FORMULATION AND EVALUATION OF CLINDAMYCIN PHOSPHATE MICROSPONGE GEL FOR TOPICAL DRUG DELIVERY

By

RICHA KADIVAR

[Enrollment Number: 220521002]

Research Guide

Ms. Rachana Joshi

M.Pharm

Assistant Professor

A Thesis Submitted to ATMIYA UNIVERSITY in Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy in Pharmaceutics

NOVEMBER – 2024

Department of Pharmaceutics

School of Pharmaceutical Sciences

"Yogidham Gurukul", Kalawad Road, Rajkot – 360005

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CERTIFICATE

This is to certify that research work embodied in this thesis entitled **"Formulation and Evaluation of Clindamycin Phosphate microsponge gel for topical drug delivery"** was submitted by **Richa Kadivar (Enrollment No. 220521002)** at **Atmiya University, Rajkot** for partial fulfillment of M.Pharm degree to be awarded by Atmiya University, Rajkot. This research work was carried out under my supervision and is to my satisfaction.

Date: 21/11/24

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Signature and Name of Supervisor

Ms. Rachana Joshi

M.Pharm

Assistant Professor

Signature and Name of Dean

Dr. Kevinkumar Garala

M.Pharm, Ph.D

Associate Professor

COMPLIANCE CERTIFICATE

This is to certify that research work embodied in this thesis entitled **"Formulation and Evaluation of Clindamycin Phosphate microsponge gel for topical drug delivery"** was carried out by **Richa Kadivar (Enrollment No. 220521002)** at **Atmiya University, Rajkot** for partial fulfillment of M.Pharm degree to be awarded by Atmiya University, Rajkot. She has complied to the comments of Mid semester Reviewer to my satisfaction.

Date: 21/11/2024

Place: Rajkot

Signature and Name of Student

Richa Kadivar

(Enrollment No: 220521002)

Signature and Name of Guide

Ms. Rachana Joshi

M.Pharm

Assistant Professor

THESIS APPROVAL

This is to certify that research work embodied in this thesis entitled **"Formulation and Evaluation of Clindamycin Phosphate microsponge gel for topical drug delivery"** was carried out by **Richa Kadivar (Enrollment No. 220521002)** at **Atmiya University, Rajkot** is approved for the award of degree M.Pharm in Pharmaceutics by Atmiya University, Rajkot.

Date: 21/11/24

Place: Rajkot

Examiners:

1) Dr. Poiya Patel

2) Ms. Rachana Joshi

3) _____

DECLARATION

I hereby certify that I am the sole author of this thesis and that neither any part of the thesis nor the whole of the thesis has been submitted for a degree to any other University or Institution.

I certify that to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violet any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

I declare that this is a true copy of my thesis, including any final revisions, as approved by thesis review committee.

Date: 21/11/24

Place: Rajkot

RK

Signature of the Student

Name of Student: Richa Kadivar

Enrollment No. : 220521002

Signature of Guide

Name of Guide: Ms. Rachana Joshi

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Last but not the least, I pay my reverence to ***Atmiya University, Department of Pharmaceutical Sciences.*** *I am undeniably proud to be associated with this university.*

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RK.

NOVEMBER 2024

RICHA KADIVAR

Dedicated to my

Beloved Parents

and Brother

CONTENTS

ABSTRACT

Clindamycin is a potent lincosamide antibiotic against susceptible anaerobic bacteria and gram-positive aerobes. Furthermore, CLN also has anti-inflammatory activities. It is applied topically in the management of acne vulgaris.The current research was focused on gel loaded for the topical delivery to Clindamycin phosphate to overcome short half life problem of the drug and to minimize the side effects of drug with conventional gel. The FTIR study revealed that there was no interaction between drug and polymer and the combination is suitable for preparation of microsponges. Clindamycin phosphate loaded microsponges were prepared by oil in oil emulsion solvent diffusion method as the drug is highly water soluble and belongs to BCS class III. The microsponges were prepared by using ethyl cellulose as the polymer and incorporated into carbopol gel. The microsponges were evaluated for visual inspection, % yield, % drug content, % entrapement efficiency and particle size. The effect of formulation variables like stirring speed and drug:polymer ratio were evaluated on microsponges. The optimized formulation with drug:polymer ratio of 1:1, stirring speed of 1000rpm had the drug content of 78.69% and percent entrapement efficiency was found to be 87.94% The results concluded that as the drug:polymer ratio increases, drug content and entrapement efficiency decreases. The particle size of optimized batch of microsponges was found to be 37.44μm. The results of particle size analysis concluded that as the drug:polymer ratio increases, particle size increases. The microsponges of batch CLPA1-CLPA9 were subjected to in-vito drug release study by Franz diffusion cell apparatus. The batch showed the controlled release of 95.49% of drug release at 10 hrs. The results of in-vitro drug release showed that as the drug:polymer ratio increases, more controlled release formulation is obtained. The microsponges were incorporated into carbopol gel was evaluated for

visual inspection, pH, spreadability and stability of gel at room temperature for 1 month. The transparent white microsponge gel had the pH of 6.38 with spreadability of 7.05 g cm/s. The gel was found to be stable after 1 month. In-vitro antibacterial activity was observed for microsponge gel and compared with marketed 1%w/w CLP gel (Clindac A).

KEY WORDS: microsponges, acne vulgaris, oil in oil emulsion solvent diffusion, Clindamycin phosphate, short half life

LIST OF ABBREVIATIONS

Abs	Absorbance
Conc.	Concentration
°C	Degree centigrade
TDDS	Transdermal Drug Delivery System
TDS	Transdermal Delivery System
DDS	Drug Delivery System
FT-IR	Fourier Transform Infrared Spectroscopy
G	Gram
Hrs.	Hours
mg	Milligram
Fig	Figure
Min.	Minutes
RPM	Revolutions per minute
w/w	Weight by weight
MDS	Microsponge Delivery System
β-CD	Beta-cyclodextrin
BCS	Biopharmaceutical Classification System
CLP	Clindamycin Phosphate
CLN	Clindamycin
EC	Ethyl cellulose
μm	Micrometer
mm	Millimeter

ml	Milliliter
Sec	Second
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
PCS	Photon correlation spectroscopy
O / O	Oil in oil
PBS	Phosphate buffer Saline
HCL	Hydrochloric acid
pH	Negative logarithm of hydrogen ion concentration
B.E.T	Brunauer-Emmett-Teller
API	Active Pharmaceutical Ingredient
DSC	Differential scanning Calorimetry
ICH	International Council for Harmonisation
USP	United States Pharmacopeia
$t_{½}$	Half life
% CDR	percentage cumulative drug release
UV	Ultra violet
λ_{max}	Maximum wavelength
µg	Microgram
%	Percentage

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CHAPTER 1

1.0 INTRODUCTION

Delivery of drug is the method of administering a drug to attain a therapeutic outcome in human or animals. For many decades the acute and long-lasting illness are cured with pharmaceutical dosage form like tablet, pills, capsules, ointments, creams, liquids, injectable, suppositories, and gels, which are considered as conventional dosage forms.[1]

Conventional transdermal uses of gels are to discharge the drug upon application, which leads to accumulation and irritation of drug in layers of the skin. The active moiety can be applied directly to the skin, either in a substantially pure form or dispersed in liquid vehicle. Such direct application of active moiety leads to rapid evaporation of volatile active substance, extensively pure active form can often lead to unexpected increase in active moiety in the blood plasma levels, causing serious adverse reactions, such as allergic reactions and toxicity. The adverse reaction can be reduced by dilution of the drug in an appropriate liquid carrier, and moreover, further dilution of drug resulting in the reduction of the effectiveness of the final product. Therefore, precaution should be taken during dilution of drug, which may show subtherapeutic effect. [2]

For this above reason, it would be desirable to optimize the composition, so that the drug could be accomplished of providing prolonged delivery of finished products, while application of drug to the skin. Desirably, these delivery systems could control toxicity of active substance. Due to enzymatic degradation, and poor design module fails to reach systemic circulation for certain period of time,

whichfrequently results in poor patient compliance. This problem can be overwhelmed by design of novel drug delivery systems. [3]

Recently new approaches in the novel drug delivery systems introduced are:

- Oral Drug Delivery System (DDS)
- Mucosal DDS
- Nasal DDS
- Parenteral DDS
- Vaginal DDS
- Intrauterine DDS
- Ocular DDS
- Transdermal DDS

Among the various delivery systems, transdermal delivery of drugs is suitable which can increase bioavailability, decreases side effects, by pass hepatic first pass metabolism, and desirably shows local action.

1.1 TOPICAL DELIVERY

Topical delivery includes two basic types

- External topical delivery on to the skin (spread, sprayed, or dispersed)
- Internal topical delivery that are intended to apply on the mucosal membranes.

Several advanced modules were developed and adopted for delivering directly to the systemic circulation by transdermal drug delivery system (TDDS) using the skin as an effective surface area for entry. TDS has enhanced the efficacy of many drugs that are administered through skin with safety. For sustained and local action

of drugs on that skin and dermis, no efficient carrier system has been developed.Application of topically applied drugs can causes many problems such as unappealing, oiliness, tackiness, that results into lack of poor patient compliance. The high concentration of drug is required for its low efficiency of delivery system, and results into irritation, allergic reactions. Thus there is the necessity to prolong the drug release and to maximize amount of time for active ingredient to be present either on superficial layer of the skin or deeper layers.[4]

Delivering the drug from dosage form into the epidermis remains localized at that region, and allow drug enter into the blood with limited amount, it means of controlling side effects that are related to drugs. The need for delivery systems is to shows its action either on the skin or deeper layers of the skin, the drug molecule can penetrate by three potential ways into the layers of the skin as shown in Fig: 1.1.

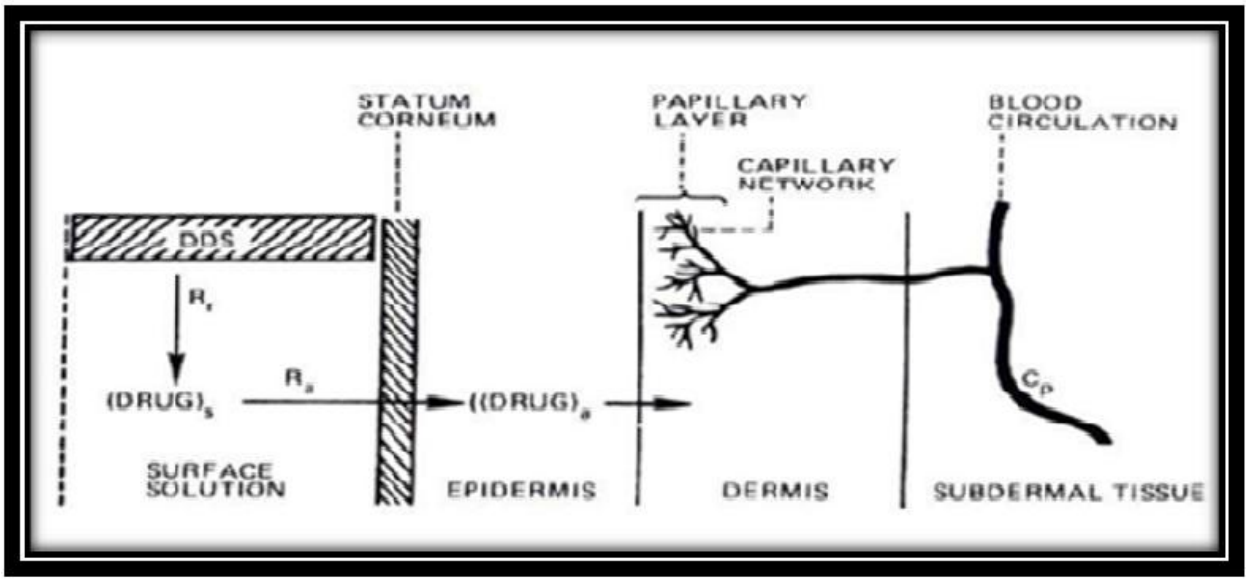


FIG: 1.1 Trans Appendgeal of Skin

There are three possible ways that drug molecules can pass through stratum corneum.

- Transfollicular route.
- Transcellular route.
- Intercellular route.[1]

To deliver the drug from transdermal route, carrier systems are essential for safe and effective delivery of active substance.

Table No: 1.1 Carrier Systems for Drug Delivery

Carrier systems	**Types**	
	Vesicular system	**Microparticulate system**
Colloidal carriers	Liposomes Niosomes Pharmacosomes Virosomes Immunoliposomes	Nanocapsules Nanoparticles Magnetic microspheres Microparticales Microsponges
Cellular carriers	Resealed erythrocytes, serum albumin, immunoglobulins, platelets, leucocytes	
Supramolecular delivery systems	Micelles, are composed of reverse, mixed, polymeric, liquid crystals, lipoproteins	
Polymer based system	Signal sensitive, muco-adhesive, bioerodible, soluble synthetic, polymer carrier.	
Macromolecular carriers	Mabs, fab fragments, proteins, viral envelops.	

1.2 MICROSPONGE: AN APPROACH FOR TOPICAL DELIVERY

1.2.1 Microsponge Technology

In recent years, micro particular drug delivery system has shown a great interest in the pharmaceutical area to improve novel microsponge based drug delivery systems, in order to adapt release behaviour of the drugs by incorporation into a carrier system. This can alter the therapeutic index, duration of the activity of drugs, and its therapeutic efficacy, with reduction of side effects. Carrier technology in the microparticulate system is adopted to solve the problems related to above challenges to achieve targeted and sustained release of drugs. Each microsponge comprises of interconnecting voids spaces, inside a non-collapsible structure, with large porous surface area. Microsponges belongs to polymeric delivery systems consisting of porous nature surrounded to microspheres, that can protect by entrapping wide range of active ingredients such as, anti-infective, anti-fungal, and anti-inflammatory agents. The microsponge technology was developed by Won in 1987, and the original patents were assigned to advanced polymer systems.[5]

1.3 STRUCTURE OF MICROSPONGES

The microsponges size can be varied from 5-300 μm in diameter, although the microsponge sphere can have upto 2,50,000 pores, this results in a larger surface area to diffuse the drug from each microsponge. The microsponge particles are relatively larger than skin pores as they can't go into the skin, rather they are entrapped in tiny hooks and stay in the layers of the skin. The slow release of entrapped drug takes place, this adds a point of safety to these microsponge by preventing bacterial contamination and bacterial cannot enter into the pores of the microsponges.[6]

In general, the release of drug from topical formulation over an extended period of time is difficult, the vehicles process high concentration of drug for effective therapy because of their low efficiency in designing the module, resulting in devoid of allergic reactions and irritation, and upholding efficacy. Their high grade of cross linking result in particles that are insoluble, inert of sufficient strength to stand up to the high shear and stress, commonly used in manufacturing of creams, lotion, and powder. [7]. The typical structure of microsponge is shown in Fig. 1.2.

Their characteristic feature is the capacity to adsorb an active material into the particles and on its surface. Its capacity for entrapment is up to three times of its weight. [8].

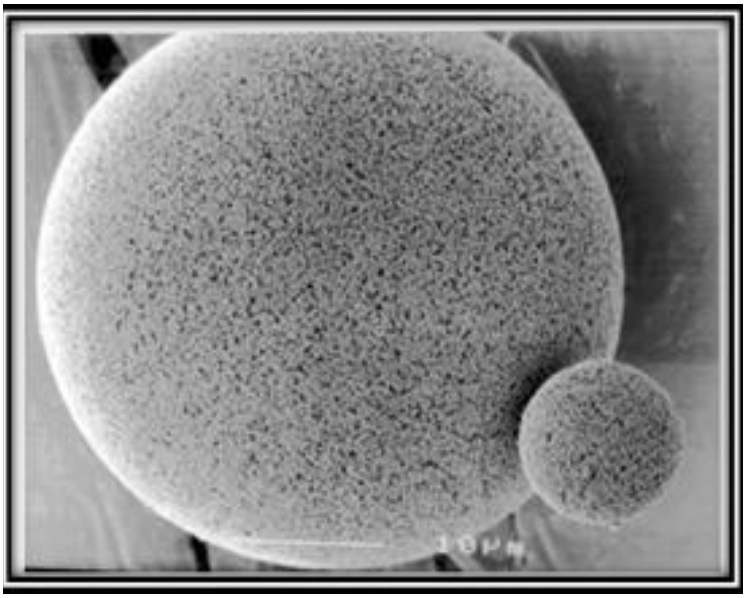


Fig: 1.2 Typical Structure of Microsponges

Table No: 1.2 Drugs Delivered using Microsponges

Category	Drug(s)
Antifungal	Terbinafine, Fluconazole, Itraconazole,Miconazole nitrate, Luliconazole Tioconazole
Anti-bacterial	Mupirocin
Anti-inflammatory	Ibuprofen, Paracetamol, Curcumin, Flurbiprofen, Ketoprofen, Diclofenac, Lornoxicam, Indomethacin, Etodolac
Antiviral	Acyclovir
Anti-cholinergic	Dicyclomine
Anti-pyretic	Paracetamol
Anti-acne treatment	Benzoyl peroxide, Tretinoin
Antibiotic	Erythromycin, Clindamycin

1.4 MECHANISM OF ACTION OF TYPICAL MICROSPONGES

The selected drug is added to the continuous vehicle as its solubility is merely soluble. The microsponges have an effective porous nature where the drug is allowed to move in and out from the pores of the microparticles and into the vehicle until the equal concentration is achieved. When the vehicle becomes saturated with the microsponge then the diffusion is stopped. Due to this typical property of the formulated microsponges are applied superficially to the skin, the active drug that is there in the vehicle will be absorbed first into the skin where continuous vehicle can deliver the initial dose and followed by prolonged release. For that initial therapy, the vehicle should be treated with the drug, when the drug is released into the vehicle from the microsponges. As, microsponges are scattered into the vehicle, it delivers the drug to diffuse out.[9]. The mechanism of drug release from microsponges shown in Fig. 1.3.

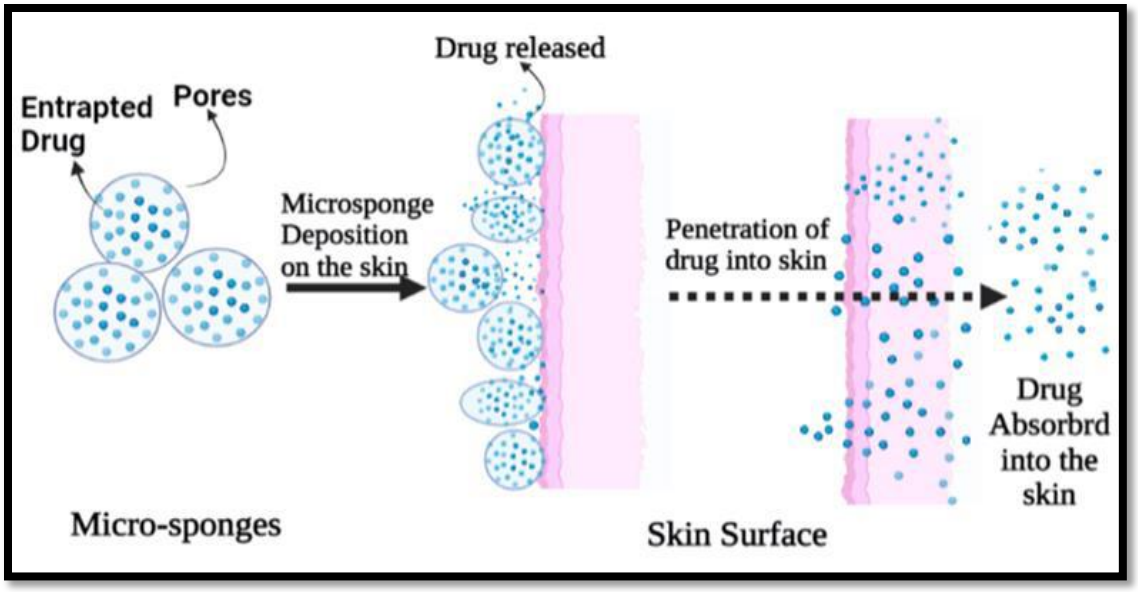


Figure 1.3 : Mechanism of drug release from microsponges

Release mechanisms of microsponges:

- Pressure – rubbing or pressure applied will release active ingredients from microsponge onto the skin.
- Temperature – with increased skin temperature, the rate of flow is increased and thus release is enhanced.
- pH – triggering the pH – based release of the active ingredient is occurred by modifying the coating on the microsponges.
- Solubility – microsponges loaded with hydrophilic active ingredients like antiseptics, deodorants amd antiperspirants will be discharged in the presence of liquid media. Microsponges releases drug in the presence of water.[10]

1.5 POLYMERS USED IN MICROSPONGE PREPARATION

Usually monomers like styrene, di vinyl benzene, ethyl vinyl benzene and methyl methacrylate are engaged in liquid-liquid suspension polymerization technique. Whereas, Eudragit RS-100 and Carbopol were employed for quasi emulsification technique. Eudragit polymers are copolymers synthesis from esters of acrylic and methacrylic aids, whose physicochemical properties are recognized by functional groups. Eudragit RS100 polymer is available in a wide range of various physical forms. Eudragit RS100 is employed for quasi emulsification technique**.**Internal phase consists of Eudragit RS 100 in organic solvent and external phase consists of polyvinyl alcohol in water.[3]

1.6 FORMULATION CONSIDERATION

Following considerations are taken into brief explanation in order to achieve desired finished product. The solubility of drugs must be limited in the vehicle; otherwise the encapsulation is major problem. Formulation of microsponges much be integrated more than 10 to 12 % w/w to escape cosmetic problem. Optimizations of polymer are studied to incorporate desired concentration levels of active into the microsponges to attain required release for given period [11].

1.7 METHOD OF PREPARATION OF MICROSPONGES

Based on the preparation of microsponges, method is divided in to two ways:

i) One stage process or liquid-liquid suspension polymerization

ii) Two stages process or quasi-emulsion diffusion

1.8 EFFECT OF FORMULATION VARIABLES ON PHYSICAL PROPERTIES OF MICROSPONGES

1.8.1 Composition of internal and external phase and their effects

The particle size of microsponges were directly proportional to the viscosity of dispersed phase. Greater the difference among viscosity of dispersed (internal phase) and continuous phase (external phase), the internal phase volume has higher viscosity, can effects in the globules size formed, resulting in an increase in mean particle size. Microsponges can be produced when 3 to 5 ml of the internal phase is utilized, microsponges can be influenced by internal phase volume. As the measure of the internal phase increases from 5 to 15 ml, it shows the decreased impact on percentage yield and drug content of microsponge, this is because of the greater concentrations of internal phase volume and lower concentration of the drug.[12].

1.8.2 Effect of drug to polymer ratio

When the quantity of polymer is kept constant but the drug to polymer ratio is varied, the drug containing capacity is not much effected by drug to polymer ratio but the production of microsponges yield can be extremely changed from minimum ratio to maximum one. Another parameter is achieved from the drug to polymer ratio change is particle size. Particle size of the microsponges increases as amount of drug increased. [13].

1.8.3 Effect of stirring rate in the formation on the physical properties of microsponges

As the stirring rate has influence on size of the microsponges are obtained, increase in the stirring rate decrease the production yield but the drug content increased as stirring rate is increasing. This is due to the turbulence produced within the

external phase due to which polymer gets detected at the paddle region and production yield gets reduced. [14].

1.9 APPLICATION OF MICROSPONGE SYSTEM

Microsponges are porous polymeric microspheres that are used normally for topical or oral administration. It has shown an alternative to develop cosmetic products. Microsponges are intended to deliver drugs which shows effective therapy at the minimum dose and also to improve stability, with reduced adverse effects.[15]

1.10 BENEFITS OVER CONVENTIONAL FORMULATION

Conventional formulations of topically applied drugs are executed to show its action on the outer stratum of the skin. Such finished products release their drug upon application, creates an extremely concentrated layer of drug that is quickly absorbed. When compared to the modify release system microsponge can prevent unnecessary accumulation of active agent within the layers of the skin. Actually, the microsponge system can diminish the irritation of the drugs without reducing their therapy.[16]

1.11 BENEFITS OVER MICROENCAPSULATION AND LIPOSOMES

Liposomes suffer from lower encapsulation efficiency, difficulty in formulation design, limited chemical and microbial stability. It has ability to stay stable at pH of 1 to 11, and withstands temperature up to 130 °C, compatible with most continuous phase, due to presence of pores it is self-sterilizing as average pore size is 0.25 μm where small bacteria cannot penetrate into the carrier system. [17]

1.12 BENEFITS OVER OINTMENTS

Ointments are often aesthetically unattractive, oiliness, tackiness etc. that frequently results into poor patient compliance. These continuous phase (vehicles) need high concentrations of drugs for therapy because of their low ability in delivery system chosen, ultimately results into allergic reactions. moreover, the disadvantage of topical formulation has uncontrolled evaporation of active ingredient likely continuous phase has incompatibility with the drug. Microsponge system has the ability to uphold the active ingredient for a maximum period either on the superficial layer of the skin or within the layers, while limiting its entry into transdermal system. [18]

The recent technologies in microsponges drug delivery system were made by modifying the method to form β-CD microsponges that can be used for highly hydrophobic drugs, for oral administration of BCS class II drugs. Future predictions of microsponge drug delivery system holds a unique opportunity in various pharmaceutical applications in the forthcoming future as it, has distinctive properties to develop novel product forms [19].

1.13 NEED OF THE CURRENT STUDY

Among the several categories of active ingredients, antibiotic drug is chosen for its need of delivery at local region. The drug with topical delivery is used for local action. The drug delivery onto the skin is considered an effective means of treatment. The therapeutic efficacy increases effectiveness after delivering the drug onto the skin, it is essential to utilize absorption enhancers. An attempt has been made, to prolong the drug release of Clindamycin phosphate.

Many of marketed conventional dosage forms have quick drug absorption and more dosing frequency through skin, proposed to act very quickly while producing much concentration, hence there is a demand for delivery system for enhancing or prolonging its action through transdermal route, to deliver the drug in least dose and diminish side effects.

The current research was focused on development of clindamycin phosphate loaded microsponges and finally incorporate into gel, to sustain the drug release over a period of time and to diminish the side effects of the conventional gel. The drug has several side effects when applied onto skin. Further this drug is having low permeability, high solubility, belongs to BCS class III. Hence, the research work was considered to prolong drug release by gel loaded with Clindamycin phosphate microsponges topically and evaluate duration of action of drug when compared with marketed formulations available as conventional gel.

In this research formulation, and evaluation of gel loaded with microsponges of Clindamycin phosphate, for the topically delivery by using polymer like ethyl cellulose by adopting oil in oil emulsion solvent diffusion technique.

CHAPTER 2

REVIEW OF LITERATURE

Younis M A, *et al.*, 2020 have formulated Sulpiride (SUL), floating microsponges by quasi-emulsion solvent diffusion method and characterized its physico-chemical properties. In addition, Taguchi design of experiment was utilized to optimize some independent variables affecting microsponges performance. The optimized SUL microsponges showed a yield of 79.82 ± 2.37%, an encapsulation efficiency of 89.11 ± 2.28% and *in vitro* time & presence of microsponges are observed under X-ray studies for 8 hrs. Additionally, pharmacokinetics studies were investigated in rabbits and compared with the commercial SUL formulation. Optimized SUL microsponges showed 2fold increase in oral bioavailability compared with commercial product. It could be concluded that the floating microsponges could be useful as an oral platform to enhance the Sulpiride absorption and bioavailability.[20]

Othman M H, *et al.*, 2020 have developed 5-Fluorouracil (5-FU) loaded microsponges (MS) compressed in enteric-coated tablets of the colon targeting for colorectal cancer. Hydroxypropyl methylcellulose and pectin were used as polymer. *In vitro* drug release behavior was studied in different pH media, while the X-ray imaging was used to monitor the *in vivo* movement of prepared tablets containing 5- FU-MS throughout the GI system. The results that the encapsulation efficiency was from 71.80 ± 1.62% to 101.3 ± 2.60%, the particle size were ranged from 53.11 to118.12 nm. The *in vivo* human volunteer study of X-ray has shown that tablets ultimately reached the colon without disturbing in the upper gastro intestinal system. The obtained carrier formulation is considered as a novel system to deliver 5-FU to the colon tumor with 100% targeting without any drug release in the upper GIT or first-pass metabolism.[21]

Hans M, *et al.*, 2019 have worked on formulation and evaluation of Fluconazole microsponge by using quasi emulsion solvent diffusion method at different stirring rate at 500, 800, 1000, 1200 and 1500 rpm. Particle size of prepared microsponge was observed in the range of 76.2 to 32.5 μm. SEM results revealed the porous, spherical nature of the microsponges and production yield, entrapment efficiency and drug content were found to be 78.24%, 82.76%, 81.36%. The impact of Drug: Polymer ratio and process variables like stirring speed and stirring time on the physical features of microsponges like production yield, mean particle size, entrapment efficiency was examined. The formulation with higher drug to polymer ratio 1:8 (F4) was chosen to investigate the effect of stirring rate on the morphology of microsponges. The dispersion of the drug and polymer within the aqueous phase was found to be dependent on the agitation speed. As the speed was increased the size of microsponges was reduced and the microsponges were found to be spherical and uniform.[22]

Dua J S, *et al.*, 2019 have fabricated and evaluated Itraconazole loaded microsponges using Eudragit for the controlled release formulation. Microsponges containing Itraconazole were prepared by using quasi emulsion solvent diffusion method at different stirring rate at 500, 800, 1000, 1200 and 1500 rpm corresponding to observed particle size of 78.43 to 23.18 μm. The production yield, entrapment efficiency, and drug content were found to be 80.88%, 84.53% and 82.89%. The formulation with higher drug to polymer ratio 1:10 (F5) were chosen to investigate the effect of stirring rate on the morphology of microsponges and found to be optimized with variables. The cumulative percentage drug release upto 8 hrs for F5 was 89.54% and the mechanism of drug release from the formulations during the dissolution was determined using the zero order, first

order, Higuchi equation and Peppas equation. All formulations were best fitted to zero order and Peppas plot. The best formulation F5 follows zero order release.[23]

Bhatia M, *et al.*, 2018 have done research to improve the release rate of Curcumin by microsponges prepared through quasi-emulsion solvent diffusion technique using ethylcellulose and polyvinyl alcohol as carriers. The microsponges were characterized by FTIR, DSC, XRD and SEM studies followed by determination of total drug content and entrapment efficiency. The prepared microsponges were filled in hard gelatin capsule shell and loaded in carbopol gel to evaluate its potential in oral and topical drug delivery. Further, it was observed from the studies on release rate that microsponges was 93.2% in hard gelatin capsules and 77.5 % release in carbopol gel for 24 hrs. The estimated drug remained in the skin was207.61 ± 5.03 μg/cm2 as determined by a Franz diffusion cell. The drug release profile data were found to be fitted best into the zero-order model with anomalous transport mechanism of drug release in both cases.[24]

Maheshwari R, *et al.*, 2017 have fabricated Nifedipine sustained release microsponges and then formulated to tablets by using different compositions of nifedipine and polymer in various molar ratios using emulsion solvent diffusion technique. MF-3 batch was found optimized as revealed by analyzing its surface morphology, and showed better flow. Different batches of tablets were formulated, batch MF-3, 30 % of MCC, 20 % of starch and 2 % of talc (TF-33), showed 92.73 ±2.19 % drug release during 24 hr *in vitro* release study in comparison to other batches including commercial formulation which was found to be released completely in 20 hr. Further, stability analysis revealed good drug retention of loaded nifedipine as well as consistent *in vitro* release pattern over a period of 90 days at 40 °C and 75% RH.[25]

Patel S S, *et al.*, 2017 have developed once-daily sustained release microsponges of Nicorandil by using quasi-emulsion solvent diffusion method. SEM studies revealed that the microsponges of nicorandil with Eudragit-RSPO and HPMC K100M were smooth, porous, glossy and discrete spherical. The actual drug content and encapsulation efficiency of batch M1 to M9 were obtained in range of 62.05 ±0.31 to 80.69 ± 0.43 and 64.41 ± 1.71 to 70.58 ± 1.12, respectively. The microsponges formulations were subjected to *in vitro* release studies and the results were evaluated kinetically and statically. The best fitted model was found to be Korsmeyer-Peppas model (R2 = 0.9992) for M6 batch. The "*n*" value for Korsmeyer-Peppas model was between 0.5 and 1.0 which is indicative of non-Fickian diffusion.[26]

Kuchekar M, *et al.*, 2016 have developed Voriconazole gel for treatment of fungal infection. Carbopol based gel was developed and the formulation studies were aimed to keep all other ingredients constant and only change in carbopol 940 concentrations. Gel formulations were characterized for physical evaluation, rheological studies. The results were found satisfactory for all the parameters studied.[27]

Kumar P M *et al.*, 2015 have formulated metronidazole microsponges by emulsion solvent evaporation method. Box-Behnken experimental design was used to optimize the formulation parameters. The particles were characterized in terms of drug entrapment efficiency (DEE), particle size, pore characteristics by mercury intrusion porosimetry, scanning electron microscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry and powder X-ray diffraction study. The drug release from the microsponge loaded gel was studied by modified Franz diffusion cell. *In vitro* dermal toxicity was evaluated by cell lines. SEM analysis confirmed spherical shape of microsponges and the topical application of

optimized gel enhanced drug residence time in the skin and therapeutic drug concentration was maintained up to 24 hrs.[28]

Sareen R, *et al.*, 2014 have developed and optimized the Curcumin microsponges by quasi emulsion solvent diffusion method with 3^2 full factorial designs. Prepared microsponges were optimized in order to analyze for the encapsulation efficiency, particle size, and drug release. The optimized formulation was subjected to *in vivo* study using acetic acid induced colitis model in rats. The F7 was selected as optimized formulation based on particle size of 41.63 μm, percentage entrapment efficiency of 78.13%, and % cumulative drug release of 84.12%, and desirability factor of 0.83. The drug release profile of F7 formulation was subjected to different kinetic models and based upon the best correlation coefficient the release was found to follow Higuchi model. Pharmacodynamic study showed that curcumin loaded microsponges causes a significant decrease in edema, necrosis, and hemorrhage of colon as compared to free curcumin.[29]

Kumar, *et al.*, 2011has reviewed that microsponge as a novel drug delivery system for controlled release of medicinally active ingredients. It was generally prepared by polymerization or quasi emulsion solvent diffusion technique and has been successfully used for targeted drug delivery. Microsponges are preferred over the microspheres owing to their better drug loading capacity. The article deals with recent advancements in the microsponge preparation and its pharmaceutical applications and commercial utility considering patents.[30]

Gangadharappa H V, *et al.*, 2013 has reviewed that microsponge as a microscopic sphere having 10 -25µm in size and capable of absorbing skin secretions, therefore reducing the oiliness of the skin. Conventional topical preparations have various disadvantages due to irritancy, odour, greasiness and

patient compliance. In many topical dosage forms fail to reach the systemic circulation in sufficient amounts in few cases. These problems overcome by the usage of formulation as microsponge in the areas of research. Drug release in microsponge was done by the external stimuli like pH, temperature and rubbing. It has several advantageous over the other topical preparations in being non-allergenic, non-toxic, non-irritant and non-mutagenic. These microsponges are used in the sun screens, creams, ointments, over-the-counter skin care preparations.[31]

Jadhav N, *et al.*, 2013 has reviewed that microsponges are at the head of the rapidly developing field of novel drug delivery technology. Microsponge drug delivery technology holds a great promise for reaching the goal of controlled and sitespecific drug delivery and hence, has attracted wide attention of researchers. The article presents a broad review of microsponges delivery system discussing the principles and preparation methods. Appropriate analytical techniques for characterization of microsponges like particle size and its distribution, surface morphology, porosity, density are covered. Advantages, limitations and their possible remedies of the microsponge drug delivery are also mentioned. The microsponges are used in the sunscreens, creams, ointments, over-the-counter skin care preparations, which are meant for topical application. Microsponge drug delivery can provide increased efficacy for topically active agents with enhanced safety, extended product stability.[32]

Abdelmalak NS, *et al.*, 2012 have formulated topical microsponge containing Fluconazole (FLZ) for controlled release of the drug by using ethyl cellulose (EC) and eudragit RS 100 were prepared by quasi-emulsion solvent diffusion method. 24 factorial design was employed for to study the effect of external phase. Results revealed that a selected FLZ microsponge (F3, containing FLZ and EC in 1:1 ratio

and prepared using 0.75% PVA and methylene chloride) was incorporated in carbopol gel formulation and evaluated for its *in vitro* release characteristics. The developed microsponges were spherical and porous. There was no interaction between drug and polymer molecules. The drug release through cellulose dialysis membrane showed Fickian release pattern.[33]

Jain V, *et al.*, 2011 have done research on designing microsponge based colon specific drug delivery system containing paracetamol by using Eudragit RS-100 containing drug in varying amounts were prepared using quasi-emulsion solvent diffusion method. DSC and FTIR studies indicated compatibility of the drug in various formulations. Shape and surface morphology of the microsponges were examined using scanning electron microscopy. The formulations were subjected to *in vitro* release studies showed a bi-phasic pattern with an initial burst effect. The cumulative percent release at the end of 12 hrs was noted to be between 74-98 %. The release kinetics showed that the data followed Higuchi model and the main mechanism of drug release was diffusion. The colon specific tablets were designed to release at specific site by coating with pectin: HPMC mixture. *In vitro* study showed release of drug at 6 hr.[34]

Dash S, *et al.*, 2010 has reviewed that the mathematical models used to determine the kinetics of drug release from drug delivery systems. The quantitative analysis of the values obtained in dissolution/release rates is easier when mathematical formulae are used to describe the process. The mathematical modeling can ultimately help to optimize the design of a therapeutic device to yield information on the efficacy of various release models.[35]

Vikas J, *et al.*, 2010 have formulated Dicyclomine-loaded Eudragit-based microsponges by using a quasi-emulsion solvent diffusion method. The

compatibility of the drug with formulation components was established by differential scanning calorimetry (DSC) and Fourier transform infra-red (FTIR) found to be compatible. Shape and surface morphology of the microsponges were examined using scanning electron microscopy. Furthermore, the drug was stable in all the formulations by increase in drug: polymer ratio resulted decreases in the release rate of the drug from the microsponges. Kinetic analysis showed that the main mechanism of drug release was by Higuchi matrix controlled diffusion. Drug release was bi-phasic with an initial burst effect with 16 – 30 % of the drug was released in the first hour. Cumulative release for the microsponges over 8 hours ranged from 59 - 86 %.[36]

Amrutiya N, *et al.*, 2009 have developed and evaluated microsponges containing Mupirocin were prepared by emulsion solvent diffusion method. The effect of formulation and process variables such as internal phase volume and stirring speed on the physical characteristics of microsponges were examined on optimized drug/polymer ratio by 32 factorial design. The optimized microsponges were incorporated into an emulgel base. *In vitro* and *ex vivo* drug release and drug deposition, further *in vivo* antibacterial activity of mupirocin-loaded formulations were studied. The optimized formulations were stable and non-irritant to skin as demonstrated by Draize patch test. Microsponges-based emulgel formulations showed prolonged efficacy in mouse surgical wound model infected with *S. aureus*. Mupirocin was stable in topical emulgel enhanced retention in the skin indicating better potential treatment of skin infection with the delivery system.[37]

Jain V, *et al.*, 2010have done research on preparation of Paracetamol loaded Eudragit based microsponges by using quasi emulsion solvent diffusion method. The compatibility of the drug with various formulation components was

established and found to be stable. Surface morphology of the microsponges were examined using SEM. The colon specific formulations were prepared by compression coating of microsponges in to tablets with pectin: hydroxypropyl ethylcellulose. The *in vitro* dissolution studies were done on all formulations and the results release at 6 hr, followed Higuchi matrix with diffusion mechanism.[38]

D'souza J I, *et al.*, 2008 have investigated that Fluocinolone Acetonide (FA) microsponges to reduce skin inflammation and relieve itching by controlled release of drug to the skin could reduce the side effect while reducing percutaneous absorption. FA entrapped microsponges prepared by quasi emulsion solvent diffusion method and is incorporated into gel to release the drug to the skin. Compatibility of drug with reaction adjuncts was studied by FTIR and DSC and found to be stable. Production yield, loading efficiency and particle size analysis and surface morphology of microsponges were performed found to within the limits. Free flowing powder microsponges were spherical in shape, between 31.34 and 82.26 μm in diameter. Drug release was observed controlled with comparative anti-inflammatory activity with the gels containing free drug.[39]

Nokhodchi A, *et al.*, 2007 have formulated Benzoyl peroxide (BPO) microsponge using emulsion solvent diffusion technique, by adding an organic internal phase containing benzoyl peroxide, ethyl cellulose and dichloromethane into a stirred aqueous phase containing polyvinyl alcohol (PVA). and explore the parameters affecting the morphology and other characteristics of the resultant products employing scanning electron microscopy (SEM). The SEM micrographs of the BPO microsponges enabled measurement of their size and showed that they were spherical and porous. Results showed that the morphology and particle size of microsponges were affected by drug: polymer ratio, stirring rate and the amount of emulsifier used. The results obtained also showed that an increase in the ratio of

drug: polymer resulted in a reduction in the release rate of BPO from the microsponges. The release data showed that the highest and the lowest release rates were obtained from lotions containing plain BPO particles.[40]

Comoglu T, *et al.*, 2007 have fabricated and evaluated the Ketoprofen incorporated into modified release microsponge tablets of profenid retard 200 mg was studied *in vitro* and *in vivo*. The formulation containing Ketoprofen microsponges yielded good modified release tablets. An *in vivo* study was designed to evaluate the pharmacokinetic parameters and to compare them with the commercially available. Ketoprofen retard tablets containing the same amount of the active drug that is available commercial Ketoprofen retard tablets showed a more rapid absorption rate than modified release tablets and peak levels were reached within almost 3.6 h after administration. the new modified release tablets showed a slower absorption rate and peak levels were reached 8 h after administration thus prolonged action of the drug is achieved.[41]

Orlu M, *et al.*, 2006 have designed a novel colon using specific drug delivery system containing Flurbiprofen (FLB) microsponges were prepared by Eudragit RS100 by quasi emulsion solvent diffusion method. various control parameters are done on the physical characteristics of microsponges were investigated. Surface morphology, particle size and pore structure of microsponges were examined. *In vitro* dissolution studies were done on all formulations and the results were kinetically and statistically evaluated. The microsponges were spherical in shape, between 30.7 and 94.5 μm in diameter and showed high porosity values (61–72%). *In vitro* studies exhibited that compression coated colon specific tablet formulations started the drug release by modified pattern at the 8th hr.[42]

Jelvehgari M, *et al.*, 2006 have formulated ethylcellulose microsponges containing BPO were prepared by using emulsion solvent diffusion method. Adding an organic internal phase containing benzoyl peroxide, ethyl cellulose and dichloromethane into a stirred aqueous phase containing polyvinyl alcohol. Drug content, particle size analysis and loading yield were determined in the prepared microparticles. BPO microparticles were then incorporated into standard vehicles for release studies. Scanning electron microscopy was used to study the shape and morphology of the microsponges found to be spherical and porous for diffusion of solvents. It was shown that the various parameters influence the particle size and drug release behavior of the formed microsponges are satisfactory. The results showed that, generally, an increase in the ratio of drug: polymer resulted in a reduction in the release rate of BPO from microsponges.[43]

Grimes P E, *et al.*, 2004 has reviewed that hyperpigmentation such as melasma and post inflammatory hyperpigmentation (PIH) are treated by a new formulation of HQ 4% with retinol 0.15% entrapped in microsponge reservoirs was developed for the treatment of melasma and PIH. Microsponges were used to release HQ gradually to prolong exposure to treatment and to minimize skin irritation. Patients applied the microentrapped HQ 4% formulation to the full face twice daily for 4, 8, and 12 weeks. The microentrapped HQ 4%/retinol 0.15% formulation produced improvement at all study endpoints. Hence microentrapped HQ 4% with retinol 0.15% was safe and effective.[44]

Comoglu T, *et al.*, 2003 have formulated that microsponges containing Ketoprofen and Eudragit RS 100 were prepared by quasi-emulsion solvent diffusion method. The effects of different mixing speeds, drug–polymer ratios, and solvent– polymer ratios on the physical characteristics of the microsponges as well as the *in vitro* release rate of the drug from the microsponges were investigated. All the factors

studied had an influence on the physical characteristics of the microsponges. *In vitro* dissolution results showed that the release rate of Ketoprofen was modified in all formulations.[3]

Moin A, *et al.*, 2016 developed and investigated a novel microsponge based gel as a topical carrier for the prolonged release and cutaneous drug deposition of fluconazole (FLZ); destined for facilitated fungal therapy. Compatibility studies results reflected no sign of any chemical interaction between the drug and polymers used. Whereas, varied drug-polymer ratios and emulsifier concentration indicated significant effect on production yield, drug content, encapsulation efficiency, particle size and drug release. Spherical microsponges with a porous surface and 29.327 ± 0.31 μm mean particle size were evident from SEM micrographs. In vitro release outcomes, from microsponge loaded gels depicted that F1 formulation was more efficient to give extended drug release of 85.38% at the end of 8 h, while conventional formulation by releasing 83.17% of drug got exhausted incredibly earlier at the end of 4 h merely. Moreover, microsponge gels demonstrated substantial spreadability and extrudability along with promising antifungal activity.[45]

Potulwar A, *et al.*, 2021reviewed all the methods available for preparation and development of Micro sponges. This review gives information reguarding all the methods existing for preparation and development of Micro sponges. Some of the entrapment limitations have overcome with different new techniques in current scenario. These methods are used with toxin controlling with use of excipients.[46]

Yadav V, *et al.*, 2017designed novel drug delivery system containing oxiconazole nitrate microsponges and to prepare microsponge gel. Oxiconazole nitrate is an antifungal drug used in the treatment of fungal infection having a poor aqueous

solubility, side effects and adverse reactions. The Fourier transform infrared radiation measurement (FTIR) and Differential scanning colorimetry (DSC) of drug and excipient confirm compatibility. Results revealed that quasi-emulsion solvent diffusion method is a suitable technique for the preparation of microsponges as most of the formulations were discrete and spherical in shape with a good production yield of 61.44% to 80.45% and the highest drug release for F3 and F9 formulation was found to be 87.77 % and 83.24 % respectively for the 8 h. The microsponge gel formulation MGI (F3) showed the controlled release of oxiconazole nitrate for 12 h. The drug release data of optimised batch MGI (F3) were fitted into different kinetic models and showed that the drug release from gel formulation follows zero order release.[48]

Guenther L C, *et al.*, 2003have researched that the efficacy and tolerability of Tazarotene 0.1% gel and Tretinoin 0.1% microsponge gel were evaluated with double-blind, randomized, parallel-group study in patients with mild-to-moderate inflammatory facial acne vulgaris. A total of 169 patients were randomized to once daily applications of one of these topical retinoids for 12 weeks. The mean amount of medication applied by the patients was 0.28 g per application with tazarotene and 0.41 g per application with tretinoin microsponge, resulting in cost-effectiveness for treatment success with tazarotene and treatment success with tretinoin microsponge. Tazarotene was observed to have greater efficacy and comparable tolerability and to be a cost-effective alternative to tretinoin 0.1% microsponge gel.[49]

Comoglu T, *et al.*, 2002 have worked on Ketoprofen microsponges were prepared by quasi emulsion solvent diffusion method with Eudragit RS 100. The Ketoprofen tablets prepared with microsponges were subjected to direct compression method. Different pressure values were applied to the tablet powder mass in order to

determine the optimum pressure value for compression of the tablets. Results indicated that microsponge compressibility was much improved over the physical mixture of the drug and polymer and owing to the plastic deformation of sponge like structure, microsponges produce mechanically strong tablets.[50]

Chen G, *et al.*, 2000 has reviewed that biodegradable hybrid sponge of poly(D-L-lacticcoglycolic acid) (PLGA) and collagen was fabricated by forming microsponges of collagen in the pores of PLGA sponge. Observation of the PLGA– collagen hybrid sponge by scanning electron microscopy showed that microsponges of collagen. The formation of collagen microsponges was dependent on collagen concentration, the effective range of which was from 0.1 to 1.5 (w/v) %. The mechanical strength of the hybrid sponge was higher than that of either PLGA or collagen sponges, in both dry and wet states. The wettability with water was improved by hybridization with collagen, which facilitated cell seeding in the hybrid sponge. Mouse fibroblast L929 cells attached well and spread on the surfaces of the microsponges of collagen. Use of the PLGA sponge as a skeleton facilitated formation of the hybrid sponge into desired shapes with high mechanical strength while collagen microsponges contributed good cell interaction and hydrophilicity.[51]

2.1 OVERVIEW OF MICROSPONGES

2.1.1 Definition

Microsponges are polymeric delivery system, composed of porous microspheres; they are tiny sponge like spherical particles with process a large porous surface area.

2.1.2 Advantage of the microsponges delivery system (MDS) [52]

- MDS has stability over the wide range of pH from 1 to11.

- MDS can withstand at high temperature up to 130°C.
- Loading efficiency of MDS is up to 60%.
- MDS act as good adsorbent over the skin and bypass first pass hepatic metabolism.
- In microsponges dosage form it cannot undergo any unwanted reaction.
- It can resist attack by moisture, oxidation & reduction.
- MDS processes relatively longer half-life.

2.1.3 Disadvantages

- Poor stability in presence of light and high temperature.
- Cannot incorporate water soluble drugs.
- Cannot penetrate into the deeper layers of the skin.

2.1.4 Differentiate between microsponges and microspheres

Microsponges are polymeric delivery systems composed of porous microspheres typically 5-300 μm. They are tiny sponge-like spherical particles with a large porous surface that are used mostly for topical purpose and have recently been used for oral administration.

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm (1 mm)). Microspheres are sometimes referred to as spherical microparticles. In general microspheres are solid or hollow and do not have a fluid inside, as opposed to microcapsules.

2.1.5 Method of Preparation

2.1.5.1 Oil in oil (O/O) emulsion solvent diffusion method

Microsponges were prepared by utilizing oil in oil emulsion solvent diffusion technique. This method involves dissolving a given amount of polymer in a dispersing solvent such as acetone or dichloromethane to generate a clear solution, adding a deflocculating agent such as magnesium stearate, and ultrasonically mixing the mixture to obtain a homogenous dispersion. The mixture is then introduced into continuous oil phase, similar to liquid paraffin, and stirred continuously till the evaporation of the solvent. The microsponges formed are filtered, washed with n-hexane and dried at room temperature.[53]

2.1.6 Polymers used in Preparation of Microsponges

Ethyl cellulose
Carbopol 940

2.1.7 Solvents used in Preparation of Microsponges

Dichloromethane
Liquid paraffin

2.1.8 Evaluation of Microsponges

2.1.8.1 Visual inspection[21]

The formulated microsponges were subjected for visual inspection for the parameters like color, appearance and the nature of microsponges.

2.1.8.2 Percent yield[54]

The formulated microsponges were subjected to percentage yield by calculating practical weight of microsponges obtained to the theoretical weight (polymer + drug)

2.1.8.3 Drug content [45][55]

% drug content = actual amount of drug in microsponges/weighed quantity of microsponges * 100

2.1.8.4 Determination of entrapment efficiency[45][55]

The entrapement efficiency (%) is calculated using the following equation:

% entrapement efficiency = actual amount of drug in microsponges / theoretical drug content * 100

2.1.8.5 Particle size analysis[56]

Particle size and size distribution are evaluated using an optical microscope or electron microscope. This is a fundamental step; the size of the particles greatly affects the surface of the formulation and its stability. Particle size analysis of loaded and unloaded microsponges can be performed by laser light diffractometric or any other suitable method. The values of (d50) can be stated for all formulations as mean size range. Cumulative percentage drug release from microsponges of different particle size will be plotted against time to study effect of particle size on drug release from microsponges.

2.1.8.6Morphology and surface topography of microsponges[57]

For morphology and surface topography, various techniques have been used like photon correlation spectroscopy (PCS), Scanning electron microscopy (SEM), transmission electron microscopy (TEM) etc. SEM is used extensively for which prepared microsponges are coated with gold–palladium under an argon atmosphere

at room temperature and then the surface morphology of the microsponges is studied.

2.1.8.7Characterization of pore structure[58]

Pore volume and diameter are dynamic in regulatory the concentration and duration of efficacy of the active ingredient. Pore diameter also affects the migration of active ingredients from microsponges into the vehicle. The rate of drug release from microsponges can affect by pore diameter of microsponges. Numerous porosity parameters of microsponges such as total pore surface area, intrusion-extrusion isotherms, pore size distribution, average pore size diameters, shape and morphology of the pores, bulk and apparent density are also being studied

.2.1.8.8Determination of pore diameter[59]

The conventional method of measuring and expressing pore sizes, the pore diameter is calculated by B.E.T. nitrogen multipoint analysis and from the measurement of the pore volume by the mercury intrusion method.

2.1.8.9Drug-Polymer Compatibility studies[42]

The drug-excipients compatibility studies are carried out in order to ensure that there is no reaction between the two ingredients, when formulated into a dosage form. These studies are commonly carried out by recording the differential scanning Calorimetry (DSC) of the chemicals. API and excipients individually and together and checking for any addition or deletion of peaks or troughs. For DSC approximately 5 mg samples can be accurately weighed into aluminum pans and sealed and can be run at a heating rate of 15 °C/min over a temperature range 25-430 °C in atmosphere of nitrogen. (FTIR) spectroscopy can also reveal the incompatibilities between the chemical moieties.

2.1.8.10 *In vitro* Release Studies[60]

In vitro release studies must be being carried out using dissolution apparatus USP XXIII equipped with a modified basket consisted of 5µm stainless steel mesh. Dissolution rates were measured at 37 °C under 150 rpm speed. The dissolution medium is selected while considering solubility of active ingredients to ensure sink conditions. Sample aliquots were withdrawn from the dissolution medium and analyzed the content by suitable analytical method (UV-Visible spectrophotometer) at regular intervals of time.

2.1.9 Evaluation of Microsponges Gels

2.1.9.1 Visual Inspection[45]

The organoleptic properties, are to be examined for color, texture, consistency, homogeneity, and physical appearance of gel containing microsponges were checked by visual observation.

2.1.9.2 pH Measurement[45]

The finalized gel formulation pH was recorded using digital pH meter. 5 g gel was dispersed in 45 ml distilled water at 27 °C and solution pH was measured.

2.1.9.3 Spreadability Studies[61]

Spreadability was determined wooden block and glass slide apparatus. It consists of two slides upper movable slide and lower non-movable slide. Weights of about 20 gm were added to the pan and time was noted for upper slide to separate completely from the fixed slides. Spreadability was then calculated by using the formula:

$$S = M.L/T$$

Where, S= Spreadability; w= weight tide to upper slide; L= length moved on the glass slide;

T= time taken to separate the slide completely from each other.

2.1.9.4 Stability studies[62]

The optimized gel formulation was subjected to stability testing as per ICH norms. Gel was filled in clean, lacquered, collapsible aluminum tubes, and various replicates were kept at 40 ± 2 °C and 75 ± 5 % relative humidity in a humidity chamber. Gel was assessed for change in appearance, pH and *in vitro* release profile at an interval of 30, 60 and 90 days.

2.1.9.5 Viscosity[63]

The viscosity of the gel formulation was measured with a Brookfield viscometer using 1x model and cone number 01, with an angular velocity of 5 rpm at 25 ºC. An average of five readings was used to calculate viscosity.

2.1.9.6 *In vitro* Release Studies[54]

The *in vitro* release of gel formulations should be carried out using Franz diffusion cells. The cellophane membrane (pore size 0.45µm) or egg membrane was mounted onto the diffusion cell with 25 ml receptor compartment volume. PBS(pH7.4) was used as receptor medium, and the system was thermostatic to 37 ± 1 °C under stirring with magnetic bead inside. Aliquots of 1 ml volume were withdrawn at specific time intervals by maintaining sink condition simultaneously. Withdrawn aliquots were then diluted using receptor medium and analyzed by UV spectrophotometer.

Table No: 2.1 List of Marketed Products based on Microsponges

Product Name	Pharmaceutical uses	Manufacturer
Retinol cream	Helps maintain healthy skin	Biomedic
Salicylic Peel 20	Excellent exfoliation	Biophor
Ultra Guard	Protects baby's skin	Scott Paper Company
Oil-free matte block SPF 20	Sunscreen	Dermalogica
Retin A Micro	Acne vulgaris	Ortho-McNeil-Pharmaceutical, Inc

Glycolic Acid Moisturizer w/SPF 15	Anti-Wrinkles	AMCOL Health & Beauty Solution
EpiQuin Micro	Hyper pigmentation	Skin Medica Inc

Table No: 2.2 List of Patented Products based on Microsponges

Inventor	Patent number	Publication year
Won	US4690825	1987
Dean *et al*	US 4863856	1989
Straub *et al.*	US 6395300	1999
Halliday	US20080160065	2008
Ray	US5725869	1996
Robert *et al.*	US5316774	1994
Katz *et al.*	US5135740	1992

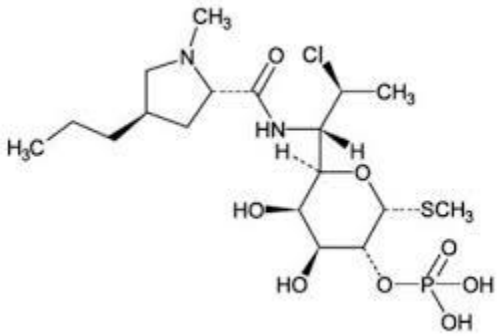
2.1.9.8 Over-all Conclusion

The overview of microsponges as delivery system with advantage and disadvantages, difference between microsponges and microspheres, its method of preparation with list of polymers used in preparation of microsponges and the solvents used in preparation of microsponges were discussed. And also the evaluation of microsponges and the gels also were discussed. Further the commercially availablemarketed microsponges and the list of patented products on microsponges were discussed in this chapter.

From the literature review, dosage form selected for its unique property of releasing the drugs with microparticulate carrier (microsponge) which is developed by different techniques using internal and external phase with different polymers and various ratio of organic solvents. Microsponges are selected for its release of drug for prolonged period of time for BCS class III drugs which can reduce the side effects when applied transdermally.

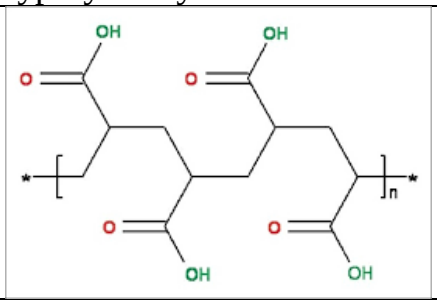
2.2 DRUGS, EXCIPIENTS, SOLVENTS AND CHEMICALS PROFILE[64]

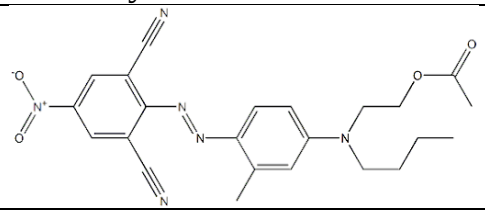
2.2.1 Drug Profile

Drug	Clindamycin Phosphate
IUPAC name	(2R,3R,4S,5R,6R)-6-[(1S,2S)-2-Chlor-1-({[(2S,4R)-1-methyl-4-propylpyrrolidin-2-yl]carbonyl}amino)propyl]-4,5-dihydroxy-2-(methylsulfanyl)tetrahydro-2H-pyran-3-yldihydrogen-phosphat
Structure	
Molecular formula	C18H34ClN2O8PS
Molecular weight	504.96g/mol
Solubility in water	Water soluble
BCS Class	III
Category	Lincosamide antibiotic
Treatment	Acne vulgaris
Dosage	1% twice daily
Mechanism of action	Topically it reduces free fatty acid concentration on the skin And suppresses the growth of Propionibacterium acnes (Corynebacterium acnes), an aneaerobe found in sebaceous glands and follicles
Pharmacokinetics **Metabolism** **Excretion**	 Hepatic (CYP2C9) Urine 10%, faces 3.6%
Half life	Mean effective – 2.4hrs. depending upon age Adults – 3hrs. Children – 2.5hrs. Elderly – 4hrs.
Log P	-0.57
pKa	7.56
Protein binding	92-94%

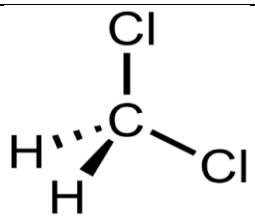
Side effects	Drying,itching,scaling,burning,redness of skin
Market formulations	Clindamycin phosphate 1.0% gel, Clindamycin phosphate 1.0% lotion, Clindamycin phosphate 1.0% solution

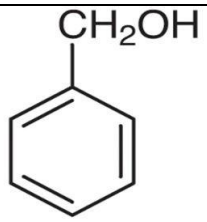
2.2.2 Excipients Profile

Carbopol 940	
Synonyms	Acritamer 940, Acrypol 940.
Description	Carbopol as a gelling polymer produced an opaque gel
Chemical name	Carboxypolymethylene
	
Molecular formula	C3H4O2
Molecular weight	72.06266 g/mol
Density	Carbopol weighs 1.0511 g/cc or 1 051.1 kg/cc
Melting point	Decomposition occurs at 260 °C.
Solubility	Soluble in water, and after neutralization in ethanol(95%),and glycerin
Applications	The readily water swellable carbopol polymers are used in a diverse range of pharmaceutical applications to provide controlled release in tablets, bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications. Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs.
Storage condition	Exposure to light causes oxidation. Carbomer powder should be stored in an airtight, corrosion resistant container in a cool, dry, place.

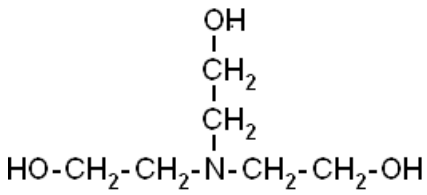
Ethyl cellulose	
Synonyms	Aqua coat ECD, Ethocel, ethyl cellulosum,
Description	Ethylcellulose is a tasteless, free-flowing, white to light tancolored powder.
Chemical name	Cellulose ethyl ether
	
Molecular formula	C20H38O11
Molecular Weight	454.5 g/mol
Density	1.07-1.18 g/cm²
Melting point	240-255 °C.
Solubility	Practically insoluble in water, glycerol, propane-1,2-diol, and freely soluble in ethanol, methanol, toluene, chloroform, ethyl acetate.
Applications	Ethylcellulose is widely used in topical pharmaceutical formulations, and used to produced water-insoluble film.
Storage	Ethyl cellulose should be stored at a temperature not exceeding32 °C in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

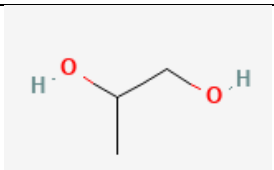
Dichloromethane	
Synonyms	Methylene chloride.
Description	Organochloride compound with the formula CH2Cl2. This colorless, volatile liquid with a chloroform-like, sweet odour is widely used as a solvent. Although it is not miscible with water, it is polar, and miscible with many organic solvents.
Chemical name	Methylene chloride, Methylene dichloride

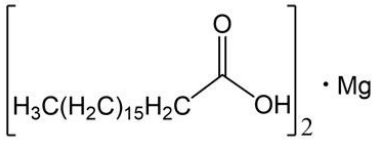
	
Molecular Formula	CH2Cl2
Molecular Weight	84.93 g/mol
Density	1.3266 g/cm^2
Melting point	-96.7 °C (-140.1 °F)
Solubility	Miscible in ethyl acetate, alcohol, hexane, benzene, carbon tetra chloride, diethyl ether, CHCl3
Applications	Dichloromethane is used as a solvent in food technology. It is used in aerosol formulations. Used as a solvent in the manufacturing of pharmaceutical products.
Storage condition	Dichloromethane is highly volatile and should be stored in a cool, dry area in tightly closed, labeled containers. This chemical needs to be kept away from metals, light and any source of heat or ignition.

Benzyl alcohol	
Synonyms	Benzene, Benzyl alcohol
Description	Benzyl alcohol is a monochromatic primary alcohol widely used as a solvent and as an intermediate in cosmetic formulation.
Chemical name	Phenyl methanol
	
Molecular Formula	C6H5CH2OH
Molecular Weight	108.14 g/cc
Density	3.7
Melting point	-15 °C
Solubility	Soluble in water, ethanol and ether

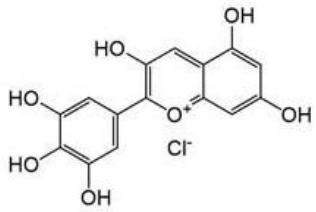
Applications	Benzyl alcohol is an aromatic alcohol and in wide verity of cosmetic formulation as a fragrance, pH adjuster, preservative and viscosity decreasing agent.
Storage condition	Store in a cool, dry, well-ventilated, locked store room away from incompatible materials.

Triethanolamine	
Synonyms	Trihydroxy tri ethylamine
Description	Triethanolamine as a variable mixture of alkanolamines consisting largely of triethanolamine, diethanolamine, and monoethanolmine. Triethanolamine is a clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacal odor.
Chemical name	Trolamine
	
Molecular formula	C6H15NO
Molecular Weight	149.19 g/mol
Density	1.13 g/cm³
Melting point	21.60 °C
Solubility	Triethanolamine is miscible in ethanol (95%), methanol and water.
Applications	Triethanolamine is widely used in topical pharmaceutical formulations primarily in the formation of emulsions.
Storage condition	It should be stored away from light

Propylene glycol	
Synonyms	1,2-dihydroxypropane, 1,2-propanediol, methyl glycol, and trimethyl glycol.
Description	*Viscous, colorless liquid, which* is nearly odorless but possesses a faintly sweet taste.
Chemical name	propane-1,2-diol
	
Molecular Formula	$C_3H_8O_2$
Molecular Weight	76.09 g/mol
Density	1.04 g/cm³
Melting point	-59 °C
Solubility	Completely soluble in water and it has the feature of dissolving many organic compounds such as fragrances, essential oils and resins
Applications	Commonly used drug solubilizer in topical, oral, and injectable medications. It is used as stabilizer for vitamins, and as a water-miscible cosolvent.
Storage condition	Store in a dry, cool and well-ventilated place.

Magnesium stearate	
Synonyms	Magnesium distearate or Dibasic Magnesium stearate
Description	Fine white powder that sticks to your skin and is greasy to the touch.
Chemical name	Magnesium Octadecanoate
	
Molecular Formula	Mg(C18H35O2)2
Molecular Weight	591.27 g/mol

Density	1.026 g/cm^3
Melting point	88.5 °C
Solubility	Negligible in ether and alcohol; slightly soluble in benzene
Applications	As a lubricant.It also forms a barrier between the medicines and the machines that make them.
Storage condition	Store in a dry, cool and well-ventilated area, away from incompatible materials

Liquid paraffin	
Synonyms	Paraffinum liquidum, paraffin oil, liquid paraffin oil or Russian mineral oil
Description	A clear, flammable liquid distilled from petroleum
Chemical name	paraffin oil or kerosene
	
Molecular Formula	$C_{15}H_{11}ClO_7$
Molecular Weight	338.69664g/mol
Density	0.85 g/mL at 20 °C
Melting point	-24 °C
Solubility	Freely soluble in chloroform, in ether, in volatile oils, and in most warm fixed oils.
Applications	Used as a moisturizer to treat or prevent dry, rough, scaly, itchy skin and minor skin irritations.
Storage condition	Store in tightly-closed, original container in a dry, cool and well-ventilated place.

CHAPTER 3

SCOPE OF THE RESEARCH WORK

The drug clindamycin phosphate has not been formulated as gel loaded with microsponges by utilizing oil in oil emulsion solvent diffusion technique. The marketed conventional gel of the drug is not a controlled release formulation and also has the side effects like drying, iching, scaling, burning and redness of skin. Hence, there is a demand to deliver the drug through transdermal route by reducing the frequency of application and diminish side effects.

To overcome the side effects of conventional dosage form, current research is focused to formulate and evaluate the gel loaded with microsponges of clindamycin phosphate by using oil in oil emulsion solvent diffusion technique. Transdermal route is the best way to overcome such adverse effects due to larger surface area of drug administration.

Microsponges of particle size range of 5 to 300 microns that can entrap wide range of active ingredients and release them over extended period of time.[42] Hence, microsponges technology was adapted in this research as a versatile drug delivery system. To control the drug delivery rate of clindamycin phosphate to a predetermined site in any model and to minimize the potential risk of side effects, the present study was designed with objective to extend short half life through gel loaded with microsponges.

Since half life is very short for clindamycin belongs to BCS III drug. So present study was carried out to minimize the above problem of conventional dosage form.

Hence, present study was focused to formulate the topical delivery of drugs by gel loaded with microsponges. Oil in oil emulsion solvent diffusion technique was

employed to prepare gel loaded with microsponges due to its ease of preparation and economical.

Hence, the aim of the study was designed to formulate and evaluate the gel loaded with microsponges of clindamycin phosphate for the topically delivery by using oil in oil emulsion solvent diffusion technique as a novel topical approach to sustained the drug therapy.

CHAPTER 4

AIM AND OBJECTIVES

4.1 AIM

The aim of the study is to formulate and evaluate the gel loaded with microsponges of Clindamycin phosphate for the topically delivery by using oil in oil emulsion solvent diffusion technique as a novel topical approach to sustained the drug therapy.

4.2 OBJECTIVES

The current research was focused on gel loaded for the topical delivery to Clindamycin phosphate to overcome short half life problem of the drug and to minimize the side effects of drug with conventional gel.

The following are objectives of the study:

1. Selection of drug and polymer for the formulations
2. Preformulation studies of Clindamycin phosphate (CLP)
3. Formulation of gel loaded with CLP microsponges and its evaluation.

a) Preparation Clindamycin microsponges (CLP).

b) Evaluation of CLP.

c) Preparation of gel loaded with CLP.

d) Evaluation of gel loaded with CLP.

CHAPTER 5

MATERIAL AND EXPERIMENTAL METHODS

5.1 MATERIALS

5.1.1 List of Chemicals Used in the Experiment

Sr. No.	Materials
1	Carbopol 940
2	Ethyl cellulose
3	Clindamycin phosphate
4	Dichloromethane
5	Liquid paraffin
6	Magnesium stearate
7	Triethanolamine
8	Benzyl alcohol
9	Propylene glycol
10	Sodium chloride
11	Distilled water
12	Potassium dihydrogen phosphate
13	Disodium hydrogen phosphate
14	Methanol

5.1.2 List of Instruments Used in the Experiment

Sr. No.	Instruments
1	Photomicroscope
2	Digital balance
3	UV-Visible Spectrophotometer
4	Triple blade stirrer
5	Ultrasonicator
6	Magnetic Stirrer
7	Digital pH meter
8	FT-IR

5.2 EXPERIMENTAL METHODS

5.2.1 Formulation and Evaluation of Gel Loaded with Clindamycin phosphate Microsponges

5.2.1.1 Selection of Clindamycin phosphate

Clindamycin (CLN) is a potent lincosamide antibiotic against susceptible anaerobic bacteria and gram-positive aerobes.Furthermore, CLN also has anti-inflammatory activities.It is applied topically in the management of acne vulgaris.

Acne vulgaris is a widespread follicular skin disorder that typically affects the Pilosebaceous unit of the face and upper part of the body and is triggered by different factors involving follicular hyperkeratosis, excess sebum production, Propionibacterium acnes hyperproliferation and inflammation.These factors produce non-inflammatory or inflammatory lesions that can be serious and leave scars.

Topical treatment is the first line therapy for acne, and CLN, as a gel or a solution, is one of the most common and efficacious topical anti acne agents.Due to anti-bacterial and anti-inflammatory properties of CLN, it targets two key factors in acne pathogenesis: P. acnes colonization and inflammation.

Conventional topical formulations of CLN immediately release the drug upon application in excessive concentrations over a short period of time, and then they release the drug in relatively low concentrations. Thus, resulting in two critical problems, the first one is cutaneous side effects such as irritation, dryness, oiliness, itching, erythema and peeling. The other problem is the short duration of drug action, thereby, the drug should be applied two times daily.

As a result, limitations of conventional topical preparations of CLN may lead to poor patient compliance and reduction in treatment efficacy.Therefore, it is prudent

to develop a new technique for delivering CLN that overcomes the disadvantages of its marketed products.[65]

5.2.1.2 Preformulation studies of Clindamycin phosphate (CLP)

5.2.1.2.1 Determination of melting point of CLP

The melting point of the CLP was determined by capillary method by introducing the drug into a small capillary tube to the height of 3.0mm which was sealed at one end and open at the other end, attaching this to the stem of the thermometer centered in a heating bath, heating the bath slowly and observing the temperatures at which the melting begins and is complete.Average of three readings were taken. Melting point indicates the identity and purity of drug sample.

5.2.1.2.2 Determination of UV absorption maxima of CLP

100 mg of CLP, was dissolved in 100 ml of phosphate buffer pH 7.4 was considered as standard stock solution I, from the stock solution I, one ml pipetted out andwas further diluted to 100ml with phosphate buffer pH 7.4 to get 10 μg/ml, the solution wascarried out for filtration with Whatman filter paper and finally checked formaximum absorbance using UV-Visible spectrophotometer in the range from 200to 400 nm and average of triplicate reading was taken.The same procedure was followed for methanol as a solvent.

5.2.1.2.3Saturation solubility studies of CLP

Saturation solubility of CLP was carried out in distilled water, methanol and phosphate buffer ph 7.4. 5ml of respective solvent was taken and excess quantity of drug was added and dissolved until supersaturation.Then it was filtered and diluted. The absorbance was measured at 210nm in UV spectrophotometer.[66]

5.2.1.2.4 Preparation of standard graph of CLP in PBS pH 7.4

The calibration curve was prepared by dissolving 100 mg of CLP in 100 ml ofpH 7.4 phosphate buffer saline considers as working standard stock I. From thisStock-I solution , 1 ml was taken and made up to 100 ml with phosphatebuffer saline pH 7.4, and this was working standard stock-II. From this stockII,1,2,3,4,5,6,7,8,9 ml was taken and made up to 10 ml with pH 7.4 phosphatebuffer. This gave concentration 1,2,3,4,5,6,7,8,9 µg/ml. Absorbance was measuredat 210 nm spectrophotometrically against pH 7.4 phosphate buffer saline as blank.

5.2.1.2.5Compatibility studies of the drug-polymer physical mixture by using FTIR spectroscopy

The drug-excipients compatibility studies are supported out in order to ensureand safeguard that there was no reaction between the drug, and polymer, when theyare physically mixed together.[45] These studies are commonlycarried out by recording the FTIR of CLP, ethyl cellulose as polymer, drug-polymer physical mixtureindividually and together for checking any addition or deletion of peaks, bycomparing with pure CLP. FTIR spectroscopy can also reveal the compatibility orincompatibilities between the chemical moieties used in formulation.

5.2.1.3 Study on Formulation of Clindamycin Microsponges (CLP)

Oil in oil(O/O) emulsion solvent diffusion technique was utilized for the preparation of CLP microsponges. The internal phase composed of appropriate ratio of drug and polymer is dissolved in dichloromethane. Magnesium stearate is added to it which is then subjected to ultrasonication for homogenous dispersion. The external phase composed of liquid paraffin.Then internal phase is then poured in a dropwise manner into the external phase and subjected to agitation at 500-1000rpm for 180mins.The microsponges are formed by diffusion of organic

solvent out of the droplets.The formed microsponges are filtered and washed with petroleum ether and n- hexane mixture and air dried overnight.[53]

5.2.1.4 Experimental design

During development of pharmaceutical formulations, numerous formulation and process variables involving effectiveness and usefulness should be optimized concurrently. 3-level factorial design has been applied using Statease Design Expert software to optimize the formulation variables with basic prerequisite of understanding interaction of independent variables. Preliminary investigations of the process parameters revealed that factors like drug to polymer ratio (X1) and stirring speed (X2) shows significant effect on particle size, % entrapement efficiency and % drug release.

Table 5.2 Composition of Clindamycin phosphate Microsponges with Design of Experiment

Formulation Code	Drug: polymer (mg)	Polymer	Solvent(mL)	RPM
CLPA1	1:1	Ethyl cellulose	Dichloromethane	750
CLPA2	1:1	Ethyl cellulose	Dichloromethane	1000
CLPA3	1:1	Ethyl cellulose	Dichloromethane	1250
CLPA4	1:2	Ethyl cellulose	Dichloromethane	750
CLPA5	1:2	Ethyl cellulose	Dichloromethane	1000
CLPA4	1:2	Ethyl cellulose	Dichloromethane	1250
CLPA7	1:3	Ethyl cellulose	Dichloromethane	750
CLPA8	1:3	Ethyl cellulose	Dichloromethane	1000
CLPA9	1:3	Ethyl cellulose	Dichloromethane	1250

5.2.1.4 Evaluation Studies of CLP

5.2.1.5.1 Visual inspection of CLP[21]

The formulated microsponges were subjected for visual inspection for the parameters like color, appearance and nature of microsponges.

5.2.1.5.2 Determination of percentage yield[54]

The formulated microsponges were subjected to percentage yield by calculating practical weight of microsponges obtained to the theoretical weight (polymer + drug)

5.2.1.5.3 Drug content[55]

A sample equivalent to 100mg of CLP was dissolved in 100ml PBS pH 7.4. pipette out 1ml and dilute upto 100ml with same solvent. Measure absorbance in UV.

% drug content = actual amount of drug in microsponges/weighed quantity of microsponges * 100

5.2.1.5.4 Percentage entrapment efficiency[54]

% entrapement efficiency = actual amount of drug in microsponges / theoretical drug content * 100

5.2.1.5.5 Particle size analysis

The microscope was fitted with a stage micrometer to calibrate the eye piece micrometer. On the stage micrometer powdered microsponges were uniformly spread on glass slide and particle size was determined by using stage and eyepiece micrometer. The effect of drug:polymer ratio was studied for particle size analysis.

5.2.1.5 Formulation of Gel Loaded with CLP

Carbopol 940 is soaked in distilled water overnight and dispersed by agitation at 600rpm. Then add propylene glycol, benzyl alcohol with gradual stirring. Add

triethanolamine and mix. the microsponges equivalent to 1%w/w of drug are uniformly dispersed in gel. [21]

Table 5.3 Composition of Gel Loaded with Clindamycin phosphate microsponges (1 %w/w)

Ingredients	Formulation		
	F1	**F2**	**F3**
Microsponges	Equivalent to 0.1g drug	Equivalent to 0.1g drug	Equivalent to 0.1g drug
Carbopol 940	0.05g	0.1g	0.15g
Distilled water	10ml	10ml	10ml
Propylene glycol	1ml	1ml	1ml
Benzyl alcohol	0.5ml	0.5ml	0.5ml
Triethanolamine	3 drops	3 drops	3 drops

5.2.1.6 Evaluation Studies of Gel Loaded with CLP

5.2.1.7.1 Visual inspection of gel loaded with CLP[45]

The formulation was inspected for color, consistency, homogeneity and uniformity.

5.2.1.7.2 pH Measurement[45]

5g gel was dispersed in 45ml distilled water and pH of solution was measured using digital pH meter.

5.2.1.7.3 Spreadability Studies[61]

2gm gel was placed between two glass slides and other end tied to weight pan. 1000gm weight was placed on top of the two slides for 5mins for uniform thickness. Excess of gel was scrapped off from the edges. The top plate was subjected to pull off at 80gm with help of string attached to the hook and the time in sec. required by the top slide to cover the distance of 7.5cm is noted. A shorterinterval indicates better spreadibility.

Spreadibility can be measured by using followingequation:

$$S = ML/T$$

Where, M = weight placed on upper slide

L = length of slide

T = time taken to separate slide completely from each other

5.2.1.7.4 Stability studies

Stability testing was carried out by keeping 50g of gel at room temperature for 1month. It was checked for visual disturbance, pH and spreadability.

5.2.1.7.5 In-vitro antimicrobial activity[68]

Agar Cup method - The antimicrobial activity of the marketed gel and microsponge gel of formulation CLPA5, CLPA6, CLPA7 was compared with each other by agar well diffusion method using staphylococcus aureus. In this method, previously autoclaved nutrient agar medium was poured into sterilized assay plates. These plates were allowed to cool down on the leveled surface. After solidification, surface was inoculated with suspension of *Staphylococcus aureus, Bacillus subtilis and Escherichia coli* and kept for 30mins. at room temperature for

complete diffusion of microbial suspension. In the plates, 4 well were excavated with sterile borer. Into each well formulation was introduced. The plated were then incubated for 24 hours at 37°C. Antibacterial activity of the formulations were compared with the market formulation, 1% Clindamycin Phosphate gel (Clindac A). Average of the three measurements are recorded by taking the readings at different angles to ensure accuracy.

5.2.1.7.6 *In-vitro* Diffusion studies by using Franz Diffusion cell[67]

Clindamycin phosphate microsponges loaded gel was subjected to *in vitro* release studies of batches CLPA1-CLPA9 to examine release pattern of the drug. The study was carried out using Franz diffusion cell. The cellophane membrane (pore size 0.45µm) was mounted onto the diffusion cell with 20ml receptor compartment volume. PBS pH 7.4 was used as receptor medium and donor compartment containing gel loaded with CLP microsponges and the system was thermostatically maintained at 37°C under stirring condition with magnetic stirrer at 50rpm. Aliquotes of 1ml volume were withdrawn at specific time intervals and same volume was replaced for maintain sink conditions. Withdrawn aliquots were then diluted using PBS pH 7.4 and analyzed by UV spectrophotometer against blank.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 RESULTS OF FORMULATION AND EVALUATION OF GEL LOADED WITH CLINDAMYCIN PHOSPHATE MICROSPONGES

6.1.1 Results of Preformulation studies of Clindamycin phosphate (CLP)

6.1.1.1 Results of melting point of CLP

Table no. 6.1 Results of melting point of CLP

Sr. No.	Melting point (°C)
1	112 -116
2	110 - 114
3	114 - 116
Average	**112 - 115**

6.1.1.2 Results of determination of UV absorption maxima of CLP

Table no. 6.2 Results of Determination of λ Max in Phosphate buffer pH 7.4

Sr. No.	Solvent	λmax (nm)	λmax (nm) Average
1	Phosphate buffer pH 7.4	210	**210**
2	Phosphate buffer pH 7.4	209	
3	Phosphate buffer pH 7.4	210	

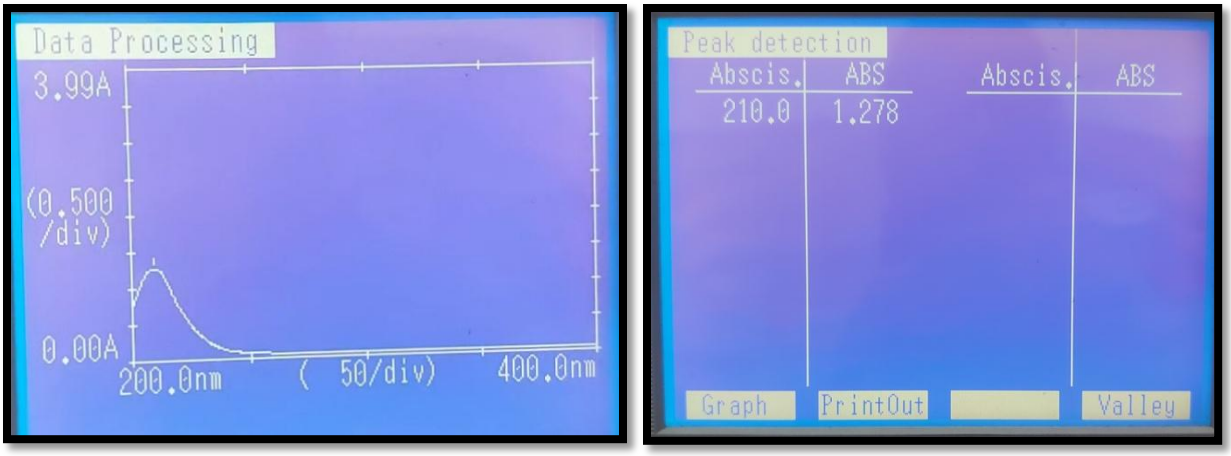


Figure 6.1 Absorption spectra of clindamycin phosphate in Phosphate buffer

Table no. 6.3 Results of Determination of λ Max in Methanol

Sr. No.	Solvent	λmax (nm)	λmax (nm) Average
1	Methanol	209	**210**
2	Methanol	210	
3	Methanol	210	

Spectral scanning was done for CLP in distilled water and methanol at 10µg/ml concentration individually. The average of triplicate was taken.

6.1.1.3 Results of determination of solubility studies of CLP

Table no: 6.4 Results of Saturation solubility studies of CLP

Solvent	Amount present (mg/100ml)*
Distilled water	80.5
Methanol	15.0
Phosphate buffer saline ph 7.4	88.5

*All readings are shown in mean (n=3)

Saturation solubility of CLP was carried out in distilled water, methanol, phosphate buffer ph 7.4.5ml of respective solvent was taken and excess quantity of drug was

added and dissolved until supersaturation. Then it was filtered and diluted. The absorbance was measured at 210nm in UV spectrophotometer.

6.1.1.4 Results of preparation of standard graph of CLP in PBS pH 7.4

Table no: 6.5 Results of Absorbance of CLP in PBS pH 7.4

Concentration(µg/ml)	Absorbance *
0	0
1	0.052
2	0.182
3	0.271
4	0.395
5	0.466
6	0.598
7	0.766
8	0.898
9	0.948

*All readings are shown in mean (n=3)

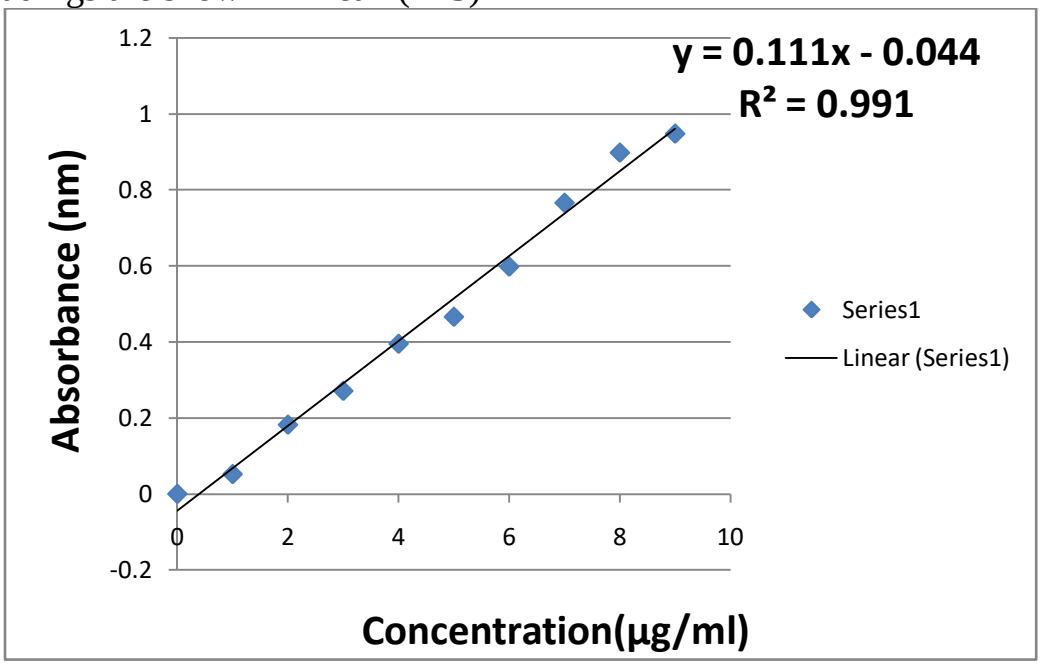


Figure 6.3 : Result of standard graph of CLP in PBS pH 7.4

The calibration curve of CLP was done by using PBS pH 7.4 was analyzed spectrophotometrically (SHIMADZU UV-1700) and obtained curve was found to be linear over a concentration range of 0 to 10 ug/ml with R^2 =0.991. the best fit linear equation obtained is y = 0.111x – 0.044.

6.1.1.5 Results of drug-polymer physical mixture interaction studies by FTIR

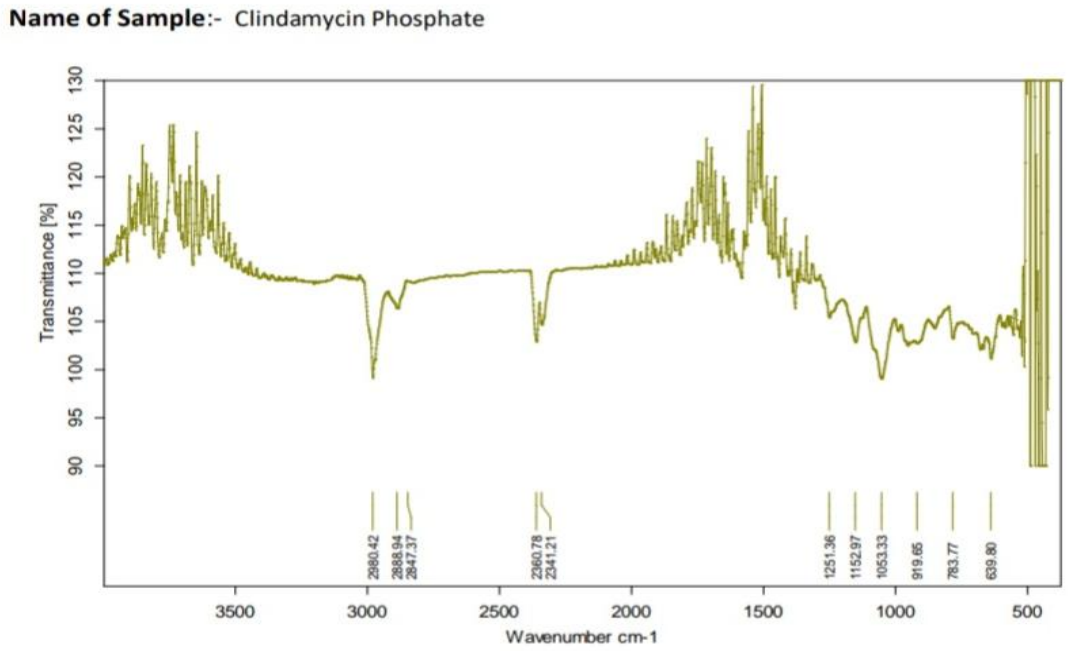


Figure 6.4: Results of FTIR of drug CLP

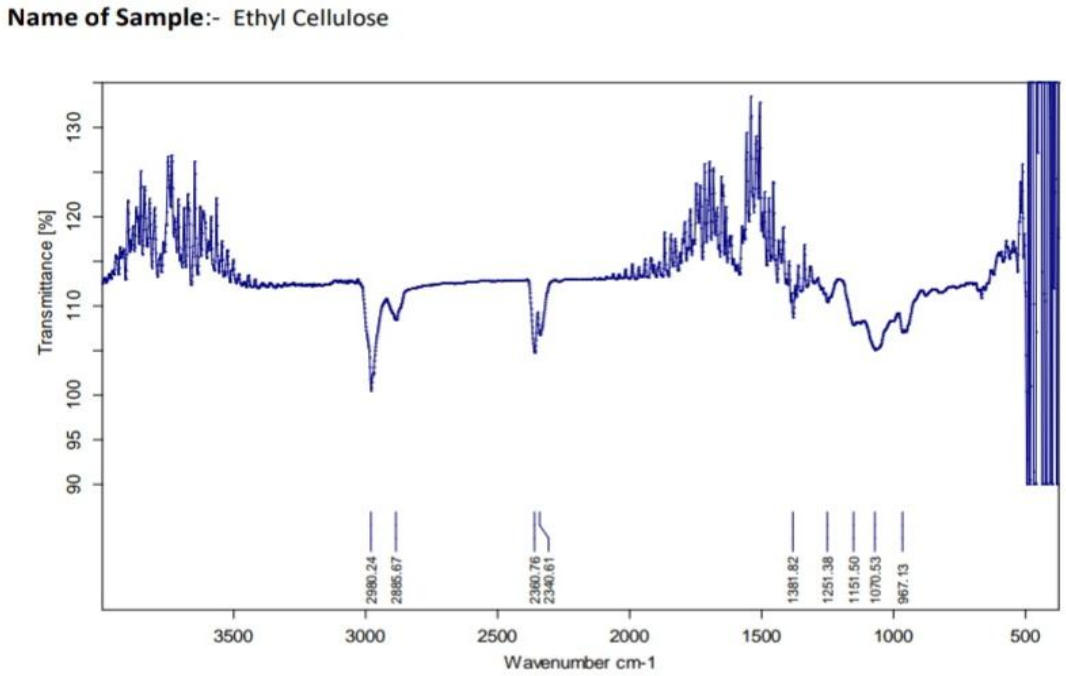


Figure 6.5: Results of FTIR of polymer Ethyl cellulose (EC)

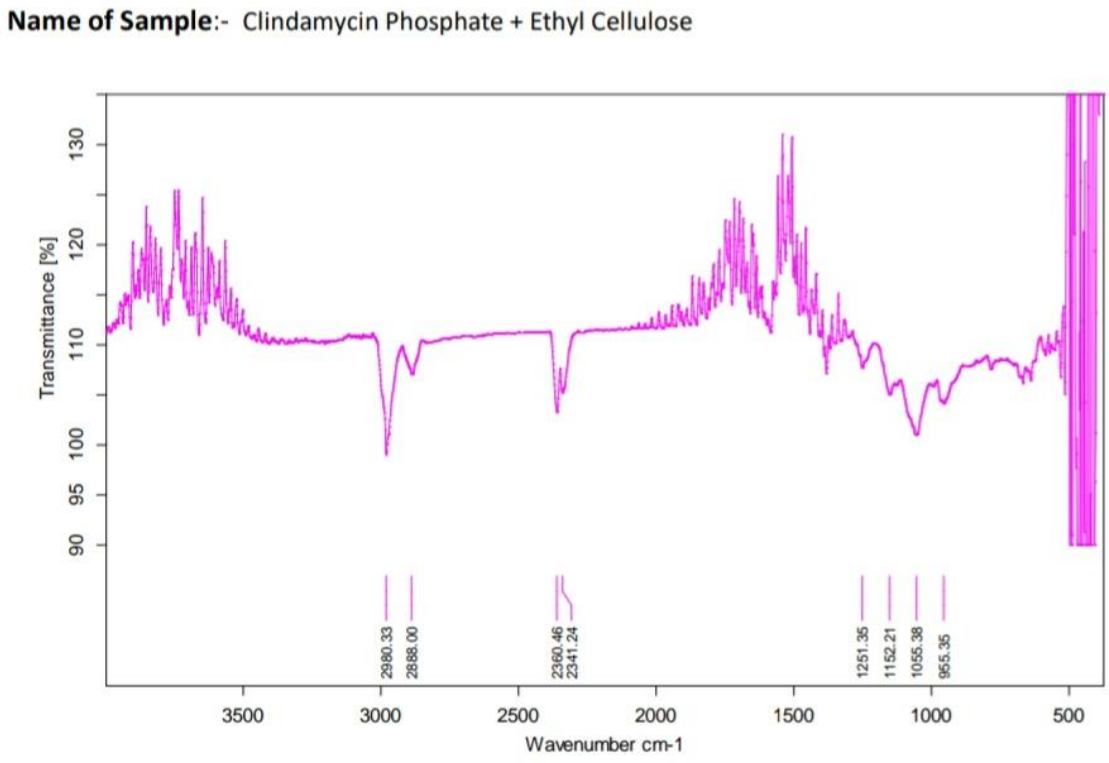


Figure 6.6 : Results of FTIR of physical mixure of CLP + EC

Table No. 6.6 Compatibility study of drug and polymer using FTIR

Functional group	Wavenumber (cm^{-1})			
	Standard peaks	**CLP**	**EC**	**CLP + EC**
C-H stretch (aliphatic)	2990 -2850	2980.42	2980.24	2980.33
C-F stretch	1000-1400	1152.97	1151.50	1152.21
C-O stretch	1300 -1000	1053.33	1070.53	1055.38
=C-H bend	995 – 685	919.65	967.13	955.35
C-N stretch (amine)	1250 -1000	1251.36	1251.38	1251.35

FTIR spectrum of drug CLP , polymer ethyl cellulose and drug-polymer physical mixture were recorded and interpretation was done. The results reveal the purity of CLP. It concludes that CLP does not interact with polymer in the preformulation studies.

6.1.2 Results of formulation studies of Clindamycin phosphate microsponges (CLP)

Oil in oil emulsion solvent diffusion technique was utilized for the preparation of CLP microsponges. The internal phase composed of 200 mg of CLP, 200 mg of Ethyl cellulose as a polymer and 3% w/v of magnesium stearate as a droplet stabilizer. The above internal phase polymer was dissolved in 5 ml of dichloromethane. The external phase consists of 50ml of liquid paraffin. The internal phase was poured drop wise into external phase under continuous stirring

at 1000 rpm for180 mins. The product obtained was filtered and washed with petroleum ether and n- hexane and left for drying overnight.

Table 6.7 Results of Composition of Optimized Formulation

Specification	Optimum values
Drug: polymer	1:1
CLP	100mg
Amount of Ethyl cellulose	100mg
Magnesium stearate	3%w/v
Inner phase solvent	Dichloromethane
Quantity of internal phage solvent (ml)	5ml
Quantity of liquid paraffin in external phage (ml)	50ml
Stirrer type	Three blades
Stirring rate	1000rpm
Stirring time	180mins

6.1.3 Results of Evaluation Studies of CLP microsponges

Table no. 6.8 Results of visual inspection of CLP microsponges (drug:polymer ratio)

Drug : Polymer ratio	Appearance	Colour	Nature
1:0.5	Not formed	-	-
1:1	Porous	White	Powdered
1:2	Porous	White	Powdered
1:3	Porous	White	Powdered
1:4	Non-Porous	White	Powdered

The porous microsponges formed looked fluffy and successfully floated in PBS pH 7.4 in waterbath at 37°C.

Table no. 6.9 Results of visual inspection of CLP microsponges (stirring speed)

Stirring speed (rpm)	Appearance	Colour	Nature
500	Non-Porous	White	Clumps
650	Non-Porous	White	Clumps
750	Porous	White	Powdered
900	Porous	White	Powdered
1000	Porous	White	Powdered
1250	Porous	White	Powdered
1300	Porous	White	Uneven shape

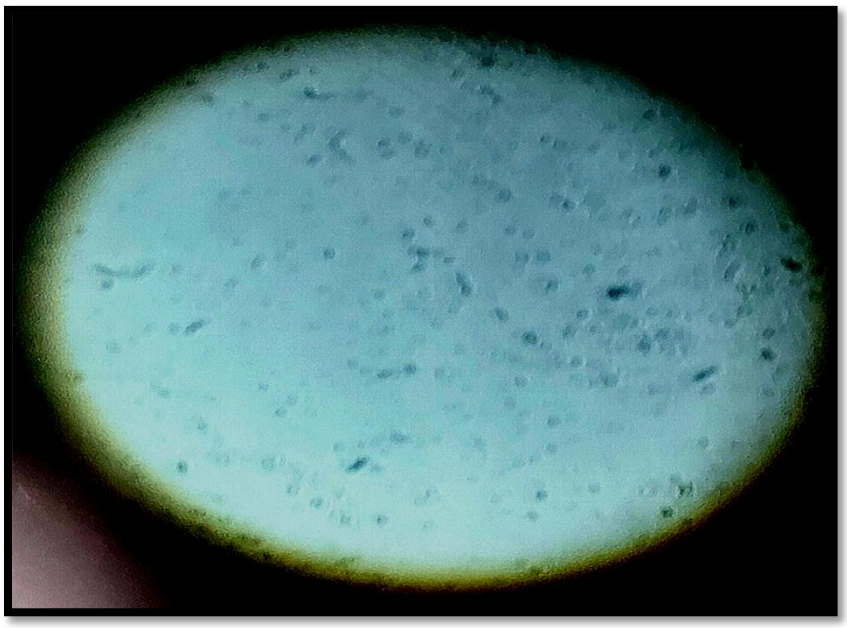


Figure 6.7 Spherical microsponges viewed under photomicroscope

6.1.3.2 EFFECT OF FORMULATION VARIABLES:

Table 6.9 Effect of formulation variables on microsponges :

Batch	Drug : polymer (mg)	Stirring speed (rpm)	% yield	Drug content*	Entrapement efficiency*
CLPA1	1:1	750	70.08%	77.41%	87.34%
CLPA2	1:1	1000	75.81%	79.81%	88.62%
CLPA3	1:1	1250	73.44%	79.82%	88.09%
CLPA4	1:2	750	81.22%	69.79%	83.58%
CLPA5	1:2	1000	84.69%	72.91%	84.91%
CLPA6	1:2	1250	83.11%	71.37%	84.12%
CLPA7	1:3	750	85.49%	66.63%	74.11%
CLPA8	1:3	1000	89.66%	68.83%	74.82%
CLPA9	1:3	1250	88.42	68.04%	73.62%

***All readings are shown in mean (n=3)**

As the drug:polymer ratio increases, drug content and entrapement efficiency was found to decrease. As the polymer concentration increases, entrapement efficiency increases when compared to drug content.

6.1.3.3 Results of particle size analysis by Photomicroscopic method

Table No.6.10 Results of particle size analysis

Drug: polymer ratio has the effect on the size of microsponges.The results concludes as the polymer concentration increases, particle size increases. This might be due to fact that the polymer available at high drug: polymer ratio is in considerable amount per microsponge, thus increasing the polymer surrounding the drug. This consequently results in the formation of larger microsponges.

The particle size of the optimized formulation batch was found to be **37.44µm.**

6.1.4 Results of Evaluation Studies of Gel Loaded with CLP microsponges

6.1.4.1 Results of Visual Inspection of Gel Loaded with CLP microsponges

Table No. 6.12 Results of visual inspection of gel loaded with CLP microsponges

Carbopol 940 (g)	Appearance	pH	Spreadability	Uniformity	Homogeneity
0.05	Transparent white	6.51	6.81	Good	Good
0.1	Transparent white	6.84	7.08	Good	Good
0.15	Transparent white	6.92	6.33	Poor	Good

Lesser time indicates better spreadability of the gel formulation. The optimized batch of the gel with 0.1g of carbopol 940 has the highest spreadability with pH 6.84. The gel formulation was found to have good uniformity and homogeneity with no phase separation.

6.1.4.2 Results of pH Measurement

Table No. 6.13 Results of pH of CLP loaded microsponge gel

Sr.No.	pH
1	6.37
2	6.39

3	6.37
Average	**6.38**

The pH of microsponge gel was found to be 6.38. The pH of gel loaded was measured using pH meter. The formulation is considered as safe and non-irritating, since their pH was within the normal skin pH that is 6.7.

6.1.4.3 Results of Spreadability Studies

Table No. 6.14 Results of spreadability study of CLP loaded microsponge gel

Sr.No.	Spreadability (g.cm/s)
1	7.09
2	7.64
3	6.42
Average	**7.05**

6.1.4.4 Results of Stability Studies

Table 6.15 Effect of parameters on microsponge gel

Parameters	After 1 month
Visual	Transparent white
pH*	6.41
Spreadability*	7.13 g cm/s

***All readings are shown in mean (n=3)**

The stability studies showed that there was no significant changes in pH and spreadability. There was no change in colour and feel of the gel.

6.1.4.5 Results of antimicrobial activity

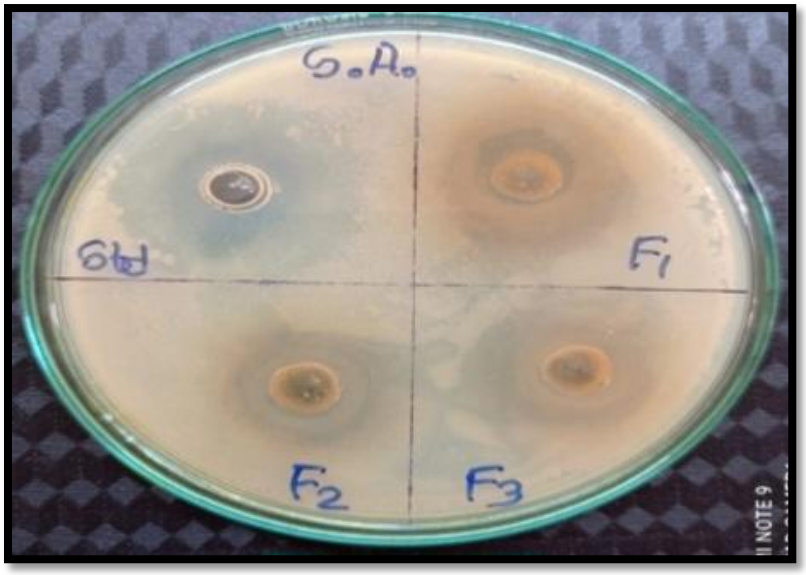


Figure 6.10 In-vitro antibacterial activity against *S.aureus*

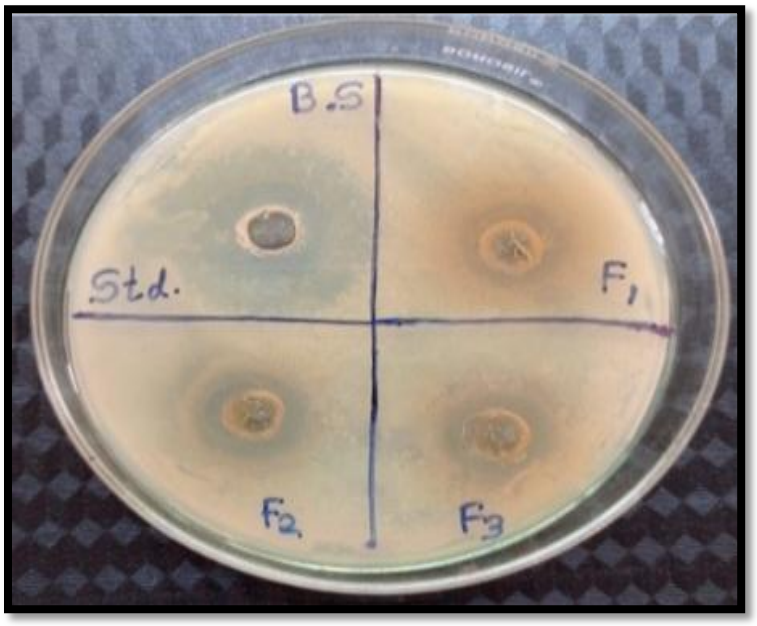


Figure 6.11 In-vitro antibacterial activity against *B.subtilis*

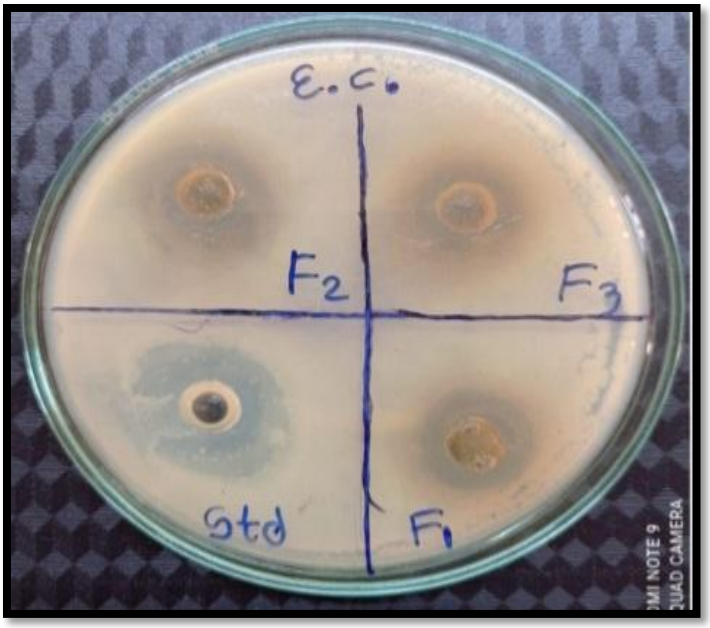


Figure 6.12 In-vitro antibacterial activity against *E.coli*

Table no. 6.16 Results of in-vitro antibacterial activity

Sample code	Zone of inhibition(mm)*
Std: Clindac A gel (1%w/w)	23.7
F1: Microsponge gel loaded with CLPA5	16.8
F2: Microsponge gel loaded with CLPA6	11.4
F3: Microsponge gel loaded with CLPA7	9.6

*All readings are shown in mean (n=3)

Table no. 6.20 shows diameter of zone of inhibition produced. From the said table it has been observed that the maximum zone of inhibition was in the following order: Marketed CLP gel > Microsponge gel loaded with CLP microsponges with drug : polymer ratio of 1:1 at 1000rpm> gel loaded with CLP microsponges of drug polymer ratio of 1: 2 at 1000rpm> gel loaded with CLP microsponges with drug polymer ratio of 1:3 at 1000rpm.

6.1.4.6 Results of in vitro Drug Release Studies of Gel Loaded with CLPA1-CLPA9 using Franz Diffusion Cell

Time mins	CLPA1	CLPA2	CLPA3	CLPA4	CLPA5	CLPA6	CLPA7	CLPA8	CLPA9
0	0	0	0	0	0	0	0	0	0
60	36.25	33.34	30.56	31.07	30.82	34.61	29.81	29.93	29.55
120	47.74	46.23	46.35	45.97	44.08	46.35	38.90	36.63	36.63
180	54.82	57.85	55.83	55.45	57.09	54.82	45.09	42.56	43.70
240	62.65	64.92	67.57	62.14	65.17	63.41	52.80	48.25	52.04
300	69.97	72.13	77.17	71.62	69.97	70.23	56.08	55.07	56.46
360	78.44	77.55	79.20	83.49	75.03	77.55	61.76	61.51	63.03
420	81.47	80.33	80.33	85.89	80.46	81.97	67.20	70.23	70.23
480	90.56	90.56	84.25	90.56	89.81	89.91	70.73	77.81	81.47
540	94.23	91.70	86.65	92.33	90.94	92.46	76.92	84.25	82.98
600	95.26	97.34	92.11	95.42	96.41	94.14	88.86	91.32	84.33

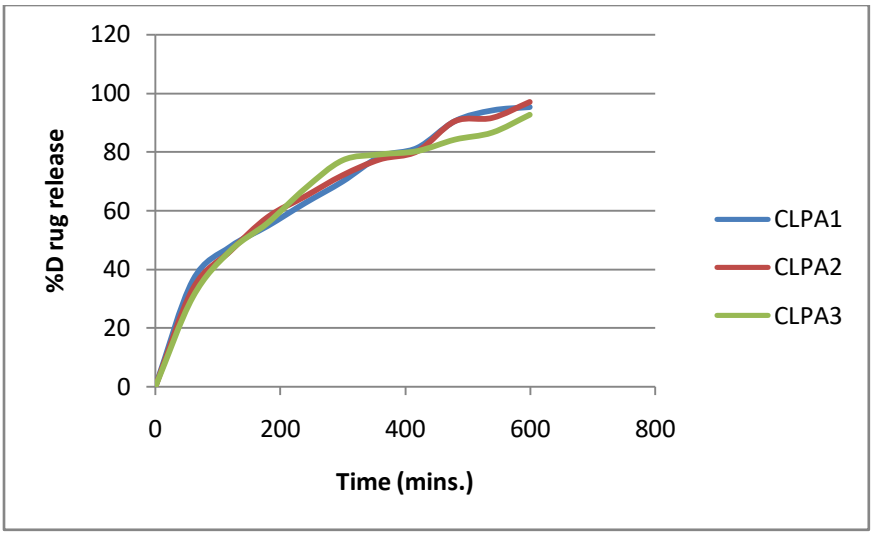


Figure 6.13 : Results of comparative in vitro drug release of CLPA1-CLPA3

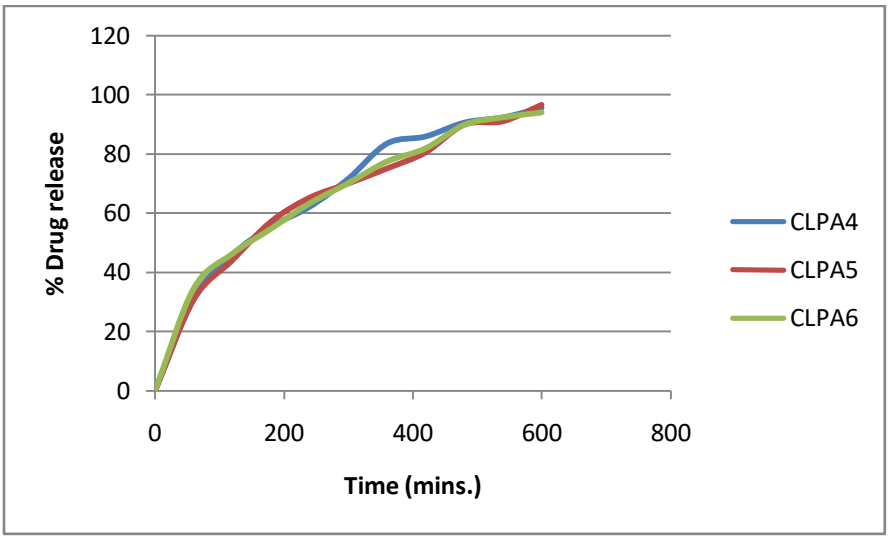


Figure 6.14 : Results of comparative in vitro drug release of CLPA4-CLPA6

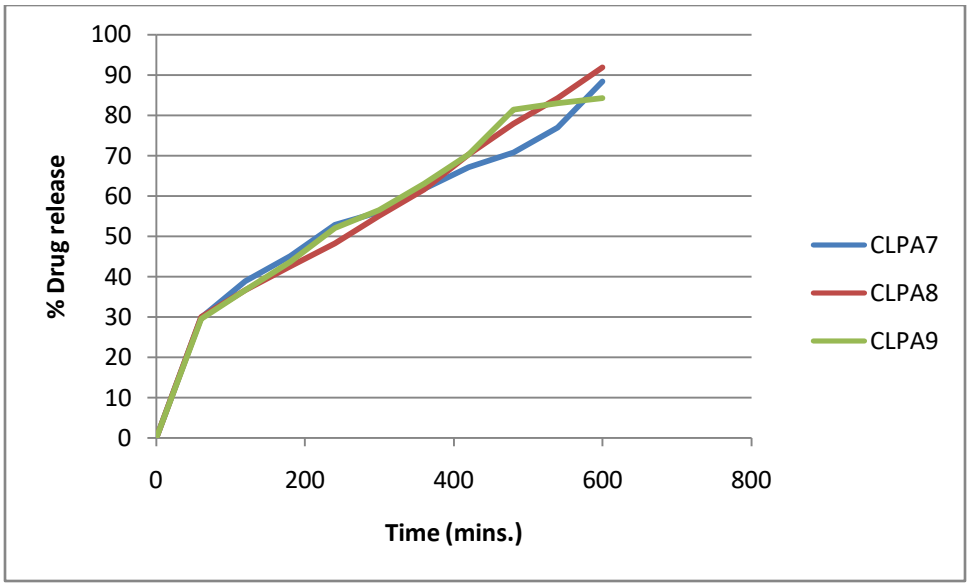
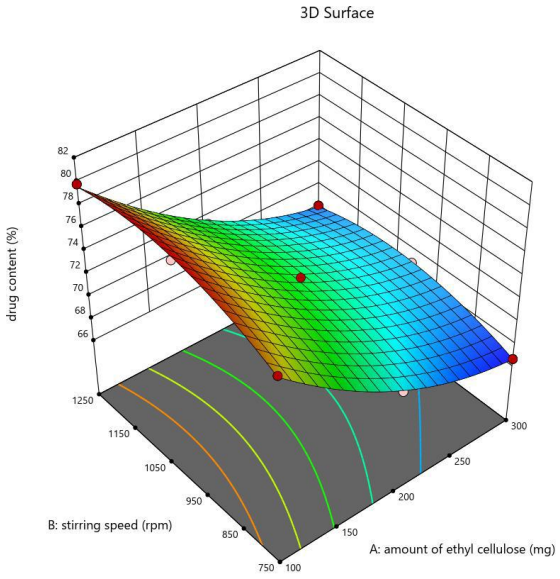
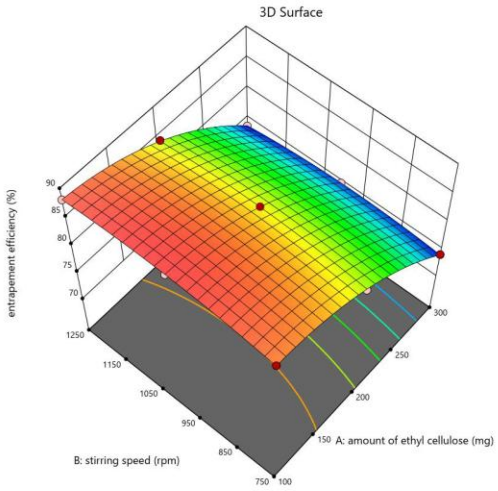


Figure 6.15 : Results of comparative in vitro drug release of CLPA7-CLPA9



A)



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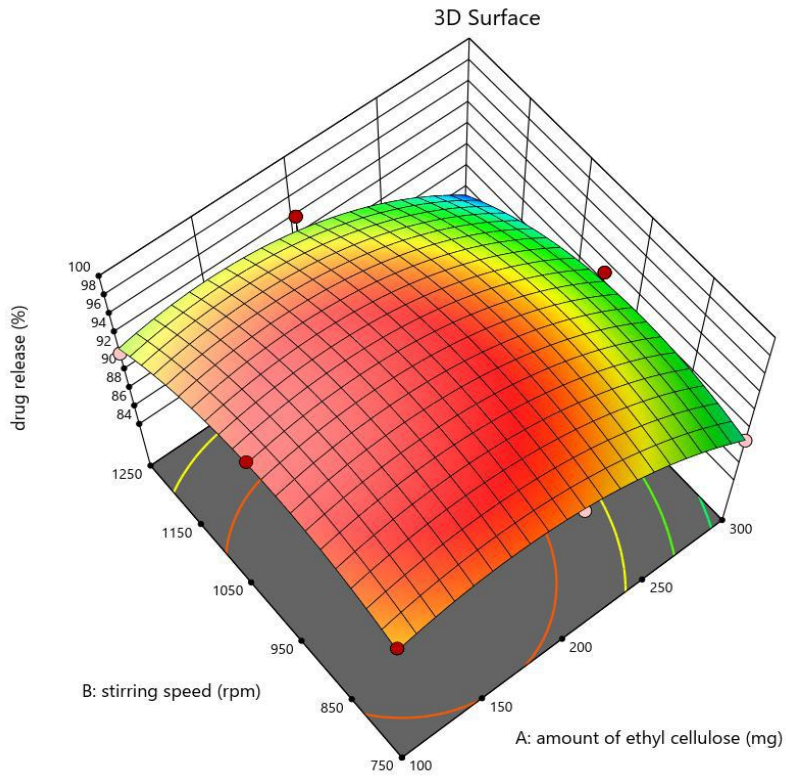

C)

Figure 6.17 : Influence of formulation variable drug polymer ratio (X1) and stirring speed on (X2) on A) drug content B) Entrapement efficiency C) Percentage drug release

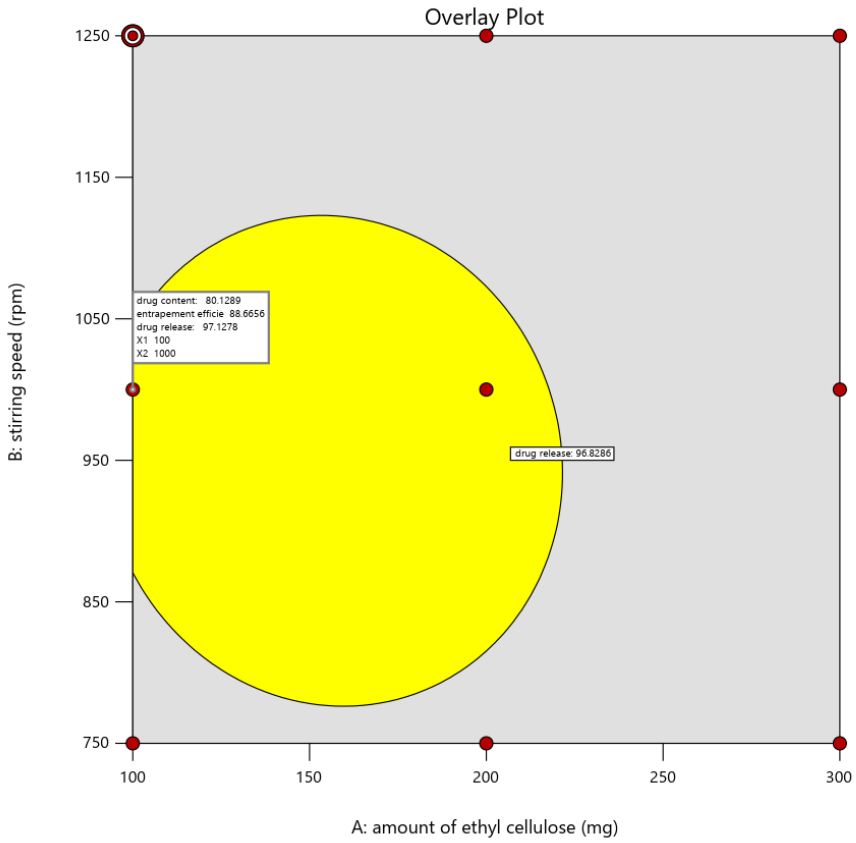

Figure 6.18 Overlay plot of clindamycin phosphate microsponge formulation

Table 6.1.4.7 : Predicted and Experimental values obtained based on optimized formula for preparation of microsponges

Predicted values based on optimized formula				
Factors		**Responses**		
Amount of ethyl cellulose (mg)	Stirring speed (rpm)	Drug content (%)	Entrapement efficiency (%)	In vitro drug release (%)
100	1000	80.13	88.67	97.13
Experimental values based on optimized formula				
Amount of ethyl cellulose (mg)	Stirring speed (rpm)	Drug content(%)	Entrapement efficiency (%)	In vitro drug release (%)
100	1000	78.69	87.94	95.49

CHAPTER 7

CONCLUSION

7.1 CONCLUSION

The microsponges delivery system is a unique technology for the sustained release of porous microspheres, loaded with active ingredient, offering potential reduction in side effects. Incorporating antibiotic drug showed better retention of the drug in the skin signifying well improved delivery system as compared with marketed formulation.

In the present work microsponges of Clindamycin phosphate are prepared by oil in oil (O/O) emulsion solvent diffusion method using polymer ethyl cellulose for controlled release of the drug. The prepared microsponges were incorporated into gel of Carbopol 940.

The study leads to the following conclusions:

- FTIR study indicates that the drug is compatible with the polymer.
- Particle size analysis to determine particle size of prepared microsponges.
- Drug content of prepared microsponges to determine actual drug content in microsponges.
- Percentage entrapement efficiency to determine the percent of drug successfully entrapped in microsponges.
- pH of microsponge gel to avoid irritation or erythema on skin.
- Visual inspection of gel for colour, appearance, texture and homogeneity.
- Spreadability test to determine ease of application of gel on the skin.
- Stability test as the evidence of quality of the product at specific conditions and time intervals.

The present study shows that topical application of antibiotic microsponge gel may exhibits better control of inflammation more effectively reversing the complication associated with inflammation release than normal gel application. Thus, gel bearing microsponges of Clindamycin phosphate was designed. This approach was found to be a novelty in the drug delivery system, offering in treatment of acne vulgaris.

7.2 SCOPE FOR FUTURE WORK

Further studies are needed for the followings

1. Laboratory scale to pilot plant scale up technique to increase the batch size.
2. studies in human volunteers after ethical clearance for its efficacy
3. Analyzing the pharmacokinetic parameters in animals and humans.
4. Feasibility to check with other polymers to extend more release.

CHAPTER 8

SUMMARY

8.1 SUMMARY

Microsponges are particulate drug delivery system composed of porous nature. They are small tiny sponges like spherical particles with large porous surface moreover they may heighten the stability by modifying the drug release pattern with reduced side effects. Microsponges Delivery System (MDS) can suspend or entrapped a wide variety of substance which act as carrier. Gel loaded microsponges are unique technology, where drug is dispersed in gel and also entrapped in microsponges, on application of gel loaded microsponges the active drug moves from the gel to skin as initial dose, when the drug is exhausted in the gel, the movement of drug from the microsponges start, this mechanism will continue until the complete drug is absorbed.

For development of microsponges, oil in oil (o/o) emulsion solvent diffusion method was employed due to its better microsponges formation technique. Moreover, they may enhance stability, reduce side effects and modify drug release mechanism. An attempt was made to develop the dosage form to delivery through the skin in the form of microsponges gel, to shows better sustain release of drug compare to conventional gel.

Chapter 1 describes about the drug delivery system, types of dosage form, new approaches in drug delivery, topical delivery is technique to overcome the side effects related to oral delivery. microsponge: an approach for topical delivery, these are polymeric device to show sustained action with structure of microsponges contain pores to facilitate the movement of drug from vehicle to device as its mechanism of action of typical microsponges are does not penetrate into the skin

rather they release the drug in sustained manner. polymers used in microsponge preparation, help in formation of porous polymeric and devices during formulation consideration, drug should have low solubility in water. These methods were employed for the preparation of microsponges.

The chapter also elaborates the preparation of microsponges technology, structure of microsponges and drug delivery using microsponges with a detailed illustrated figure. Further the mechanism of action of microsponges with the polymers used in the formulation consideration with various methods of preparation like liquid-liquid suspension polymerization and quasi emulsion evaluation diffusion technique are discussed and also detailed effects of formulation variables on physical properties of microsponges, stirring rate applications and benefits over other transdermal products are discussed. Finally, the need of their current study was also discussed.

Chapter 2 discuss about the literature review which explore the earlier research work on formulation of microsponges, based on the literature review, various techniques for the preparation of microsponges are discussed. Further it also explores the material and methods to be adopted were noted.

The overview of microsponges as delivery system were discussed in this chapter. The drug Clindamycin phosphate has not been formulated as gel loaded with microsponges by utilizing oil in oil emulsion solvent diffusion technique.

Clindamycin phosphate as conventional formulations has various side effects and is required to be applied twice daily due to short half life of drug.Hence, there is a demand to deliver the drug through transdermal route with minimum possible dose and diminish side effects. To overcome the side effects of conventional dosage form, current research was focused to formulate and evaluate the gel loaded with

microsponges of Clindamycin phosphate by using oil in oil emulsion solvent diffusion technique.

Chapter 3 briefs about scope of research for selection of dosage form with antibiotic drug. Clindamycin phosphate conventional dosage forms available in market has many side effects with dose of daily twice due to short half life of drug. Clindamycin phosphate as microsponge gel helps to reduce side effects and prolong duration of action of drug by controlled release.

Chapter 4 describe the aim and objectives of the research work.

Chapter 5 describes the materials and instruments used in the research work. It describes the methodology involved in selection of drug. It further explains about evaluation studies for gel loaded with Clindamycin phosphate microsponges , with proper literature reported methods.

Chapter 6 elaborates the results and discussion of the proposed research work planned in preformulation studies, preparation of microsponges, formulation of gel loaded microsponges, and their evaluation studies with results and discussion. Preformulation studies Clindamycin phosphate is carried out for its melting point, UV absorption maxima, solubility studies, Standard graph, and identification of drug by FT-IR showed the identification, quality and purity of the drugs.

Microsponges were prepared and evaluated using design expert software.

Further microsponges are loaded into the gel and was evaluated for the visual inspection of gel loaded microsponges of CLP observed for color, appearance, pH, spreadabilty, stability, in vitro antimicrobial studies and franz diffusion ctest to examine release pattern of the drug.

Chapter 7 describes the conclusion and future scope of the research work to be carried out for formulation and evaluation of gel loaded with Clindamycin Phosphate microsponges. Thus, gel bearing microsponges of Clindamycin phosphate was designed in this approach was found to be challenging as a novel delivery system, offering in treating of acne vulgaris.

CHAPTER 9

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