# DEVELOPMENT, OPTIMIZATION AND SCALE-UP OF 5-FLUOROURACIL LOADED MICROSPONGE DELIVERY SYSTEM BY RESPONSE-SURFACE METHODOLOGY

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***Title of the thesis***

DEVELOPMENT,OPTIMIZATION AND SCALE-UP OF 5-FLUOROURACIL LOADED MICROSPONGE DELIVERY SYSTEM BY RESPONSE-SURFACE METHODOLOGY.

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# DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

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## *CERTIFICATE OF APPROVAL*

This is to certify that the thesis entitled "**Development, Optimization and Scale-up of 5-Fluorouracil loaded Microsponge Delivery Sysyem by Response-Surface methodology**" submitted by **Shahjaman Halder**, of Jadavpur University, for the course of **Master of Pharmacy** is absolutely based upon his own work under the supervision of **Dr. Jasmina Khanam**, Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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## DECLARATION BY THE CANDIDATE

I do hereby declare that the work incorporated in the thesis entitled "**Development, Optimization and Scale-up of 5-Fluorouracil loaded Microsponge Delivery System by Response-Surface methodology"** has been carried outby me in the Department of Pharmaceutical Technology, Jadavpur University under the supervision of Dr. Jasmina Khanam, Professor, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032. Neither the thesis nor any part therefore has been submitted for any other degree.

Date: Signature of the candidate

Place: Kolkata, India (SHAHJAMAN HALDER)

Dedicated to My Family and My Guide

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Date: SHAHJAMAN HALDER

# PREFACE

The present thesis work entitled "**Development, Optimization and Scale-up of 5-Fluorouracil loaded Microsponge Delivery System by Response-Surface methodology"** deals with development of control release microsponge particles which was developed and optimized by statistical modeling (response-surface) methodology. Quasi-emulsion solvent evaporation method was used to prepare microsponge particles. Three factors were taken in design polymer, stirring rate and surfactant. Four responses were checked (%Yield, Particle size, Entrapment efficiency and Release of drug at 8hr) by software and optimized the formulation.

The introduction part of the thesis (Chapter 1) describes about the characteristics and types of preparation of microsponges and thereafter gel preparation with these particles.Some discussions about the model drug and polymer chosen were highlighted. Chapter 2 represents a careful selection and presentation of recent published works relatedto the present topic of work. The aims and objectives of this work are laid sequentially and logically in Chapter 3. Chapter 4 consists of the preparation, characterization and optimization of microsponges by response-surface methodology. The conclusions drawn from the entire work are detailed in Chapter 5. Chapter 6 gives comprehensive list of referenceswhich were cited in the text.

# Contents

# *Chapter 1*

INTRODUCTION

## 1. Introduction:-

Microsponge particle may be defined as hard sponge in which drug molecules are entrapped within polymeric porous multi channeled structure. 'Microsponge Drug delivery system' consists of tiny sponge like round shaped particles with a large porous surface. This drug loaded structured body causes enhanced stability, reduced side effects and modified drug release favorably. Microsponge technology possesses favorable characteristics which makes this porous structure as multifaceted drug delivery vehicle. These porous microspheres entrapping a variety of substances can then be incorporated into a suitable vehicle such as a gel, cream, liquid or powder to make it effective as topically dosage form. Microsponge delivery system can provide increased efficacy for topically active agents with enhanced safety, extended product stability and improved appealing properties in an efficient manner. Many drugs exist already with reliability for systemic delivery of drugs under the heading of 'Transdermal delivery system' using the skin as a portal of entry. It has improved the efficacy and safety of many drugs that may be better administered through skin. But TDDS is not practicable for delivery of materials whose final target is skin itself. In that case drug molecules should be chosen judiciously for dermal dosage form as permeation through the skin into systemic circulation is not desirable in significant amount. Restriction of its activity in the skin only cannot be ensured fully. Microsponges consist of non-collapsible structures with porous surface through which active ingredients are released in controlled manner. Depending upon the average particle size of microsponge, the total pore length may range amazingly up to 10 feet and pore volume up to 1 mL/g. When it is applied to the skin, the 'microsponge drug delivery system' (MDS) releases its active ingredient on a time mode and also in response to other stimuli such as rubbing, temperature, and pH. Microsponges have the capacity to absorb or load a high degree of active materials into the particle or onto its surface.

Its large capacity for entrapment of active ingredients up to 3 times its weight makes microsponges distinguished from other types of dermatological delivery systems. It acts as storehouse while releasing drug in slow rate. Mostly microsponge is used for topical/transdermal drug delivery system, besides this it can be applied for oral delivery too specially for colon targeted drug. It can be used to absorb skin secretion.

As microsponge particles are tiny, inert and indestructible spheres so it cannot pass through the skin. But these can reside in the tiny pores of the skin and slowly release the entrapped drug, as the skin needs it. The size of the microsponges can be varied, usually from 5-300 μm in diameter.

Research interest has been focused recently on the dosage form in which the drug remains primarily localized and does not enter the systemic circulation in significant quantities. No efficient vehicles exist truly for controlled and localized delivery of drugs into the stratum cornium and underlying skin layers and not ahead of the epidermis. Patient compliance is often compromised owing to unappealing, greasy and sticky nature of topical dosage forms. It is continuous endeavor to develop a suitable dermal preparation. The vehicles of topical formulations often are loaded with high concentrations of active agents to achieveadequate therapeutic response because of the low efficiency of carrier gel system in which uncomfortable side effects may arise such as irritation / allergic reactions in significant users. Some other problems often may evolve out with traditional ointment/cream such as uncontrolled evaporation of active ingredient, obnoxious odor and potential in-compatibility of drugs with the vehicles. The microsponge delivery system may be useful in prolonging the residence time of drug either on skin surface or within the epidermis, while reducing its trans-dermal penetration into the body. It has improved the efficacy and safety of many drugs that may be better administered through skin. But TDDS is not practically appropriate for delivery of materials whose final target is skin itself. The terminology ‘Transdermal delivery’ should be restricted to the therapeutic condition in which a solute (potent active agent) permeates from surface through the various layers of the skin and into the systemic circulation for the treatment of various ailments. Dermal (topical) delivery system provides localized action on the pathological sites within the skin and it should ensure minimal systemic absorption intending to cure dermatological ailments such as skin cancer, psoriasis, eczema, and microbial infections.

In past years, there has been extensive emphasis given to the development of microsponge base novel drug delivery systems, in order to modify and control the release behavior of the drugs. In this micro porous structure leaching happens as drug molecules diffuse through micro tortuous channels, and diffusion rate depends on average particle size, pore size, length of channels and properties of vehicle, drug molecules and temperature. By incorporation of these microsponge

particles into a carrier system, it is possible to amend the therapeutic index and duration of the activity of drugs intended for dermal ailment and systemic effect (colon targeted drugs)[Patil et al, 2016; N.H.Aloorkar et al, 2012].

The microsponge technology was developed by Won in 1987, and the original patents were assigned to Advanced PolymerSystems, Inc. This company developed a large number of variations of the procedures and those are applied to the cosmetic as well as over-the-counter (OTC) and prescription pharmaceutical products.

The scanning electron microscopy of the microsponge particle reveals that its internal structure as the "bag of marbles". The porosity is due to the interstitial spaces between the marbles. The interstitial pores can entrap many wide ranges of active ingredients such as emollients, fragrances, essential oils, sunscreens, anti-infective and anti-inflammatory agents. These entrapped microsponges may then be integrated or formulated into product forms, such as creams, lotions, powders, soaps, capsules and tablets. When these products are applied the entrapped material gets delivered to the skin in a controlled time release pattern or a pre-programmed manner through the use of several different 'triggers', rubbing or pressing the Microsponge after it has been applied to the skin, elevates skin surface temperature introducing solvents for the entrapped materials such as water, alcohol or even perspiration and controlling the rate of evaporation. Active ingredients entrapped in the porous polymeric structure display altered behavior, with respect to their release, which is restricted and prolonged.

## 2. Dermal Drug Delivery System (DDDS) [Marc B.Brown and Gary P.Martin et al. 2008]:-

Innovations in the area of drug delivery are takingplace at a much faster pace as compared with thelast two decades. Improved patient compliance andeffectiveness are inextricable aspects of new drugdelivery systems. A more radical approach has beento explore newer interfaces on the body for introducingtherapeutics. One such approach, dermal drug delivery system should only be used to define a targeting to pathological sites within the skin, which involves ensuring minimal systemic absorption. This delivery system is important in the treatment of dermatological conditions such as skin cancer, psoriasis, eczema and microbial infection where the disease is located in the skin. Generally semi-solid dosage forms are applicable for this delivery system. Semi-solids constitute a significant proportion of pharmaceutical dosage forms.

They serve as carriers for drugs that are topically delivered by the way of the skin. Because of their peculiar rheological behavior, semi-solids can adhere to the application surface for sufficiently long periods before they are washed off. This property helps prolong drug delivery at application site. A semi-solid dosage form is advantageous in terms of its easy application, rapid formulation and ability to deliver topically a wide variety of drug molecules. Semi-solids are available as wide range of dosage forms, each having unique characteristics. Recent approaches in dermal drug delivery are biphasic vesicles.

## 2.1 Benefits and limitations associated with dermal or cutaneous delivery system:-

**Benefits:-**

- The avoidance of first pass metabolism and other variables associated with the GI tract such as pH, gastric emptying time [Cleary 1993; Henzel and Loomba 2003; Kormic et al, 2003].
- Sustained and controlled delivery over a prolong period of time [Varvel et al. 1989; Yang et al. 2004].
- Reduction in side effects associated with systemic toxicity i.e, mimization of peaks and troughs in blood-drug concentration [Cramer and Saks et al. 1994; Kormic et al. 2003].
- Improved patient acceptance and compliance [Payne et al. 1998; Jarupanichet al. 2003; Archer et al. 2004].
- Direct access to target or diseased site, e.g., treatment of skin disorders such as psoriasis, eczema, and fungal infections [Colin Long 2002].
- Ease of dose termination in the event of any adverse reaction either systemic or local.
- Convenient and painless administration [Cleary 1993; Henzel and Loomba 2003].
- Ease of use may reduce overall health care treatment cost [Whittington and Faulds 1995; Frei et al. 2003].
- Provides an alternative in circumtences where oral dosing is not possible (in unconscious and nauseated patients) [Kormic et al. 2003].

**Limitations:-**

- A molecular weight less than 500 Da is essential to ensure ease of diffusion across the SC (Stratum Cornium) [Bos and Meinardi 2000], since solute diffusivity is inversely related to its size.
- Sufficient aqueous and lipid solubility, a Log P (octanol/water) between1-3 is required for the permeant to successfully traverse the SC and its underlying aqueous layers for systemic delivery to occur [Yano et al. 1986; Lee et al. 1994].
- Pre systemic metabolism; the presence of enzymes in the skin such as peptidases and esterases might metabolize the drug into a form that is therapeutically inactive, thereby reducing the efficacy of the drug [Steinstrasser and Merkle 1995].
- Skin irritation and sensitization; referred to as the "Achilles heel" of dermal and transdermal delivery. The skin as an immunological barrier may be provoked by exposure to certain stimuli, this may include drugs, excipients, or components of delivery devices resulting in erythema,oedema, etc. [Hogan and Maibach 1990; Carmichael 1994; Toole et al. 2002; Murphy and Carmichael 2000].

## 2.2 List of Microsponge drugs approved by USFDA (Table 1.1):-

| Approved Year | Drugs | Trade name, company | Application |
|---|---|---|---|
| 1997 | Retinol | Retin-A-Micro; Ortho-McNeil Pharmaceutical Corporation | Used as Anti-acne agent |
| 1999 | 5- Fluorouracil | Carac cream 0.5%; Dermic Laboratories, Inc. | Used as Actinic Keratoses(AK), Pre cancerous skin cond. |
| 2017 | Benzoyl peroxide, Clindamycin Phosphat | Benzoyl peroxide topical gel; Tolmar Laboratories | Used as Anti-acne agent |
| 1990 | Fluconazole | Diflucan; Pfizer | Used as Anti-fungal drug |

| | | | |
|---|---|---|---|
| 2009 | Ibuprofen | Ibuprofen; Bionpharma, Inc. | Used as Anti-inflammatory drug |
| 2009 | Ketoprofen | Nexcede; Novartis Consumer Health, Inc. | Used as Anti-inflammatory drug |
| 2000 | Terbinafine HCL | LamisilAT; Glaxosmithkline | Used as Anti-fungal drug |
| 2013 | Acyclovir | Acyclovir; Mylan Pharms, Inc. | Used as Anti-viral drug |
| 1995 | Clotrimazole | Clotrimazole; Teva Pharmaceuticals | Used as Anti-fungal drug |

## 3. Skin structure and functions [Montagna et al, 1978; Berner B et al, 1987]:-

The skin is the largest organ in the body, comprising about 15% of body weight. The total skin surface of an adult ranges from 12-20 square feet. In terms of chemical composition the skin is about 70% water, 25% protein and 2% lipids.

The skin consists of three main layers Epidermis, Dermis and Subcutaneous tissue.

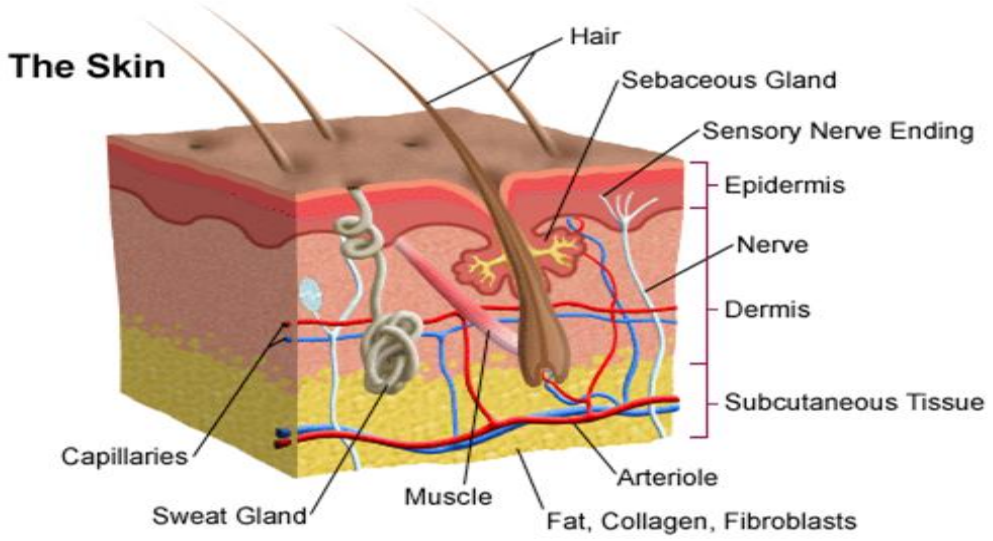



**Fig. 1.1 :- Structure of skin**

## Epidermis

The epidermis is the most superficial layer of the skin and is composed of *stratified keratinised squamous epithelium*, which varies in thickness in different parts of the body. It is thickest on the palms of the hands and soles of the feet. There are no blood vessels or nerve endings in the epidermis, but its deeper layers are bathed in interstitial fluid from the dermis, which provides oxygen and nutrients, and drains away as lymph.

There are several layers (strata) of cells in the epidermis which extend from the deepest *germinative layer* to the most superficial *stratum corneum* (a thick horny layer). The cells on the surface are flat, thin, non-nucleated, dead cells, or *squames*, in which the cytoplasm has been replaced by the fibrous protein *keratin*. These cells are constantly being rubbed off and replaced by cells that originated in the germinative layer and have undergone gradual change as they progressed towards the surface. Complete replacement of the epidermis takes about a month.

Melanin, a dark pigment derived from the amino acid tyrosine and secreted by *melanocytes* in the deep germinative layer, is absorbed by surrounding epithelial cells. The amount is genetically determined and varies between different parts of the body, between people of the same ethnic origin and between ethnic groups. The number of melanocytes is fairly constant so the differences in colour depend on the amount of melanin secreted. It protects the skin from the harmful effects of sunlight. Exposure to sunlight promotes synthesis of melanin.

Langerhans cells are a specialized population of dendritic cells that are found in the epidermis of the skin. They help to drive protective of different infection of skin. They prevent unwanted foreign substances from penetrating the skin.

## Dermis

The dermis is tough and elastic. It is formed from connective tissue and the matrix contains *collagen fibres* interlaced with *elastic fibres*. Rupture of elastic fibres occurs when the skin is overstretched, resulting in permanent striae or stretch marks, that may be found in pregnancy and obesity. Collagen fibres bind water and give the skin its tensile strength, but as this ability declines with age, wrinkles develop. Fibroblasts, macrophages and mast cells are the

main cells found in the dermis. Underlying its deepest layer there is areolar tissue and varying amounts of adipose (fat) tissue. The structures in the dermis are: blood vessels,lymph vessels, sensory (somatic) nerve endings, sweat glands and their ducts, hairs, arrectorpili muscles and sebaceous glands.

**Blood and lymph vessels**

Arterioles form a fine network with capillary branches supplying sweat glands, sebaceous glands, hair follicles and the dermis. Lymph vessels form a network throughout the dermis.

**Sensory nerve endings**

Sensory receptors (specialized nerve endings) sensitive to *touch, temperature, pressure* and *pain* are widely distributed in the dermis. Incoming stimuli activate different types of sensory receptors. The Pacinian corpuscle is sensitive to deep pressure. The skin is an important sensory organ through which individuals receive information about their environment. Nerve impulses, generated in the sensory receptors in the dermis are conveyed to the spinal cord by sensory nerves, then to the sensory area of the cerebrum where the sensations are perceived.

**Sweat glands**

These are widely distributed throughout the skin and are most numerous in the palms of the hands, soles of the feet, axillae and groins. They are formed from epithelial cells. The bodies of the glands lie coiled in the subcutaneous tissue. There are two types of sweat gland. The commonest type opens onto the skin surface through tiny pores, and the sweat produced here is a clear, watery fluid important in regulating body temperature. The second type opens into hair follicles, and is found, for example, in the axilla. Bacterial decomposition of these secretions causes an unpleasant odour. A specialized example of this type of gland is the ceruminous gland of the outer ear, which secretes earwax.

The most important function of sweat, which is secreted by glands, is in the regulation of body temperature. Excessive sweating may lead to dehydration and serious depletion of sodium chloride unless intake of water and salt is appropriately increased. After 7 to 10 days exposure to

high environmental temperatures the amount of salt lost is substantially reduced but water loss remains high.

**Hairs**

These are formed by a down growth of epidermal cells into the dermis or subcutaneous tissue, called *hair follicles*. At the base of the follicle is a cluster of cells called the *papilla* or *bulb*. The hair is formed by multiplication of cells of the bulb and as they are pushed upwards, away from their source of nutrition, the cells die and become keratinised. The part of the hair above the skin is the *shaft* and the remainder, the root.

**Arrectorpili**

These are little bundles of smooth muscle fibres attached to the hair follicles. Contraction makes the hair stand erect and raises the skin around the hair, causing 'goose flesh'. The muscles are stimulated by sympathetic nerve fibres in response to fear and cold. Erect hairs trap air, which acts as an insulating layer. This is an efficient warming mechanism, especially when accompanied by shivering, i.e. involuntary contraction of skeletal muscles.

**Sebaceous glands**

These consist of secretory epithelial cells derived from the same tissue as the hair follicles. They secrete an oily substance, *sebum*, into the hair follicles and are present in the skin of all parts of the body except the palms of the hands and the soles of the feet. They are most numerous in the skin of the scalp, face, axillae and groins. In regions of transition from one type of superficial epithelium to another, such as lips, eyelids, nipple, labia minora and glans penis, there are sebaceous glands that are independent of hair follicles, secreting sebum directly onto the surface.

Sebum keeps the hair soft and gives it a shiny appearance. On the skin it provides some waterproofing and acts as a bactericidal and fungicidal agent, preventing infection. It also prevents drying and cracking of skin, especially on exposure to heat and sunshine. The activity of these glands increases at puberty and is less at the extremes of age, rendering the skin of infants and older adults prone to the effects of excessive moisture (*maceration*).

## Subcutaneous layer

Subcutaneous tissue is the innermost layer of the skin located under the dermis consisting of connective tissue and fat molecules. Subcutaneous fat acts as a shock absorber and heat insulator protecting underlying tissues from cold and mechanical trauma.

The loss of subcutaneous tissue, often accounting with age, leads to facial sagging and wrinkles.

## Functions of the skin [Rooks et al, 2010]:-

Human skin has multifunctional activities.

• Acts as protective barrier againstthe ingress of foreign materials (pathogenic microbes and chemical agents) and it provides safety against physical injury.

• Helps to balance fluid and restricts loss of endogenous materials such as water.

• Helps to prevent excessive water absorption by imparting water resistance to the skin.

• Helps to transport active solute molecules by passive diffusion.

• It is involved in temperature regulation of the body.

• It is the body's main sensory organ for temperature, pressure, touch and pain.

• Provides protection from UV light.

• Plays a key role in metabolism, including vitamin D synthesis and biotransformation of some chemicals. Lack of vitamin D can lead to soft bones and many associated problems.

## 3.1 Physiological and Pathological conditions of skin [Montngna et al, 1978; Shabbir et al, 2013]:-

- **Reservoir effect of horny layer-** Skin's deeper layer or horny layer has a reservoir effect which can bind irreversibly with a part of drug.
- **Lipid Film-** The lipid film on the skin surface prevents the removal of moisture from the skin and maintains skin's barrier function.
- **Skin hydration-** Skin can be hydrated by covering or occluding with a plastic sheet, hence accumulation of sweat can enhance the penetration.

- **Skin temperature and pH-** Increased temperature can cause easier penetration as energy required for diffusion is easily achieved. The pH of the normally acidic ranging from pH-4-6. If the formulation has very low or very high pH value then it may cause destruction of the skin.
- **Regional variation-** Variation in climates of different region and the thickness can affect the permeability.
- **Pathological injuries to skin-** Injury that disrupt the continuity of the skin layers can cause easy permeation, caused by increased vasodilatation.
- **Cutaneous self-metabolism-** Epidermis or cutaneous layer having catabolic enzymes may render the drug inactive by metabolism as well as bioavailability of the topical drug.
- **Skin barrier properties in young and adult-** The pH of the skin of neonates or newborns is higher than adult skin. Skin surface is slightly hydrophobic in case of young infants. Whereas, the adult's skin is smooth and rigid, moisture content decreases within the age.
- **Race-** Racial differences between white and black skin have different anatomical and physiological functions. Black skin is having increased intracellular cohesiveness, lipid content and electrical resistance.

**Table 1.2**

## 4. Factors to be concerned for Microsponge dose calculation:-

| **Physicochemical** | **Pharmacokinetic** | **Biological** |
|---|---|---|
| Solubility | Half life | Site of application |
| Crystallinity | Total body clearance | Skin toxicity |
| Molecular weight | Peak plasma concentration | Skin metabolism |
| Polarity | Volume of distribution | Allergic reaction |
| Melting point | Bioavailability | Skin barrier properties |

## 4.1 Characteristics of Microsponges [Jadhav et al, 2013; Pavani et al, 2017]:-

When these are applied to the skin, the microsponge releases its active ingredient gradually to the skin on a time mode and also in response to stimuli such as rubbing, temperature and pH effect etc. with excellent efficacy and minimal irritation. Characteristics of microsponges are as follows: -

**i.** Microsponge formulations are stable over range of pH 1 to 11.

**ii.** Microsponge formulations are stable at the temperature up to 1300ºC.

**iii.** Microsponge formulations are compatible with most vehicles and ingredients.

**iv.** Microsponge formulations are self- sterilizing as their average pore size is about 0.25μm where the bacteria cannot penetrate the pores.

**v.** Microsponge formulations have high entrapment upto 50 to 60%.

**vi.** Microsponge formulations are free flowing and can be cost effective.

**vii.** Microsponge particles themselves are too large so they are difficult to be absorbed into the skin and this adds a measure of safety to these microsponge materials by avoiding the side effects of the microsponge adjuvant.

**viii.** Microsponge's formulations can be cost effective even for the cosmetic mass market use where the cost of the material is important.

**ix.** Microsponges can absorb oil up to 6 times its weight without drying.

**x.** It provides continuous action up to 12 hours i.e. extended release.

**xi.** They have superior formulation flexibility.

## 4.2 Characteristics of materials entrapped in Microsponges [Emanuele and Dinarvand, 1995; Aritomi et al, 1996]:-

Most liquid or soluble ingredients can be entrapped inthe particles. Actives that can be entrapped inmicrosponges must meet following requirements-

- It should be either fully miscible in monomer or capable of being made miscible by addition of small amount of a water immiscible solvent.
- It should be water immiscible or at most only slightly soluble.
- It should be inert to monomers.

- It should be stable in contact with polymerization catalyst and conditions of polymerization.
- The solubility of actives in the vehicles must be limited to avoid cosmetic problems; not more than 10 to 12% w/w microsponges must be incorporated into the vehicle. Otherwise the vehicle will deplete the microsponges before the application.
- It should be inert to monomers and should not increase the viscosity of the mixture during formulation.
- The solubility of active ingredients in the vehicle should be low; otherwise the microsponge will be diminished by the vehicle before application.
- Payload and polymer design of the microsponges for the active must be adjusted to obtain the desired release rate of a given period of time.

## 4.3 Advantages of Microsponges [Shasha Li et al, 2013]:-

Microsponges have several advantages which are explained below:-

- **High surface area:-**A 25 μ sphere can have a total pore length of about 10ft with a pore volume of about 1 mL/g and can have up to 25,000 pores. This provides an extensive surface area for high entrapment [Embil and Nacht, 1996] (Figure 2).

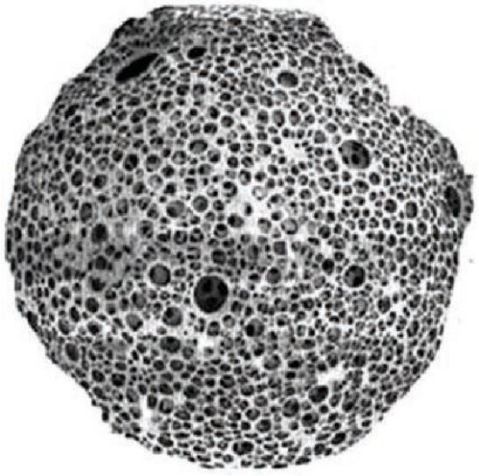


**Fig. 1.2: - Picture showing the highly porous nature of a microsponge.**

Because of the entrapment and adsorption of activesonto the polymeric cage, the release of actives issustained.

- **Simple production methodology: -** The production of such microsponges is relativelysimple in scaling up and hence there is a higher potentialfor commercialization.
- **Range:-**Microsponges can be customized to modulate theirproperties and make them suitable for a specific purpose.The various parameters that can be changed includeparticle size, pore characteristics and hardness.

## 4.4 Release mechanisms of Microsponge Technology [Christensen and Natch, 1983]:-

- **Pressure triggered systems:-**Microsponge system releases the entrapped material when pressurized; the amount released depends upon various characteristics of the sponge. By varying the type of material and different process variables, the microsponge best suited for a given application may be optimized. When compared with mineral oil containing microcapsules, mineral oil containing microsponge, the latter showed much more softening effect. The duration of emolliency was also much more for the microsponge systems.
- **Temperature triggered systems:-**It is possible to modulate the release of substances from the microsponge by modulation of temperature. Forexample, viscous sunscreens were found to show higherrelease from microsponges when exposed to highertemperatures; thus a sunscreen would be released from a microsponge only upon exposure to the heat from the sun.
- **pH triggered systems: -** Triggering the pH-based release of the active can be achieved by modifying the coating on the microsponge. Although this has many applications in drug delivery, only a few applications are possible for cosmetic delivery.
- **Solubility triggered systems:** - Presence of an aqueous medium such as perspiration can trigger the release rate of active ingredients. Ingredients such as antiseptics, deodorants and antiperspirants may be formulated in such types of systems. Release may be achieved based on the ability of the external medium to dissolve the active, the concentration gradient or the ability to swell the microsponge network.

## 4.5 Drugs entrapped in Microsponge drug delivery system:-

- Ketoprofen

- Benzoyl peroxide
- Retinol
- 5-Flurouracil
- Fluconazole
- Ibuprofen
- Tretinoin
- Trolamine
- Meloxicam
- Hydroquinone
- Miconazole
- Ammonium lactate
- Salicylic acid
- Hydroxyzine Hydrochloride
- Terbinafine Hydrochloride

## 4.6 Method of preparation of Microsponge [Tadwee et al, 2011; Junhui Hong et al, 2015]:-

Microsponge drug delivery system can be prepared in two ways, one-step process or by two-step process that is Liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques that is based on physicochemical properties of drug to be loaded.

### A. Liquid-liquid suspension polymerization

This is Bottom-up approach starting with monomer. Microsponges were conveniently prepared by free radical suspension polymerization in an emulsified liquid-liquid system. Particles forming polymerization mixtures are usually two phase systems. The monomers are referred to as “monomer phase” or “dispersed Phase”; the immiscible liquid phase containing the dispensed (or dissolved) monomer is defined as “Polymerization medium.” In addition to the monomers and polymerization medium, another liquid (miscible with the monomer and immiscible with the medium) may also be added to the monomer to form a pore network. This liquid is known as “monomer diluents” or “porogen” and belongs to the category of inert , nonpolar organic solvents when added to

the polymerization reaction, polymeric beads with open, porous structures can be obtained and they look just like sponges under SEM, hence the name "Microsponges". For preparing Microsponge, the requirements are monomer namely Styrene, PHEMA, Cross linking agents is Divinyl Benzene and Porogen is Toluene.

It is important to maintain the temperature for most efficient operation.

Once polymerization is complete the solid particles that result from the process are recovered from the suspension. The particles are then washed and processed until they are substantially ready for use. Particle formation and incorporation of the functional substance is thus performed as a single step. This may be termed as one step process. When the material is sensitive to the polymerization conditions, polymerization is performed using substitute porogen. The porogen is then removed and replaced by contact absorption assisted by solvents to enhance absorption rate.

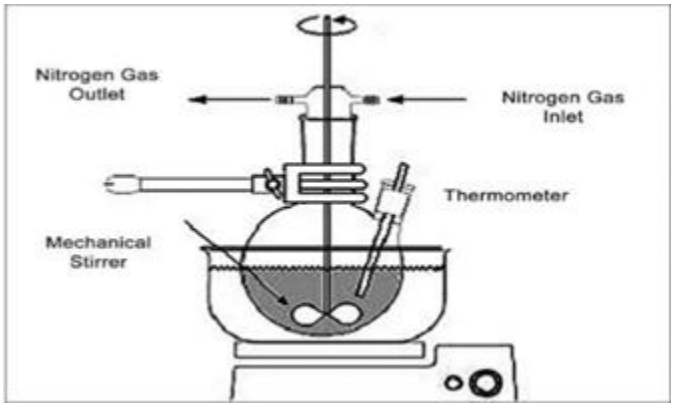



**Fig. 1.3:- Liquid-liquid suspension polymerization**

## B. Quasi-emulsion solvent diffusion:-

This is top-down approach starting with preformed polymer. This process involved formation of quasi-emulsion of two different phases' i.e. internal phase and external phase. The internal phase of drug-polymer solution made in a volatile solvent like ethanol or acetone or dichloromethane was added to external phase comprising the aqueous polyvinyl alcohol (PVA) solution with vigorous stirring. Triethylcitrate (TEC), which was added at an adequate amount in order to facilitate plasticity. Stirring lead to the formation of discrete emulsion globules called quasi-emulsion globules. Solvent was then extracted out from these globules to form insoluble, rigid microparticles i.e.

microsponges. Following sufficient stirring, the mixture was then filtered to separate the microsponges. The microsponges were then dried in an air heated oven.

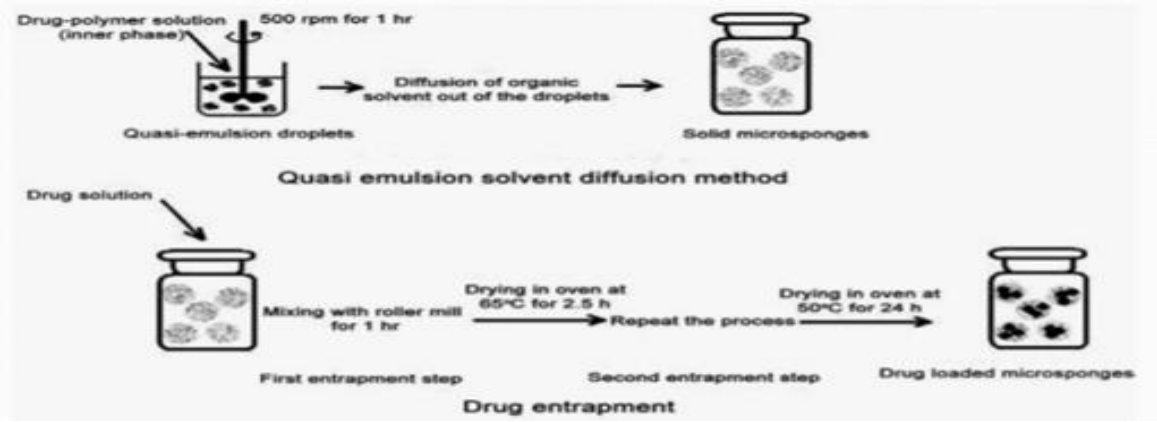



**Fig. 1.4:- Quasi emulsion solvent diffusion**

## 4.7 Evaluation parameters of Microsponges [Ingale et al, 2012; N.Shah et al, 2014]:-

Various method are used for the evaluation of the Microsponge drug delivery system they are following-

A. Particle size and size distribution.
B. Morphology and Surface Topography.
C. Determination loading efficiency and production yield.
D. Determination of true density.
E. Characterization of pore structure.
F. Compatibility studies.
G. Polymer/Monomer composition.
H. Resiliency.
I. Drug Release.
J. In vitro study and determination of kinetics of release.

**A. Particle size and size distribution-**

Particle size and size distribution are evaluated using either an optical microscope or an electron microscope. This is an extremely crucial step, as the size of the particles greatly affects the texture of the formulation and its stability. Free-flowing powders with fine aesthetic attributes are possible to obtain by controlling the size of particles during polymerization. Particle size analysis of loaded and unloaded microsponges can be performed by laser light diffractometry or any other suitable method. The values (d50) can be expressed 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.

**A. Morphology and Surface topography of SPM-**

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 widely 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.

**C. Determination of loading efficiency and production yield-**

The loading efficiency (%) of the Microsponges can be calculated according to the following equation:

%loading efficiency= (actual drug content in Microsponges)/(theoretical drug content) ×100

The production yield of the microparticles can be determined by calculating accurately the initial weight of the raw materials and the last weight of the SPM obtained.

%Production yield=(Production yield)/theoretical mass (polymer+drug) ×100

**D. Determination of true density-**

The true density of Microsponges can be measured using an ultra-pycnometer under helium gas and is calculated from a mean of repeated determinations.

**E. Characterization of pore structure-**

Pore volume and diameter are vital in controlling the intensity and duration of effectiveness of the active ingredient. Pore diameter also affects the migration of active ingredients from Microsponges into the vehicle in which the material is dispersed. Mercury intrusion porosimetrycan be employed to study effect of pore diameter and volume with rate of drug release from Microsponges.

Porosity parameters of Microsponges include intrusion–extrusion isotherms. Pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density can be determined by using mercury intrusion porosimetry. Incremental intrusion volume scan be plotted against pore diameters that represented pore size distributions. The pore diameter of Microsponges can be calculated by using Washburn equation:

$D=-4\gamma\cos\Theta/P$

Where, D is the pore diameter (μm); γ the surface tension of mercury (485 dyne $cm^{-1}$); θ the contact angle (130º); and P is the pressure (psia).

Total pore area ($A_{tot}$) was calculated by using equation,

$A_{tot}=1/\gamma\cos\theta\int P.dV$

Where, P is the pressure (psia); V volume (mL $g^{-1}$); Vtot is the total specific intrusion volume (mL $g^{-1}$).The average pore diameter (Dm) was calculated by using equation,

$Dm=4V_{tot}/A_{tot}$

Envelope (bulk) density ($\rho_{se}$) of the Microsponges was calculated by using equation,

$$\rho_{se}=Ws/(Vp-V_{Hg})$$

Where, Ws is the weight of the SPM sample (g); Vpthe empty penetrometer (mL); $V_{Hg}$ is the volume of mercury (mL).

Absolute (skeletal) density ($\rho_{sa}$) of Microsponges was calculated by using equation,

$$\rho_{sa}=Ws/(V_{se}-V_{tot})$$

Where $V_{se}$ is the volume of the penetrometer minus the volume of the mercury (mL).

Finally, the % porosity of the sample was found from equation,

$$\text{Porosity \%} =(1-\rho_{se}/\rho_{sa})\times 100$$

**F. Compatibility studies-**

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

incompatibilities between the chemical moieties. Compatibility of drug with reaction adjuncts can also be studied by thin layer chromatography (TLC) and FT-IR.Effect of polymerization on crystallinity of the drug can be studied by powder X-ray diffraction (XRD) and Differential Scanning Colorimetry (DSC).

**G. Polymer/ Monomer composition-**

Factors such as particle size, drug loading, and polymer composition govern the drug release from Microsponges. Polymer composition of the Microsponges Drug Delivery system can affect partition coefficient of the entrapped drug between the vehicle and the Microsponges system and hence have direct influence on the release rate of entrapped drug. Release of drug from Microsponge systems of different polymer compositions can be studied by plotting cumulative % drug release against time. Release rate and total amount of drug released from the system composed of methyl methacrylate/ ethylene glycol dimethacrylate is slower than styrene/divinyl benzene system. Selection of monomer is dictated both by characteristics of active ingredient ultimately to be entrapped and by the vehicle into which it will be dispersed. Polymers with varying electrical charges or degrees of hydrophobicity or lipophilicity may be prepared to provide flexibility in the release of active ingredients. Various monomer combinations will be screened for their suitability with the drugs by studying their drug release profile.

**H. Resiliency-**

Resiliency (viscoelastic properties) of Microsponges can be modified to produce beadlets that is softer or firmeraccording to the needs of the final formulation. Increased cross-linking tends to slow down the rate of release. Hence resiliency of Microsponges is studied and optimized as per the requirement by considering release as a function of cross linking with time.

**I. Drug Release-**

Dissolution profile of Microsponges can be studied by use of dissolution apparatus with a modified basket consisted of 5μm stainless steel mesh. The speed of the rotation is 150 rpm. The dissolution medium is selected while considering solubility of actives to ensure sink conditions. Samples from the dissolution medium can be analyzed by suitable analytical method at various intervals.

K. **Kinetics of release-**

To determine the drug release mechanism and to compare the release profile differences among microsponges, the drug released amount versus time is used. The release data are analyzed with the following mathematical models:

$Q= k_1t^n$ or $\log Q= \log k_1 + n \log t$ ……Equation (1)

Where Q is the amount of the released at time (h), n is a diffusion exponent which indicates the release mechanism, and k1 is a constant characteristic of the drug–polymer interaction. From the slope and intercept of the plot of log Q versus log t, kinetic parameters n and $k_1$ were calculated.

For comparison purposes, the data was also subjected to Equation (2), which may be considered a simple, Higuchi type equation:

$Q = k_2t^{0.5} +C$ …… Equation (2)

Equation (2), for release data dependent on the square root of time, would give a straight line release profile, with $k_2$ presented as a root time dissolution rate constant and C as a constant.

## 4.8 Applications of Microsponge [Ahmed et al, 2018; Charde et al, 2013]:-

Microsponge delivery systems are used to enhance the safety, effectiveness and aesthetic quality of topical prescription, over-the-counter and personal care products. Microsponges can be used in variety of applications. It is used mostly for topical and recently for oral administration. Several patents have reported that it can be used as excipients owing to its high loading capacity and sustained release ability. It offers the formulator a range of alternatives to develop drug and cosmetic products. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release. Over-the-counter products that incorporate microsponge drug delivery system include numerous moisturizers, specialized rejuvenative products, and sunscreens.

## Table 1.3

## Application of Microsponges with respect to their advantages:-

| Sl. No. | Application | Advantages |
|---|---|---|
| 1 | Sunscreens | Long lasting product efficacy, with improved |

| | | |
|---|---|---|
| | | protection against sunburns and sun related injuries even at elevated concentration and with reduced irritancy and sensitization. |
| 2 | Anti-acne<br>e.g.- Benzoyl Peroxide | Maintained efficacy with decreased skin irritation and sensitization |
| 3 | Anti-inflammatory<br>e.g. hydrocortisone | Long lasting activity with reduction of skin allergic response and dermatoses. |
| 4 | Anti-dandruffs<br>e.g. zinc pyrithione, selenium sulfide | Reduced unpleasant odour with lowered irritation with extended safety and efficacy |
| 5 | Antipruritics | Extended and improved activity |
| 6 | Skin depigmenting agents<br>e.g. hydroquinone | Improved stabilization against oxidation with improved efficacy and aesthetic appeal |

## 4.9 Examples of Microsponge drug delivery system with their formulation:-

**Table 1.4**

| **Microsponge Delivery system** | **Drug** | **Disease** |
|---|---|---|
| | Benzoyl peroxide | Anti-acne treatment |
| | Fluconazole | Inflammation |
| | Mupirocin | Anti-bacterial activity |
| **Gels** | Diclofenac sodium | Inflammation |
| | Acyclovir | Viral infections |
| | Hydroxyzine HCL | Urticaria and atopic dermatitis |
| | Tebinafine HCL | Anti-fungal |

| | | |
|---|---|---|
| **Lotions** | Benzoyl peroxide | Anti-acne treatment |
| **Creams** | Hydroquinone and Retinol | Melanoma |
| | Indomethacine | Inflammation |
| | Paracetamol | Anti-pyretic |
| **Tablets** | Chlorpheniramine maleate | Hay fever |
| | Ketoprofen | Muscoskeletal pain |
| | Fenofibrate | Gout |
| | Flubiprofen | Metabolic ratio |
| | Dicyclomine | Anti-cholinergic |
| **Tablets** | Meloxicam | Arthritis |
| | Paracetamol | Colon targeting |
| **Implants** | Poly (DL-lactic-co-glycolic acid) | Skin tissue engineering |
| **Grafts** | Poly (lactic-co glycolic acid) | Cardiovascular surgery |
| **Injections** | Basic fibroblast growth factor | Growth factor |
| **Others** | Mefenamic acid | Rheumatoid arthritis |
| | Ibuprofen | NSAIDS |

## 4.10 Recent advances of Microsponge drug delivery system [Junhui et al, 2015; Othman et al, 2017]:-

Various advances were made by modifying the methods to form nanosponges, nanoferrosponges and porous microbeads.

β-CDnanosponges were also developed that can be used for hydrophobic as well as hydrophilic drugs, in contrast to polymeric micro or nanosponges. These advanced systems were studied for oral administration of dexamethasone, flurbiprofen, doxorubicin hydrochloride, itraconazole and serum albumin as model drug. These nanosponges were developed by cross-linking the β-CD molecule by reacting with diphenyl carbonate.

Some researchers also observed the nanosponges as good carrier for the delivery of gases. Researchers also observed that incorporating a cytotoxic in a nanosponge carrier system can

increase the potency of the drug suggesting that these carriers can be potentially used for targeting the cancerous cells.

Nanoferrosponge, a novel approach constituted the self-performing carriers having better penetration to the targeted site owing to the external magnetic trigger which enforces the carriers to penetrate to the deeper tissue and then causing the removal of magnetic material from the particle leaving a porous system.

Due to the improved characteristics of porous microspheres, process was developed to produce the porous micro beads. This method (High internal phase emulsion, HIPE) consisted of the monomer containing continuous oil phase, cross linking agent and aqueous internal phase.They also observed an improved stability of RNA and the relatively effective encapsulation process of siRNA. The approach could lead to novel therapeutic routes for siRNA delivery.

## 5. Future prospect [Othman et al, 2017; Patil et al, 2012]:-

Microsponge drug delivery system holds a promising opportunity in various pharmaceutical applications in the upcoming future as it has unique properties like enhanced product performance and elegancy, extended release, improved drug release profile, reduced irritation, improved physical, chemical and thermal stability which makes it flexible to develop novel product forms. The real challenge in future is the development of the delivery system for the oral peptide delivery by varying ratio of polymers. The use of biodegradable polymers for the drug delivery is enabling it for the safe delivery of the active material. As these porous systems have also been studied for the drug delivery through pulmonary route which shows that these system can show effective drug release even in the scarce of the dissolution fluid thus colon is an effective site for targeting for drug release. These carriers also require to be developed for alternative drug administration routes like parenteral and pulmonary route. These particles can also be used as the cell culture media and thus can also be employed for stem cell culture and cellular regeneration in the body. Due to their elegance, these carrier systems have also found their application in cosmetics. These developments enabled researchers to utilize them variably. These novelties in formulation also open new ways for drug deliver.

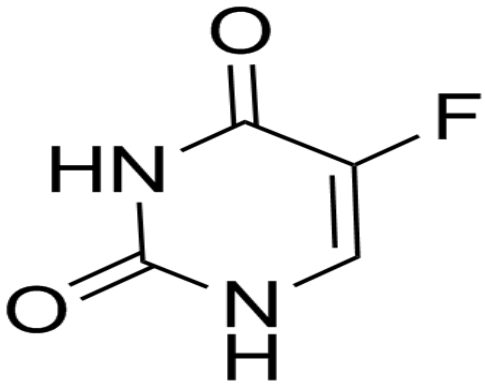
The proposed work for M.Pharm. dissertation is based on the hypothesis that microsponge delivery system is effective controlled delivery system for any type of skin ailment or cosmetic

purpose. Drug to be delivered with slow release in colon area this MDS can serve the purpose effectively. The drug materials should be selected judiciously so that the dermal preparation of MDS can be prepared.

## DRUG CANDIDATES

### 5-Fluorouracil

5-FU is an anti-neoplastic anti-metabolite. Chemically defined as **5-Fluoro-2,4(1H,3H)-pyrimidinedione.**




**Structure**

Molecular Formula: $C_4H_3FN_2O_2$

Molecular Weight: 130.078 gm/mol.

**Physicochemical data of 5-FU**

**App. –** White crystalline powder.

**M.W. –**130.078 gm/mol.

**Melting Point: -** 280-282ºc.

**Log P-** 0.89.

**Water solubility -** Soluble in water; Insoluble in chloroform, ether, benzene.

**pKa -** 8.02.

**Stability-** Stable when exposed to air.

**$T_{1/2}$ -** 16 min.

**Storage**: - Store in 2-5°C cool temperature.

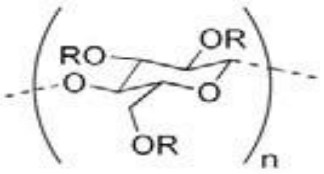
**Mechanism of action**: -

5-FU acts in several ways, but principally as a Thymidine synthese inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine, which is a nucleoside required for DNA replication. Thymidine Synthese methylatesdeoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP). Administration of 5-FU causes a scarcity in dTMP, so rapidly dividing cancerous cells undergo cell death via thymine less death. Calcium folinate provides an exogenous source of reduced folinates and hence stabilises the 5-FU-TS complex, hence enhancing 5-FU's cytotoxicity.

## Ethyl cellulose:-

**Synonyms**: Cellulose, ethyl ether, ethylated cellulose, ethylcellulose.

**Structure:**




R = H or $CH_2CH_3$

**Physical properties**:

Physical State:Solid

Appearance: white to light tan Odor: odorless

Melting point: 240°C (464°F) Density: 1.07-1.18 g/$cm^3$

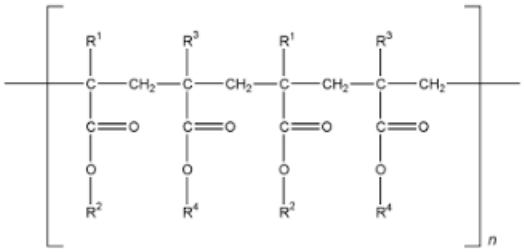
Solubility: easily soluble in coldwater

Toxic effects: Rat:LD50=5mg/kg, skin rabbit: LD50=5mg/kg

Uses: used as a food additive.

**Eudragit RS 100**

Eudragit polymers are copolymers derived from esters of acrylic and methacrylic acid, whose physicochemical properties are determined by functional groups (R). Eudragit polymers are available in a wide range of different physical forms (aqueous dispersion, organic solution granules and powders).



**Structure**

**Appearance: -** Colorless to transparent crystals.

**Melting point**: - 394.91 $^{o}$C.

**Solubility:** - Miscible with methanol, ethanol, acetone, dichloromethane ethyl acetate and methylene chloride.

# *Chapter 2*

# AIMS AND OBJECTIVES

## Aims and Objectives

Microencapsulation is widely accepted technology for control drug delivery system. The drug should be delivered to specific target sites at a rate and concentration to get therapeutic efficacy with minimum side effects. Now a day's microencapsulation dosage forms provide prolonged release of single dose. Drug is diffused slowly over a period of time with control rate and reducing adverse effects. Among various methods quasi-emulsion solvent diffusion method was used to develop microsponges. Primary step of microencapsulation method is emulsion formation. Stable emulsion is formed considering some chemical factor as well as physical factor.

Following are the aims and objectives to be implemented in my M. Pharm. dissertation work.

1. To develop 'microsponge particle–gel'- a new delivery system of 5-Fluorouracil following emulsification method, intended to be used for dermal ailments.

2. To optimize preparation of 'microsponge particle' by Response Surface Methodology, a computerized statistical method assisted by Design Expert and an attempt to scale up the method.

**Plans of work:**

i. To identify various physical and chemical properties of drug (5- fluoro uracil) required for the formulation of Microsponge drug delivery system as a part of pre formulation study

ii. To understands type of emulsification (w/o/w or o/w/o) depending on the properties of the drug and chosen polymer. In order to formulate new stable microsponge particles with desired characteristics by quasi-emulsion solvent diffusion method it is necessary to identify different physicochemical controlling parameters in order to design new Dermal Drug Delivery System of 5-Fluorouracil by experimental design method.

iii. To optimize new formulation by Design Expert software.

iv. An attempt to develop of a scale-up method for the optimized formulation of 5-flouro uracil microsponge particles in laboratory scale.

v. Validation of dosage form (microsponge particles) by various tests and studies (solid-state characterizations).

v. To prepare gel with microsponge particles, and check different properties of microsponge loaded gel and study of *in vitro*drug release from this gel form.

# *Chapter 3*

Literature Survey

# LITERATURE SURVEY

The present work entitled **"Preparation and Characterization of 5-FU loaded microsponge gel by Response-surface design and its release studies"** is needed to be discussed based on background work.

In the year **2018, A.H.Abdellatif, M.Zyed,** formulated Eudragit RS 100 microsponges loaded with albendazole using oil in oil emulsion solvent diffusion method to certainly target parasitic worms in both human and animals. Activity of ALBZ microsponge was estimated in goats experimentally infected with Haemonchuscontortus in comparison with the free ALBZ. Encapsulation efficiency, Particle size and *in vitro* release study were studied of MS-ALBZ. ALBZ loaded microsponges achieved more Area Under Curve (AUC) than drug suspension. Fecal egg reductions were 100% in both formulations. But it can be used for treatment of parasitic worms in sustain release oral dosage form.

In the year **2018, Salah, Awad,** developed a novel miconazole microsponges gel as an attractive dosage form for vaginal candidiasis. It was prepared by Quasi-emulsion solvent diffusion using Eudragit RS 100 and PVA used as an external phase. Production yield, particle size, encapsulation efficiency and release rate were optimized. Microsponges incorporated in a carbopol gel and check viscosity and bioadhesion were examined. After *in vivo* release study it showed that MCZ microsponge gel was more effective than marketed product. It was reported after histopathological study of infected rats.

In the year **2017, Othman, Zayed,** developed Eudragit RS 100 based 5-FU Microsponges for treatment of colon cancer. It is oral dosage form. Oil in oil emulsion solvent diffusion method was used for preparation. Encapsulation efficiency, production yield, drug polymer interaction and drug release profiles were characterized. HCT 116 and CACO2 cell lines were used for determination of cell viability by MTT assay. It shows5-FU loaded Microsponge was more effective than 5-FU itself.

In the year **2017, Shahzad, Saeed, d**eveloped ketoprofen loaded microsponge with hydrophobic and hydrophilic polymers. Various ratio and amount of the polymers and surfactants were used. They had reported significant impacts on encapsulation efficiency and in-vitro drug release of cellulosic microsponges.

In the year **2017, Pavani, Vinod and Anantha** formulated ketoconazole loaded microsponge based gel for proficient treatment of fungal infections. It was prepared by quasi-emulsion solvent diffusion and formulation variables such as drug-polymer ratio; stirring speed on physical characteristics of microsponges was examined. Physical parameters of gel like pH, spreadability, *in-vitro* diffusion were reported. Drug release of prepared gel was more controlled than gel prepared with the pure drug.

In the year **2017, V.S and Kuriachan f**ormulated and evaluated allylamine classes of anti-fungal terbinafine hydrochloride loaded microsponge based gel for topical sustained delivery. It was prepared by quasi-emulsion solvent diffusion with different concentration of ethyl cellulose. Drug loaded microsponges were incorporated in carbopol gel, and pH, drug content, spreadability, skin irritancy, *in vitro* diffusion, rheological behavior were reported.

In the year **2016, Dhote, Mishra and Chandel** reported about development, optimization and characterization of anti-viral compound valcyclovir loaded microsponge based gel for skin infections. Different concentrations PVA and ethyl cellulose were used in external and internal phase. Maximum drug release was showed by formulation where concentration of PVA and ethyl cellulose was more. Particle sizes of six formulations are in the range between 28 to 44 µm. Permeation enhancer was added in carbopol gel to increase release rate of formulations.

In the same year, **Bhandare and Katti** formulated resperidoneHCl loaded microsponge. Ethyl cellulose and Eudragit RS 100 based microsponge were prepared by quasi-emulsion solvent diffusion, design suitable formula to investigate effect of drug-polymer ratio, stirring rate, inner solvent, external phase. Formulation containing both ethyl cellulose and Eudragit RS 100 gave better drug release and encapsulation efficiency as compared to single use in formulation.

In the same year, **Patel, Padia, Vadhgama, Rava and Seth** formulated and evaluated fluconazole containing microsponges gel for topical fungal therapy. Physical characteristics of MS like production yield, encapsulation efficiency and particle size were investigated. Effect of drug-polymer ratio, stirring speed and stirring time were optimized in $3^2$ factorial designs. Optimized formulation was demonstrated control release and no irritancy to rat skin in anti-fungal therapy.

In the year **2016, Pandit, Patel, Bhanushali, Kulkarni and Kakad** formulated nebivolol-loaded microsponge gel for diabetic wound healing. Maximum polymer and inner solvent containing formulation showed maximum*invitro* drug release within 8hr period.*Invivo* study was performed using streptozotocin induced diabetic rats and excision wound model showed wound healing closure activity within 10thday. Drug-polymer ratio, internal volume phase of emulsion was optimized by factorial design.

In the year **2016, Mahesh Kumar and Ghosh** developed and evaluated silver sulfadiazine loaded microsponge based gel for partial thickness burn wounds. It was prepared by w/o/w solvent evaporation method and optimized by $3^2$ factorial designs. FTIR, DSC, PXRD and particle size were analyzed of optimized formulation. Safety of optimized gel assessed by MTT assay using epidermal keratinocyte (HaCaT) and mouse embryonic fibroblast (NIH-3T3) cell lines. Anti-inhibitory efficiency of the optimized gel was compared to that of commercial product against the *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It was showed reduced frequency of application, no skin irritation and low cytotoxicity in dermal cell lines.

In the year **2015, M.Osmani, H.Aloorkar, Ingale, Kulkarni, Hani and Bhosale** formulated microsponge based novel drug delivery system for augmented arthritis therapy. Diclofenac diethylamine was used which is mostly used for rheumatoid arthritis, juvenile arthritis, osteoarthritis, ankylosing spondylitis and other musculoskeletal ailments. Dibutyl-phalate was used in inner solvent to increase the plasticity of eudragit-RS 100. Microsponges were characterized by SEM, FTIR, DSC, PXRD and particle size analysis. They were incorporated in the gel which exhibited viscous modulus along with pseudo plastic behavior. *Invitro* drug release of gel was greater than that of conventional formulation after 8hr.

In the year **2012, Srivastava, Kumar and Pathak** developed calcium-pectinate matrix tablets for colon targeted delivery of meloxicam microsponges. It was formulated based on $3^2$ full factorial designs with two independent variables, volume of DCM and amount of polymer. Optimized formulation was developed into colon targeted matrix tablet. The optimized tablet in gastrointestinal region and selectively delivered in colon was visualized by *invivo* fluoroscopy of rabbit. Pharmacokinetic evaluation in rabbit's revealed appearance of drug appeared in plasma after a lag time of 7hr, $t_{max.}$ in 30hr. presented a formulation suitable for targeted colon delivery.

In the year **2011, Rizkalla, Aziz and Soleman** developed and evaluated *in-vitro* and *in-vivo* study of hydroxyzine hydrochloride contained microsponge for topical delivery. This drug is used for the treatment of urticaria, atopic dermatitis. Eudragit RS 100- drug loaded formulation was carried out by oil in oil solvent diffusion method. Magnesium stearate was added to disperse phase to prevent flocculation of microparticles. It showed 96% encapsulation efficiency and 60-70% porosity were produced. Sucrose and pregelatinized starch were used to enhance the rate of drug release. Pharmacodynamic effect was tested on the shaved back of histamine-sensitized rabbit.

In the year **2010, Jain and Singh** prepared and characterized of dicyclomin loaded eudragit based microsponge with potential for colonic delivery. Triethylcitrate was added to drug – polymer mixture DCM solvent to increase plasticity of polymer. Several formulations were carried out with fixed amount of polymer. Increase drug-polymer ratio causes decrease in particle size and increased *in vitro* cumulative drug release. Drug releases of microparticles were 16-30% in the first hour for initial burst effect. Cumulative release after 8hr. ranged from 56-89%.

In the year **2009, Amrutiya, Bajaj and Madan** developed mupirosin contained microsponges for topical delivery to evaluate sustained release and enhanced drug deposition in skin. The effect of formulationand process variables such as internal phase volume and stirring speed on the physical characteristics ofmicrosponges were examined on optimized drug-polymer ratio by $3^2$ factorial designs. Optimized formulation was incorporated in emulgel base; release through cellulose dialysis membrane and deposition studied using rat abdominal skin which exhibit significant retention by 24 hr. It was also applied mouse surgical wound infected with *S.aureus*. The formulation showed enhanced retention of drug in skin, indicating better potential as compared to marketed and conventional mupirocin dosage form.

In the year **2005, M.Jelvehgari, Siahi-Shadbad, Azarmi, Martin and Nokodchi** prepared, characterized and studied release of benzoyl peroxide loaded microsponge drug delivery system. This drug commonly is used in acne causing bacteria. Microparticles were prepared by emulsion solvent diffusion using ethyl cellulose polymer in internal solvent, PVA used in external phase.

Several formulations were carried out by increasing amount of drug only. An increase in the ratio of drug-polymer resulted in reduction in release rate and decrease internal porosity also.

# *Chapter 4*

PREPARATION, CHARACTERIZATION, OPTIMIZATION AND SCALE-UP OF DRUG LOADED MICROSPONGE GEL

# PREPARATION, CHARACTERIZATION, PROCESS OPTIMIZATION AND SCALE UP OF 5-FU LOADED MICROSPONGE BY RESPONSE SURFACE METHODOLOGY

## 4.1 INTRODUCTION

5-Fluorouracil, an anti-metabolite (pyrimidine antagonist), widely used for anal, breast, colorectal, esophageal, stomach, pancreatic and skin cancer. It has also been given topically for actinic keratoses, skin cancer and Bowen's disease. It is chemically defined as 5-fluoro-1*H*-pyrimidine-2, 4-Dione. It has very short plasma half-life 16 min. which is useful for topical preparation. When topically 5-FU enters cell, it undergoes ribosylation and phosphorylation. Then it binds to thymidylate synthase using a co-factor 5, 10 methylene tetrahydrofolate.

Thymidylate synthase is inhibited and cannot convert deoxyuridine nucleotides to thymidine nucleotides. The depletion of thymidine leads to reduced synthesis of DNA, reduced cell growth, and cell death. Because 5-FU interferes with DNA replication, rapidly proliferating cells are most sensitive to its cytotoxic effect (Moore et al., 2009).

In this study, Eudragit RS 100 and Ethyl cellulose were selected to achieve targeted and sustained release of drug. Formulations were designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effect and modify drug release profiles (Amrutiya et al., 2009).

Generally microsponges are prepared by quasi-emulsion solvent diffusion technique because it is a simple laboratory scale process, economic and well controlled method. It gives preliminary idea about control factors, process variables for the production of microsponge (Naik et al., 2013). Choice of proper mode of emulsification (o/w, o/o, w/o/w, and o/w/o), composition of dispersion phase, composition of dispersed phase, stirring rate of impeller and drug-polymer ratio should be optimized. Many trials are necessary to make an efficient formulation. In this study, w/o/w emulsification method was used because 5-FU is aqueous soluble drug which required double emulsification process. The objective of the present study was to develop, optimize and scale-up by using a three factor three-level Face centered Central Composite Designing of 5-FU loaded microsponge prepared by quasi-emulsion solvent diffusion method

using mixed solvent system( DCM: Ethanol 1:1 v/v), and aqueous alginate solution with Tween 80, as dispersion medium. Filtered microsponge incorporated in carbopol gel for *invitro* diffusion study.

## 4.2. Materials and Methods

### 4.2.1. Materials

5-Fluorouracil (Yarrow Chem. India), Eudragit RS 100 (Yarrow Chem. India), Ethyl Cellulose (Quest Chemicals, Kolkata), Sodium Alginate (Quest Chem. Kolkata), Carbopol 934 (LobaChemie, Mumbai, India), Tween 80 (Quest Chem. Kolkata), Dichloromethane, Ethanol and Triethanolamine (Quest Chemicals) were purchased. All the reagents are of analytical grade.

### 4.2.2. Pre-formulation Studies

#### 4.2.2.1. Determination of $\lambda_{max.}$ of 5-Fluorouracil

Accurately weighed 10 mg of 5-Fluorouracil (M.W. 130 gm/mol, $C_4H_3FN_2O_2$) was dissolved in 100 ml phosphate buffer (PBS pH 5.5 and 7.4) to obtain solution of 100 µg/ml (stock solution). The solution was sonicated for 20 minutes. This stock solution was scanned between 200-400 nm PBS as a blank in a double beam UV spectrophotometer (ANALAB UV-180) to determine $\lambda_{max}$of 5-Fluorouracil. It showed maximum absorbance at 265 nm.

#### 4.2.2.2. Preparation of Standard curve of 5-FU in Phosphate buffer solution of pH 5.5 and 7.4

A series of dilutions 2-10 µg/ml in 10 ml volumetric flask were made from stock solution (100µg/ml) using PBS 5.5 and 7.4. The absorbance of this solution should be measured at $\lambda_{max}$265 nm against PBS 5.5 and 7.4 as blank using UV spectrophotometer. Standard curve was obtained by plotting concentration against absorbance.

#### 4.2.2.3. Melting point determination

Melting point of drug sample drug sample was determined by melting point apparatus. It was compared to the published I.P. data.

#### 4.2.2.4. Determination of Partition Coefficient (log P)

Partition coefficient of drug sample was determined in both n-octanol-buffer systems. 10 mg of drug was added in 15ml of n-octanol and it was continuously shaken for 20 minutes. Then 15 ml buffer (pH 5.5 and 7.4) was added and mixture was shaken in mechanical stirrer for 24 hr. After 24 hr. both phases were separated and concentration of aqueous phase was determined by UV-spectrophotometer. Dilution is required if needed. Log P value is calculated using this formula:-

$$Partition\ Coefficient\ (Log\ P) = \frac{Conc.of\ drug\ in\ organic\ phase}{Conc.of\ drug\ in\ aqueous\ phase}$$

**4.2.2.5. Drug- Polymer Compatibility studies**

**4.2.2.5.1. Fourier Transform Infrared Spectroscopy (FTIR)**

IR spectrum of drug, polymer, physical mixture of drug-polymer and drug loaded microsponge gives information on presence of functional group and interaction between drug and polymer used (Kuriachan et. al. 2017). All samples should be dried; 5-10 mg was used on disc which was scanned within a range 600- 4000 wave number ($cm^{-1}$) and % transmittance using Bruker FTIR, Model- Alpha, Germany.

**4.2.2.5.2. Differential Scanning Calorimetric analysis (DSC)**

DSC of sample was done to check thermal behavior and crystallinity of samples. 2 to 5 mg samples were heated in sealed aluminum pans from 30-500 ºC at a scanning rate 10ºC/min under nitrogen atmosphere. Graph was plotted temperature versus % weight using Pyris diamond TG/DTA (Perkins Elmer Instruments, Mumbai).

**4.2.3. Method of preparation of Microsponges**

Accurately weight 50 mg of drug was mixed with Ethyl cellulose and Eudragit RS 100 (1:1) and then dispersed in 15 ml of DCM: Ethanol solvent system (1:1 v/v) (**Inner phase**). Inner phase was sonicated for 30 minutes for homogeneous dispersion. 100 ml of Aqueous sodium alginate solution (0.4% w/v) was prepared and then added Tween 80 (0.5-2% w/w) (**Outer phase**). 1% (v/v) aqueous outer phase was added to inner phase and mixed by cyclo mixer (Remi, CM 101) to prepare w/o emulsion. Then it was addeddrop wise to outer aqueous phase which was stirred continuously in a mechanical stirrer (Remi motor RQT-124A) at specific speed for a period of 4

hr. After 4 hr following complete evaporation of solvent, microsponge particles were isolated, hardened and filtered (Whatman- 150 mm filter paper) and dried in hot air oven for a period of 6 hr. It was stored in desiccators for further study.

### 4.2.4. Method of preparation of Microsponges incorporated Gel

Gel was made up of with polymer Carbopol 934 which is water soluble polymer. Gel was prepared with accurately weighed carbopol 934 (0.25% w/v) with Double Distilled Water (DDW) using magnetic stirrer at 1200-1400 rpm for 45 min. Then 10 mg of microsponges were incorporated in 1 gm gel with slow stirring for equal distribution. Then triethanolamine was added 1 to 2 drop to adjust pH 5.5-6. Microsponges were soaked overnight in carbopol gel then it was used for *in-vitro* release study.

### 4.2.5. Experimental Design

Experimental design is the process of planning a study to meet specified objectives. Planning an experiment properly is very important in order to get reproducible data. In this study Design Expert 7 trial version (Stat-Ease, USA) was used to generate design, ANOVA, Fit summary, Diagnostics, Model graphs of each responses and optimized formulation. In this study, several batches of 5-FU loaded microsponges were prepared based on 3-factor, 3-levels Face Centered Central Composite Design (FC-CCD). Drug-polymer ratio ($A_1$), Stirring speed ($A_2$) and Concentration of Tween 80 ($A_3$) were selected as independent variables. Dependent variables or Responses were the %Yield ($R_1$), Particle size ($R_2$), Entrapment efficiency ($R_3$) and %Release in 8hr. ($R_4$). Selected independent process control variables with their levels are given in Table (4.1).

**Table 4.1** Selected independent process control variables and their levels for the preparation of 5-FU loaded microsponges.

| **Independent Variables Levels** | **Low (-1)** | **Medium (0)** | **High (+1)** |
|---|---|---|---|
| Polymer range (mg) ($A_1$) | 200 | 400 | 600 |
| Stirring speed (rpm) | 800 | 1000 | 1200 |

| | | | |
|---|---|---|---|
| ($A_2$) | | | |
| Tween 80 conc.(%w/w) ($A_3$) | 0.5 | 1.25 | 2 |

### 4.2.6 Characterization of Microsponges

#### 4.2.6.1. Determination of Yield (%)

Dried microsponges were weighed accurately and yield was calculated as a percentage using the following equation:-

$$Yield(\%) = \frac{weightofmicrosponges}{weightofpolymer + weightofdrug} * 100$$

#### 4.2.6.2. Determination of Drug Entrapment Efficiency

Drug entrapment efficiency was determined by adopting solvent extraction method and amount of drug was estimated in a UV-VIS spectrophotometer. Accurately weighed 10 mg of microsponge particles was dissolved in 5 ml methanol in magnetic stirrer for 20 min. After a clear solution was formed, 20 ml fresh buffer was added to it and heated to45-50°C. After evaporation of methanol, it was cooled down to room temperature, 25°C and filtered. The concentration of drug was calculated by UV spectroscopy at $\lambda_{max}$ 265 nm with suitable dilutions (PBS 7.4). Concentration of drug was calculated by standard curve plot. Drug Encapsulation efficiency was calculated by following formula:-

$$DEE\ (\%) = \frac{Actual\ drug\ content\ of\ microsponges}{Theoretical\ drug\ content\ of\ microsponges} * 100$$

#### 4.2.6.3. Particle size analysis

The average particle size of microsponges for 50 particles was measured by optical microscope (GOKO- Miamb, Japan). Microsponge was taken on a glass slide and particle size was measured by calibrated optical-stage micrometer. Average particle size was calculated by using following formula:-

$$P.S.(avg.) = \sum D / \sum f$$

D= Particle size; f= no. of particle.

#### 4.2.6.4. *In-vitro* drug release (diffusion) for gel

*In-vitro* drug release studies were carried out using Franz's diffusion cell (Remco, India) at 37°C±0.5. Study was carried out at two pH conditions, pH 5.5 (skin) and pH 7.4 (systemic circulation). Drug from Gel was diffused through cellophane membrane (0.45 µm) which was attached with the donor cell. 1gm gel was used in donor cell. 10 mg microsponge was incorporated in 1 gm gel. So, Drug encapsulated in 10 mg microsponge formulation was considered as loaded drug sample. 50 ml PBS was used in receptor cell with 450 rpm magnetic stirring. Samples of 5 ml were withdrawn at suitable time interval and immediately replaced with same volume of fresh PBS. Time intervals are 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hr at same temperature and stirring rate. The aliquots were assayed in a UV spectrophotometer (ANALAB UV-180) for determination of drug concentration at $\lambda_{max}$265 nm. The cumulative percent release (CPR) was plotted against time (hr). Each experiment was carried out thrice.

#### 4.2.6.5. Kinetics of release

To determine the drug release mechanism and to compare the release profile differences among microsponges, plot was constructed with the drug released amount versus time is used. The release data are analyzed with the following mathematical models:

$Q= k_1t^n$ or $\log Q= \log k_1 + n \log t$ ……Equation (1) (First order equation)

Where Q is the amount of the released at time (h), n is a diffusion exponent which indicates the release mechanism, and k1 is a constant characteristic of the drug–polymer interaction. From the slope and intercept of the plot of log Q versus log t, kinetic parameters n and $k_1$ were calculated.

For comparison purposes, the data was also subjected to Equation (2), which may be considered a simple, Higuchi type equation:

$Q = k_2t^{0.5} + C$ …… Equation (2)

Equation (2), for release data dependent on the square root of time, would give a straight line release profile, with $k_2$ presented as a root time dissolution rate constant and C as a constant

Korsmeyer-peppas model: $M_t/M_\alpha = K_pt^n$ ……Eqn. (3)

Where $M_t/M_\alpha$ is the fraction of drug release at time t, $K_p$ is the Korsmeyyer-peppas release constant and n is release exponent.

Hixson-Crowell equation: $Q_0^{1/3} – Q_t^{1/3}= K_{HC}t$ …….Eqn. (4)

Where $Q_0$ is the initial amount of drug, $Q_t$ is the amount of drug at time t and $K_{HC}$ is the Hixson-Crowell rate constant.

#### 4.2.6.6. Scanning Electron Microscopy (SEM)

The surface morphology, shape and size of microsponges were analyzed using Scanning Electron Microscopy (Carl-Zeiss, SEM, Tokyo, Japan). Particles were mounted on metal stub with conductive tape. Particles were coated with a thin coating of platinum with vacuum evaporator.

### 4.2.7 Micrometric properties of microsponge

#### 4.2.7.1. Bulk Density

Bulk density is defined as weight of particles divided by the total volume. Specific quantity of particles was poured into a 5 ml graduated measuring cylinder and volume of initial packing was noted. The Bulk Density was measured by the following formula:-

$$Bulk\ density\ (B.D) = \frac{weight\ of\ microsponge\ particles}{volume\ of\ initial\ packing}$$

#### 4.2.7.2. Tapped Density

Tapped density of the particles is the ratio of the mass of the powder to the volume occupied by the particles after it has been tapped for a defined period of time. Tapping was continued until no further change in volume. It was measured by the following formula:-

$$Tapped\ Density\ (T.D) = \frac{weight\ of\ microsponge\ particles}{volume\ of\ packing\ after\ tapping}$$

#### 4.2.7.3. Hausner's Ratio

Hausner's ratio is a number that is correlated to the flowability of a particle. It was calculated by following formula:-

$$Hausner's\ ratio\ (H.R) = {}^{Tapped\ density}/_{Bulk\ density}$$

#### 4.2.7.4. Carr's Index

The carr's index is an indication of the compressibility of particles. It was calculated by the following formula:-

$$Carr's\ Index\ (C.I) = \frac{B.D - T.D}{B.D} * 100$$

**Table 4.2; Relationship between particles flowability and % Compressibility (Carr's Index)**

| Carr's Index (% Compressibility) | Flowability |
|---|---|
| 5- 11 | Excellent |
| 12- 16 | Good |
| 17-21 | Fair to passable |
| 22- 35 | Poor |
| More than 35 | Very Poor |

### 4.2.8. Characterizations of microsponge incorporated gel

#### 4.2.8.1. Viscosity of Gel

Viscosity of carbopol gel was measured by Oswald viscometer (BSU size c). Viscosity of sample was measured by following formula:-

$$\eta(Y) = \eta(W)\frac{d(Y)t(y)}{d(w)t(w)}$$

Where, $\eta(Y)$- viscosity of gel, $\eta(W)$- viscosity of water, $d(Y)$- density of gel, $t(y)$- time run off gel, $d(w)$- density of water and $t(w)$- time runoff water.

#### 4.2.8.2. pH of gel

1% aqueous solution of each formulation was prepared and pH in pH meter (Sartorius pH meter) was checked.

#### 4.2.8.3. Rheology of gel

Rheology of gel was measured by Rheometer (Anton Paar, Austria). It was checked strain vs. viscosity graph, strain vs. G` (storage modulus) and G`` (loss modulus) and angular frequency vs. G` and G`` using Rheoplus/32 Version 3.

### 4.2.9. Optimization, development of mathematical model and model graphs in experimental design software

The effects of factors or dependent variables and development of best fit mathematical models were performed using Design Expert 7 trial version (Stat-ease Inc., USA). Total 20 experimental runs were generated (8-Factorial points, 6-Center points and 6-Axial points). This software analyzed the effect of main factors and their interactions with responses. After putting the values of independent variables (Responses), it was suggested to fit quadratic model. Polynomial equation of quadratic model is following-

$R_1 = f_0 + f_1A + f_2B + f_3C + f_4AB + f_5AC + f_6BC + f_7A^2 + f_8B^2 + f_9C^2$

Where, $R_1$ is a measured response, $f_0$ is an intercept of the polynomial equation which represent coefficients of model, $f_1$ to $f_9$ represent regression coefficients of main effects (A, B and C), interacting effects (AB, AC and BC) and quadratic effects ($A^2$, $B^2$ and $C^2$). The responses are: - $R_1$ (%Yield), $R_2$ (Particle size), $R_3$ (Entrapment Efficiency) and $R_4$ (drug release at 8 hr, $Rel_{8hr}$). After running the design fitted with response data, Analysis of variance (ANOVA) was generated which displayed b-coefficients, sum of squares, mean square, F-value and p-value. Other statistical parameters like lack of fit, adjusted $R^2$, predicted $R^2$ and equations in terms of coded and actual factors. After elimination of insignificant terms ($p \geq 0.05$) mathematical model equations were obtained. Response surface plot and contour plot were observed. After diagnosis of each responses predicted and actual value were spotted. The optimized formulation of 5-FU loaded microsponge was selected based on the response target. Targets were to attain average value of production yield, minimize particle size, maximize entrapment efficiency (%EE) and maximum release at 8hr which were set by numerical optimization. After applying these constraints 19 solutions were generated by software. From these solutions which desirability function was more or near to 1 is called optimized formulation. Three CPF (Check Point Formulations) also carried out from solutions to check actual and predicted values.

**Table4.3. Design matrix and Measured responses of 5-FU loaded microsponge**

| | | Factor1 | Factor2 | Factor3 | | | | |
|---|---|---|---|---|---|---|---|---|
| **Run** | **Block** | **Polymer** | **SS** | **SA ,%** | **Yield,%** | **Particle** | **EE ,%** | **$Rel_{8hr}$** |

| | | (mg) | ($min^{-1}$) | | | size,µm | | ,% |
|---|---|---|---|---|---|---|---|---|
| 1 | Center | 400 | 1000 | 1.25 | 74.88 | 163.43 | 68.28 | 57.57 |
| 2 | Fact | 600 | 1200 | 0.5 | 60.25 | 254.07 | 57.42 | 89.35 |
| 3 | Fact | 600 | 800 | 0.5 | 72.33 | 273.9 | 78 | 71.44 |
| 4 | Axial | 400 | 1000 | 0.5 | 70.48 | 233.99 | 75.63 | 50.14 |
| 5 | Axial | 400 | 1200 | 1.25 | 72.17 | 168.29 | 61.39 | 65.85 |
| 6 | Axial | 200 | 1000 | 1.25 | 79.68 | 166.73 | 55.46 | 73.37 |
| 7 | Fact | 200 | 1200 | 2 | 60 | 144.36 | 79.87 | 58.87 |
| 8 | Axial | 600 | 1000 | 1.25 | 73.9 | 158.34 | 51 | 88.93 |
| 9 | Fact | 600 | 800 | 2 | 79.28 | 270.34 | 82.36 | 67.82 |
| 10 | Center | 400 | 1000 | 1.25 | 86.97 | 141.84 | 55.83 | 62.21 |
| 11 | Axial | 400 | 1000 | 2 | 68.22 | 218.75 | 85.38 | 53.86 |
| 12 | Center | 400 | 1000 | 1.25 | 76.31 | 164.86 | 61.64 | 62.83 |
| 13 | Fact | 600 | 1200 | 2 | 63.49 | 147.035 | 75.98 | 72.81 |
| 14 | Center | 400 | 1000 | 1.25 | 75.42 | 223.57 | 71.23 | 69.17 |
| 15 | Center | 400 | 1000 | 1.25 | 78.36 | 236.41 | 65.72 | 65.98 |
| 16 | Center | 400 | 1000 | 1.25 | 78.39 | 229.22 | 63.12 | 71.31 |
| 17 | Axial | 400 | 800 | 1.25 | 90.4 | 236.52 | 69.29 | 55.85 |
| 18 | Fact | 200 | 1200 | 0.5 | 67.89 | 262.05 | 55.95 | 58.87 |
| 19 | Fact | 200 | 800 | 2 | 86.16 | 276.97 | 79.42 | 56.88 |
| 20 | Fact | 200 | 800 | 0.5 | 90.64 | 295.88 | 70.93 | 50.28 |

SS- stirring speed, SA – concentration of Surface active agent

## 4.3 RESULTS AND DISCUSSIONS

### 4.3.1. Standard curve of 5-FU in Phosphate buffer pH 5.5 and 7.4

For determination of drug entrapment efficiency and release of drug from microsponge, a standard calibration curve was plotted using PBS at pH 5.5 and 7.4. The linear curve with regression coefficients should be near to 1 which was obtained from the calibration curve (Fig. - 4.1 & 4.2) and corresponding absorbance is shown in Table 4.4. The line equation for PBS at pH 5.5 was found to be: $Y= 0.066x + 0.009$ ($R^2= 0.998$); for PBS at pH7.4 was found to be: $Y= 0.128x + 0.022$ ($R^2= 0.997$).

**Table 4.4: Absorbance of various concentrations of 5-FU at $\lambda_{max}$ 265 nm**

| Concentration (µg/ml) | OD (PBS 5.5) | OD (PBS 7.4) |
|---|---|---|
| 0 | 0 | 0 |
| 2 | 0.1388±0.0007 | 0.287± 0.0028 |
| 4 | 0.28995±0.0019 | 0.5413±0.0004 |
| 6 | 0.4088±0.0008 | 0.8185±0.0007 |
| 8 | 0.54665±0.0019 | 1.0668±0.0025 |
| 10 | 0.6588±0.0007 | 1.2755±0.0021 |

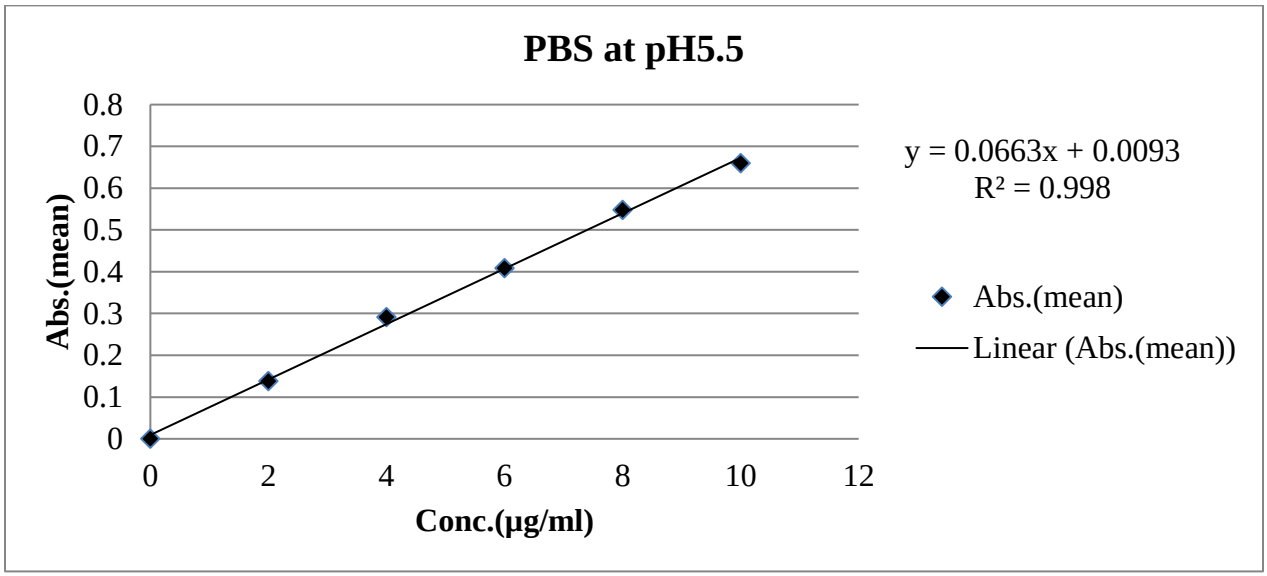



**Fig. 4.1: Standard Calibration curve of 5-FU PBS (Phosphate buffer saline), pH 5.5**

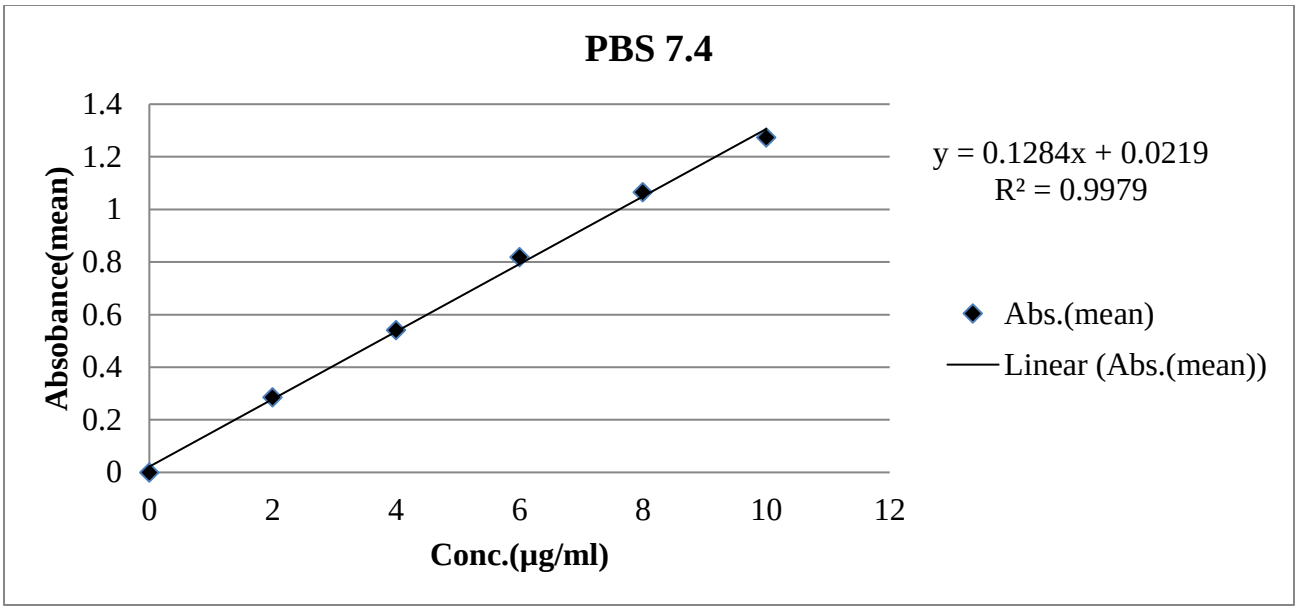



**Fig. 4.2: Standard Calibration curve of 5-FU (PBS 7.4)**

### 4.3.2. Melting point determination

The melting point of 5-FU was found to be 280º C and melting range was found to be 280-285º C.

### 4.3.3. Determination of partition coefficient

The partition coefficient value in n-octanol-PBS (7.4) system was found to be 0.85. Partition coefficient value in n-octanol-PBS (5.5) system was found to be 0.86. Each was studied in triplicate.

### 4.3.4. Drug-polymer solid state characteristics

#### 4.3.4.1. FTIR (Fourier Transform Infrared Spectroscopy)

The FTIR spectra of drug, polymer, drug-polymer physical mixture and standard formulation are given in figures below. Figure 4.3 (A) shows the FTIR of 5-FU (drug), (B-1) Ethyl Cellulose, (B-2) Eudragit RS 100. Figure 4.4 (C) shows the FTIR of physical mixture of drug-polymer (1:1) and (D) Microsponge formulation. Each graph was plotted Wavenumber ($cm^{-1}$) vs. %Transmittance.

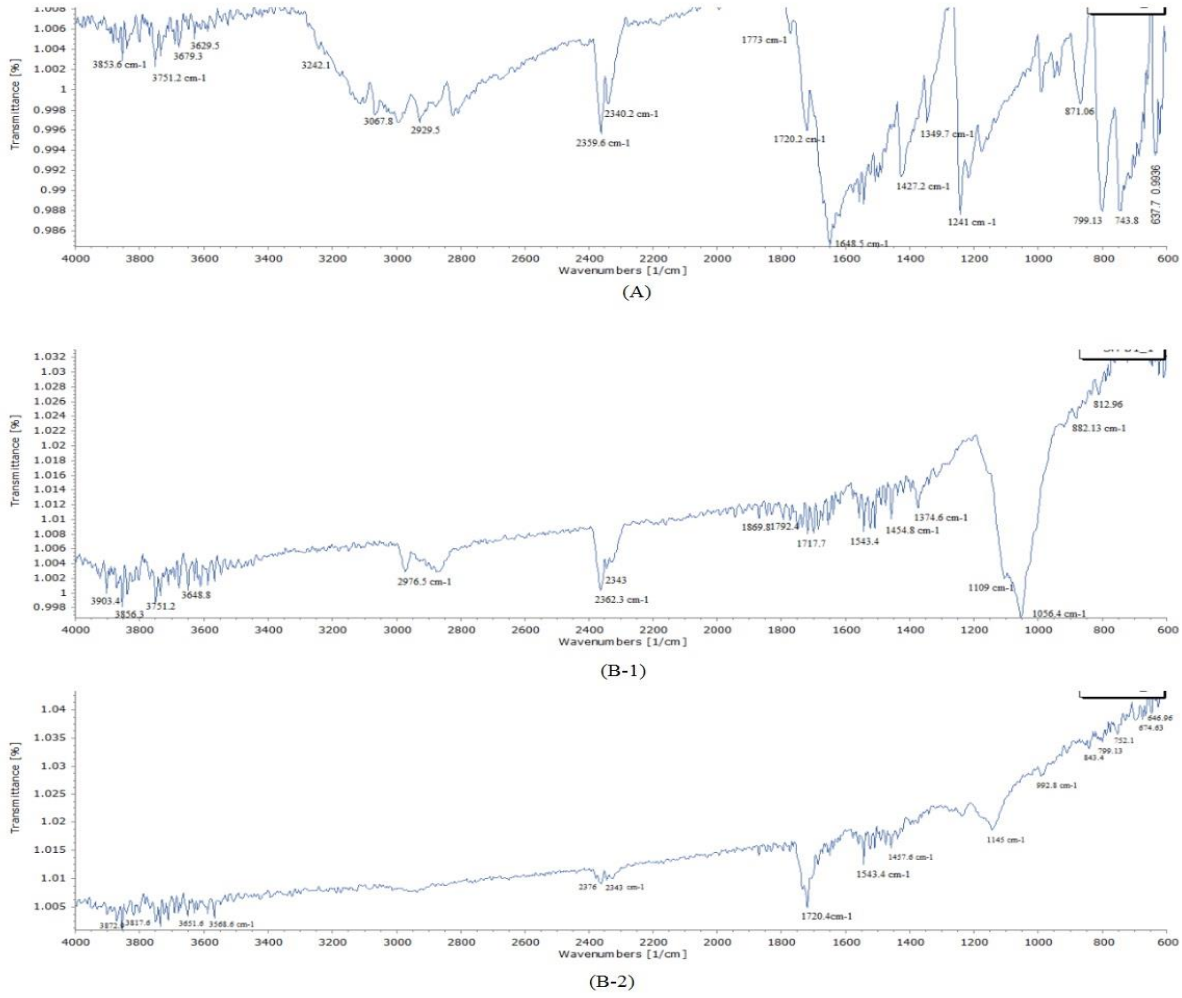



**Figure 4.3: FTIR spectra of (A) 5-FU, (B-1) Ethyl Cellulose and (B-2) Eudragit RS100.**

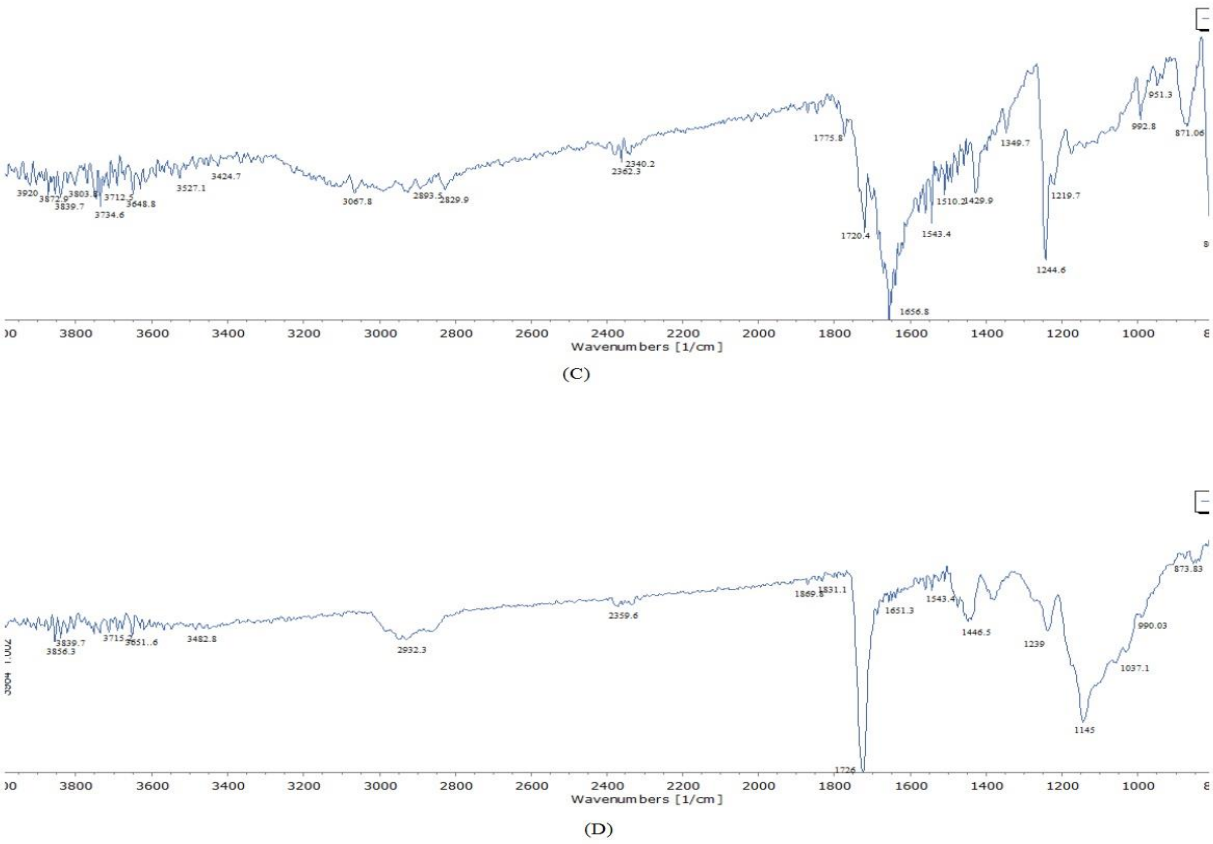



**Figure 4.4: FTIR spectra of (C) Physical Mixture (1:1) and (D) MS 3 Formulation.**

The spectrum of pure 5-FU (Fig. 4.3 A) showed characteristics peaks of 1648.5 $cm^{-1}$ and 3067.8 $cm^{-1}$ owing to N-H bending and stretching. Characteristics peaks were seen at 1241 $cm^{-1}$ and 1349.7 $cm^{-1}$ owing to C-F stretching. Peak at 1720.2 $cm^{-1}$ indicates C=O stretching, 1427.2 $cm^{-1}$ indicates C=C stretching of carboxylate groups. Characteristics peaks at 2929.5$cm^{-1}$ and 743.8 $cm^{-1}$ showed due to C-H stretch of alkane and aromatics (out of plane blend).

The FTIR spectra of Ethyl Cellulose (Fig. 4.3 B-1) showed peaks at 3648.8 and 2976.5 $cm^{-1}$ owing to presence of O-H and C-H groups. Peaks at 1454.8 and 1374.6$cm^{-1}$ indicates the stretching of $CH_2$ and $CH_3$ groups. C-O-C stretching at 1109 $cm^{-1}$ and peaks at 1056.4$cm^{-1}$ showed O-H bend. Peaks showed at 882.13 $cm^{-1}$ owing to N-H bending.

The FTIR spectra of Eudragit RS 100 (Fig. 4.3 B-2) showed peaks at 2346 and 2376 $cm^{-1}$ represent the stretching of C-H groups. Peak at 3568.6 $cm^{-1}$ showed N-H stretching, peak at

1720.4 $cm^{-1}$ showed stretching of C=O groups and a peak 1457.6 $cm^{-1}$ represent $CH_3$ stretch. Peaks showed at 1145 and 992.8 cm-1 owing to C-H bending and C-O stretching vibrations.

The FTIR spectra of Physical mixture (Fig. 4.4 C) showed some characteristics peaks (3067.8, 1656.8, 1720.4 and 1420.9 cm-1) of the drug and characteristics peaks (871.06, 3648.5, 1429.9, 2340.2 and 992.8) of the polymers. Therefore, it states that no incompatibility between the drug and the polymer.

The FTIR spectra of formulation (Fig.4.4 D) showed resemblance with the FTIR spectra of physical mixture. It also characteristics peaks with slight shifts of drug

### 4.3.4.2 DSC (Differential Scanning Calorimetry)

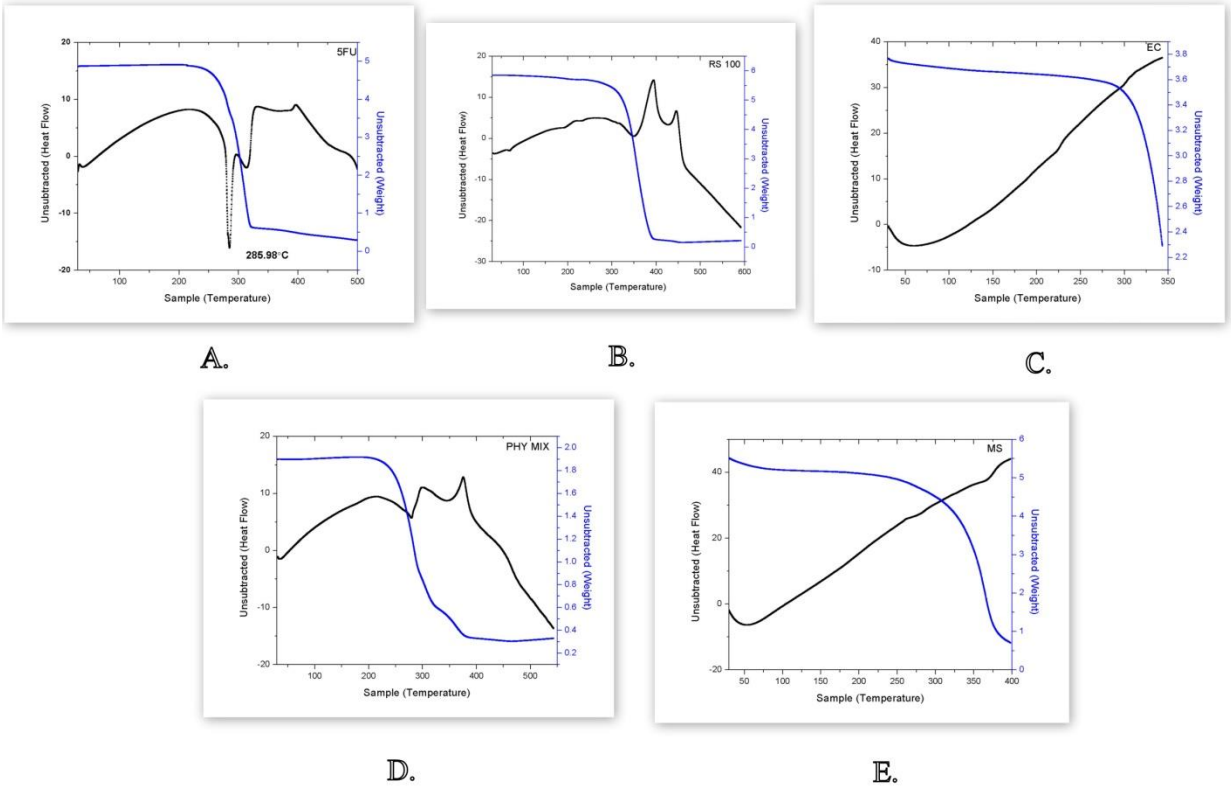



**Fig. 4.5 DSC of (A) pure 5-FU, (B) Eudragit RS-100, (C) Ethyl Cellulose, (D) Physical mixture of drug-polymer (1:1), (E) Microsponges.**

Differential scanning calorimetry or DSC is a technique in which the difference in the amount of heat required to increase temperature (30-500°C) of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same

temperature throughout the experiment under nitrogen purge of 25ml/min. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time.

The DSC thermogram of pure 5-FU is shown in the Figure 4.5 (A). It shows sharp endothermic peak at 282°C which corresponds to meting point. Melting points of Eudragit RS 100 (B) 398°C and ethyl cellulose (C) 220°C were much beyond the melting point of drug alone or in formulation. DSC graph of physical mixture (D) were showed melting points of drug, ethyl cellulose and eudragit RS 100. The absence of 5-FU crystalline peak, which should have been appeared at ~ 282°C, proved that the drug was in an amorphous state in drug loaded microsponges (E). From the above studies, it may be confirmed that the drug has no chemical incompatibility with the polymer. Second plot in each graph was stand for TGA (Thermogravimetric Analysis).

### 4.3.5 *In vitro* drug release studies

Cumulative percent release of 5-FU loaded microsponges (Run 1-20) in Phosphate buffer (pH 7.4). Amount of drug loaded in each formulation was equivalent to drug encapsulated in 10 mg microsponges.

Run 1:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 757.9 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.037667±0.0015 | 0.13020833 | 6.510416667 | 0.651041667 | 6.510416667 | 0.859007 |
| 1 | 0.122667±0.0016 | 0.79427083 | 39.71354167 | 3.971354167 | 40.36458333 | 5.325846 |
| 2 | 0.220333±0.0005 | 1.55729167 | 77.86458333 | 7.786458333 | 82.48697917 | 10.88362 |
| 3 | 0.327667±0.0014 | 2.39583333 | 119.7916667 | 11.97916667 | 132.2005208 | 17.443 |
| 4 | 0.418333±0.0005 | 3.10416667 | 155.2083333 | 15.52083333 | 179.5963542 | 23.69658 |
| 5 | 0.542667±0.0015 | 4.07552083 | 203.7760417 | 20.37760417 | 243.6848958 | 32.15264 |
| 6 | 0.66±0.001 | 4.9921875 | 249.609375 | 24.9609375 | 309.8958333 | 40.88875 |
| 7 | 0.716333±0.005 | 5.43229167 | 271.6145833 | 27.16145833 | 356.8619792 | 47.08563 |
| 8 | 0.850333±0.0015 | 6.47916667 | 323.9583333 | 32.39583333 | 436.3671875 | 57.57583 |

Run 2:- (Polymer- 600 mg, SS- 1200 rpm, SA- 0.5% and Drug loaded- 441.69 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.022333±0.0025 | 0.010416667 | 0.520833333 | 0.052083333 | 0.520833333 | 0.117918 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 0.114667±0.002 | 0.731770833 | 36.58854167 | 3.658854167 | 36.640625 | 8.295552 |
| 2 | 0.124±0.001 | 0.8046875 | 40.234375 | 4.0234375 | 43.9453125 | 9.949356 |
| 3 | 0.244±0.001 | 1.7421875 | 87.109375 | 8.7109375 | 94.84375 | 21.47292 |
| 4 | 0.337333±0.0005 | 2.471354167 | 123.5677083 | 12.35677083 | 140.0130208 | 31.69939 |
| 5 | 0.483667±0.002 | 3.614583333 | 180.7291667 | 18.07291667 | 209.53125 | 47.43853 |
| 6 | 0.588±0.001 | 4.4296875 | 221.484375 | 22.1484375 | 268.359375 | 60.7574 |
| 7 | 0.711333±0.0015 | 5.393229167 | 269.6614583 | 26.96614583 | 338.6848958 | 76.67932 |
| 8 | 0.785667±0.0015 | 5.973958333 | 298.6979167 | 29.86979167 | 394.6875 | 89.35849 |

Run 3:- (Polymer- 600 mg, SS- 800 rpm, SA- 0.5% and Drug loaded- 599.99 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.024±0.001 | 0.0234375 | 1.171875 | 0.1171875 | 1.171875 | 0.195316 |
| 1 | 0.036±0.001 | 0.1171875 | 5.859375 | 0.5859375 | 5.9765625 | 0.99611 |
| 2 | 0.138333±0.0015 | 0.916666667 | 45.83333333 | 4.583333333 | 46.53645833 | 7.756206 |
| 3 | 0.250667±0.002 | 1.794270833 | 89.71354167 | 8.971354167 | 95 | 15.8336 |
| 4 | 0.360333±0.0016 | 2.651041667 | 132.5520833 | 13.25520833 | 146.8098958 | 24.46872 |
| 5 | 0.477333±0.0015 | 3.565104167 | 178.2552083 | 17.82552083 | 205.7682292 | 34.29528 |
| 6 | 0.583667±0.0025 | 4.395833333 | 219.7916667 | 21.97916667 | 265.1302083 | 44.1891 |
| 7 | 0.715±0.001 | 5.421875 | 271.09375 | 27.109375 | 338.4114583 | 56.40285 |
| 8 | 0.874±0.0017 | 6.6640625 | 333.203125 | 33.3203125 | 427.6302083 | 71.27289 |

Run 4:- (Polymer- 400 mg, SS- 1000 rpm, SA- 0.5% and Drug loaded- 839.49 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.035333±0.0022 | 0.11197917 | 5.598958333 | 0.559895833 | 5.598958333 | 0.666948 |
| 1 | 0.051667±0.002 | 0.23958333 | 11.97916667 | 1.197916667 | 12.5390625 | 1.493652 |
| 2 | 0.189333±0.0015 | 1.31510417 | 65.75520833 | 6.575520833 | 67.51302083 | 8.042147 |
| 3 | 0.371±0.0035 | 2.734375 | 136.71875 | 13.671875 | 145.0520833 | 17.2786 |
| 4 | 0.465667±0.0021 | 3.47395833 | 173.6979167 | 17.36979167 | 195.703125 | 23.31214 |
| 5 | 0.546333±0.001 | 4.10416667 | 205.2083333 | 20.52083333 | 244.5833333 | 29.13475 |
| 6 | 0.662±0.001 | 5.0078125 | 250.390625 | 25.0390625 | 310.2864583 | 36.9613 |
| 7 | 0.746667±0.002 | 5.66927083 | 283.4635417 | 28.34635417 | 368.3984375 | 43.8836 |
| 8 | 0.808667±0.0011 | 6.15364583 | 307.6822917 | 30.76822917 | 420.9635417 | 50.14515 |

Run 5:- (Polymer- 400 mg, SS- 1200 rpm, SA- 1.25% and Drug loaded- 681.42 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.022667±0.0015 | 0.013020833 | 0.651041667 | 0.06510417 | 0.651041667 | 0.095542 |
| 1 | 0.037667±0.0021 | 0.130208333 | 6.510416667 | 0.65104167 | 6.575520833 | 0.964973 |
| 2 | 0.144333±0.0015 | 0.963541667 | 48.17708333 | 4.81770833 | 48.89322917 | 7.175197 |
| 3 | 0.269667±0.0011 | 1.942708333 | 97.13541667 | 9.71354167 | 102.6692708 | 15.06696 |
| 4 | 0.332±0.002 | 2.4296875 | 121.484375 | 12.1484375 | 136.7317708 | 20.06571 |
| 5 | 0.470667±0.0015 | 3.513020833 | 175.6510417 | 17.5651042 | 203.046875 | 29.79761 |
| 6 | 0.694667±0.0016 | 5.263020833 | 263.1510417 | 26.3151042 | 308.1119792 | 45.21616 |
| 7 | 0.820333±0.0015 | 6.244791667 | 312.2395833 | 31.2239583 | 383.515625 | 56.28183 |
| 8 | 0.905±0.0026 | 6.90625 | 345.3125 | 34.53125 | 447.8125 | 65.71755 |

Run 6:- (Polymer- 200 mg, SS- 1000 rpm, SA- 1.25 % and Drug loaded- 1109.2 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.032±0.0026 | 0.0859375 | 4.296875 | 0.4296875 | 4.296875 | 0.387385 |
| 1 | 0.167±0.001 | 1.140625 | 57.03125 | 5.703125 | 57.4609375 | 5.180395 |
| 2 | 0.284333±0.0011 | 2.057291667 | 102.8645833 | 10.28645833 | 108.9973958 | 9.826667 |
| 3 | 0.353667±0.0028 | 2.598958333 | 129.9479167 | 12.99479167 | 146.3671875 | 13.19574 |
| 4 | 0.554333±0.002 | 4.166666667 | 208.3333333 | 20.83333333 | 237.7473958 | 21.43413 |
| 5 | 0.855±0.0017 | 6.515625 | 325.78125 | 32.578125 | 376.0286458 | 33.90089 |
| 6 | 1.165±0.002 | 8.9375 | 446.875 | 44.6875 | 529.7005208 | 47.75519 |
| 7 | 1.406±0.0043 | 10.8203125 | 541.015625 | 54.1015625 | 668.5286458 | 60.27124 |
| 8 | 1.641667±0.0011 | 12.66145833 | 633.0729167 | 63.30729167 | 814.6875 | 73.44821 |

Run 7:- (Polymer- 200 mg, SS- 1200 rpm, SA- 2% and Drug loaded- 1597.4 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.237333±0.0011 | 1.690104167 | 84.50520833 | 8.450520833 | 84.50520833 | 5.290172 |
| 1 | 0.457±0.001 | 3.40625 | 170.3125 | 17.03125 | 178.7630208 | 11.19087 |
| 2 | 0.564333±0.0023 | 4.244791667 | 212.2395833 | 21.22395833 | 237.7213542 | 14.88177 |
| 3 | 0.716333±0.0005 | 5.432291667 | 271.6145833 | 27.16145833 | 318.3203125 | 19.9274 |
| 4 | 0.966±0.001 | 7.3828125 | 369.140625 | 36.9140625 | 443.0078125 | 27.73305 |
| 5 | 1.113333±0.0015 | 8.533854167 | 426.6927083 | 42.66927083 | 537.4739583 | 33.6468 |
| 6 | 1.330667±0.0017 | 10.23177083 | 511.5885417 | 51.15885417 | 665.0390625 | 41.63259 |
| 7 | 1.541333±0.0015 | 11.87760417 | 593.8802083 | 59.38802083 | 798.4895833 | 49.98683 |
| 8 | 1.733667±0.0011 | 13.38020833 | 669.0104167 | 66.90104167 | 933.0078125 | 58.4079 |

Run 8:- (Polymer- 600 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 392.3 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.027667±0.0023 | 0.05208333 | 2.604166667 | 0.26041667 | 2.604166667 | 0.66382 |
| 1 | 0.036667±0.0012 | 0.12239583 | 6.119791667 | 0.61197917 | 6.380208333 | 1.62636 |
| 2 | 0.125667±0.0005 | 0.81770833 | 40.88541667 | 4.08854167 | 41.7578125 | 10.64436 |
| 3 | 0.210333±0.0017 | 1.47916667 | 73.95833333 | 7.39583333 | 78.91927083 | 20.11707 |
| 4 | 0.333667±0.0015 | 2.44270833 | 122.1354167 | 12.2135417 | 134.4921875 | 34.28299 |
| 5 | 0.450667±0.001 | 3.35677083 | 167.8385417 | 16.7838542 | 192.4088542 | 49.04636 |
| 6 | 0.530333±0.002 | 3.97916667 | 198.9583333 | 19.8958333 | 240.3125 | 61.25733 |
| 7 | 0.618±0.004 | 4.6640625 | 233.203125 | 23.3203125 | 294.453125 | 75.05815 |
| 8 | 0.697667±0.0023 | 5.28645833 | 264.3229167 | 26.4322917 | 348.8932292 | 88.93531 |

Run 9:- (Polymer- 600 mg, SS- 800 rpm, SA- 2% and Drug loaded- 633.53 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.03±0.001 | 0.0703125 | 3.515625 | 0.3515625 | 3.515625 | 0.554926 |
| 1 | 0.104667±0.0025 | 0.65364583 | 32.68229167 | 3.268229167 | 33.03385417 | 5.214253 |
| 2 | 0.214333±0.0015 | 1.51041667 | 75.52083333 | 7.552083333 | 79.140625 | 12.49201 |
| 3 | 0.317333±0.0011 | 2.31510417 | 115.7552083 | 11.57552083 | 126.9270833 | 20.0349 |
| 4 | 0.427667±0.0015 | 3.17708333 | 158.8541667 | 15.88541667 | 181.6015625 | 28.66503 |
| 5 | 0.537±0.001 | 4.03125 | 201.5625 | 20.15625 | 240.1953125 | 37.9138 |
| 6 | 0.609333±0.0021 | 4.59635417 | 229.8177083 | 22.98177083 | 288.6067708 | 45.55534 |
| 7 | 0.733±0.0014 | 5.5625 | 278.125 | 27.8125 | 359.8958333 | 56.80802 |
| 8 | 0.841667±0.0017 | 6.41145833 | 320.5729167 | 32.05729167 | 430.15625 | 67.89832 |

Run 10:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 619.71 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.0025 | 0.067708333 | 3.385416667 | 0.338541667 | 3.385416667 | 0.54629 |
| 1 | 0.065333±0.002 | 0.346354167 | 17.31770833 | 1.731770833 | 17.65625 | 2.849115 |
| 2 | 0.122±0.001 | 0.7890625 | 39.453125 | 3.9453125 | 41.5234375 | 6.700463 |
| 3 | 0.218333±0.0015 | 1.541666667 | 77.08333333 | 7.708333333 | 83.09895833 | 13.40933 |
| 4 | 0.337667±0.0005 | 2.473958333 | 123.6979167 | 12.36979167 | 137.421875 | 22.17519 |
| 5 | 0.443667±0.0016 | 3.302083333 | 165.1041667 | 16.51041667 | 191.1979167 | 30.8528 |
| 6 | 0.514667±0.0017 | 3.856770833 | 192.8385417 | 19.28385417 | 235.4427083 | 37.9924 |
| 7 | 0.603333±0.0022 | 4.549479167 | 227.4739583 | 22.74739583 | 289.3619792 | 46.69313 |
| 8 | 0.791333±0.0017 | 6.018229167 | 300.9114583 | 30.09114583 | 385.546875 | 62.21408 |

Run 11:- (Polymer- 400 mg, SS- 1000 rpm, SA- 2% and Drug loaded- 947.72 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.132±0.001 | 0.8671875 | 43.359375 | 4.3359375 | 43.359375 | 4.575173 |
| 1 | 0.255667±0.0015 | 1.833333333 | 91.66666667 | 9.166666667 | 96.00260417 | 10.12996 |
| 2 | 0.362±0.002 | 2.6640625 | 133.203125 | 13.3203125 | 146.7057292 | 15.48002 |
| 3 | 0.481333±0.0011 | 3.596354167 | 179.8177083 | 17.98177083 | 206.640625 | 21.8042 |
| 4 | 0.588±0.0005 | 4.4296875 | 221.484375 | 22.1484375 | 266.2890625 | 28.09816 |
| 5 | 0.654±0.002 | 4.9453125 | 247.265625 | 24.7265625 | 314.21875 | 33.15558 |
| 6 | 0.760333±0.0023 | 5.776041667 | 288.8020833 | 28.88020833 | 380.4817708 | 40.14749 |
| 7 | 0.822±0.0024 | 6.2578125 | 312.890625 | 31.2890625 | 433.4505208 | 45.73662 |
| 8 | 0.939±0.0013 | 7.171875 | 358.59375 | 35.859375 | 510.4427083 | 53.86064 |

Run 12:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 684.2 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.054333±0.0011 | 0.26041667 | 13.02083333 | 1.30208333 | 13.02083333 | 1.903074 |
| 1 | 0.177667±0.0025 | 1.22395833 | 61.19791667 | 6.11979167 | 62.5 | 9.134756 |
| 2 | 0.288±0.001 | 2.0859375 | 104.296875 | 10.4296875 | 111.71875 | 16.32838 |
| 3 | 0.365667±0.0017 | 2.69270833 | 134.6354167 | 13.4635417 | 152.4869792 | 22.2869 |
| 4 | 0.507±0.001 | 3.796875 | 189.84375 | 18.984375 | 221.1588542 | 32.32371 |
| 5 | 0.583333±0.002 | 4.39322917 | 219.6614583 | 21.9661458 | 269.9609375 | 39.45644 |
| 6 | 0.641667±0.0017 | 4.84895833 | 242.4479167 | 24.2447917 | 314.7135417 | 45.9973 |
| 7 | 0.731667±0.0013 | 5.55208333 | 277.6041667 | 27.7604167 | 374.1145833 | 54.67913 |
| 8 | 0.801333±0.0021 | 6.09635417 | 304.8177083 | 30.4817708 | 429.0885417 | 62.71391 |

Run 13:- (Polymer- 600 mg, SS- 1200 rpm, SA- 2% and Drug loaded- 584.46 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.025333±0.0015 | 0.033854167 | 1.692708333 | 0.169270833 | 1.692708333 | 0.289619 |
| 1 | 0.057667±0.0018 | 0.286458333 | 14.32291667 | 1.432291667 | 14.4921875 | 2.479586 |
| 2 | 0.142±0.0021 | 0.9453125 | 47.265625 | 4.7265625 | 48.8671875 | 8.361083 |
| 3 | 0.276±0.002 | 1.9921875 | 99.609375 | 9.9609375 | 105.9375 | 18.12571 |
| 4 | 0.400667±0.0017 | 2.966145833 | 148.3072917 | 14.83072917 | 164.5963542 | 28.16212 |
| 5 | 0.509333±0.0022 | 3.815104167 | 190.7552083 | 19.07552083 | 221.875 | 37.96239 |
| 6 | 0.668667±0.0023 | 5.059895833 | 252.9947917 | 25.29947917 | 303.1901042 | 51.87525 |
| 7 | 0.716667±0.0018 | 5.434895833 | 271.7447917 | 27.17447917 | 347.2395833 | 59.41204 |
| 8 | 0.841667±0.001 | 6.411458333 | 320.5729167 | 32.05729167 | 423.2421875 | 72.41594 |

Run 14:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 790.65 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.028667±0.0005 | 0.05989583 | 2.994791667 | 0.29947917 | 2.994791667 | 0.378776 |
| 1 | 0.155667±0.0015 | 1.05208333 | 52.60416667 | 5.26041667 | 52.90364583 | 6.691159 |
| 2 | 0.252667±0.002 | 1.80989583 | 90.49479167 | 9.04947917 | 96.0546875 | 12.14883 |
| 3 | 0.381±0.001 | 2.8125 | 140.625 | 14.0625 | 155.234375 | 19.63377 |
| 4 | 0.54±0.0032 | 4.0546875 | 202.734375 | 20.2734375 | 231.40625 | 29.26785 |
| 5 | 0.664667±0.0021 | 5.02864583 | 251.4322917 | 25.1432292 | 300.3776042 | 37.99122 |
| 6 | 0.771667±0.0016 | 5.86458333 | 293.2291667 | 29.3229167 | 367.3177083 | 46.45769 |
| 7 | 0.900333±0.0024 | 6.86979167 | 343.4895833 | 34.3489583 | 446.9010417 | 56.52325 |
| 8 | 1.073667±0.003 | 8.22395833 | 411.1979167 | 41.1197917 | 548.9583333 | 69.43127 |

Run 15:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 729.49 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.046±0.0017 | 0.1953125 | 9.765625 | 0.9765625 | 9.765625 | 1.338692 |
| 1 | 0.153667±0.0005 | 1.036458333 | 51.82291667 | 5.182291667 | 52.79947917 | 7.237862 |
| 2 | 0.281333±0.002 | 2.033854167 | 101.6927083 | 10.16927083 | 107.8515625 | 14.78452 |
| 3 | 0.356667±0.0015 | 2.622395833 | 131.1197917 | 13.11197917 | 147.4479167 | 20.21247 |
| 4 | 0.442333±0.0023 | 3.291666667 | 164.5833333 | 16.45833333 | 194.0234375 | 26.59713 |
| 5 | 0.531±0.0027 | 3.984375 | 199.21875 | 19.921875 | 245.1171875 | 33.60117 |
| 6 | 0.682333±0.0011 | 5.166666667 | 258.3333333 | 25.83333333 | 324.1536458 | 44.43565 |
| 7 | 0.823667±0.0017 | 6.270833333 | 313.5416667 | 31.35416667 | 405.1953125 | 55.54501 |
| 8 | 0.933667±0.002 | 7.130208333 | 356.5104167 | 35.65104167 | 479.5182292 | 65.73335 |

Run 16:- (Polymer- 400 mg, SS- 1000 rpm, SA- 1.25% and Drug loaded- 700.63 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.0015 | 0.06770833 | 3.385416667 | 0.338541667 | 3.385416667 | 0.483196 |
| 1 | 0.050333±0.0017 | 0.22916667 | 11.45833333 | 1.145833333 | 11.796875 | 1.683752 |
| 2 | 0.146±0.001 | 0.9765625 | 48.828125 | 4.8828125 | 50.3125 | 7.181037 |
| 3 | 0.335333±0.0011 | 2.45572917 | 122.7864583 | 12.27864583 | 129.1536458 | 18.43393 |
| 4 | 0.494667±0.0022 | 3.70052083 | 185.0260417 | 18.50260417 | 203.671875 | 29.06982 |
| 5 | 0.619667±0.001 | 4.67708333 | 233.8541667 | 23.38541667 | 271.0026042 | 38.67985 |
| 6 | 0.764667±0.0025 | 5.80989583 | 290.4947917 | 29.04947917 | 351.0286458 | 50.10186 |
| 7 | 0.827667±0.003 | 6.30208333 | 315.1041667 | 31.51041667 | 404.6875 | 57.76052 |
| 8 | 0.991333±0.0032 | 7.58072917 | 379.0364583 | 37.90364583 | 500.1302083 | 71.38293 |

Run 17:- (Polymer- 400 mg, SS- 800 rpm, SA- 1.25% and Drug loaded- 769.11 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.002 | 0.067708 | 3.385417 | 0.338542 | 3.385417 | 0.440173 |
| 1 | 0.050333±0.0017 | 0.229167 | 11.45833 | 1.145833 | 11.79688 | 1.533835 |
| 2 | 0.146±0.0018 | 0.976563 | 48.82813 | 4.882813 | 50.3125 | 6.541652 |
| 3 | 0.335333±0.0021 | 2.455729 | 122.7865 | 12.27865 | 129.1536 | 16.79261 |
| 4 | 0.494667±0.002 | 3.700521 | 185.026 | 18.5026 | 203.6719 | 26.4815 |
| 5 | 0.519667±0.0011 | 3.895833 | 194.7917 | 19.47917 | 231.9401 | 30.15695 |
| 6 | 0.662667±0.0025 | 5.013021 | 250.651 | 25.0651 | 307.2786 | 39.9525 |
| 7 | 0.727667±0.0015 | 5.520833 | 276.0417 | 27.60417 | 357.7344 | 46.51277 |
| 8 | 0.841±0.001 | 6.40625 | 320.3125 | 32.03125 | 429.6094 | 55.85799 |

Run 18:- (Polymer- 200 mg, SS- 1200 rpm, SA- 0.5% and Drug loaded- 1119 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.0029 | 0.067708 | 3.385417 | 0.338542 | 3.385417 | 0.302539 |
| 1 | 0.150333±0.001 | 1.010417 | 50.52083 | 5.052083 | 50.85938 | 4.545074 |
| 2 | 0.246±0.0021 | 1.757813 | 87.89063 | 8.789063 | 93.28125 | 8.336126 |
| 3 | 0.435333±0.0024 | 3.236979 | 161.849 | 16.1849 | 176.0286 | 15.73089 |
| 4 | 0.594667±0.0005 | 4.481771 | 224.0885 | 22.40885 | 254.4531 | 22.73933 |
| 5 | 0.719667±0.0011 | 5.458333 | 272.9167 | 27.29167 | 325.6901 | 29.10546 |
| 6 | 0.964667±0.002 | 7.372396 | 368.6198 | 36.86198 | 448.6849 | 40.09695 |
| 7 | 1.127667±0.0015 | 8.645833 | 432.2917 | 43.22917 | 549.2188 | 49.08121 |
| 8 | 1.281333±0.0011 | 9.846354 | 492.3177 | 49.23177 | 652.474 | 58.30866 |

Run 19:- (Polymer- 200 mg, SS- 800 rpm, SA- 2% and Drug loaded- 1588.4 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.129667±0.002 | 0.848958 | 42.44792 | 4.244792 | 42.44792 | 2.672369 |
| 1 | 0.250333±0.0024 | 1.791667 | 89.58333 | 8.958333 | 93.82813 | 5.907084 |
| 2 | 0.396±0.0029 | 2.929688 | 146.4844 | 14.64844 | 159.6875 | 10.05336 |
| 3 | 0.535333±0.002 | 4.018229 | 200.9115 | 20.09115 | 228.763 | 14.4021 |
| 4 | 0.794667±0.002 | 6.044271 | 302.2135 | 30.22135 | 350.1563 | 22.04459 |
| 5 | 0.919667±0.0021 | 7.020833 | 351.0417 | 35.10417 | 429.2057 | 27.02126 |
| 6 | 1.264667±0.0017 | 9.716146 | 485.8073 | 48.58073 | 599.0755 | 37.71566 |
| 7 | 1.527667±0.0015 | 11.77083 | 588.5417 | 58.85417 | 750.3906 | 47.24192 |
| 8 | 1.741±0.001 | 13.4375 | 671.875 | 67.1875 | 892.5781 | 56.19354 |

Run 20:- (Polymer- 200 mg, SS- 800 rpm, SA- 0.5% and Drug loaded- 1418.6 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.0018 | 0.067708 | 3.385417 | 0.338542 | 3.385417 | 0.238645 |
| 1 | 0.050333±0.0021 | 0.229167 | 11.45833 | 1.145833 | 11.79688 | 0.831586 |
| 2 | 0.146±0.001 | 0.976563 | 48.82813 | 4.882813 | 50.3125 | 3.54663 |
| 3 | 0.335333±0.0013 | 2.455729 | 122.7865 | 12.27865 | 129.1536 | 9.104303 |
| 4 | 0.594667±0.0022 | 4.481771 | 224.0885 | 22.40885 | 242.7344 | 17.11084 |
| 5 | 0.819667±0.002 | 6.239583 | 311.9792 | 31.19792 | 353.0339 | 24.88607 |
| 6 | 1.064667±0.0017 | 8.153646 | 407.6823 | 40.76823 | 479.9349 | 33.83159 |
| 7 | 1.287667±0.0015 | 9.895833 | 494.7917 | 49.47917 | 607.8125 | 42.84594 |
| 8 | 1.441±0.001 | 11.09375 | 554.6875 | 55.46875 | 717.1875 | 50.55601 |

Different batches of microsponges prepared under some constraints possess characteristics such as: particle size/interfacial area, particle size distribution, drug distribution in polymer and surface texture which have major role in release of drug. Lowest drug releases were (50.14-53.86%) showed by run 4 and 20 where polymer range was minimum to average and surface active agent was low. Maximum drug releases were (88-89.35%) showed by run 2 and 8 where polymer range was maximum but surface active agent was lowest to moderate and SS was moderate to higher.

Other runs were showed moderate cumulative percent release (58.4- 73.44 %).

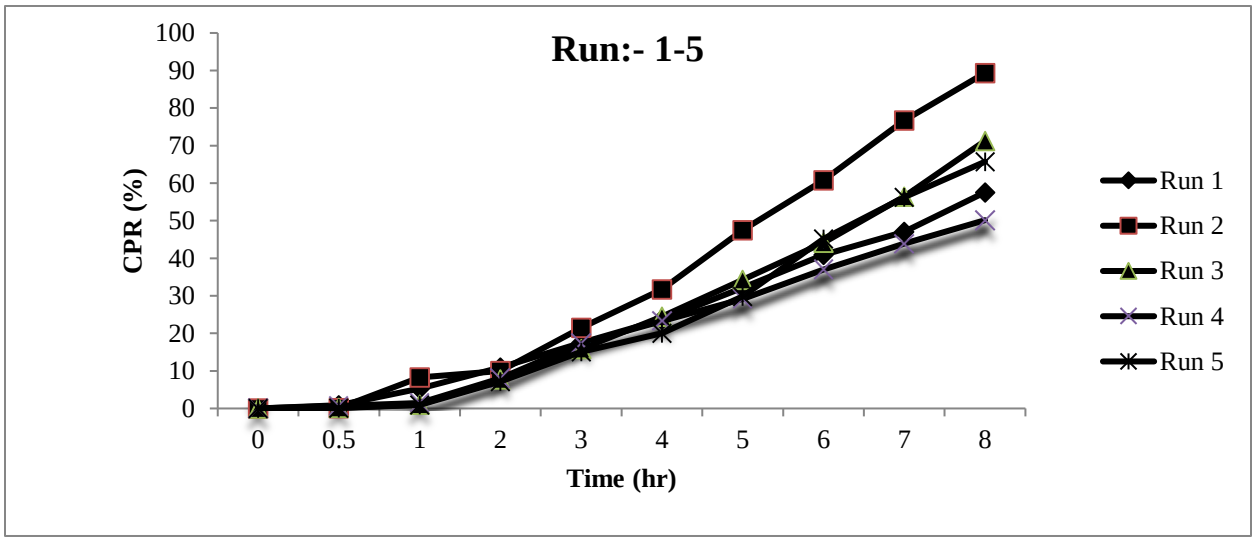



**Fig. 4.6 Drug Release Profiles (Run1-5)**

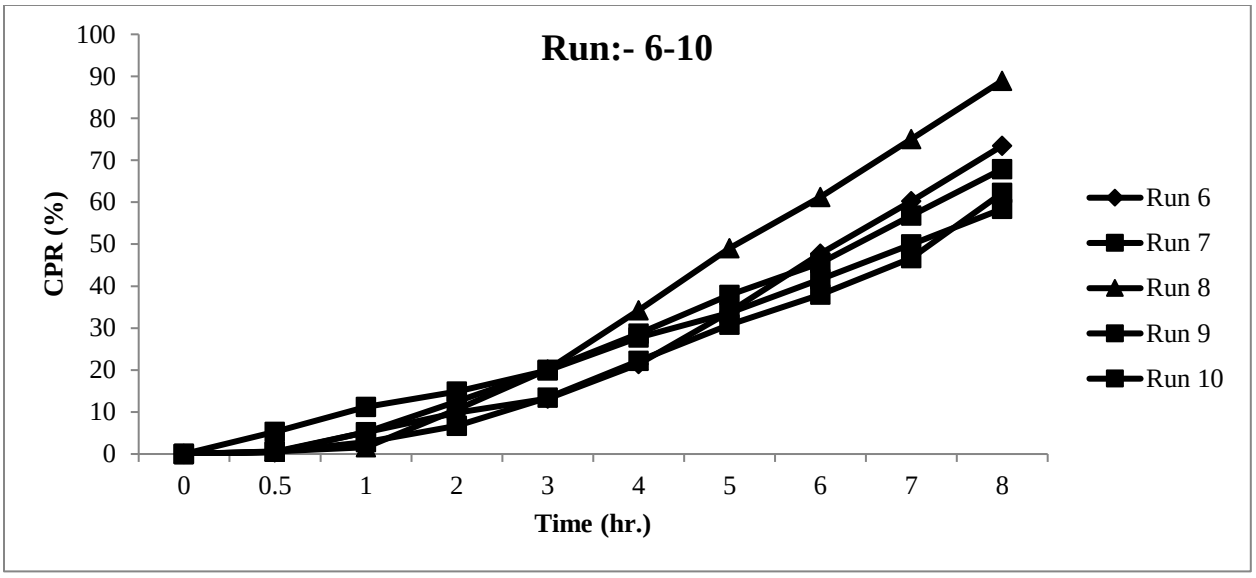



**Fig. 4.7 Drug Release Profiles (Run 6-10)**

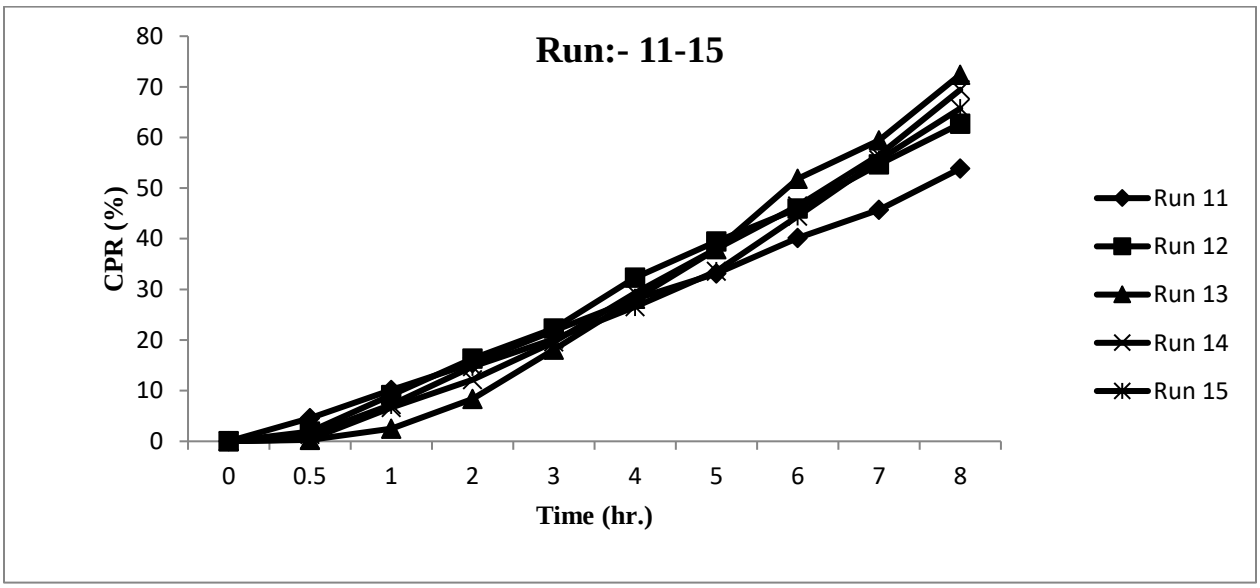



**Fig. 4.8 Drug Release Profiles (Run 11-15)**

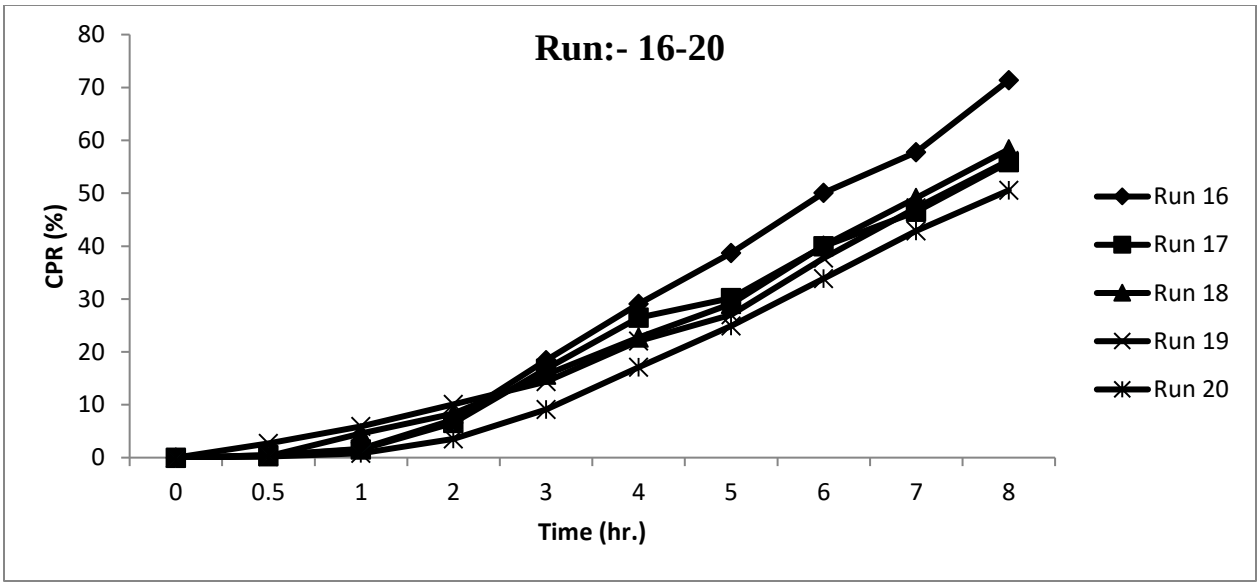



**Fig. 4.9 Drug Release Profiles (Run 16-20)**

### 4.3.6. Evaluation of Micrometric properties of particles

**Table 4.5 Micrometric properties of 5-FU Microsponges**

| Batch code | Bulk Density (gm/ml) | Tapped Density (gm/ml) | Carr's Index (%) | Hausner Ratio |
|---|---|---|---|---|
| Run 1 | 0.31 | 0.33 | 6.06 | 1.033 |
| Run2 | 0.33 | 0.36 | 8.33 | 1.1019 |
| Run 3 | 0.25 | 0.27 | 5.55 | 1.1034 |
| Run 4 | 0.2 | 0.2173 | 7.96 | 1.08 |
| Run 5 | 0.111 | 0.133 | 16.54 | 1.20 |
| Run 6 | 0.141 | 0.166 | 15.06 | 1.17 |
| Run 7 | 0.25 | 0.2857 | 12.49 | 1.14 |
| Run 8 | 0.34 | 0.39 | 12.82 | 1.14 |
| Run 9 | 0.2857 | 0.33 | 13.42 | 1.16 |
| Run 10 | 0.166 | 0.181 | 8.28 | 1.095 |
| Run 11 | 0.181 | 0.222 | 18.46 | 1.227 |
| Run 12 | 0.2 | 0.22 | 9.09 | 1.11 |
| Run 13 | 0.19 | 0.2 | 5 | 1.05 |

| | | | | |
|---|---|---|---|---|
| Run 14 | 0.181 | 0.21 | 13.8 | 1.163 |
| Run 15 | 0.180 | 0.211 | 14.69 | 1.17 |
| Run 16 | 0.182 | 0.210 | 13.33 | 1.15 |
| Run 17 | 0.222 | 0.25 | 11.2 | 1.126 |
| Run 18 | 0.145 | 0.163 | 11.04 | 1.12 |
| Run 19 | 0.142 | 0.166 | 14.45 | 1.173 |
| Run 20 | 0.1384 | 0.1636 | 15.4 | 1.182 |

In Table 4.5, Micrometric properties such as bulk densities, tapped densities, Carr's index and Hausner's ratio were shown. No significant difference between bulk and tapped densities was found, suggesting uniform particle size and more sphericity. Compressibility index of 12 formulations was excellent and 8 formulations were good to fair. Hausner's ratio of all runs was less than 1.25 which indicates better flow properties. In consideration of Hausner's ratio microsponges appeared to be of good flow ability.

### 4.3.7. Surface Morphology of microsponges (SEM)

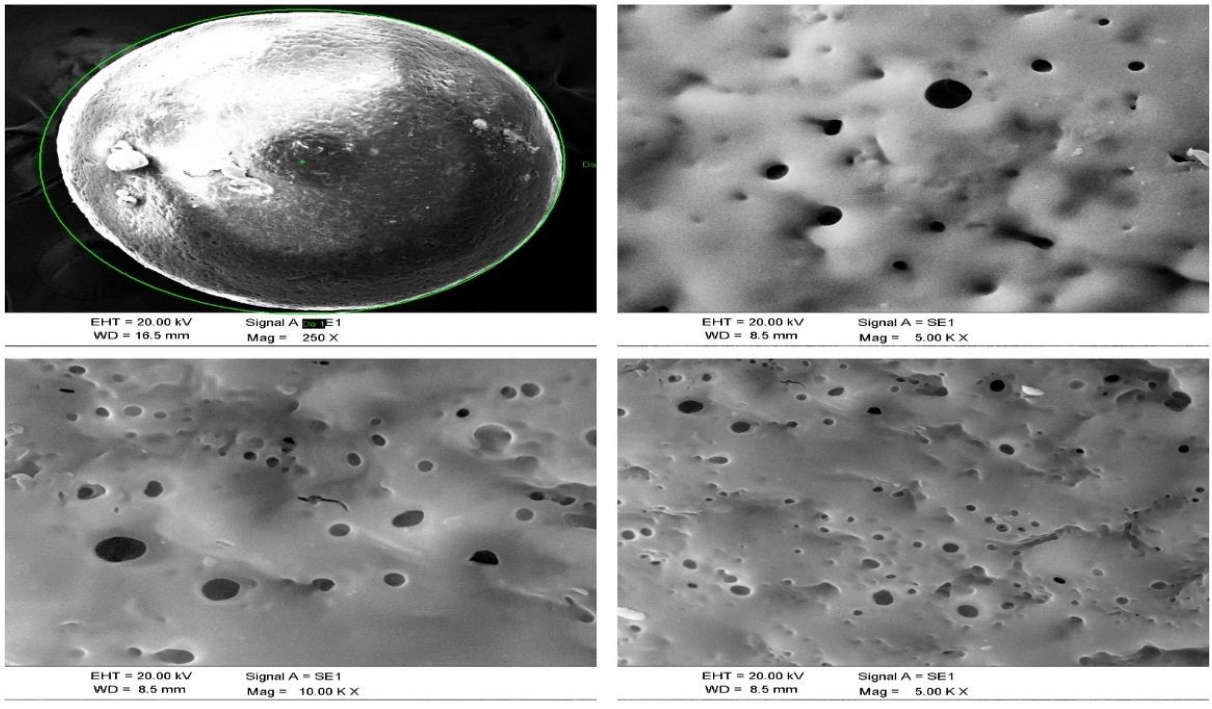



**Figure 4.10- SEM images of MS (Run 18) showing shape and porous surface of the MS formed.**

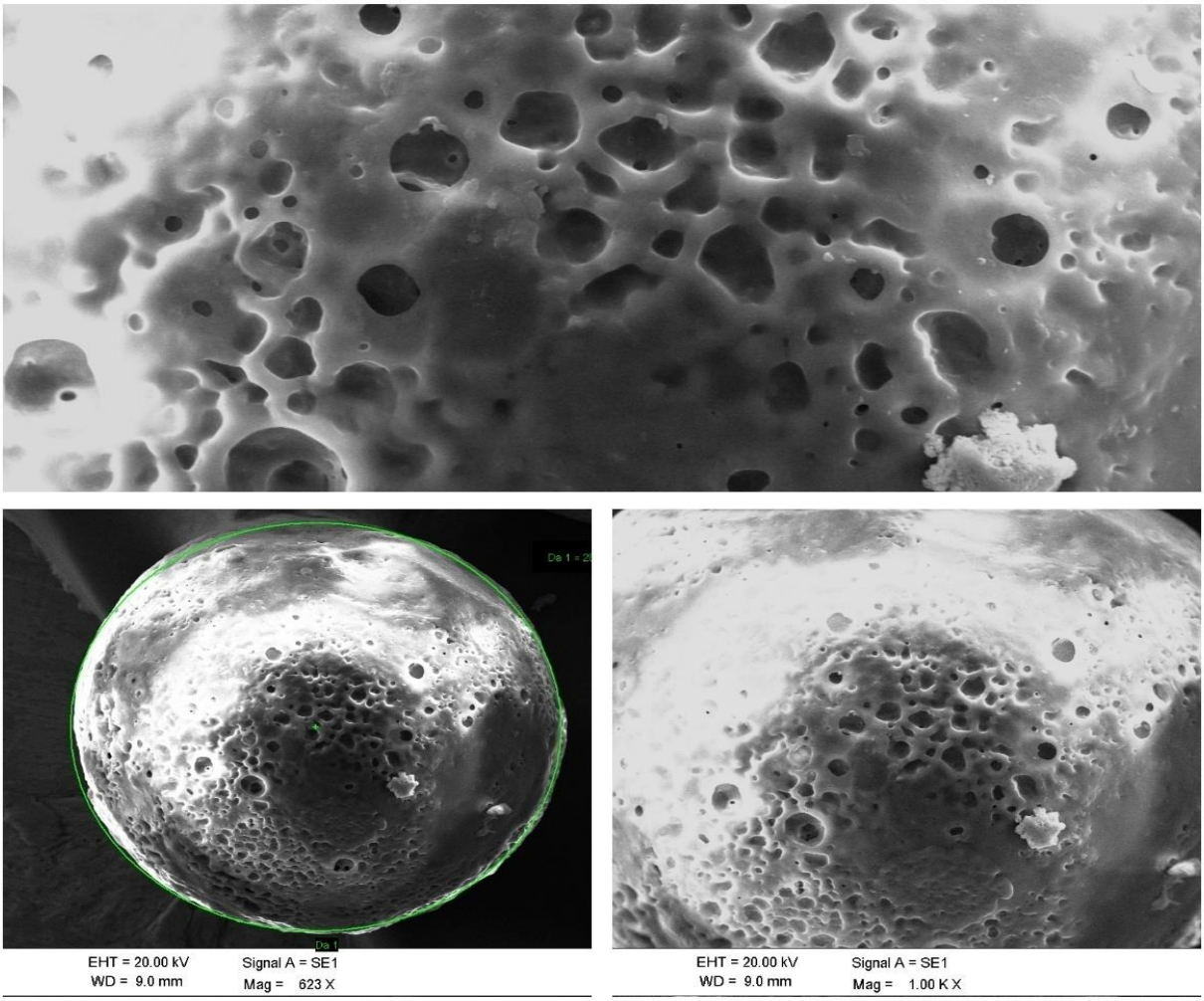



**Fig.4.11. SEM images of MS 18 showing shape and surface of MS after 1hr release.**

Figure 4.10 shows different sizes of microsponges with smooth and fine porous surface. The rough surface of microsponges is due to high polymer content, shearing effect and solidification of large sized globules in the emulsion at high rate of evaporation of solvent. Figure 4.11 shows after 1hr drug release, pores were non-uniform and irregular in shape.

### 4.3.8. Characterization of microsponges incorporated Gel

#### 4.3.8.1. pH of Gel

pH of gel was measured in a Sartorius pH meter. pH of each formulations wasobserved as 5.6-5.9. Each experiment was carried out in triplicate.

### 4.3.8.2. Rheology of Gel

Rheology is the study of flow of matter, primarily in a liquid state, but also as soft solids. It is a branch of physics which deals with the deformation and the flow of materials.Many of the materials we use each day are structured fluids. Number of soft semisolid materials also falls under the category of structured fluids since they have a multiphase structure and exhibit complex flow behavior.Many factors affect the stability of structured fluids. The viscosity of the liquid phase in dispersions usually plays an important role on the flow properties of the material. Dispersions have wide variations in performance depending on particle size, shape, concentration, and any attraction with the continuous phase in which they are suspended. When there is a repulsive electrostatic or steric force between particles they tend not to settle rapidly, instead forming a network structure which will stabilize the suspension if undisturbed. Shearing or even Brownian motion can destroy this delicate structure and break down the fluids viscosity. Structured fluids do not obey a simple linear relationship between applied stresses and flow (Newtonian fluid behavior). The rheological property of gel were investigated three test methods flow behavior, amplitude/strain sweep and frequency sweep.

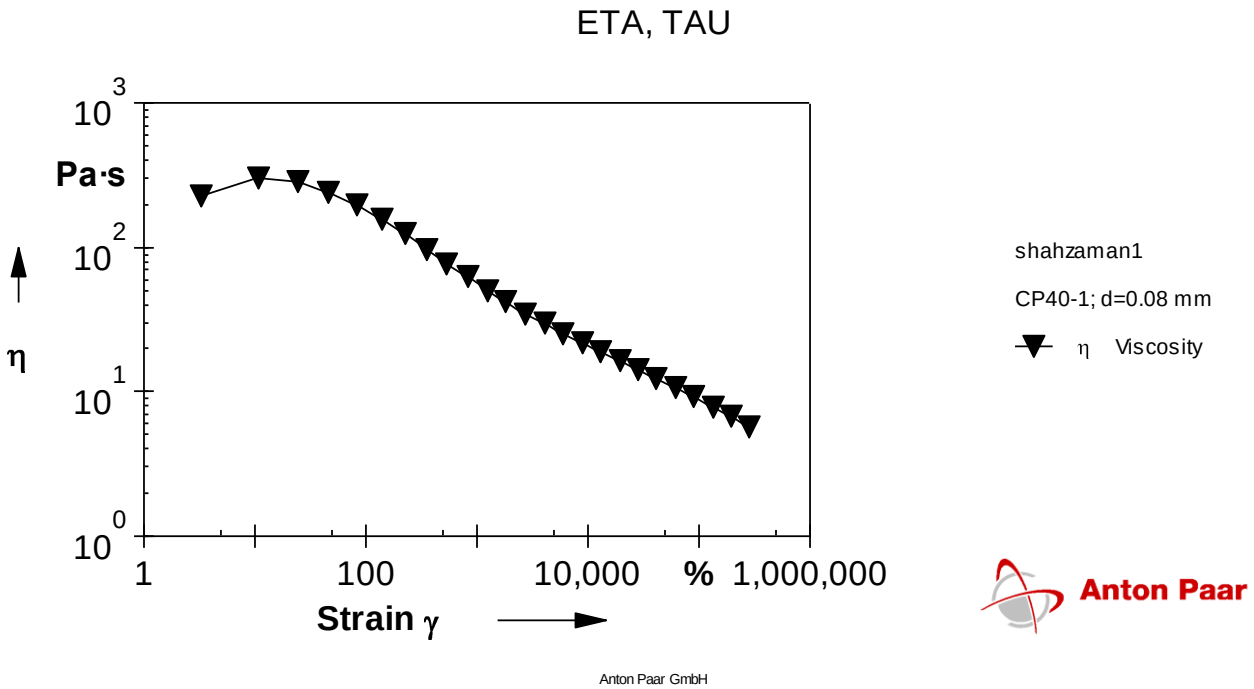



**Fig 4.12 Effect in viscosity after applying strain (Flow Curve)**

In Fig 4.12 flow curve of gel is shown. Viscosity of gel was high at low shear rate (953 Pa.s at 0.0018 sec $^{-1}$ ). Viscosity is dropped at higher rates of shear rate (9.93 Pa.s at 100 sec $^{-1}$ ). This is

the ideal phenomenon of gel (shear thinning) which becomes progressively larger as the shear rate increases. It showed pseudo plastic properties because viscosity decreased after increasing shear rate which causes better drug release.

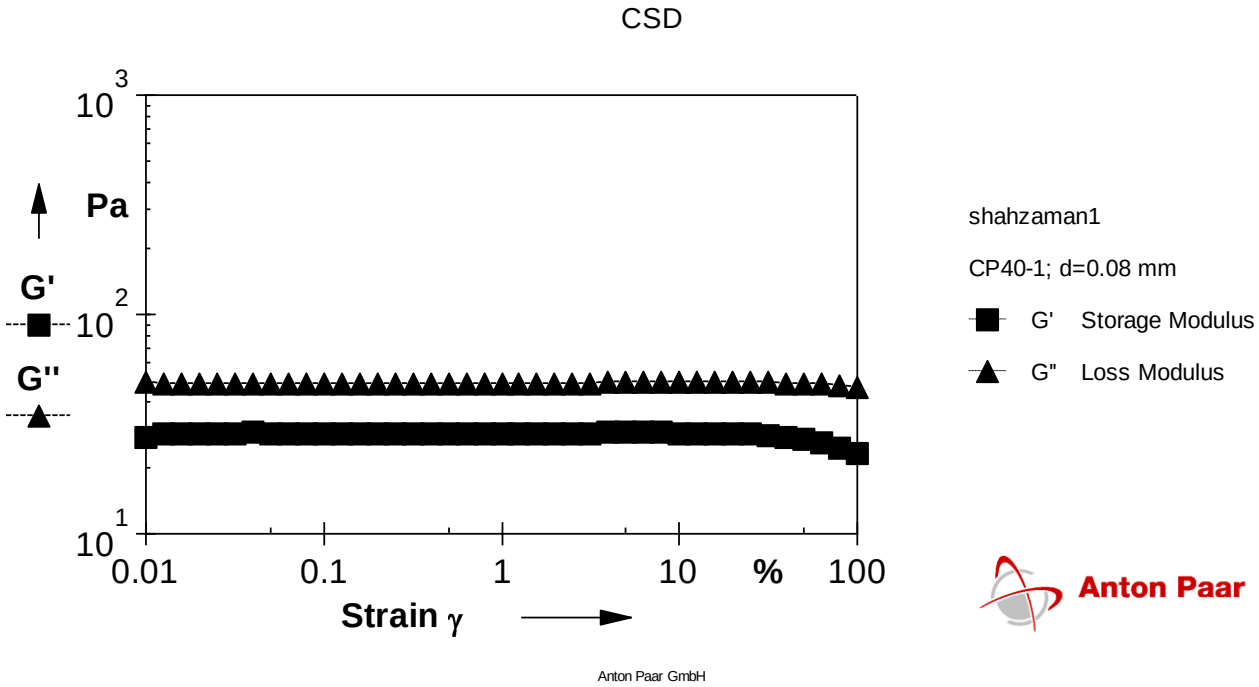



**Fig 4.13 Strain vs. Storage Modulus (G') and Loss Modulus (G") (Amplitude sweep curves).**

In Fig. 4.13 showed amplitude sweep analysis. It was performed to assess the linear viscoelastic range and viscoelastic properties of polymer. The range of applied strain within which G' and G" remain constant represent the linear viscoelastic range (LVE). Strength of the gel was so high that's why it's longer linear. At certain strain (50.1%) G' was declined (2.69 Pa). It was stated that from this amount strain breakdown of structure started. G" is greater than G' indicated that gel was highly structured with elastic characteristics. Usually the rheological properties of a visco-elastic material are independent of strain up. Beyond this critical strain level, the material's behavior is non-linear and the storage modulus declines. So, measuring the strain amplitude dependence of the storage and loss moduli (G', G") is a good first step taken in characterizing visco-elastic behavior: A strain sweep will establish the extent of the material's linearity. In this graph G" is greater than G' indicating the gel becomes progressively more fluid like and the module decline.

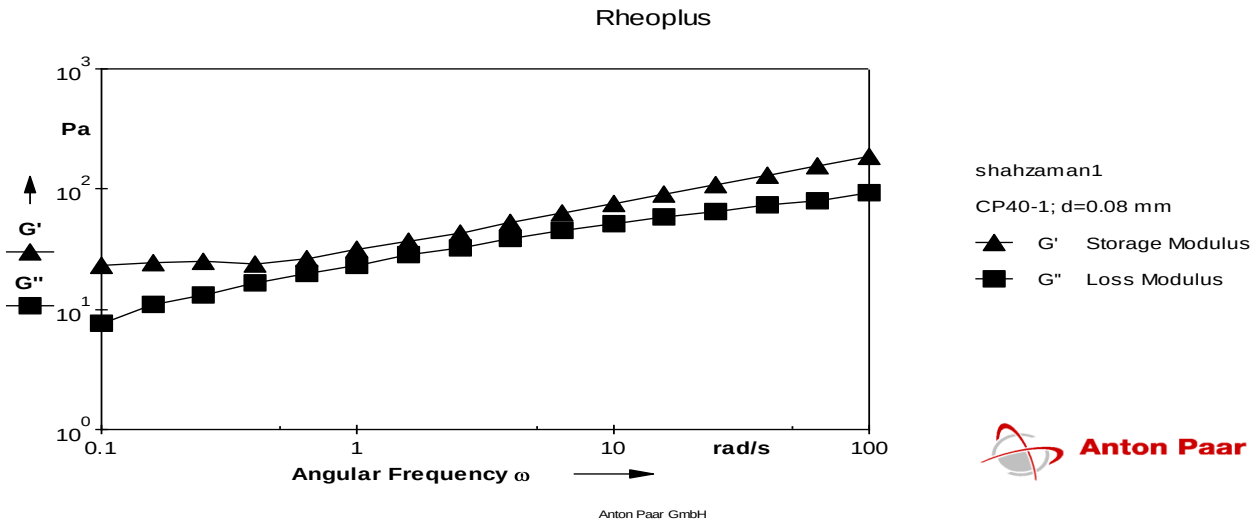



**Fig 4.14 Angular Frequency (ώ) vs. Storage (G') and Loss Modulus (G") (Frequency sweep)**

Frequency sweep analysis within the LVE range obtained from amplitude sweep test indicates the structural integrity and mechanical strength of material more precisely and accurately (Chakravorty et al. 2016) (Fig 4.14). The structural integrity of the sample was determined by the structural response to deformation at longer and shorter oscillatory stress (100-0.1 rad/sec). Higher values of storage modulus (G') over the loss modulus indicate a strong elastic gel. It was show higher yield stress due to the sample unable to show any kind of crossover point. Moreover absence of any crossover region indicated absence of gel to solid transformation. After the fluid's linear visco-elastic region has been defined by a strain sweep, its structure can be further characterized using a frequency sweep at a strain below the critical strain $\gamma_c$. This provides more information about the effect of colloidal forces, the interactions among particles or droplets. In a frequency sweep, measurements are made over a range of oscillation frequencies at a constant oscillation amplitude and temperature. Below the critical strain, the elastic modulus G' is often nearly independent of frequency, as would be expected from a structured or solid-like material. The more frequency dependent the elastic modulus is, the more fluid-like is the material. In Fig 4.14 high strain amplitudes showed better fluid like behavior (G">G').

### 4.3.9 Optimization data analysis and model-validation

**Table 4.6.**ANOVA results for the dependent response variable (% Yield) ($R_1$)

| Source | Sum of Squares | d.f. | Mean Square | F-Value | P-Value | |
|---|---|---|---|---|---|---|
| Model | 1418.368 | 9 | 157.5964 | 15.28336 | < 0.0001 | significant |
| A-Polymer | 123.3414 | 1 | 123.3414 | 11.96138 | 0.0061 | |
| B-SS | 902.69 | 1 | 902.69 | 87.54091 | < 0.0001 | |
| C-SA | 1.97136 | 1 | 1.97136 | 0.191178 | 0.6712 | |
| AB | 55.3352 | 1 | 55.3352 | 5.366287 | 0.0430 | |
| AC | 63.6192 | 1 | 63.6192 | 6.169651 | 0.0323 | |
| BC | 6.3368 | 1 | 6.3368 | 0.614529 | 0.4513 | |
| A^2 | 2.343728 | 1 | 2.343728 | 0.22729 | 0.6438 | |
| B^2 | 35.08418 | 1 | 35.08418 | 3.402388 | 0.0949 | |
| C^2 | 192.3427 | 1 | 192.3427 | 18.65298 | 0.0015 | |
| Residual | 103.1164 | 10 | 10.31164 | | | |
| Lack of Fit | 4.031678 | 5 | 0.806336 | 0.040689 | 0.9984 | not significant |
| Pure Error | 99.08468 | 5 | 19.81694 | | | |
| Cor Total | 1521.484 | 19 | | | | |

| | | | | |
|---|---|---|---|---|
| Std. Dev. | 3.211174 | | R-Squared | 0.932226 |
| Mean | 75.261 | | Adj R-Squared | 0.87123 |
| C.V. % | 4.266717 | | Pred R-Squared | 0.899113 |
| PRESS | 153.498 | | Adeq Precision | 13.89931 |

P<0.05 considered as significant

**Table 4.7** ANOVA results for the dependent response variable (Particle size) ($R_2$)

| Source | Sum of Squares | d.f. | Mean Square | F-Value | P-Value | |
|---|---|---|---|---|---|---|
| Model | 40152.96 | 9 | 4461.44 | 4.518375 | 0.0137 | significant |
| A-Polymer | 178.9713 | 1 | 178.9713 | 0.181255 | 0.6793 | |
| B-SS | 14273.66 | 1 | 14273.66 | 14.45582 | 0.0035 | |
| C-SA | 6887.213 | 1 | 6887.213 | 6.975105 | 0.0247 | |
| AB | 67.89038 | 1 | 67.89038 | 0.068757 | 0.7985 | |
| AC | 84.5325 | 1 | 84.5325 | 0.085611 | 0.7758 | |
| BC | 5113.386 | 1 | 5113.386 | 5.178641 | 0.0461 | |
| A^2 | 861.7024 | 1 | 861.7024 | 0.872699 | 0.3722 | |
| B^2 | 1351.455 | 1 | 1351.455 | 1.368702 | 0.2692 | |
| C^2 | 5852.801 | 1 | 5852.801 | 5.927493 | 0.0352 | |
| Residual | 9873.992 | 10 | 987.3992 | | | |
| Lack of Fit | 1459.855 | 5 | 291.9711 | 0.1735 | 0.9614 | not significant |
| Pure Error | 8414.137 | 5 | 1682.827 | | | |

| Cor Total | 50026.95 | 19 | | | | |
|---|---|---|---|---|---|---|
| Std. Dev. | 31.42291 | | R-Squared | 0.802627 | | |
| Mean | 213.3278 | | Adj R-Squared | 0.62499 | | |
| C.V. % | 14.72987 | | Pred R-Squared | 0.596228 | | |
| PRESS | 20199.5 | | Adeq Precision | 6.317713 | | |

P<0.05 considered as significant

In the Table 4.6 and 4.7 response variables 'Yield' ($R_1$) and 'Particle size' ($R_2$) suggested a quadratic relationship in which some of the terms are significant. Other cubic model is aliased. The regression equation best represents the responses after eliminating non-significant terms. The ANOVA result of $R_1$ showed the main effects (A- Polymer, B- Stirring speed), interaction effects (AB, AC) and quadratic effects ($C^2$) as significant. The ANOVA result of $R_2$ showed the main effects (B, C), interaction effect (BC) and quadratic effects ($C^2$)as significant.It showed other statistics measured $R^2$, Adjusted $R^2$, Predicted $R^2$, Standard Deviation, Mean and Adequate precision. Adjusted and Predicted $R^2$ were reasonable agreement with value less than 1. The desired lack of fit of two responses was not significant.

**Table 4.8** ANOVA results for the dependent response variable (% Entrapment efficiency) ($R_3$)

| Source | Sum of Squares | d.f. | Mean Square | F-Value | P-Value | |
|---|---|---|---|---|---|---|
| Model | 1812.794 | 9 | 201.4215 | 11.61973 | 0.0003 | significant |
| A-Polymer | 0.97969 | 1 | 0.97969 | 0.056517 | 0.8169 | |
| B-SS | 243.9372 | 1 | 243.9372 | 14.0724 | 0.0038 | |
| C-SA | 423.5406 | 1 | 423.5406 | 24.43347 | 0.0006 | |
| AB | 19.31311 | 1 | 19.31311 | 1.114146 | 0.3160 | |
| AC | 11.25751 | 1 | 11.25751 | 0.64943 | 0.4391 | |
| BC | 109.7421 | 1 | 109.7421 | 6.330869 | 0.0306 | |
| A^2 | 293.8887 | 1 | 293.8887 | 16.95403 | 0.0021 | |
| B^2 | 8.637614 | 1 | 8.637614 | 0.498292 | 0.4964 | |
| C^2 | 788.8958 | 1 | 788.8958 | 45.51029 | < 0.0001 | |
| Residual | 173.3445 | 10 | 17.33445 | | | |
| Lack of Fit | 27.25394 | 5 | 5.450789 | 0.186555 | 0.9554 | not significant |
| Pure Error | 146.0905 | 5 | 29.21811 | | | |
| Cor Total | 1986.138 | 19 | | | | |
| Std. Dev. | 4.163466 | | R-Squared | 0.912723 | | |
| Mean | 68.195 | | Adj R-Squared | 0.834173 | | |

| | | | | |
|---|---|---|---|---|
| C.V. % | 6.105236 | | Pred R-Squared | 0.800629 |
| PRESS | 395.9783 | | Adeq Precision | 11.58146 |

P<0.05 considered as significant

**Table 4.9** ANOVA results for the dependent response variables (Rel. 8hr.) ($R_4$)

| Source | Sum of Squares | d.f. | Mean Square | F-Value | P-Value | |
|---|---|---|---|---|---|---|
| Model | 2112.984 | 9 | 234.776 | 14.19104 | 0.0001 | significant |
| A-Polymer | 871.7957 | 1 | 871.7957 | 52.69571 | < 0.0001 | |
| B-SS | 183.3552 | 1 | 183.3552 | 11.08291 | 0.0076 | |
| C-SA | 10.94116 | 1 | 10.94116 | 0.661339 | 0.4350 | |
| AB | 21.2552 | 1 | 21.2552 | 1.284771 | 0.2835 | |
| AC | 82.30445 | 1 | 82.30445 | 4.974895 | 0.0498 | |
| BC | 43.71125 | 1 | 43.71125 | 2.642128 | 0.1351 | |
| A^2 | 778.1773 | 1 | 778.1773 | 47.03695 | < 0.0001 | |
| B^2 | 35.30778 | 1 | 35.30778 | 2.13418 | 0.1747 | |
| C^2 | 420.3327 | 1 | 420.3327 | 25.40702 | 0.0005 | |
| Residual | 165.4396 | 10 | 16.54396 | | | |
| Lack of Fit | 36.5011 | 5 | 7.30022 | 0.283089 | 0.9038 | not significant |
| Pure Error | 128.9385 | 5 | 25.7877 | | | |
| Cor Total | 2278.424 | 19 | | | | |

| | | | | |
|---|---|---|---|---|
| Std. Dev. | 4.067426 | | R-Squared | 0.927389 |
| Mean | 65.086 | | Adj R-Squared | 0.862038 |
| C.V. % | 6.249311 | | Pred R-Squared | 0.754292 |
| PRESS | 559.826 | | Adeq Precision | 14.52359 |

P<0.05 considered as significant

In the Table 4.8 and 4.9 response variables 'Entrapment efficiency' ($R_3$) and 'Rel. at 8hr.' ($R_4$) suggested a quadratic relationship in which some of the additional terms are significant. Other cubic model is aliased. The regression equation best represents the responses after eliminating non-significant terms. The ANOVA result of $R_3$ showed the main effects (B- Stirring speed, C-Surface active agent), interaction effects (BC) and quadratic effects ($A^2$, $C^2$) as significant. The ANOVA result of $R_4$ showed the main effects (A, B), interaction effect (AC) and quadratic effects ($A^2$, $C^2$) as significant. It showed other statistics measured $R^2$, Adjusted $R^2$, Predicted $R^2$, Standard Deviation, Mean and Adequate precision. Adjusted and Predicted $R^2$ were reasonable agreement with value less than 1. The desired lack of fit of two responses was not significant.

Model equations obtained from ANOVA results after removing insignificant terms:-

**For %Yield ($R_1$)**

Final Equation in Terms of Coded Factors:

Yield =78.12-3.51*A-9.50*B+2.63*A*B+2.82*A*C-8.36* $C^2$

Final Equation in Terms of Actual Factors:

Yield =224.04227-0.088346*Polymer-0.24498*SS+6.57500E-005*Polymer*SS+0.018800*Polymer*SA-14.86788*$SA^2$

**For Particle size ($R_2$)**

Final Equation in Terms of Coded Factors:

Particle size =188.03-37.78*B-26.24*C-25.28*B*C+46.13*$C^2$

Final Equation in Terms of Actual Factors:

Particle size =869.96690-1.11577*SS-80.15121*SA-0.16855*SS*SA+82.01495*$SA^2$

**For Entrapment efficiency ($R_3$)**

Final Equation in Terms of Coded Factors:

EE =64.01-4.94*B+6.51*C+3.70*B*C-10.34*$A^2$+16.94*$C^2$

Final Equation in Terms of Actual Factors:

EE=138.60823-0.12864*SS-88.12777*SA+0.024692*SS*SA-2.58443E-004*$Polymer^2$+30.11071*$SA^2$

**For Rel. at 8Hr. ($R_4$)**

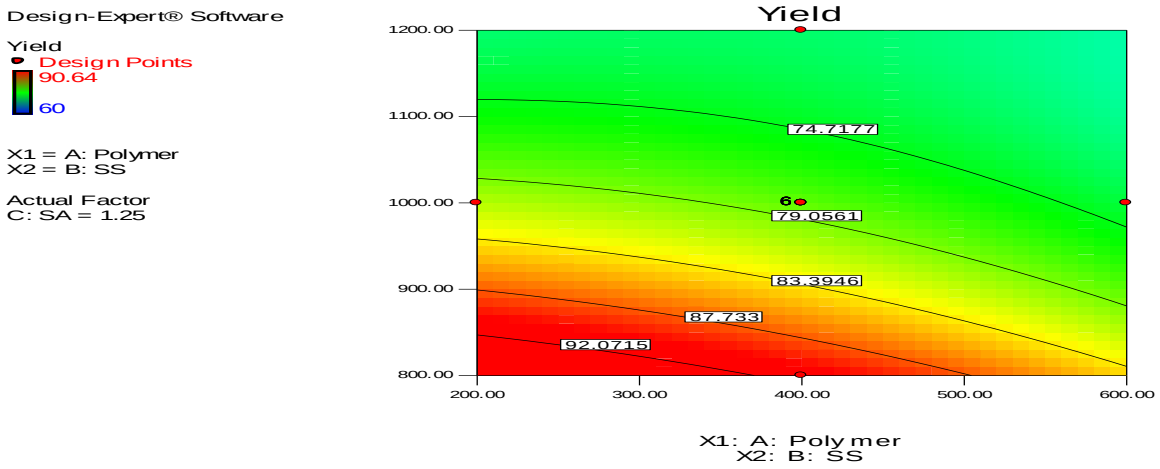
Final Equation in Terms of Coded Factors:

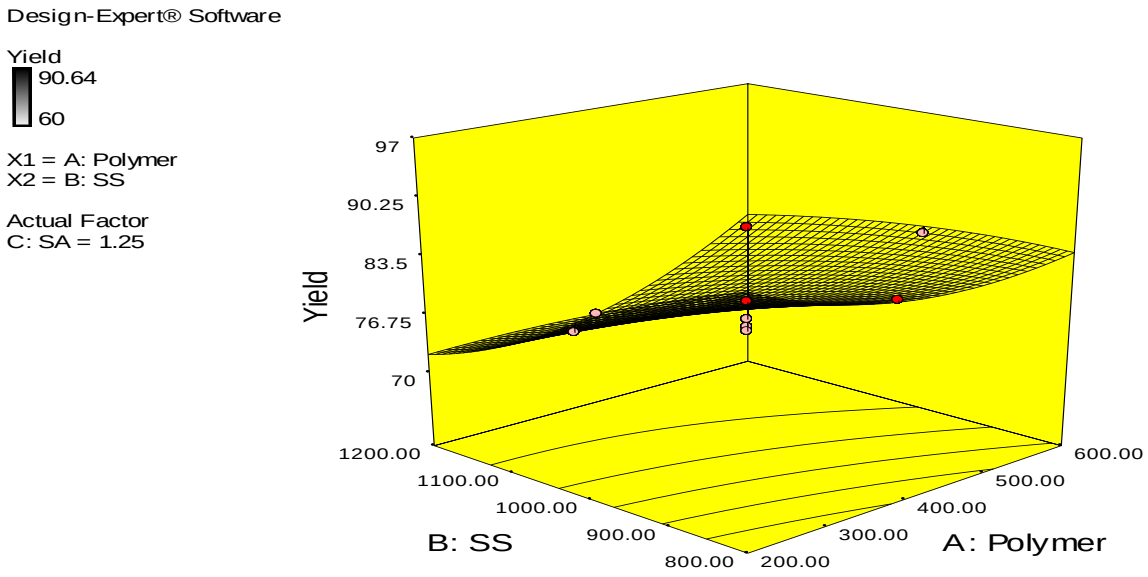
$Rel_{8hr}$ =64.65+9.34*A+4.28*B-3.21*A*C+16.82*$A^2$-12.36*$C^2$

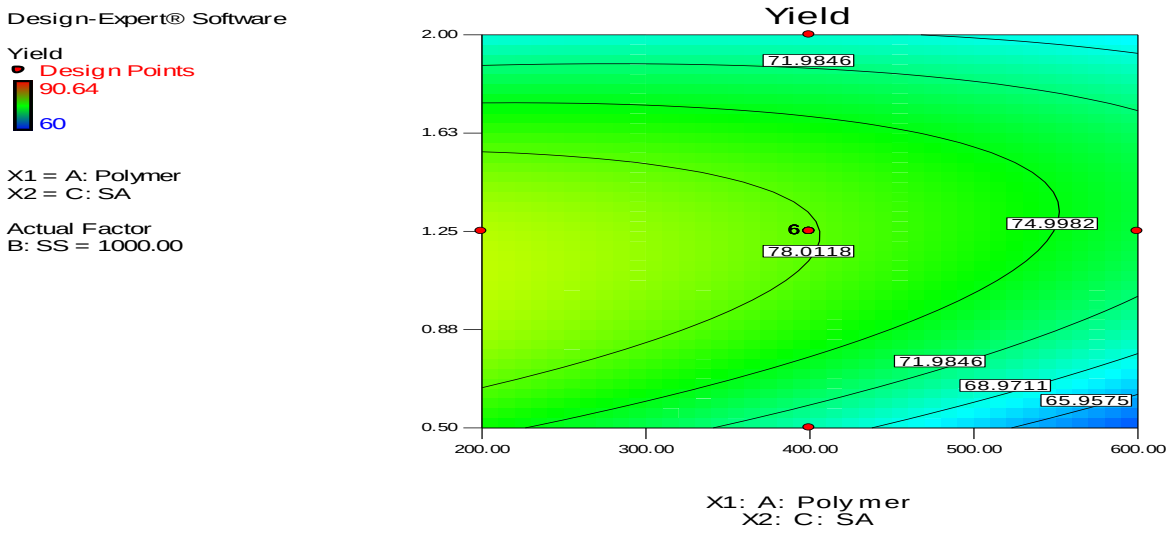
Final Equation in Terms of Actual Factors:

Rel 8hr=-44.19767-0.30377*Polymer+0.20375*SS-0.021383*Polymer*SA+4.20545E-004*$Polymer^2$-21.97899*$SA^2$

## 4.3.10 Contour and Response Surface plot analysis





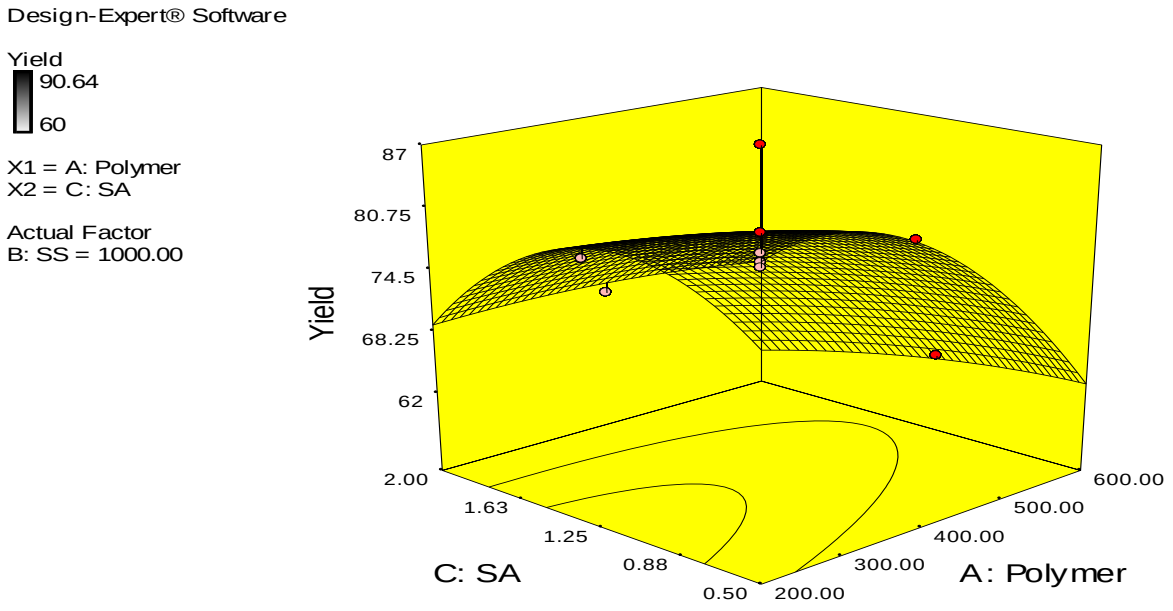




**Fig. 4.15. Contour and Response surface plots showing the effect of polymer ratio, Stirring speed and Surface active agent on Yield (%).**

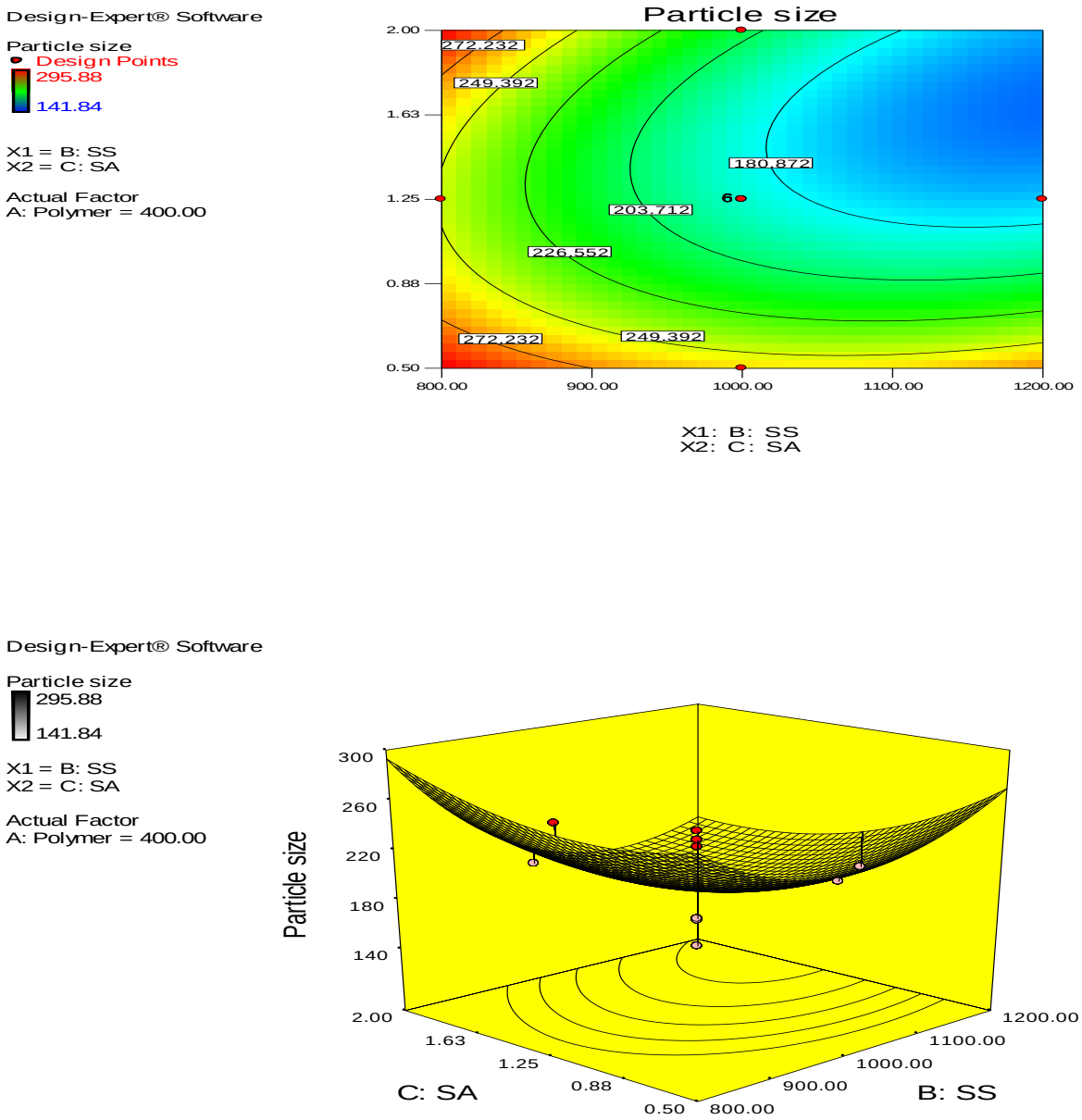
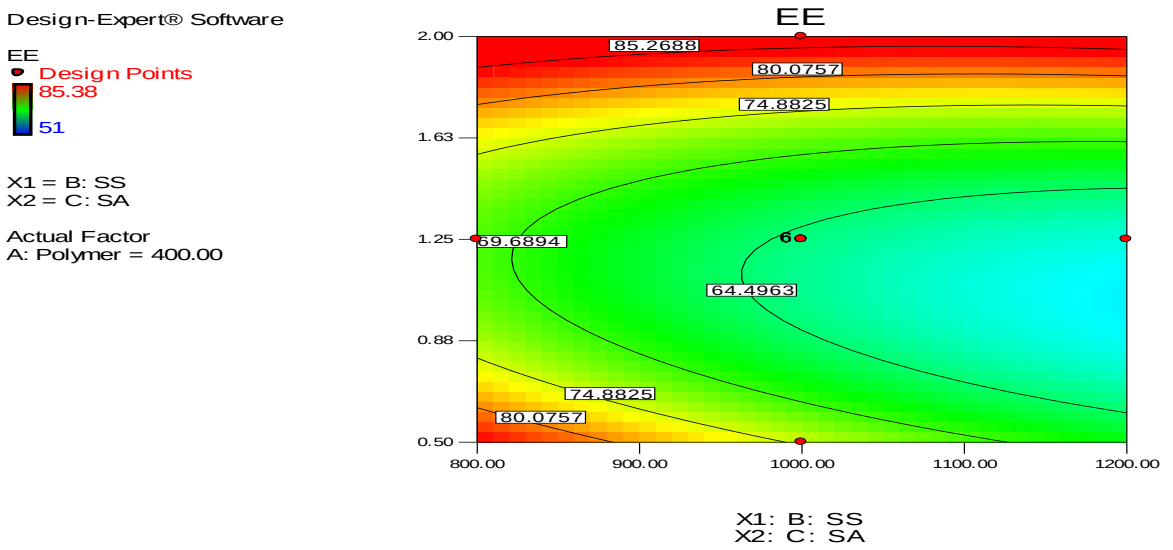



**Fig.4.16. Contour and Response surface plots showing the effect of polymer, stirring rate and surface active agent on Particle size (µm).**



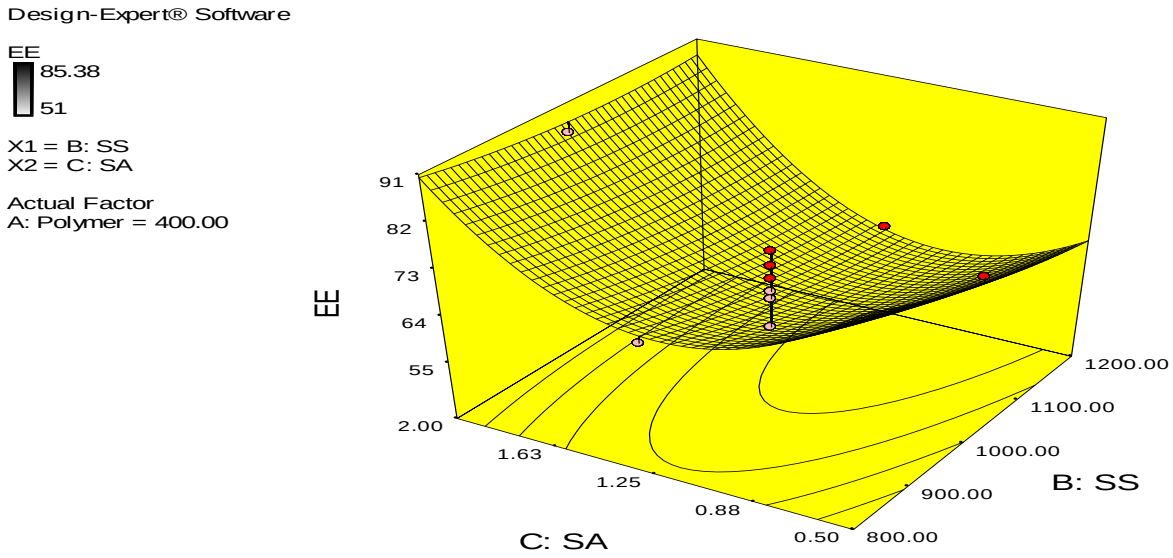
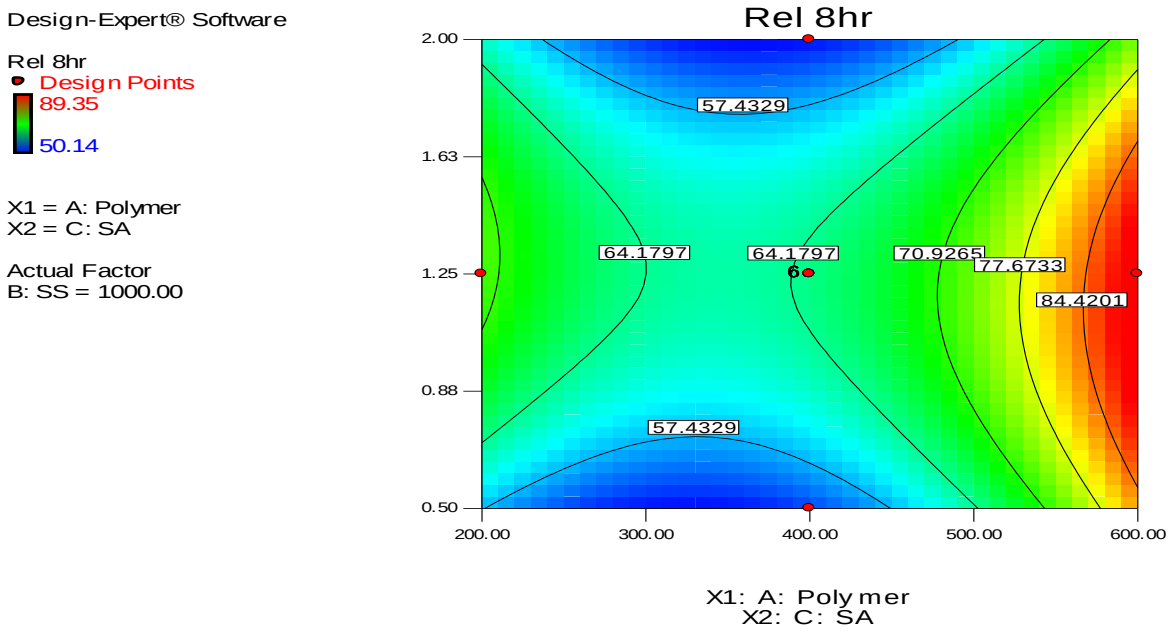




**Fig.4.17. Contour and Response surface plots showing the effect of polymer, stirring rate and surface active agent on Entrapment efficiency (%).**



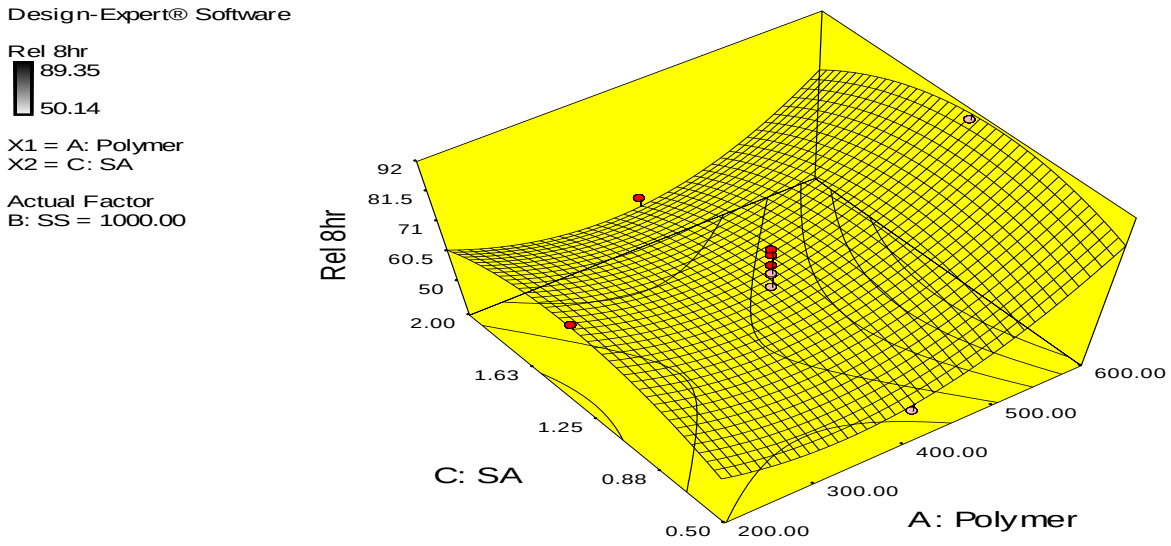




**Fig.4.18. Contour and Response surface plots showing the effect of polymer, stirring rate and surface active agent on Rel. 8hr. (%).**

The contour and response surface plots were generated by Design Expert software (version 7) which helps to show the effects of process parameters on the response variable. Percentage yield was increased with decreasing level of polymer ($X_1$) and decreased at higher level of stirring speed ($X_2$). Average particle size was decreased with increasing level of stirring speed ($X_1$) and decreased most at the mid level of Surface active agent ($X_2$).

Similarly, Entrapment efficiency was increased due to increasing level of Surface active agent (X2) at a fixed level of stirring speed ($X_1$); andat higher level of Surface active agent ($X_2$), EE is not dependent much on stirring rate. Drug release at 8 hr was increased with increasing level of polymer ($X_1$) from mid to high level .$Release_{8\ hr}$ was increased from low to mid level and then decreased from mid to high level of Surface active agent ($X_2$).It is highest at the centre level of surface active agents concentration.

### 4.3.11. Validation of the developed models

Response surface- Central composite design was validated by choosing randomly three check point formulations (CPF) prepared with same conditions within experimental ranges as suggested by solution given by software. Compositions of the CPFs are shown in Table 4.10. For each response, the average of three experimental reading was taken and this value was compared with that of predicted value as obtained from statistically obtained correlation (Table 4.10). Low percentage error (<5%) between the experimental and predicted values indicated that the developed models are adequate and predicted results are in good agreement. The drug release profiles for CPFs are depicted in Table 4.11, 4.12, 4.13 and Fig. 4.19. Predicted and actual values of all responses are also showed.

**Table 4.10 Validation test Results**

| Formulation | Experimental composition | | | Response Variables | Exp. value | Pred. value | Percentage Error |
|---|---|---|---|---|---|---|---|
| | Polymer (mg) | SS (rpm) | SA (%w/v) | | | | |
| | | | | Yield (%) | 64.31 | 63.50 | -1.25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CPF 1 | 597.97 | 1199.97 | 2 | Part. size(µm) | 150.22 | 151.5 | 0.85 |
| | | | | EE (%) | 74.86 | 75.45 | 0.78 |
| | | | | $Rel_{8\ hr}$ (%) | 72.29 | 73.76 | 2.03 |
| CPF 2 | 200 | 1137.93 | 1.9 | Yield (%) | 63.12 | 64.48 | 2.15 |
| | | | | Part. size(µm) | 151.4 | 150.8 | -0.39 |
| | | | | EE (%) | 74.01 | 73.27 | -0.99 |
| | | | | $Rel_{8\ hr}$(%) | 64.99 | 63.55 | -2.21 |
| CPF 3 | 200 | 1136.27 | 1.8 | Yield (%) | 63.72 | 64.63 | 1.42 |
| | | | | Part. size(µm) | 152.57 | 150.97 | -1.04 |
| | | | | EE (%) | 72.81 | 73.08 | 0.37 |
| | | | | $Rel_{8\ hr}$(%) | 62.14 | 63.67 | 2.46 |

**Table 4.11 Drug release of CPF 1 (dissolution medium-PBS at pH 7.4)**

| time(h) | OD±SD | conc. (µg/ml) | amt/µg in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.029667±0.0015 | 0.067708 | 3.385417 | 0.338542 | 3.385417 | 0.540888 |
| 1 | 0.050333±0.002 | 0.229167 | 11.45833 | 1.145833 | 11.79688 | 1.884786 |
| 2 | 0.146±0.0026 | 0.976563 | 48.82813 | 4.882813 | 50.3125 | 8.038425 |
| 3 | 0.335667±0.003 | 2.458333 | 122.9167 | 12.29167 | 129.2839 | 20.65567 |
| 4 | 0.494667±0.0031 | 3.700521 | 185.026 | 18.5026 | 203.6849 | 32.54272 |
| 5 | 0.619667±0.0021 | 4.677083 | 233.8542 | 23.38542 | 271.0156 | 43.30015 |
| 6 | 0.764667±0.001 | 5.809896 | 290.4948 | 29.04948 | 351.0417 | 56.0859 |
| 7 | 0.827667±0.002 | 6.302083 | 315.1042 | 31.51042 | 404.7005 | 64.65897 |
| 8 | 0.869333±0.0017 | 6.627604 | 331.3802 | 33.13802 | 452.487 | 72.29381 |

**Table 4.12 Drug release of CPF 2 (dissolution medium-PBS at pH 7.4)**

| time(h) | OD±SD | conc. (µg/ml) | amt/µg in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.154333 | 1.041667 | 52.08333 | 5.208333 | 52.08333 | 2.815315 |
| 1 | 0.350333 | 2.572917 | 128.6458 | 12.86458 | 133.8542 | 7.23536 |
| 2 | 0.496 | 3.710938 | 185.5469 | 18.55469 | 203.6198 | 11.00648 |
| 3 | 0.836 | 6.367188 | 318.3594 | 31.83594 | 354.987 | 19.18849 |
| 4 | 1.196333 | 9.182292 | 459.1146 | 45.91146 | 527.5781 | 28.51774 |
| 5 | 1.523 | 11.73438 | 586.7188 | 58.67188 | 701.0938 | 37.89696 |
| 6 | 1.864667 | 14.40365 | 720.1823 | 72.01823 | 893.2292 | 48.28266 |
| 7 | 2.027667 | 15.67708 | 783.8542 | 78.38542 | 1028.919 | 55.61726 |
| 8 | 2.271 | 17.57813 | 878.9063 | 87.89063 | 1202.357 | 64.99226 |

**Table 4.13 Drug release CPF 3 (dissolution medium-PBS at pH 7.4)**

| time(h) | OD±SD | conc. (µg/ml) | amt/µg in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.229667±0.0015 | 1.630208 | 81.51042 | 8.151042 | 81.51042 | 4.461435 |
| 1 | 0.450333±0.0017 | 3.354167 | 167.7083 | 16.77083 | 175.8594 | 9.625582 |
| 2 | 0.696±0.002 | 5.273438 | 263.6719 | 26.36719 | 288.5938 | 15.79605 |
| 3 | 0.835333±0.0023 | 6.361979 | 318.099 | 31.8099 | 369.388 | 20.21828 |
| 4 | 1.096±0.0026 | 8.398438 | 419.9219 | 41.99219 | 503.0208 | 27.53261 |
| 5 | 1.322667±0.003 | 10.16927 | 508.4635 | 50.84635 | 633.5547 | 34.67732 |
| 6 | 1.565667±0.0017 | 12.06771 | 603.3854 | 60.33854 | 779.3229 | 42.65588 |
| 7 | 1.825±0.0015 | 14.09375 | 704.6875 | 70.46875 | 940.9635 | 51.5032 |
| 8 | 2.142333±0.0021 | 16.57292 | 828.6458 | 82.86458 | 1135.391 | 62.14508 |

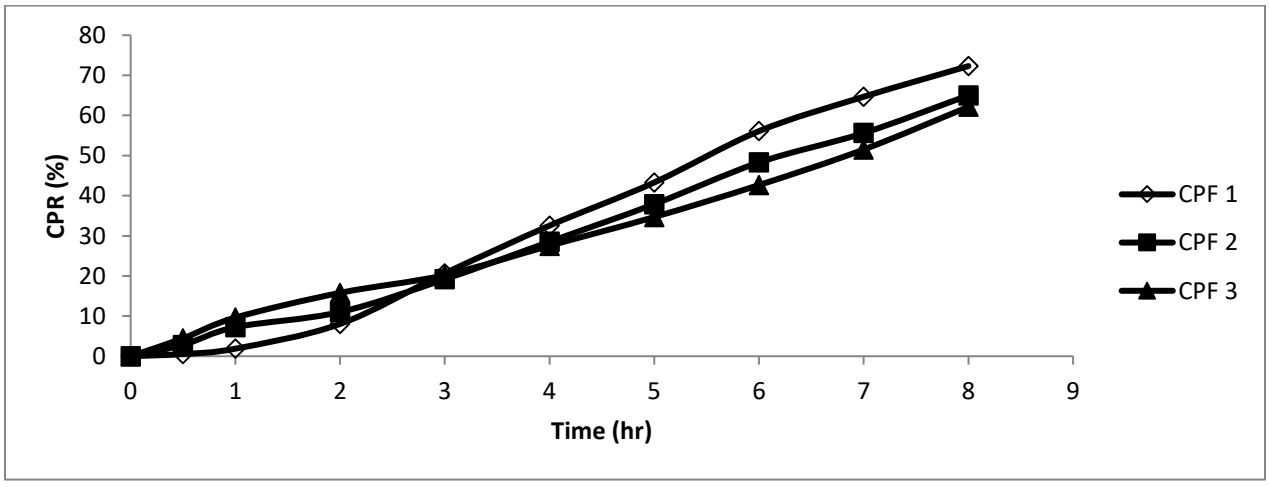


**Fig. 4.19 Release graphs of CPF formulations**

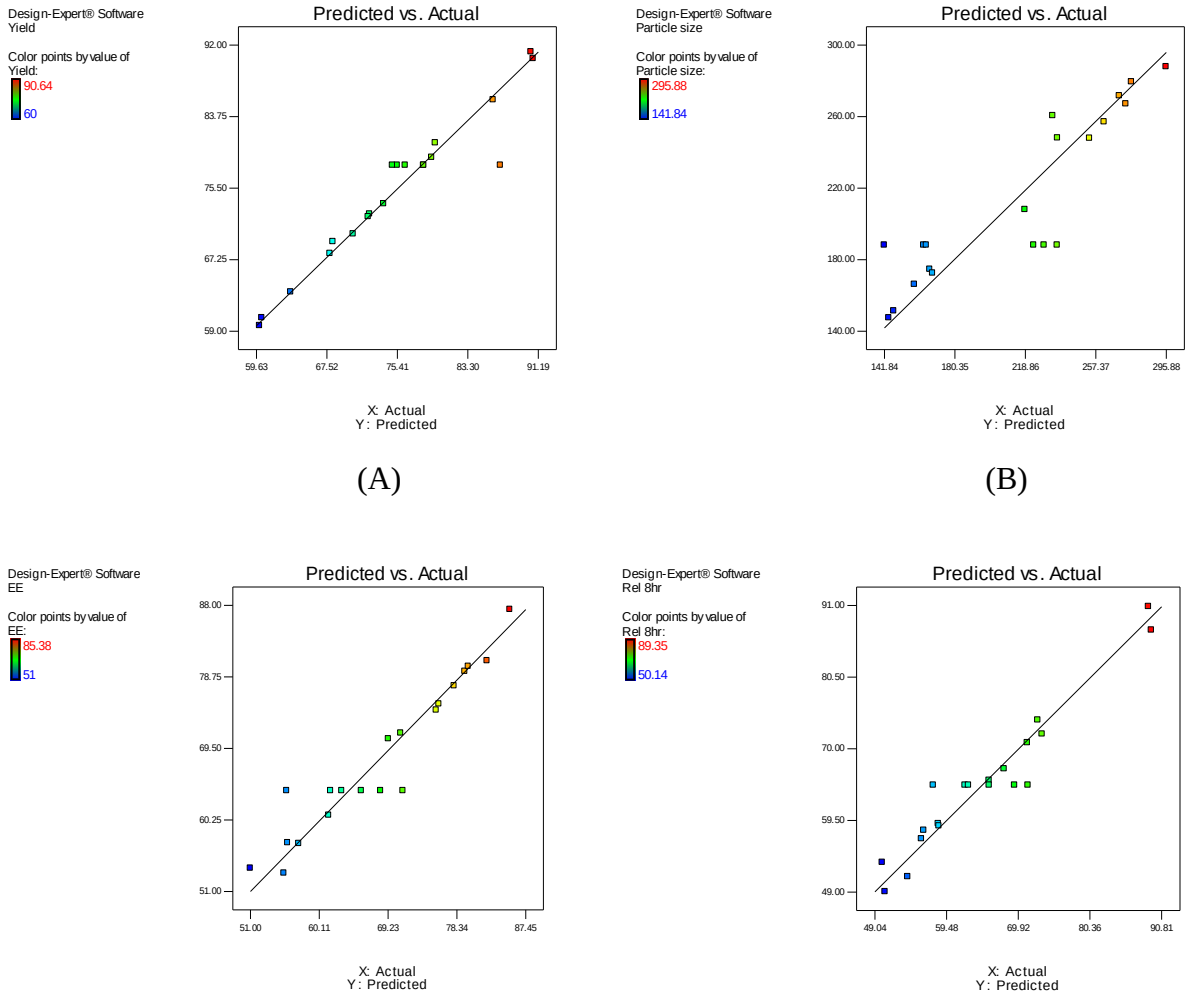



**Fig. 4.20 Plots of actual vs. predicted responses of (A) Yield, (B) Particle size, (C) Entrapment efficiency and (D) Rel. at 8hr.**

### 4.3.12. Optimization by desirability function

Optimization of a process was performed with the help of desirability function from the solutions given by software (Design Expert, Version 7). Four responses were optimized simultaneously by numerical optimization. A high value of desirability coefficient ($0<y<1$) indicates that the operating point can produce acceptable formulation. The responses: Yield ($R_1$), Particle size ($R_2$), Entrapment efficiency ($R_3$) and $Rel_{8hr}$ ($R_4$) were transformed into desirability function.

Among them $R_1$ had to be in range (60-90.64), $R_2$ minimized because it is better for topical delivery, $R_3$ maximized and $R_4$ maximized. The maximum value of desirability coefficient δ= 0.7402 was obtained at the chosen constraints, polymer amount of 600 mg, stirring speed of 1196.79 rpm and surfactant concentration of 2% (w/v) (**Table 4.14**). The results were comparing the experimentally obtained and model predicted values of all four responses in **Table 4.14**. Experimental values are very close to the predicted values which suggested that the optimized formulation was reasonable and reliable.

**Table 4.14 Criteria for numerical optimization**

| Parameter | Goal | Lower limit | Upper limit | Lower Wt. | Upper Wt. | Importance |
|---|---|---|---|---|---|---|
| A1- Polymer (mg) | In range | 200 | 600 | 1 | 1 | 3 |
| A2- SS | In range | 800 | 1200 | 1 | 1 | 3 |
| A3- SA | In range | 0.5 | 2 | 1 | 1 | 3 |
| $R_1$- Yield | In range | 60 | 90.64 | 1 | 1 | 3 |
| $R_2$- Particle Size | Minimized | 141.84 | 291.88 | 1 | 1 | 3 |
| $R_3$- EE | Maximized | 51 | 85.38 | 1 | 1 | 3 |
| $R_4$- Rel. 8h. | maximized | 50.14 | 89.35 | 1 | 1 | 3 |

| A1- Polymer (mg) | A2- SS (rpm) | A3- SA (%w/v) | Responses | Experimental value | Predicted value | %Error | Desirability (δ) |
|---|---|---|---|---|---|---|---|
| 600 | 1197.54 | 2 | Yield (%) | 64.17 | 63.51 | -1.02 | 0.7402 |
| | | | Particle size (µm) | 152.63 | 151.45 | -0.66 | |
| | | | EE (%) | 75.05 | 75.21 | 0.21 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | $Rel_{8hr}$ (%) | 75.75 | 74.07 | -1.34 | |

**Optimized batch formulation**

### 4.3.13. Scale up for large batch production of 5-FU microsponge gel

Reproducibility of a method can be checked for higher product size by scale up technique. To convert this formulation prepared in laboratory scale into higher scale production, geometric similarities were maintained as much as possible in consideration with power law approach. Stirring speed had been maintained in higher scales. Specifications maintained in scale up were displayed in **Table 4.15**. System geometry of scale up were beakers diameter (6.8, 9.6 and 13.4 cm), impellers diameter (3.7, 5.2 and 7.3 cm) and clearance (1.13, 1.586 and 2.226 cm). Volumes of emulsions were taken as per scale up. Shape factor should be same on each batch. Batch produced at each scale showed similar characteristics as that of optimized batch such as yield, particle size, entrapment efficiency and $Rel_{8hr}$ **Table 4.16**.

**Table 4.15** Specifications maintained in scale up process

| Parameters | Optimized | Batch- 1 | Batch- 2 |
|---|---|---|---|
| 5-FU (mg) | 50 | 100 | 400 |
| Polymer (mg) | 600 | 1200 | 4800 |
| Volume of dispersion phase (ml) | 15 | 30 | 120 |
| Volume of continuous phase (ml) | 100 | 200 | 800 |
| Impeller dia.($D_a$-cm) | 3.7 | 5.2 | 7.3 |
| Beaker diameter (T-cm) | 6.8 | 9.6 | 13.4 |
| $S_1$ ($D_a$/T) | 0.544 | 0.542 | 0.544 |
| Impellorclearance from bottom (E-cm) | 1.13 | 1.586 | 2.226 |
| $S_2$ (E/ $D_a$) | 0.305 | 0.305 | 0.305 |
| Stirring rate ($sec^{-1}$),N | 20 $sec^{-1}$ | 20 $sec^{-1}$ | 20 $sec^{-1}$ |
| Density of dispersion phase ρ (gm/ml) | 1.06 | 1.06 | 1.06 |
| Viscosity of dispersion phase η(cp) | 1.55 | 1.55 | 1.55 |
| Reynolds no. ($N_{re}=ND_a^2\rho/\eta$) | 187.24 | 369.83 | 728.86 |

**Table 4.16** Characterizations of batches and optimized 5-FU microsponges

| Scale up | Yield (%) | Particle size(µm) | EE (%) | Rel. 8hr. (%) |
|---|---|---|---|---|
| Optimized | 64.17 | 152.63 | 75.05 | 75.75 |
| Batch 1 | 64.03 | 151.69 | 75.24 | 74.58 |
| Batch 2 | 63.91 | 152.18 | 74.87 | 76.08 |

### 4.3.14. *In vitro* study of optimized and scaled up batches

*In vitro* study of drug diffusion is an important step to assure its release from microsponges. The data and profiles of 5-FU release in PBS at pH 7.4 from the optimized and scaled up batches were shown in Table 4.17, 4.18, 4.19 and Fig. 4.21. According to skin pH drug release of optimized and scaled up batches observed in PBS at pH 5.5; which were shown in Table 4.20, 4.21, 4.22 and Fig 4.22. Therefore, release pattern according to these profiles were observed identical.

**Table 4.17** Cumulative release of optimized formulation

(Polymer- 600 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 625 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.049667±0.0015 | 0.223958 | 11.19792 | 1.119792 | 11.19792 | 1.791667 |
| 1 | 0.150333±0.0025 | 1.010417 | 50.52083 | 5.052083 | 51.64063 | 8.2625 |
| 2 | 0.246±0.001 | 1.757813 | 87.89063 | 8.789063 | 94.0625 | 15.05 |
| 3 | 0.385333±0.0023 | 2.846354 | 142.3177 | 14.23177 | 157.2786 | 25.16458 |
| 4 | 0.504667±0.0017 | 3.778646 | 188.9323 | 18.89323 | 218.125 | 34.9 |
| 5 | 0.649667±0.002 | 4.911458 | 245.5729 | 24.55729 | 293.6589 | 46.98542 |
| 6 | 0.764667±0.001 | 5.809896 | 290.4948 | 29.04948 | 363.138 | 58.10208 |
| 7 | 0.827667±0.0015 | 6.302083 | 315.1042 | 31.51042 | 416.7969 | 66.6875 |
| 8 | 0.892±0.0013 | 6.804688 | 340.2344 | 34.02344 | 473.4375 | 75.75 |

**Table 4.18** Cumulative release of Batch 1 formulation

(Polymer- 1200 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 626 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.039±0.002 | 0.140625 | 7.03125 | 0.703125 | 7.03125 | 1.123203 |
| 1 | 0.172±0.0015 | 1.179688 | 58.98438 | 5.898438 | 59.6875 | 9.534744 |
| 2 | 0.265333±0.0025 | 1.908854 | 95.44271 | 9.544271 | 102.0443 | 16.301 |
| 3 | 0.395333±0.0023 | 2.924479 | 146.224 | 14.6224 | 162.3698 | 25.93767 |
| 4 | 0.504667±0.0027 | 3.778646 | 188.9323 | 18.89323 | 219.7005 | 35.09593 |
| 5 | 0.641333±0.0013 | 4.846354 | 242.3177 | 24.23177 | 291.9792 | 46.64204 |
| 6 | 0.764667±0.001 | 5.809896 | 290.4948 | 29.04948 | 364.388 | 58.20895 |
| 7 | 0.827667±0.001 | 6.302083 | 315.1042 | 31.51042 | 418.0469 | 66.78065 |
| 8 | 0.872±0.0015 | 6.648438 | 332.4219 | 33.24219 | 466.875 | 74.58067 |

**Table 4.19** Cumulative release of Batch 2 formulation

(Polymer- 4800 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 623 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.060333±0.0025 | 0.307292 | 15.36458 | 1.536458 | 15.36458 | 2.466225 |
| 1 | 0.150333±0.0015 | 1.010417 | 50.52083 | 5.052083 | 52.05729 | 8.355906 |
| 2 | 0.262±0.0026 | 1.882813 | 94.14063 | 9.414063 | 100.7292 | 16.16841 |
| 3 | 0.385333±0.002 | 2.846354 | 142.3177 | 14.23177 | 158.3203 | 25.41257 |
| 4 | 0.513±0.0027 | 3.84375 | 192.1875 | 19.21875 | 222.4219 | 35.70175 |
| 5 | 0.662667±0.0013 | 5.013021 | 250.651 | 25.0651 | 300.1042 | 48.17081 |
| 6 | 0.742333±0.002 | 5.635417 | 281.7708 | 28.17708 | 356.2891 | 57.18926 |
| 7 | 0.817333±0.0017 | 6.221354 | 311.0677 | 31.10677 | 413.763 | 66.41461 |
| 8 | 0.892±0.001 | 6.804688 | 340.2344 | 34.02344 | 474.0365 | 76.08932 |

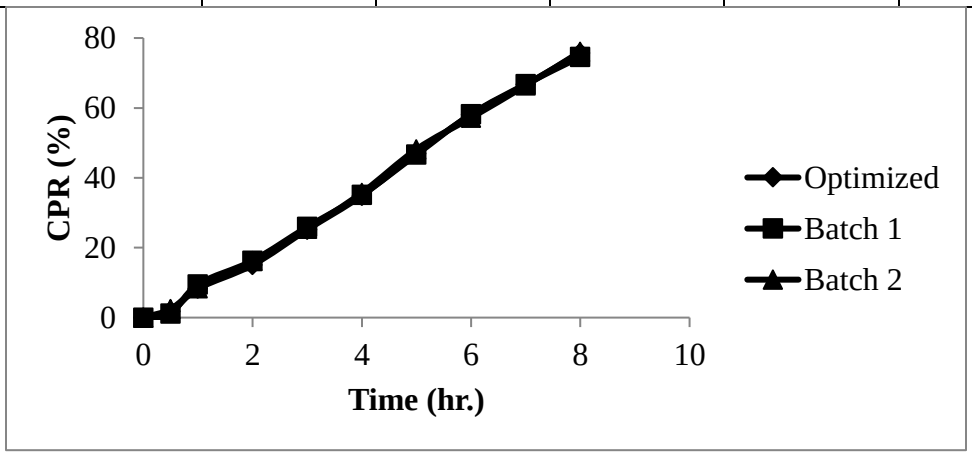



**Fig. 4.21 Release graph of optimized and batch formulations**

**Table 4.20** Cumulative release of optimized formulation (PBS 5.5)

(Polymer- 600 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 625 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.018±0.002 | 0.13636364 | 6.818181818 | 0.681818182 | 6.818181818 | 1.090909 |
| 1 | 0.064667±0.003 | 0.84343434 | 42.17171717 | 4.217171717 | 42.85353535 | 6.856566 |
| 2 | 0.135333±0.0021 | 1.91414141 | 95.70707071 | 9.570707071 | 100.6060606 | 16.09697 |
| 3 | 0.202667±0.0025 | 2.93434343 | 146.7171717 | 14.67171717 | 161.1868687 | 25.7899 |
| 4 | 0.255667±0.0013 | 3.73737374 | 186.8686869 | 18.68686869 | 216.010101 | 34.56162 |
| 5 | 0.311667±0.001 | 4.58585859 | 229.2929293 | 22.92929293 | 277.1212121 | 44.33939 |
| 6 | 0.363667±0.0025 | 5.37373737 | 268.6868687 | 26.86868687 | 339.4444444 | 54.31111 |
| 7 | 0.423333±0.0015 | 6.27777778 | 313.8888889 | 31.38888889 | 411.5151515 | 65.84242 |
| 8 | 0.481667±0.002 | 7.16161616 | 358.0808081 | 35.80808081 | 487.0959596 | 77.93535 |

**Table 4.21** Cumulative release of Batch 1 formulation (PBS 5.5)

(Polymer- 1200 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 626 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.020333±0.0005 | 0.171717 | 8.585859 | 0.858586 | 8.585859 | 1.371543 |
| 1 | 0.070333±0.001 | 0.929293 | 46.46465 | 4.646465 | 47.32323 | 7.559622 |
| 2 | 0.125±0.0013 | 1.757576 | 87.87879 | 8.787879 | 93.38384 | 14.91755 |
| 3 | 0.202667±0.0017 | 2.934343 | 146.7172 | 14.67172 | 161.0101 | 25.72046 |
| 4 | 0.244333±0.0015 | 3.565657 | 178.2828 | 17.82828 | 207.2475 | 33.10663 |
| 5 | 0.311667±0.002 | 4.585859 | 229.2929 | 22.92929 | 276.0859 | 44.10317 |
| 6 | 0.374333±0.002 | 5.535354 | 276.7677 | 27.67677 | 346.4899 | 55.34982 |
| 7 | 0.433±0.0017 | 6.424242 | 321.2121 | 32.12121 | 418.6111 | 66.87078 |
| 8 | 0.494333±0.0023 | 7.353535 | 367.6768 | 36.76768 | 497.197 | 79.42444 |

**Table 4.22** Cumulative release of Batch 2 formulation (PBS 5.5)

(Polymer- 4800 mg, SS- 1197.54 rpm, SA- 2% and Drug loaded- 623 µg)

| time(h) | OD±SD | conc. (µg/ml) | Amt(µg) in donor cell | Sample in µg | Actual amt in µg | CPR (%) |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 0.017333±0.003 | 0.126263 | 6.313131 | 0.631313 | 6.313131 | 1.013344 |
| 1 | 0.069333±0.0015 | 0.914141 | 45.70707 | 4.570707 | 46.33838 | 7.437943 |
| 2 | 0.122667±0.002 | 1.722222 | 86.11111 | 8.611111 | 91.31313 | 14.657 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 0.198667±0.0011 | 2.873737 | 143.6869 | 14.36869 | 157.5 | 25.2809 |
| 4 | 0.242333±0.0017 | 3.535354 | 176.7677 | 17.67677 | 204.9495 | 32.89719 |
| 5 | 0.311667±0.002 | 4.585859 | 229.2929 | 22.92929 | 275.1515 | 44.16557 |
| 6 | 0.372±0.001 | 5.5 | 275 | 27.5 | 343.7879 | 55.18265 |
| 7 | 0.439333±0.0015 | 6.520202 | 326.0101 | 32.60101 | 422.298 | 67.78459 |
| 8 | 0.502333±0.001 | 7.474747 | 373.7374 | 37.37374 | 502.6263 | 80.67837 |

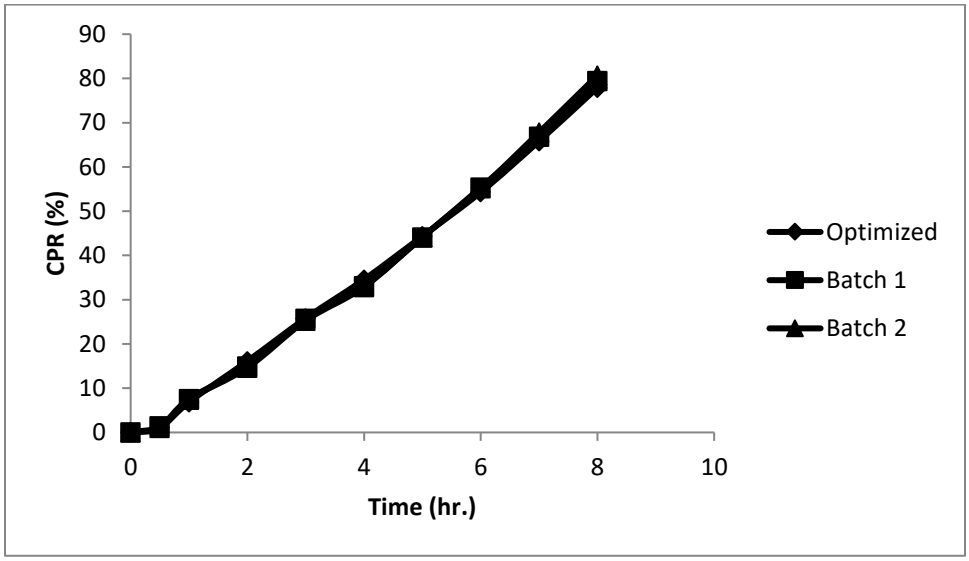



**Fig. 4.22 Release graph of optimized and batch formulations (PBS 5.5)**

# *Chapter 5*

# SUMMARY AND CONCLUSION

## SUMMARY AND CONCLUSION

In the microencapsulation approach it is difficult to enacapsulate5-Fluorouracil (water soluble drug)with single emulsion process. This active ingredient mainly used as dosage form (tablet, capsule and injections) for colon cancer, esophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer.

The main objective of the present study is to develop anti-neoplastic drug (5-FU) containing microsponge gel by avoiding oral route to treat actinic keratoses on skin. Development of new DDDS (Dermal Drug Delivery System) formulation is suggested by w/o/w double emulsion to maximize encapsulation and release rate.

Before preparation of the topical delivery system pre-formulation studies are necessary for drug and polymers used in formulation. Characteristics were compared for physical mixture and formulation. For the drug candidate melting point was determined. Partition co-efficient of drug was performed. The $\lambda_{max}$ of 5-FU was determined and standard curve was plotted. Instrumental analysis (FTIR and DSC) was performed for drug, polymers, physical mixture and prepared microsponges to check any interaction among ingredients.

From DSC study, it was concluded that crystalline drug may have converted to amorphous phase owing to its homogeneous dispersion within formulation additives, so it did not show any endothermic peak in the DSC thermogram like pure drug, but this does not indicate anychemical incompatibility in drug-polymer matrix. In physical mixture, thermogram reflects presence of short melting thermogram of drug and it was shifted. TGA also confirmed that compatibility of the drug and polymer used.

From FTIR spectra it was concluded that there was no chemical interference between drug and polymers. Some of the peaks of drug are not visible in the FTIR spectrum of Microspong formulation. It suggests that some functional groups of drugs form weak Vander Waals force with that of polymers.In the spectrum of physical mixture some of peaks of drug and polymers are visible.

Next step to work was to optimize the process variables, which are involved in the preparation of 5-FU loaded microsponges using response-surface methodology (RSM-CCD).

The response-surface optimization was carried out to optimize levels of the dependent factors (polymer ratio, stirring speed and surfactant concentration) to achieve the desired responses. The ANOVA results showed that polymer, SS and SA had strongest effects on the percentage yield, particle size, entrapment efficiency and release in 8 hr. A numerical optimization technique was used to find optimize formulation by desirability function. Targets were set to find optimized formulation usingindependent variables (polymer ratio, stirring speed and concentration of tween 80) in range and dependent variables percentage yield (in range), particle size (minimize),maximize entrapment efficiency and rel. in 8hr. 19 solutions were found by the software. Maximum desirability coefficient should be considered as parameter of optimized formulation. The combination of independent variables levels polymer (600 mg), stirring speed (1197 rpm) and surfactant (2% w/v) were found to give a desirability value of 0.7402 ( by design generated statistical method), showing yield (63.51%), average particle size (151.56 µm), entrapment efficiency (75.21 %) and release in 8hr (74.24%). Final formulation was gel that's why viscosity and rheological characteristics also were studied. Rheological characteristics of gel had to show shear thinning property, which is ideal for drug release.

In the present study an approach of scale-up work was highlighted using composition of optimized formulation. It was done by power law approach coupled with fixed different shape factors. It was limited to laboratory scale. Development of technology of this type is difficult under the laboratory facility. There is still plenty of scope for up gradation of this method upto large scale by pharmaceutical industry.

# *Chapter 6*

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