EXPERIMENTAL INVESTIGATIONS IN ENCAPSULATION OF PHARMACEUTICALLY ACTIVE INGREDIENTS

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Chemical Engineering

by

Yashawant Pralhad Bhalerao
Enrollment No. 129990905005

under supervision of

Dr. Shrikant J. Wagh

GUJARAT TECHNOLOGICAL UNIVERSITY

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The viva-voce of the PhD Thesis submitted by **Shri Yashawant Pralhad Bhalerao** (Enrollment No.129990905005) entitled **Experimental Investigations in Encapsulation of Pharmaceutically Active Ingredients** was conducted on Monday, 14/09/2020 at Gujarat Technological University.

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Abstract

Microencapsulation comes as an important protection, storage technique, and controlled release tool for several Pharmaceutically Active Ingredients (PAI), food, cosmetic, medical and other products. Purposes for encapsulation of PAI like thymol may be numerous, such as its controlled and targeted controlled release, protection/preservation, economic utilization, formulations, and modification/hiding undesirable property such as taste, odour and touch. Controlled release helps to maintain an adequate drug concentration in blood or in target tissues at a desired value as long as possible and, for this, they are able to control drug release rate, hence control release of PAI (thymol) is important. Controlled release formulation can be obtained by various methods such as spray drying, solvent diffusion, nanoprecipitation etc. Several parameters which influence the property of the micro particle are Drug (PAI) solubility in the solvent, Drug to polymer ratio, polymer and surfactant concentration

The main objective of this study is to encapsulate thymol in biodegradable shell and determine its controlled or sustained release by emulsification solvent diffusion, nanoprecipitation and emulsified beads techniques. In this work, the effect of concentration of thymol (the core material), sodium alginate and ethyl cellulose (the shell material), surfactant concentration on sustained release and encapsulation efficiency of thymol loaded beads and microparticles has been studied.

For method optimization the statistical design approach using 3 level 2 factors design and Plackett–Burman factorial Design (PBD) was applied using the Design-Expert® (DoE) software. Cumulative percent drug release for emulsification solvent diffusion runs are in the range of 83.43% to 94.38% while in nanoprecipitation technique 84.91% to 98.71 %. Obtained formulation of thymol loaded ethyl cellulose microparticles shows maximum 98% drug release in 10 h. on the other hand, Formulation of thymol loaded calcium chloride – sodium alginate beads showed maximum 95.18±0.43 % drug release in 12 h. Both methods are suitable for the microparticles preparation. No chemical interaction was found in FTIR study and particles obtained are spherical and distinct in nature.

This study observed that nanoprecipitation method gives quite better results than the solvent diffusion method and seems to be promising for sustained delivery of thymol. The solvent diffusion method shows better in terms of encapsulation efficiency compare with nanoprecipitation.

Acknowledgement

Beyond everything and anything, I would first like to thank my parents for their grace, guidance, and blessings.

During this wonderful journey of my doctoral research, I have really been fortunate enough to gain unconditional support from some really kind people who inspired, motivated and helped me throughout the milestones of this journey. Words would never be enough for expressing my gratefulness to all these special people in my life who made this research possible.

I am really very thankful to my research supervisor, guide and mentor **Dr. Shrikant J. Wagh**, especially for his constant motivation, support and encouragement throughout the past six years. His enthusiasm, constructive criticism, research ideas, patience, motivation, and immense knowledge, healthy discussions have made the completion of my research possible and highly enjoyable. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. I am privileged to have worked under his guidance.

The completion of this work would not have been possible without, the Doctoral Progress Committee (DPC) members: Dr. S. A. Puranik– Director & Environmental Auditor, Env. Audit Cell, AITS, Atmiya University, Rajkot and Dr. Sachin Parikh HOD, Chemical Engineering Department, LDCOE, Ahmedabad. I am thankful for their rigorous reviews and valuable suggestions during my research.

I gratefully acknowledge the funding received towards my PhD from Shroff S R Rotary Institute of Chemical Technology (SRICT), Ankleshwar. Thanks to Mr. Ashok Panjwani, Vice-Chairman, Ankleshwar Rotary Education Society, for his encouragement and support.

The Ph.D. Department at the Gujarat Technological University (GTU) has been an excellent place to conduct research, and all the members of staff and colleagues have been encouraging and helpful whenever needed. My gratitude goes out to the assistance and support of Dr. Akshai Aggarwal, Ex-Vice Chancellor of GTU, Dr. Rajul Gajjar, Ex. Dean, Ph.D. Programme, Dr. Navin Sheth, Present Vice-Chancellor of GTU, Dr. N. M. Bhatt, Dean, Ph.D. Programme, and other staff members of Ph.D. Section, GTU.

My sincere thanks also goes to Dr. Vanadana B. Patravale, Professor of Pharmaceutics, Dr. Shreerang V. Joshi, Professor, Head of Department, Pharmaceutical Sciences and

Technology, ICT, Mumbai, and Dr. Sandip Gite, and Dr. Dhiraj Lote who provided me an opportunity to join their team and who gave access to the laboratory and research facilities in ICT, Mumbai. Without their precious support it would not be possible to conduct this research.

Most importantly none of these would have been ever possible without my loving, supportive, encouraging, and caring wife Sushma. Her unceasing faith and support helped me survive all the stress for all these years and let me not give up. She has been non-judgmental of me and instrumental in instilling confidence in me. Also not to forget my cute and little Master Asit and princess Avni, my biggest inspiration and motivation for my final stages of the research.

I would like to give a special mention for the support given by my colleagues and fellow Ph.D. Scholars, especially Mr. Robinson Timung, Mrs. Ujvala Christian, Dr. Sandeep Rai, Dr. Suhas Patil, Dr. Avinash Chaudhari, Mr. Rajesh Nagotkar and Dr. Satish Billewar. They have all extended their support in a very special way, and I gained lot from them, through their personal and scholarly interactions, their suggestions at various points of my research.

I would like to address special thanks to the reviewers of my thesis, for accepting to read and review this thesis and giving approval of it. I would like to appreciate all the researchers whose work I have used, initially in understanding my field of research and later for updates. I am very grateful to the authors of various articles on the Internet, for helping me become aware of the research currently ongoing in this field.

I also extend my hearty thanks to all those who have directly or indirectly helped me in the adventurous journey of my doctorate research.

Thank you.

I dedicate this thesis to

My parents, my wife and my lovely children for their constant support and unconditional love

I love you all dearly.

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List of Abbreviations

Abbreviation	Full Form
PBD	Plackett Burman factorial Design
PAI	Pharmaceutically Active Ingredients
DD	Drug Delivery
O/W	Oil-in-Water
W/O	Water-in-Oil
O/O	Oil-in-Oil
SDS	Sodium Dodecyl Sulfate
W/O/W	Water-in-Oil-in-Water
O/W/O	Oil-in-Water-in-Oil
PLA	Polylactic Acid or Polylactide
PLGA	Polylactic-co-glycolic acid
PCL	Poly e-caprolactone
FNP	Flash Nanoprecipitation
NSAID	Nonsteroidal Anti-Inflammatory Drug
HIV	Human Immunodeficiency Virus
pH	Potential of Hydrogen
EC	Ethyl cellulose
RESS	Rapid Expansionof SupercriticalFluid
IP	Interfacial Polycondensation
CD	Cyclodextrin
DMAEMA	Dimethyl Aminoethyl Methacrylate
EPO	Exclusive Provider Organization
GI	Gastro-Intestinal
HPMCP	Hydroxypropyl Methylcellulose Phthalate
RH	Relative Volatility
WPI	Whey Protein Isolate
PG	Propylene Glycol
MD	Maltodextrin
DSC	Differential Scanning Calorimetry

ZB	Zygosaccharomyces Bailii
MCT	Medium Chain Triglycerides
MIC	Minimum Inhibitory Concentration
SC	Sodium Caseinate
ND	Nanodispersions
DPPH	1,1-Diphenyl-2-Picrylhydrazyl
HLB	Hydrophilic-Lipophilic Balance
FCPC	Fast Centrifugal Partition Chromatography
LPLC	Low Pressure Liquid Chromatography
HPLC	High Pressure Liquid Chromatography
SE	Spissum Extract
MPO	Myeloperoxidase
COLTHY	collagen-based containing thymol
DoE	Design of Experiments
DCM	Dichloromethane
RPM	Revolutions Per Minute
FTIR	Fourier Transforms Infrared Spectroscopy
DOE	Design Of Experiments
XRD	X-Ray Diffraction
FE-SEM	Field-Emission Scanning Electron Microscopy
UV–Vis	Ultraviolet–Visible
EE	Encapsulation Efficiency
ANOVA	Analysis of variance
CMC	Critical Micelle Concentration
DR	Drug Release
PDI	Polydispersity Index
PSD	Particle Size Distribution

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CHAPTER-1

Introduction

1.1 Introduction

The encapsulation process allows the core compound to be encapsulated in a small sphere called a microsphere/microcapsule. This technique involves coating of particles ranging dimensionally from several tenth of a micrometer to about 5000 micrometer.

The most important characteristic of microcapsules is their tiny size, which allows a large surface. This huge surface is available for diffusion, desorption and adsorption, chemical reactions and light scattering [1].

Microencapsulation technique has been used in a variety of chemical, pharmaceuticals, cosmetics and printing. In pharmaceuticals industry different release mechanisms of encapsulated materials provide controlled, persistent or targeted release of core substance. The microencapsulation system offers potential advantages over conventional drug delivery systems. Microcapsules are unique delivery systems for many pharmaceutical products and can be adapted to aim targeted tissue systems. Therefore, the microcapsules can be used not only for controlled release, but also for the targeted delivery of drugs to a specific point of the body. Micro particulate drug delivery offers several applications for drug showing poor bioavailability. Several of pharmaceutically encapsulated active ingredients are at present in the market, such as glucotrol, cefadroxil, aspirin, theophylline and its subordinates, nutrients, antihypertensive, potassium chloride, progesterone and its hormone blends.

The main objective of this study is to encapsulate thymol in biodegradable shell and determine its controlled or sustained release by emulsification solvent diffusion, nanoprecipitation and emulsified beads techniques. In this work, the effect of concentration of thymol (the core material), sodium alginate and ethyl cellulose (the shell material), surfactant concentration on sustained release and encapsulation efficiency of thymol loaded beads and microparticles has been studied. For method optimization the statistical design approach using 3 level 2 factors design and Plackett–Burman factorial Design (PBD) was applied using the Design-Expert® (DoE) software.

Microencapsulation is a multidisciplinary field, which is viewed as the blend of numerous branches, for example, material science, colloid chemistry, polymer and physical science.

[1,2]. The chemical engineering fundamentals of mass transfer, reaction kinetics, reaction engineering and chemical engineering thermodynamics play very important role in basic understanding and pave a strong pathway for its application in various fields, such as pharmaceutical or therapeutic treatment that is the main objective of the present work. A wide number of techniques have been accounted for to form targeted microcapsules. As per the arrangement devise of the shell material and the essential conditions for the planning of the microcapsules, they can be characterized into three branches: physico-chemical methods, physical methods and chemical methods. A portion of these are recorded in Figure 1.1. Physical methods like fluidized bed coating [3] provide an alternative for the fabrication of agents in oil drilling or other correlated areas, in addition to interfacial polymerization as a chemical method and solvent evaporation and coacervation as physico-chemical methods. Fluidized bed coating is one of the little superior technologies capable of coating particles with mostly any type of shell material [4]. This method has the advantage of simple equipment, low cost, convenient operation, and it is also favourable for scale-up [5, 6]

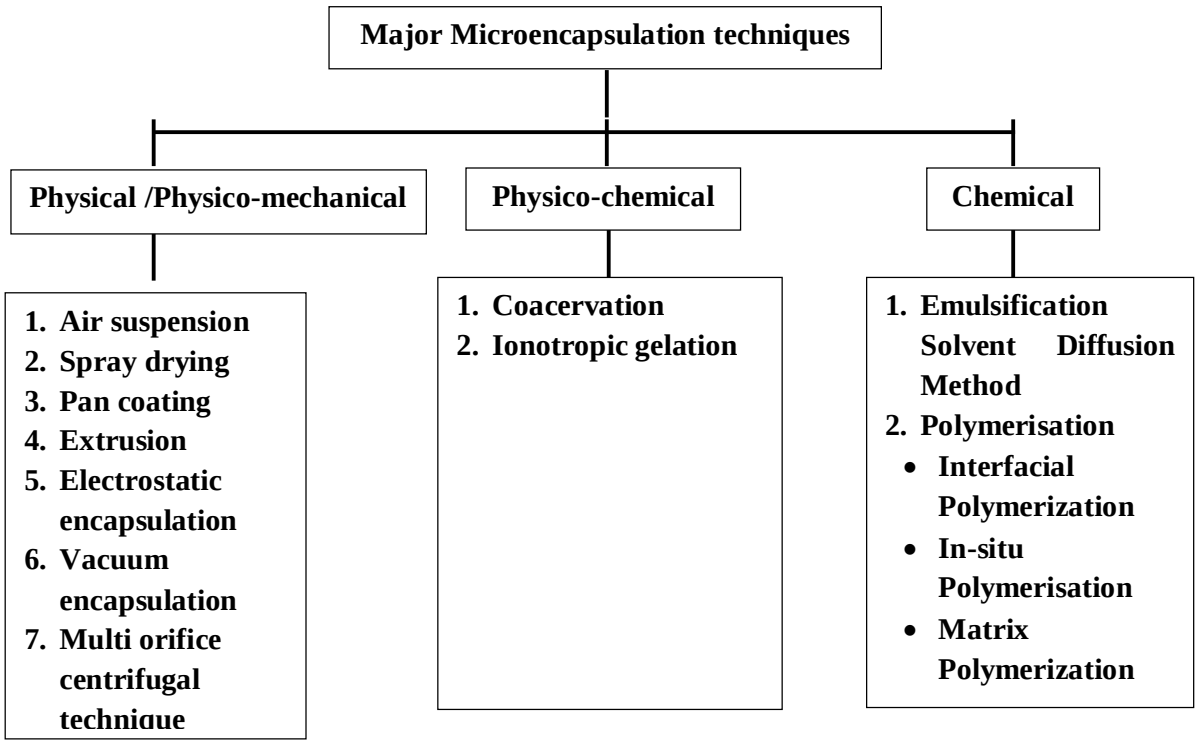


Figure 1.1:Major microencapsulation methods

Microencapsulation techniques have been used in many industries such as agrochemicals, cell localization, beverage processes, pharmaceuticals, protection of molecules against other compounds, drug delivery, quality and safety as well as in agriculture and the environment. Pharmaceutically Active Ingredients (PAI) is encapsulated for various reasons. Some of them are controlled drug release, preservation and protection of the active compounds, targeted controlled release, convenient packaging and smart option for storage, easy handling and formulation, hiding /modify undesired properties like odour, touch and taste. This technology has many advantages along with some disadvantages and it is listed in table 1.1

Table 1.1: Merits and demerits of Microencapsulation

Sr. No.	Merits of Microencapsulation	Demerits of Microencapsulation
1.	Food products have greater nutritional and wellbeing benefits	Due to foreign ingredients in foods customers with allergies may not be aware
2.	Wider range of specific products for customers to choose from	More expertise and information is required to utilize this progressed and complex innovation
3.	Microencapsulated ingredient do not interfere with other ingredients	probable cross reaction that may take place between the core and wall material selected
4.	Consumers are unable to taste the added capsules	Difficult to achieve uninterrupted and consistent film
5.	The microencapsulated core material can be added at any time in the processing and remains unaffected	Cost of production is very high
6.	Shelf life may be increased	Shelf life of hygroscopic drugs is reduced

1.2 Organisation of the thesis

The research work on topic "**experimental investigations in encapsulation of pharmaceutically active ingredients**" gives a brief description on i) Introduction ii) Literature review iii) Materials and Methodology iv) Results and Discussion v) Conclusion and future scope vi) Bibliography.

The introduction and literature review discuss merits, demerits, challenges, and factors affecting microencapsulation, scope, aims & objectives of PAI microencapsulation, organization of thesis, information about Emulsion Technique, Solvent diffusion, Nanoprecipitation, Beads, Biodegradable polymers.

The experimental methodology is to prepare thymol loaded EC micro particles using solvent diffusion and nanoprecipitation techniques and methods of analysis is to study the effect of concentration of thymol, sodium alginate, stirring speed on synthesis, and sustained release properties of thymol loaded sodium alginate beads using emulsion microencapsulation technique.

The chapter on Results and Discussion presents all experimental observations in the form of various graphs and their interpretation. The effect of variables studied, as mentioned in the scope has been substantiated with the help of logical reasoning. The chapter also gives basic information about all analytical techniques and instruments used in this work.

The chapter on conclusion and future scope discuses conclusive remarks on the experimental work of the present study of thymol encapsulation.

1.3 Encapsulation techniques

The major microencapsulation techniques used in the encapsulation of the thymol with biodegradable polymers are discuss here

1.3.1 Emulsion Technique

Emulsion is a group of scatter systems comprising of two immiscible liquids. The liquid droplets (the scatter phase) are dispersed in a liquid medium (the constant phase). A few classes of emulsion might be recognized, in particular oil-in-water (O/W), water-in-oil (W/O) and oil-in-oil (O/O). The last class might be exemplified by an emulsion comprising of a polar oil (for example propylene glycol) scattered in a nonpolar oil (paraffinic oil), and vice versa. So as to scatter two immiscible fluids a third ingredient is required, in particular the emulsifier; the decision of emulsifier is vital for the development of the emulsion but also for its long-term stability [7-9].

A few procedures identifying with the breakdown of emulsions may happen on capacity, depending on:

- The particle size distribution and the density difference between the droplets and the medium.
- The magnitude of the attractive versus repulsive forces, which determines flocculation. The solubility of the disperse droplets and the particle size distribution, which in turn determines Ostwald ripening.
- The stability of the liquid film between the droplets, which determines coalescence; and
- Phase inversion.

Emulsions may be categorized according to the nature of the emulsifier or the arrangement of the system (see Table 1.2). The most straightforward sort is particles, for example, - OH that can be explicitly adsorbed on the emulsion bead in this way delivering a charge. Electrostatic repulsion can be provided when electrical double layer can be developed. This has been exhibited by very dilute O/W emulsions through expel any acidity. Certainly that technique is not efficient. W/O and O/W emulsifiers are emulsify by non-ionic surfactant the most powerful emulsifiers. Moreover against the coalescence and flocculation they preserve the emulsion. For O/W emulsification many ionic surfactant like Sodium Dodecyl Sulfate (SDS) may be used, but in presence of electrolytes the system is sensitive. For stabilization of the emulsion many permutations and combinations are possible for surfactants like ionic and non-ionic, or mixtures of non-ionic surfactants can be better impressive in emulsification and stabilization. Polymeric surfactant such as pluronics, it is types of non-ionic polymers are more useful in stabilization of the emulsion; the main difficulty observed is that they produce tiny droplets, unless tremendous energy is applied for the technique. Polyelectrolytes, for example, poly (methacrylic corrosive) can likewise be applied as emulsifiers. Blends of polymers and surfactants are perfect in accomplishing simplicity of emulsification and adjustment of the emulsion. Lamellar fluid crystalline stages that can be delivered utilizing surfactant blends are exceptionally powerful in emulsion adjustment. Strong particles that can accumulate at the O/W interface can likewise be utilized for emulsion adjustment. These are referred to as Pickering emulsions, whereby particles are made halfway wetted by the oil stage and by the fluid stage [10].

Types of emulsion system:

i. Macroemulsions or common emulsions (O/W and W/O): this type normally involve a dimension of 0.1 - 5 μm with a typical of 1 - 2 μm.
ii. Microemulsions or Micellar emulsions: microemulsions are normally having the dimension range of 5 - 140 nm. They are thermodynamically steady [11].
iii. Nanoemulsions: this type normally involves a diameter sphere of 20 - 100 nm. Like macroemulsions, they are just actively consistent and steady [12].
iv. Complex or Double and various emulsions: this types also known for emulsions-of-emulsions, O/W/O and W/O/W structures are the examples.
v. Mixed emulsions: These types of emulsions comprise of two distinctive scatter beads which do not blend in a ceaseless intermediate.

Table 1.2: various types of emulsion systems

Sr. No.	**character of emulsifier**	**system**
1	Plain molecules	Macromolecules
2	Ionic surfactants	Macroemulsions
3	Mixed polymers and surfactants	Mixed emulsions
4	Non-ionic polymers	Bilayer droplets
5	Surfactant mixtures	Micellar emulsions (microemulsions)
6	Polyelectrolytes	Double and multiple emulsions

The physical phenomena engaged with every breakdown procedure are not straightforward, and requires an examination to be made of the different surface powers included. Also, the above procedures may happen simultaneously rather than consecutively which in turn complicates the analysis. Model emulsions, with monodisperse beads, can't be effectively produced and subsequently any hypothetical treatment must consider the impact of droplet size circulation. Theories that take into account the polydispersity of the system are complex, and in many cases only numerical solutions are possible. In addition, the measurement of surfactant and polymer adsorption in an emulsion is not simple, and such information must be extracted from measurements made at a planar interface.

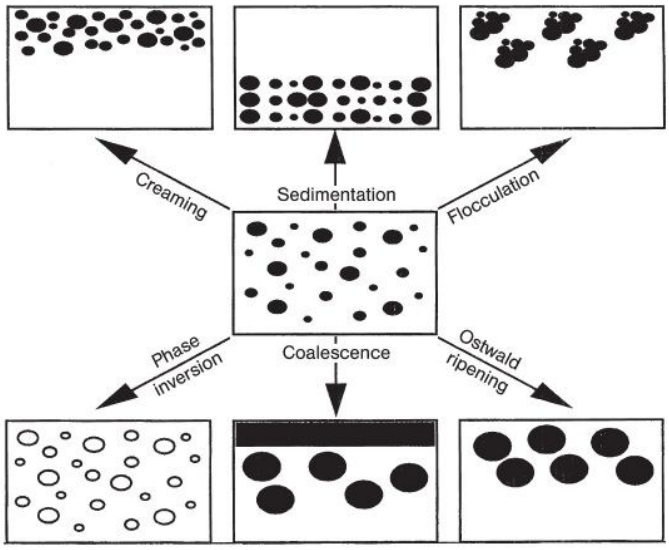


Figure 1.2: Stages in Emulsions processes

(Source:Emulsion Formation and Stability, First Edition. Edited by Tharwat F. Tadros. Published 2013 by Wiley-VCH Verlag GmbH & Co. KGaA.)

An outline of each of the above breakdown processes (as shown in Figure 1.2) is provided in the following sections, together with particulars of each process and methods for its prevention.

Creaming and Sedimentation

This procedure happen because of gravitational impact and both of them are referred as gravitational partition. They are regularly observed forms of instability in emulsions. Depending on relative densities of scattered and uninterrupted phase, creaming, or sedimentation may take place. If the density of scattered phase is lower than continuous phase, droplets shift upward and get detached from uninterrupted phase forming a separate layer at the top of the continuous phase. This event is termed as creaming. When density of dispersed phase is higher than uninterrupted phase forcing droplets to move down and forming a layer at the bottom of continuous phase and the phenomena is called sedimentation [13].

Flocculation

Flocculation relate to aggregation of the beads (with no adjustment in initial bead diameter) into lumps or flocks. It prevails after effect of the van der Waals force of attractions that are all inclusive with all scatter frameworks. Clustering happens when

around no adequate repulsion to keep the beads separated at separations where the van der Waals attraction is powerless. Flocculation might be either 'solid' or fragile, depending on the magnitude of the attractive energy involved [14].

Ostwald Ripening (Disproportionate)

Ostwald ripening is the increase growth of one emulsion droplet on the expense of a smaller one because of the distinction in chemical ability of the material inside the droplets [15, 16]. This difference arises from the distinction in the radius of curvature of the drops. Ostwald Ripening results from the limited solubility of the fluid phase. Non soluble or miscible fluids which are referred to as being immiscible frequently have shared solubility are which are not inappropriate. With emulsions which are typically polydisperse, the lesser droplets will have a more prominent solubility as contrasted with bigger droplets (because of shape effects). With moment, the minor droplets vanish and their atoms scatter to the mass and become stored on the bigger drops. With time, the bead size conveyance movements to bigger qualities.

Coalescence

Coalescence is the process of diminishing and interruption of the fluid film between the globules, with the result that combination of at least two beads occurs to form larger beads. The precautionary case for coalescence is the entire division of the emulsion into two definite liquid phases. The main driving force for coalescence is the surface or film fluctuations; this outcome in a close path of the droplets whereby the van der Waals forces is effective and prevents their partition [17].

Phase Inversion

In phase inversion process there will be a trade between the disperse stage and the medium. For instance, an O/W emulsion may with time or change of condition upset to a W/O emulsion. As a rule, stage reversal goes through a transformation state whereby numerous emulsions are created.

A number of industrial systems consist of emulsions of which the following important are revealing:

- Food emulsions, for example, mayonnaise, mixed greens creams, butter, sweets and drinks.

- Pharmaceuticals, for example, analgesics, lotion, lipids, vanillin in liquid paraffin emulsion.
- Personal care and corrective items, for example, hand-creams, moisturizers, hair-splashes and sunscreens.
- Bitumen emulsions are readied stable in their compartments at the same time, when applied the street chippings, they should blend to shape a uniform film of bitumen.
- Agrochemicals - self-emulsifiable oils which produce emulsions on weakening with water, emulsion concentrates (beads scattered in water; EWs) and yield oil splashes.
- Paints, for example, emulsions of alkyd gums and latex emulsions.
- Dry-cleaning plans; these may contain water beads emulsified in the dry-cleaning oil, which is important to expel soils and muds.
- Emulsions in the oil business - numerous unrefined oils contain water beads (for example North Sea oil); these must be expelled by mixture pursued by division.
- Oil smooth dispersants – oils pilled from tankers must be emulsified and afterward isolated.

1.3.2. Nanoprecipitation

Nanoprecipitation is also referred as solvent displacement or interfacial deposition. It is the simplest method for preparation of polymeric nanoparticles containing drug. It is amongst the primary developed approach for encapsulation of the drugs molecules [18]. It is exclusively and widely used to encapsulate the hydrophobic drug molecules in nanospheres or nanocapsules or nanoparticles. For encapsulation mainly biodegradable polymers such as poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA), and poly(e-caprolactone) (PCL) are use for specific reason. Nanocapsules are vesicular patterns that showcase core-shell structure wherein the pharmaceutical active ingredients is specifically restricted to a container or within a cavity surrounded by means of a polymer membrane. Nanospheres are, however, matrix style colloidal molecules where the pharmaceutical compound is dissolved or dissipated inside the polymer matrix. The drug molecule might

be also adsorbed at the basement of the nanocarrier [19]. It is a simple and regenerative process that has been commonly used in the formation of nanoparticles.

Nanoprecipitation has many advantages over other encapsulation techniques:

- Excellent reproducibility
- Large quantity of contaminated solvents are avoided
- Ease of operation and scalability
- Less amount of energy input required
- submicron particle sizes obtaining with narrow size distribution[20]

Modified nanoprecipretation method (as shown in Figure 1.3) developed by Bilati et al. (2005) is basically for hydrophilic molecules [21, 22]. Since the invention of the system, numerous actions have been made to improve its reproducibility, adaptability, and security. Improvement of reproducibility could limit between group varieties while improvement of adaptability permits the getting of details which are effectively appropriate in the pharmaceutical trade. Protection could be given by maintaining a strategic distance from the utilization of poisonous organic solvents. They comprised of the employment of inventive mixing tools, for example, "T"- shape blender, film contactor, microfluidics (Bally) or flash nanoprecipitation method[26].

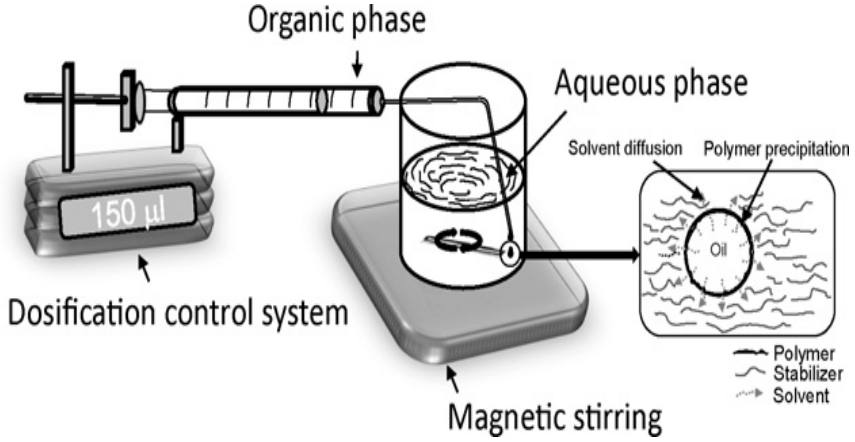


Figure 1.3: The nanoprecipitation technique.

Steps involved in Nanoprecipitation method

It is a very easy, reproducible and replicable method that creates particles along narrow size distribution over a broad range of processing factors. It calls for miscible phases: an organic/oil phase and an aqueous phase. The method of particle development inside this method may contain various includes three steps:

i. Nucleation,
ii. Growth,
iii. Aggregation.

Behind this process of nanoprecipretation the main driving force is super saturation. It is proportion of polymer concentration to the solubility of the polymer in the appropriate solvent mainly organic. Super saturation is significant on the grounds that it likewise decides the nucleation rate. Here, fluid dynamics and mixing of phases play an important role. Truth be told, they influence super saturation and due to the speed of molecule arrangement process, they decide likewise the nucleation rate. In fact, poor blending produces barely any enormous or big nanoparticles (low nucleation rate) while good mixing conditions give birth to high nucleation rates, i.e., larger population of smaller nanoparticles [23]. Variation in surface tension also plays an important role in formation of nanoparticles. This result became based totally on studies carried out through Davies on mass transfer among liquids and on the Gibbs–Marangoni impact [24]. Actually in reality, a fluid with a high surface tension pulls more firmly on the nearby fluid than one with a low surface tension. This variation between surface energy of the fluid and the oil stage causes interfacial instability and thermal imbalances in the framework. This prompts the stable development of vortices of solvent at the interface of the two fluids. The organic solvent diffuses from regions of low surface tension which causes gradual precipitation of the polymer on the oil surface and forms nanocapsules[19].

The oil phase involves on an organic solvent in which water get dissolve completely, for instance, acetone or ethanol. The normal stage contains similarly the polymer and the hydrophobic drug. Distinctive blends possibly will be added to the dissolvable, for instance, vegetable or mineral oils, or hydrophobic surfactants, triglycerides. Use of mineral or vegetable oils allow gaining nanocapsules instead of nanospheres. Surfactants hamper the gathering of the particulate possessor. The fluid stage is typically water however some different excipients, for example, hydrophilic surfactants could be added to

maintain a strategic distance from particles collection. These surfactants could be regular or engineered. Likely, a few polymers could be added to liquid stage as covering materials. Hydrophilic medications could be disintegrated in the dilute phase.

One of the straight forward nanoprecipitation strategies called Flash Nanoprecipitation (FNP) with a traditional procedure brings about heterogeneous blending ensuing about in polydispersed molecule sizes. FNP, however, is a scalable process that could be used to prepare nanoparticles with controlled size distribution and a high drug loading rate. This system was first depicted by Johnson and Prud'homme to create nanoparticles encapsulating hydrophobic medications. FNP produces nanoparticles with a narrow size distribution ranging from 80 to 1 μm [25]. Rapid precipretation method is used to obtained the nanoparticles, the other advantages of the FNP are it offers encapsulation of the multiple drugs in same nanoparticles, provides maximum drug loading capacity. A few effective uses of FNP have been accounted for encapsulation of different hydrophobic medications/ drugs, imaging specialists, peptides, or a blend of the two therapeutics and inorganic colloids. [26].

Strength, stability, undesirable taste concerns, bioavailability are some of the issues related with many hydrophobic or hydrophilic drugs. Encapsulation of such molecules in nanoparticles could be a very interesting alternative to solve these problems in order to enhance the efficacy of such molecules and promote patient compliance. Nanoprecipitation is a system that has been generally utilized for the readiness of polymer nanoparticles expected for a few biomedical applications since its innovation. Working conditions must be all around figured out how to get nanoparticles with appropriate properties for the biomedical applications they are intended for. Different works concentrated on the improvement of its versatility, reproducibility, and security with few research works have been made to utilize nanoprecipitation in a regular manner. Flash nanoprecipitation, Film innovation and micro fluidics, were acquainted with accomplish such purposes. Advantages of submicron carriers prepared by nanoprecipitation in the biomedical field have been confirmed in vivo by numerous studies. These accomplishments include improved bioavailability, better focusing on and resilience, targeted and sustained discharge rate, and upgraded assimilation of the medication through biological barriers.

1.3.3. Beads

A drug delivery system releases the drug inside the specific frame compartment on the controlled rate required for a particular treatment. At the moment maximum available drug distribution structures use bio-degradable, biocompatible and herbal bio-polymers and are able to rate controlled drug release system. Now diverse research efforts are being implemented on oral sustained drug release system, this device are for solid dosage form researchers developed numerous sustained and controlled release of dosage by using entrapped the drug in natural polymer and forming a gel [27].

Micro-beads are defined as the monolithic sphere distributed the whole matrix as a molecular dispersion of particle and molecular dispersion defined as the drug particle are dispersed in to the continuous phase of one or more than one miscible polymers [28]. It is a small spherical particle with diameters in the micro-meter range (greater than 0.1µm and less than equal to 5mm) which can vary in chemical composition, size, shape, density, and function. Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects. Beads loaded with antibiotics to be beneficial for oral delivery to treatment of various kind diseases that is peptic ulcer and for the ulcerative colitis, carcinomas and infections of the intestine. The managed systemic absorption particularly in the intestinal region offers exciting opportunities for the treatment of diseases which include allergies, arthritis or inflammation [29].

Sodium alginate is commonly used in different controlled release dosage form due to its natural, biodegradable and hydro-gel forming properties. Alginates are natural polysaccharide polymers obtained from brown algae, consisting of two different unit β-D-mannuronic acid and 1,4 α-L-guluronic acid. These residues are arranged in homo-polymeric blocks and in hetero-polymeric blocks. Alginates show gelling properties in the presence of divalent cations such as Ca^{2+}, Sr^{2+} or Zn^{2+}**[**30**].**Different researches have shown the feasibility of alginate-based micro-beads for transplantation of laboratory cell lines as well as of xenogeneic tissue by forming micro-beads. The micro-beads are prepared by ionotropic gelation technique where the gelation of natural anionic polysaccharide sodium alginate, the natural polymer was reacted with oppositely charged calcium ions, acting as cross linker or counter ion, to form immediately micro-beads**.** Various parameters like swelling, mechanical stability of the beads and degradation will be

studied [31].Natural polysaccharides, which are mainly used in the food and pharmaceutical industry, act as dietary fiber and they are not invested in the gastrointestinal tract. An anionic polysaccharide, alginic acid were used as a medicine for stomach ulcers and food additive as a result of it given orally it shows defensive impact on the gastric mucosa. Alginic acid undergoes a sol-gel conversion by the presence of cations such as the calcium particle and ions immediately form a gel matrix in the presence of sodium alginate [32]. Over the past two decades, a number of micro-beads prepared by different natural polymer (e.g., alginate, agar, chitin, agarose, gellangum, chitosan) have been developed and analysed in pharmaceutical field. Various synthetic polymers are also used in preparation of micro- beads but some advantages and disadvantages are associated with it.

Advantages of the beads are as follows

• The synthetic polymers have capability to tailor mechanical properties and biodegradability.

• They are also appealing because they may be fabricated into various shapes.

• Polymers may be designed with precise compound functional groups.

• Simple and easy to manufacture [33].

Disadvantages of the beads are as follows

• It is always higher in cost; the production cost is also much in higher compared with natural polymer.

• Some synthetic polymers are not biocompatible and produce toxicity.

• Synthesis of artificial polymer causes environment pollution.

• It also produces side effects (acute and chronic effect).

• And poor patient compliance [34].

Method of preparation for beads

Beads can be synthesized by the following methods-

a) Ionotropic Gelation Technique
b) Cross-Linking
c) Emulsification Gelation Technique

Above three methods show many advantages

- It is Easy and mild, inexpensive preparation techniques.
- No organic solvent or high shear force to be used.

- These method would be used for broad category of drugs such as protein, macromolecules etc.
- More Stable [35].

Ionotropic gelation technique

Preparation of Micro-beads by Ionotropic Gelation Technique: Steps involved in are explained as follows

1. Weigh the raw materials as sodium alginate, drug used and calcium chloride.
2. Required amount of distilled water is gradually added to the weighed quantity of sodium alginate allowed to warmness for 10-15 minutes on a hot plate
3. After that, distilled water is slowly introduced in the weighed quantity of calcium chloride to form a solution.
4. The paste of sodium alginate is then stirred in a magnetic stirrer at an appropriate pace for several minutes till it became homogeneous.
5. The Drug is dispersed in the paste of sodium alginate and stirred at suitable pace within the magnetic stirrer.
6. By adding the calcium chloride solution in it through a syringe with the help of a needle, micro-beads are formed.
7. The micro-beads are filtered with whatman filter paper & washed thoroughly with distilled water.
8. The micro beads were dried at room temperature subsequently for few hours [36].

Cross-linking

Preparation of beads by cross-linking: Steps involved in are explained as follows

1. The cross-linking polymer solutions of diverse concentrations are prepared by dissolving in water under slow agitation.
2. Drug was slowly added in the polymer solution under constant mixing with the stirrer for 5 min for uniform distribution throughout the solution.
3. Finally, drug and polymer solution was extruded drop wise through a 1.2 mm needle into a continuously stirred calcium chloride solution at room temperature.
4. As soon as the micro-beads were formed they were allowed to remain in the stirred solution for 10 min curing time.
5. The micro-beads are filtered with whatman filter paper and several times washed with distilled water and then it is dried at room temperature [37].

Emulsification Gelation Method:

Preparation of beads by emulsification gelation method: Steps involved in are explained as follows

1. In emulsion gelation technique polymer sodium alginate was dissolved in the water.
2. The drug was slowly added in to the sodium alginate polymer solution till it becomes uniform.
3. The polymer solution was then added in a thin string of heavy liquid paraffin solution contained in a beaker.
4. Then calcium chloride homogeneous solution was added into the emulsion and continuously stirred for 15 min to formed spherical micro-beads.
5. The micro-beads were collected and separate it by decantation and washed it with petroleum spirit or ether.
6. The obtained micro-beads were air dried to get discrete microcapsules [38].

The beads are extensively use for many purposes some of the applications of Beads are as follows

1. Non-Steroidal Anti-inflammatory Drug NSAID like Diclofenac micro-beads, show reduced release in the stomach. It reduces the adverse effects and avoids direct contact between the drug and the mucous membrane of stomach [39].
2. The micro-beads loaded with the antibiotics (like Oxytetracycline) are useful for the oral administration in a treatment of gastric and intestinal ailment [40].
3. Lamivudine is a synthetic nucleoside analog that is being more and more used because the centre of an antiretroviral routine for the remedy of HIV contamination and it formulated in the alginate beads so that their controlled release can be acquired for the extended therapeutic impact [41].
4. Ranitidine, peptic ulcer drug, it designed in micro-bead shape in one of these way that it will likely be retained in abdomen for adequate time. So, it is able to open new treatment of gastric ulcer and acidity.
5. Sustained discharge of Prednisolone from chitosan gel beads and it will increases the therapeutic efficacy and decreases ill effects by means of minimizing the accomplishing of the drug to the systemic circulate towards infection [42].

6. Theophylline, a poorly water soluble bronchodilator and the focused drug for controlled transport, and decreases drug release under physiologically simulated pH conditions (acidic and neutral) so, it is able to be formulated into modified dosage form [43].

7. Fluorouracil entrapped alginate micro-beads for the remedy of breast cancer can be formulated in the alginate beads so that their controlled release can be obtained for the prolonged healing effect [44].

8. Metformine Hydrochloride, an antidiabetic medication, using albumin are degraded in to acidic medium whilst it given orally. Formulating them in the modified alginate micro-beads can deliver them in to intestinal region without degradation in the stomach location [45].

9. Insuline, an antidiabetic drug, formulating it inside the modified alginate beads can at once deliver them within the intestinal region without drug degradation in the stomach [46].

10. Piperine turned into fabricated into alginate beads using sodium alginate. The primary goal to expand the sustained launch of piperine from alginate beads with the aid of in vitro evaluation. The drug release studies have been showed that the alginate beads sustained the release [47].

11. Rifampicine, first line drug used to treat tuberculosis, so the sustained and the controlled release of ampicillin can be useful to overcome its brief half of-existence [48].

12. Salbutamol sulphate is a quick-performing β2-adrenergic receptor agonist used for the comfort of bronchospasm in situations including bronchial asthma, so their healing impact improved by way of the use of the sodium alginate interpenetrating network beads [49].

13. Microspheres are also being used in cancer treatment. Cancer microsphere technology is the latest trend in cancer therapy [50].

14. Developing of calcium alginate–gelatin based totally microspheres for managed launch of Endosulfan act as a model pesticide because of lowering environmental impact of insecticides within the macromolecular community of calcium alginate and gelatin based microspheres [51].

1.4 . Biodegradable polymers

Biodegradable polymers are described as materials whose chemical and physical characteristics go through deterioration and completely degrade when exposed to microorganisms, bacterial decomposition method, cardio and anaerobic process. Even if

plastics is best for many purposes which includes in packaging, building materials and commodities, as well as in hygiene merchandise, can result in waste disposal issues inside the case of conventional petroleum-derived plastics, as these substances are not completely biodegradable and due to their resistance to microbial degradation, they assemble as it is inside the atmosphere. Further nowadays oil cost have improved noticeably. These facts have helped to stimulate interest in biodegradable polymers and in particular biodegradable biopolymers. A number of the widely used biodegradable polymers in food and prescription drugs enterprise are poly lactic acid, Ethyl Cellulose (EC), sodium alginate and many more.

Biodegradable plastics and polymers were first brought in 1980s. There are numerous resources of biodegradable plastics, from artificial to natural polymers. Synthetic polymers are created from non-renewable petroleum sources while natural polymers are available in big portions from renewable resources. Biodegradation happens via the action of enzymes and/or chemical deterioration related to residing organisms. This event takes place in steps. The initial is the separation of the polymers into small molecular mass species by using both biotic reactions, i.e. degradations through microorganisms and abiotic reactions, i.e. oxidation, photo degradation or hydrolysis. This is accompanied by means of bio assimilation of the polymer fragments via microorganisms and their mineralisation. Biodegradability depends not only on the origin of the polymer but also on its chemical character and the environmental degrading situations. Mechanisms and estimation techniques of polymer biodegradation were reviewed [52]. The mechanical behaviour of biodegradable materials relies upon on their chemical composition [53, 54], the production, the storage and processing characteristics [55, 56], the ageing and the application conditions [57].

1.4.1. Ethyl cellulose (EC)

EC is widely used for encapsulation because of its several versatile properties, such as white or light brown granular fine particles or odorless and tasteless substance. The range of melting temperatures is 240-255 °C; insoluble in water, but soluble in several organic solvents such as ketone, alcohol, ester, and ether; biocompatible and companion able among several celluloses, resins and almost all plasticizers; resistant to light, heat, oxygen, moisture and chemicals; The ability to take up pressure to protect the coating from disintegrate under compression.EC is soluble in a variety of solvents, which facilitates its

use. Suitable solvents include esters, aromatic hydrocarbons, alcohols, ketones and chlorinated solvents. EC is more soluble in a solvent that has almost the same energy density or cohesive solubility parameters as the material itself. EC is the most stable cellulose derivative. It is alkali resistant, both diluted and concentrated, but it is sensitive to acids. Humid air or immersion requires very little water, leaving the evaporation of EC unchanged. EC produces a good film with excellent tensile, flexibility and elongation properties; but these films paint sweetness. As a result, EC alone flows at a temperature that is too high to be practical in molding or other applications requiring a good thermoplastic material.

The EC is utilized to encapsulate different pharmaceutical ingredients so as to stabilize them from dynamic environment, hydrolysis and oxidation. It is likewise utilized as a lattice and/or shell agent to increases discharge properties. On the other hand, the determination of a reasonable polymer and the advancement in encapsulation is a lengthy and complex procedure, requiring in detail knowledge over inside and out information on the physicochemical properties of different polymers and drugs.

This polymer is insoluble at any pH value that is observed in organism, however in the presence of gastric juice it experiences the swelling, then penetrable for water and allows prolonged modified drug release. Hence consider to be the ideal polymer for modified drug release. [58-61].

EC is a stable, non-poisonous, compressible, inert, non-bio degradable, bio-compatible hydrophobic polymer that has been broadly used to plan for pharmaceutical dosage. Now a days it is mainly utilized in encapsulation of Pharmaceutical active substances, controlled release, taste and flavour masking, binding a tablet, and as a sustain release coating for pills and beads. It is extracted from plant fibre (cellulose, cotton) and is then synthetically changed; EC is prepared from wood pulp or cotton by means of treatment with base and ethylation of the alkali dealt with fibre with ethyl chloride. The resulting product is then additionally steamed and dried.

1.4.2. Sodium Alginate (SA)

The most desire material of encapsulation is alginates extracted from brown algae/seaweeds consisting of two monomeric structures: D-mannuronic (M) acid and L-guluronic (G). Moreover, alginate is broadly utilized in tissue designing and biotechnological ventures on account of its biocompatibility, low consumption and

harmless nature. Alginate can shape ionic bonds with polyvalent subtitles, for instance, calcium particles, making a cross associated arrangement along with the L-guluronic social gatherings of different alginate chains, encircling a polymeric layer. [62].

1.5 Scope of the thesis

Encapsulation of thymol in solid microparticles, nano- and microemulsions, liposomes and polymeric nanoparticles represents a promising approach for overcoming thymol's limitations, lowering their dosage and increasing long-term protection of thymol. Low dose of thymol are also found to be effective to lower its long term effects on various parts of body [63].

Standardization is necessary in terms of purity of product and stability. Microencapsulation formulation can provide an effective alternative for thymol administration in relatively high or low dosage depending upon application.

Thymol has potentials for maintaining and promoting health, it additionally prevent and potentially treating some diseases (hookworm, cardiovascular, rheumatic and skin disease) [64]. On the other hand low water solubility, stability as well as the high volatile nature and the aftereffect associated with its use has limited their application in medicine. Encapsulation is a new approach that has prospective applications in health and medical research. A practical and alternative method for encapsulation is a sol-gel silica base that takes place at an ambient temperature where the decomposition of compounds is inexpensive because capital investment in production is very low and environmentally friendly. Microencapsulation is very useful tool for increasing the compounds stability in the presence of air, moisture, low and high temperatures. In addition, the micro particles permit easier and safer handling of liquid substance by converting them into solid powder, determining the trapping of volatile substances and masking the taste, prepare controlled and sustain release , sequential release of various active ingredients, reduce poisonous/toxic side effects, and improve solubility of hydrophobic ingredients and boosting bioavailability and effectiveness. The present review not only enlists several works on microencapsulation and controlled release of thymol, but also opens up newer pathways for formulation methods for PAIs. Thus the broad scope of this work is to study the microencapsulation of thymol in naturally occurring and biodegradable polymers.

1.6 Objective of the thesis

The objectives of the thesis are given below

i. To investigate suitable encapsulation techniques (Emulsion solvent diffusion (emulsification diffusion), Nano-precipitation) for thymol.

ii. To optimize the important process variables (Process time, stirring speed for synthesis (RPM), Polymer concentration, surfactant concentration) for encapsulation efficiency and controlled release by Emulsion solvent diffusion and Nano-precipitation techniques and their comparative study.

iii. To study the sustained release of thymol from Sodium Alginate beads synthesized by emulsion solvent diffusion microencapsulation.

1.7. Executive Summary

The main objectives of this work are to study the effect of concentration of thymol (the core material), and that of sodium alginate and ethyl cellulose (the shell material) on encapsulation efficiency of thymol in biodegradable polymeric shell through the methods such as emulsification solvent diffusion and nanoprecipitation, and to determine its controlled / sustained release. This PhD Thesis will help in understanding the effective techniques for encapsulation of thymol in biodegradable polymers with their control release and encapsulation efficiency.

CHAPTER – 2

Literature Review

2.1. Introduction

The comprehensive literature review on encapsulation of thymol through various techniques has been reported in this chapter. The entire literature review is based on the theme of encapsulation techniques, biodegradable polymers, control release of thymol and various therapeutic properties of thymol. Literature on emulsification technique followed by its review on nanoprecipitation technique has been discussed thoroughly. In emulsification process, solvent diffusion with different single and double solvent diffusion techniques like O/W, W/O, O/W/O, W/O/W has been accounted. In O/W single emulsion solvent diffusion technique, the polymer is mainly dissolved in an appropriate water miscible organic solvent, and drug solution is emulsified in this polymeric solution. On the other hand, in O/W solvent emulsion technique the oil (organic solvent) is the dispersed medium at the same time as water is serves as continuous aqueous medium. In emulsification technique encapsulation product is mostly obtained in the form of beads or miroparticles.

2.2. General Background

The important issues ought to be take in to consideration whenever encounter with encapsulation of Pharmaceutically Active Ingredients; some of them are health compatibility with the biodegradability of shell material, half life period of active ingredients and information on microstructure formation. Formulation refers to when diverse compounds such as inner active ingredients in core along with outer shell materials will together deliver a therapeutic compound or pharmaceutical drug. Several important applications of microencapsulations are controlled release, a crucial preservation methodology, efficient storage system, and regulated release mechanism for diverse Pharmaceutically Active Ingredients, different food materials, beauty products and other pharmaceutical commodities. Volatile structure, significant reactivity, and low shelf life of active material are some of the logic behind choosing the technique of encapsulation. Various thought process for encapsulation of critical ingredients in the pattern of shell-core or matrix particles has been researched; for example for example security from oxidative disintegration and vanishing, protection of essence, or simply to ensure

controlled release. So as to adjust for various sorts of pharmaceutically active ingredients and shell materials distinctive microencapsulation techniques have been created, producing particles with adaptable shell thicknesses, tailor made capsules, range of sizes and penetrability, and give a device to change the discharge pace of the pharmaceutically active ingredients [65].Various parameters and conditions at the time of synthesis of microparticles such as temperature, concentration ratio, blending rate, types of solvents used has a significant impact on the shell of polymer produced around the core material. The goal of the encapsulation is to controlled delivery of the PAI captivated in the shell. However encapsulation can be organised as per the choice and need of the user. Advancement in pharmaceutical, food, cosmetics, biotechnology with the incorporation of microencapsulation though innovation, promoted useful attributes and hence added significance to products. Keeping this perspective it is noteworthy to promote state-of-the-art techniques, or streamline existing ones for encapsulation of active ingredients for pharmaceutical, food and biotechnological industry, as a consequence strengthening towards inventive and included importance to the products in response to human desires and requirements.

Encapsulation techniques and framework for core and shell:

When a small sphere surrounded by wall homogeneously covered around it is called microcapsule. Material inside the capsule is also called as inner phase, centre, core or fill, while the cover is referred to as shell, coating, outside layer, film. Most of the microcapsules diameters are in the range among micrometers and millimeters [66]. When a small molecules or fluid droplets trapped inside the covering the technique is known as microencapsulation [67]. Normally, the most reduced molecule size of microcapsules is 1μm and the biggest size is 1mm. Microcapsules comprise of a center and a divider (or shell). The association of the center can be a round or irregular molecule, a hard suspension to a liquid phase, sturdy network, discrete strong and totals of solids or liquid forms.

Objectives during encapsulation of PAI may be several, including supervised discharge, focused targeted administered deliverance, conservation/safety, commercial usage, handy packaging, and clever choice for repository, simple portability and formulation, alteration/shielding unwanted attribute such as savour, flavor, smell, and contact. The capsulated active agent can be delivered by varied driving processes, such as, mechanical, hotness, scattering or dispersion, pH, biological degradation and disintegration. Many

packaging methods are based on the central part (fluid or solid fine particles appearance) and are enveloped via carriers in a vaporous or fluid stage by applying different physic substance strategies [68]. For ease of comparison, various physical and chemical techniques of microencapsulation are given in tabulated form [69] (Table 2.1) the other literature is reviewed in conventional manner pointing out salient features of all the research works perused for the purpose of the present work.

Table 2.1: Different Encapsulation Techniques

Techniques	Mechanism	(Core) and shell
Spray-drying	The procedure includes atomization of a fluid into splashes of beads with an atomizer with spray nozzle. Emulsions and suspensions can be utilized for spray drying. The beads quickly lose moisture and dry when they encounter a stream of hot gas. The drying happens in a protected chamber.	(Aroma, Food) herbal gum Arabic, alginates (anhydrous milk) whey protein, sodium caseinate [70]
Fluid bed coating	The liquid bed coating process comprises of showering a covering arrangement into a fluidized bed of strong particles. The principle parameters influencing the procedure are flow rate and pressure of the spraying liquid, composition and rheology of the covering solution, stream rate and temperature of the fluidizing air.	(Pharmaceuticals, probiotic cells) maltodextrins[71]
Spray chilling technology	The process of solidifying an atomized liquid spray into particles is called Spray chilling. Also known as spray cooling and spray congealing technology, or prilling, this process is suitable for making particles from a few microns to several millimeters.	(Minerals) lipid carriers such as wax [72]
Emulsification	It is two phase system, organic phase separated from aqueous phase. The knowledge of two phases, interface, and emulsifiers are required for proper formulation and design of system. Various ways such as homogenizer, high shear mixer, and high speed stirrer are commonly used in emulsification	(drug, pharmaceutically active compound) PLA, PLGA [73]
emulsions with multilayer	Multilayer emulsions are framed by adding polyelectrolytes to an emulsion containing oppositely charged beads so they adsorb and structure a nano-overlaid covering. This technique can be repetitive a numeral of times to shape multilayer coatings around the oil beads.	(soy oil) β-lactoglobulin (βLG) and pectin layers. [74]

Techniques	Mechanism	(Core) and shell
Coacervation	Coacervation method is essentially used for encapsulation of hydrophilic molecules and so for proteins. The technique employs encapsulation of proteins and peptides within the polymer-rich segment acquired via liquid–liquid phase separation of charged macromolecule from organic solvent.	(Essential oil, linseed oils)chitosan. [75]
microspheres via extrusion or dropping	When a core active substance encapsulating in the biopolymer gel network it is called as microspheres. It is generally prepared inside the presence of the active; but post loading of blank microspheres containing oil droplets	(probiotics, pharmaceutically active agent, enzymes) Calcium-alginate gel. [76]
microspheres via emulsification	When in the emulsion of alginate solution and vegetable oil by adding calcium chloride solution breaks the emulsion and formation of micro beads takes place. By means of the gelation of the alginate beads or as alginate calcium (in the shape insoluble, alongside calcium carbonate) might be found in water emulsion.	(Vegetable oil) chitosan, gelatine. [77]
Co-extrusion	During co-extrusion encapsulation, one of the above core solutions from a glass syringe and the shell fluid (1 % alginate solution) from a plastic syringe were extruded simultaneously through the nozzle of the encapsulator into droplets with an outer (shell) and inner (core) structure.	(probiotics dispersed in oil) calcium-alginate or potassium-carrageenan. [78]
Inclusion complexation	Incorporation complexation, which is the development of host–visitor consideration complex by weak intermolecular communication, has been demonstrated to be a promising strategy in upgrading solvency and bioavailability of inadequately water dissolvable medications	(thyme oil,) hydroxyproply-β-cyclodextrin. [79]

Techniques	Mechanism	(Core) and shell
Liposome entrapment	Liposomes have an exceptional vesicular structure. These vesicles are made out of a lipid bilayer that structures looking like an empty circle including a watery stage. All things considered, any payload of interest can be capsulated inside liposomes in either the aqueous compartment (in the event that it is water-dissolvable/hydrophilic) or inside the lipid bilayer (if fat-solvent/lipophilic).	(lecithin) cholesterol. [80]
Rapid Expansion of Supercritical Solution (RESS)	Rapid Expansion of Supercritical Solution (RESS) is utilized to deliver fine particles for the nourishment, beautifiers, and pharmaceutical ventures. Natural material is broken down in supercritical carbon dioxide and is utilized to quickly grow the supercritical arrangement through expansion nozzle.	(Proteins or volatile flavours) cellulose, hydroxypropyl methylcellulose. [81]
Freeze Vacuum Drying	a procedure whereby an item is dried under low temperature and vacuum. The water in the sample is first solidified to a solid and afterward evacuated straightforwardly by transforming the ice into fume. This is done under vacuum and without going through the fluid stage.	(Fish oil) Gum Acacia and Sodium Alginate. [82]
Interfacial Polycondensation	Two reactive monomers that are soluble in their respective immiscible phases come into contact at the interface. The process consists in the dispersion of one phase containing a reactive monomer, into a second immiscible phase to which is added a second monomer. Both monomers react at the droplet surface (interface), forming a polymeric membrane.	Polyurethanes, polyamides, polyureas, polysulfonamides and polyphenyl esters. [83]

2.3 Review of Literature:

2.3.1. Encapsulation of thymol:

Nieddu M. et al., (2014) have assessed encapsulation of thymol in a shell made of Cyclodextrin (CD) alongside thepolymer was synthesized fromDimethyl Aminoethyl Methacrylate (DMAEMA). Enhancing taste-masking, solubilisation, and powderisation properties are the intentionbehind encapsulation. Sealed-heating and co-precipitation techniques are used for thymol-beta cyclodextrin complex. They demonstrates with the purpose of undesirable sensorial properties of thymol can be hiding by incorporating thymol in formulation established on a complex of Eudragit® EPO with cyclodextrin; the formulation can be boosted to feed supplements of a model diet. Products of Sealed heating techniques had been acquired via sealing combinations of thymol and beta-CD. The consideration complex arranged via fixed warming, utilizing a 1:1 molar proportion amongthymol and beta-CD, can expand the disintegration pace of thymol, which breaks up gradually in Gastro-Intestinal (GI) replicated liquid; this impact is because of the outstanding impact of beta-CD going about as a solubilised of substances which are inadequately water solvent. Encapsulating thymol in beta cyclodextrin using sealed heating technique shows proper methodology with incredible loading capability; thymol volatility is control by means of blending the complex in with the DMAEMA copolymer. Beta Cyclodextrin quickens the in vivo thymol retention rate contrasted and the free medication; the thymolhalf life is still long [84].

Rassu G. et al., (2014) discover the main purpose behind the thymol encapsulation was formulations for universal and limited delivery of herbal drug as a alternative to existing drugs to treat numerous human and animal infections. The thymol encapsulation with diverse biodegradable polymers for hiding undesirable taste and expand its tastefulness.

The natural biodegradable polymers hydroxypropyl methylcellulose phthalate (HPMCP) and methylcellulose are used for thymol encapsulation. Spray drying techniques to be used to synthesised microspheres. It is noticed that the half life time declines however its bioavailability raises intensely contrasted with encapsulation in HPMCP. Consequently, it is recommended for thymol in low doses mode for efficient management, and in alternative situation, for local treatment of intestinal diseases because of remarkably limited penetration rate. [85].

Bose A. et al., (2019) studied the necessary relations between thymol with α and β cyclodextrins (CDs); which are successfully recognized drug delivery agents. The measure

of encapsulation has been established by utilizing several spectroscopic facilities. Interpreting the data also reveal impression of binding of Thymolalong with the β CD. The molecular structure exhibited that thymol successfully trapped inside both with α and βCD. In NMR studies and molecular dynamic ultimately concluded that solvated thymol molecules are the main participants in the interaction with CDs which is responsible for these fascinating natures. Antioxidant assay performed to reveal the practical application of such encapsulation. It has been discovered that on encapsulation there is an improvement of the antioxidant activity of thymol. Antibacterial assay demonstrate the consistent antibacterial properties of thymol on encapsulation. Hence thymol can be safely delivered through CDs in living organization without inhibiting its valuable properties. [86].

Yibo Zhang et al., (2019) in their present work demonstrated the method of coaxial electrospinning in which core–shell nanofibers prepared with thymol as an antibacterial agent. Thymol encapsulated into the poly (lactide-co-glycolide) by means of nanofiber film the growth of bacteria on the surface of food restrained with the controlled release of thymol takes place in between nanofiber and food. Nanofiber film for fruit preservation and antibacterial activities tested on strawberries. Experiments revels the the growth of bacteria, fungi, and yeast are successfully and effectively inhibited and extend the shelf life of fruit. This innovative antibacterial packaging material with great biocompatibility, biodegradability, and exceptional sustained discharge behavior would have a extensive application probability in the area of food preservation [87].

Peggy A. et al., (2010) outlined the manufacturing of thymol and cinnamaldehyde incorporation composites with b-cyclodextrin (b-CD) after blending the parts in liquid intermediate. Co- precipretation techniques are used to form compound of thymol and cinnamaldehyde. The work examined the connection between the sorption attributes of b-CDs and multifaceted compound shaped with thymol and cinnamaldehyde and their release. The result demonstrated that the incorporation compound between thymol-b-CD and cinnamaldehyde-b-CD remain stable during long repository occasions. By and large, the dynamic active ingredient releases from the b-CD incorporation compound were distinguishable in the zone of the water adsorption isotherm at which a quick increase of water content happened the release of dynamic atoms was in this manner administered by the type of the water sorption isotherm [88].

2.3.2. Controlled release of thymol:

Isabel M. et al., (2012) examined the release of thymol and p-cymene employ as center components within Poly Lactic Alcohol (PLA) microcapsules. Coacervation process was employed to get the microcapsules. The outcome revealed that the released of thymol and p-cymene is speedy in the main hour managing controlled release of thymol approximately constant in the subsequent times. The release reviews pointed out for a slower release rate for thymol when used as single center material. Diffusion method clarified the release of oils from the PLA microcapsules [89].

Antilisterial activity of thymol:

Dan Xiao et al., (2011) developed Spray-dried microparticles from zein with the equal concentration of nisin and thymol but with changing the concentration of Tween 20. They acknowledge that the non-ionic surfactants can efficiently raise the antimicrobial action in food materials. Spray drying is a most advanced technology for the manufacturing of antimicrobial particles, which is accomplished with using surfactant such as Tween 20. Microstructure of capsules and release properties of encapsulated antimicrobials can hamper by inclusion of intrinsic surfactant hence impacting interactions with capsule constituents [90].

Kang Pan et al., (2013) Sodium caseinate polymers with high shear homogenization techniques utilized for encapsulation of thymol. The straightforward scattering at impartial pH was steady for 30 days at room temperature .The marginally diminished molecule measurement during capacity shows the nonattendance of Ostwald ripening. The typified thymol demonstrated the altogether improved antilisterial movement in milk with various fat levels when contrasted with thymol gems, coming about because of the snappier blending and expanded solvency in the milk serum. The straightforward thymolnanodispersions have promising applications to improve microbiological security and nature of nourishments. [91].

Antioxidant activity of thymol:

C. Liolios et al., (2009) successfully encapsulated thymol and other components embodied in phosphatidyl choline-based liposomes. It was previously extracted by hydro-refining method. On selected microorganism their antioxidant and antimicrobial actions was tested. dispersion technique were used to check antimicrobial properties of the oils against four

gram positive and four gram negative pathogens and three human pathogenic parasites, just as the nourishment borne microorganism, Listeria monocytogenes. In application to investigate all conceivable unpleasant outcomes between thymol/carvacrol and c-terpinene/carvacrol, the antimicrobial exercises of the mix were likewise dissected when liposome epitome. Every tried composite introduced propelled antimicrobial conduct after the exemplification. The cancer prevention agent movement of the blends: carvacrol/thymol and carvacrol/c-terpinene was assessed utilizing Differential Scanning Calorimetry (DSC) ahead of time of and after embodiment in liposomes. [92].

Mina D. et al., (2017) his research experiments revels dispersion of potato starch in thymol in order to enhance the useful property, though potato starch itself shows of antioxidant and antibacterial activity but in presence of polysorbate and thymol in combination it shows great effect against microorganism. The antibacterial activity of thymol can be endorsed when encapsulated in starch chain. It also causes a decrease in viscosity, particle size and significantly increases zeta potential. The encapsulated polysorbatethymol shows decline in tensile force, swelling along with stiffness it also enhance the elasticity, permeability with solubility of starch. The experiments also carried out in dispersion of polysorbate, citric acid, glycerol and potato for encapsulating thymol. The Starch polysorbate, citric acid, glycerol and potato formulation had extremely small antioxidant and antibacterial action, but it reveals strong antioxidant and antibacterial capacities when including thymol. Eventually, the revised antibacterial / antioxidant capability of starch dispersal recommend that it is probable function as excipients in many edible materials [93].

Antimicrobial activity of thymol:

Yuhua C. et al., (2012) arranged stable nanoemulsion of thymol oil in combination with ripening inhibitor has been successfully tested as a prominent antimicrobial agent. The thyme oil in water nanoemulsion was extremely unsteady for phase separation and droplet growth, hence physically stable nanoemulsion could be the answer and formulated with thyme oil and ripening inhibitor like Medium Chain Triglycerides (MCT) / corn oil. Types of ripening inhibitor along with their concentration had significant effect to the antimicrobial activity of the thyme oil nanoemulsion. However mixing the inhibitors diminishes the antimicrobial action of the thyme oil in the nanoemulsions. The maturing inhibitor category and fixation experienced an apparent effect with antimicrobial

movement in nanoemulsions of the thyme oil. When all is said in done, expanding the maturing inhibitor levels in the lipid stage diminished the antimicrobial viability of the nanoemulsions, with the impact contingent upon aging inhibitor type. At a similar fixation in the lipid stage, MCT diminished the antimicrobial viability of thyme oil more than corn oil. It is along these lines critical to enhance the maturing inhibitor type and level to augment both physical soundness and antimicrobial action [94].

Bhavini Shah et al., (2012) fabricated Whey protein isolate–maltodextrin conjugates are used for thymol encapsulated transparent Nanodispersions (ND). The main aim for this study is to illustrate antimicrobial performance of nanodispersion against free thymol. Tests were led for Escherichia coli at different pHs and temperatures. Results show that the MIC for nanodispersed and free thymol against all strains of both gram-negative and gram-positive pathogens tried. No noteworthy impact of temperature on antimicrobial action was watched. The present investigation exhibited that straightforward nanodispersions of thymol have promising antimicrobial action against an expansive range of foodborne pathogens. ND thymol stayed useful against both gram-positive and gram-negative microbes [95].

Ulloa P. et al., (2017) fabricated Matrices from soy protein (SP) and maltodextrin (MD) are being used to encapsulate the characteristic antimicrobial agent. The possible activities were assessed for food packaging coatings. Microcapsules were set up by oilin-water (O/W) emulsions at various focuses. Comparative outcomes were acquired for carvacrol with a similar centralization of MD. Korsmeyer–Peppas and Weibull numerical models were effectively fitted to the arrival of the AM specialists, portraying the Fickian dissemination arrival of the parts. Distinctive discharge rates were gotten as a component of the synthetic idea of the exemplification material and its focus [96].

Antispasmodic activity of thymol:

Jonas E. et al., (2012) extracted thymol fluid concentrate by High Pressure Liquid Chromatography (HPLC), Low Pressure Liquid Chromatography (LPLC) and Fast Centrifugal Partition Chromatography (FCPC) composite separated were recognized with spectroscopic procedures. Bioassay testing was done by estimation of antispasmodic activity in the preconstricted rat. Thymol-denied Spissum Extract (SE) had extraordinary antispasmodic activity. Fractionation guided by bioassay exhibited that rosmarinic destructive and apigenin didn't include with this effect. Luteolin basically added to

anticonvulsant development. Thyme expels have antispasmodic development, which is at any rate a result of synergistic effects of phenolic precarious oil blends and the flavone luteolin. Conclusions of thyme-containing courses of action should insinuate this flavone despite focusing on the unsteady phenols [97].

Anti-inflammatory activity of thymol:

K. Riella et al., (2012) explored to evaluate the anti-inflammatory and wound recuperating exercises of thymol in rodents. They showed that thymol displays mitigating impacts and the inclusion of this monoterpene into collagen-based dressing films was fruitful in improving injury soothing. Along these lines, the pharmacological activities of Lippiagracilis in famous prescription practices might be identified with the existence of thymol as significant segment in the basic oil. Consequently, we recommend that thymol is a promising compound to be utilized in treatment of provocative procedures just as scarhealing [98].

2.4. Knowledge gap:

With various techniques for encapsulation of thymol in solid microparticles, liposomes, nano-and microemulsions, and polymeric nanoparticles addresses a hopeful procedure for beating thymol's limitations, cutting down their segment and growing long stretch security of thymol. Small doses of thymol are additionally seen as successful to bring down its long haul symptoms on different pieces of body.

In terms of purity of product and strength Standardization is demanding. Technique of Microencapsulation formulation can strengthen an impressive opportunity for thymol administration in comparatively large or tiny dosage depending upon remedy.

Thymol potentially for keeping up and promotes healthiness, also prohibiting and probably treating some diseases. Nonetheless, the most important part low water dissolvability, stability as the high instability and reactions related with their utilization has restricted their utilization in medication. Encapsulation is advance technique that has prospective function in drugs, biotechnology, and health research. A efficient and substitute technique for microencapsulation is a sol-gel silica base that takes place at an ambient temperature where the disintegration of mixtures is economical in light of the fact that capital interest underway is low and naturally amiable. Encapsulation is particularly helpful instrument for expanding the substance solidness in the presence of light, air, humidity, and raised

temperatures. apart from it , the microparticles allow smoother and secure managing of fluid materials by modifying them into hard fine particles, regulating the trapping of unstable/volatile materials and hiding the taste, make targeted release and/or subsequent release of diversify pharmaceutically active elements, minimize harmful side reactions, and upgrade water solubility of hydrophobic elements and strengthen bioavailability and accomplishment. The current literature survey not solely engages different endeavors on microencapsulation and controlled release of thymol, but in addition to opens up recent pathways for formulation processes for PAIs.

From the above literature review it has been observed that thymol has a great potential as a drug and its formulation needs to be systematically studied. The encapsulation technique of thymol has not been experimentally and extensively studied by research community in chemical engineering context. From the process synthesis point of view, there is definitely a need for taking investigative approach and conducting an experimental study on thymol encapsulation and go deeper in to controlled release part of it. Therefore, the present work deals with thymol encapsulation. As a choice for the shell material, Ethyl Cellulose and Sodium Alginate are used because they are easily available, relatively cheap and essentially biodegradable. As to the process selection aspect, emulsification solvent diffusion, nanoprecipitation and polymer bead formation methods have been selected because of their ease of operation and scalability. We believe that the work adds to enrich the technical literature as regards to thymol formulation in pharmaceutical world.

CHAPTER – 3

Materials and Methodology

As per the objectives planned and the gaps found from the literature review on thymol formulation, mainly two types of experiments were planned and conducted.

i. Experiments for thymol encapsulation
ii. Experiments for thymol release from polymer shell

While there are several encapsulation methods as described in the earlier chapter, are in practice, we have studied in detail the following one because of their less complexity, ease of operation, and greater possibility of easier scalability of operation.

a) Emulsification Solvent Diffusion Method
b) Nanoprecipitation techniques
c) Emulsified beads techniques

As regards to the controlled release part of the study, a standard approach as described in literature [90] has been followed.

This chapter covers details of the above along with materials, equipment, instruments and sequences of various steps in all experiments.

In order to check the reproducibility of the experimental data all the runs reported here were repeated in entirely.

3.1 Materials

Thymol (refined Thymol crystal 99% Extra Pure, M.W. 150.22), Tween-80 (Tween -80 Extra Pure, Poly oxyethylene (20) sorbitan monooleate) are procured at Loba Chemie, Mumbai, India. EC (ETHOCEL™ High Productivity (HP) is acquired from Colorcon, India. Ethanol, sodium alginate, Calcium Chloride dehydrates, DCM (Organic solvents) and all other chemicals, and excipients are of analytical standards and are purchased from Merck limited, Mumbai, India. Tween 20 was purchased from SDFCL, Mumbai, India. Glutaraldehyde 25% solution (v/v) solution was purchased from Rankem, Mumbai, India.

The main objective of this study is to prepare thymol loaded EC nano particles by using Emulsification Solvent Diffusion Method, Nanoprecipitation techniques, and Emulsified beads techniques. In the following description thymol is the drug being encapsulated in the polymer shell of EC. The organic solvents used are ethanol and dichloromethane.

In the experiments several parameters which effect the properties of the micro particle includes

a) Drug (PAI) solubility in the solvent
b) Drug to polymer ratio
c) Microencapsulation efficiency
d) Control release of thymol

Analytical Techniques used for analysis, characterization of micro particle are

a) SEM
b) Particle size analysis (Zeta Potential)
c) FTIR
d) XRD

3.2. Experiments for encapsulation

3.2.1. Techniques of encapsulation

Emulsification Solvent Diffusion Method

Preparation of micro-capsules by the Emulsification Solvent Diffusion method allows both lipophilic and hydrophilic active substance encapsulation. Thymol loaded EC nanoparticles were formulated by single emulsion (O/W) solvent diffusion technique. Firstly, few trial batches for different polymer to drug ratio with varying concentrations of surfactant were done. For this purpose, 400 mg of thymol and desired quantity of EC (according to design) was dissolved in 10-15 ml of ethanol (solution A). The continuous aqueous phase was prepared by dissolving tween 80 (0.2%, 0.6%, 1%) in 100 ml of distilled water (Solution B). Solution A was added drop wise in the solution B under constant stirring 6500 rpm (±10) using T 25 digital ULTRA-TURRAX high speed homogenizer using a syringe. The homogeneous solution was stirred magnetically at room temperature (28 ^{0}C) for 2 h at a speed of 400 rpm (±5) to evaporate the organic solvent. After evaporation of organic solvent (ethanol), the suspension was formed. Obtained suspension was filtered using Whatman filter paper to obtain the particles and dried to room temperature for 48 hours the schematic representation of this process is shown in Figure 3.1 & 3.1 (a).

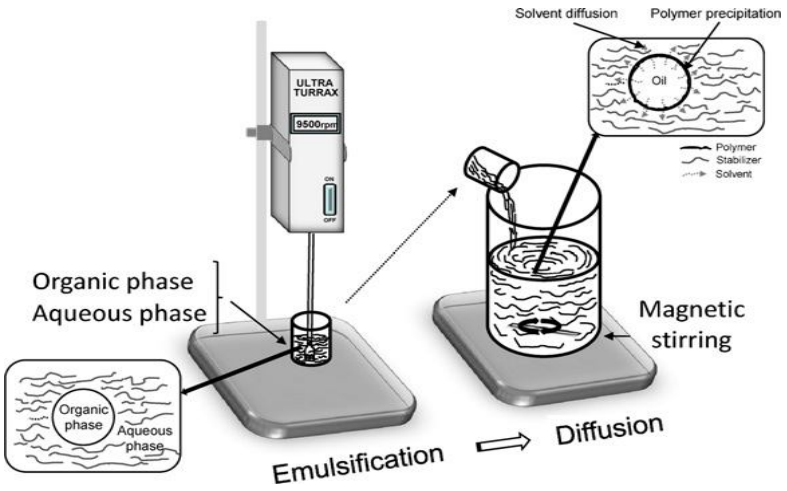


Fig 3.1:Set-up used for preparation of micro-capsules by the emulsion–diffusion method.

Steps by step experimental procedure:

Drug (Core Material-PAI): Thymol

Polymer (Shell Material): Ethyl Cellulose (EC)

Stabilizer (surfactant): Tween-80

Organic solvent (for oil phase): Ethanol

Organic phase (Oil phase): it consists of thymol, EC and ethanol in proportion of 6 mg thymol +6 mg EC+ 5 ml Ethanol.

Aqueous Phase (Stabilizer solution): (1 %) the solution was prepared (v/v) in proportion of 1 ml of Tween-80 is added in 99 ml of distilled water.

For batch size of 20 ml and 1:1 ratio of drug: polymer the initial experiments were conducted

Step I: 6 mg of thymol is added with 6 mg of EC and the mixture is dissolve in 5 ml of ethanol.

Step II: keep the solution for five minutes in Ultrasonic Bath (Sonicator) for complete dissolution/ homogenisation of the solid particles in solvent ethanol.

Step III: 15 ml of aqueous phase (1 % Tween-80) was taken in to another beaker.

Step IV: now organic phase was slowly added to aqueous phase under the constant stirring using T 25 digital ULTRA-TURRAX high speed homogenizer at 6500 RPM(±10).

Step V: thymol, EC and ethanol mixture (oil phase) is emulsified in aqueous phase to form an oil in water emulsion.

Step VI:oil phase Nano particles are formed and emulsion was stabilized which will be shows with light pale blue color.

Step VII: freshly prepared stabilized particles are taken for particle size analysis in MARVERN zeta sizer.

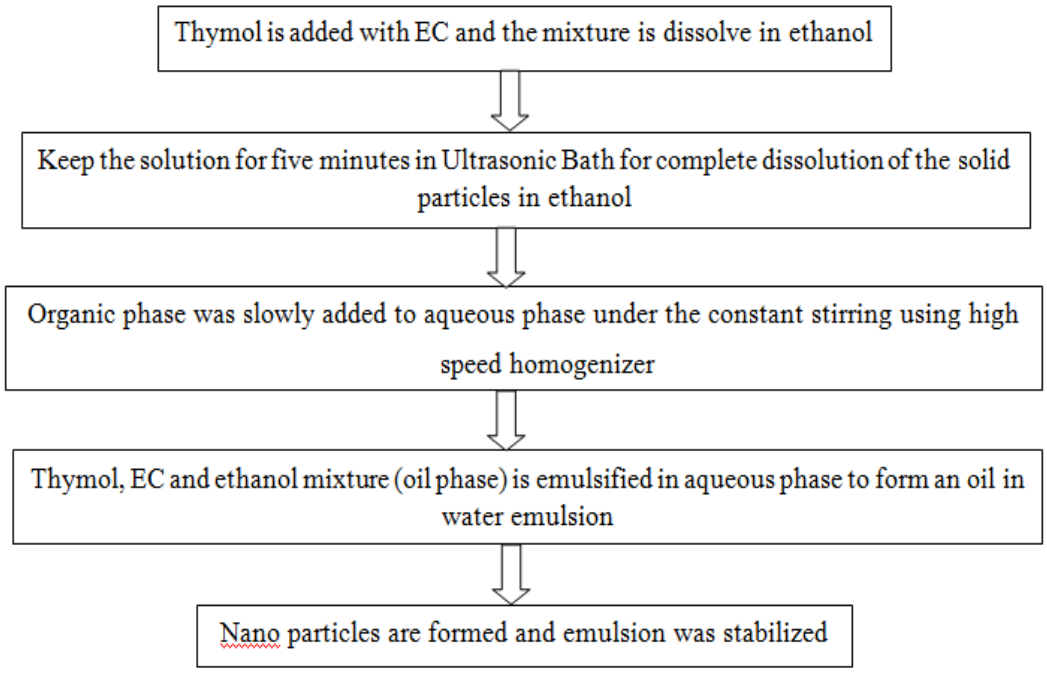


Figure 3.1(a): The schemes/flow chart of preparation of particles using Emulsification Solvent Diffusion Method

Nanoprecipitation techniques

Ethyl cellulose nanoparticles loaded with thymol get ready by means of the nanoprecipitation technique using the same concentrations of drug and other excipients in the above method. In this technique, diffusion phase (Solution A) was prepared by dissolving the drug and polymer in DCM (Dichloromethane). It was subsequently inserted into Tween-80 and distilled water solution (aqueous dispersing medium) with a syringe at 700 rpm. The polymer (EC) deposit forms a colloidal suspension resting on the interface among the aqueous and the organic solvent. The colloidal particles are isolated by filter paper and desiccated for XRD, SEM, and FTIR analysis [99].

Steps by step experimental procedure:

Drug (Core Material-PAI): Thymol

Polymer (Shell Material): Ethyl Cellulose (EC)

Stabilizer (surfactant): Tween-80

Organic solvent (for oil phase): Dichloromethane

Organic phase (Oil phase): it consists of thymol, EC and Dichloromethane in proportion of 500 mg thymol + 500 mg EC + 10 ml Dichloromethane.

Aqueous Phase (Stabilizer solution): (1 %) the solution was prepared (v/v) in proportion of 0.5 ml of Tween-80 is added in 50 ml of distilled water.

For batch size of 50 ml and 1:1 ratio of drug to polymer the initial experiments were conducted.

Step I: 500 mg of thymol is added with 500 mg of EC and the mixture is dissolve in 10 ml of Dichloromethane.

Step II: keep the solution for 5 minutes in Ultrasonic Bath (Sonicator) for complete dissolution/ homogenisation of the solid particles in solvent Dichloromethane.

Step III: 50 ml of aqueous phase (1 % Tween-80) was taken in to beaker.

Step IV: the organic phase was slowly added by syringe to aqueous phase under the constant stirring using stirrer/homogenizer at 700 RPM.

Step V: micro particles are formed from the small droplets in to tiny particles with continuous stirring for 2 hr.

Step VI: after 2 hr, the solution is filtered, dried and for XRD, SEM, and FTIR analysis.

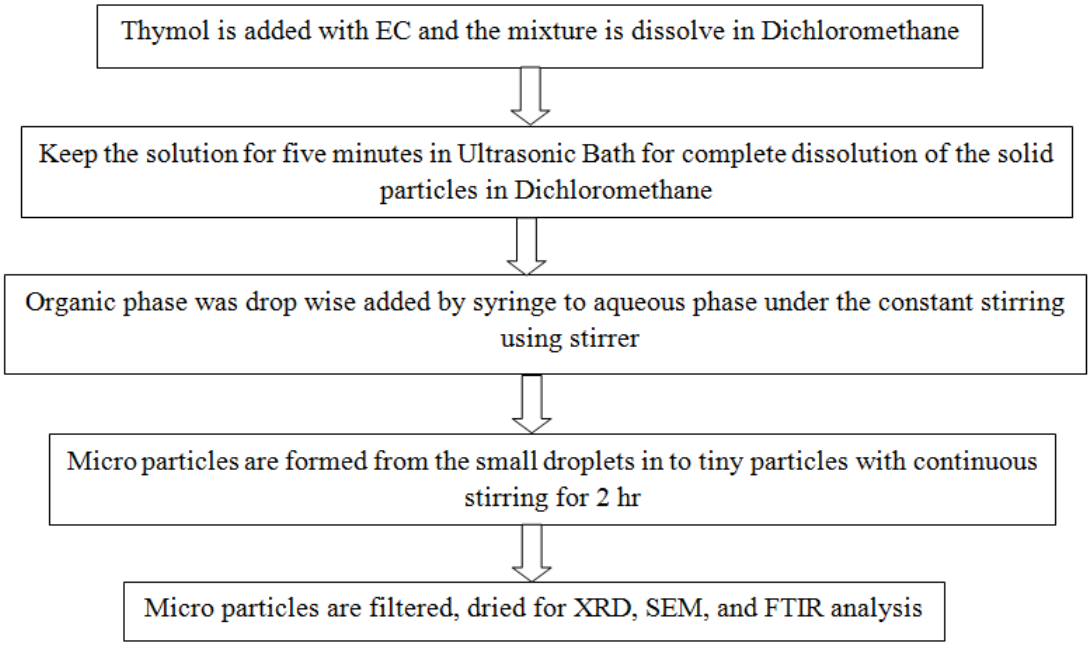


Figure 3.2: The schemes/flow chart of preparation of particles using Nanoprecipitation method

Emulsified beads techniques (Calcium-alginate emulsified Beads)

2% and 4% of sodium alginate powder was dispersed in 100 ml hot water for 1 hr at 70-80^{0}C. Thymol (100-200 mg) pure drug was first dissolved in hot water (10 ml, 50 ^{0}C) and then added to a solution of sodium alginate (2 - 4%) and homogeneously dispersed at 50 ^{0}C. An air-bubble free suspension was forced through a 0.8 mm needle into 200 - 400 ml of the calcium chloride solution (0.2 M) at a flow rate of 10-20 drops/min. The mixture was stirred using a mechanical stirrer at 400-500 rpm for 90 min. Alginate gel beads were allowed to stand in calcium chloride solution for 24 hr until they fully grew. The beads were then separated by filtration through Whatman filter paper to obtain the particles which were washed three times with 100 ml deionised water, and allowed to dry at room temperature for 24 hr. The prepared beads were analysed by XRD, SEM, and FTIR.

Steps by step experimental procedure:

Drug (Core Material-PAI): Thymol

Polymer (Shell Material): 2% and 4% of Sodium Alginate (SA)

Stabilizer (surfactant): Tween-20, Calcium Chloride

Organic solvent (for oil phase): hot water

Organic phase (Oil phase): it consists of thymol, SA and Hot water in proportion of 100 mg thymol + 2% of SA + 0.5 ml glutaraldehyde as cross linking agent.

Aqueous Phase (Stabilizer solution): (0.2 molal) solution of calcium chloride was prepared (v/v) + 1 ml of Tween-20 +200 ml of distilled water.

Sodium alginate solution (2%): it is prepared by dispersing 2 gm of sodium alginate powder in 100 ml of water having temperature of 70-80 ^{O}C.

Step I: 100 mg of thymol is added with 2% of SA and 0.5 ml glutaraldehyde

Step II: keep the solution for 5 minutes in Ultrasonic Bath (Sonicator) for complete dissolution/ homogenisation of the solid particles.

Step III: ice cooled 200 ml of aqueous phase was taken in to beaker.

Step IV: now organic phase was slowly added by insulin syringe (U-40) to aqueous phase under the constant stirring using stirrer/homogenizer at 700 RPM.

Step V: micro particles are formed from the small droplets in to tiny particles with continuous stirring for 2 hr., which will help homogenize the micro particles and achieve uniformity in size.

Step VI: after 2 hr, the solution is filtered, dried and for XRD, SEM, and FTIR analysis.

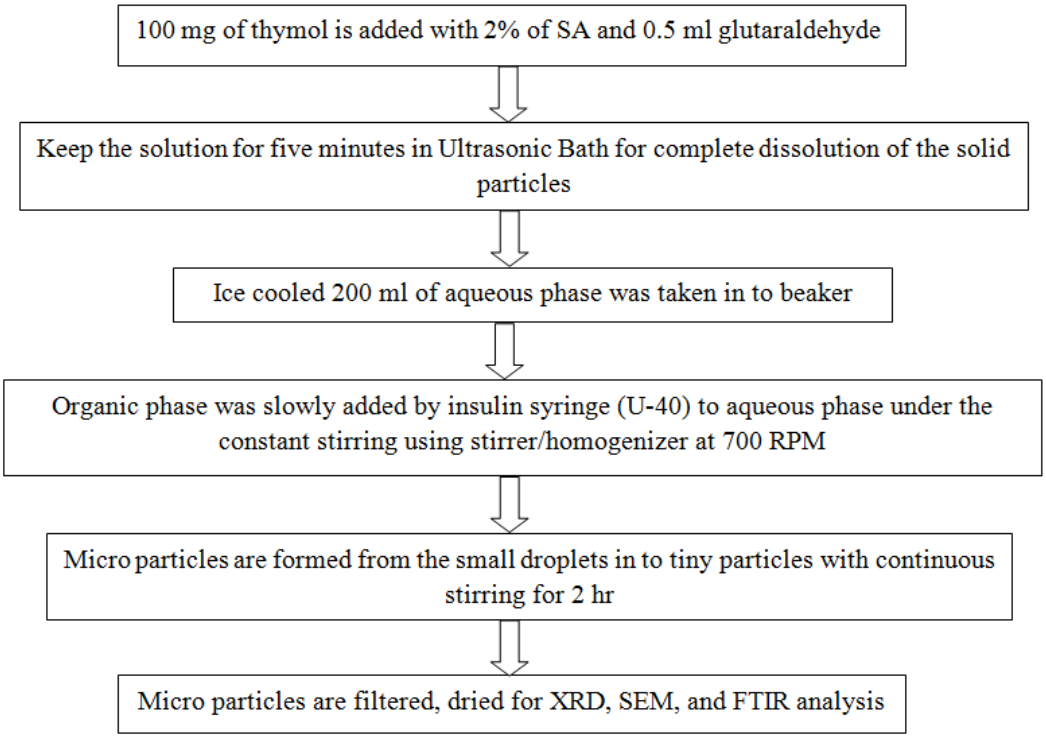


Figure 3.3: The schemes/flow chart of preparation of particles using Emulsified beads techniques

3.3. Experiments for control release

Controlled drug release is one which delivers the drug at a fixed rate, for locally or systemically, for a specified period of time. The controlled release of drug uses drug encapsulating devices from which controlled rate therapeutics can be used for long periods of days to months. Such systems offer many advantages over traditional drug delivery methods, including adjustment of drug delivery rate, protection of sensitive drugs, and increased patient comfort and compliance.

3.4. Characterization equipment and analytical instruments

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) is a sensitive technique used to identify an infrared spectrum of absorption of a solid, liquid and gas. It can also be used to identify both pure and simple mixtures of some inorganic compounds. It accumulates wide spectral range of high- spectral-resolution data. Infrared light from the light source passes through interferometer. The intensity of the interference light is recorded in an interferogram, with the optical path difference recorded along the horizontal axis. To examine the synergy

between the drug and polymer, FTIR range of pure thymol, pure Sodium Alginate and formulations 2% and 4% loaded Sodium Alginate were obtained using Fourier transform infrared spectrophotometer (FTIR 4800: Shimadzu, Asia Pacific Pvt. Ltd., Singapore) using Potassium bromide (KBr) pellet method. The KBr disc is prepared by compressing approximately 40 mg of KBr with 1-2 mg of sample to be analyzed and the spectra were recorded in the wavelength region of 4000 cm^{-1} to 400 cm^{-1}.

X-ray diffraction (XRD) analysis

X-ray powder diffraction (XRD) is a rapid, Non-destructive analytical technique primarily used for determining the crystal structure, physical properties and chemical composition of materials. It is based on scattered intensity of an X-ray beam as a function of wavelength, polarization, incident and scattered angle. XRD provides a wide spectrum for analysing solids; quantitative and qualitative analysis to crystal structure. The crystalline/amorphous nature of thymol, pure Sodium Alginate and formulations 2% and 4% loaded Sodium Alginate beads were examined using X-ray diffract meter. The X-ray tube was operated at voltage 40 kV and current 40 mA with Cu Kα radioactivity (λ=1.5 Å). Sample run was investigated at 2 theta angles in range between 5- 80 ^{0}.The powder diffraction measurements were carried out by using Lab XRD-6100 Shimadzu with Cu-Ka1 radiation.

Field-emission scanning electron microscopy (FE-SEM)

FE-SEM is an electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. It provides ultra-high resolution imaging at low accelerating voltages and small working distances. Electrons interact with atoms producing various signals containing information about composition and surface topography of the sample. The surface morphology and shape of formulated beads were examined by FE-SEM (FE-SEM S 4800, Type-II, Hitachi, Japan) at working distance of 8.6 – 8.8 mm and at accelerated voltage of 10 kV. To obtain the image, the gold coated beads were mounted on metal stub using double side adhesive tape under vacuum. Gold coating is applied to make beads electrically conductive.

Ultraviolet–visible (UV–vis) analysis

The EE and controlled released of encapsulated drug were examined using UV–vis spectrophotometer (Hitachi U2900). 10 mg encapsulated drug as beads mixed with 5 ml

dichloromethane and then extracted in phospapshate buffer solution of pH 6.8. A continuous stirring for 30 min at room temperature was done to facilitate the evaporation of organic solvent. The drug content after suitable dilution was ascertained in UV -vis spectrophotometer at 274 nm. The % EE of thymol was calculated using Eq. 3.1.

$$Encapsulation\ Efficiency = \frac{(amout\ of\ drug\ in\ nanoparticles)}{(intial\ amount\ of\ drug)} \times 100 \ldots \textbf{(3.1)}$$

For drug disintegration testing advanced USP Dissolution -Type II Apparatus at 37 ± 0.5°C and100 rpm with 900 ml phosphate buffer solution having pH 6.8 was performed to determine the drug release from beads. 10 ml test samples from phosphate buffer were taken out at distinct time interims and refilled again with equivalent volume of new disintegration media to keep up the sink condition. It was then filtered through standard filter paper. The thymol composition after appropriate dilution was ascertained in UV -vis spectrophotometer at the standard value λ_{max} of 274 nm using a 1 cm cell.

3.5. Design of Experiments (DoE) for process optimisation

Full Factorial design using Design-expert was used to optimize the process variables i.e. Polymer Concentration, Surfactant concentration in the microencapsulation process of biodegradable polymer. 3 levels 2 factor designs with total 13 runs were carried out for each method to study the outcome of variables on the response. A second order polynomial model was generated to express the responses as a function of the independent factors. The model equation generated was as follows (Eq. (3.2)):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1+i}^{k} \beta_{ij} x_i x_j \qquad \textbf{(3.2)}$$

Where, Y is predicted response, $\beta_o$is intercept, β_i, β_{ii}, β_{ij} is coefficients for linear, quadratic and interaction effect, and x_i, x_j is the independent variables. The significance of the model and the effect of process variables towards the response were determined using coefficient of determination (R^2) and probability value (P-value).

Factorial Design

The 2-Level Factorial Design Builder dialog box two-level full factorial and partial factorial structures. You can research 2 to 21 components utilizing 4 to 512 runs. This assortment of plans gives a compelling way to screening through numerous components to locate the basic few.

Full two-level factorial plans (design) might be run for up to 9 components (factors). These plans allow estimation of every main effects and all collaboration impacts (aside from those confounded with sections.)

Design-Expert offers a wide variety of fractional factorial designs. Design Expert ascertains point by point data on the assumed name structure, which you ought to investigate to be certain that the selected design estimates the interactions of interest.

You will discover the goals, or expected alias pattern of each fragmentary factorial by taking a gander at both colour and documentation on the 2-Level Factorial Design show.

Plackett-Burman designs

Design-Expert offers a selection of Plackett-Burman designs. The number of factors allowed is up to one less than the number of runs (for example 11 factors in 12 runs.) Choose the design with the number of factors equal to or just larger than the number you actually have. Fill in the factor names, units, type, and actual low and high levels. Factors can be specified as numerical or categorical. If you have unused factors, leave them lettered, or name them "dummy". The next screen is the alias structure for that design. The last screen is for the response information. Enter the response names and units. When this is complete, the design will be created.

The Plackett–Burman design (PBD) was used to design microencapsulation experiments minimize the process parameter errors. The calcium-alginate concentrations were selected as independent factor and EE and DR were the response. Drug concentration was kept constant in all the experimental runs [100]. In PBD, 11 most reduced components and 47 most noteworthy elements can be utilized. In most minimal 11 components, 6 definition process factors were researched so as to improve the EE of the globules and staying 5 variables were dummied. PBD plots were made of 12 runs containing variation value of factors. 6 independent variables such as amount of Thymol drug (A, mg), Sodium alginate (B, %), Calcium chloride (C, ml), Mechanical stirrer (D, revolution per minute, rpm), Tween (E, %) and Glutaraldehyde (F, ml) were considered. DoE examines the input data, shows response plots and reveal the effects of variables to response [101].

CHAPTER – 4

Results and Discussion

This chapter summarises the outcomes on various encapsulation and control release experiment of thymol with different biodegradable polymers. The optimization of the important process variables, Polymer concentration on encapsulation efficiency and control release by emulsion solvent diffusion and nano-precipitation techniques was done. The comparative studies of both the methods were discussed in detail.

The comparison between Emulsification Solvent Diffusion Method and Nanoprecipitation techniques for thymol loaded EC nanoparticles is discussed in section (4.1). For method optimization of this study, the statistical design approach with 3 level 2 factors design was used. On the other hand, the formulation and sustained release of thymol from sodium alginate beads synthesized by Emulsified beads techniques of encapsulation studied separately is described in section (4.2). This work focuses mainly on the effect of concentration of thymol, sodium alginate, stirring speed on synthesis, and sustained release properties of thymol loaded sodium alginate beads using emulsion microencapsulation technique. For method optimization of the above study, the statistical design approach using Design-Expert® software was employed.

4.1. Thymol loaded microparticles formulation using Emulsification Solvent Diffusion and Nanoprecipitation techniques

The experiments conducted in this work allow us to compare the characteristics of thymol loaded EC nanoparticles by Emulsification Solvent Diffusion Method and Nanoprecipitation techniques. This work can therefore give certain information about controlled release from the EC shell of nanoparticles of thymol by both the methods. The relation between independent variables and dependent variables can thus be studied in both the techniques. Independent variables selected in this study are the polymer concentration and the surfactant concentration. The dependent variables are Encapsulation Efficiency and Drug Release.

EE is a factor that speaks about the effectiveness of the process (method) of encapsulation. While certain chemical components used in different techniques are different, the sequence of physical operations has its own standing in captivating the PAI in polymer shell of EC.

In all the methods used, the size of capsules and their distribution, their morphological characteristics are largely decided by the physical steps. Therefore EE is taken as one of the important parameter to give adequate idea about the success of the techniques used.

The % drug release and the rate of drug release are the main objectives of the experiments. They give clean idea about the probable use of the product as a formulation, and also give important pointers towards making the process(es) really commercial as the future scope of the work.

4.1.1. Effect of surfactant and polymer concentration on encapsulation efficiency and drug release

The effect of surfactant concentration (0.2 – 1.0 %) at different polymer concentration (400 – 1200 mg) on EE (%) and DR (%) of thymol in Emulsification Solvent Diffusion Method and Nanoprecipitation techniques has been studied. The surfactant concentration effects on EE (%) and DR (%) at 400 mg polymer concentration in both techniques are represented in Figure 4.1(a)-(c) and Figure 4.2(a)-(c) respectively.

In both the processes, for all polymer concentrations, similar trends are observed for EE and DR. both EE and DR increases monotonously with increasing surfactant concentration but the trends of increase for both parameters are different.

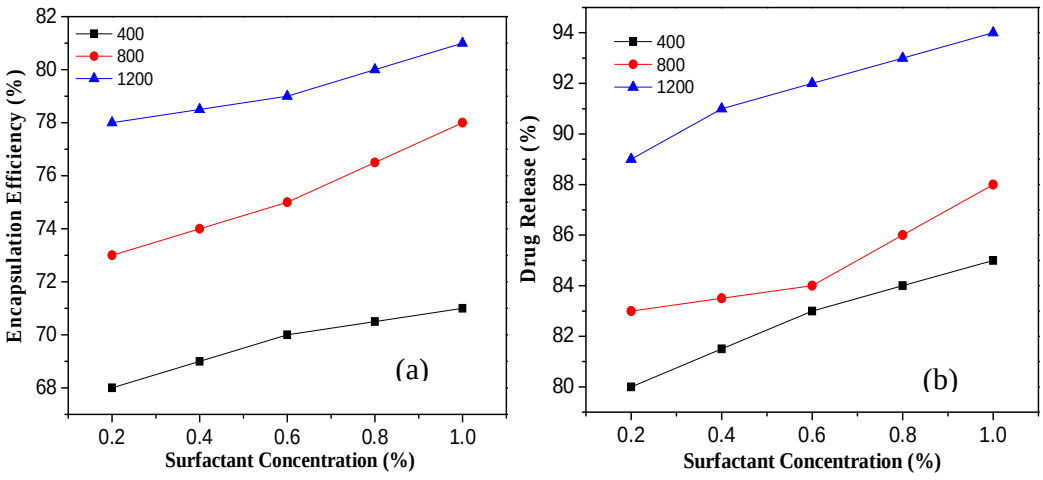


Figure 4.1: Effect of surfactant concentration at different polymer concentration (400, 800 and 1200 mg) on: (a) encapsulation efficiency (%) and (b) drug release (%) in Emulsification Solvent Diffusion Method

Figures 4.1(a)-(b) depicted that as we increase the concentration of the surfactants (0.2 to 1.0 %) the encapsulation efficiency and drug release also increases in emulsification solvent diffusion process. Similarly with increasing the polymer concentration (400 to 1200 mg) the EE and DR also increases in the process. The increase in surfactant (Tween 80) concentration from 0.2 to 1.0 % raise the EE from 68.0 % to 71.0 % at 400 mg polymer concentration; 73% to 78% at 800 mg polymer concentration and 78% to 81% at 1200 mg polymer concentration as shown in Figure 4.1(a). On the other hand, DR increases from 80.0 % to 85.0 % at 400 mg polymer concentration; 83% to 88% at 800 mg polymer concentration and 89% to 94% at 1200 mg polymer concentration as shown in Figure 4.1(b), when we increase the surfactant concentration from 0.2 to 1.0 %.

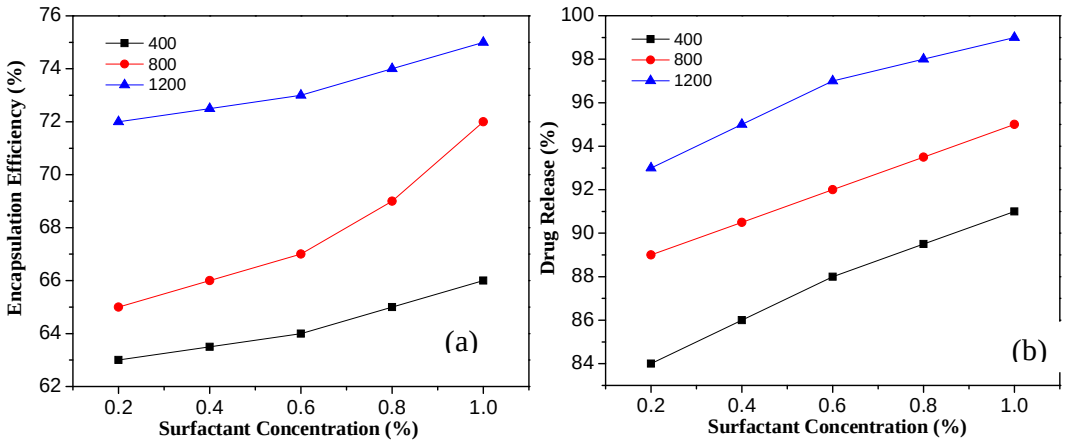


Figure 4.2: Effect of surfactant concentration at different polymer concentration (400, 800 and 1200 mg) on: (a) encapsulation efficiency (%) and (b) drug release (%) in nanoprecipitation technique

In nanoprecipitation technique Figures 4.2(a)-(b) represent that as we increase the concentration of the surfactants (0.2 to 1.0 %) the encapsulation efficiency and drug release also increases in emulsification solvent diffusion process. Similarly with increasing the polymer concentration (400 to 1200 mg) the EE and DR also increases in the process. The increase in surfactant concentration from 0.2 to 1.0 % raise the EE from 63.0 % to 66.0 % at 400 mg polymer concentration; 65 % to 72 % at 800 mg polymer concentration and 72 % to 75 % at 1200 mg polymer concentration as shown in Figure 4.2(a). On the other hand, DR increases from 84.0 % to 91.0 % at 400 mg polymer concentration; 89 % to 95 % at 800 mg polymer concentration and 93 % to 99 % at 1200 mg polymer concentration as shown in Figure 4.2(b), when we increase the surfactant concentration

from 0.2 to 1.0 %.The above results reveal that increasing polymer concentration (400 to 1200 mg) also increases the EE and DR.

The polymer concentration (mg) effect on EE (%) and DR (%) at different % of surfactant concentration (0.2 to 1.0) in Emulsification solvent diffusion and nanoprecipitation technique is represented in Figure 4.3(a)-(b) and Figure4.4(a)-(b) respectively.

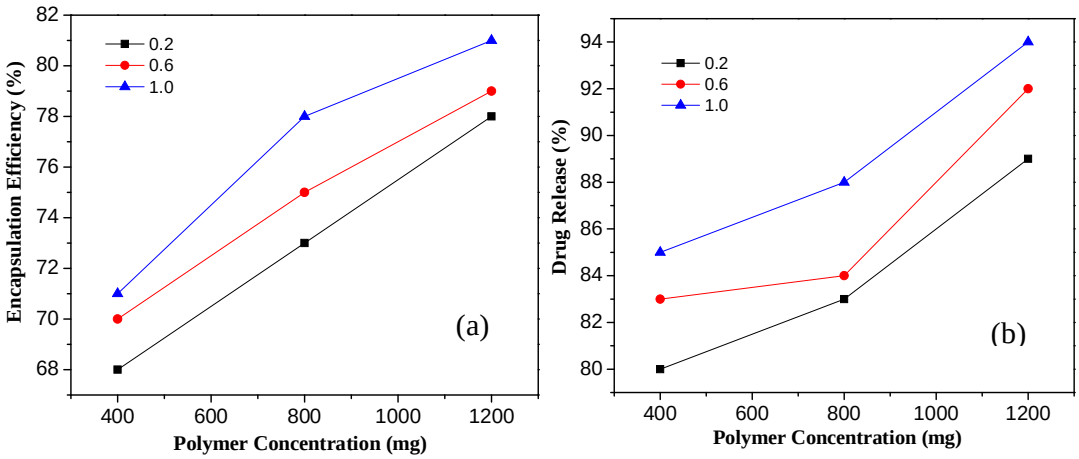


Figure 4.3: Effect of polymer concentration at different surfactant concentration (0.2%, 0.6% and 1.0 %)on: (a) encapsulation efficiency (%) and (b) drug release (%) in Emulsification Solvent Diffusion Method

The above Figures 4.3(a)-(b) represent polymer concentration (mg) effect on encapsulation efficiency (%) and drug release (%) at 0.2-1.0% surfactant concentration in solvent diffusion. Figures 4.3(a) shows that the increasing polymer concentration from 400 to 1200 mg increased the encapsulation efficiency from 68.0 % to 78.0 % at 0.2 % surfactant concentration; 70 % to 79 % at 0.6 % surfactant concentration and 71 % to 81 % at 1.0 % surfactant concentration. On other hand DR increases from 80 % to 89 % at 0.2 % surfactant concentration; 83 % to 92 %at 0.6 % surfactant concentration and 85 % to 94 % at 1.0 % surfactant concentration as presented in Figure 4.3(b). The above results reveal that increasing polymer concentration (400-1200 mg) increases the encapsulation efficiency and drug release.

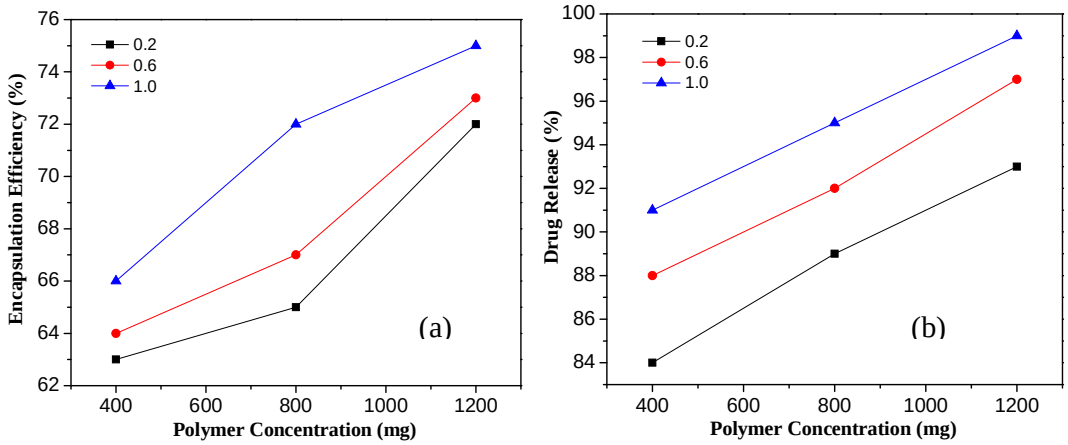


Figure 4.4: Effect of polymer concentration at different surfactant concentration (0.2%, 0.6% and 1.0 %) on: (a) encapsulation efficiency (%) and (b) drug release (%) in nanoprecipitation technique

Figures 4.4(a)-(b) depicted that, increasing polymer concentration from 400 to 1200 mg increases the EE and DR in Nanoprecipitation process. Similarly with increasing the concentration of the surfactants from 0.2 to 1.0 % the EE and DR also increases in the process.

Figures 4.4(a) shows that the increasing polymer concentration from 400 to 1200 mg increased the encapsulation efficiency from 63.0 % to 72.0 % at 0.2 % surfactant concentration; 64 % to 73 % at 0.6 % surfactant concentration and 66 % to 75 % at 1.0 % surfactant concentration). On other hand DR increases from 84 % to 93 % at 0.2 % surfactant concentration; 88 % to 97 % at 0.6 % surfactant concentration and 91 % to 99 % at 1.0 % surfactant concentration as presented in Figure 4.4(b).

From the above results of the preliminary study the matrix for process variables has been projected accordingly for factorial design approach as discussed and optimized in the following section.

For any given polymer loading % DR substantially and continually increases with increase in surfactant concentration. However, % EE generally shows a decrease when surfactant concentration is 0.6 % and then an increase at surfactant concentration of 1.0 %. This is quite typical of both the methods of encapsulation. The increase in % DR with concentration of surfactant can be explicated as follows. Within certain range of surfactant concentration (below Critical Micelle Concentration (CMC)) the surface tension are expected to be very influential which reduces the bead size and hence increases the total surface area. Thus % DR increases with increasing concentration of surfactant.

It also indicates that the morphology undergoes a regular transformation of gradual increase in membrane permeability.

But % EE decreases a little bit at surfactant concentration of 0.6 % and then increases at surfactant concentration of 1.0 %. It means that the drug entrapment decrease first and then increases with increase in concentration of surfactant at the same polymer loading. There seems to be other factor at work apart from surface tension effect. It is roughly guessed to be because of slight decrease in the solubility of the drug, thymol, in the organic solvent at this surfactant concentration. For any firm conclusion to be made, certain more analysis is warranted.

Table 4.1: Percent encapsulation efficiency and percent drug release by emulsification Solvent Diffusion

Surfactant Concentration	Polymer Concentration					
	400 mg		800 mg		1200 mg	
	EE	DR	EE	DR	EE	DR
0.2	70.19	80.21	73.00	82.75	78.16	89.33
0.6	69.11	83.62	71.91	85.29	77.45	92.71
1.0	72.12	85.11	78.63	88.28	81.30	94.38

Table 4.2: Percent encapsulation efficiency and percent drug release by Nanoprecipitation techniques

Surfactant Concentration	Polymer Concentration					
	400 mg		800 mg		1200 mg	
	EE	DR	EE	DR	EE	DR
0.2	63.12	84.91	65.00	89.11	71.09	93.16
0.6	62.90	87.97	65.19	92.13	71.13	97.63
1.0	66.33	91.33	72.61	95.27	75.47	98.71

EE of nanoparticles (thymol loaded Ethyl Cellulose) had revealed that at high polymer concentration, EE is higher. The EE obtained in the range of 69.11 % to 81.3 % for the solvent diffusion method and 63.12 % to 75.47 % for the nanoprecipitation technique. Encapsulation efficiency is directly proportional to concentration of polymer.

4.1.2. Optimisation of the process parameters using factorial design

The process parameters involved in the emulsification solvent diffusion techniques plays an important role in the controlled release of the drug. Hence the factors affecting the emulsification process such as Polymer Concentration, Surfactant concentration was optimized using 3 levels 2 factor designs in this study.

4.1.2.1. Emulsification Solvent Diffusion Method

Full Factorial design was employed to determine the independent and interaction effect of the process parameters towards encapsulation efficiency and drug release; and to obtain the optimium condition for microencapsulation process of thymol into EC. According to the obtained prelininary experimental results, matrix was designed for the low and high level of each factor using Full Factorial design. The coded level for independent factors; Polymer Concentration (400 – 1200 mg), Surfactant concentration (0.2 – 1.0 %) is represented in Table 4.3. The design matrix and their corresponding experimental and predicted values are shown in Table 4.4.

Table 4.3. Factors and coded levels for Full Factorial design.

Factor		Low	Medium	High
A	Polymer Concentration	400	800	1200
B	Surfactant concentration	0.2	0.6	1.0

Effect of process parametes on Encapsulation Efficiency

Table 4.4. Full Factorial design matrix of the factors and its corrresponding experimental and predicted responses (Encapsulation Efficiency (%)) for emulsification solvent diffusion method.

Std. Order	Run Order	Polymer Concentration (mg)	Surfactant concentration (vol %)	Encapsulation efficiency (%)	
				Actual Value	Predicted Value
1	4	400	0.2	70.19	69.75
2	1	800	0.2	73	74.13
3	7	1200	0.2	78.16	77.47
4	6	400	0.6	69.11	68.80
5	5	800	0.6	71.91	73.74
6	11	1200	0.6	77.45	77.63
7	3	400	1	71.12	71.87
8	10	800	1	78.63	77.37
9	12	1200	1	81.3	81.80
10	8	800	0.6	75.83	73.74
11	13	800	0.6	72.47	73.74
12	9	800	0.6	74.776	73.74
13	2	800	0.6	73.6	73.74

From Table 4.4, it can be observed that within the range of parameters studied, the highest encapsulation efficiency (81.3 %) was obtained at highest polymer concentration (1200 mg) and at maximum surfactant concentration (1%). On the other hand the smallest encapsulation efficiency (69.11%) was found at lowest polymer concentration (400 mg) and intermediate surfactant concentration (0.6%). Hence this study shows that the encapsulation efficiency increases with increasing polymer concentration, whereas, when surfactant concentration was decreases the encapsulation efficiency gets reduced. The optimum condition of the process variables generated in emulsification solvent diffusion method for encapsulation efficiency by the model was 1184.16 mg polymer concentration and 1.0 % surfactant concentration. Triplicate experiments were performed at the optimum

condition and the average value (80.51 ± 0.21%) was reported which is close to the predicted value (81.65%) suggesting a good fit of the model.

Analysis of variance (ANOVA) of quadratic model for encapsulation efficiency

The parameters affecting the encapsulation efficiency can be ascertained from F-test of ANOVA as represented in Table 4.5. The significance of each coefficient was determined by F-values and P-values. The large F-value and corresponding low P-value depicts that the variable has high effect in the process, and those with P-value > 0.05 shows that the factors are not affecting the response.

Table 4.5. Analysis of variances (ANOVA) for response surface quadratic model : encapsulation efficiency.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	Remarks
Model	145.1797	5.00	29.03594	13.61	0.0017	significant
A-Polymer Concentration (mg)	116.9534	1.00	116.9534	54.83	0.0001	
B-Surfactant concentration (vol %)	15.68167	1.00	15.68167	7.35	0.0301	
AB	1.221025	1.00	1.221025	0.57	0.4740	
A^2	0.766058	1.00	0.766058	0.36	0.5679	
B^2	11.14	1.00	11.14	5.22	0.0562	
Residual	14.93196	7.00	2.133137			
Lack of Fit	4.511763	3.00	1.503921	0.58	0.6601	not significant
Pure Error	10.4202	4.00	2.605049			
Cor Total	160.1117	12.00				

It can be clearly observed from Table 4.5 that the model developed is highly significant with P-value < 0.0017. Also the parameters such as Polymer Concentration (A), Surfactant concentration (B) with P-value < 0.05 has high individual effect towards the response. The quadratic factors such as B^2 with P-value <0.056 also shows minuscule significance which reveals that the encapsulation efficiency increases with increasing polymer concentration.

The mutual interactions between the factors towards the response can be observed from the curvature of 3D response plot as shown in Figure 4.5.

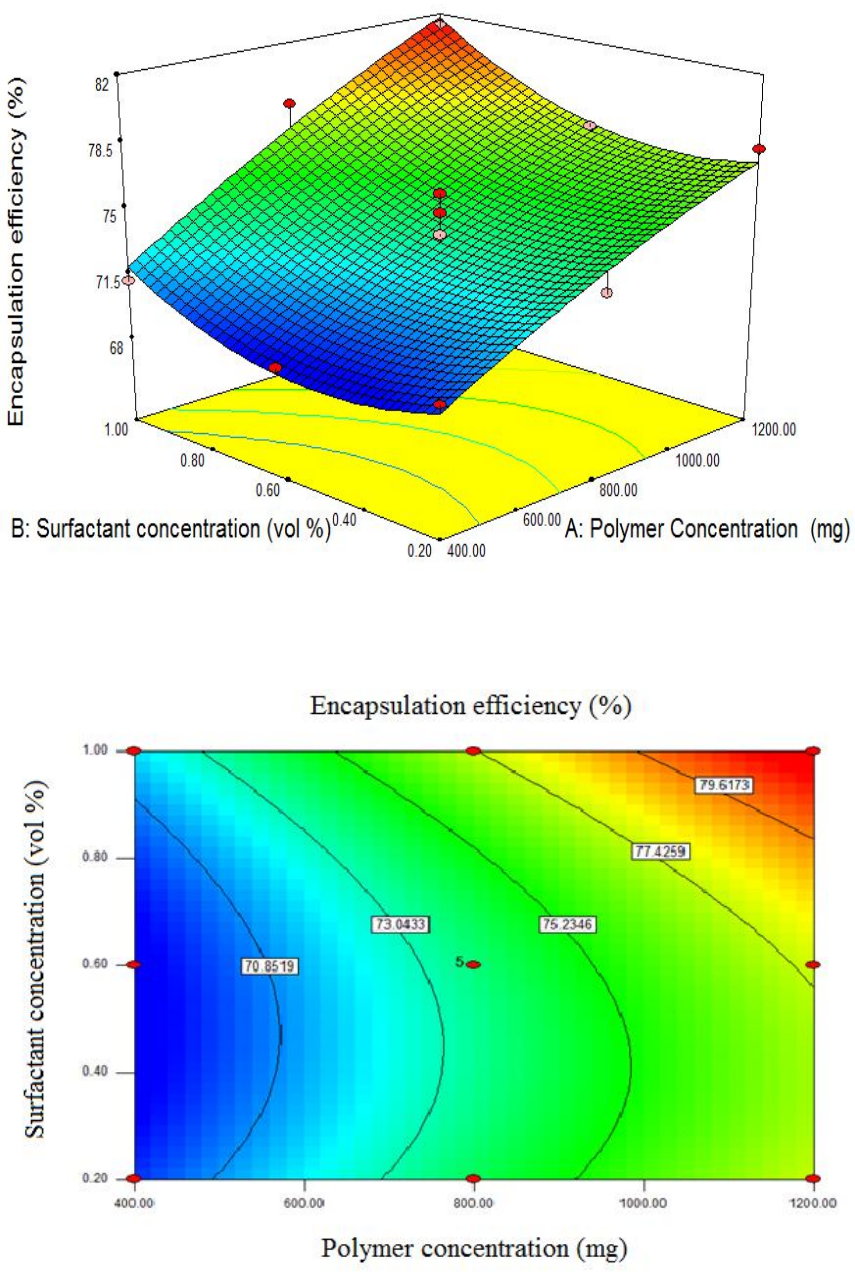


Figure 4.5: 3D and contour plots representing the interaction effects between polymer and surfactant concentration in emulsification solvent diffusion method for encapsulation efficiency

The model equation developed for the microencapsulation technique for drug polymers in terms of both coded and experimental values are given in Eq. (2) and Eq. (3) respectively.

$$Y = 73.74 + 4.42 * A + 1.62 * B + 0.55 * A * B - 0.53 * A^2 + 2.01 * B^2 \quad (4.1)$$

$$Y = 66.56 + 0.014 * A - 13.78 * B + 0.003 * A * B - 3.29E - 06 * A^2 + 12.552 * B^3 \quad (4.2)$$

Model Validation for encapsulation efficiency

The significance of the model is checked by the determination coefficient (R^2). For a good fit of a model, R^2 value should be a minimum of 0.80. The statistical parameters of the developed model such as R^2 = 0.9067 and F-value of 13.61 with low probability (P <0.0017) shows a highly significant model. The coefficient of variance (CV = 1.96 %), which measures the residual variation of the data relative to mean indicates a good accuracy and reliability of the experiments. The standard deviation of the model is 1.46, and the non-significant lack of fit (P-value = 0.6601) demonstrates a good fitness of the data to the model. The comparison of the actual value and predicted value of the response (Encapsulation efficiciency (%)) as presented in Figure 4.6 also depicts a good fitness of the data to the model.

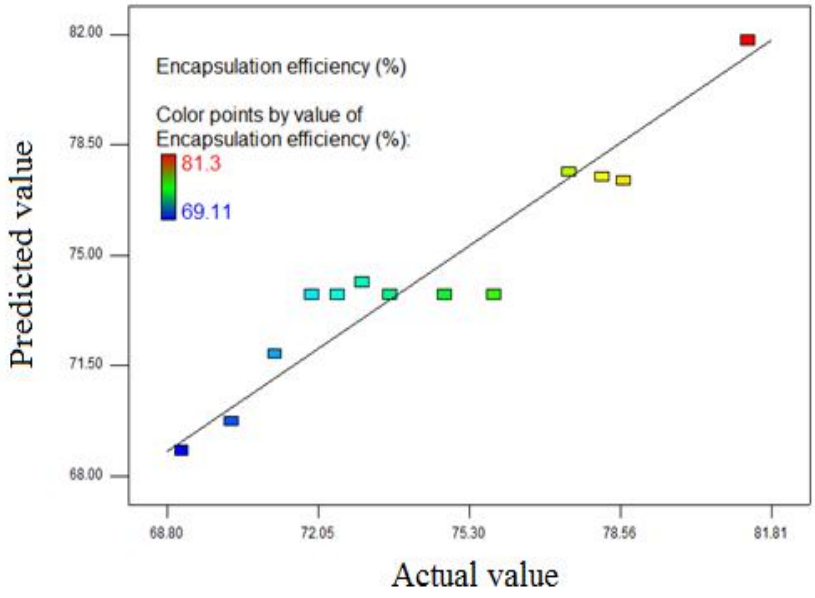


Figure 4.6: Comparison plot between experimentally obtained value (Actual value) and Predicted value towards the response in emulsification solvent diffusion method for encapsulation efficiency.

The optimum condition of the process variables generated in emulsification solvent diffusion method for drug release by the model was 1193.63 mg polymer concentration

and 0.97 % surfactant concentration. Triplicate experiments were performed at the optimum condition and the average value (93.59 ± 0.74%) was reported which is close to the predicted value (94.45 %) suggesting a good fit of the model.

Effect of process parametes on Drug Release

Table 4.6. Full Factorial design matrix of the factors and its corrresponding experimental and predicted responses (Drug Release (%)) for emulsification solvent diffusion method.

Std. Order	Run Order	Polymer Concentration (mg)	Surfactant concentration (vol %)	Drug Release (%)	
				Actual Value	Predicted Value
1	4	400	0.2	80.21	80.34
2	1	800	0.2	82.75	82.53
3	7	1200	0.2	89.33	89.42
4	6	400	0.6	83.62	83.18
5	5	800	0.6	85.29	85.42
6	11	1200	0.6	92.71	92.34
7	3	400	1	85.11	85.42
8	10	800	1	88.28	87.69
9	12	1200	1	94.38	94.66
10	8	800	0.6	84.72	85.42
11	13	800	0.6	83.46	85.42
12	9	800	0.6	86	85.42
13	2	800	0.6	86.82	85.42

From Table 4.6, it can also be observed that within the range of parameters studied, the highest drug release (94.38 %) was obtained at highest polymer concentration (1200 mg) and at maximum surfactant concentration (1%). On the other hand the smallest drug release (80.21%) was found at lowest polymer concentration (400 mg) and lowest surfactant concentration (0.2%). Hence this study shows that the drug release increases with increasing polymer concentration, whereas, when surfactant concentration was decreases the drug release gets reduced.

Analysis of variance (ANOVA) of quadratic model for drug release

The parameters affecting the Drug Release can be ascertained from F-test of ANOVA as represented in Table 4.7. The significance of each coefficient was determined by F-values and P-values. The large F-value and corresponding low P-value depicts that the variable has high effect in the process, and those with P-value > 0.05 shows that the factors are not affecting the response.

Table 4.7. Analysis of variances (ANOVA) for response surface quadratic model : drug release.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	Remarks
Model	182.1054	5	36.42107	33.74	< 0.0001	significant
A-Polymer Concentration (mg)	125.8584	1	125.8584	116.61	< 0.0001	
B-Surfactant concentration (vol %)	39.9384	1	39.9384	37.00	0.0005	
AB	0.005625	1	0.005625	0.01	0.9445	
A^2	15.18555	1	15.18555	14.07	0.0072	
B^2	0.257217	1	0.257217	0.24	0.6403	
Residual	7.555244	7	1.079321			
Lack of Fit	1.041564	3	0.347188	0.21	0.8826	not significant
Pure Error	6.51368	4	1.62842			
Cor Total	189.6606	12				

It can be clearly observed from Table 4.7 that the model developed is highly significant with P-value < 0.0001. Also the parameters such as Polymer Concentration (A), Surfactant concentration (B) with P-value < 0.05 has high individual effect towards the response. The quadratic factors such as A^2 with P-value <0.007 also shows highly significance effect towards the response which reveals that the drug release increases with increasing polymer concentration. The mutual interactions between the factors towards the response can be observed from the curvature of 3D response plot as shown in Figure 4.7.

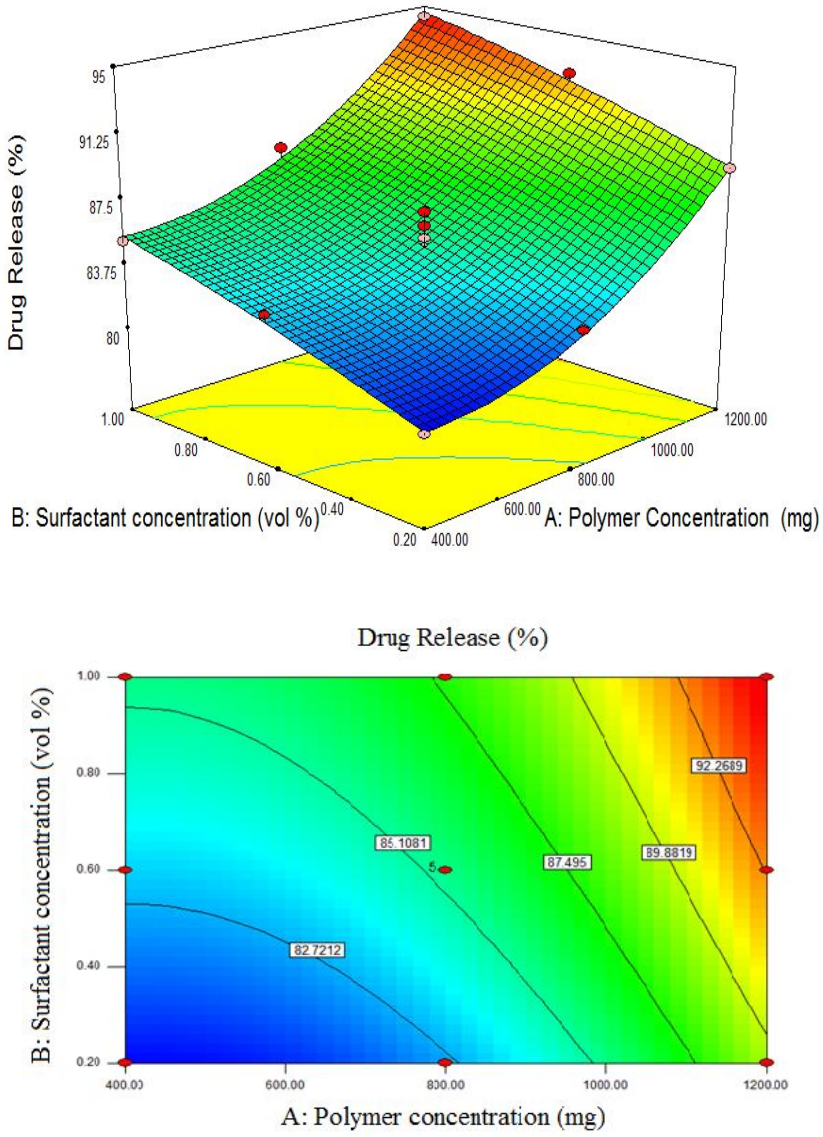


Figure 4.7: 3D and contour plots representing the interaction effects between polymer and surfactant concentration in emulsification solvent diffusion method for drug release.

The model equation developed for the microencapsulation technique for drug polymers in terms of both coded and experimental values are given in Eq. (4.3) and Eq. (4.4) respectively.

$$Y = 85.42 + 4.58 * A + 2.58 * B + 0.04 * A * B - 2.34 * A^2 - 0.31 * B^2 \quad (4.3)$$

$$Y = 81.19 - 0.01 * A - 8.55 * B + 2.34375E\text{-}004 * A * B + 1.46552E\text{-}005 * A^2 - 1.90733 * B^3 \quad (4.4)$$

Model Validation for drug release

The significance of the model is checked by the determination coefficient (R^2). The statistical parameters of the developed model such as R^2 = 0.9602 and F-value of 33.74 with low probability (P <0.0001) shows a highly significant model. The coefficient of variance (CV = 1.20 %), which measures the residual variation of the data relative to mean indicates a good accuracy and reliability of the experiments. The standard deviation of the model is 1.04, and the non-significant lack of fit (P-value = 0.8826) demonstrates a good fitness of the data to the model. The comparison of the actual value and predicted value of the response (Drug Release (%)) as presented in Figure 4.8 also depicts a good fitness of the data to the model.

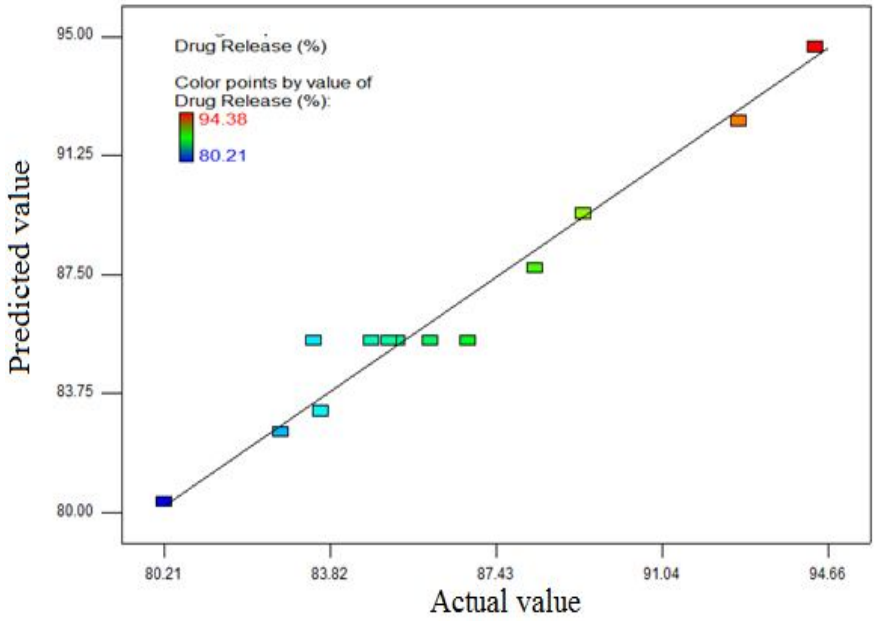


Figure 4.8: Comparison plot between experimentally obtained value (Actual value) and Predicted value towards the response in emulsification solvent diffusion method for drug release.

The optimum condition of the process variables generated in emulsification solvent diffusion method for drug release by the model was 1198.49 mg polymer concentration and 1.0 % surfactant concentration. Triplicate experiments were performed at the optimum condition and the average value (94.09 ± 0.27%) was reported which is close to the predicted value (94.60 %) suggesting a good fit of the model.

4.1.2.2. Nanoprecipretation Method

Similar to emulsification techniques the process parameters involved in the nanoprcipritation method also plays an important role in the controlled release of the drug. Hence the factors affecting the nanoprcipritation process such as Polymer Concentration, Surfactant concentration was optimized using 3 levels 2 factor designs in this study.

Effect of process parametes on Encapsulation Efficiency

Table 4.8. Full Factorial design matrix of the factors and its corrresponding experimental and predicted responses (Encapsulation Efficiency (%)) for nanoprecipitation method

Std. Order	Run Order	Polymer Concentration (mg)	Surfactant concentration (vol %)	Encapsulation efficiency (%)	
				Actual Value	Predicted Value
1	7	400	0.2	63.12	62.54
2	13	800	0.2	65	66.28
3	1	1200	0.2	71.09	70.40
4	5	400	0.6	62.9	62.80
5	12	800	0.6	69.23	66.83
6	11	1200	0.6	71.13	71.24
7	2	400	1	66.33	67.02
8	6	800	1	72.61	71.34
9	3	1200	1	75.47	76.05
10	10	800	0.6	68.97	66.83
11	4	800	0.6	64.63	66.83
12	9	800	0.6	66.16	66.83
13	8	800	0.6	65.19	66.83

From Table 4.8, it can be observed that within the range of parameters studied, the highest encapsulation efficiency (75.47 %) was obtained at highest polymer concentration (1200 mg) and at maximum surfactant concentration (1%). On the other hand the smallest encapsulation efficiency (62.90 %) was found at lowest polymer concentration (400 mg) and intermediate surfactant concentration (0.6%). Hence this study shows that the

encapsulation efficiency increases with increasing polymer concentration, whereas, when surfactant concentration was decreases the encapsulation efficiency gets reduced.

The optimum condition of the process variables generated in Nano precipitation method for encapsulation efficiency by the model was 1178.14 mg polymer concentration and 1.00 % surfactant concentration. Triplicate experiments were performed at the optimum condition and the average value (74.52 ± 0.34%) was reported which is close to the predicted value (75.70 %) suggesting a good fit of the model.

Analysis of variance (ANOVA) of quadratic model for encapsulation efficiency

The parameters affecting the encapsulation efficiency can be ascertained from F-test of ANOVA as represented in Table 4.9. The significance of each coefficient was determined by F-values and P-values. The large F-value and corresponding low P-value depicts that the variable has high effect in the process, and those with P-value > 0.05 shows that the factors are not affecting the response.

Table 4.9. Analysis of variances (ANOVA) for response surface quadratic model : encapsulation efficiency.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	Remarks
Model	159.5367	5	31.90734	9.63	0.0048	significant
A-Polymer Concentration (mg)	107.0193	1	107.0193	32.28	0.0007	
B-Surfactant concentration (vol %)	38.50667	1	38.50667	11.62	0.0113	
AB	0.342225	1	0.342225	0.10	0.7574	
A^2	0.097545	1	0.097545	0.03	0.8687	
B^2	10.80515	1	10.80515	3.26	0.1140	
Residual	23.20477	7	3.314968			
Lack of Fit	4.886853	3	1.628951	0.36	0.7889	not significant
Pure Error	18.31792	4	4.57948			
Cor Total	182.7415	12				

It can be clearly observed from Table 4.9 that the model developed is significant with P-value < 0.0048. Also the parameters such as Polymer Concentration (A), Surfactant concentration (B) with P-value < 0.05 has high individual effect towards the response. However the interaction between the parameters was not significant as can be observed from the ANOVA table. The mutual interactions between the factors towards the response can be observed from the curvature of 3D response plot as shown in Figure 4.9.

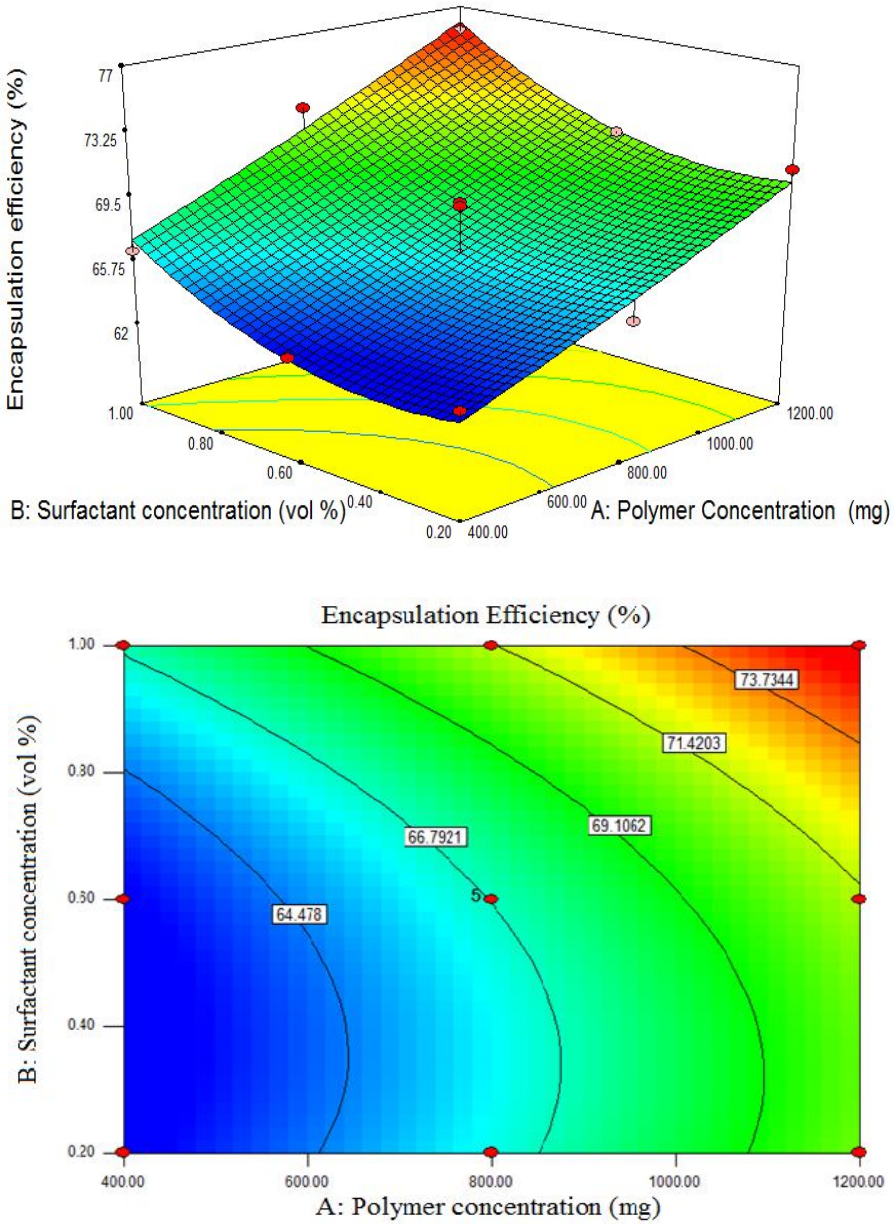


Figure 4.9: 3D and contour plots representing the interaction effects between polymer and surfactant concentration in nanoprecipitation method for encapsulation efficiency.

The model equation developed for the microencapsulation technique for drug polymers in terms of both coded and experimental values are given in Eq. (6) and Eq. (7) respectively.

$$Y = 66.83 + 4.22 * A + 2.53 * B + 0.29 * A * B - 0.19 * A^2 + 1.98 * B^2 \quad (4.5)$$

$$Y = 60.67 + 0.007 * A - 9.96 * B + 0.002 * A * B + 1.18\ \text{E-006} * A^2 + 12.37 * B^3 \quad (4.6)$$

Model Validation for encapsulation efficiency

The significance of the model is checked by the determination coefficient (R^2). For a good fit of a model, R^2 value should be a minimum of 0.80. The statistical parameters of the developed model such as R^2 = 0.8730 and F-value of 9.63 with low probability (P <0.0048) shows a highly significant model. The coefficient of variance (CV = 2.68 %), which measures the residual variation of the data relative to mean indicates a good accuracy and reliability of the experiments. The standard deviation of the model is 1.82, and the non-significant lack of fit (P-value = 0.7889) demonstrates a good fitness of the data to the model. The comparison of the actual value and predicted value of the response (Encapsulation Efficiency (%)) as presented in Figure 4.10 also depicts a good fitness of the data to the model.

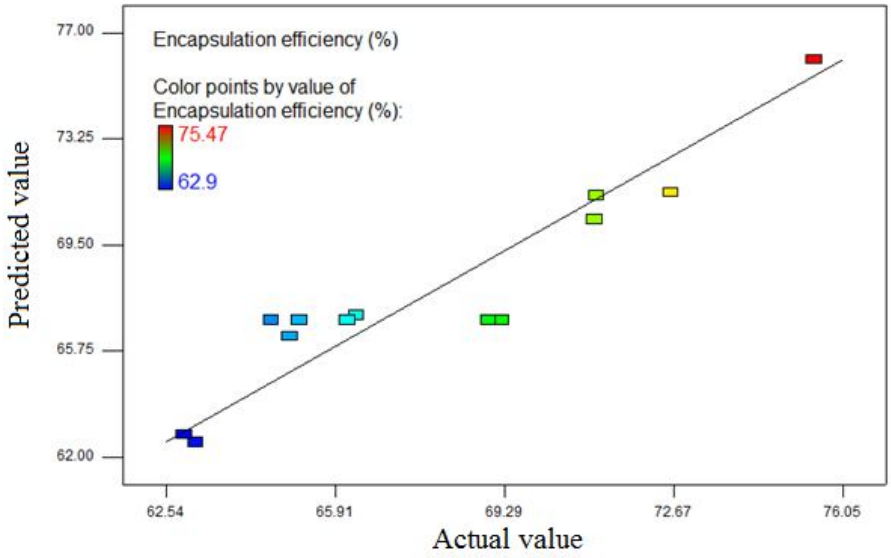


Figure 4.10. Comparison plot between experimentally obtained value (Actual value) and Predicted value towards the response in nanoprecipretation method for encapsulation efficiency.

Effect of process parametes on Drug Release

Table 4.10. Full Factorial design matrix of the factors and its corrresponding experimental and predicted responses (Drug Release (%)) for nanoprcipritation method.

Std. Order	Run Order	Polymer Concentration (mg)	Surfactant concentration (vol %)	Drug Release (%)	
				Actual Value	Predicted Value
1	7	400	0.2	84.91	84.90
2	13	800	0.2	89.11	88.52
3	1	1200	0.2	93.16	93.76
4	5	400	0.6	87.97	87.94
5	12	800	0.6	90	91.35
6	11	1200	0.6	97.63	96.37
7	2	400	1	91.33	91.37
8	6	800	1	95.27	94.57
9	3	1200	1	98.71	99.37
10	10	800	0.6	93.21	91.35
11	4	800	0.6	91.03	91.35
12	9	800	0.6	89.09	91.35
13	8	800	0.6	92.13	91.35

From Table 4.10, it can be observed that within the range of parameters studied, the highest drug release (98.71 %) was obtained at highest polymer concentration (1200 mg) and at maximum surfactant concentration (1%). On the other hand the smallest drug release (84.91%) was found at lowest polymer concentration (400 mg) and lowest surfactant concentration (0.2%). Hence this study shows that the drug release increases with increasing polymer concentration, whereas, when surfactant concentration was decreases the drug release gets reduced.

Analysis of variance (ANOVA) of quadratic model for drug release

The parameters affecting the drug release can be ascertained from F-test of ANOVA as represented in Table 4.11. The significance of each coefficient was determined by F-values and P-values. The large F-value and corresponding low P-value depicts that the variable

has high effect in the process, and those with P-value > 0.05 shows that the factors are not affecting the response.

Table 4.11. Analysis of variances (ANOVA) for response surface quadratic model : drug release.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob> F**	**Remarks**
Model	164.1723	5	32.83445	16.04	0.0010	significant
A-Polymer Concentration (mg)	106.5974	1	106.5974	52.09	0.0002	
B-Surfactant concentration (vol %)	54.78282	1	54.78282	26.77	0.0013	
AB	0.189225	1	0.189225	0.09	0.7699	
A^2	1.789783	1	1.789783	0.87	0.3808	
B^2	0.105021	1	0.105021	0.05	0.8273	
Residual	14.32551	7	2.046501			
Lack of Fit	3.557828	3	1.185943	0.44	0.7367	not significant
Pure Error	10.76768	4	2.69192			
Cor Total	178.4978	12				

It can be clearly observed from Table 4.11 that the model developed is highly significant with P-value < 0.0001. Also the parameters such as Polymer Concentration (A), Surfactant concentration (B) with P-value < 0.05 has high individual effect towards the response. However the interaction between the parameters was not significant as can be observed from the ANOVA table. The mutual interactions between the factors towards the response can be observed from the curvature of 3D response plot as shown in Figure 4.11.

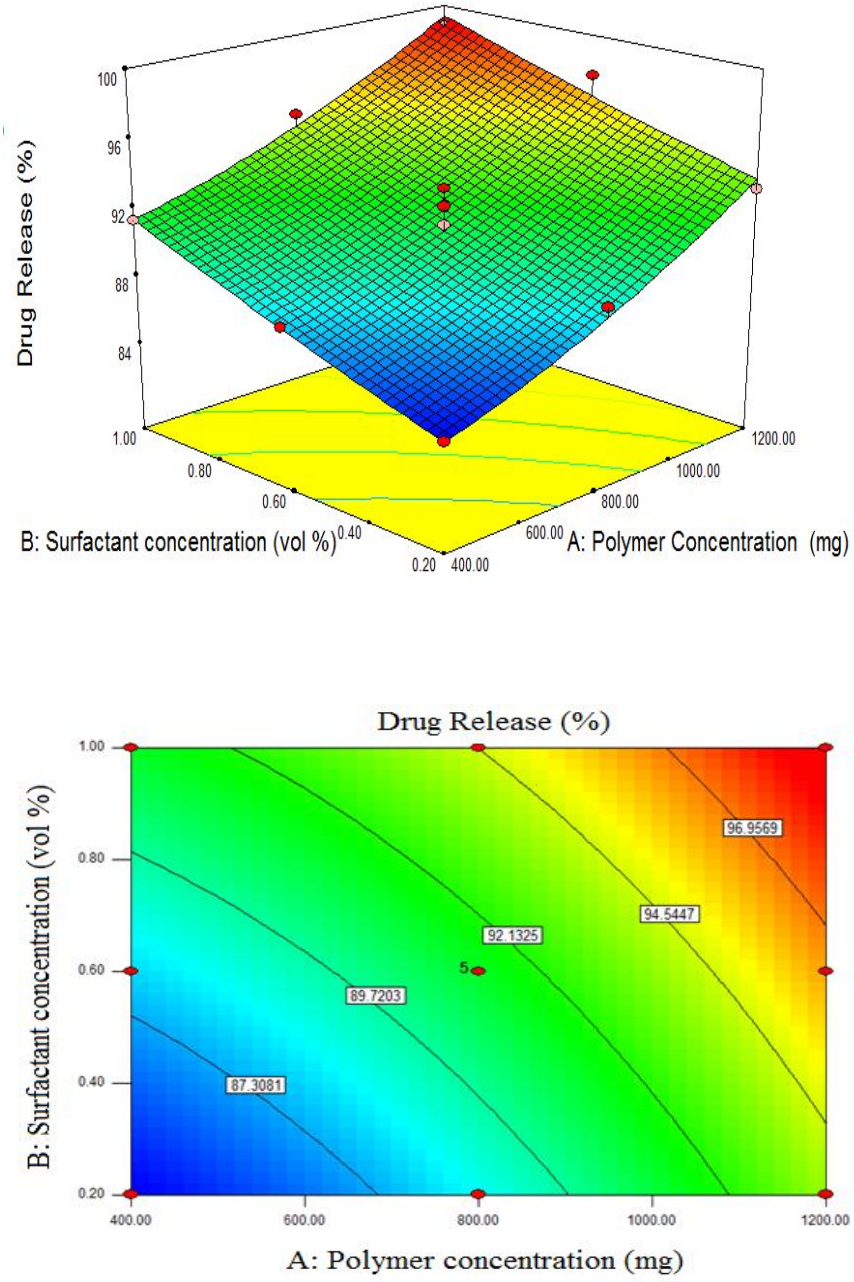


Figure 4.11: 3D and contour plots representing the interaction effects between polymer and surfactant concentration

The model equation developed for the microencapsulation technique for drug polymers in terms of both coded and experimental values are given in Eq. (8) and Eq. (9) respectively.

$$Y = 91.35 + 4.21 * A + 3.02 * B - 0.22 * A * B + 0.80 * A^2 + 0.20 * B^2 \quad (4.7)$$

$$Y = 81.39 - 0.003 * A + 7.18 * B - 0.003 * A * B + 5.03125\text{E-}006 * A^2 + 1.22 * B^3 \quad (4.8)$$

Model validation for drug release

The significance of the model is checked by the determination coefficient (R^2). For a good fit of a model, R^2 value should be a minimum of 0.80. The statistical parameters of the developed model such as R^2 = 0.9197andF-value of 16.04 with low probability (P <0.0010) shows a highly significant model. The coefficient of variance (CV = 1.56 %), which measures the residual variation of the data relative to mean indicates a good accuracy and reliability of the experiments. The standard deviation of the model is 1.43, and the non-significant lack of fit (P-value = 0.7367) demonstrates a good fitness of the data to the model. The comparison of the actual value and predicted value of the response (Drug Release (%)) as presented in Figure 4.12 also depicts a good fitness of the data to the model.

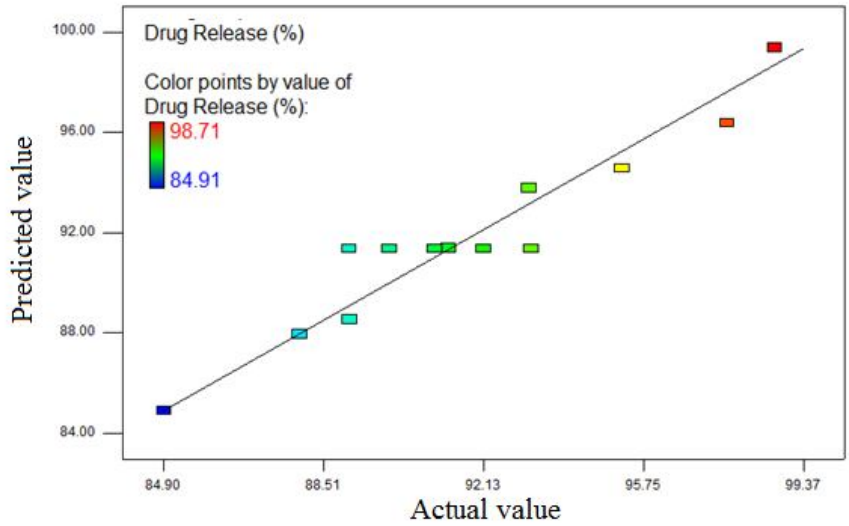


Figure 4.12: Comparison plot between experimentally obtained value (Actual value) and Predicted value towards the response.

The optimum condition of the process variables generated in Nano precipitation method for drug release by the model was 1164.34 mg polymer concentration and 0.99 % surfactant concentration. Triplicate experiments were performed at the optimum condition and the average value (97.911 ± 0.34%) was reported which is close to the predicted value (98.77 %) suggesting a good fit of the model.

4.1.3. Drug release behaviour of microparticles

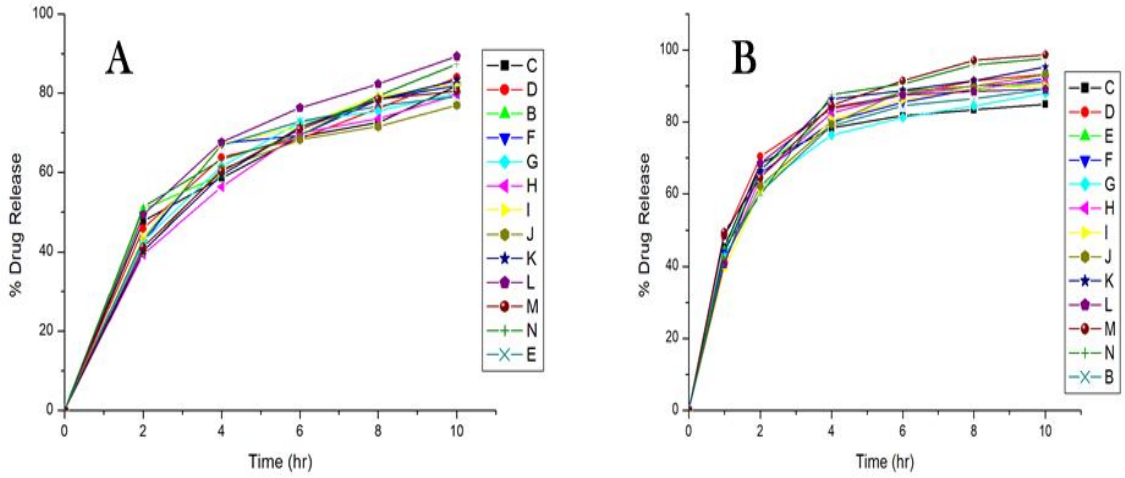


Figure 4.13: Display of the release behavior of nanoparticles by emulsification Solvent Diffusion method (A) and nanoprecipitation method (B).

Drug release profile for all the runs was carried out using *in-vitro* release study at pH 6.8. Cumulative percent drug release for emulsification Solvent Diffusion runs are in the range of 83.43% to 94.38% while in nanoprecipitation technique 84.91% to 98.71 % in 10 h shows control release (Figure 4.13).

4.1.4. Characterization of micro particles

In this section the detail discussion of micro particles obtained in solvent diffusion and nanoprecipitation has been discussed which are characterized using FTIR, XRD, FESEM and particle size distribution.

Fourier transforms infrared spectroscopy (FTIR)

The chemical structure of the material as well as core-polymer interaction and degradation of core during microencapsulation were evaluated by FTIR. Figure 4.14 (a-d) represents the FTIR spectra of pure thymol (A) ethyl cellulose (B), nanoparticles prepared by solvent diffusion (C) and nanoparticles prepared by nanoprecipitation (D) method. To examine the interaction in between the drug with polymer FTIR spectra for pure thymol, ethyl cellulose and formulation were obtained using Fourier transform infrared spectrophotometer (FTIR 4800: Shimadzu, Asia Pacific Pvt. Ltd., Singapore) using Potassium bromide (KBr) pallet. The KBr disc is prepared by compressing the approximately 40 mg of KBr with 1-2 mg of

sample to be analyzed and the spectra were recorded in the wavelength region of 4000 cm^{-1} to 400 cm^{-1}.

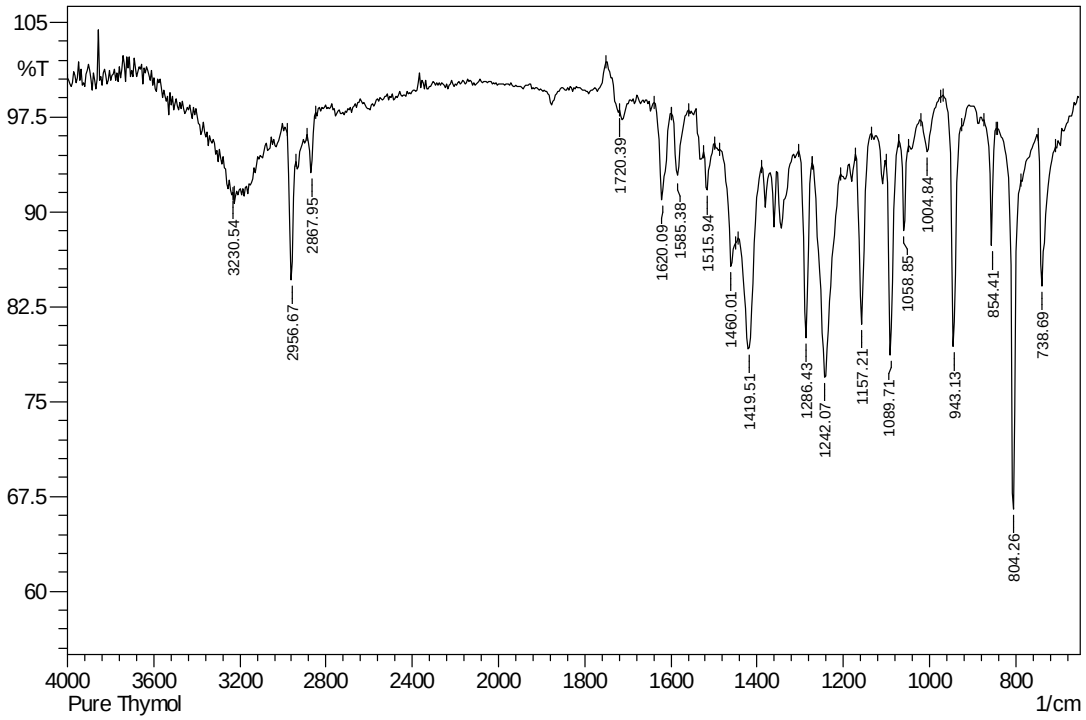


Figure 4.14 (a): FTIR spectra of pure thymol

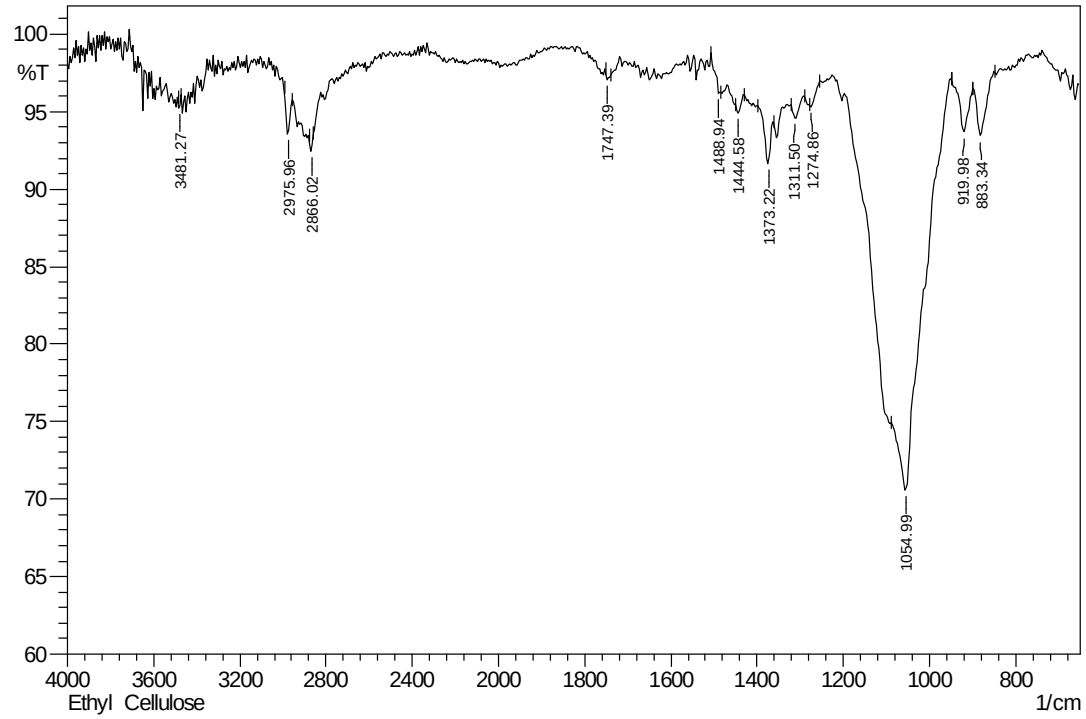


Figure 4.14 (b): FTIR spectra of ethyl cellulose

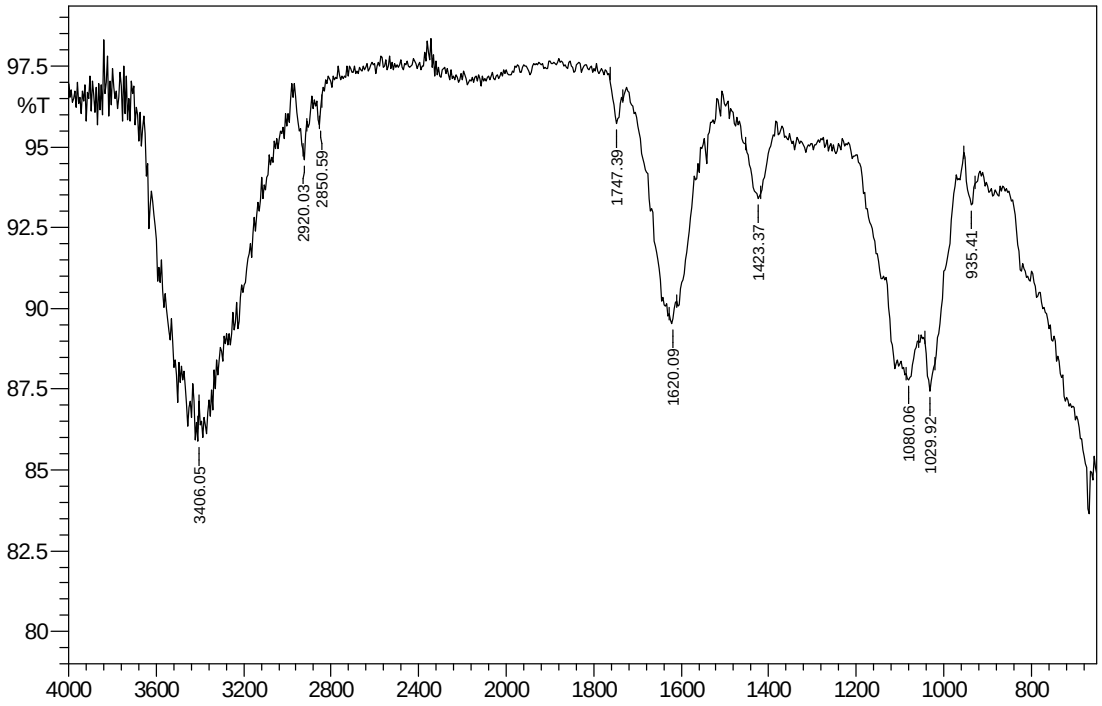


Figure 4.14 (c): FTIR spectra of nanoparticles prepared by solvent diffusion method

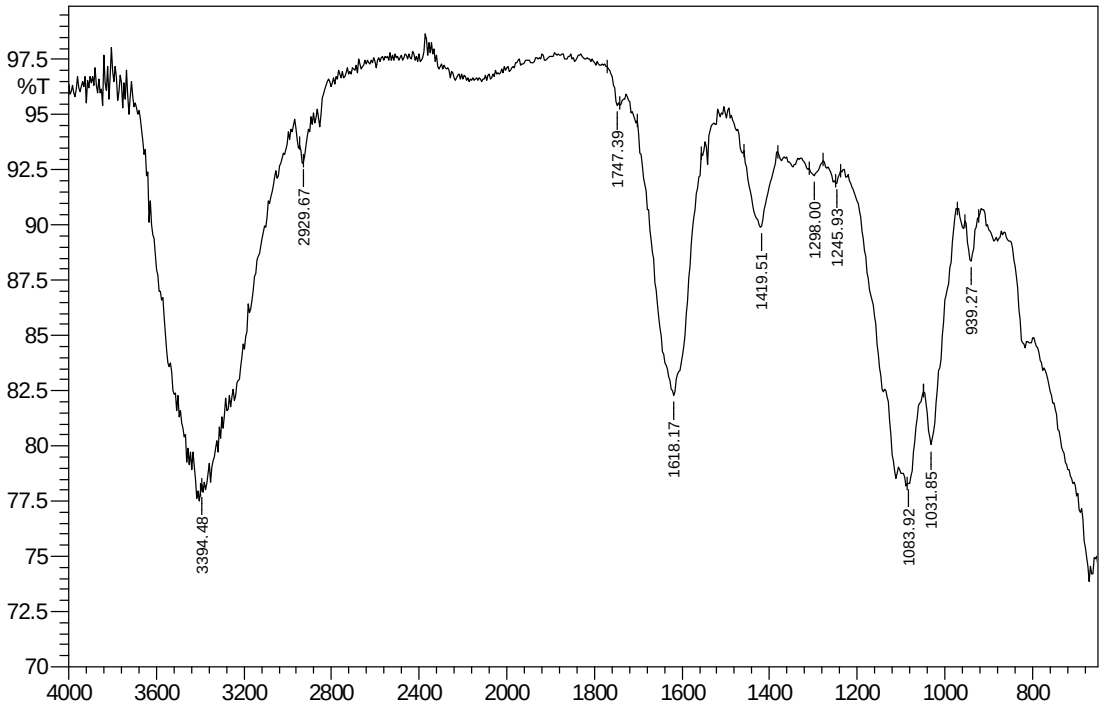


Figure 4.14 (d): FTIR spectra of nanoparticles prepared by nanoprecipitation method

The corresponding functional groups of pure thymol, EC, nanoparticles prepared by solvent diffusion and nanoparticles prepared by nanoprecipitation are given in Table 4.12. The analysis revealed that the functional groups of EC on the surface of microparticles have the similar chemical characteristics. No substantial variation was observed between spectra of EC and thymol loaded particle. There was no shift of spectrum after the formulation of thymol loaded particle that revealed no strong chemical interaction of EC with thymol, and thymol was physically dispersed in the matrix of polymeric. The results thus obtained from these characteristic peaks indicated that thymol was encapsulated by EC.

Table 4.12: Major functional groups

Wavelength range (cm^{-1})	**Functional groups**	**thymol**	**Ethyl cellulose**	**nanoparticles prepared by solvent diffusion**	**nanoparticles prepared by nanoprecipitation**
3600-3200	alcohol OH stretch	3230.54	3481.27	3406.05	3394.48
3600-2500	carboxylic acid OH stretch	2956.67	2975.96	2920.03	2929.67
2900-2800	-C-H aldehydic	2867.95	2866.02	2850.59	
1750-1720	C=O ester	1720.39	1747.39	1747.39	1747.39
1680-1600	C=C alkene	1620.09		1620.09	1618.17
1700-1500	C=O amide	1585.38			
1600-1400	C=C aromatic	1515.94	1488.94	1423.37	1419.51
1480-1440	CH2 bend	1460.01	1444.58		
1600-1400	C=C aromatic	1419.51			
1400-1000	C-F Alkyl Halide	1286.43	1373.22, 1054.99	1029.92	1298
1080-1360	C-N Amine	1089.71	1311.50, 1274.86	1080.06	1245.93, 1083.92, 1031.85
675-1000	=C-H Alkene	943.13	919.98, 883.34	935.41	939.27
600-800	C-Cl Alkyl Halide	738.69			

X-ray diffraction analysis

XRD patterns of pure thymol (A), ethyl cellulose (B) and thymol loaded EC nanoparticles prepared (C) are displayed in Figure 4.15. XRD spectra of pure thymol produced peaks at various 2 theta (Θ) angles and found to be crystalline in nature while, in case of EC and formulation less intense peaks are observed than the XRD spectra of pure thymol. This result revealed maximum entrapment and dispersion of the drug within the polymer. The crystalline/amorphous nature of thymol, EC and EC loaded thymol nanoparticles was examined using X-ray diffractometer (Bruker, D8 Advanced, Germany). The X-ray tube was operated at 40 kV and 40 mA with Cu K α radiation (λ=1.5 Å). Sample run to analyze at 2 theta angle range 5-800.

Thymol exhibited several characteristic intense peaks, which are attributed to its crystalline nature. For EC, there were only single peaks at 2Θ = 20°. M. Davidovich-Pinhas et al., (2014) have reported that EC in nature is semi crystalline which can also be observed in this study as presented in Figure 4.15.

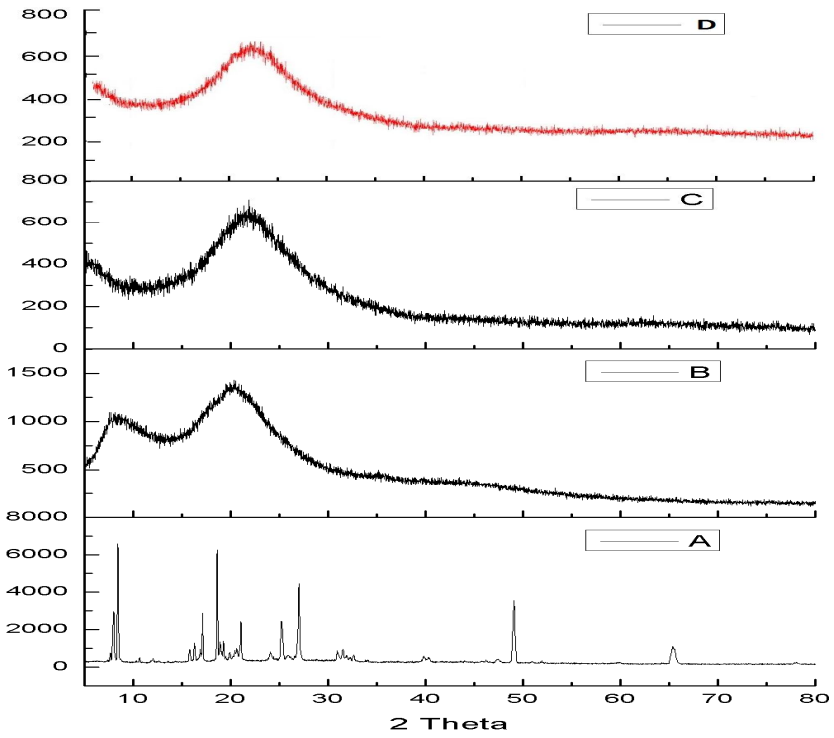


Figure 4.15: Showing the XRD spectra of pure Thymol (A), Ethyl Cellulose (B), Thymol loaded ethyl cellulose nanoparticles (C).

Field-Emission Scanning Electron Microscopy and particle size

The surface morphology of thymol loaded EC nanoparticles was analysed using FE-SEM. Figure 4.16(A) depicts the microscopic images of particles obtained by solvent diffusion method whereas Figure 4.16(B) shows that for particles obtained by nanoprecipitation method. The obtained nanoparticles are smooth, discrete, and spherical with sharp edges without any cracks or erosion at the surface. The particles prepared by solvent diffusion method are in the range of 6 to 19 µm ($6x10^3$ to 9×10^3 nm) and particles prepared by nanoprecipitation method have size range 27 to 38 µm ($27x10^3$ to 38×10^3 nm).

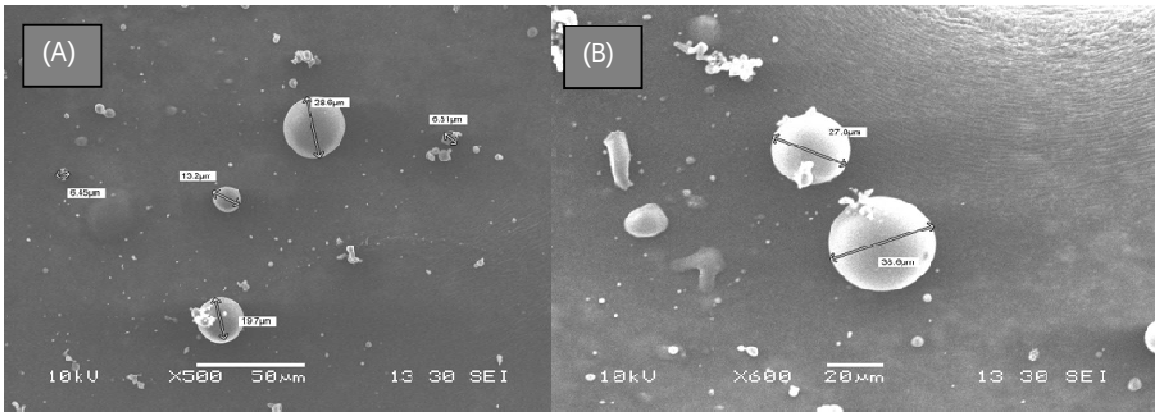


Figure 4.16: Field-Emission Scanning Electron Microscopy of particles obtained by (A), Emulsification Solvent Diffusion method and (B) nanoprecipitation method

Polydispersity Index (PDI)

Decrease in particle size shows increment in rate of dissolution. The PDI of optimized batch for solvent diffusion method is 0.122 while the PDI of batch for nanoprecipitation method is 0.223 (nm) (Figure 4.17). The results indicate that the particles obtained by the solvent diffusion method shows narrow particle size distribution than the particles obtained by nanoprecipitation method (Figure 4.18).

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 261.3	Peak 1:	285.0	100.0	96.20
PdI: 0.122	Peak 2:	0.000	0.0	0.000
Intercept: 0.946	Peak 3:	0.000	0.0	0.000

Result quality : Good

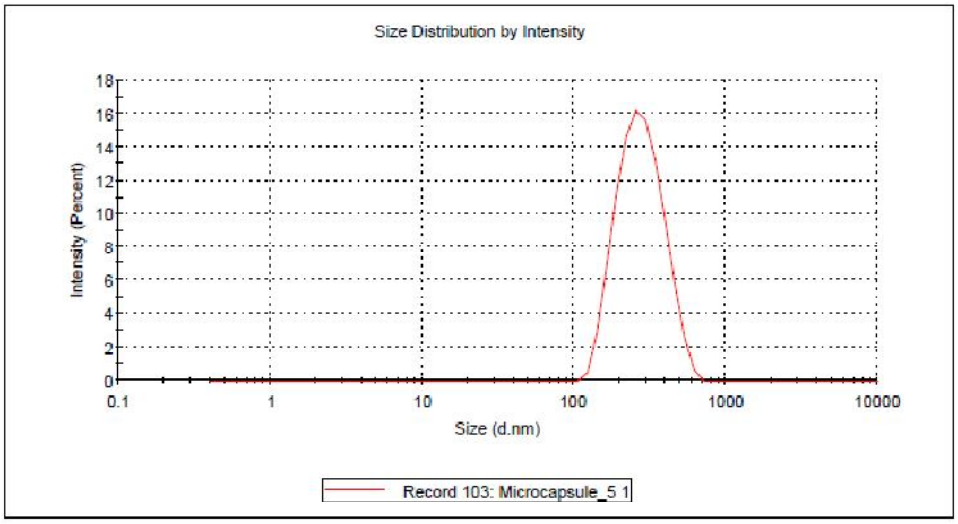


Figure 4.17: Showing the particle size distribution of optimized batches by Emulsification Solvent Diffusion Method

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 356.0	Peak 1:	363.7	98.5	110.6
PdI: 0.223	Peak 2:	5393	1.5	314.3
Intercept: 0.953	Peak 3:	0.000	0.0	0.000

Result quality : Good

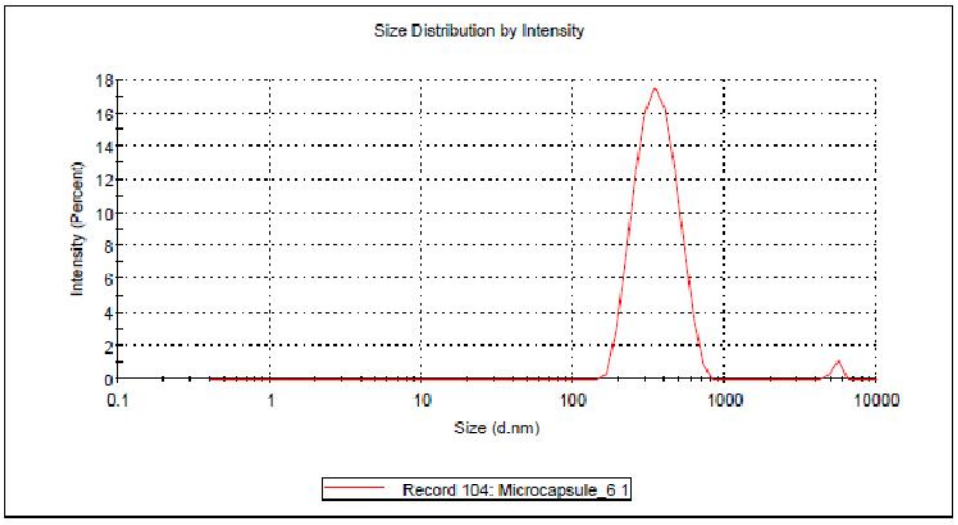


Figure 4.18: Showing the particle size distribution of optimized batches by Nanoprecipitation method

4.1.5. Summary

In this work, the entrapment of thymol in EC by emulsification solvent diffusion and nanoprecipitation method was studied. DR from polymer matrix was noticed in the profile of in-vitro release up to 10 h. Both methods are suitable for the microparticles preparation. No chemical interaction was found in FTIR study and particles obtained are spherical and distinct in nature. Comparison of two methods showed that emulsification solvent diffusion method gives better encapsulation efficiency results while particles prepared by nanoprecipitation method gives the more controlled action in in-vitro release.

Formulation shows maximum 98 % drug release in 10 h. Thymol loaded EC microparticles were successfully prepared by emulsification solvent diffusion as well as nanoprecipitation method without any incompatibility. This study observed that nanoprecipitation method gives quite better results than the solvent diffusion method and appears to be promising for sustained delivery of thymol.

4.2. Thymol loaded beads

4.2.1 Thymol loaded Sodium Alginate Beads synthesized by Emulsified beads techniques

The objective of this work was to prepare thymol loaded sodium alginate beads using microencapsulation emulsion technique and study its release behavior. This work evaluated the effect of concentration of thymol, sodium alginate, stirring speed on synthesis, and sustained release properties of thymol loaded sodium alginate beads using emulsion microencapsulation technique. For optimization method, the statistical design approach of Plackett–Burman factorial design (PBD) was employed. Preparation of thymol loaded calcium chloride – sodium alginate beads was carried out using the emulsion microencapsulation technique.

4.2.2Statistical design and analysis

The PBD factorial structure of analyses was performed utilizing Design-Expert®. In PBD, 11 most reduced components (factors) and 47 most elevated components can be utilized. In most minimal 11 variables, 6 plan process factors were researched so as to upgrade the EE of the beads and staying 5 components were dummied. PBD plots were made of 12 runs containing variation value of factors. 6 independent variables such as amount of Thymol drug (A, mg), Sodium alginate (B, %), Calcium chloride (C, ml), Mechanical stirrer (D, revolution per minute, rpm), Tween (E, %) and Glutaraldehyde (F, ml) were considered.

Table 4.13: Screening factors and their levels in the Plackett-Burman Design

Factors	Name of	Unit	Low Actual	High Actual
A	Thymol	mg	100	200
B	Sodium alginate	%	2	4
C	Calcium chloride	ml	200	400
D	Mechanical stirrer	Rpm	400	500
E	Tween 20	%	15	20
F	Glutaraldehye	Ml	0.5	1.0
G	G	-	-1	1
H	H	-	-1	1
J	J	-	-1	1
K	K	-	-1	1
L	L	-	-1	1

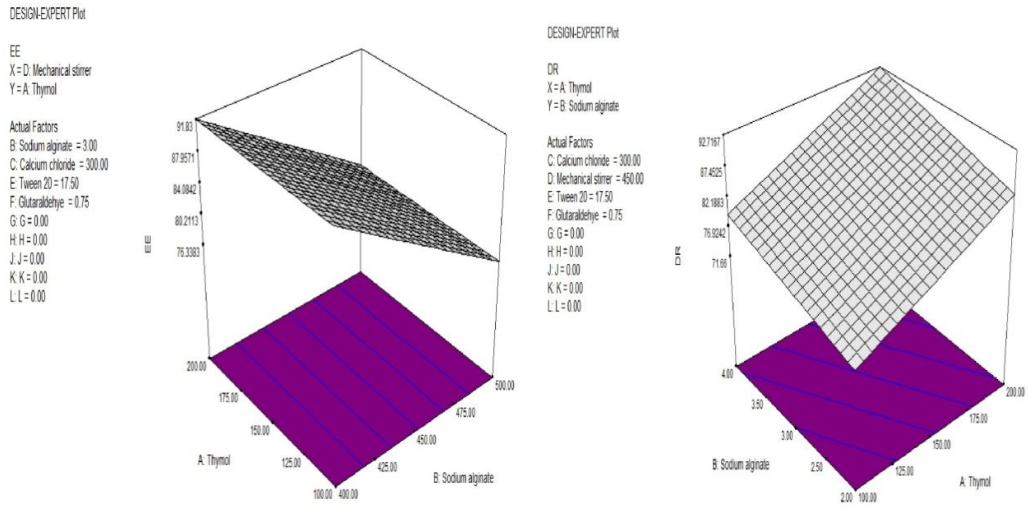
Table 4.14: The Plackett - Burman Experimental Design framework (in coded level) and trial results

Runs	Variables											Responses in	
	A	B	C	D	E	F	G	H	J	K	L	EE (%)	DR (%)
1	-1	1	1	-1	-1	1	1	-1	1	-1	-1	65.31± 0.56	63.44±0.54
2	-1	1	1	-1	1	1	-1	1	-1	-1	-1	96.81± 1.59	87.03±0.65
3	-1	1	-1	-1	1	1	1	1	-1	1	1	91.86± 2.74	92.9±0.74
4	-1	-1	-1	-1	-1	-1	1	-1	-1	-1	-1	95.04± 2.12	30.06±0.73
5	1	1	-1	1	-1	-1	-1	1	1	1	-1	96.20± 1.25	95.07±0.72
6	-1	-1	-1	1	-1	-1	-1	1	-1	1	-1	87.79± 2.55	79.96±0.92
7	1	-1	1	-1	1	1	1	-1	1	1	-1	94.66± 0.57	78.98±0.12
8	1	-1	-1	-1	1	-1	1	1	-1	-1	1	80.27± 2.43	95.18±0.43
9	1	1	-1	1	1	-1	1	-1	1	-1	1	93.24± 1.86	85.72±0.70
10	1	-1	-1	1	1	1	-1	1	1	-1	1	31.18± 0.94	94.63±0.54
11	-1	1	1	-1	1	-1	-1	-1	1	1	1	92.34± 0.83	91.26±0.43
12	1	-1	1	1	-1	1	-1	-1	-1	1	1	84.31± 2.24	92.03±0.87

The levels of variables assessed in this study are given in Table 4.13. DoE examines the input data, shows response plots and reveal the effects of variables to response. The results obtained in PBD are shown in Table 4.14.

Encapsulation efficiency (% EE)

The response (EE) of different experimental runs is presented in Table 4.14. The impact of variable on every response and 3D response surface plots (Figure 4.19) were developed. The % EE of the beads was calculated to be in the range 31.18 % to 96.81 %.



(A) **(B)**

Figure 4.19: 3D surface plots showing independent and interaction effect of thymol and sodium alginate on (A) encapsulation efficiency; (B) drug release, prepared by microencapsulation.

In vitro drug dissolution studies

Figure 4.20 depicts the cumulative % DR as a function of the dissolution time from the thymol loaded beads. The release of thymol was evaluated using phosphate buffer (pH 6.8) as the release medium. Sustained DR was observed in the range of 30.06±0.73 to 95.18±0.43 % in 12 h.

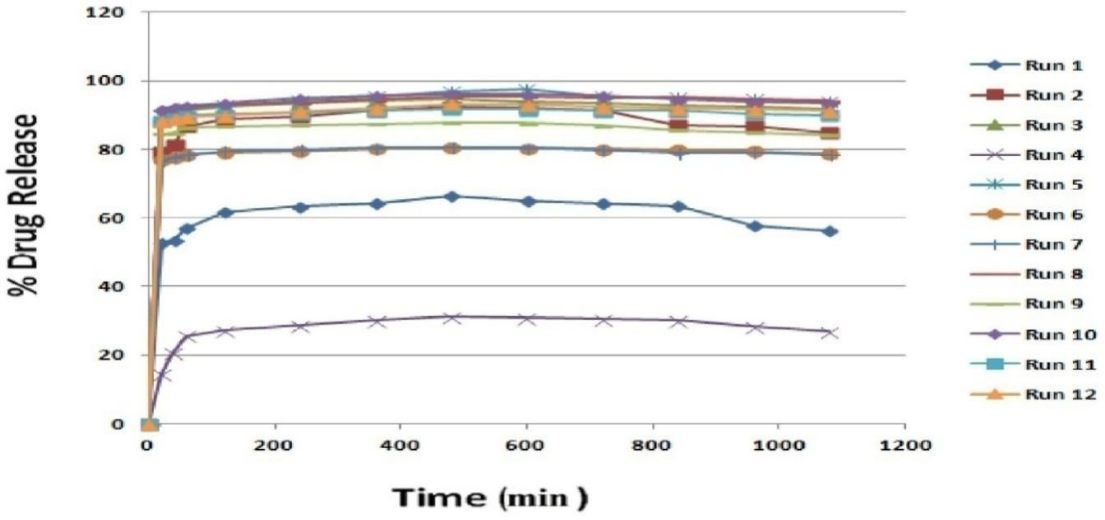


Figure 4.20: Cumulative % drug release.

4.2.3 Characterization of the beads

The beads obtained were characterized using FTIR, XRD, and FESEM. To examine the interaction between the drug with polymer, FTIR spectra of pure thymol, pure Sodium Alginate and formulations 2% and 4% loaded Sodium Alginate were obtained using Fourier Transform Infrared Spectrophotometer. The crystalline/amorphous nature of thymol, pure Sodium Alginate and formulations 2% and 4% loaded Sodium Alginate beads were examined using X-ray diffractometer. The structure of formulated beads was examined by FE-SEM.

Fourier Transform-Infrared Spectroscopy (FTIR)

Figure 4.21 represents the FTIR spectra of pure thymol (a), sodium alginate (b), formulated beads 2% loaded sodium alginate prepared by microencapsulation method (c) and formulated beads 4% loaded sodium alginate prepared by microencapsulation method (d). In the spectra of sodium alginate (b), the absorption bands around 1610 cm^{-1}, 1416 cm^{-1}, and 1306^{-1} cm are attributed to stretching vibrations of asymmetric and symmetric bands of carboxylate anions, respectively. The intensity band at 3430 cm^{-1} corresponds to stretching vibrations of hydroxyl groups. The peak at 3419 cm^{-1} is due to O-H stretching vibrations.

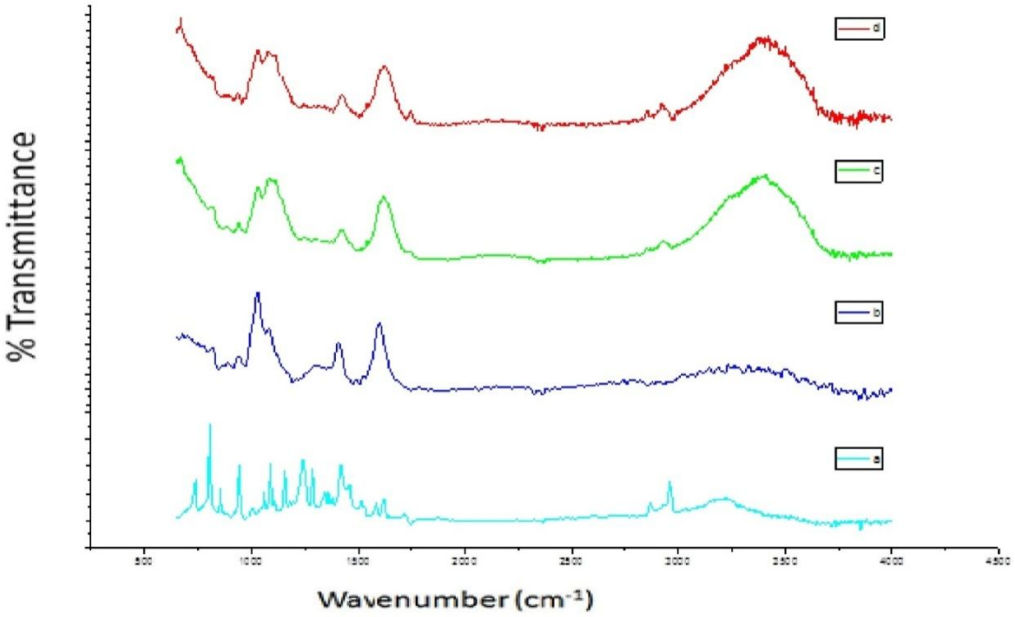


Figure 4.21: FTIR spectra (a) pure thymol (b) sodium alginate (c) 2% sodium alginate beads and (d) 4% sodium alginate beads

X-ray diffraction analysis

X-RD patterns of pure thymol (a), sodium alginate (b), formulated beads 2% loaded sodium alginate (c) and formulated beads 4% loaded sodium alginate both prepared by microencapsulation method (d) are displayed in Figure 4.22 X-Ray diffractogram of pure thymol produced peaks at different 2Ɵ angles show crystalline nature while in case of sodium alginate and the formulations, less intense peaks are observed than that of pure thymol. The XRD designs arranged by the two strategies indicated 43.7% crystalline and 56.3 % amorphous regions. Along these lines, showing lack of diverse diffraction peaks of thymol.

This result tells that majority of the drug was entrapped within the polymer and is dispersed homogeneously at molecular level

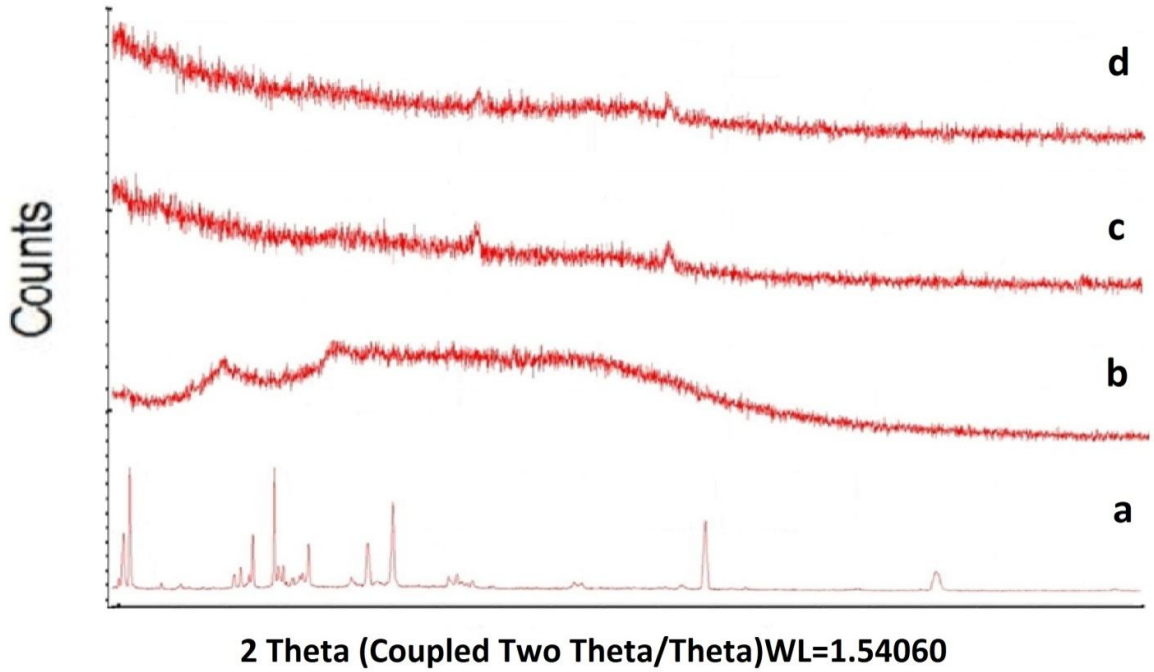


Figure 4.22: XRD of pure Thymol (a), sodium alginate (b), 2% sodium alginate beads (c) and 4% sodium alginate beads (d)

Field-emission scanning electron microscopy

The structure of thymol loaded 2% sodium alginate beads was examined by FE-SEM as depicted in Figure 4.23 (A & A1). Thymol loaded 4% sodium alginate beads were examined by FE-SEM Figure 4.23 (B & B1). The obtained beads are smooth, discrete, and spherical with sharp edges without any cracks or erosion at the surface before drying (Figure 4.23 (A & B)), but after drying, an irregular and rough surface was observed

(Figure 4.23 (A1 & B1)). The beads prepared by microencapsulation method are in the range of 6 to 38 μm.

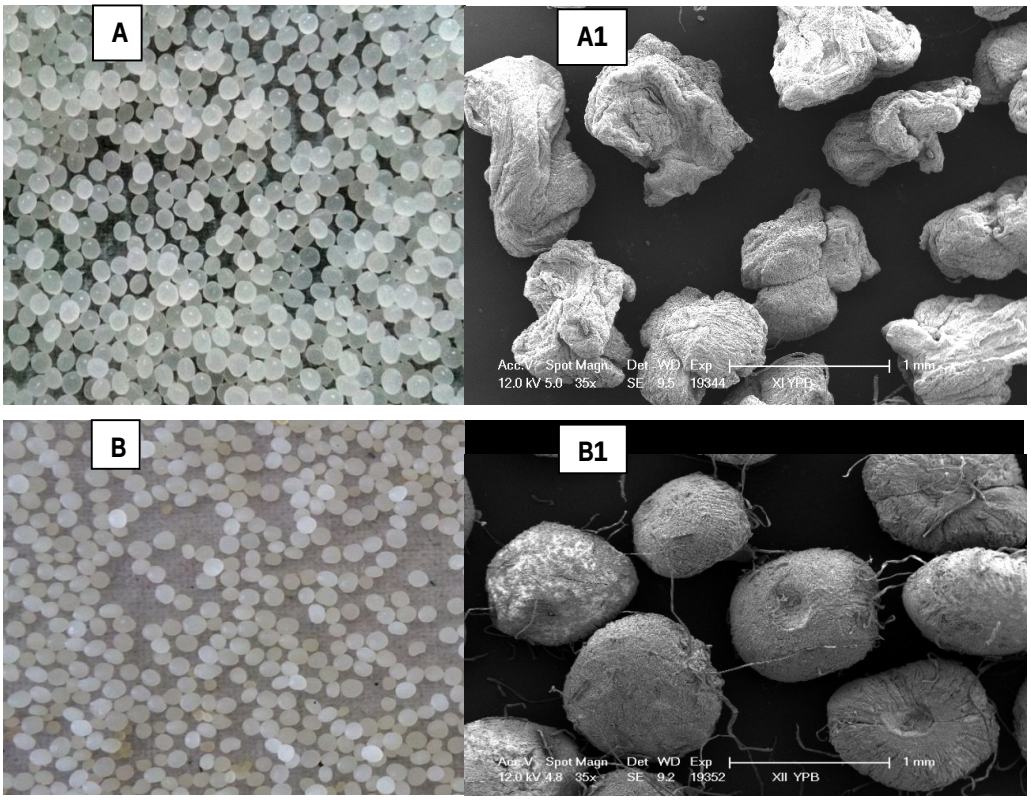


Figure 4.23: Microscopic analysis of beads obtained by microencapsulation : A, A1 before and after drying at 2% sodium alginate beads respectively; and B, B1 before and after drying at 4% sodium alginate beads respectively

4.2.4 Summary of Thymol loaded beads

This work studied successful entrapment of thymol in the sodium-alginate by microencapsulation method. The % EE of the beads ranged from 31.18 % to 96.81 %. DR from polymer matrix was noticed in the profile of in-vitro release up to 12 h. Product formulation indicated that microencapsulation strategy gives increasingly continued activity in-vitro discharge. It also shows highest i.e. 95.18±0.43 % drug release were observed in 12 h. in FTIR study no chemical relations were found and globules acquired were round and particular in nature.

Thymol loaded sodium-alginate beads were prepared successfully by microencapsulation method. This work revealed that microencapsulation method gives satisfactory results and gives assurance for sustained delivery of thymol. As the concentration of thymol increases,

the EE also increases. On the other hand, increasing the concentration of sodium alginate the EE slightly decreases. Whereas an increasing pattern of DR was observed with increase in the concentration of thymol and sodium alginate.

CHAPTER – 5

Conclusion and future scope

5.1 Conclusion

- Microencapsulation of thymol is successfully carried out with three methods.For optimization method, using software Design-Expert® (DoE) the statistical design approach of factorial design and Plackett–Burman of experiment was performed.
- Experiments and analysis with sophisticated equipments support the truth of the optimisation carried out by design of experiment software.
- All systems were successfully completed with repeat run in triplication for each system.

In the experimental investigation of thymol was effectively encapsulated in the biodegradable polymer shell of Ehtyl Cellulose by emulsification solvent diffusion and nanoprecipitation methods. Drug release from prepared polymer matrix was detected slowly in in-vitro release analysis up to 10 h.

Both methods are suitable for the microparticles preparation. No chemical interaction was found in FTIR study and particles obtained are spherical and distinct in nature. When we compare both the methods with respect to the encapsulation efficiency and drug release, both methods have their own better results i.e. solvent diffusion method gives better encapsulation efficiency while particles prepared by nanoprecipitation method gives more controlled action in *in-vitro* release.

In study microencapsulation method was productively used to encapsulate thymol in the calcium-alginate by emulsified beads. The % EE of the beads ranged from 31.18 % to 96.81 %. The prepared polymeric shell microparticles gives slow *in-vitro* Drug Release profile up to 12 h. Formulation showed that microencapsulation method gives more sustained action in *in-vitro* release. Formulation shows maximum 95.18±0.43 % drug release in 12 h. No chemical interaction was found in FTIR study and beads obtained were spherical and distinct in nature.

Without any incompatibility calcium-alginate beads loading thymol were successfully synthesized by microencapsulation method. The study observed that microencapsulation

method gives agreeable results and seems to be shows potential for controlled delivery of thymol. As the thymol concentration enhance, the EE too increases. On the other hand with increasing concentration of sodium alginate the EE slightly decreases. Whereas an increasing outline of drug release was observed with enhance in the concentration of thymol and sodium alginate. In both the methods it is observed that the surfactant concentration plays a very important role in dictating EE and DR.

5.2 Future scope

5.2.1 Size distribution study

The particle size distribution (PSD) and the stability of nanoparticles enabled Pharmaceutical Active Ingredients in complex environments are key attributes to assess their quality, safety and efficacy.

5.2.2 Loading of thymol as per therapeutic recommendations

Physiochemical and pharmacokinetic properties of thymol as well as bioavailability, absorption, eradication rate, solubility are the major blockades in the medication plan and delivery of thymol. Various ways for instance modification in structure, microparticles using cellular derivatives, encapsulation, solid dispersion, complexation and nanoparticles formulation which could prepare to control drug conveyance choices for thymol.

5.2.3 *In vivo* study of thymol release

The discoveries from different examinations evaluated in this demonstrated the job of thymol in the avoidance of different kinds of maladies through its multi-pharmacological properties from cell reinforcement to against tumor ones. Thymol containing plants have been utilized in customary drug for the executives of different sicknesses, for example, numerous malignancy types, cardiovascular ailments, diabetes, and neurodegenerative ailments. Different pharmacological and atomic instruments of activity for its preventive and helpful impacts have been exhibited dependent on its sub-atomic targets recognized in various investigations. While an incredible number of in vitro investigations for various ailments including malignant growth and cardiovascular infections have been accounted for, additional in vivo examinations ought to be embraced to affirm the in vitro discoveries. Moreover, there is an inconsistency between in vitro fixations and in vivo portions in specific sorts of malignancy. Consequently, pharmacokinetics and pharmaceutical

investigations are expected to decipher the irregularity between in vitro and in vivo outcomes.

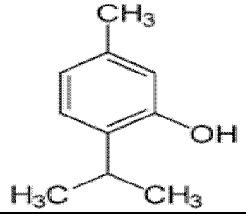
5.2.4 Study of Carvacrol: an isomer of thymol

Another compound known for its similar properties like thymol is Carvacrol, an isomer of thymol. Carvacrol has received considerable attention as an anti microbial agent showing very high antifungal activity. Several attempts have been made to develop active packaging systems in which Microbial Agents are incorporated into polymeric materials and are slowly released onto the food surface. Carvacrol as the core material or in combination of thymol for encapsulation is recommended for future study.

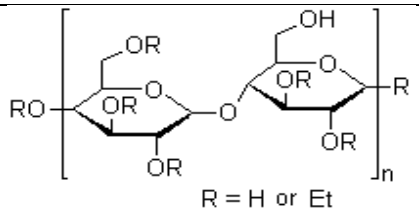
Appendix A

Drug, polymer and excipients profile

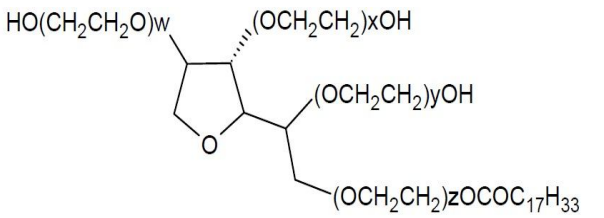
1. Thymol

Sr. No.	Particulars	Details
1	Common name	Thymol
2	Chemical Names	Thymol; 89-83-8; 2-Isopropyl-5-methylphenol; 5-Methyl-2-isopropylphenol; Thyme camphor; 3-p-Cymenol
3	Molecular Formula	$C_{10}H_{14}O$
4	Molecular Weight	150.221 g/mol
5	IUPAC Name	5-methyl-2-propan-2-ylphenol
6	Colour	Colorless, translucent crystals or plates from ethyl acetate, acetic acid or dimethyl carbonate
7	Boiling Point	232.5 °C
8	Melting Point	51.5 °C
9	Water Solubility	900 mg/L (at 20 °C)
10	Therapeutic Uses	Anti-Infective Agents, Antifungal Agents. Thymol, a naturally occurring monocyclic phenolic compound derived from Thymus vulgaris (Lamiaceae), it is one of the most frequently occurring constituents of Essential Oils; it is obtained principally from thyme species. It has been reported to exhibit anti-inflammatory property in vivo and vitro. thymol is antibacterial, antifungal, antiviral, antiseptic, antitumor, and anti-inflammatory properties. It also acts as an antioxidant (free radical scavenger, anti-lipid per-oxidative agent, etc.) and as a biocide by causing disruption of the bacterial membrane. Thymol also provides the distinctive, strong flavour of the culinary herb thyme. Thymol is a part of naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol.
11	structure	

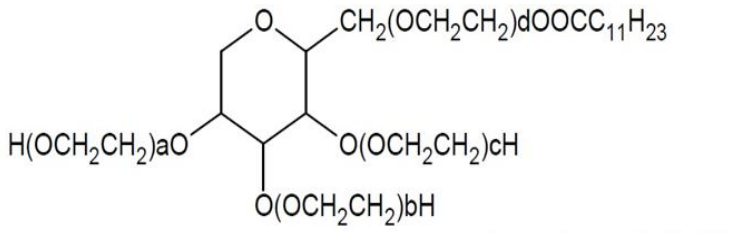
2. Ethyl cellulose

Sr. No.	Particulars	Details
1	Non-proprietary Names	Ethyl cellulose
2	Chemical Names	Aquacoat ECD; Aqualon; Ashacel; E462; Ethocel; ethylcellulosum; Surelease
3	Molecular/Empirical Formula	$C_{12}H_{23}O_6$ $(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$,where n can vary to provide a wide variety of molecular weights
4	Molecular Weight	454.5 g/mol
5	IUPAC Name	2-[4,5-diethoxy-2-(ethoxymethyl)-6-methoxyoxan-3-yl]oxy-6-(hydroxymethyl)-5-methoxyoxane-3,4-diol
6	Colour	White to light tan-colored powder.
7	Boiling Point	--
8	Melting Point	240-255 °C
9	Water Solubility	Insoluble
10	Bulk Density	0.4 g/cm^3
11	Glass transition temperature	129–133 ˚C
12	Moisture content	Ethylcellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily
13	Solubility	Ethylcellulose is practically insoluble in glycerine, propylene glycol, and water. Ethylcellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.
14	Stability & Storage condition	Ethylcellulose is a stable, slightly hygroscopic material may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340nm range. Ethylcellulose should be stored at a temperature not exceeding 32 ˚C (90 ˚F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.
	Therapeutic Uses	Ethylcellulose is widely used in oral and topical pharmaceutical formulation. The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. High-viscosity grades of ethyl cellulose are used in drug microencapsulation (10% - 20 %).
	structure	 R = H or Et

3. Tween 80

Sr. No.	Particulars	Details
1	Common/Non-proprietary Names	Tween 80
2	Chemical Names / Synonyms	Polysorbate 80, Montanox 80,Alkest TW 80,Tween 80 ,PS 80
3	Molecular/Empirical Formula	$C_{64}H_{124}O_{26}$
4	Molecular Weight	1310 g/mol
5	IUPAC Name	Polyoxyethylene (20) sorbitanmonooleate
6	Colour	light yellow to amber oily viscose liquid
7	Boiling Point	>100 °C
8	Melting Point	--
9	Water Solubility	easily soluble in water
10	Bulk Density	1.08 g/mL at 20 °C
11	Solubility	soluble in ethanol, vegetable oil, ethyl acetate, methanol, and insoluble in mineral oil
12	Stability & Storage condition	Tween-80 should be stored in cool, dry and draughty place. Shelf life is 2years. Then, Tween-80 can still be used if qualified after re-check
13	Therapeutic Uses	used as emulsifier, wetting agent, penetrating agent, diffusing etc
14	structure	 Tween 80 Sum of z,x,y & z is 20

4. Tween 20

Sr. No.	Particulars	Details
1	Common/Non-proprietary Names	Tween 20
2	Chemical Names / Synonyms	Polysorbate 20, Polyoxyethylene 20 Laurate.
3	Molecular/Empirical Formula	$C_{58}H_{114}O_{26}$
4	Molecular Weight	1128 g/mol
5	IUPAC Name	
6	Colour	Yellow colored oily liquid,
7	Boiling Point	> 100 °C (212 °F; 373 K)
8	Melting Point	--
9	Water Solubility	dissolves in water at 100 mg/mL, giving a clear yellow solution
10	Bulk Density	1.1 g/mL
11	Solubility	It is also miscible with alcohol, dioxane, and ethyl acetate; practically insoluble in liquid paraffin and fixed oils.
12	Stability & Storage condition	Stored in a closed container.
13	Therapeutic Uses	Non-ionic surfactant used as emulsifying agent in the preparation of emulsions, as wetting agent in pharmaceutical formulations and as solubilizing agent.
14	structure	 Sum of a,b,c & d is 20 Tween 20

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