itching skin on the legs or feet
- Stasis ulcers (or venous stasis ulcers)
- If CVI is not treated, the pressure and swelling increase until the tiniest blood vessels in the legs (capillaries) burst.

  When this happens, the verlying skin takes on a reddish-brown color and is very sensitive to being broken if bumped or scratched.
- At the least, burst capillaries can cause local tissue inflammation and internal tissue damage. At worst, this leads to ulcers, open sores on the skin surface. These venous stasis ulcers can be difficult to heal and can become infected. When the infection is not controlled, it can spread to surrounding tissue, a condition known as cellulitis.
- CVI is often associated with varicose veins, which are twisted, enlarged veins close to the surface of the skin. They can occur almost anywhere, but most commonly occur in the legs.

Risk Factors For Chronic Venous Insufficiency[11.12]:

- Deep vein thrombosis (DVT)
- Varicose veins or a family history of varicose veins
- Obesity
- Pregnancy
- Inactivity
- Smoking

- Extended periods of standing or sitting
- Female sex
- Age over 50

CVI Diagnoses[13,14]:

- To diagnose CVI, a complete medical history and physical examine legs.
- A test called a vascular or duplex ultrasound may be used to examine the blood circulation in legs. During the vascular ultrasound, a transducer (small hand-held device) is placed on the skin over the vein to be examined. The transducer emits sound waves that bounce off the vein. These sound waves are recorded, and an image of the vessel is created and displayed on a monitor.

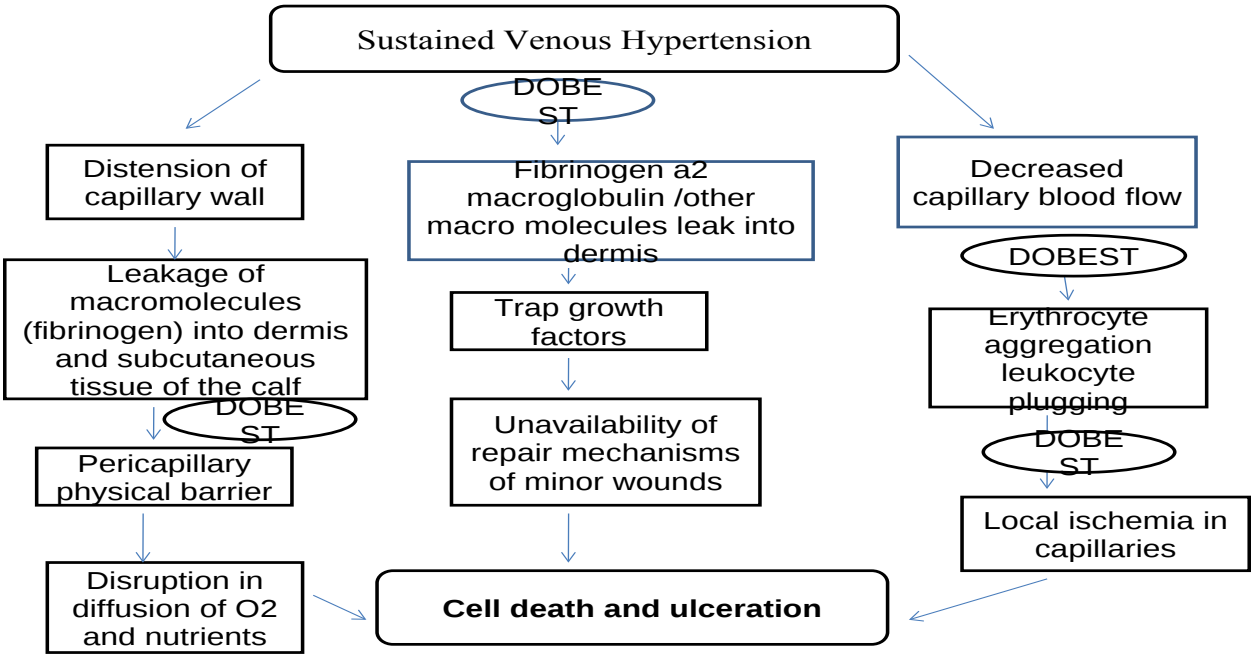
VARICOSE VEINS, VENOUS ULCERS, VARICOSE ULCERS – PROMISING ROLE OF CALCIUM DOBESILATE[15,16,17,18,19,20]:

Varicose veins and their accompanying secondary complications, namely venous ulcers and stasis dermatitis are associated with chronic morbidity, economic loss and reduction in the patient's quality of life. Venous ulcers constitute the majority of leg ulcers, accounting for up to 80%8. The overall prevalence of venous ulcers in the general population is in the range of 2%. Stasis dermatitis commonly accompanies venous ulcers and is severely disabling to treat.

Venous ulcers incur substantial costs.

Calcium Dobesilate is very effective in the conditions of chronic venous insufficiency (CVI), otherwise known as "heavy leg syndrome" or venous varicosities. It acts at various levels of the disease process as shown

Fig. No.2: Pathogenesis of chronic venous disease:-



Calcium Dobesilate has a comprehensive mode of action. It increases endothelial nitric oxide levels by enhancing the activity of nitric oxide synthase and decreasing capillary hyper permeability. Calcium Dobesilate shows anti-platelet and fibrinolytic activities by inhibiting platelet activation factor (PAF) and enhancing the release of tissue plasminogen activator (tPA), thereby improving the local blood flow to tissues, otherwise inhibited due to thrombosis. Calcium Dobesilate also inhibits the two pathophysiological reactions in diabetes, viz. polyol pathway and glycation of proteins, due to its inhibitory effects on aldose reductase. Calcium Dobesilate acts on the endothelial layer and basement membrane of the capillaries. It reduces histamine and bradykinin-induced hyperpereability. It increases red blood cell membrane flexibility and reduces capillary fragility. Calcium Dobesilate can reduce the platelet aggregation stimulated by collagen and thrombin, but not by arachidonic acid. Calcium

Dobesilate may also inhibit the formation of sorbitol, thus providing another possible mechanism for its usefulness in diabetic retinopathy. Glucose inhibits the formation of both type I and type II collagen formation. Calcium Dobesilate does not affect type I inhibition by glucose but accelerates type II collagen

fibrillogenesis, a major structural component of the arterial wall. Calcium Dobesilate has angioprotective action by reducing the permeability and fragility of microvessels, which should restrict fluid extravasation into the cardiac interstitium. Itsantiplatelet effect counteracts thrombosis and its reduction of plasma viscosity preventsstasis.

Introduction to Targeted Drug Delivery System[44,45,46]:

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and then to maintain the desired drug concentration. Conventional drug delivery system achieves as well as maintains the drug concentration within the therapeutically effective range needed for treatment only when taken several times a day. This results in a significant fluctuation in drug level. The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlich, who proposed drug delivery to be as a "magic bullet".

Controlled and Novel delivery envisages optimized drug in the sense that the therapeutic efficacy of a drug is optimized, which also implies nil or minimum side effects. It is expected that the 21st century would witness great changes in the area of drug delivery. The products may be more potent as well as safer. Target specific dosage delivery is likely to overcome much of the criticism of conventional dosage forms. The cumulative outcome could be

summarized as optimized drug delivery that encompasses greater potency & greater effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance.

Sustained Release (SR):

It indicates an initial release of drug sufficient to provide a therapeutic dose soon after administration and then a gradual release over an extended period of time. It is substantially affected by the external environment.

Advantages

- Decreased local and systemic side effects.
- Better drug utilization.
- Improved efficiency in the treatment.
- Improved patient compliance.
- Increased margin of safety of high potency drugs.

Disadvantages:

- Possibility of dose dumping.
- Poor *in vitro-in vivo* correlation.
- Adjustment of dosage regimen is difficult to the physician.
- Retrieval of drug is difficult in case of toxicity, poisoning and hypersensitivity.

Prolonged Release [33,34]:

Provide the slow release of a drug at a rate, which will provide longer duration of action than its single dose in a conventional dosage form.

Controlled Release (CR):

Delivers the drug at constant predetermined rate locally (or) systemically for a specified period of time and independent of external environment, controlled by the design of the system itself.

INTRODUCTION OF MICROSPHERES[63,64]:

There is growing interest in the development of homogenous monolithic drug release systems for various routes of administration. One very attractive type of such dosage from is micro spheres.

- Flexibility in design and development.
- Attractive in appearance.
- Better, improve the safety and efficiency of bio-active agents.
- Desired release pattern can be engineered**.**

WHAT IS A MICROSPHERE?

"Microspheres are defined as solid spherical particles containing dispersed drug in either solution or micro-crystalline form". "A plastic compound used in some dermal fillers for the correction of wrinkles that are filled with a substance and released as the shell disintegrates", "Small, hollow glass spheres used as fillers n epoxy and polyester compounds to reduce density"

ADVANTAGE OF MICROSPHERE

- Controlled release delivery Biodegradable microspheres are used to control drug release rates thereby decreasing toxic side effects, and eliminating the inconvenience of repeated injections.
- Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.
- PLGA copolymer is one of the synthetic biodegradable and biocompatible polymers that have reproducible and slow-release characteristics in vivo.

Types Of Microspheres :

1.Fluorescent Microspheres

Fluorescent Microspheres are round spherical particles that emit bright colors when illuminated by UV light. Ability to emit intense color under UV (black light) illumination provides contrast and visibility of Microspheres relative to background materials. For example, fluorescent micro beads are often used as traces to simulate spread of viruses in medical research.

Fluorescent spheres have a unique ability to appear translucent (clear) and practically invisible under ordinary light, and emit intense visible color when energized by ultraviolet (UV) light. This effect allows scientists and engineers to design blind tests and controlled experiments (e.g. simulate spread of viruses) This unique feature of fluorescent Microspheres has numerous applications in biomedical research and process troubleshooting.

2.Glass Microsphere

Glass Microspheres are microscopic spheres of glass manufactured for a wide variety of uses in research, medicine, consumer goods and various industries. Glass Microspheres are usually between 1 to 1000 micrometers in diameter, although the sizes can range from 100 nanometers to 5 millimeters in diameter. Hollow spheres are used as a light weight filler in composite materials such as syntactic foam and concrete. Hollow spheres also have uses ranging from storage and slow release of pharmaceuticals and radioactive tracers to research in controlled storage and release of hydrogen.

3.Paramagnetic Microsphere

They have the ability to increase in magnetization with an applied magnetic field and lose their magnetism when the field is removed. Neither hysteretic nor . This property allows efficient washing steps, low background and good reproducibility.One use of paramagnetic Microspheres as large as 1mm in diameter to simulate salmon eggs, Scientists are able to place them in a natural habitat, observe how they move with the water currents and then use their magnetic properties to clean them up.

Different Form Of Polymeric Microspheres

Albumin Microspheres:-

The albumin is a widely distributed natural protein. The particulate or the colloidal form of albumin is considered as the potential carrier of drug for either there sites specific localization or their local application into anatomical discrete sites. Albumin microspheres loaded with anticancer drug such as misogynic-C were found to be more effective than the drug alone. Burger et al., 1985 observed that cisplatin- loaded microspheres are 10 times more potent in targeting the drug to the patient with hyper vascular liver carcinoma.

Gelatin Microspheres:-

It is a biodegradable polymer obtained from the partial hydrolysis of the collagen derived from the skin, connective tissue & bones of animals. The acid treated collagen is called type-A & the alkali treated is referred as type B.Gelatin microspheres.

The gelatin microspheres being susceptible for the macrophages recognition can be used as carrier for the antigens. The antigens from microspheres are released within the macrophages upon their degradation leading to enhanced production of antigen specific antibody.

Material used in the preparation of microspheres :-

1. Synthetic polymer
2. Non –biodegradable.
3. PMMA
4. Acrolein
5. Glycidyl methacrylate
6. Epoxy polymers

Biodegradable :-

Lactides and glycosides and these copolymers Poly-alkyl cyano acrylates, poly-anhydrides.

Natural materials:-

- Protein
- Gelatin
- Collagen

- Carbohydrates
- Agarose
- Carrageenan
- Chitosan
- Chemically modified carbohydrates
- DEAE cellulose
- Poly (acryl) dextran
- Poly (acryl) starch

Method Of Preparation

Solvent evaporation/ double emulsion technique:

It is most extensively used method of microencapsulation. A buffered or plain aqueous solution of the drug (may contain a viscosity building or stabilizing agents) is added to an organic phase consisting of the polymer solution in solvents like dichloromethane (or ethyl acetate or chloroform) with vigorous stirring to form the primary water in oil emulsion. This emulsion is then added to a large volume of water containing an emulsifier like PVA or PVP to form the multiple emulsions (w/o/w). The double emulsion, so formed is then subjected to stirring until most of the organic solvent evaporates, leaving solid microspheres. The microspheres can then be washed, centrifuged and lyophilized to obtain the free flowing and dried microspheres.

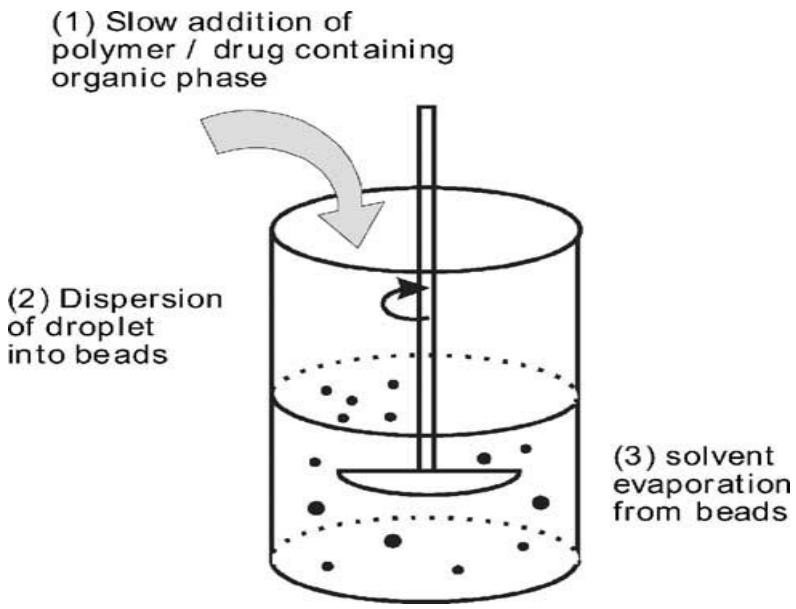


Fig. No.3: Depiction of sphere formation by solvent evaporation

Single emulsion technique:

The micro particulate carriers of natural polymers, i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in the non-aqueous medium e.g., oil. In the second step of preparation, cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using chemical cross linkers. The chemical cross-linking agents used include glutaraldehyde, formaldehyde, etc.

Polymerization techniques:-

The polymerization techniques conventionally used for the preparation of the micro spheres are mainly classified as

Normal polymerization:-

The two processes are carried out in a liquid phase. Normal polymerization proceeds and carried out using different technique as bulk suspension precipitation, emulsion and micelle polymerization process. In bulk polymerization, a monomer or a mixture of monomer along with the initiator is usually heated to initiate the polymerization and carry out the process. One catalyst or the initiator is added to the reaction mixture to facilitate or accelerate the rate of the reaction. The polymer so obtained may be molded or fragmented as micro spheres. For loading of drug, adsorptive drug loading or adding drug during the process of polymerization may be opted. They have the ability to increase in magnetization with an applied magnetic field and lose their magnetism when the field is removed. Neither hysteretic nor . This property allows efficient washing steps, low background and good reproducibility. One use of paramagnetic Microspheres as large as 1mm in diameter to simulate salmon eggs, Scientists are able to place them in a natural habitat, observe how they move with the water currents and then use their magnetic properties to clean them up.

Interfacial polymerization:-

Interfacial polymerization essentially proceeds involving reaction of various monomer at the interface between the two immiscible liquid phased to form a film of polymer that essentially envelops the dispersed phase. In this technique two reacting monomers are employed, one of which is dissolved in the continuous phase while the other being dispersed in the continuous phase. The continuous phase is generally aqueous in nature through which the second monomer

is emulsified. The monomers present in either phase diffuse rapidly and polymerize rapidly at the interface.

Phase separation co-acervation techniques:

Phase separation method is specially designed for preparing the reservoir type of the system i.e. to encapsulate water soluble drugs like peptides and proteins. In this technique the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution.

Spray drying:

In this process, the drug may be dissolved or dispersed in the polymer solution and spray dried. The quality of spray-dried microspheres can be improved by the addition of plasticizers, e.g. citric acid, which promote polymer coalescence on the drug particles and hence promote the formation of spherical and smooth surfaced microspheres.

The size of microspheres can control by the rate of spraying, the feed rate of polymer drug solution, nozzle size, and drying temperature. This method of microencapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible and easy to scale up.

Solvent extraction:

This method involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the microspheres.

Route of Administration:-

Micro spheres can be used for the delivery of drugs via different routes. Route of administration is selected depending on the drug properties, disease state being treated and the condition of the patient. Desirable properties of the microspheres used for the delivery will also change depending on the route of administration.

Oral Delivery:-

Oral delivery is the simplest way of drug administration. In oral drug delivery, the microspheres have to pass through frequently changing environments in the GI tract. There is also patent variation in GI content, so much emptying time and peristaltic activity. Although constrains of the oral route are numbers, on the whole, it less potential danger than the pretrial route.

The relatively brief transit time of about 12 h through the GI tract limits the duration of action that can be expected via the oral route. Recently, it has been reported that microspheres of less than 10 un in size are taken up by the payer's patches and may increase the retention time in the stomach. Eldrige el al. (1990) found that oral administration of poly-lactide co-glycodine microspheres containing staphylococcal enter toxin B is effective in including disseminated muccal Iga antibody response.

Parentral delivery:-

Most of the micro spheres based controlled delivery system are developed was the aim of using them for parental administration. Drug released is completely absorbed in this case. Micro spheres used for parental delivery should be sterile and should be dispersible in a suitable vehicle for injection hydrophilic micro spheres have the potential advantage of aqueous dispensability surfactants in small concentrations are often necessary for reconstituting hydrophobic particles for injection is aqueous vehicles which are reported to case adverse tissue reactions and affect the incorporated drug.

Mechanism of drug release:-

Theoretically, the release of drug from biodegradable micro spheres can he classified into four different categories. But actual, the mechanism is more complex and interplay of different mechanism may operate.

Application of Microspheres

For biodegradable: -

Degradation controlled monolith system:-In degradation controlled monolithic microspheres system, the drug is dissolved in the matrix is in degradation controlled monolithic microspheres system, the dissolved and is released only on degradation of the matrix. The diffusion of the drug is slow compared with the degradation of the matrix. When degradation as by homogeneous bulk mechanism, drug release is show initially and increase rapidly when repaid bulk degradation starts. Drug release from such type of device in independent of the geometry of the device if the degradation is by homogeneous mechanism, degradation is confined to the surface. Hence rate of release is affected by the geometry of the device.

Diffusion controlled monolith system:-

Here the active is released by diffusion prior to or concurrent with the degradation of the polymer matrix. Degeneration of the polymer matrix affects the rate of release and to be taken into account. Rate of release also depends on whether the polymer degrades by homogeneous or heterogeneous mechanism.

Erodable polyagent system:-

In this case the active agent is chemically attach to matrix & the rate of biodegradation of matrix is slow compared to the rate of hydrolysis of drug-polymer bond. Assuming that the rate of diffusion of active agent from the matrix to the surrounding is rapid, the rate limiting step is the rate of cleavage of bond attaching drug to polymer matrix. This type of delivery is obtained in the release of norethindrone-17-chlorofirmate which is then attached to the –OH group of polymer. In vitro studies in rats using labeled drug polymer conjugate showed that a fairly constant release is obtained during the time of observation which was 5 months

Utilization of Microspheres in Body:-

Microparticulate carrier system can be administered through different routes such as i.v, ocular, i.m, oral, intra arterial .etc. Each routes has it's own biological significance, limitation & pharmaceutical feasibility. The micro particles are intended to be administration through different routes to achieve desired activity of either sustained action or targeting or both.

Through different routes different mechanisms of uptake, transport & fate of trans located particles have been proposed. Biodegradable micro particulate carriers are of interest for oral delivery of drugs to improve bioavailability, to enhance drug absorption, to target particular organ 7 to reduce toxicity to improve gastric tolerance of gastric irritant to stomach & as a carrier for antigen. The polystyrene microspheres administered orally are reported to be taken up by Peyer's Patch. They are subsequently trans located to discrete anatomical compartments such as mecentric lymph vessels, lymph nodes & to a lesser extent in liver & spleen.

The particulates matters gain entry into follicle associated epithelium through Peyer`s patches. After the uptake of particulate carriers via different mechanism their fate become more important. Some uptake mechanism avoids the lysosomal system of the enterocytes. The particles following uptake by enterocytes are transported to the mecentric lymph, followed by systemic circulation & subsecuently phagocyosized by the Kupffer cells of liver. However, after uptake by enterocytes, some particulate carriers may be taken up into vacuoles & discharged back into gut lumen. Microsperes can also be designed for the controlled release to the gastrointestinal tract. The release of drug contents depends on the size of micro particles & the drug content within microspheres. The release of the drug could be regulated by selecting an appropriate hydrophilic/lipophilic balance of the matrix such as in case of matrix of polyglycerol, ester of fatty acids.

Micro particles of mucoadhesives polymers get attached to the mucous layer in GIT & hence, prolong the gastric residence time & functionally offer a sustained release. The microspheres of particle size less then 0.87 μm are taken to the general circulation. The fluid environment of the GIT can affect the number & rate of particles translocation.

In enteric release dosage form.

Drugs which are irritant to the stomach & other side effects like aspirin, pancrelipase & erythromycin, salbutamol sulphate can be incorporated in microspheres for their selective release in intestine.

To protect reactive materials against environment.

It is useful for drugs vitamins. Aspirin which are sensitive to oxygen & water.

To mask bitter of unpleasant taste of the drug.

E.g. for drugs such as quinidine, nitrofurantion, paracitamol prednisolone, metronidazole, fish oils, sulpha drugs, clofibrate, alkaloids & salts.

For drug targeting.

E.g. casein & gelatin microspheres containing Adriamycin & iterferons respectively were magnetically delivered to tumour site. Albumin microspheres used for anti-inflammatory agents for directing against knee joints.

As a topical drug delivery system.

E.g. Microspheres of benzoyl peroxide for their bactericiday activity against acne.

As an antidote in the poisoning of heavy metals.

E.g. Polymercaptal microspheres as an antidote against mercury poisoning.

As antigen carrier. E.g: PLGA microspheres of varying composition have used to improve the ability of the antigens to provoke a mucosal immune response.

To reduce gastric irritation.

Hard gelatin capsule containing microspheres liberate in stomach & spread in the overall GIT, thus ensuring more reproducible drug absorption with less local irritation.[8]

2.LITERATURE REVIEW

1. [61]**Hervt Allain *et al,*** Safety of Calcium Dobesilate in Chronic Venous Disease, Diabetic Retinopathy & Haemorrhoids, The aim of the present review is to consider the adverse effects and the safety profile of calcium dobesilate. Calcium dobesilate (DoxiumTM)is a veno-tonic drug, which is widely prescribed in more than **60** countries. Used chronic venous disease , diabetic retinopathy and the symptoms of haemorrhoidal attack. calcium dobesilat did not occur very frequently and had the following distributionin terms of frequency: fever (26%), gastrointestinal disorders (12.5%), skin reactions **(8.2%),** arthralgia (4.3%), and agranulocytosis (4.3%). No deaths were attributed to calcium dobesilate in the PMS report. Most adverse events are type B, i.e. rare and unrelated to the phartnacological properties of calcium dobesilate. This review concludes that the **rjsk of** an adverse effect with calcium dobesilate 500-1500 mg/day is low and constant over time. The recently raised problem of agranulocytosis (a total of 13 known cases drawn from **all** data sources) **appears** to be related to methodological bias. Such a review reinforces the need for a strong international pharmacovigilance organisation using similar methods to detect andanalyse the adverse effects of drugs.

2. [(47)]**Pathak Naresh *et al,*** Formulation & evaluation of floating microspheres of Lansoprazole, the aim of the work is The drug and polymer in different proportions are weighed the polymer was co dissolved into previously cooled mixture of Ethanol **:** Dichloromethane at room temperature. The mixture was stir vigorously to form uniform drug polymer dispersion. The above organic phase was slowly added to100 ml of distilled water & 0.1 HCL containing 0.01% Span 80 by maintain the temperature at 20**°C** to 30 **°C** and emulsified by stirring at 1200 rpm for 30 min . The formed Microspheres were filtered & washed with water and sieved between 30-50 mesh size, and dried overnight for 40 °C**.**

3. [62]**Hiteshkumar D Patel1 *et al,*** Calcium Dobesilate in the symptomatic treatment of hemorrhoidal disease**:** An interventional study: Hemorrhoidal disease is one of the commonest ailments that affects mankind and is currently believed to be caused by distal displacement and structural distortion of anal cushions, which are physiologic structures

randomized, double blind, controlled study was conducted to investigate the efficacy of oral and local calcium dobesilate therapy in treating acute attacks of internal hemorrhoids. Fiftynine (59)adult patients with first or second-degree internal hemorrhoids were treated with calcium dobesilate for six weeks, while 56 patients received only a high fiber diet to serve as control. Both symptoms and anoscopic inflammation were scored on a scale from 0 to 2 before and six weeks after treatment.

4. **GD Guptha[48]** ***et al:*** To prepare and evaluate floating microspheres of silymarin for prolonged gastric residence time and increased drug bioavailability.Cellulose microspheres – formulated with hydroxylpropyl methylcellulose (HPMC) and ethylcellulose (EC) – and Eudragit microspheres – formulated with Eudragit® S 100 (ES) and Eudragit® RL (ERL) - were prepared by an emulsion-solvent evaporation method. Mean particle size increased while drug release rate decreased with increasing EC and ES contents of cellulose and Eudragit microspheres, respectively. The microspheres exhibited prolonged drug release for 12 h while still remained buoyant. Drug release kinetics, evaluated using the linear regression method, followed Higuchi kinetics and drug release mechanism was of the non-Fickian type. The developed floating microspheres of silymarin exhibited prolonged drug release in simulated gastric fluid for at least 12 h, and, therefore, could potentially improve the bioavailability of the drug as well as patient compliance.

5. **2012 Swait[49]** ***et al:*** The purpose of this study was to prepare and characterize microspheres loaded by Aceclofenac. To achieve this goal Chitosan and Sodium alginate microspheres loaded by Aceclofenac were prepared by emulsification and ionic gelation methods. Morphology, size, encapsulation efficiency and drug release from these microspheres were evaluated. Microscopic evaluation of microspheres showed that microspheres were spherical in shape. The size analysis results indicated that size range varied from 1 to 13 μm. Encapsulation efficiency of microspheres was increased by increasing drug to polymer ratio. Drug release was found to be Zero order. In conclusion, microspheres loaded with Aceclofenac were prepared that could be used for control delivery of Aceclofenac.

6. **2014 Bharat W[50] *et al:*** The aim of the present work is to develop the Metoclopramide hydrochloride microsphere using Eudragit RL 100 and hydroxyl propyl methyl cellulose (HPMC K100) as a polymer by solvent evaporation method for Sustained effect. For the preparation of Metoclopramide Hydrochloride Microsphere the solvent system i.e., (Dichloromethane and ethanol) and the drug–polymer ratio are use in various concentrations, to obtain the desire sustained formulation. Various formulation of metoclopramide hydrochloride microsphere was formulated by using Eudragit RL 100 and HPMC K100 polymers. The E-6 batch microsphere prepared from the Eudragit RL100 polymer in that the drug-polymer ratio is 01:1.5, and 01:01 Solvent system (DCM: Ethanol), using 2% span 80 as dispersing agent. Metoclopramide hydrochloride microsphere E-6 formulation releases the maximum drug i.e., 95.87 ± 0.70 for 12 hrs. The kinetic study was carried out and the best fitted kinetic model for E6 optimised batch was Korsmeyer peppas have R value 0.998 and k value was 13.62.

7. **2010 Ghodaka[51] *et al:*** The present study involves preparation and evaluation of floating microspheres with Metformin Hydrochloride as model drug for prolongation of gastric residence time. The microspheres were prepared by the emulsification solvent diffusion technique using polymers Hydroxypropyl methyl cellulose K4M and Eudragit RS100.. In vitro drug release studies were performed and drug release kinetics was evaluated using the linear regression method. Effects of the stirring rate during preparation, polymer concentration, solvent composition and dissolution medium on the size of microspheres and drug release were also observed. The prepared microspheres exhibited prolonged drug release (8 h). The mean particle size increased and the drug release rate decreased at higher polymer concentration. No significant effect of the stirring rate during preparation on drug release was observed. In most cases good in vitro floating behavior was observed and a broad variety of drug release pattern could be achieved by variation of the polymer and solvent ratio, which was optimized to match target release profile.

8. **2010 Alireza Mahboubian[52] *et al:*** Prepared And Evaluated The Controlled Release PLGA Microparticles Containing Triptorelin. The present study describes the formulation of a sustained release microparticulate drug delivery system containing triptoreline acetate, using poly (D,L lactide-co-glycolide) (PLGA). Biodegradable microspheres were prepared using 50:50 PLGA by a water in-oil-in-water (w/o/w) double emulsion-solvent

evaporation procedure. Effect of critical process parameters and formulation variables; *i.e*. volume of inner water phase, addition of NaCl to the outer aqueous phase (W2), addition of different types and amounts of emulsifying agents on microsphere characteristics; were investigated. Microspheres prepared were spherical with a smooth surface, but addition of poloxamer to the first emulsion produced microspheres with large pores. Increasing the inner water phase volume resulted in larger particles with lower encapsulation efficiency. Low concentrations of Span 20 decreased triptoreline release rate, whereas the addition of poloxamer or high concentrations of Span 20 increased the drug release rate. In conclusion, by selecting an appropriate level of the investigated parameters, spherical microparticles with encapsulation efficiencies higher than 90% and a prolonged triptoreline release over 45 days were obtained.

9. **2006 Ana Rita C.[53]** ***et al:*** prepared controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs. Ethylcellulose/methylcellulose blends were produced using different precipitation techniques and impregnated with naproxen, a non-steroidal anti-inflammatory drug (NSAID). Solvent-evaporation technique was used not only for the preparation of ethylcellulose/methylcellulose microspheres but also to encapsulate naproxen. Supercritical fluid (SCF) impregnation was also performed to prepare naproxen loaded microspheres. In vitro release profiles at pH 7.4 and 1.2, of naproxen-loaded microspheres were evaluated and the results were modelled Fick's law of diffusion and Power law. Miscrospheres prepared by supercritical antisolvent have a higher loading capacity and present a slower release profile. The systems studied present a release mechanism controlled by drug diffusion which complies Fick's law of diffusion.

10. **2010 B.Appa Rao** ***et al:*** Reported prolonged-release microcapsules of diclofenac sodium (DS) were prepared by employing ethyl cellulose as a polymer in various ratios of 1:1, 2:3 & 2:1, by emulsion solvent evaporation technique. Scanning electron microscope photographs of samples revealed that all prepared microcapsules were almost spherical in shape and have a slightly smooth surface. The encapsulation efficiency was found to be in the range of 66.17 -72.99%. The *In-vitro* release profile of diclofenac indicates that all the batches of microcapsules showed controlled and prolonged drug release over an extended period of 10 h. The release kinetics study reveals that the drug follows first order kinetics

and the mechanism of drug release was diffusion controlled type. The author concluded that Sustained release DS microcapsules could be formulated by using ethyl cellulose as a release retardant by emulsion solvent evaporation technique. Increasing the polymer concentration in microcapsule formulation decreases the rate of drug release dramatically.

11. **2000 J.M. Teijon[55]** ***et al:*** studied the Chitosan microspheres in PLG films as devices for cytarabine release. Cytarabine was included in chitosan microspheres and several of these microspheres were embedded in a poly (lactide-co-glycolide) (PLG) film to constitute a comatrix system, to develop a prolonged release form. Chitosan microspheres, in the range of 92±65 μm, having good spherical geometry and a smooth surface incorporating cytarabine, were prepared. The cytarabine amount included in chitosan microspheres was 43.7μg of ara-C per milligram microsphere. The incorporation efficiency of the cytarabine in microspheres was 70.6%. Total cytarabine release from microspheres *In-vitro* was detected at 48h. Inclusion of cytarabine-loaded microspheres in poly (lactide-co-glycolide) film initiated a slower release of the drug and, in this way, the maximum of cytarabine released (80%) took place in vitro at 94.5 h.
12. **2005 Sinha VR[56]** ***et al:*** formulated and characterized the Ketorolac tromethamine biodegradable microspheres. Ketorolac tromethamine has to be given every 6 hr intramuscularly in patients for acute pain, so to avoid frequent dosing and patient inconvenience we found it to be a suitable candidate for parenteral controlled delivery by biodegradable microspheres for the present study. Ketorolac tromethamine-loaded microspheres were prepared by o/w emulsion solvent evaporation technique using different polymers: polycaprolactone, poly lactic-co-glycolic acid (PLGA 65/35), and poly lactic-co-glycolic acid (PLGA 85/15). In pure PLGA65/35 and PLGA85/15, particle size was 28 micron and 8 micron, respectively. Surface topography was studied by scanning electron microscopy that revealed a spherical shape of microspheres. From our study it as concluded that with careful selection of different polymers and their combinations, we can tailor the release of ketorolac tromethamine for long periods.

13. **2008 Xia CHEN[57]** ***et al:*** Prepared and characterized biodegradable Polylactide (PLA) Microspheres Encapsulating Ginsenoside Rg3. The microspheres surface was smooth. Particle size analysis results showed that the diameters of the microspheres encapsulating ginsenoside Rg3 were even larger than those of the non encapsulated ones and they were rather homogeneous in size. For *in vitro* drug release experiments, the dialysis dynamic

method was applied. Among the experimental methods available for determining the *in vitro* release profiles from colloidal suspension, this method was the most suitable, to release the drugs rapidly and completely in the releasing medium. Ginsenoside Rg3 PLA microspheres were prepared by using the emulsion solvent evaporation method. The controlled release of ginsenoside Rg3 from PLA microspheres could be explained by a diffusion mechanism, which was in good agreement with the Heller-Baker Model.

14. **2012 Yagnesh Bhatt[58] *et al:*** reported influence of additives on fabrication and release from protein loaded PLGA microparticles**.** The encapsulation efficiency was reduced by adding PEG 1450 into oil phase during the emulsification. The Encapsulation efficiencies of BSA within microparticles were 43% ± 0.63, 36% ± 0.85, 52% ± 1.02 for PLGA/PEG ratios 1:1, 1:2 and 2:1 respectively, where PLGA microparticles without PEG 1450 shown slightly higher encapsulation efficiencies (56% ± 0.23). The control microparticles showed a smooth, nonporous surface while the microparticles with PEG exhibited a highly porous surface. These studies have shown that the incorporation of additives PEG 1450 significantly increased the early-stage release of BSA from PLGA microparticles in comparison to the control. There was no improvement in encapsulation efficiency. In the case of surfactants, PVA was found to be the most efficient surfactant in very less concentration (0.25–0.5 % w/v) considering both the encapsulation efficiency and the size reducing effect in comparison of polaxomer 407.

15. **2003 Yilmaz Capan[59] *et al:*** assessed the physicochemical properties of a controlled release formulation of recombinant human growth hormone (rHGH) encapsulated in poly (D,L-lactide-co-glycolide) (PLGA) composite microspheres. rHGH was loaded in poly(acryloyl hydroxyethyl) starch (acHES) microparticles, and then the protein-containing microparticles were encapsulated in the PLGA matrix by a solvent extraction/evaporation method. rHGH-loaded PLGA microspheres were also prepared using mannitol without the starch hydrogel microparticle microspheres for comparison. The composite microspheres were spherical in shape (44.6±2.47 μm), and the PLGA-mannitol microspheres were 39.7±2.50 μm. Drug-loading efficiency varied from 93.2% to 104%. The composite microspheres showed higher overall drug release than the PLGA/mannitol microspheres. FTIR analyses indicated good stability and structural

integrity of HGH localized in the microspheres. The PLGA-acHES composite microsphere system could be useful for the controlled delivery of protein drugs.

16. **2004 Y. Yeo[60] *et al:*** studied the control of encapsulation efficiency and initial burst in polymeric microparticle systems. Initial burst is one of the major challenges in protein-encapsulated microparticle systems. Since protein release during the initial stage depends mostly on the diffusional escape of the protein, major approaches to prevent the initial burst have focused on efficient encapsulation of the protein within the microparticles. For this reason, control of encapsulation efficiency and the extent of initial burst are based on common formulation parameters. The present article provides a literature review of the formulation parameters that are known to influence the two properties in the emulsion-solvent evaporation/extraction method. Physical and chemical properties of encapsulating polymers, solvent systems, polymer-drug interactions, and properties of the continuous phase are some of the influential variables. Most parameters affect encapsulation efficiency and initial burst by modifying solidification rate of the dispersed phase. In order to prevent many unfavorable events such as pore formation, drug loss, and drug migration that occur while the dispersed phase is in the semi-solid state, it is important to understand and optimize these variables.

3.AIM AND OBJECTIVE

AIM OF PRESENT STUDY:

Calcium Dobesilate is controlled release microspheres are gaining prominence as new targeted drug delivery system. This dosage form has to be administered orally for controlling the drug release. In this study, an effort has been made to formulate controlled release microspheres using polymer HPMC K100, Ethyl Cellulose, Eudragit L100, Sodium Alginate. Controlled release microspheres are gaining prominence as new targeted drug delivery system. In this study we aim to formulation and evaluation of Calcium Dobesilate microspheres for the treatment of chronic venous disease.

Hence The Calcium Dobesilate as design controlled release microspheres provided following benefits

1. Microspheres in improve treatment efficacy while reducing toxicity.
2. The microspheres continue to protect the encauplasting agent after administration.
3. site specific drug can be achieved.
4. The microspheres release encapsulation molecules over extended time intervals up to 24 hrs.
5. drug is having Short half life, high water solubility to prolong the pharmacological action ideal candidate for design of controlled release microspheres formulation.
6. Constant drug releases for better therapeutic action.
7. In order to improve patient compliance .
8. Maintain therapeutic window, obtain controlled Drug release.
9. To reduce cost effect
10. To reduce side effects.
11. To reduce dosage frequency.
12. Long duration of action.

OBJECTIVE OF PRESENT STUDY

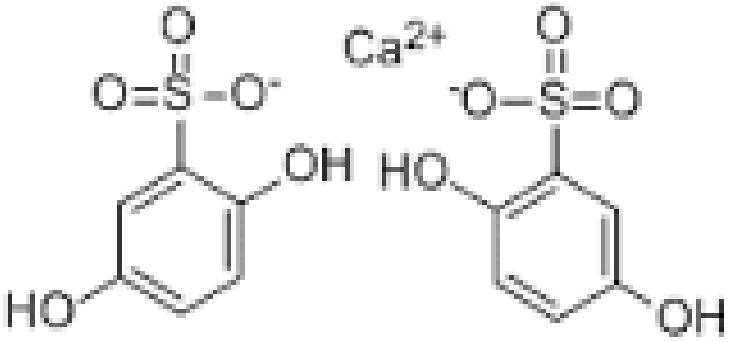
Following objectives to develop to the formulation development and evaluation of Calcium Dobesilate microspheres.

- To perform pre formulation studies.
- To prepare the microspheres by using different methods- Ionic Gelation, Emulsion Solvent Evaporation method, Emulsification Ionic Gelation Method.
- Selection of appropriate method for preparation of microspheres.
- Study effect of various formulations and process variables on Microspheres size, entrapment efficiency and *In-vitro* release studies.
- Evaluate the effect of different independent variables such as polymer concentration, Calcium chloride concentration and stirring speed.
- To determine the compatibility of drug with the polymer by FTIR studies.
- Study effect of various formulations for *In-vitro* drug release and release kinetics.
- To carry out stability studies of Calcium Dobesilate microspheres.

4.DRUG AND EXCIPIENT PROFILE

1.CALCIUM DOBESILATE:-

Fig. No.4:Chemical structure of CALCIUM DOBESILATE:-



Calcium Dobesilate **:** Calcium di(2,5-dihydroxybenzenesulphonate) monohydrate

Chemical IUPAC Name : Calcium di(2,5-dihydroxybenzenesulphonate) monohydrate

Trade Name **:** Doxium

Category **:** Venotonic,

Chemical Formula **:** $C_{12}H_{10}CaO_{10}S_2,H_2O$

Molecular Weight **:** 418.41g/mol

Melting Point **:** 251**°C**

Mechanism of Action[22,23,24]:

Calcium dobesilate has a comprehensive mode of action. It increases endothelial nitric oxide levels by enhancing the activity of nitric oxide synthase and decreasing capillary hyperpermeability. Calcium dobesilate shows anti-platelet and fibrinolytic activities by inhibiting platelet activation factor (PAF) and enhancing the release of tissue plasminogen activator (tPA), thereby improving the local blood flow to tissues, otherwise inhibited due to thrombosis. Calcium dobesilate also inhibits the two pathophysiological reactions in diabetes, viz. polyol

pathway and glycation of proteins, due to its inhibitory effects on aldose reductase. Calcium dobesilate acts on the endothelial layer and basement membrane of the capillaries. It reduces histamine and bradykinin-induced hyperpermeability. It increases red blood cell membrane flexibility and reduces capillary fragility. Calcium dobesilate can reduce theplatelet aggregation stimulated by collagen and thrombin, but not by arachidonic acid.Calcium dobesilate may also inhibit the formation of sorbitol, thus providing another possible mechanism for its usefulness in diabetic retinopathy.Glucose inhibits the formation of both type I and type II collagen formation. Calciumdobesilate does not affect type I inhibition by glucose but accelerates type II collagen fibrillogenesis,a major structural component of the arterial wall. Calcium dobesilate hasangioprotective action by reducing the permeability and fragility of microvessels, which should restrict fluid extravasation into the cardiac interstitium. Itsantiplatelet effect counteracts thrombosis and its reduction of plasma viscosity preventastasis.

Physical data[24]:

Colour : White Powder

Odour : None

Taste : Bitter

Solubility : very soluble in water ,freely soluble in Ethanol, Slightly soluble in 2propanol, soluble in Methanol , practically insoluble in methylene chloride.

Table No.1:

Pharmacokinetic data[25]:

Protein binding	**25%**
Metabolism	**Kidney**
Half life	**2 hours**

Indications:

Calcium dobesilate is indicated for the treatment of chronic venous disease diabetic retinopathy, haemmeriods (piles)

Clinical particulars[26,27,28]:

Therapeutic indications

Microangiopathies, in particular diabetic retinopathy. Clinical signs of chronic venous insufficiency in the lower limbs (pain, cramps, paresthesia, oedema, stasis dermatosis), as adjuvant in superficial thrombophlebitis. Haemorrhoidal syndrome, microcirculation disorders of arteriovenous origin.

Posology and method of administration

Generally 500 to 1000 mg – 1 capsule once or twice a day - to be taken with the main meals. Treatment duration, which is generally between a few weeks and several months, depends on the disease and its evolution. Dosage should be adapted individually according to the severity of the case.

Contra-indications

Hypersensitivity towards calcium Dobesilate.

Special warnings and special precautions for use

Dosage should be reduced in case of severe renal insufficiency requiring dialysis.

In very rare cases (0.32/million patients), incidence estimated on the basis of spontaneous reports, the intake of calcium dobesilate may induce agranulocytosis, probably linked to a hypersensitivity reaction. This condition may be expressed by symptoms such as high fever, oral cavity infections (tonsillitis), sore throat, anogenital inflammation and accompanying symptoms, that are often signs of an infection. The patient should be told that by any sign of infection he/she must immediately inform his/ her physician. In that case, it is essential to control without delay the blood formula and leucogram and to discontinue the treatment.

Interactions with other medicinal products and other forms of interaction

No interaction is known up to now.

At therapeutic doses, calcium dobesilate may interfere with creatinine assay by giving lower values.

Pregnancy and lactation

Pregnancy category *C:* studies in pregnant women or animals are not available. As it is not known whether calcium dobesilate crosses the placental barrier in humans, the drug should only be administered if the potential benefit justifies the potential risk to the foetus. Calcium dobesilate enters the maternal milk in very low quantities (0,4 µg/ml after intake of 3x500 mg). As a precaution, either the treatment or the breastfeeding should be stopped.

Effects on ability to drive and use machines

Doxium 500 has no effect upon driving capacity and managing of machines.

Gastrointestinal disorders

Rare : nausea, diarrhoea, vomiting.

Skin and subcutaneous tissue disorders

Rare: pruritus,rash.

General disorders and administration site conditions

Rare: fever,chills.

Musculoskeletal disorders

Rare : arthralgia.

Cardiac disorders

Uncommon: tachycardia.

Blood and lymphatic system disorders:

Isolated cases of agranulocytosis have been reported mainly in elderly patients and in combination with other drugs. These reactions are generally reversible when stopping treatment course. In case of gastrointestinal disorders, the dosage should be reduced or the treatment temporarily withdrawn. In case of skin reactions, fever, articular pain or change in blood formula, the treatment must be stopped and the treating physician informed as this may constitute hypersensitivity reactions.

Pharmacodynamic properties[29,]:

Regulator of capillary functions. Calcium dobesilate acts on the capillary walls by regulating its impaired physiological functions - increased permeability and decreased resistance. It increases erythrocyte flexibility, inhibits platelet hyperaggregation and, in diabetic retinopathy, it reduces plasma and blood hyperviscosity, thus improving blood rheological properties and tissue irrigation. These effects allow to correct capillarydysfunctions either of functional origin or caused by constitutional or acquired metabolic disorders. Calcium dobesilate contributes to reduce oedema.

Pharmacokinetic properties[30,31]:

After oral administration of 500 mg of calcium dobesilate, its blood level is above 6 µg/ml between the 3rd and 10th hour, with a maximum (Cmax) of 8 µg/ ml on the average after 6 hours (tmax). Twenty four hours after intake blood level is about 3 µg/ml. The rate of protein-binding is 20 - 25%. In animals, calcium dobesilate does not cross the haematoencephalic or the placental barrier, but it is not known whether this is also the case in humans. Calcium dobesilate enters the maternal milk in very low quantities (0,4 µg/ml after intake of 1500 mg as observed in one study). Calcium dobesilate does not enter the enterohepatic cycle and is excreted mainly unchanged with only 10% being excreted as metabolites. About 50% of the orally administered dose are eliminated in the first 24-hour urine and about 50% in the faeces. Plasma half-life is around 5 hours. *Kinetics in particular clinical situations* It is not known to what extent renal function disorders influence the pharmacokinetic properties of calcium dobesilate (see "Precautions").

Preclinical safety data[32]:

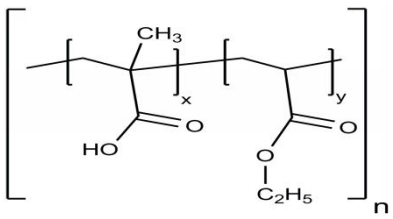
Acute and chronic toxicity studies, foetotoxicity and mutagenicity studies on calcium dobesilate have not revealed any toxic effect.

2.EUDRAGIT L 100-55 [40] :

EUDRAGIT® L 100-55 contains an anionic copolymer based on methacrylic acid and ethyl acrylate. It is prepared by Spray drying of Eudragit L 30 D-55

Physical properties: It is a white free flowing powder 95% dry redispersible in water to form a latex ratio of free –COOH groups to ester groups is 1:1. Films dissolve above pH 5.5 forming salts with alkalis thus affording coatings which are insoluble in gastric media, but are soluble in Small Intestine

Figure. No.5:Chemical structure of EUDRAGIT® L 100-55 [40] :



Targeted Drug Release Area: duodenum

Dissolution: above pH 5.5

Characteristics:

- Effective and stable enteric coatings with a fast dissolution in the upper Bowel
- Granulation of drug substances in powder form for controlled release
- Site specific drug delivery in intestine by combination with EUDRAGIT® S grades
- Variable release profiles

Solubility: 1 g of EUDRAGIT® L 100-55 dissolves in 7 g methanol, ethanol, isopropyl alcohol and acetone, as well as in 1 N sodium hydroxide to give clear to cloudy solutions. It is practically insoluble in ethyl acetate, methylene chloride, petroleum ether and water.

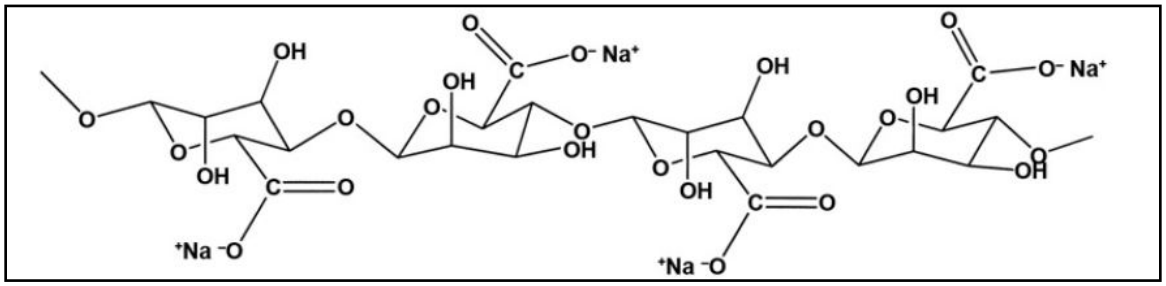
Viscosity / Apparent viscosity: 100 - 200 mPa.s

Storage: Store at controlled room temperatures (USP, General Notices). Protect against moisture. Any storage between 8 °C and 25 °C fulfils this requirement.

Stability: Minimum stability dates are given on the product labels and batch-related Certificates of Analysis. Storage Stability data are available upon request.

3.SODIUM ALGINATE[35,36,43]:

Figure. No.6:Chemical structure of SODIUM ALGINATE:



Empirical formula: $(C_6H_7O_6Na)_n$

Synonym: Alginic acid, algin, sodium poly nuronate, Kelcosol, Keltone, Sodium polymannuronate.

Description: Sodium alginate (ALG) is the purified carbohydrate product extracted from brown sea weed by the use of dilute alkali. It consists chiefly of the sodium salt of alginic acid, a polyuronic acid composed of β-D-mannuronic acid residues so that the carboxyl group of each unit is free while the aldehyde group is shielded by a glycosidic linkage. Sodium alginate occurs as white to pale yellowish brown colour powder which is odourless and tasteless. Sodium alginate is practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures

in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3, poorly soluble in water, forming a viscous colloidal solution. The powder may be coarse and having viscosity of 20-400cps at 20^0c (1% aqueous solution) and of 1% w/v aqueous solution is having pH 7.2.

Storage: Sodium alginate is a hygroscopic material. It should be stored at low relative humidity and a cool temperature. Numerous studies have indicated sodium alginate to be quite safe allergy tests have shown it to be non allergenic.

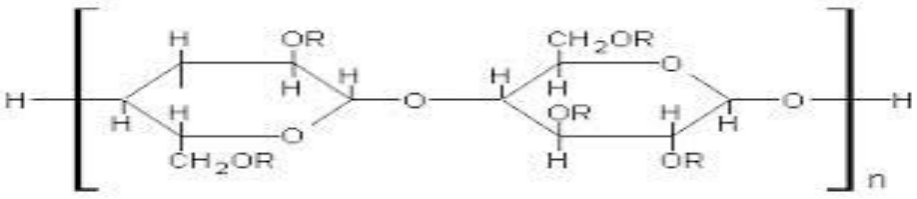
Incompatibility: It is incompatible with acridine derivatives, crystal violet, phenyl mercuric nitrate and acetate, calcium salts, alcohol in concentrations greater than 5% and heavy metals.

Applications

- Used as stabilizing agent in emulsions, as suspending agent, thickening agent, tablet disintegrating agent and tablet binder.
- Used as haemostatic agent in surgical dressings.
- Used for the aqueous microencapsulation of drugs in contrast with the more conventional microencapsulation techniques used in combination with an H_2 receptors antagonist in the management of gastro oesophageal reflex.
- Used in the preparation of sustained release oral formulations, since it can delay the dissolution of the drugs from tablet and aqueous suspensions.
- Used in cosmetics and food products.
- Used in the modulation of gastrointestinal transit time and delivery of biomolecules like DNA, proteins and cells.[14-22]

4.HYDROXY PROPYL METHYL CELLULOSE K100[37,38,42]:

Figure. No.7:Chemical Structure of HYDROXY PROPYL METHYL CELLULOSE:



Non Proprietary Name:

British pharmacopoeia : Hypromellose

United State Pharmacopoeia : Hydroxy Propyl Methyl cellulose

Synonyms **:** Methocel, HPMC

Chemical Name **:** Cellulose, 2-Hydroxypropyl methyl ether methanol

Stability **:** Stable in dry condition from pH 3.0 to 11.0

Storage Condition **:** It is hygroscopic in nature. Should be stored in well-closed container, in a cool and dry place.

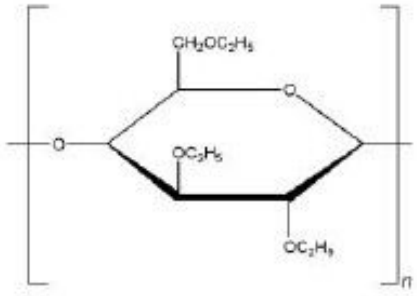
Incompatibilities **:** Incompatible with some oxidizing agents. Since it's nonionic, hydroxy methyl cellulose will not complex with metallic effect.

Safety **:** It's generally regarded as a nontoxic and non-irritant material although excessive oral consumption may have a laxative effect.

Application **:** HPMC is widely used in oral and topical pharmaceutical formulations. In oral products, it primarily used tablet binder.[25]

5.ETHYL CELLULOSE[39,40,41]:

Figure. No.8:Chemical structure of ETHYL CELLULOSE:



Synonyms: Ethocel

Nonproprietary Names: BP: Ethylcellulose

PhEur: Ethylcellulosum

Chemical Name **:** Cellulose ethyl ether

CAS Registry Number : 9004-57-3

Empirical Formula: C12H23O6 (C12H22O5)n-2 C12H23O5

Functional category: Coating agent; flavoring fixative; tablet binder; tablet filler; viscosity-increasing agent.

Description: Ethyl cellulose is a tasteless, free-flowing, white to light tan-colored powder.

Solubility: 40 Insoluble in water and glycerin, but soluble in certain organic solvents, depending upon ethoxy content.

Stability and Storage Conditions: It is resistant to alkalis, both dilute and concentrated, and to salt solutions. It is more sensitive to acidic materials than are cellulose esters. However, the material can withstand dilute acids for a limited period of exposure. Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures.

This may be prevented by use of an antioxidant and a compound with light absorption properties between 230-340 nm. Ethyl cellulose should be stored between 7° and 32°C in a dry area away from all sources of heat. Store in a well-closed container.

Incompatibilities: Incompatible with paraffin wax and microcrystalline wax.

Safety: Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. It is also used in food products. Ethyl cellulose is generally regarded as a nontoxic, non allergenic, and nonirritating material. As ethyl cellulose is not considered to be a health hazard, the WHO has not specified an acceptable daily intake. [26]

5.PLAN OF WORK

Plan of Work

- Literature survey
- Selection of drug
- Standard calibration curve of Calcium Dobesilate.
- Preparation of Calcium Dobesilate microspheres
- Evaluation of Microspheres
  - Preformulation studies
    - FT-IR studies
  - Evaluation parameters
    - Determination of percentage yield
    - Drug entrapment efficiency
    - Particle size analysis
    - *In-vitro* drug release
    - *In-vitro* drug release kinetics

6.MATERIALS AND METHODS

Materials used in the present research work:

Table No.2:

S.NO	NAME OF THE PRODUCT	NAME OF THE SUPPLIER
1	Calcium Dobesilate	Qualikems fine chem...pvt, Ltd
2	HPMC K100	Qualikems fine chem...pvt, Ltd
3	Eudragit L100	Otto chemika-biochemica reagent
4	Ethyl Cellulose	Suvidhnath Laboratories,Baroda
5	Sodium alginate	Finars Reagents,Ahmedabad
6	Ethanol	Suvidhnath Laboratories,Baroda
7	Methanol	Qualikems fine chem...pvt, Ltd
8	Span 80	Samir tech-chem pvt.Ltd Vadodara
9	Potassium di hydrogen ortho phosphate	Qualikems fine chem...pvt, Ltd
10	Sodium Hydroxide	Qualikems fine chem...pvt, Ltd
11	Calcium chloride	Indian Research Products IRP
12	Dichloromethane	Karnataka fine chemical ,Bangalore
13	Groundnut oil	Vk. Pharamavceuticals, Hydrabad
14	HCL	Qualikems fine chem...pvt, Ltd

Equipment used in present work:

Table No.3:

S.NO	Name of the Instrument	Model and Manufacture/supplier
1	High precision balance	Sartorius-bsa224s-CW, Mombay
2	U.V/Visible spectrophotometer	LAB INDIA
3	Magnetic stirrer	LAB INDIA
4	Dissolution test apparatuses	LAB INDIA , Navi Mumbai
5	p^H meter	LAB INDIA,
6	Melting point apparatuses	LAB INDIA, Mumbai
7	Tapped density apparatuses	LAB INDIA
8	Glass ware	Borosil

PREFORMULATION STUDIES:

Preformulation studies are the first step in the rational development of dosage form of drug substance. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone when combined with excipients.

The following Preformulation studies were performed for Calcium Dobesilate

1. Solubility
2. Melting point Determination.
3. Density
4. Carr's Index
5. Hauser's Ratio
6. Angle of repose
7. FT-IR Spectral studies
8. Stability Studies

1.Solubility:

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into dissolution medium and consequently, the therapeutic efficiency of the pharmaceutical product. The solubility of the molecules in various solvents is determined as a first step. Solubility is usually determined in variety of commonly used solvents and some oils if the molecules are lipophilic. The solubility of material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged until equilibrium is achieved. A known quantity of solute was dispersed in the solvent and based on following table the solubility was determined.

A known quantity of solute was dispersed in the solvent and based on following table the solubility was determine

Solubility chart

Table No.4:

Descriptive term	Approximate volume of solvent in milliliters per gram of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	More than 10,000

2.Melting Point Determination:

A characteristic of a pure substance is a defined melting point or melting range. If not pure, the substance will exhibit a change in melting point. This phenomenon is commonly used to determine the purity of a drug substance and in some cases the compatibility of various substances before inclusion in the same dosage form. it is determined by capillary tube method.

3.Density:

1.Bulk Density (Db):

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/ml and is given by

$$D_b = \frac{M}{Vb}$$

Where M = is the mass of powder.

Vb =is the bulk volume of the powder.

2.Tapped Density (Dt):

Fig. No.9: Tapped Density:

It is the ratio of total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder for 750 times and the tapped volume was noted if difference between these two volumes is less than 2%. If it is more than 2%,tapping is continued for 1250 times and tapped volume was noted. Tapping was continued until the difference between successive volumes is less than 2% (in a bulk density apparatus).

It is expressed in gm/ml and is given by

$$D_t = \frac{M}{Vt}$$

M= is the mass of powder.

V_t=isthetapped volume of the powder

4.Carr's Index (I): (or) % compressibility:

It indicates the ease with which a material can be induced to flow. It is expressed in percentage and is given by

I=[Dt-Db/Dt] X100

Where,

D_t=is the tapped density of the powder. D_b=is the bulk density of the powder.

Relationship between % compressibility and Type of flow

Table No.5:

Carr's index (%)	Type of flow
5-12	Excellent
12-18	Good
18-23	Fair to passable
23-35	Poor
35-38	Very Poor

>40	Extremely Poor

5.Angle of Repose (θ):

Fig. No.10:

The friction forces in a loose powder can be measured by the angle of repose θ. It is defined as maximum angle possible between the surface of a pile of powder and the horizontal plane. The powder mixture was allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose was the calculated by measuring the height and radius of the heap of powder formed.

$$\tan \theta = \frac{h}{r}$$

$$\theta = \tan^{-1}(h/r)$$

Where, θ is the Angle of repose,

h is height of pile,

r is radius of pile,

Table No.6: Angle of Repose as an Indication of Powder Flow Properties I.P standard values:

ANGLEOF REPOSE (In degrees)	TYPEOF FLOW
<25	Excellent
25-30	Good
30-40	Passable

>40	Very Poor

6.Hauser's ratio:

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula

Hausner's ratio = Dt/Db

Where, DT is the tapped density.

D_b is the bulk volume.

Table No.7: Hausner's ratio values:

Hausner'sratio	**Typeof flow**
<1.25	Good
1.25-1.5	Moderate
> 1.5	Poor

7.FT-IR Spectral studies:

The IR spectra for the formulation excipients and pure drugs were recorded on BRUKER FT-Infrared spectrophotometer using ATR technique at the resolution rate of 2-2.5µm Spectrum was integrated in transmittance mode at the wave number range 400-4000 cm^{-1}

Analytical method Development by UV/Visible Spectrophotometer:

Standard calibration with 6.8 pH phosphate buffer

Preparation of Stock solution

100 mg of Calcium Dobesilate was solubilized ethanol then dissolved in 100 ml of 6.8 pH phosphate buffer in a 100 ml volumetric flask and made up to the volume with 6.8 pH phosphate buffer. From this 10 ml of solution was taken and made to 100 ml with 6.8 pH phosphate buffer.

Method

For the estimation of Calcium Dobesilate in 6.8 pH phosphate buffer the stock solution has to be diluted subsequently with 6.8 pH phosphate buffer to get a series of dilutions containing 10,20,30,40 &50 µg/ml of solution. The absorbance of these solutions were measured at 302 nm against blank.

PREPARATION OF CALCIUM DOBESILATE MICROSPHERES:

1.Orifice Ionic Gelation Method[30-31]:

Sodium alginate(1 to 6 %) and drug were dissolved in purified water 10 ml separately and mixed thoroughly with help of stirrer to form viscous dispersion. The resulting dispersion was added drop wise into 20% to 40% w/v calcium chloride solution through a syringe with needle (size no 23) with continues stirring at 900 rpm. The added droplets were retained in the calcium chloride solution for 15 minutes to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45^0C for 12 hours and stored in desiccators

2.Emulsion Solvent Evaporation[90 ,44]:

The drug and polymer in different proportions are weighed the polymer was co dissolved into previously cooled mixture of Ethanol **:** Dichloromethane at room temperature. The mixture was stir vigorously to form uniform drug polymer dispersion. The above organic phase was slowly added to100 ml of distilled water & 0.1 HCL containing 0.01% Span 80 by maintain the temperature at 20**°C** to 30 **°C** and emulsified by stirring at 1200 rpm for 30 min . The formed Microspheres were filtered & washed with water and sieved between 30-50 mesh size, and dried overnight for 40 °C**.**

3.Emulsification Ionic Gelation method[30-31]:

The Emulsification method was utilized for the preparation of microspheres followed by cross-linking with calcium chloride Core material, Calcium Dobesilate (250 mg) was dispersed in 8% aqueous solution of sodium alginate (10 ml). The aqueous phase was emulsified in Coconut oil, the ratio 1:10 containing 2% (v/v) Span 80 using a mechanical stirrer (Remi Motors, India) at 400–1000 rpm for 60 min to it 5ml of 8% calcium chloride dissolved in a mixture of methanol and isopropyl alcohol (2:3) was added slowly to the emulsion and stirred to assure efficient cross-linking. Microspheres were collected by filtration in vacuum, washed with isopropyl alcohol thrice and finally air-dried at room temperature. Various formulations of alginate microspheres were prepared using the variables.

Table No.8: FORMULATION OF MICROSPHERES IONIC GELLATION METHOD (F1-F16)

	F1	F2	F3	F4	F5	F6	F7	F8
Drug	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**
Water	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**
Sodium Alginate	**1%**	**2%**	**3%**	**4%**	**5%**	**6%**	**2%**	**3%**
Calcium Chloride	**20%w/v**	**20%w/v**	**20%w/v**	**20%w/v**	**20%w/v**	**20%w/v**	**30%w/v**	**30%w/v**

	F9	F10	F11	F12	F13	F14	F15	F16
Drug	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**	**250mg**

Water	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**	**10ml**
Sodium Alginate	**4%**	**5%**	**6%**	**2%**	**3%**	**4%**	**5%**	**6%**
Calcium Chloride	**30%w/v**	**30%w/v**	**30%w/v**	**40%w/v**	**40%w/v**	**40%w/v**	**40%w/v**	**40%w/v**

FORMULATION OF MICROSPHERES EMULSIFICATION IONIC GELLATION METHOD BY USING COCONUT OIL:

Table No.9:

Ingredients	**F17**
Drug	**1g**
Water	**10ml**
Sodium Alginate	**8%**
Span80	**2%v/v**
Methanol	**2ml**
Isopropyl Alcohol	**3ml**
Coconut oil	**100ml**
Calcium Chloride	**6%w/v**

FORMULATION OF MICROSPHERES EMULSIFICATION IONIC GELLATION METHOD BY USING GROUND NUT OIL:

Table No.10:

Ingredients	**F18**
Drug	**1 g**
Sodium Alginate	**1 g**

Hpmc k100	**1 g**
Water	**50ml**
Groundnut oil	**300ml**

FORMULATION OF MICROSPHERES EMULSION SOLVENT EVAPORATION METHOD (F19-F24):

Table No.11:

Ingredients	**F19**	**F20**	**F21**	**F22**	**F23**	**F24**
Drug	**1g**	**1g**	**1g**	**1g**	**1g**	**1g**
Ethyle cellulose	**2.250g**	**2.250g**	**1.500g**		**500mg**	**1g**
Hpmc k100	**750mg**	**_**	**_**	**750mg**	**_**	**_**
Eudragit L100	**_**	**750mg**	**1.500g**	**2.250g**	**_**	**_**
Hpmc k4	**_**	**_**	**_**	**_**	**500mg**	**1g**
Dichloro Methane	**30ml**	**30ml**	**30ml**	**30ml**	**30ml**	**30ml**
Ethanol	**30ml**	**30ml**	**30ml**	**30ml**	**30ml**	**30ml**
Water	**100ml**	**_**	**_**	**_**	**_**	**_**
HCL 0.1N	**_**	**100ml**	**100ml**	**100ml**	**100ml**	**100ml**
Span 80	**0.01%**	**0.01%**	**0.01%**	**0.01%**	**0.01%**	**0.01%**

EVALUATION OF MICROSPHERES:

- Determination of percentage yield
- Drug entrapment efficiency
- Particle size analysis
- In-vitro drug release
- In-vitro release kinetics

1.Determination of percentage yield:

Microspheres dried at room temperature were weighed and the yield of microspheres was calculated using the formula.

$$Percentage\ yield = \frac{Practical\ yield\ (gm)}{Theoretical\ yield} \times 100$$

2.Drug entrapment efficiency:

The amount of drug entrapped was estimated by dissolving the 100mg of microspheres in ethanol and 6.8ph in 5:95 ratio ,under vigorous shaking for 1hr, the resultant solution is soaking with in 12 hrs, and filtered to this 1ml was taken and dilueted with 10ml of 6.8 ph buffer ,. The drug content in solution was analyzed by using UV at 302nm with further dilutions against appropriate blank.

The amount of the drug entrapped in the microspheres was calculated using the formula:

$$Drug\ entrapment\ efficiency\ (\%) = \frac{Amount\ of\ drug\ actually\ present}{Theoretical\ drug\ load\ expected} \times 100$$

3.Particle size analysis:

Determination of average particle size of Calcium Dobesilate microspheres with carrier was very important characteristic. It was measured by using optical microscope

4.In vitro dissolution studies

The *in vitro* release of drug from the micro particles was carried out in basket type dissolution containing 900 ml of 6.8 pH phosphate buffer for 24 hrs. The volume of the dissolution media was maintained at 900 ml with constant stirring (50 rpm) and temperature of bath was maintained at 37 ± 0.5°C. Aliquots (10 ml) of dissolution media were sampled at specified time intervals and replaced with fresh media immediately after sampling. Samples were analyzed for drug content by UV visible spectroscopy (ELICO).The release data obtained were fitted into various mathematical models. Dissolution studies were carried out for all the batches of the prepared formulations

i.e. F19,F20,F21,F22,F23,F24. (6 batches) and compared.

Fig. No.11: VEEGO Dissolution Apparatus:-

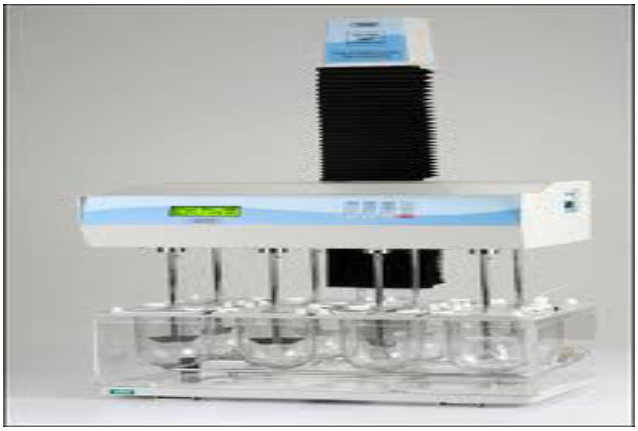


Table No.12: Description of VEEGO Dissolution Apparatus:-

Medium	**6.8 pH phosphate buffer**
Volume	**900 ml**
RPM	**50**
Apparatus	**USP I** basket type
Temperature	**37.0 ^{0}c ± 0.5^{0}c**
Time intervals	**2,4,8,12,16,20…24 hrs**

5.In-vitro release kinetics:

To analyze the *In-vitro* release data various kinetic models were used to describe the

release kinetics. The zero order rate Equation describes the systems where the drug release rate is independent of its concentration. The first order Equation describes the release from system where release rate is concentration dependent.

Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.

The results of *In-vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows:

- Zero - order kinetic model – Cumulative % drug released versus time.
- First – order kinetic model – Log cumulative percent drug remaining versus time.
- Higuchi's model – Cumulative percent drug released versus square root of time.
- Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log time.

1.Zero order kinetics:

Zero order release would be predicted by the following equation:

$$At = A_0 - K_{0t}$$

Where,

A_t = Drug release at time't'

A_0 = Initial drug concentration.

K_0 = Zero- order rate constant (hr-1)

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K_0**.**

2.First order kinetics:

First - order release could be predicted by the following equation:

$$Log\ C = \log C_0 - K_t / 2.303$$

Where,

C = Amount of drug remained at time‘t’

C_0 = Initial amount of drug.

K = First - order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant ‘K_1’ can be obtained by multiplying 2.303 with the slope value.

3.Higuchi’s model:

Drug release from the matrix devices by diffusion has been described by following Higuchi’s classical diffusion equation:

$$Q = [D\epsilon / \tau (2A - \epsilon Cs) Cst] 1/2$$

Where,

Q = Amount of drug release at time‘t’

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = Solubility of drug in the matrix.

ϵ = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assume that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation becomes:

$$Q = Kt_{1/2}$$

When the data is splitted according to equation i.e cumulative drug release versus square root of time yields a staright line, indicating that the drug was released by diffusion mechanism.the slope is equal to ‘k’.ss

4.Korsmeyer equation / Peppa's model:

To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well-known exponential equation (Korsmeyer equation/ Peppa's law equation), which is often used to describe the drug release behavior from polymeric systems.

$$Mt / M\alpha = Ktn$$

Where,

Mt / Mα = the fraction of drug released at time't'.

K = Constant incorporating the structural and geometrical characteristics of the drug / polymer system.

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides,

$$\text{Log } Mt / M\alpha = \text{Log } K + n \text{ Log } t$$

7.RESULTS AND DISCUSSION

1.Preformulation studies:

Table No.13:- Identification of API

S.No	PARAMETER	STANDARAD VALUES	OBSERVED VALUES
1.	**Appearance**	**Colour:** white or almost white hygroscopic powder **Odour:** none **Taste:** bitter	white or almost white hygroscopic powder **Odour**-none, **Taste** -bitter.
2.	**Texture**	Amorphous powder	Amorphous powder
3.	**Solubility**	Very soluble in water, freely soluble in anhydrous ethanol ,very slight soluble in2-propanol,practically insoluble in methylene chloride	Very soluble in water, freely soluble in anhydrous ethanol ,very slight soluble in2-propanol,practically insoluble in methylene chloride.
4.	**Melting point**	251°C.	247^0c
5.	**% Purity**	98%	97%
6.	**λ- max**	221nm-355nm	302nm

Table No.14: The solubility of the calcium Dobesilate is shown following:

S.No	Solubility Media	Descriptive term
1	**Water**	Soluble 100mg in 1ml
2	**Methanol**	Soluble 100mg in 3ml
3	**Ethanol**	Soluble 100mg in 1ml
4	**Ph 6.8 Buffer**	Soluble 100 mg in 10ml

2.Hausner,s ratio, Carr;s index:

On analyzing for density it was found that calcium dobesilate showed bulk density value 0.91gm/cc and tapped density value 1.04 gm/cc, The value of Carr's Index for 16.5 and drugs showed good flow characteristics ,The value of Hausner's ratio for calcium dobesilate was 1.14 drugs showed good flow characteristics

Table No.15:-Characterization of Micro Meritic parameters of Calcium Dobesilate pure drug:

S.No.	Parameters	Observations
2	Angle of repose	15.41^0
3	Bulk density	0.91gm/cc
4	True density	0.405gm/cc
5	Tapped density	1.04gm/cc
6	Compressibility index%	14.28%
7	Cares index %	16.5%
8	Hausner's ratio	1.14
9	Drug content	98%
10	Melting point	247^0 c
11	Particle size	14.28.µm

3.STABILITY STUDIES:

FTIR Spectral studies:

Fig. No.12: FT-IR Reports for API of CALCIUM DOBESILATE:

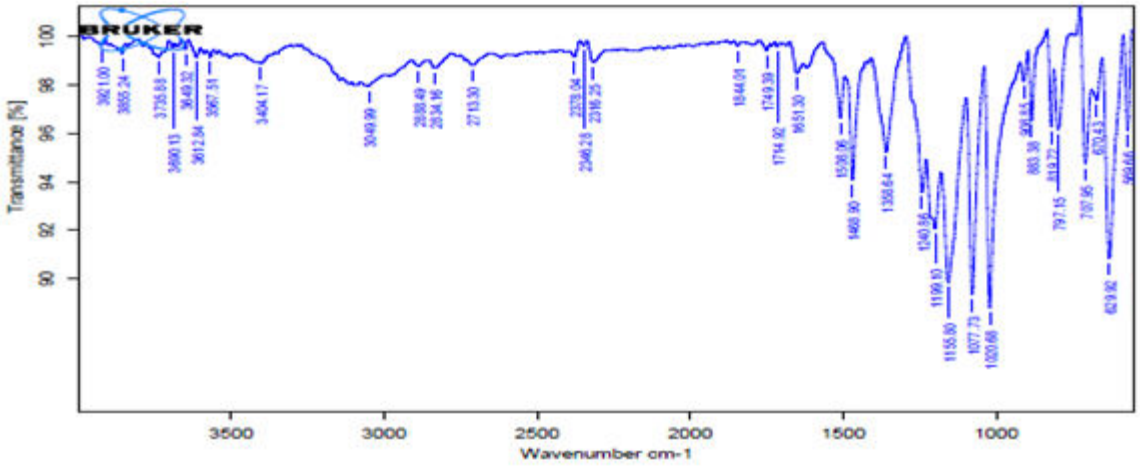


Fig. No.13: FT-IR Reports for CALCIUM DOBESILATE with HPMCK100 formulation:

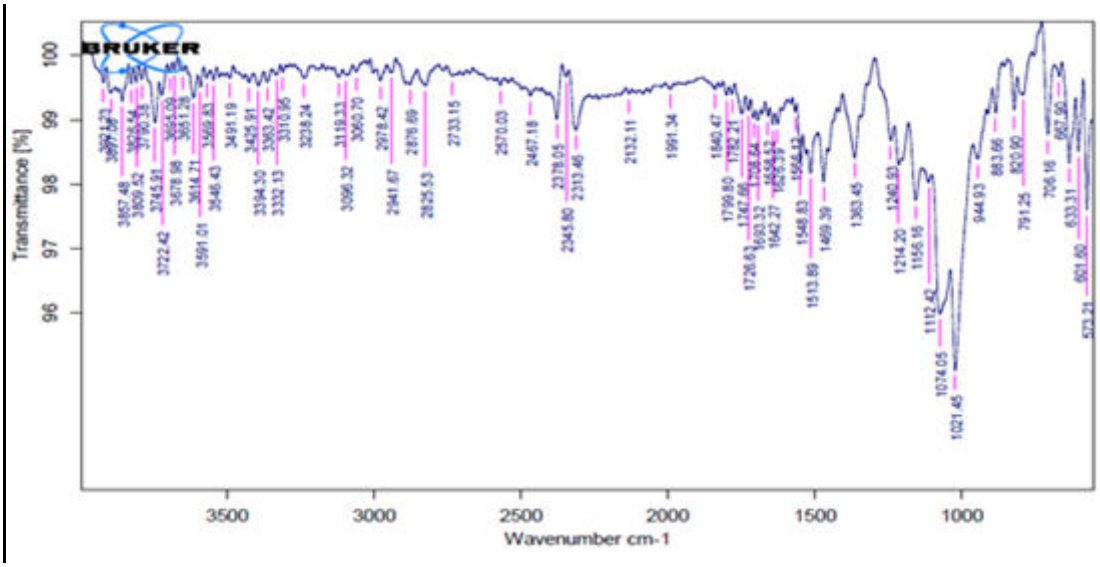


Fig. No.14: FT-IR Reports for CALCIUM DOBESILATE with Eudragit L100 formulation:

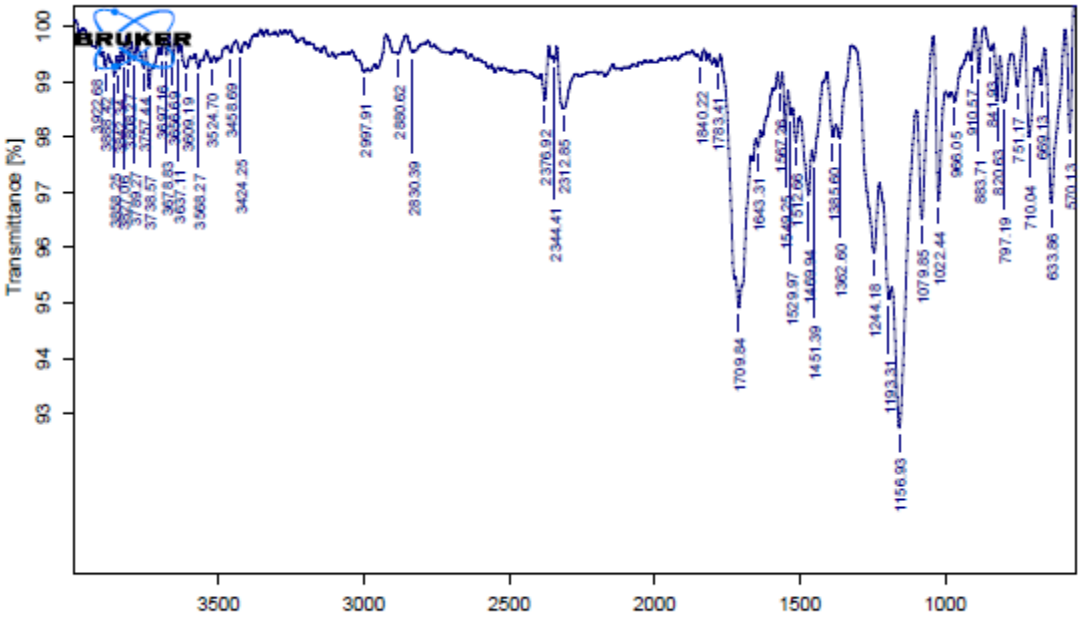


Fig. No.15: FT-IR Reports for CALCIUM DOBESILATE with Ethyl cellulose formulation:

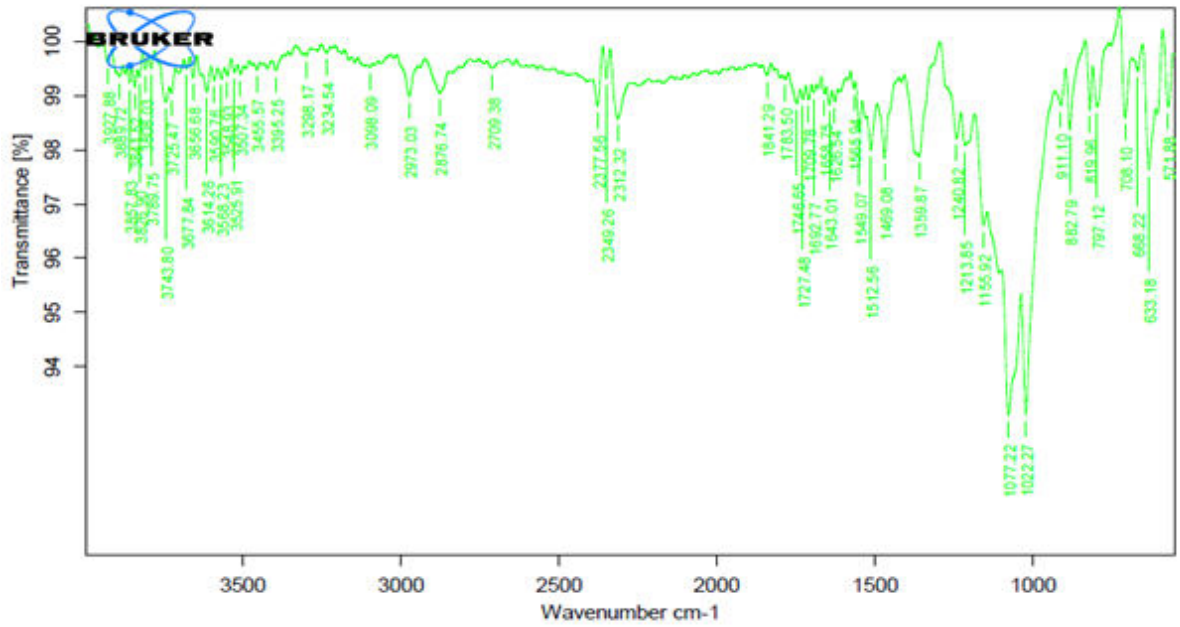
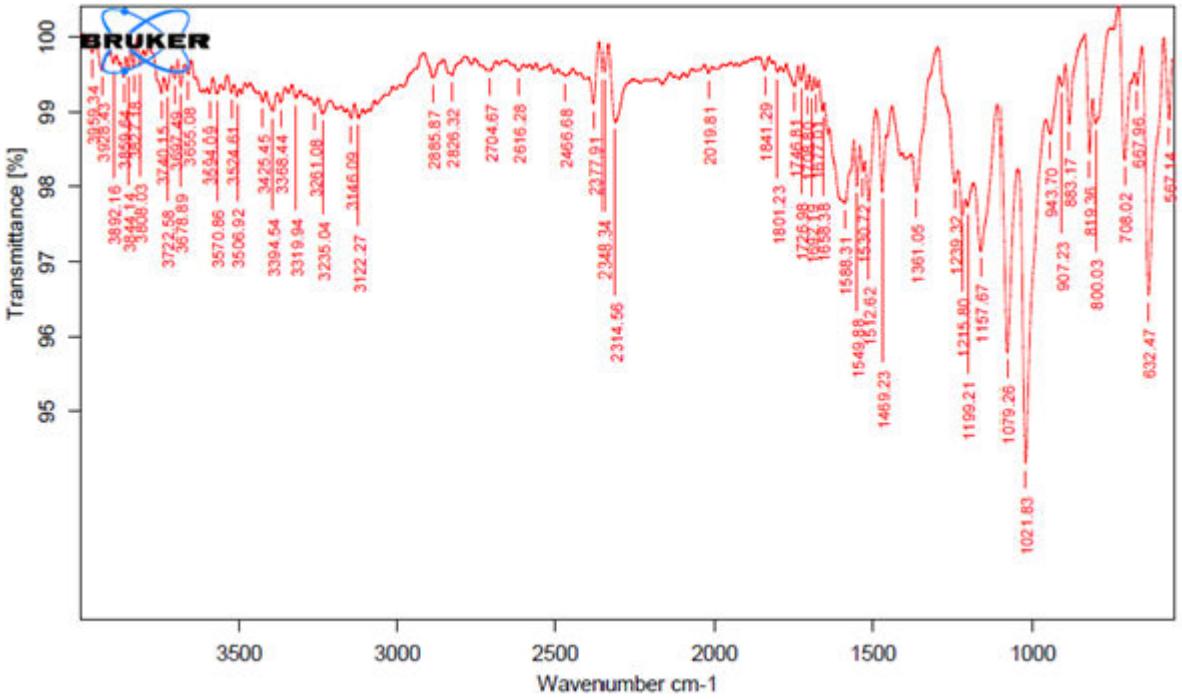


Fig. No.16: FT-IR Reports for CALCIUM DOBESILATE with Sodium alginate:

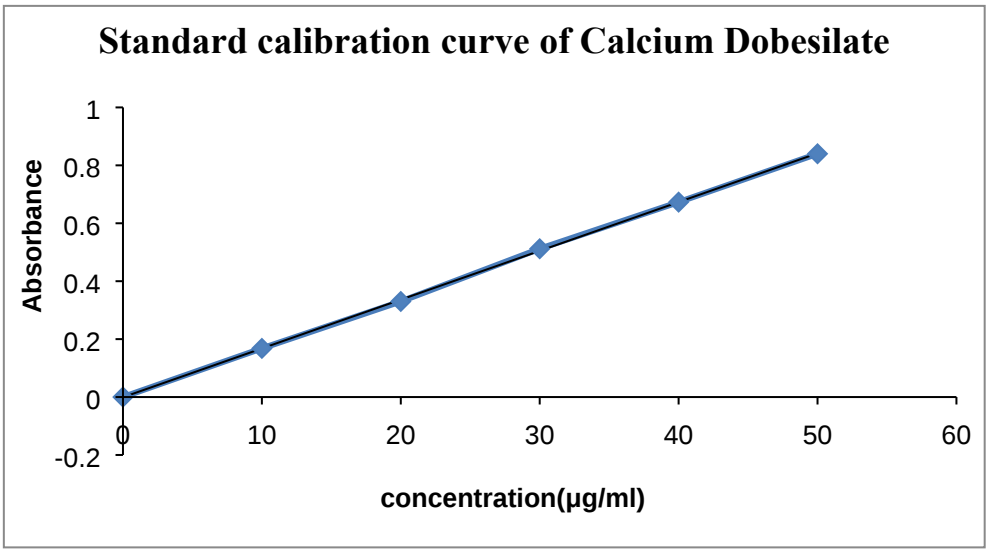


4.Analytical method:

Table No.16: Calibration curve values for estimation Calcium Dobesilate in 6.8 PH phosphate buffer:

S.no	Concentration (µg/ml)	Absorbance
0	**0**	**0**
1	10	0.168
2	20	0.330
3	30	0.512
4	40	0.673
5	50	0.840

Fig. No.17:Standard calibration curve of Calcium Dobesilate in 6.8pH at 302nm:



5.Evaluation of microspheres:

Percentage Yield, Entrapment Efficiency and Mean Particle size of Calcium Dobesilate microspheres:

Table No.17: Percentage Yield, Entrapment Efficiency and Mean Particle size of Calcium Dobesilate microspheres from formulation F19 to F24:

Batch code	Percentage yield(%)	Entrapment efficiency	Rounded mean particle size (µm)
F19	28.52	20.71	25.7
F20	55.10	35.92	27.4
F21	80.11	73.15	29.3
F22	64.01	50.20	24.1
F23	20.02	3.2	18.2
F24	33.20	12.05	23.3

Fig. No.18: Mean Particle size of Calcium Dobesilate microspheres from formulation F19 to F24:

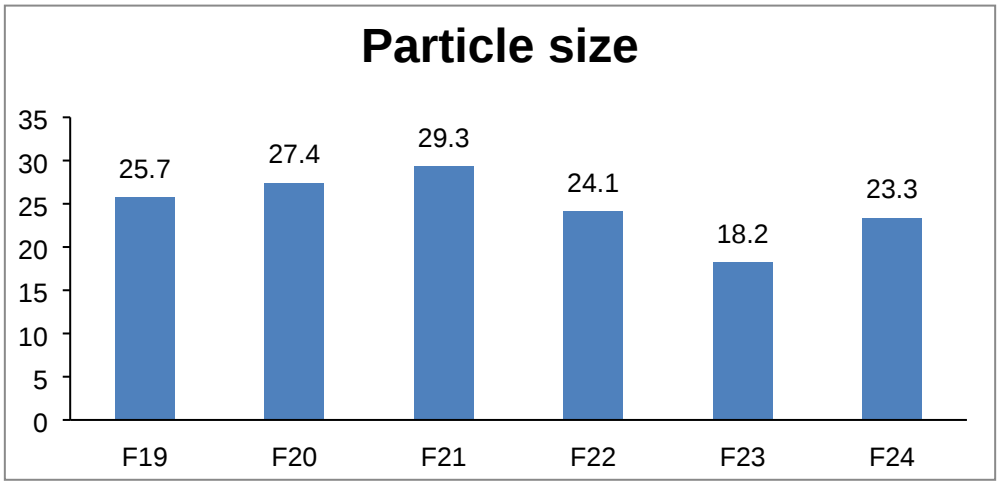
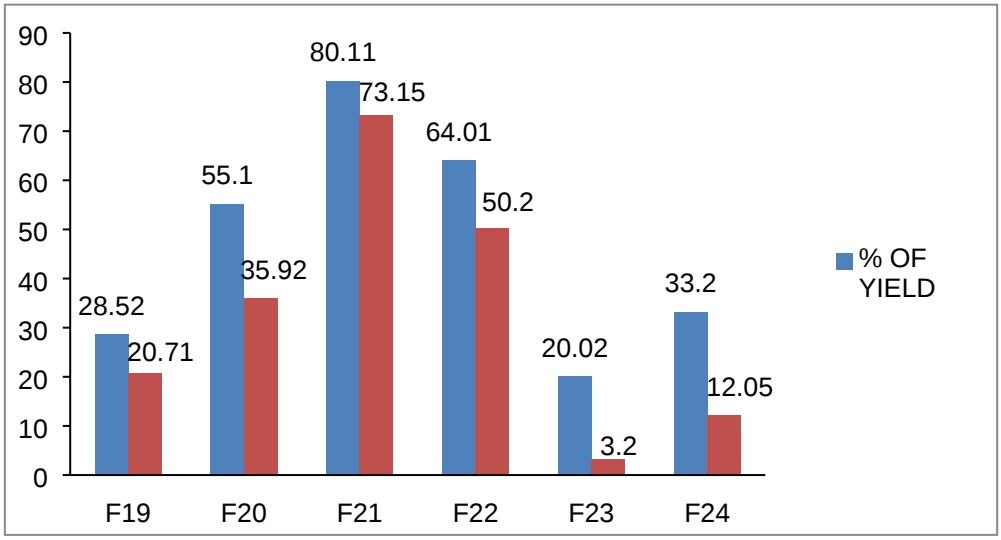


Fig. No.19: % of Yield, Entrapment efficasy of Calcium Dobesilate microspheres from formulation F19 to F24:



6.In-vitro drug release studies

Table No.18: % drug release of Calcium Dobesilate Microspheres:

Time (hr)	F19	F20	F21	F22	F23	F24
0	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
1	7.1	9.7	10.9	9.4	18.3	16.7
2	11.4	10.9	15.4	12.5	29.3	27.4
3	16.3	15.1	20.2	16.1	34.4	35.1
4	19.3	19.1	24.1	20.1	40.9	45.3
6	22.3	29.2	33.2	28.4	58.2	53.1
8	25.4	37.3	41.9	42.8	78.2	65.3
10	33.1	49.3	49.2	50.7	90.5	76.2
12	39.7	62.4	55.4	59.4	96.2	86.1
14	47.2	71.3	70.4	71.7	_	97.8
16	51.3	75.2	79.7	88.7	_	_
18	53.4	78.2	86.9	97.1	_	_
20	55.2	_	91.1	_	_	_
22	59.3	_	96.4	_	_	_
24	61.2	_	98.2	_	_	_

Fig. No.20: % OF DRUG RELEASE OF CALCIUM DOBESILATE:

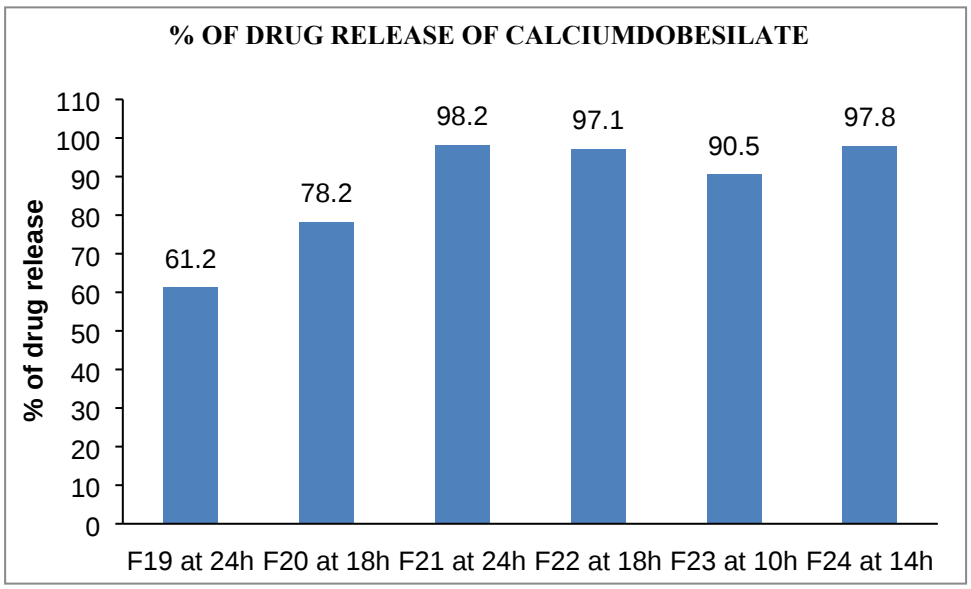


Fig. No.21: % OF DRUG RELEASE OF CALCIUM DOBESILATE:

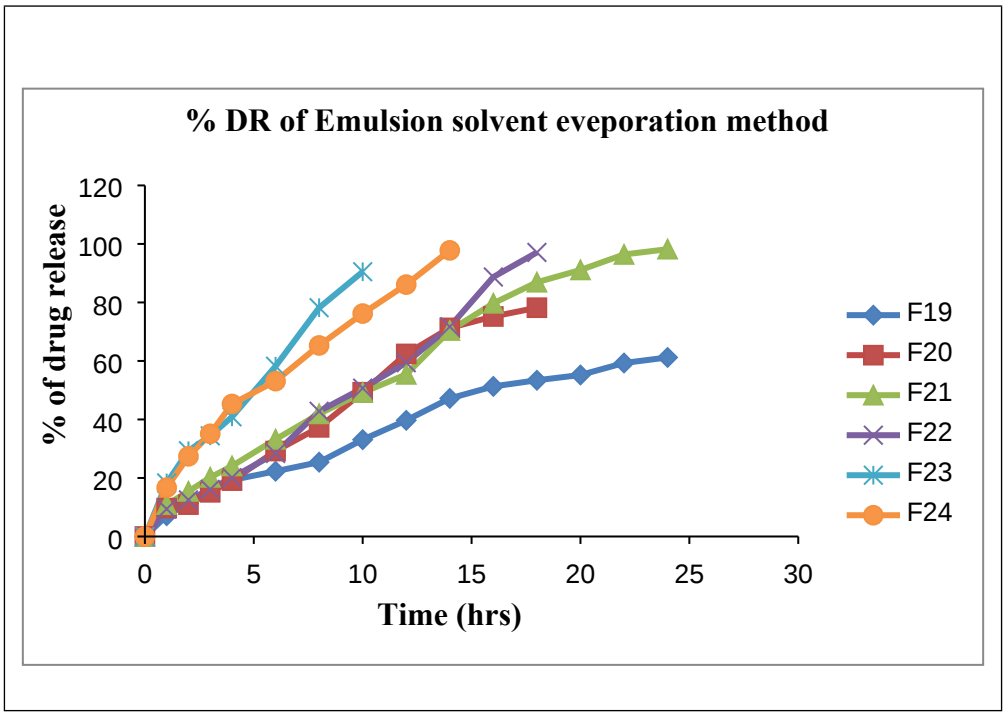


Fig No.22: Percentage drug release of optimized formula F21:

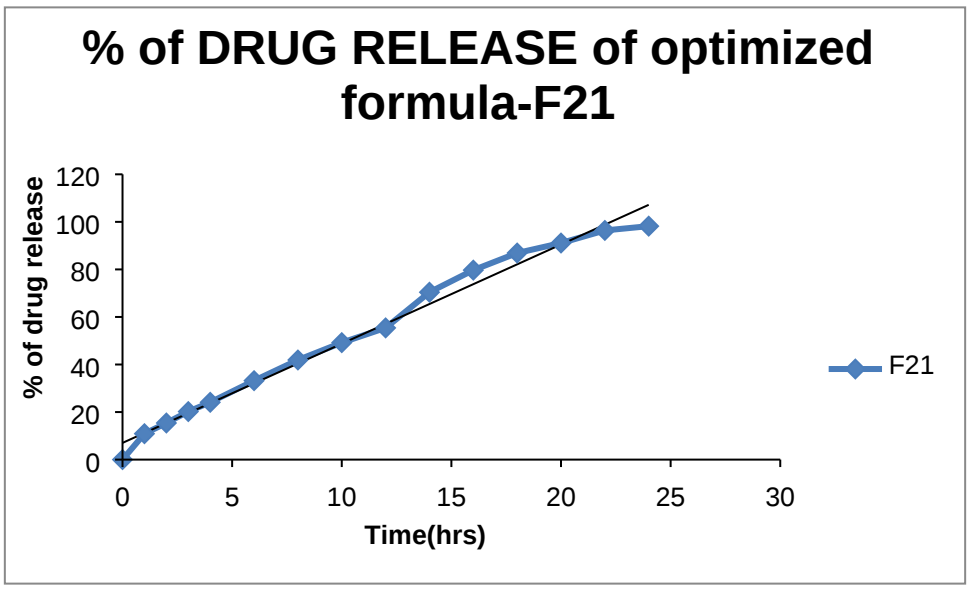


Fig. No.23: Zero order release of optimized formula F21:

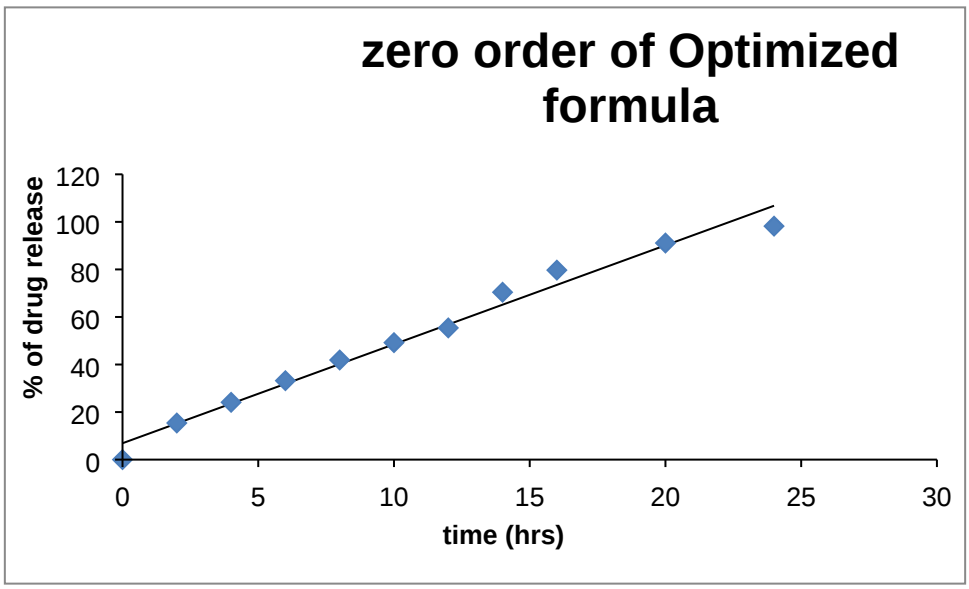


Fig.No.24: Peppas mechanism of optimized formula F21:

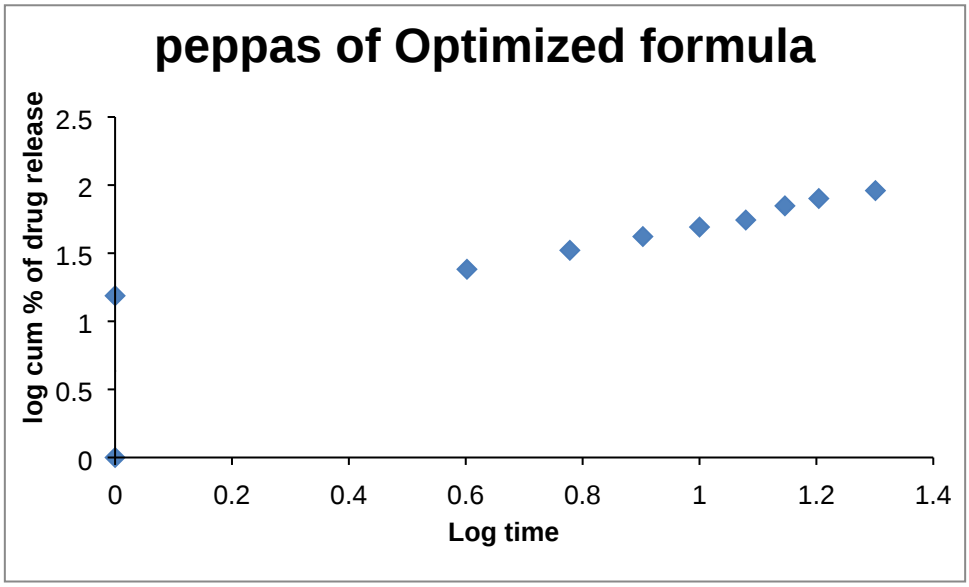


Fig. No.25: 1st Order of optimized formula F21:

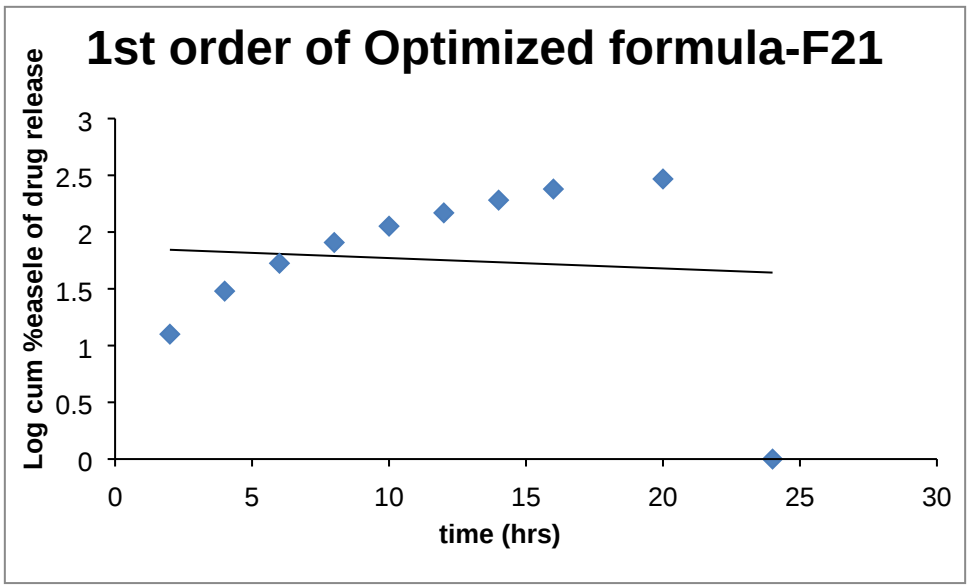


Fig. No.26: Higguchi mechanism of optimized formula F21:

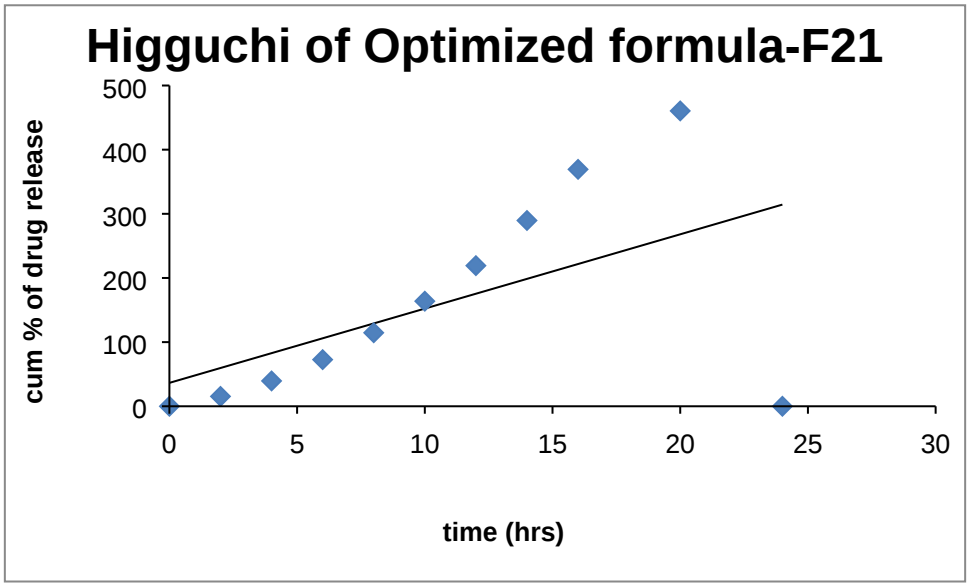
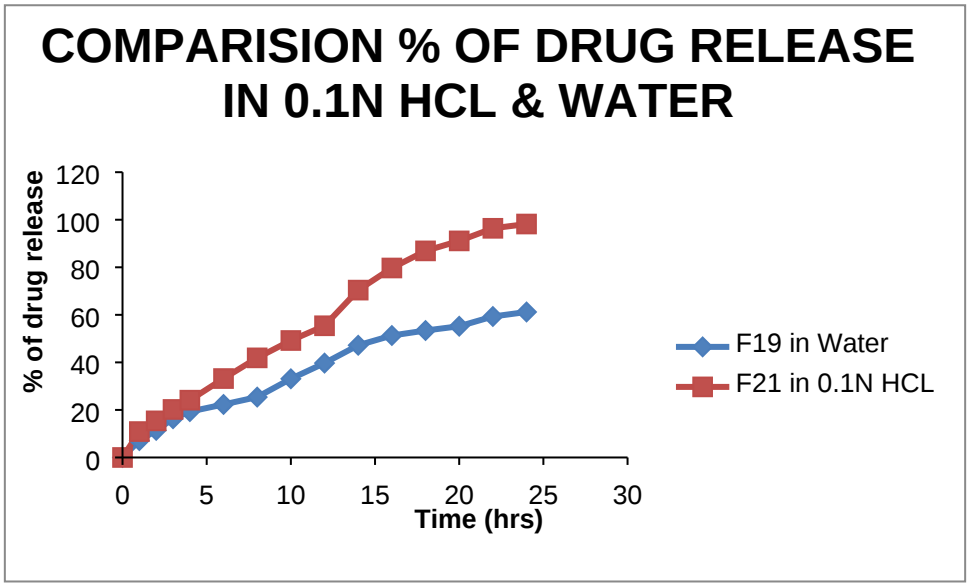


Fig. No.27: COMPARISION OF % OF DRUG RELEASE OF CALCIUM DOBESILATE:



The present research work is an attempt made to Formulation And Evaluation Of Calcium Dobesilate Microspheres for the treatment of Chronic Venous Disease by using Ionic Gelation and Emulsion Solvent Evaporation methods, Emulsification Ionic Gelation methods. For preparation of these methods by using different polymers like HPMC K100, Eudragit

L100, Ethyl Cellulose, Sodium Alginate, HPMC K4M were used. Based on the investigational reports we concluded the following results and discussions.

Preformulation Studies:

The reports indicate the exhibit good and passable flow properties. So there is no need to improve the flow of the powder. The regression value obtained from analytical method development in buffer media is 0.999, So, the drug is exhibiting linearity in concentration 10µg to 50 µg. The FT-IR spectral studies indicate good stability and no chemical interaction between the drugs and excipients used.

Identification of Calcium Dobesilate:

In identification of API it was found that Calcium Dobesilate was soluble in Methanol, Ethanol, Water .

Solubility studies:

Table No.19:Solubility of Calcium Dobesilate :

Calcium Dobesilate is soluble in methanol, ethanol, water

S.NO	Solubility media	Descriptive term
1	**Water**	Soluble 100 mg per 1ml
2	**Methanol**	Soluble 100mg per 3ml
3	**Ethanol**	Soluble 100 mg per 1ml
4	**ph 6.8 buffer**	Soluble100 mg per 10 ml

Melting Point:

It was also found Melting point of Calcium Dobesilate was 247^0c,

Density:

On analyzing for density it was found that Calcium Dobesilate showed bulk density value 0.91gm/ml and tapped density value 1.04 gm/ml,

Carr's Index:

The value of Carr's Index for 16.5 and drugs showed good flow characteristics

Compressibility Index:

The value of Compressibility Index for was 14.28, and drugs showed good characteristics.

Hausner's ratio:

The value of Hausner's ratio for Calcium Dobesilate was 1.14 drugs showed good flow characteristics.

Angle of Repose:

The studies on angle of repose showed that was 15.41 values indicated good flow properties.

Stability studies:-

FTIR studies:

From the FTIR spectra, it was concluded that similar characteristic peaks with minor difference for the drug and their formulation. Hence, it appears that there was no chemical interaction between the drugs and excipients used**.** The IR Spectra of Calcium Dobesilate with HPMC - K100, Eudragit Rs100, Ethyl cellulose, Sodium Alginate. The peaks were observed in as well as Calcium Dobesilate with excipients.

EVALUATION OF MICROSPHERES:

Determination of % yield:

The percentage yield was estimated from all the 18 formulations the results obtained between the range 80 % to 12 %.

Drug entrapment efficiency:

The drug entrapment of all formulation of Calcium Dobesilate microspheres varied from 73% to 12%

Particle size analysis:

The particle size of the microspheres of the Calcium Dobesilate formulations varied from 18 µm to 29 µm.

In vitro drug release studies:

The formulation F1 to F16 contains Sodium Alginate is polymer (1 - 6%) & Calcium Chloride is cross linking agent (20 - 40%) are prepared by Ionic Gelation method. Here we observed, the sphears are slowly dispersed in cross linking agent. May be the reason is Calcium- Calcium interaction & sodium alginate is incompatible with drug. Here very low yield value is observed. So dissolution studies were not conducted.

The formulation F17 containing 8% Sodium Alginate, Calcium Chloride containing 6 %. This method is prepared by Emulsification Ionic Gelation Method. Here emulsifying agent is used as Coconut Oil. Here also Microspheres are formed but low yield value is observed, So dissolution studies were not conducted.

The formulation F18 that containing 1g of Sodium Alginate, Calcium Chloride containing 6 % & HPMC K100 1g. This method is prepared by Emulsification Ionic Gelation Method. Here emulsifying agent is used as Ground nut Oil. Here also Microspheres are formed but very low yield because of Calcium- Calcium interaction is there & clanging of oil. So dissolution studies were not conducted.

The formulations F19 - F24 are prepared by Emulsion Solvent Evaporation method. The F19 contains EC 2.250g & HPMC K100 750mg. The volatile organic solvents are DCM, Ethanol & water is used as medium. The yield value was 28% & the drug content is 20%. Here Microspheres are formed but low yield value was observed.

The F20 contains EC 2.250g & Eudragit 750mg. The volatile organic solvents are DCM, Ethanol & 0.1N HCL is used as medium. The yield value was 55% & the drug content is 35.9%. The dissolution studies are 78.2% at 18 hrs. This formula is not reached up to the mark.

The F22 contains HPMC K100 750mg & Eudragit 2.250g. The volatile organic solvents are DCM, Ethanol & 0.1N HCL is used as medium. The yield value is 64% & the drug content is 50.2%. The dissolution studies are 97.1% at 18 hrs. This formula is not reached up to the mark because the yield value is half of the total amount of the formula.

The F23 contains EC 500mg & HPMC K_4M 500mg. The volatile organic solvents are DCM, Ethanol & 0.1N HCL is used as medium. The yield value is 20% & the drug content is 3.2%. Here the yield value is very low. This formula is failed to reached the object.

The F24 contains EC 1g & HPMC K4M 1g. The volatile organic solvents are DCM, Ethanol & 0.1N HCL is used as medium. The yield value is 33% & the drug content is 12%. This formula is not reached up to the mark because the yield value low. It is also not fulfil the object.

The F21 contains EC 1.5g & Eudragit L100 1.5g. The volatile organic solvents are DCM, Ethanol & 0.1N HCL is used as medium. The yield value is 80% & the drug content is 73%. Here we got high yield value & drug entrapment efficasy also increased. The dissolution studies are 98.2% at 24 hrs This formula is reached up to the mark . It is fulfil the object.

Finally among all the formulations F21 is full fill the object.

Effect of method of preparation

- Ionic Gelation
- Solvent evaporation

1. **On particle size :**

From formulations F1to F16 are by Ionic Gelation method.

From formulations F17-F18 are by Emulsification Ionic Gelation method.

From

F1-F18 - low yield value & highly swelling observed because of drug is incompatible with Sodium Alginate & calcium - calcium interactions.

formulations F19 to F24 are by Emulsion Solvent Evaporation method, are in desired particle size range i.e is 18μm - 29 μm.

So that Spheres obtained are smaller than by Ionic Gelation method.

2. **On Drug release:**

Drug release is controlled in formulations prepared by Emulsion solvent evaporation than Ionic Gelation method and emulsification ionic Gellation method

Therefore finally Emulsion Solvent Evaporation Method is selected to fulfil the object.

Effect of polymers

HPMC K100, Ethyl Cellulose, Eudragit L100, Sodium Alginate are 4 different polymers used as controlled release polymers.

Formulations F1 - F17 are prepared by Orifice Ionic Gelation method with single polymer that is Sodium Alginate, where as F18 is prepared by Emulsification Ionic Gelation method with dual polymers they are Sodium Alginate, HPMC K100 respectively.

Remaining all formulations F19 - F24 are combination of two each polymers of respective ratios.

HPMC K100 is hydrophilic, Ethyl cellulose is hydrophobic nature. Combination of Hydrophilic and Hydrophobic polymers is tested at different ratios to determine effect on controlled drug release.

Also HPMC K100 with coating polymer Eudragit L100 combination is tested at different ratios.

Among all these ,F21 i.e, with Eudragit L100, Ethyl Cellulose by solvent evaporation method shows desired drug release compared to all other formulations.

Therefore it is concluded that there is an effect of combination of polymers(Eudragit L100, Ethyl Cellulose) on controlled drug release of Calcium Dobesilate microspheres.

Kinetics of drug release from optimized formulation

The kinetics of the drug release was evaluated by drug release rate models namely zero order, First order. The mechanisms of drug release was evaluated by Zero order drug release & followed Pappas mechanism.

8.SUMMARY AND CONCLUSION

The present investigations were Formulation And Evaluation Of Calcium Dobesilate Microspheres for the Treatment of Chronic Venous Disease was developed to prolong action.

The summary and conclusions of investigations is as follows

1. The present study was carried out to design the controlled release microspheres for the Calcium Dobesilate for treatment of Chronic Venous Disease.
2. The microspheres were formulated for controlled release by using different polymers like HPMC K100, Eudragit L100, Ethyl Cellulose in different ratios was found to control and stable drug release.
3. Sodium Alginate & Calcium Chloride is prepared by Ionic Gelation method. Here Calcium- Calcium interacted & sodium alginate is incompatible with drug. So spheres are not formed.
4. The use of Ethyl Cellulose and Eudragit L100 polymer makes a controlled release of Calcium Dobesilate microspheres with dissolution mechanism.
5. By using the enteric polymer Eudragit L100, increases the drug entrapment efficasy & yield value in 0.1N HCL than water. The reason is Eudragit L100 is insoluble in 0.1 n HCL.
6. These concept is explained the application of fixed dose dosage form which results in cost –effectiveness and reduce multiple of dosage forms.
7. From the above observations it is concluded that by Emulsion Solvent Evaporation Technique Formulation F21 was found 98.2 % at 24 hrs drug release
8. The release characteristics of the formulation appear as to follow F21 shows near zero order drug release and Zero order- Pappas mechanism.
9. Among all the techniques the best method was found Emulsion Solvent Evaporation method for Calcium Dobesilate.

9.BIBLIOGRAPHY

1. Chen WY, Rogers AA. Recent insights into the causes of chronic leg ulceration in venous diseases and implications on other types of chronic wounds. Wound Repair Regen. 2007 Jul;15(4):434-449.
2. Kahn SR. The post-thrombotic syndrome: progress and pitfalls. Br J Haematol. 2006 Aug;134(4):357- 65. Epub 2006 Jul
3. Patel NP, Labropoulos N, Pappas PJ. Current management of venous ulceration. Plast Reconstr Surg. 2006 Jun;117(7 Suppl):254S-260S
4. Rathbun SW, Kirkpatrick AC. Treatment of chronic venous insufficiency. Curr Treat Options Cardiovasc Med. 2007 Apr;9(2):115-26.
5. Wollina U, Abdel-Naser MB, Mani R. A review of the microcirculation in skin in patients with chronic venous insufficiency: the problem and the evidence available for therapeutic options. International Journal of Lower Extremity Wounds. 2006 Sep;5(3):169-80.
6. Renner R, Gebhardt C, Simon JC, Seikowski K. Changes in quality of life for patients with chronic venous insufficiency, present or healed leg ulcers. *J Dtsch Dermatol Ges*. Nov 2009;7(11):953-61.
7. Zaniewski M, Simka M. Biophysics of venous return from the brain from the perspective of the pathophysiology of chronic cerebrospinal venous insufficiency. *Rev Recent Clin Trials*. May 2012;7(2):88-92.
8. Bryan LJ, Callas PW, Criqui MH, Cushman M. Higher soluble P-selectin is associated with chronic venous insufficiency: The San Diego Population Study. *Thromb Res*. Aug 11 2012.
9. Carrasco OF, Ranero A, Hong E, Vidrio H. Endothelial function impairment in chronic venous insufficiency:effect of some cardiovascular protectant agents. *Angiology*. Dec-2010 Jan 2009;60(6):763-71.
10. Coon WW, Willis PW 3rd, Keller JB. Venous thromboembolism and other venous disease in the Tecumseh community health study. *Circulation*. Oct 1973;48(4):839-46.
11. Schultz-Ehrenburg U, Weindorf N, Matthes U, Hirche H. [An epidemiologic study of the pathogenesis of varices. The Bochum study I-III]. *Phlebologie*. Nov-Dec 1992;45(4):497-500.

12. Chiesa R, Marone EM, Limoni C, Volonte M, Schaefer E, Petrini O. Chronic venous insufficiency in Italy: the 24-cities cohort study. *Eur J Vasc Endovasc Surg*. Oct 2005;30(4):422-9.
13. Abbade LP, Lastoria S, Rollo Hde A. Venous ulcer: clinical characteristics and risk factors. *Int J Dermatol*. Apr 2011;50(4):405-11.
14. Tsai S, Dubovoy A, Wainess R. Severe chronic venous insufficiency: magnitude of the problem and consequences. *Ann Vasc Surg*. 2005;19:705-11.
15. Racette S, Sauvageau A. Unusual sudden death: two case reports of hemorrhage by rupture of varicose veins. *Am J Forensic Med Pathol*. Sep 2005;26(3):294-6.
16. Evans GA, Evans DM, Seal RM, Craven JL. Spontaneous fatal haemorrhage caused by varicose veins. *Lancet*. Dec 15 1973;2(7842):1359-61.
17. Casian D, Gutsu E, Culiuc V. Surgical treatment of severe chronic venous insufficiency caused by pulsatile varicose veins in a patient with tricuspid regurgitation. *Phlebology*. Apr 2009;24(2):79-81.
18. Gasparis AP, Tsintzilonis S, Labropoulos N. Extraluminal lipoma with common femoral vein obstruction: a cause of chronic venous insufficiency. *J Vasc Surg*. Feb 2009;49(2):486-90.
19. Bonnetblanc JM. Leg ulcerations: a clinical appraisal. *Eur J Dermatol*. May-Jun 2005;15(3):127-32.
20. Morales-Cuenca G, Moreno-Egea A, Aguayo-Albasini JL. [General surgeons and varicose vein surgery]. *CirEsp*. Apr 2009;85(4):205-13.
21. Van den Berg PJ, Visser LH. Extra- and transcranial echo colour Doppler in the diagnosis of chronic cerebrospinal venous insufficiency. *Phlebology*. Mar 2012;27 Suppl 1:107-13.
22. Lattimer CR, Azzam M, Kalodiki E, Makris GC, Geroulakos G. Saphenous pulsation on duplex may be a marker of severe chronic superficial venous insufficiency. *J Vasc Surg*. Jul 11 2012;
23. [Guideline] Gloviczki P, Comerota AJ, Dalsing MC, et al. The care of patients with varicose veins and associated chronic venous diseases: clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum. *J Vasc Surg*. May 2011;53(5 Suppl):2S-48S.
24. Zhan HT, Bush RL. A review of the current management and treatment options for superficial venous insufficiency. *World J Surg*. Oct 2014;38(10):2580-8.

25. Vanscheidt W, Ukat A, Partsch H. Dose-response of compression therapy for chronic venous edema--higher pressures are associated with greater volume reduction: two randomized clinical studies. *J Vasc Surg*. Feb 2009;49(2):395-402, 402.e1.
26. Couzan S, Leizorovicz A, Laporte S, Mismetti P, Pouget JF, Chapelle C, et al. A randomized double-blind trial of upward progressive vs degressive compressive stockings in patients with moderate to severe chronic venous insufficiency. *J Vasc Surg*. May 15 2012;
27. Ramos-González E, Moreno-Lorenzo C, Matarán-Peñarrocha GA, Guisado-Barrilao R,Aguilar-Ferrándiz ME,Castro-Sánchez AM. Comparative study on the effectiveness of myofascial release manual therapy and physical therapy for venous insufficiency in postmenopausal women. *Complement Ther Med*. Oct 2012;20(5):291-8.
28. Nijsten T, van den Bos RR, Goldman MP, et al. Minimally invasive techniques in the treatment of saphenous varicose veins. *J Am Acad Dermatol*. Jan 2009;60(1):110-9.
29. Zimmet SE. Venous leg ulcers: modern evaluation and management. *Dermatol Surg*. Mar 1999;25(3):236-41.
30. Sadick NS. Advances in the treatment of varicose veins: ambulatory phlebectomy, foam sclerotherapy, endovascular laser, and radiofrequency closure. *Dermatol Clin*. Jul 2005;23(3):443-55, vi.
31. Navarro TP, Nunes TA, Ribeiro AL, Castro-Silva M. Is total abolishment of great saphenous reflux in the invasive treatment of superficial chronic venous insufficiency always necessary?. *Int Angiol*. Feb 2009;28(1):4-11.
32. Maksimovic ZV, Maksimovic M, Jadranin D, Kuzmanovic I, Andonovic O. Medicamentous treatment of chronic venous insufficiency using semisynthetic diosmin--a prospective study. *Acta Chir Iugosl*. 2008;55(4):53-9.
33. Encyclopedia of pharmaceutical technology, Vol. 10, pg: 1-30(1998).
34. Edith Mathiowitz (Edited by). Encyclopaedia of Controlled Drug Delivery, vol. 2.
35. HPMC K100 polymer information From Wikipedia, the free encyclopedia.
36. Indian pharmacopeia
37. United states Pharmacopeia
38. Raymond C Rowe et.al , 6th edition, Hand Book Of Pharmaceutical Excipients
39. Encyclopedia of pharmaceutical technology, Vol. 10, pg: 1-30(1998).
40. Vikrant K Nikam, Pharmacology online 1: 152-164 (2011), EUDRAGIT A VERSATILE POLYMER.

41. Raymond CR, Paul JS, Paul JW. *Hand book of pharmaceutical excipients. 4th Edn,.Pharmaceutical press. Royal Pharmaceutical Society Great Britain*;pg:462-467 (2003).
42. Harwood,R.J."Hypermelose" in Handbook of pharmaceutical excipients, 4th ed., Raymond C. R., Paul, J. S. andPaul, J.W., Pharmaceutical Press, American Pharmaceutical association, London, U.K. pg: 297-300(2003)
43. Ainley wade and Paul J Weller, Handbook of pharmaceutical excipients, 2nd edition, The Pharmaceutical Press, London, ;pg: 186-190(1994)
44. A text book of Suresh P Vyas
45. A text book of NK Jain
46. Ghodake J.D., Vidhate J.S., Formulation and evaluation of floating Microspheres containing anti-diabetic (metformin hydrochloride) drug International Journal of PharmTech ResearchVol.2, No.1, pg: 378-384, Jan-Mar 2010
47. **Pathak Naresh,** Formulation & evaluation of floating microspheres of Lansoprazole, 2011, research article.
48. B.Senthil kumar, K.L. Senthil kumar, *Design and development of PACLITAXEL - loaded mcrospears for targeted drug delivery to the colon International Journal of Biomedical Research1* [2] [2010];pg:80-98(2010)
49. Bharat W Tekade, Umesh T Jadhao, Formulation and Evaluation of Metoclopramide Hydrochloride Sustained Release Microsphere. RRJPPS | Volume 3 | Issue 1 | January - March, 2014;pg; 22-31 (2014)
50. Ghodake J.D., Vidhate J.S., Formulation and evaluation of floating Microspheres containing anti-diabetic (metformin hydrochloride) drug International Journal of PharmTech ResearchVol.2, No.1, pg: 378-384, Jan-Mar 2010
51. Alireza Mahboubian, Seyyed Kazem Hashemein, Shadi Moghadam, Fatemeh Atyabi and Rassoul Dinarvand. Preparation and In-vitro evaluation of controlled release PLGA microparticles containing Triptoreline, Iranian Journal of Pharmaceutical Research, Vol. 9 (4);pg:369-378(2010).
52. Ana Rita, C. Duarte., Mariana Sousa Costa, Ana Luisa Simplicio, Maria Margarida Cardoso, Catarina, M.M Duarte. Preparation of controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs, International Journal of Pharmaceutics, Vol. 308 (1-2), pg:168–174(2006).

53. AppaRao, B., Shivalingam, M.R., Kishore Reddy, Y.V., Sunitha, N., Jyothibasu, T., Shyam, T. *Design and evaluation of sustained release microcapsules containing diclofenac sodium*, *International Journal of Pharmaceutical and biomedical research*, Vol. 1 (3), pg:90-93(2010).
54. Balasubramaniam,V., Jaganathan, K.S. *Development and characterization of hbsag loaded PLGA microspheres for muco-adhesive delivery systems*, *The Int. J. Pharm & Ind. Res.*, Vol. 1 (4), pg:283-288,(2011).
55. Laura C. Herrera., María V. Defain Tesoriero., and Laura G. Hermida. In Vitro release testing of PLGA microspheres with Franz Diffusion Cells Dissolution Technologies, pg: 6-11(2012).
56. Wu, X.S, Wang, N. *Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers*, *J Biomater. Sci. Polym.*, Vol. 12 (1), pg: 21-34(2001).
57. Yagnesh Bhatt and Dushyant Shah. *Influence of additives on Fabrication and Release from Protein loaded PLGA microparticles, Journal of Chemical and Pharmaceutical Research*, Vol. 4 (3), pg:1708-1715(2012).
58. Yilmaz Capan, Ge Jiang, Stefano Giovagnoli, Kyu-Heum Na, and Patrick P. DeLuca. Preparation and characterization of poly(D,L-lactide-co-glycolide) microspheres for controlled release of human growth hormone, *AAPS Pharm. Sci. Tech*., Vol. 4 (2), pg:147–156(2003).
59. Yoon Yeo and Kinam Park. Control of Encapsulation Efficiency and Initial Burst in Polymeric Microparticle Systems, Arch Pharm. Res., Vol. 27 (1),pg: 1-12(2004).
60. Lumir OH, Tomas R, Valery MD, Arieh M. Myrrh commiphora chemistry**.** Biomed Papers; 149(1):pg: 3–28(2005).
61. Hervt Allain, Safety of Calcium Dobesilate in Chronic Venous Disease, Diabetic Retinopathy & Haemorrhoids, 2004, review article
62. Hiteshkumar D Patel1, Calcium Dobesilate in the symptomatic treatment of hemorrhoidal disease: An interventional study:2013, review article
63. harshad parmar, different methods of formulation and evaluation of mucoadhesive microsphere, ijabptvolume: i: issue-3: nov-dec -2010.
64. kataria sahil1, microsphere:a review,international journal of research in pharmacy and chemistry, 2011, issn: 22312781