INVESTIGATION OF DIFFERENT PARAMETERS AFFECTING PARTICLE SIZE TO DEVELOP TUNABLE POLYMERIC EMULSION (O/W) OF AMPHOTERICIN B

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"INVESTIGATION OF DIFFERENT PARAMETERS AFFECTING PARTICLE SIZE TO DEVELOP TUNABLE POLYMERIC EMULSION(O/W) OF AMPHOTERICIN B"

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CERTIFICATE OF APPROVAL

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We hereby recommend that the dissertation prepared under our supervision by: **SANA AHMED (**NUST2013362103MSMME62413F**)** Titled: **"*Investigation of different parameters affecting particle size to develop tuneable polymeric emulsion(o/w) of Amphotericin B*"** be accepted in partial fulfilment of the requirements for the award of **MS** degree with grade____.

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DEDICATION

I dedicate this thesis to my beloved parents for their never ending moral support, motivation and prayers throughout my academic life.

CERTIFICATE OF ORIGINALITY

I hereby declare that this research study has been done for partial fulfilment of requirements for the degree of Master of Science in Biomedical Sciences. The intellectual content of this thesis is a product of my own work and no portion of the work referred to in this thesis has been submitted in any other degree or other institute of learning. I also certify that the thesis has been written by me. The help I received during my research work and preparation of the thesis, itself has been acknowledged. Moreover, I certify that all sources and literature used have been indicated in the thesis.

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Sana Ahmed

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LIST OF ABBREVIATIONS

Amphotericin B	AmB
Water in oil emulsion	W/O Emulsion
Scanning Electron Microscopy	SEM
Energy Dispersive X-ray Spectrometry	EDS
Sabouraud Dextrose Agar	SDA
HydroxyPropyl MethylCellulose	HPMC
Rotations per minute	rpm
Carbon	C
Oxygen	O
millivolt	mV
micrometre	µm
Fourier Transform IR Spectroscopy	FTIR
millilitre	ml
Potassium bromide	KBr
Dimethyl sulfoxide	DMSO
microliter	µl
milligram	mg
Dalton	Da

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ABSTRACT

Oil-in-water emulsions are finding increasing scope as delivery systems to encapsulate lipophilic bioactive components in functional food, personal care, and pharmaceutical products. Toxicity related issues with simple Amphotericin B (AmB) preparations and economic issues related to liposomal preparations led to the need of developing cost effective, nontoxic and therapeutically effective Amphotericin B formulation. Oil in water emulsion of Amphotericin B was prepared using Hydroxypropylmethylcellulose (HPMC) polymer, canola oil and tween 80 as an emulsifier. This study aims for the preparation of AmB formulations, O/W emulsion utilizing HPMC and Carbopol viscosity enhancing agent to improve the stability of globules produced during the emulsification process. Influence of various parameters that include surfactant concentration, polymer concentration, stirring speed and stirring time, on the particle size of Amphotericin B-loaded emulsions and hence on stability of emulsions was investigated. Emulsion were prepared based on above parameters were subjected to various characterization techniques to understand the trend of change in particle size of emulsion and the particle size distribution. The size, shape and elemental composition of particles were analysed using particle size distribution analyser (PSD), scanning electron microscopy (SEM), energy dispersive X-Ray Spectrometry (EDS), FTIR (Fourier Transform IR Spectroscopy) analysis, zeta Potential Measurement and optical microscopy. The prepared Amphotericin B emulsions showed the antifungal activity against several fungus including Aspergillus Tubingensis, Aspergillus Flavus, Aspergillus Niger when tested. The statistical analysis was also performed for every individual parameter under study.

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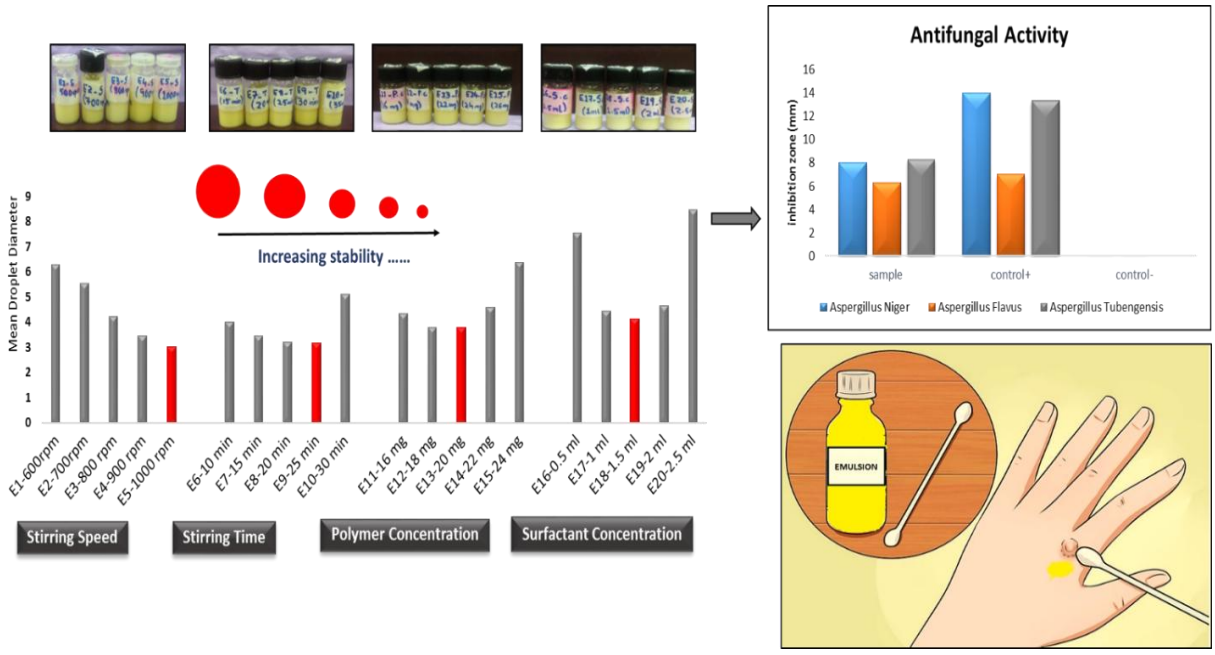
GRAPHICAL ABSTRACT



CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1 Background

The beneficial effects of amphotericin b are put in the shade due to its nephrotoxic effects that may result in complete kidney dysfunction. To reduce the noxious effects of conventional Amphotericin B, formulations incorporating amphotericin b were developed in 1990s for clinical applications. Economic issues related to liposomal preparations led to the need of developing cost effective, nontoxic and therapeutically effective Amphotericin B formulation. Currently, Amphotericin B is a considerate drug model for drug targeting due to this many drug delivery systems have been loaded and tested with Amphotericin B. [9] [14].

During recent years there has been greater interest in the use of topical vehicles that may amend the drug penetration into the dermis. Optimal dermal products tend to exert a high capacity for integrating both hydrophobic and hydrophilic drugs as well as high skin absorbance [1] [12]. To avoid the drawbacks of currently available formulations for instance high cost, several side effects and reduced drug loading capacity, the idea of development of new pharmaceutical formulations of Amphotericin B for topical administration for the treatment of invasive fungal infections was endorsed. [14]

The ability of an emulsion to possess its properties unaffected chemically or physically over a certain period of time is termed as “Emulsion Stability”. However, variations of emulsion characteristics will occur as emulsions are thermodynamically unstable. The more gradually the properties change, the more stable will be the emulsion. [51]. According to Stokes Law droplet diameter is a significant parameter in determining oil-in-water emulsion stability. The droplet size is the core parameter to characterise the stability of emulsions since instability phenomena either influence or are influenced by the particle size. [10] [30]. Therefore, factors affecting the particle size distribution of oil in water topical emulsion of Amphotericin B were evaluated and the stability of prepared emulsions was analysed as a function of particle size.

1.2 Emulsion

Emulsion is known to be a system of two non-adhesive fluids, one of which (internal phase) is homogeneously distributed as particles into the second phase / the continuous phase. [44]. Emulsions are considered thermodynamically tender due to the increase in interfacial area following emulsification. An emulsifier is used to stabilize the system by establishing a shrill film around the globules of dispersed phase. [19] [39]

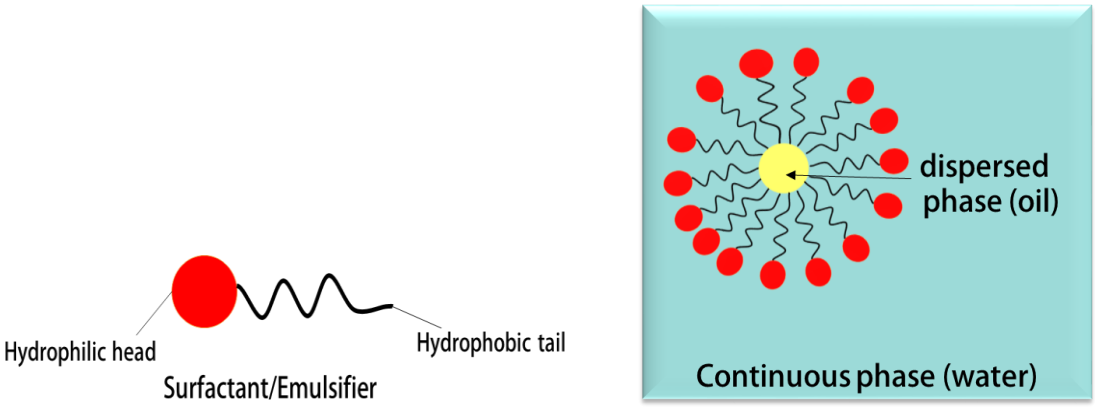


Figure 1.1: a) surfactant molecule *b) oil in water emulsion*

1.3 Types of Emulsion

Emulsions are commonly classified into two categories: [32]

1) **Simple Emulsions**

Oil in water (O/W) emulsions, wherein oil globules are distributed in an aqueous medium. *Water in oil (W/O) emulsions*, wherein water droplets are dispersed in an oil medium. [33]

2) **Multiple Emulsions**

Water in oil in water (W/O/W) emulsions comprises of water globules spread in the oil medium of an oil in water emulsion and *Oil in water in oil (O/W/O) emulsions*, in which very tiny oil particles are suspended in water particles of a water-in-oil emulsion. [33]

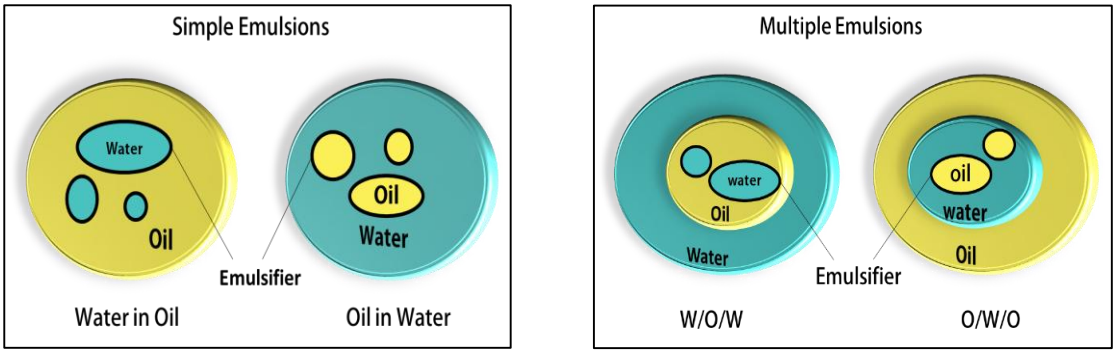


Figure 1.2: Types of Emulsions

1.4 General method of emulsification

The concept of emulsification is grounded upon research on milk. To prepare a therapeutic suspension, the prime consideration is the same as that of milk. Milk is a natural emulsion that contains a layer of casein surrounding fatty globules, suspended in water [33]. Emulsions do not form spontaneously as they are unstable. Therefore, external energy is applied to the two immiscible liquids in order to prepare emulsions, for that purpose mechanical devices are used known as homogenizers [37]. the liquids are

subjected to intense mechanical agitation in Homogenizers. Mostly, an Oil in Water suspension is developed by separating the oily medium entirely into small globules with a covering of emulsifying agent and these droplets are then suspended in aqueous stage [32]. Quite the opposite, the Water in Oil emulsion is formulated by distributing aqueous phase totally into tiny droplets with a covering of emulsifier and finally suspending the particles in the oil medium. [39]

It is possible to prepare an emulsion comprising of only oil and water, but as emulsions are thermodynamically volatile system the oil phase generally splits quickly from the aqueous phase [51]. The droplets fuse with their neighbours as they come into contact, that ultimately results in complete phase separation. All food emulsions are thermodynamically unstable system and will sooner or later collapse. Kinetic stability expresses the rate at which it will progress if it does occur. [52] [53]

1.5 Stability of Emulsion

The ability of an emulsion to possess its properties unaffected chemically or physically over a certain period of time is termed as “Emulsion Stability”. However, variations of

emulsion characteristics will occur as emulsions are thermodynamically unstable. The more gradually the properties change, the more stable will be the emulsion. [51]. Pharmaceutical emulsion products stability is characterized as an ability to retain its physical characters like elegance, odour, colour and appearance [20]

With a specific end goal to fathom emulsion adjustment components, it is critical to isolate thermodynamic strength and dynamic dependability. Thermodynamics is connected more about procedures occurring amid emulsification or after homogenisation. Energy gives data with respect to the rate at which these procedures happen [3] [6]. A hazy emulsion is framed as consequence of mixing together unadulterated oil and water. After a specific time, discrete layers of oil and water are unmistakable. The wonders of combination of oil or water globules in the above case are because of thermodynamic precariousness. The spell taken by the beads to coordinate is related to energy. [3] [51]

In spite of the fact that emulsions are thermodynamically volatile, there is a possibility to form kinetically stable emulsion for an adequate period of time, if their destabilization degree is reasonably low in comparison with the expended lifespan [39] [40] and increasing the activation energy of the system as a result of addition of emulsifiers and/or thickening agents prior to homogenization. [51]

There are numerous phenomena that that can alter emulsion physical properties to be specific combination, creaming, flocculation, Ostwald aging, and so forth. At least two of these precariousness wonders can happen all the while. It is then huge to recognize the reason for unsteadiness to choose suitable parts to frame stable emulsions. [40] [44]

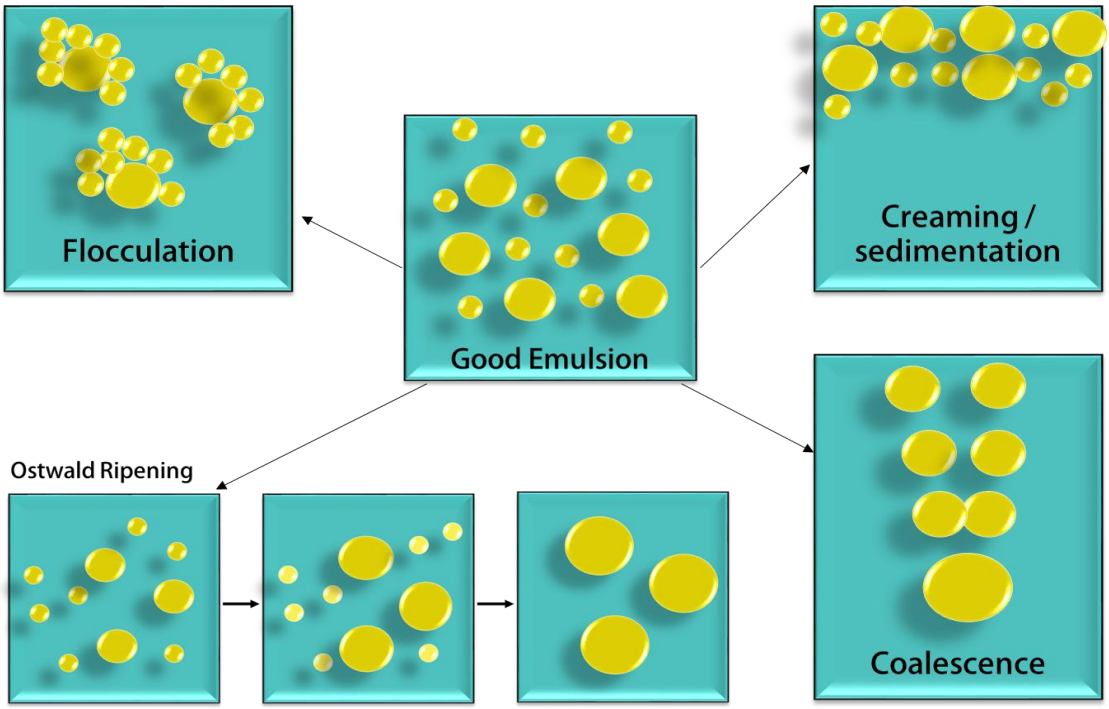


Figure 1.3: Schematic representation of instability phenomena in Emulsion systems

1.5.1 Flocculation

The association of tiny emulsion particles due to van der Waals attraction when there is not enough repulsion between the droplets to form large aggregates, that may disperse upon wobbling is termed as flocculation. It. Flocculation is thought to be a precursor of coalescence and a reversible phenomenon in which the droplets remain intact [39]. Flocculation may be minimised /eliminated by using ionic surfactant to create an electrical double layer or non-ionic surfactants or polymers to form non-electrical layer as an energy fence between the droplets. The most effective way to control the frequency and extent of flocculation is to regulate the steric, electrostatic and hydrophobic interactions between particles. [51] [52]

1.5.2 Creaming or Sedimentation

Creaming is the phenomenon in which due to the upward or downward motion of the emulsion particles having lower or higher density than the continuous phase, splits out the dispersed phase thus forming a layer on the top of the continuous phase. Creaming can be reduced by increasing the viscosity of the continuous phase [51]. Upward creaming commonly occurs in oil in water emulsions when droplets of the internal phase are not as much dense than the continuous phase. Inversely water in oil suspensions encounter downward creaming when particles of the dispersed medium are more thick than the external phase. [52]

1.5.3 Coalescence

Thinning and disruption of the liquid film between the droplets results in fusion of dispersed phase droplets to form larger droplets is known as coalescence. Partial coalescence involves partially crystalline droplets when the crystals of one droplet breach a second droplet while retaining their individual identity (as in flocculation) but there exists a molecular link between their contents (as in coalescence). Over the melting point the crystalline network is demolished and the partially coalesced droplets will unite [52] [53]. Coalescence possibly will be reduced by the addition of components with elevated boiling point or high molecular weight, to the external phase. [39]

1.5.4 Ostwald Ripening

Gradual growth of the larger droplets at the cost of smaller particles due to mass transfer of soluble dispersed phase (oil) via the continuous phase (water) is the process called Ostwald ripening. Droplet size distribution also changes due to the molecular diffusion from small to larger droplets. Ostwald ripening commonly occurs in some food emulsions like soft drink emulsions [52]. Ostwald ripening will be retarded in emulsions with a narrow droplet size distribution and by using emulsifiers that do not increase the oil solubility. [51]

1.6 Aim of Research

The goal of the present study is to carry out parametric analysis in order to develop a stable oil in water emulsion containing Amphotericin B for topical use and to evaluate its antifungal effects. Amphotericin B antifungal drug was dispersed in HPMC polymer by emulsification method and tween 80 was used as surfactant. Canola oil was used as it is loaded heavily with omega-3 fatty acids to diminish tenderness and no illustration of side effect to the dermis and moisturizes skin. Carbopol® was used to produce a wide range of viscosities and flow properties in prepared (Amphotericin B emulsion) which is already in use for the preparation of lotion and creams. The idea of this project is to analyse stability of emulsion on the basis of particle size and to study and analyse the parameters affecting the particle size distribution of prepared formulations.

CHAPTER 2
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Amphotericin B

Amphotericin B (AmB) is a yellow coloured polyene antibiotic which was derived from the culture of Streptomyces nodosus [14]. It is the first choice drug to fight deadly systemic fungal infections. Amphotericin B has been extensively used in clinical practice as invasive fungal infections are the chief cause of mortality in AIDS patients, cancer patients and in transplant recipients [63]. The beneficial effects of amphotericin b are put in the shade due to its nephrotoxic effects that may result in complete kidney dysfunction [9] [66]. To reduce the noxious effects of conventional Amphotericin B, formulations incorporating amphotericin b were developed in 1990s for clinical applications [68]. Economic issues related to liposomal preparations led to the need of developing cost effective, nontoxic and therapeutically effective Amphotericin B formulation. Currently, Amphotericin B is a considerate drug model for drug targeting due to this many drug delivery systems have been loaded and tested with Amphotericin B. [9] [10]

2.2 Chemical characteristics of Amphotericin B

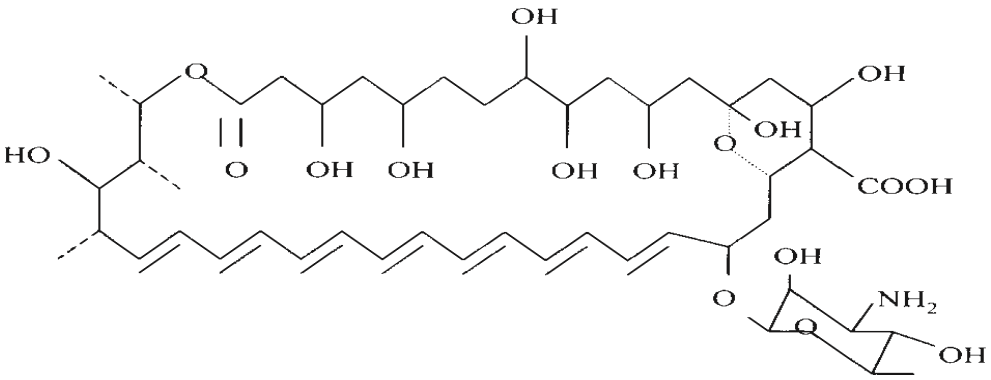


Figure 2.1: Structure of Amphotericin B [56]

Due to the structure of Amphotericin B, it exhibits two physicochemical properties, Presence of lactose ring (having polar and nonpolar ends) enables the molecule to display ***amphiphilic behaviour*** and ***Amphoteric property*** is displayed due to existence of ionisable carboxyl and amine groups [29]. Both the above mentioned properties the uneven distribution of hydrophobic and hydrophilic groups makes Amphotericin B poorly soluble in all aqueous solvents and in various organic solvents. [70] [76]

2.3 Interaction mechanism of Amphotericin B

Antifungal activity of Amphotericin B is based on its affinity for ergo-sterol which is the major sterol found in fungal cytoplasmic membranes. The interaction of the antibiotic with ergo-sterol results in the formation of porous channels in the cell membrane of the pathogen. [29]. These pores serve as a source for leakage of essential monovalent ions (K+, Na+, H+, and Cl-) and little natural atoms from the cell. This outflow of vital nutrients causes weakening of membrane hindrance work and can be considered as an essential impact prompting to cell demise [21]. Owing to the amphiphilic structure of polyenes, this Nano-pore is able to adopt an orientation in which the hydrophobic part of amphotericin b faces the lipid parts while their hydrophilic end is exposed to the channel centre allowing the outflow of water and ions through the Nano-pore. [10]

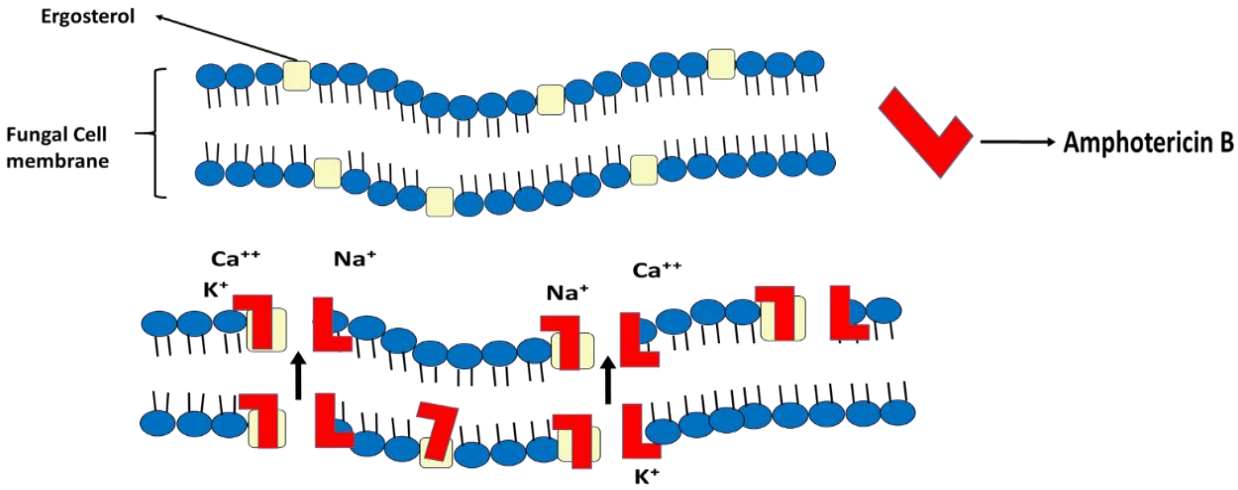


Figure 2.2: Mechanism of action of Amphotericin B [21] [18].

2.4 Amphotericin B Lipid Formulations

Different recent studies and workings have suggested that delayed and acute toxicity can be reduced by Amphotericin B associated with lipids. Substances which can dissolve in fats can be solubilized in the phase containing oil, and the lipid emulsion can also stabilize drugs which are not stable in an aqueous phase or environment. [68] [71]. Poor bioavailability of Amp B via oral route and when administered by parenteral route its benefits are undermined due to high incidence of unfavourable reactions like temperature, nausea, vomiting, headache and nephrotoxic effects leading to renal

dysfunction [45] [9] have led to the need of developing of commercial formulations of phospholipid vesicles like *AmBisome* (August 1997), *Amphotec* (December 1996) *and Abelcet* (December 1995) for therapeutic use [66] [68].Though, the efficacy of these new products is greatly restricted by their high costs so there is a need to develop low cost formulations.

2.5 Topical Delivery of Antifungals

Human skin is an efficient film and has three primary layers in particular epidermis, dermis and hypodermis. Stratum corneum (the peripheral layer of epidermis) made out of dead and keratinized cells and it is an outstanding hindrance to entrance of medications through the skin [74]. The best test for dermal conveyance is stratum corneum, and keeping in mind the end goal to enhance its penetrability, new definition approaches have been researched. Colloidal medication transporters, for example, smaller scale emulsions, vesicular bearers including liposomes, ethosomes and niosomes and, both lipidic and polymeric particulate transporter frameworks are among those new bearers to guarantee dermal organization of antifungals by dermal focusing on [28] [47].

Medication ought to infiltrate into skin layers to guarantee viable medication fixations taking after topical organization. Sorts of the plans and in addition the physio-substance attributes of medication atoms are viable parameters in topical conveyance of medications. At the point when a medication is connected on the skin, a terminal is shaped in the lipidic stratum corneum that discharges the medication step by step to the fundamental layers of skin. Henceforth, with a specific end goal to accomplish a topical impact for an antifungal medication, the discharge rate of the lipophilic medication ought to be managed by the plan to accomplish high nearby remedial fixation and to offer delayed pharmacological impact. [56]. Another imperative thought is the sub-atomic weight of the medication; this is particularly essential for antifungal medications referred to surpass 500 Da, for example, amphotericin B and ketoconazole. These contemplations have prompted to the advancement of a few transporters which were found to enhance topical medication conveyance by either finding a route into a shunt, for example, hair follicle, collecting amongst corneocytes, and blending with skin lipids, or by breaking down and converging with lipidic layers. [26] [69] Whether their size was in the micrometer or nanometer go, transporter frameworks were found to bestow attractive qualities to topical definitions of antifungal medications.

2.6 Advantages of topical administration

In topical organization, the entering of medications to systemic course is avoided or minimized. Along these lines, the systemic unfriendly impacts of medications are kept away from [25]. In addition, topical arrangements have better patient consistence due to their non-intrusiveness and, they can act naturally controlled [35] [50]. Topical operators that are expectedly utilized for the treatment of skin contagious contaminations are normally planned as creams, salves or gels. [22]. They either display fungicidal or fungistatic activities relying upon the operator being conveyed. Since the symptoms of contagious operators connected topically are not exactly their oral partners, they are the favoured specialists. [5] [11] [23]. Another preferred standpoint of topical plan is that it maintains a strategic distance from medication sedate associations, which are more regular if there should be an occurrence of oral organization. Wellbeing of treatment is to a lesser extent a sympathy toward topical drugs than oral medicines, as serum ingestion has a tendency to be negligible with topical dermatophytes treatment, subsequently making topical treatment an alluring methodology for limited diseases.

2.7 Amphotericin B Topical Formulations

During recent years there has been greater interest in the use of topical vehicles that may amend the drug penetration into the dermis. Optimal dermal products tend to exert a high capacity for integrating both hydrophobic and hydrophilic drugs as well as high skin absorbance [1]. Numerous dermal vehicles contain chemical enhancers and strong solvents to accomplish these standards. the major hindrance of chronic application is “irritation”. To deal with this problem there is a need to develop a topical vehicle system that is independent of chemical enhancers or alcohols to enable drug penetration into and through the skin. [15]

Colloidal systems and advanced drug-delivery systems such as MEs have been studied as drug delivery and targeting systems as they can remodel the bioavailability, stability and side effects of several drugs [77]. Numerous mechanisms have been suggested to explain the benefits of ME for topical drug delivery [59]. Topical delivery of drugs is dependent upon various factors that are a) drug’s affinity to the internal phase in ME, b) constituents of ME c) increased concentration gradient towards skin and the external phase acting as a reservoir, which helps to maintain a constant concentration in internal phase. [59].

To avoid the drawbacks of currently available formulations for instance high cost, several side effects and reduced drug loading capacity, the idea of development of new pharmaceutical formulations of Amphotericin B for topical administration for the treatment of invasive fungal infections was endorsed. A study was carried out by Butani, Yewale & Misra, [15] in order to evaluate the topical drug delivery potential of MEs comprised of non-irritant and pharmaceutically suitable components. Diffusion in topical drug delivery, occurs mostly via stratum corneum and the drug pursue diverse paths to penetrate the stratum corneum. Amphotericin B has poor aqueous solubility therefore it cannot penetrate the skin. [1] [75]. Thus optimal solubility of Amphotericin B is required, in both organic and inorganic phase so as to maximize its flux. Therefore, ME formulations were prepared with an objective to improve the solubility and ultimately the dermal bioavailability of the drug. It intensifies the skin penetration and permeation of Amphotericin B. ME was preferred over other colloidal analogues like liposomes and nanoparticles due to the simplicity of the process and low cost of preparation. [72]

2.8 Particle size and Emulsion stability

Particle size in emulsion is defined as the diameter of internal phase globule. The droplet size measurement is of great importance since it delivers a lot of information about the emulsion properties. Numerous techniques have been established to measure particle size distribution, the commonly used techniques are microscopy, light scattering, ultrasonic methods and more recently NMR. [51]

The droplet size is the centre parameter to portray the steadiness of emulsions since precariousness marvels either impact or are affected by the molecule estimate. the normal bead measure tends to increment because of Coalescence and Ostwald aging. In Flocculation the bead size is not influenced as beads don't coordinate. Notwithstanding, the presence of flocs may instigate an incorrect characterisation. The molecule measure specifically influences the creaming rate and wonders of creaming. [30]

According to Stokes Law droplet diameter is a significant parameter in determining oil-in-water emulsion stability. Actually, a direct correlation exists amongst particle size and emulsion stability in an emulsion in which, droplet size represented by volume and number mean diameters which are fragile to volume and number of droplets in

system, respectively [30]. Besides droplet diameter, particle size distribution is also vital for predicting emulsion stability particularly beverage emulsions as they mainly comprise of flavour agents which bear small chain fatty acids, soluble in continuous water phase, which may pass through continuous phase and merge to form large droplet. This will lead to an instability phenomena termed as Ostwald ripening. one way is to compute particle size distribution variations during time so as to evaluate the emulsion stability [37] [52].

Size of particle suspension droplets assumes a critical part in the deciding the properties of emulsion frameworks, for example appearance, security, shading, surface and so on [60]. Different reviews have uncovered that the extent of the beads depends on many variables, for example, natural stage, interfacial strain, emulsifier sort, preliminary conditions and framework pieces. [55]

Lowering droplets size will enhance the emulsion stability as emulsions having droplet diameter below 100 nm (micro emulsion) are thermodynamically stable [2]. Good stability of emulsion is associated with fine and uniform particle size. [3] [6]. Greater stability of emulsion containing smaller dispersed phase diameter could be perceived due to the facts that destabilizing processes for instance coalescence cause an increase in droplet size and another process called “ageing” decreases the stability of some emulsions remarkably. [38].

The reaction taking place at the surface of emulsion droplets accelerates the lipid oxidation therefore smaller particles are more prone to it due to their greater surface area per unit volume. [48]. Emulsions comprised of small sized particles (100 nm) are more steady than the emulsions with the bigger molecule sizes. [27]

With reference to different researchers it has been discovered that the overall stability is inversely proportional to particle size, with smaller particles providing a greater packing efficiency, thus creating a more homogenous layer. [18] [53] [62]

Emulsions with smaller molecule measure have a tendency to have higher security to gravitational division, flocculation and coalescence [41]. The light dispersing force of oil beads diminishes with lessening size so the emulsions are less turbid contrasted with equal traditional emulsions in this manner expanding bioavailability of epitomized mixes. [67].

2.9 Factors effecting particle size in Emulsion system

2.9.1 Stirring Intensity

Stirring speed is defined as the energy applied per unit total volume. Stirring is useful in order to create a stable and consistent suspension by transforming large drops to tiny droplets. It has a direct impact on particle size [13]. The droplet size is inversely related to the stirring intensity therefore raising the shear stress may reduce droplet size in emulsion system. The reason behind reduction in droplet size may be due to splitting and distribution by the applied mechanical energy of the organic and water phases so as to maintain concentration gradients at the oil–water interface [61]. By applying mechanical energy emulsification is usually achieved. Stirring is done so that the interface among the two stages is slanted to extents that bulky droplets are developed that are afterwards fragmented into minute particles to get a static and homogeneous suspension [16]. Mild mixing of the organic and aqueous phases is required during the spontaneous emulsification method to produce very fine droplets according to Saberi, Fang, & McClement, [58]. According to Gonglun and Daniel [16] a more static formulation may be formed with an elevated shear rate but lower than 2500 rpm because higher speed than 2500 rpm can cause surfactant to escape out of interface.

2.9.2 Homogenization Time

The duration (in minutes or seconds) of emulsification is defined as agitation time which is another significant factor for emulsification. Khan et al. [32] [33] reported that the droplet size declines with increasing stirring time. The surfactant becomes more operative with prolonged mixing time. Though, too long mixing time may influence the effectiveness of emulsifier negatively as the intense shearing will cause the emulsifier to escape out of the oil– water interface. Mixing time is a crucial factor during emulsification. Gonglun and Daniel, [16] reported that with the increase of mixing time, the radii of the droplets of the dispersed phase decrease. the effectiveness of emulsifying agent increases by extending mixing time, however, too much long mixing time will reduce the effectiveness as it will cause the emulsifying agents to drop out from liquids interface [2] [4]. A study carried out by Chen & Tao [16] revealed that the mean size of the particles declined very swiftly in the first few seconds and then steadily reached the limiting value after 15 min and as the emulsifying time increased further, emulsion stability reduced.

2.9.3 Surfactant Concentration

The quantity of the emulsifier used is also very crucial as the existence of surfactant at the oil-water interface administrates the efficiency of emulsion formation and stability of emulsion. Numerous investigations have verified that beyond a specific surfactant concentration emulsion stability quickly deteriorated. This is due to the reason that at low emulsifier dosage, accumulation of the oil droplets causes the emulsion to become unstable and as a consequence of high surfactant concentrations, rapid coalescence occurs that results in destabilization of emulsion. [32] [33] [57]. When the surfactant concentration was increased further, an increase in mean particle diameter was detected, which has also been reported in other studies [78] [34]. The droplet size increases above a certain surfactant level due to the formation of a highly viscous liquid crystalline phase, which makes spontaneous breakup of the oil–water interface more tough. [73]. The amount of emulsifier plays a chief role as it can elude the coalescence of the oil droplets. The molecules of the emulsifier tend to align at globule surface thus reducing the free energy between two phases at the interface and fighting coalescence of the particles. Small droplets have great surface area and thus may require more surfactant to stabilize the emulsion droplets [61].

2.9.4 Polymer Concentration

Droplet size in emulsion systems are also greatly affected by the concentration of the polymer used. The particle size increased with increase in the polymer concentration due to the reason that the viscosity of the oil phase increases that enhances the viscous forces battling droplet breakdown and thus bigger oil droplets are developed, resulting in increased particle size resulting in the formation of large sized droplets. [36].

According to a study carried out by Ayoub et al [8], polymer quantity can be an influencing factor to the physiognomies of emulsion particles and could also influence the encapsulation proficiency. All rest parameters were kept constant and the effect of change of polymer amount was analysed as a function of particle size. The results showed that polymer has not any substantial effect up to a specific proportion but after that by increasing its concentration, the particle size increases. The concentration of polymer, is a significant factor to regulate the final particle size. Different concentrations of polymer were used and rest of the parameters were kept constant. The results revealed a specific concentration of polymer suitable to formulate a long time stable emulsion. At increased concentration, the curve displaying increase in particle

size turned flat which means no further effect on particle size. Though, this higher concentration instigated increased foam in the preparation. [2]

2.10 Employment of pharmaceutical emulsion

- In pharmacy and medicine, emulsions are developed for nearly all the major routes of administration for instance oral, parenteral and dermatological. Lipid emulsions are employed for intravenous drug administration, parenteral nutrition and as oxygen carriers. [7]
- Lately, attention has been attributed on regulating the size distribution and exploring the stability phenomenon for fluid formulation systems. The foremost benefit of these systems is that they enhance the ability to transport hydrophilic drugs topically, solubility and accessibility to a site of action of medicinal drugs. [32] [40]
- Macro suspensions and micro emulsions are usually well reported as transporters for water loving and hydrophobic stimulants. Micro emulsions are blend of water, oil and emulsifier with co surfactant. They may be formed impulsively and are thermodynamically firm, they enhance bioavailability and solubility, by incorporating diverse range of drug molecules they act as potential drug delivery systems. [18]
- Multiple suspensions, exclusively W/O/W emulsions are commendable aspirant for precise and constant release of stimulants. Multiple formulations are utilized as an alternative to liposomes as transport system. [40] [42]
- Emulsions have been put to service for several centuries for treating local skin ailments. Oil in water formulations are employed for dealing with skin lacerations. The benefit of consuming the topical emulsions is to by-pass digestive atmosphere and first pass metabolic effect. [17] [42] [43]
- Emulsions may also be utilized to stabilize hydrolytically vulnerable drugs for extended release, reduced toxicity and intended transport of drugs to some organs and improved pharmaceutical effect [24].

CHAPTER 3
MATERIALS AND METHODS

3. MATERIALS AND METHOD

3.1 Materials

Amphotericin B (USP grade) obtained from Synbiotics India was used as an antifungal drug, Hydroxypropyl methycellulose CP 15 (HPMC) used to encapsulate the drug was purchased from Sigma Aldrich, Canola oil (commercially available edible oil from Season's Lahore, Pakistan) was obtained from market. Carbopol 934P used as viscosity enhancer, Sodium benzoate used as a preservative and Polyoxy ethylene sorbitan mono oleate 80 (Tween 80, density 1.08g/ml) utilized as a surfactant were purchased from Sigma Aldrich, USA. Sabouraud Dextrose Agar (SDA) media purchased from Sigma Aldrich. DMSO (Lab scan) was used for making samples for all assays. *Aspergillus niger* FCBP 0198, *Aspergillus flavus* FCBP 0064, used for antifungal testing were obtained from FCBP (fungal culture bank of Pakistan) maintained on SDA at 4^{o}C prior to use.

3.2 Emulsion preparation process

For preparation of aqueous phase (continuous phase), distilled water (approximately) 10 ml was heated to about 98°C and then 133.2 mg of carbopol was added into it. With the help of continuous stirring carbopol was completely dissolved in water. Next step involved the addition of antifungal drug amphotericin B (30 mg) and after that HPMC polymer (20 mg) was added. To ensure proper mixing continuous stirring was done throughout the process. Complete mixing of these all ingredients results in the formation of the Aqueous Phase**.** For the formation of Oil Phase (dispersed phase), canola oil (1.2 ml) and tween 80 (1.2 ml) were mixed together with the help of stirring for about 5-10 minutes. In the next step both phases were mixed together and were subjected to constant stirring in order to obtain the required emulsion. At the end sodium benzoate (50 mg) was added and stirring was done for 5-10 minutes. Following this procedure enabled the preparation of 10 ml of polymeric emulsion (O/W) of Amphotericin B as shown in figure 3.1.

3.3 Parametric analysis of Emulsion

3.3.1 Variation in Stirring Speed

The first parameter to be analyzed was the rate at which the process of emulsification was carried out. To investigate the effect of stirring speed on the size distribution of particles five different emulsions were prepared at five different stirring speeds ranging

from 600 rpm to 1000 rpm but keeping all other parameters constant as displayed in table 3.1. These five emulsions were then characterized to examine the impact on particle size of emulsion by increasing or decreasing the rate of emulsification.

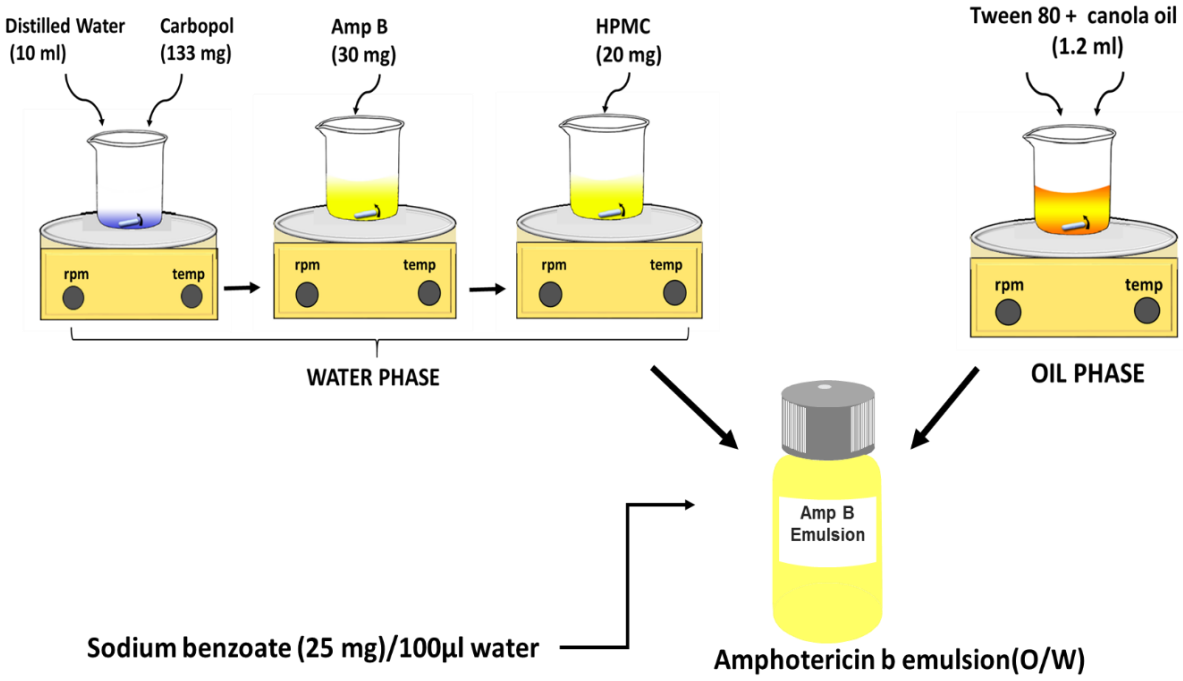


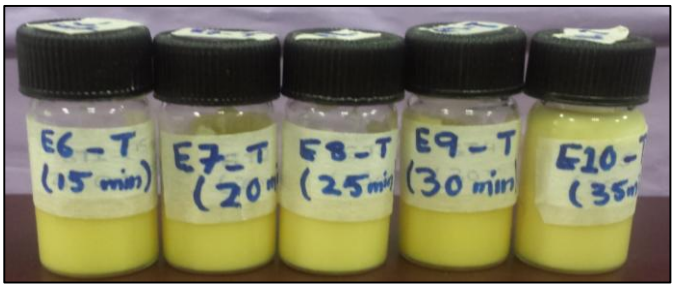
Figure 3.1: Schematic of preparation of Amphotericin B Emulsion

Emulsion name	Parameter analysed 1. stirring speed	Drug quantity	Polymer quantity	Surfactant quantity
E1-S	600 rpm	30 mg	20 mg	1.2 ml
E2-S	700 rpm	30 mg	20 mg	1.2 ml
E3-S	800 rpm	30 mg	20 mg	1.2 ml
E4-S	900 rpm	30 mg	20 mg	1.2 ml
E5-S	1000 rpm	30 mg	20 mg	1.2 ml

Table 3.1: Variations in speed of stirring

3.3.2 Variation in Stirring Time

The second parameter under study was the time of stirring provided to each step during the process of emulsification. Five emulsions were prepared provided with five different stirring times ranging from the duration of 15 minutes to 35 minutes for each stage of emulsification procedure. The prepared samples were then analyzed to study the effect of stirring time on particle size distribution of prepared emulsions and hence on their stability.

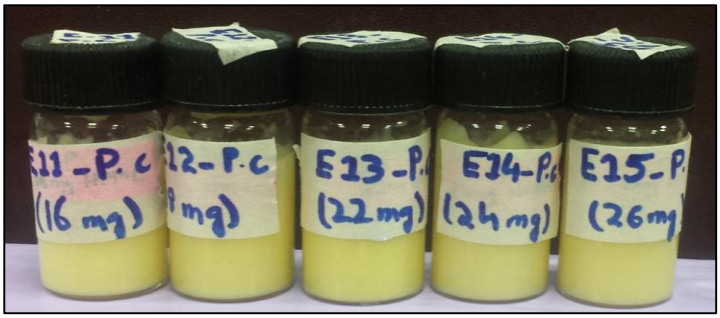


Emulsion name	Parameter analysed 2. stirring time	Drug quantity	Polymer quantity	Surfactant quantity
E6-T	10 min	30 mg	20 mg	1.2 ml
E7-T	15 min	30 mg	20 mg	1.2 ml
E8-T	20 min	30 mg	20 mg	1.2 ml
E9-T	25 min	30 mg	20 mg	1.2 ml
E10-T	30 min	30 mg	20 mg	1.2 ml

Table 3.2: Variations in time of stirring

3.3.3 Variation in Polymer Concentration

Third parameter that was examined was the polymer(HPMC) concentration used and its impact on particle size distribution of emulsion and thus on the stability of emulsion. The polymer concentrations starting from 16 mg/10ml, 18mg/10ml, 20mg/10ml, 22mg/10ml and 22mg/10ml were used. The effect on particle size as a consequence of these modifications in polymer concentrations were then observed keeping all other parameters constant in order to select the most stable emulsion with the smallest mean particle size at a particular polymer concentration used.

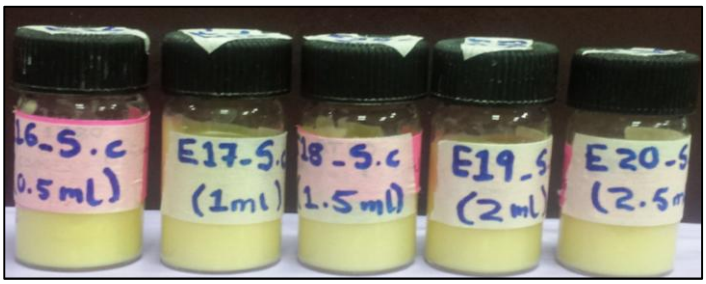


Emulsion name	Parameter analysed 3. polymer concentration	Drug quantity	Polymer quantity	Surfactant quantity
E11-P.c	16 mg	30 mg	16 mg	1.2 ml
E12-P.c	18 mg	30 mg	18 mg	1.2 ml
E13-P.c	20 mg	30 mg	20 mg	1.2 ml
E14-P.c	22 mg	30 mg	22 mg	1.2 ml
E15-P.c	24 mg	30 mg	24 mg	1.2 ml

Table 3.3: Variation in polymer concentration

3.3.4 Variation in Surfactant Concentration

Quantity of the surfactant/emulsifier (Tween 80) used was the fourth parameter of interest and its impact on particle size distribution was studied by preparing five sample emulsions with five different concentrations of the surfactant but keeping all the other parameters constant. The concentrations used were 0.5ml/10ml, 1ml/10ml, 1.5ml/10ml/, 2ml/10ml and 2.5ml/10ml.



Emulsion name	Parameter analysed 4. surfactant concentration	Drug quantity	Polymer quantity	Surfactant quantity
E16-S.c	0.5 ml	30 mg	20 mg	0.5 ml
E17-S.c	1 ml	30 mg	20 mg	1 ml
E18-S.c	1.5 ml	30 mg	20 mg	1.5 ml
E19-S.c	2 ml	30 mg	20 mg	2 ml
E20-S.c	2.5 ml	30 mg	20 mg	2.5 ml

Table 3.4: Variation in surfactant concentration

3.4 Antifungal Assay

Antifungal activity was done in triplicates via agar well diffusion method and inoculation was done by streaking. Briefly, autoclaved SDA media about 25 ml (6.5% in water), was poured into petri plate and allowed to solidify. Streaking method was used to inoculate 1ml of fungal culture on each plate. Three wells were made using micro borer. The sample dilutions were made, 1ml of amphotericin b formulation was diluted with 5ml of DMSO, blank emulsion dilution in DMSO (1ml/5ml) were prepared to be used as a negative control and 2.5mg Amphotericin b in 5ml of DMSO was used as a positive control. 100µl of each sample was poured into the corresponding labelled well (50µg of Amphotericin B per well). Incubation of petri plates for 48 hours at 28±2˚C in incubator was done and Vernier calliper was used for measurement of zones of inhibition.

3.5 Characterization

3.5.1 Optical Microscopy

Prepared emulsions were observed with a Transmission microscope - OPTIKA 600 to examine shape and external morphology of particles. A small drop of emulsion approximately 0.5ml was placed on a glass slide and spread evenly so that a smooth layer was deposited on the slide. The slide was then examined under the microscope at different magnifications 10X, 20X and 50X. No staining agents were used.

3.5.2 Fourier Transform IR Spectroscopy

Fourier Transfer Infrared Spectroscopy (FTIR) was used to investigate and predict compatibility between the drug and polymer used. Two samples were analyzed, one

was the blank emulsion (containing HPMC polymer) and second was the drug loaded emulsion (containing both HPMC and Amphotericin b). a small drop of each sample was placed on a KBr pellet and was scanned from 4000 to 450 cm-1 in FT-IR Perkin Elmer spectrometer. The IR spectrums thus obtained were compared to detect appearance or disappearance of peaks.

3.5.3 Particle Size Distribution Analysis

It gives data about the size and scope of an arrangement of particles illustrative of a material. Particle size distribution analyser (*HORIBA - LA 920, Ver.3.70)* was used to examine all the twenty sample emulsions. Sample was prepared by adding approximately 1ml of sample emulsion in 200ml of ultra-pure water and was then subjected to sonication for 30 minutes to allow complete dispersion of particles in the solvent. These samples were then analysed and the data obtained provided the information about particle size distribution of each sample.

3.5.4 Zeta Potential

Malvern Zetasizer was used to gather information on interaction between droplets, surface charge/morphology and to predict the stability of emulsion systems via adopting the electrophoretic mobility technique. Zeta potential measurement of four samples (one best emulsion from each parameter selected) was done and the zeta measurements were based on light scattering principle.

3.5.5 Scanning Electron Microscopy (SEM)

The characterization of the morphology of nanoparticles was performed by Scanning electron microscopy (SEM). For SEM analyses, the specimen was sputtered with a gold coating. SEM images of the prepared emulsion were taken by Jeol JSM at different magnifications and voltage used was and 20 kV. The particle size, shape and texture was analysed. SEM of the prepared emulsion was performed for oven dried sample. The samples were prepared by diluting 1 drop of prepared emulsion with distilled water on to the slide and expanding it on slide in the form of thin layer and it was then oven dried (FISTREEN OVA031) at 50˚C for 2 hours.

3.5.6 Energy Dispersive X-ray Spectrometry (EDS)

Quantitative analysis of elemental composition of prepared emulsion was found by Energy Dispersive X-ray Spectrometry (EDS). Same samples for EDS were used which were prepared for SEM (oven dried sample) using JEOL JSM 6490A.

3.6 Statistical Analysis

To find out the correlations between the four parameters under investigation and the particle size statistical analysis was performed. Graph pad prism 7 software was used to obtain the “p” values and “Pearson r” values according to which the level of significance and the strength of correlation was predicted respectively.

CHAPTER 4
RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Optical Microscopy

All the samples were examined under Optika 600 transmission microscope. No dilutions or staining agents were used. The microscopic images of twenty emulsions were taken at various magnifications but the best results were obtained at 50X. The images clearly displayed that all the emulsions contained fine spherical particles distributed in various sizes.

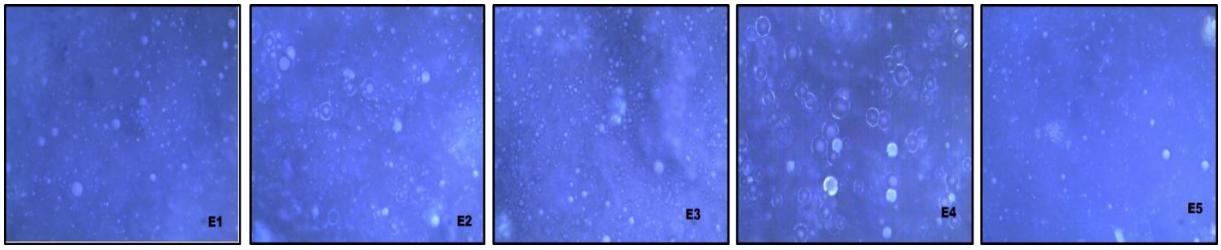


Figure 4.1: Optical microscope images of first parameter- Stirring Intensity

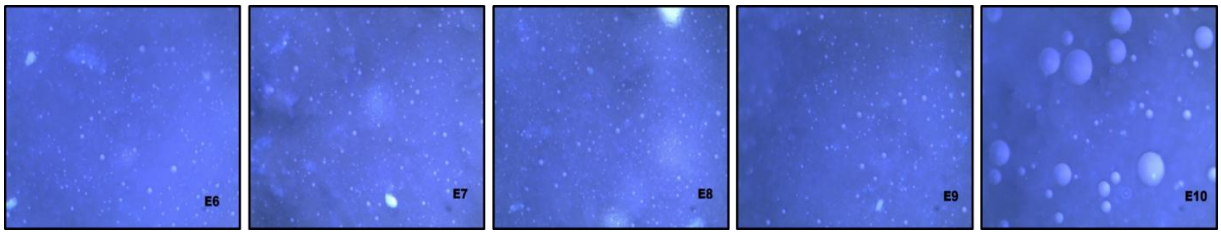


Figure 4.2: Optical microscope images of second parameter- Stirring Speed

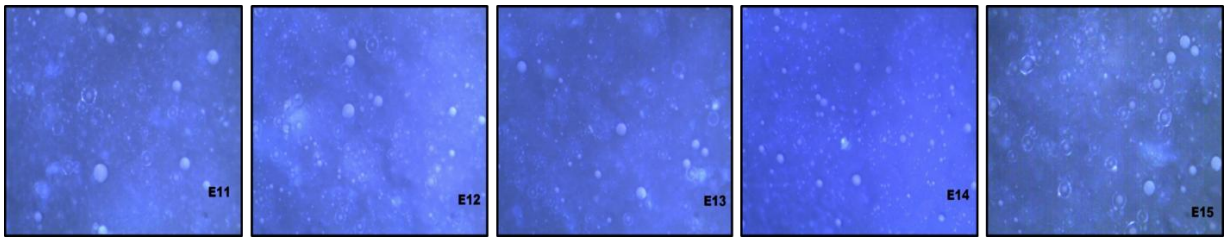


Figure 4.3: Optical microscope images of third parameter- Polymer Concentration

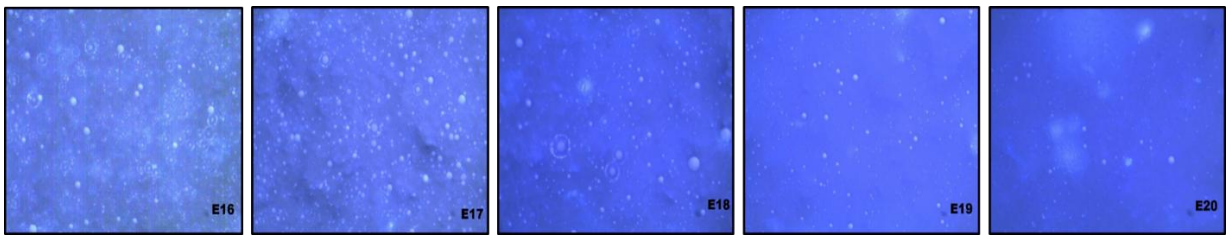


Figure 4.4: Optical microscope images of fourth parameter- Surfactant Concentration

4.2 Fourier Transform IR Spectroscopy

An FTIR analysis was carried out to check the compatibility between the drug (Amphotericin b) and selected polymer (HPMC). The spectra acquired at wavelength from 4000 cm-1 to 450 cm-1. The spectra plainly affirmed that there was no real moving, loss or appearance of useful peaks between the spectra of drug loaded emulsion containing amphotericin b and HPMC and the blank emulsion containing only HPMC (no drug). From the I.R studies it was concluded that, the selected polymer is found to be compatible with the selected drug Amphotericin B.

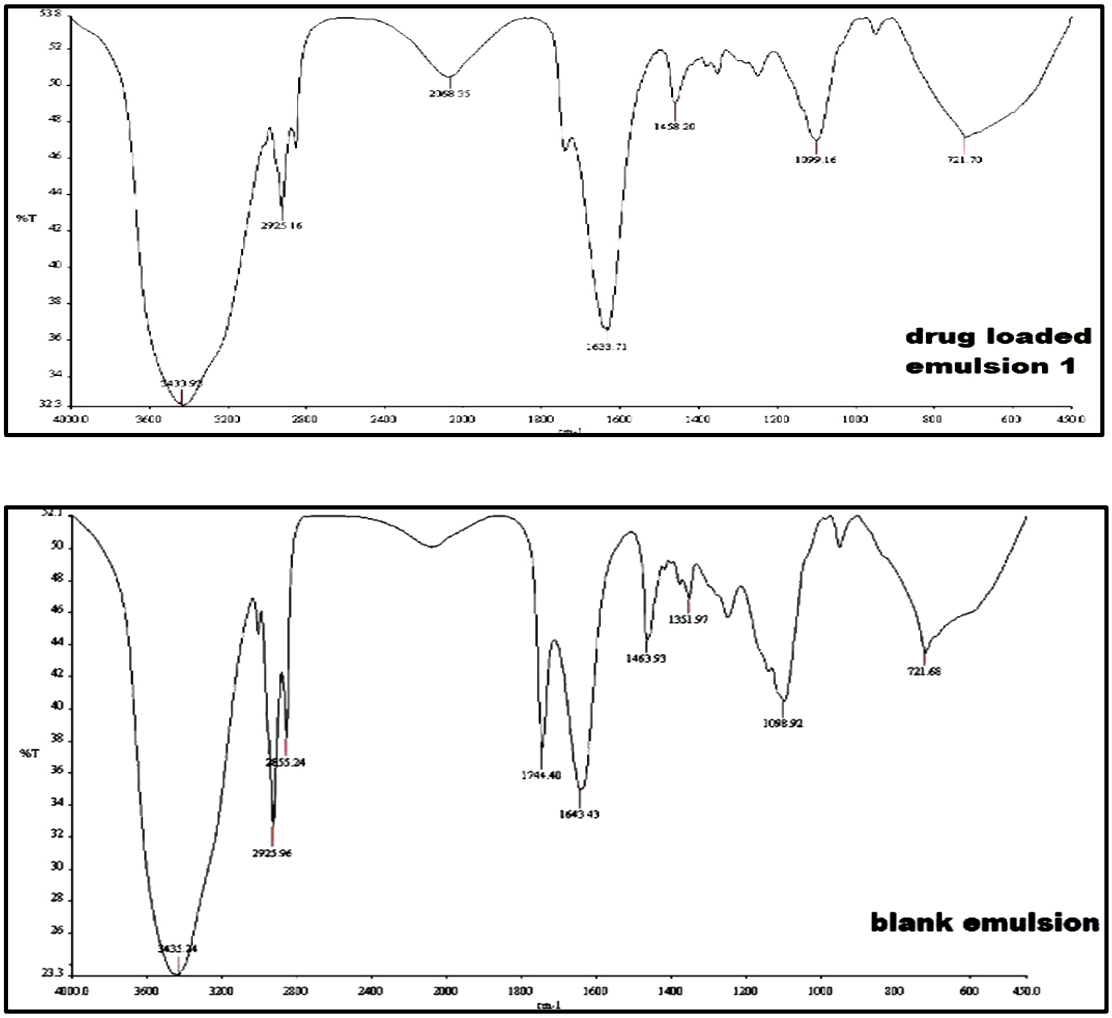


Figure 4.5: FT-IR Spectra of a) Amphotericin B loaded emulsion b) blank emulsion

4.3 Particle Size Distribution Analysis

Particle size distribution data of twenty emulsions (five emulsions for each parameter) was obtained from HORIBA - LA 920 Particle size distribution analyser. The particle size in micrometres was displayed on X-axis against the cumulative frequency (q) percentage of particles on Y-axis. The size of the droplets of all the emulsions was found to be below 100 µm and mostly the particles were abundant in the range of 1µm to 20 µm. Most of the samples displayed a single peak representing narrow range distribution of particles or mono dispersed particles. Few samples showed wide distributions/ poly dispersed particles by displaying bimodal distribution curves. The problem of poly dispersed particle distribution can be fixed by using filters (glass filters, paper filters etc.).

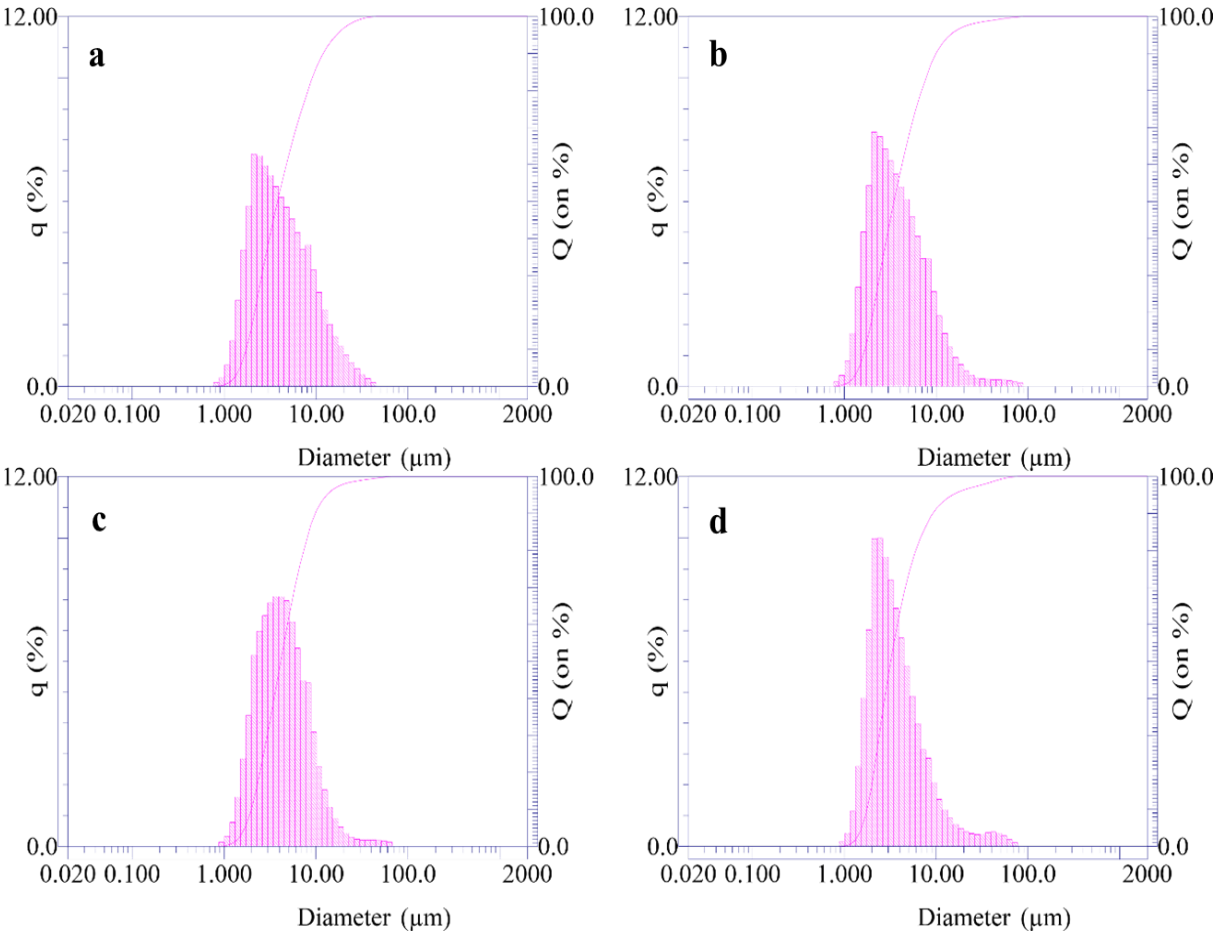


Figure 4.6: Particle Size Distribution of a) emulsion 1 from first parameter- stirring speed. b) emulsion 2 from second parameter-stirring time c) emulsion 3 from third parameter-polymer concentration d) emulsion 4 from fourth parameter-surfactant concentration

4.4 Zeta Potential Measurement

According to technical notes of Malvern instruments limited suspensions or formulations exhibiting zeta values between - 30 mV and + 30 mV are considered to be stable. If the droplet surface charges are more negative than -30 mV or more positive than +30 mV, oil droplet aggregation will be prevented in electro statically stabilized emulsions [2]. Zeta measurement of all the samples analysed displayed negative potentials in the range of -15mV to -27mV which indicates that the emulsions were quite stable. The reason for negative potential may be the strong repulsive forces between tween 80 and amphotericin b or due to the ionic contaminants of tween 80 [3] [50].

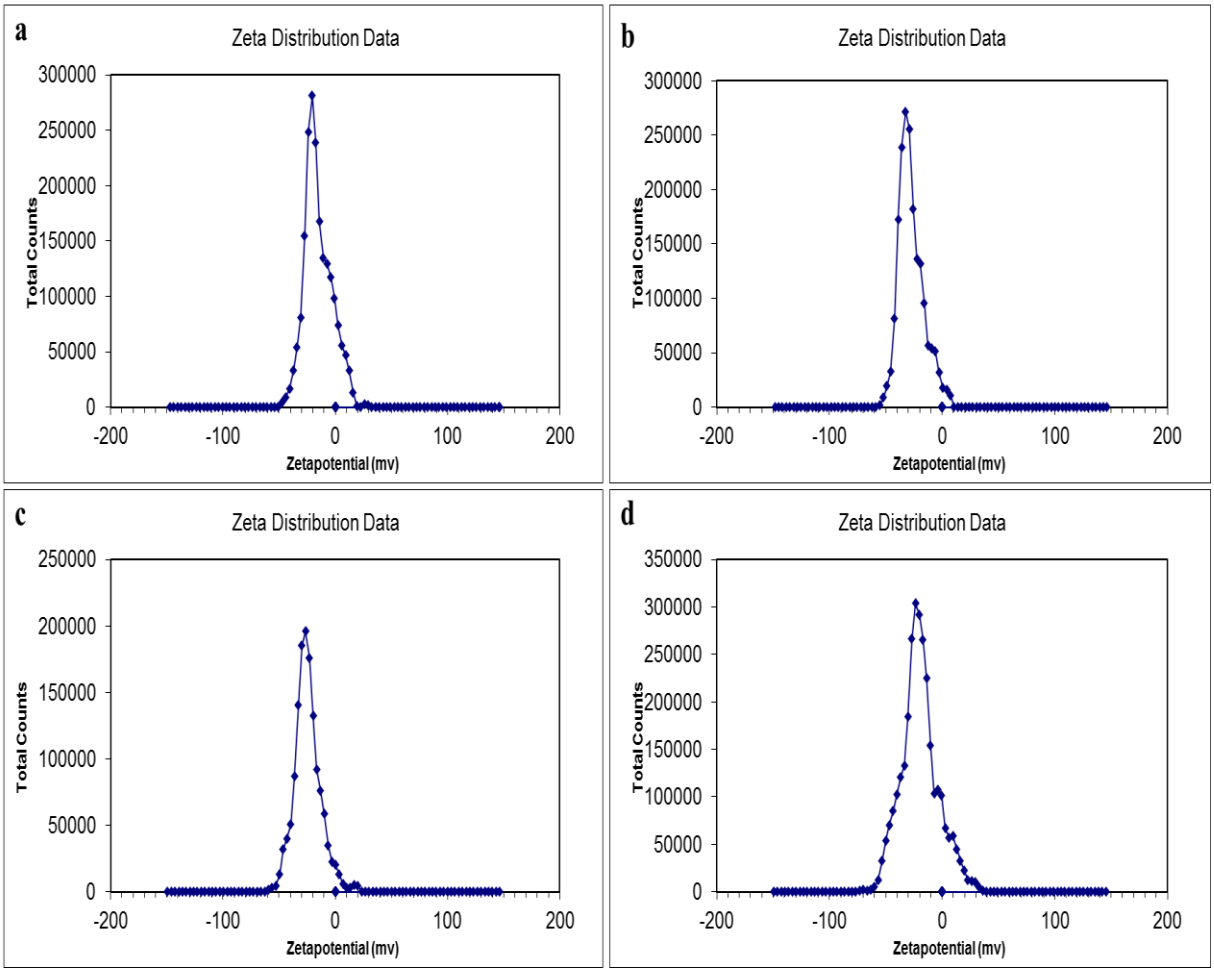


Figure 4.7: Zeta Potential Distribution of a) emulsion 1 (stirring speed) b) emulsion 2 (stirring time) c) emulsion 3 (polymer concentration) d) emulsion 4 (surfactant concentration)

4.5 SEM Analysis

The external morphology of Amphotericin B particles was examined through SEM (Jeol JSM 6490A). Particles of oven dried sample seen were in micrometres in their sizes as in Figure 4.8. SEM images of the prepared Amphotericin B-emulsion were taken at different magnifications i.e. X250, X 500, X1000,

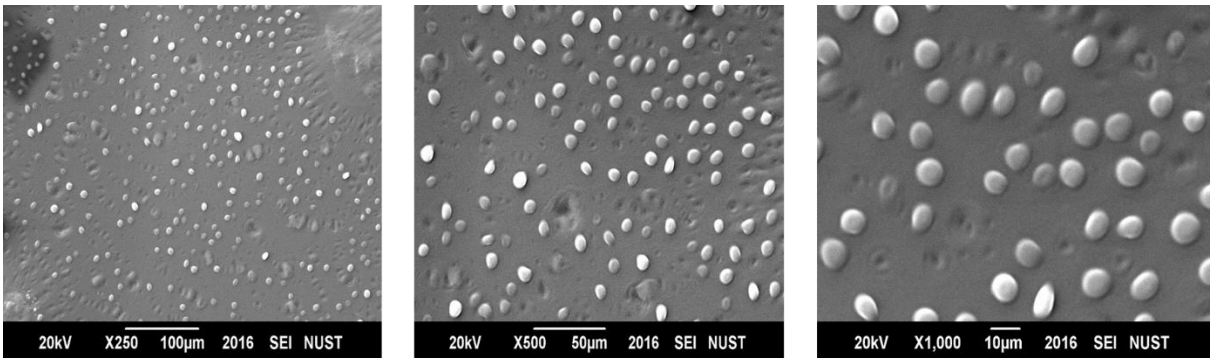


Figure 4.8: SEM Images of oven dried sample

4.6 Energy Dispersive X-ray Spectrometry (EDS)

Natural investigation of oven dried sample showed good results of the prepared emulsion only constituent elements were detected in it like carbon and oxygen which makes it organic. Their graphical representation is given in Figure 4.9

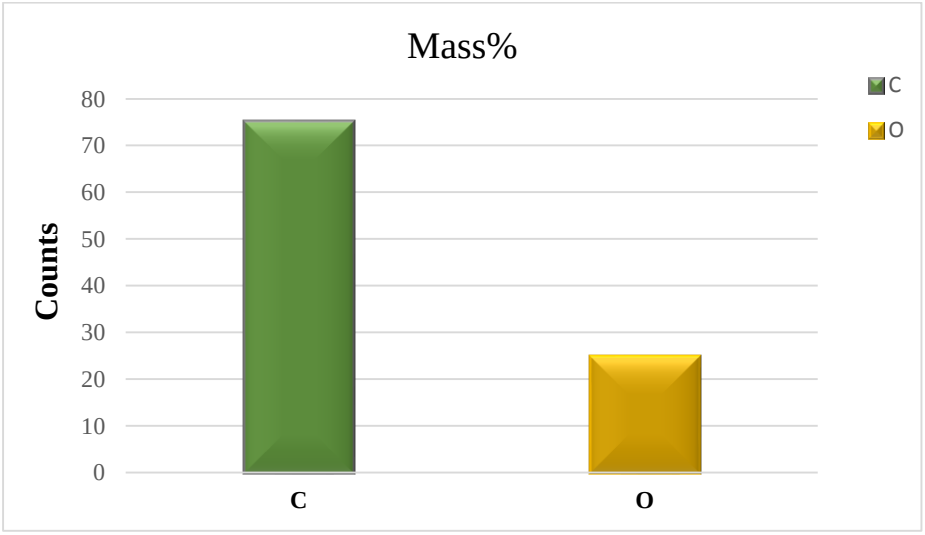


Figure 4.9: Graphical representation of mass % of elements present Oven dried sample

Element	**Mass%**
C	75.2
O	24.8
Total	100

Table 4.1: Quantitative Analysis of the elements present in oven dried sample

4.7 Parametric analysis of Emulsion

4.7.1 Effect of Stirring Speed on droplet size of emulsion

According to Gonglun and Daniel [16] more static emulsion can be formulated with an elevated stirring speed but lower than 2500 rpm due to the reason that higher than 2500 rpm will cause the emulsifier to split far from the oil water interface. The droplet size is inversely related to the stirring intensity therefore raising the shear stress may reduce droplet size in emulsion system. The reason behind reduction in droplet size may be due to splitting and distribution by the applied mechanical energy of the organic and water phases so as to maintain concentration gradients at the oil–water interface [61].

The mean particle size data obtained from particle size distribution analysis was plotted against the stirring speed to examine its effect on mean droplet size. From the results it is evident that mean dropletsize of the emulsion decreased by increasing the speed of stirring and the most stable emulsion (having smallest value of mean droplet size) was obtained at the highest speed of stirring 1000 rpm.

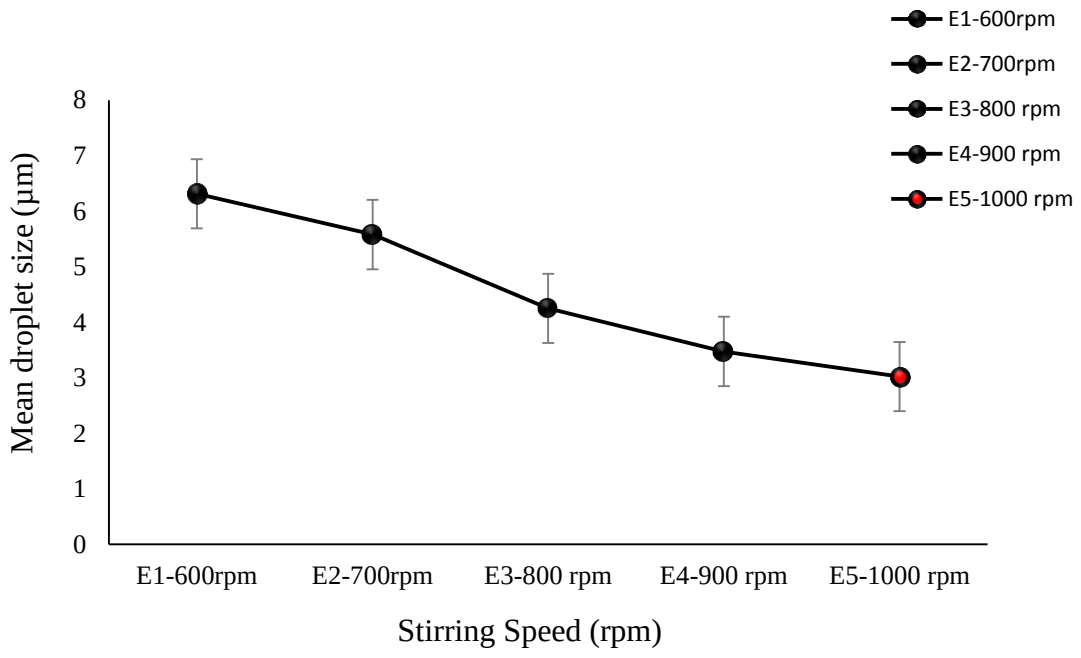


Figure 4.10: Average droplet size as a function of speed of stirring

4.7.2 Effect of Stirring Time on droplet size

The results obtained here clearly show that increasing the time of stirring proportionally decreased the mean droplet size of the emulsion particles which is in accordance with the study carried out by Chen & Tao [16] which revealed that the average size of the particles declined very swiftly in the first few seconds and then steadily reached the limiting value after 15 min and as the emulsifying time increased further, emulsion stability reduced. Increasing the time of stirring proportionally decreased the mean droplet size of the emulsion particles due to the reason that the surfactant becomes more operative with prolonged mixing time. Though, too long mixing time may influence the effectiveness of emulsifier negatively as the intense shearing will bring about the emulsifier to escape out of the oil– water interface. [32] [33]

Starting from the stirring time of 10 minutes up to the stirring time of 25 minutes, a rational decrease in particle mean size was observed. But when the time of stirring was increased up to 30 minutes a rapid increase of size in droplet diameter was reported. So it is concluded that for preparation of stable amphotericin b polymeric emulsion the recommended range of stirring time will be between 10 – 25 minutes.

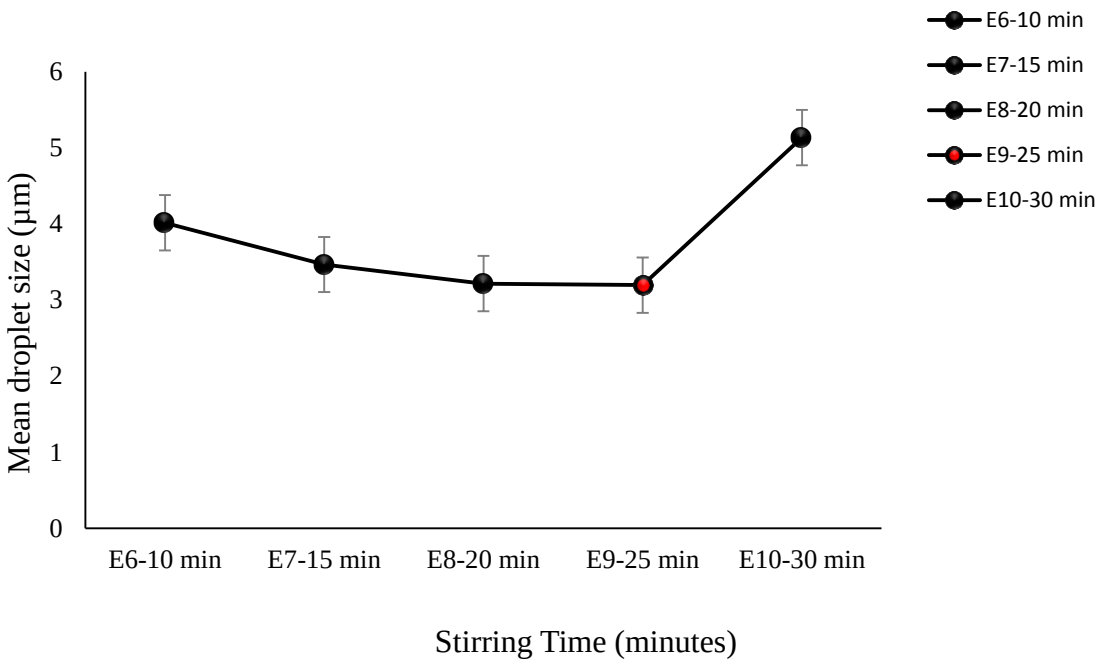


Figure 4.11: Average droplet size as a function of time of stirring

4.7.3 Effect of Polymer Concentration on mean droplet size

The concentration of polymer, is a significant factor to regulate the final particle size. Different concentrations of polymer were used and rest of the parameters were kept constant. The results revealed a specific concentration of polymer suitable to formulate a long time stable emulsion [2]. At increased concentration, the curve displaying increase in particle size turned flat which means no further effect on particle size. [8]

The results displayed an irregular pattern of polymer concentration on mean droplet size of the emulsion. Initially by increasing the concentration of the polymer (HPMC), mean particle size decreased up to the concentration of 20 mg but as we increased the polymer concentration to 22 mg, a prominent increase in the size of droplets was displayed. By further increasing the polymer concentration to 24 mg resulted in the greater mean droplet size. So it was concluded that the most suitable polymer concentration range was from 16 mg to 20 mg to formulate stable amphotericin b emulsion. The best result with the minimum particle size was obtained at 20 mg concentration of HPMC.

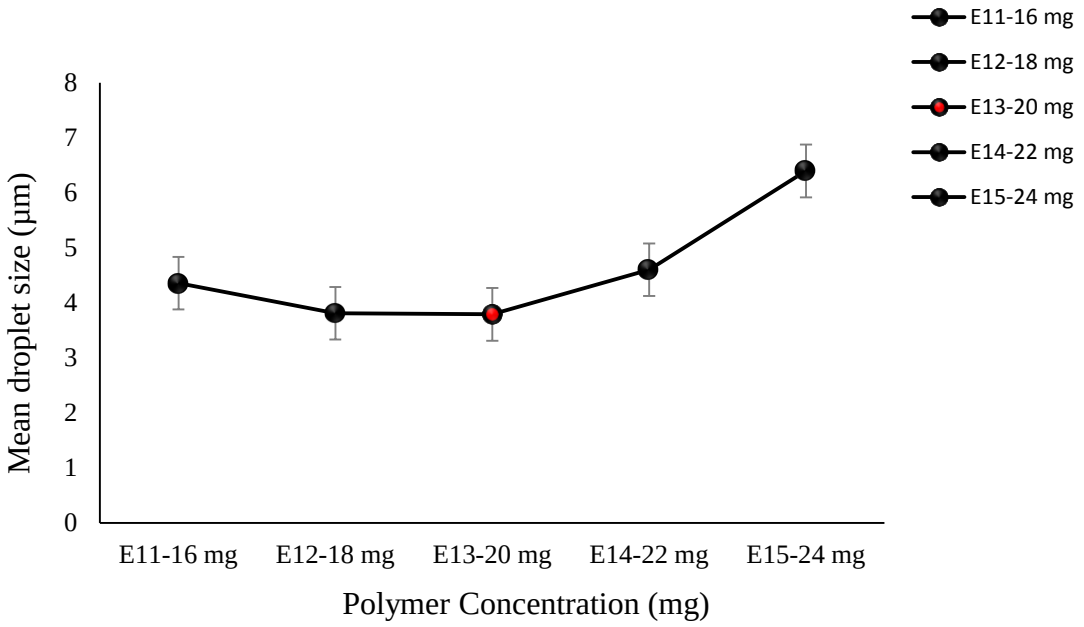


Figure 4.12: Average droplet size as a function of polymer concentration

4.7.4 Effect of Surfactant Concentration on droplet size

The surfactant used in the emulsion was Tween 80 which is a non-ionic surfactant. Various concentrations of tween 80 starting from 0.5 ml to 2.5 ml were used. A large

value of mean droplet size was obtained at 0.5 ml concentration of tween 80 but as the concentration was increased to 1ml there was a visible decrease in particle size. The mean particle diameter primarily declined with increasing surfactant concentration which is in agreement with others studies [7]. The reason for this decline in mean size may be due to greater adsorption of surfactant molecules to the oil–water interface resulting in decreased interfacial tension, which aids the formation of smaller droplets [57] or a greater quantity of surfactant particles diffusing from the oil stage to the fluid stage, in this manner prompting to the development of fine oil droplets at the limit [7]. Further no visible change in size was seen with 1.5 ml and 2ml concentration but as the concentration was increased to 2.5 ml, the mean droplet size started to rise. At the point when the surfactant fixation was expanded further, an expansion in mean molecule width was recognized, which has likewise been accounted for in different reviews [13] [78]. The droplet estimate increments over a specific surfactant level because of the arrangement of an exceptionally gooey fluid crystalline stage, which makes unconstrained separation of the oil–water interface more intense. [73]. The minimum particle mean droplet size was obtained at the 1.5 ml concentration of tween 80, so this suggests that this concentration of tween 80 was helpful in developing the most stable emulsion containing amphotericin b.

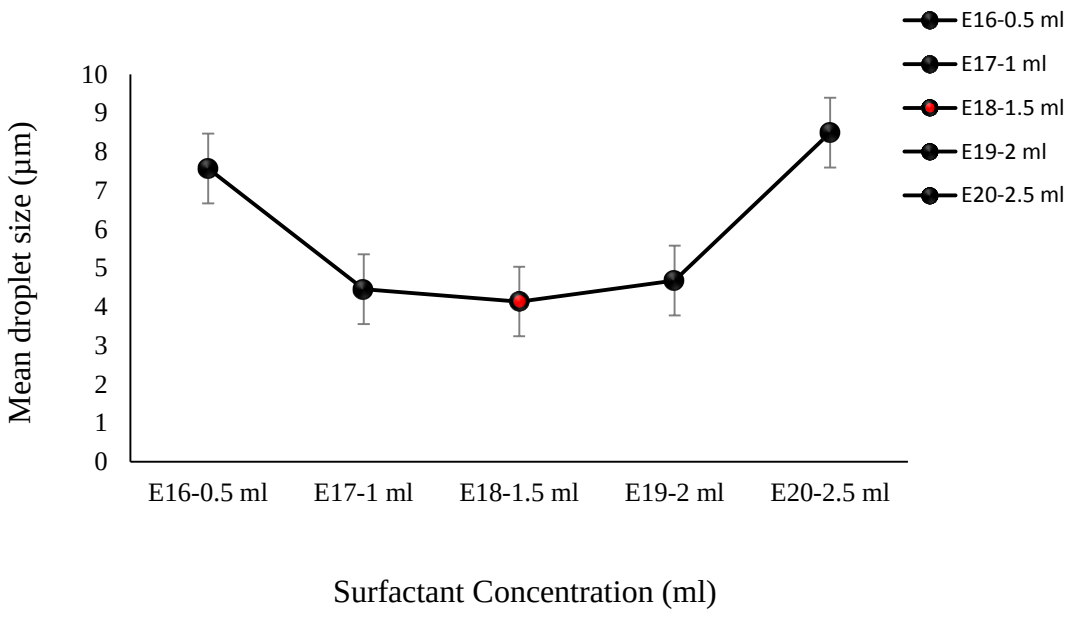


Figure 4.13: Average droplet size as a function of surfactant concentration

4.8 Antifungal Assay

The best stable emulsions from each parameter were selected for antifungal testing. These samples were tested against three fungal strains [53] (Aspergillus Niger, Aspergillus Flavus, Aspergillus Tubingensis) in triplicates. All the samples displayed good antifungal activity against all the chosen strains.

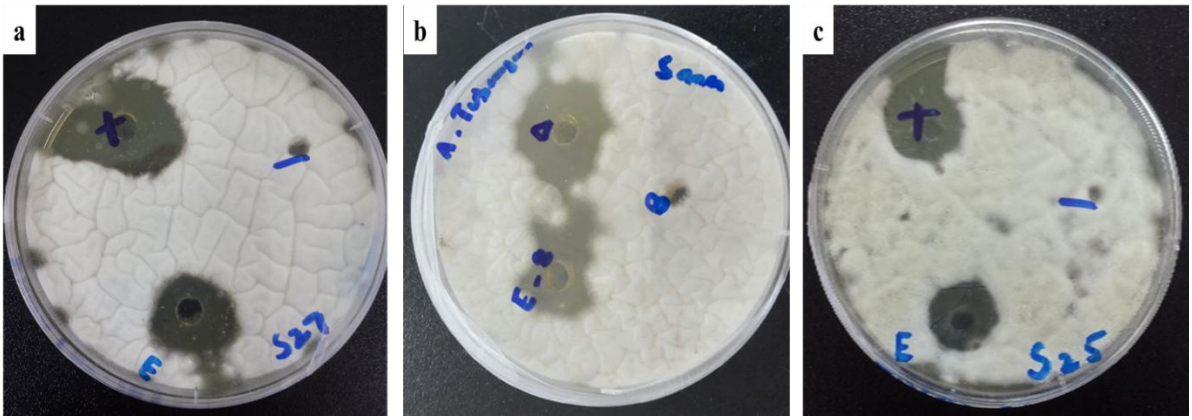


Figure 4.14: Antifungal results of Amphotericin B emulsion against a) Aspergillus Niger b) Aspergillus Tubengensis c) Aspergillus Flavus

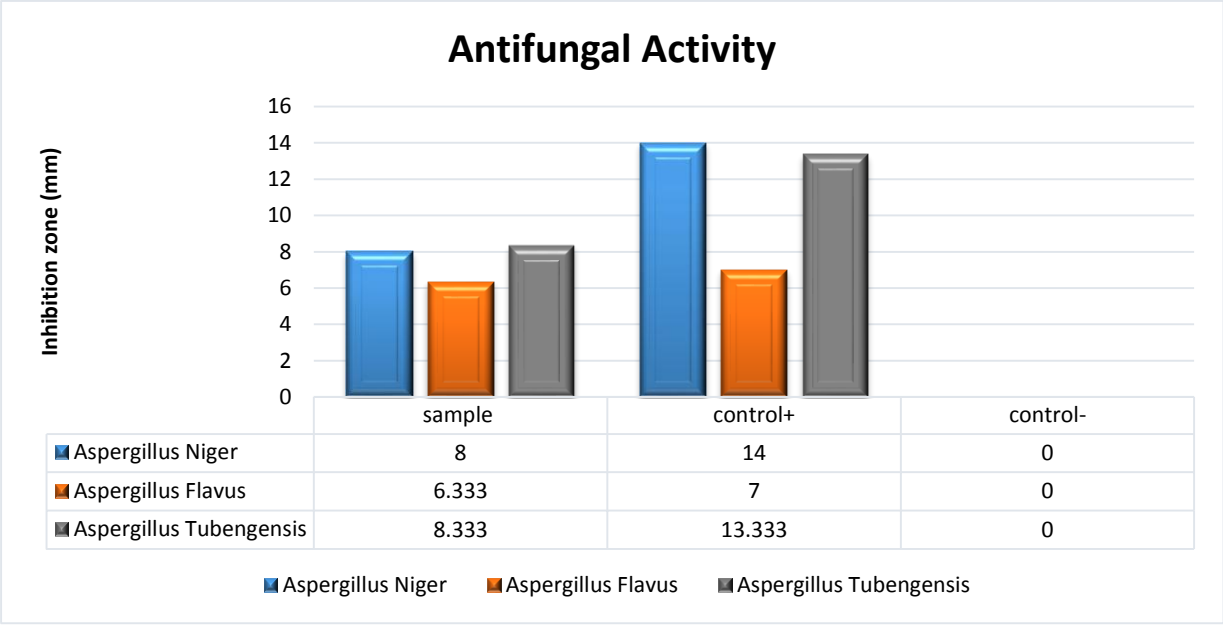


Figure 4.15: Antifungal activity results

4.9 Statistical Analysis

The data obtained from the statistical calculations revealed that for the first parameter – stirring intensity the value of correlation coefficient "Pearson r" is very close to 1 which indicates that stirring speed has a very strong correlation with the particle size of droplets. The negative sign indicates that both the variables are negatively correlated which means that by increasing one other will decrease. The p value obtained for this parameter displayed a good level of significance ($p< 0.05$). By analysing the Pearson r value and p value for the second parameter-stirring time it was concluded that the correlation is weak and not significant respectively. Polymer amount and particle size are positively correlated but p value was not found to be significant. Fourth parameter-surfactant concentration, very weakly correlated to particle size and p value was not significant.

Statistical Analysis	**Stirring Speed (rpm) vs. Particle Size**	**Stirring Time (min) vs. Particle Size**	**Polymer Amount (mg) vs. Particle Size**	**Surfactant Amount (ml) vs. Particle Size**
Pearson r	-0.9871	0.3831	0.7211	0.163
P value	0.0018	0.5244	0.1692	0.7934
P summary	**	ns	ns	ns
Significant? (alpha=0.05)	Yes	No	No	No
Number of XY Pairs	5	5	5	5

Table 4.2: Statistical values of parameters against particle size

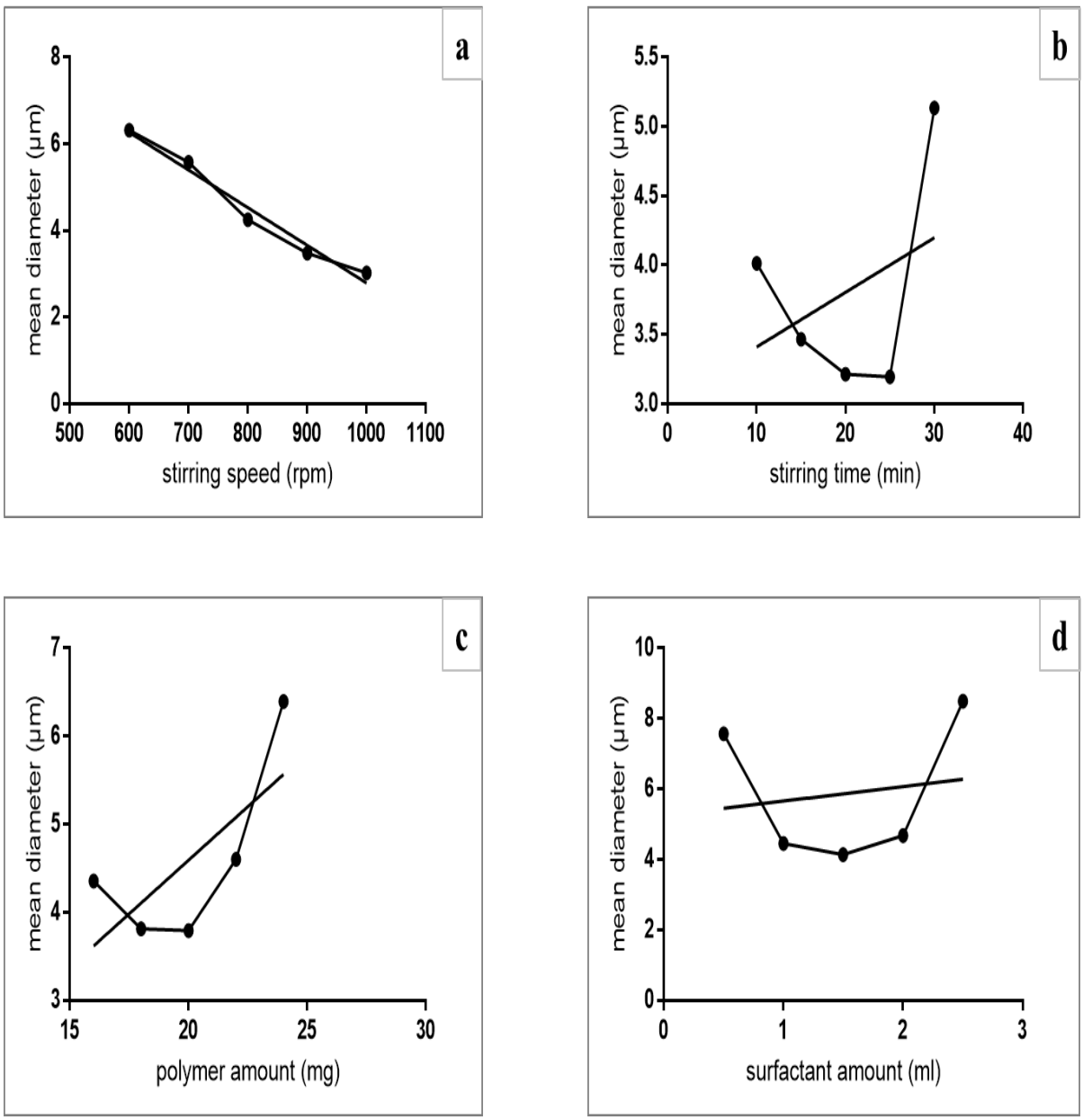


Figure 4.16: Graphical representation of statistical data from Graph pad prism 7 software.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

During recent years there has been greater interest in the use of topical vehicles that may amend the drug penetration into the dermis. Optimal dermal products tend to exert a high capacity for integrating both hydrophobic and hydrophilic drugs as well as high skin absorbance. To avoid the drawbacks of currently available formulations for instance high cost, several side effects and reduced drug loading capacity, the idea of development of new pharmaceutical formulations of Amphotericin B for topical administration for the treatment of invasive fungal infections was endorsed. To reduce the noxious effects of conventional Amphotericin B, formulations incorporating amphotericin b were developed in 1990s for clinical applications. Economic issues related to liposomal preparations led to the need of developing cost effective, nontoxic and therapeutically effective Amphotericin B formulation. Currently, Amphotericin B is a considerate drug model for drug targeting.

The parametric analysis of amphotericin b polymeric emulsion was done to investigate the impact of the four selected factors on the particle size distribution and thus on the stability of emulsions. The results depicted a negative correlation between rate of stirring and mean droplet diameter. Increasing speed decreased size. During the time interval from 10 to 25 minutes, no prominent change in particle diameter was seen but there was an increment in size when time was increased to 30 minutes. Weak correlation The mean diameter values at polymer dosage of 16mg, 18mg and 20mg (optimal range) were nearly equivalent but started to rise when concentration was increased to 22mg and continued rising. Strong positive correlation. Initially the mean value decreased by raising the surfactant dosage from 0.5ml to 1ml but no change was seen at 1ml, 1.5ml and 2ml of tween 80 concentrations. There was a rise in mean value at 2.5ml dosage. Very weak correlation. The optimal speed of stirring was 1000 rpm, stirring time range was 10-25 minutes, HPMC concentration range was 16mg – 20 mg and tween 80 concentration range was 1ml - 2ml (produced smallest droplet diameter values) to prepare a stable polymeric emulsion of Amphotericin B. Good antifungal activity was displayed by the samples analysed.

5.2 Recommendations

Simple Amphotericin B preparations are associated with relatively high toxicity and high cost of liposomal formulations necessitate the development of economical, safe and novel Amphotericin B formulation. The advancement in nanotechnology has equipped the formulator with sophisticated instruments and now the stability related features of macro and colloidal dispersion are optimized by characterizing the micro and nanoparticles present in the formulation. In vitro characterization e.g.; Drug release through Franz's diffusion Cell. After Successful in Vivo evaluation, formulation could be commercialized. Cutaneous fungal infections can be treated in a better way. Transformation of Cutaneous fungal infections into life threatening infections can be prevented. Preparation can also be used for the management of various fungal infections/diseases. Investigation of effect of pH and other factors on emulsion stability could be studied in order to improve topical use of the emulsion. Testing of emulsion on synthetic skin patches could be done in order to commercialize the product and check its topical impacts. Same formulation process can be repeated using a different drug.

CHAPTER 6
REFERENCES

6. REFERENCES

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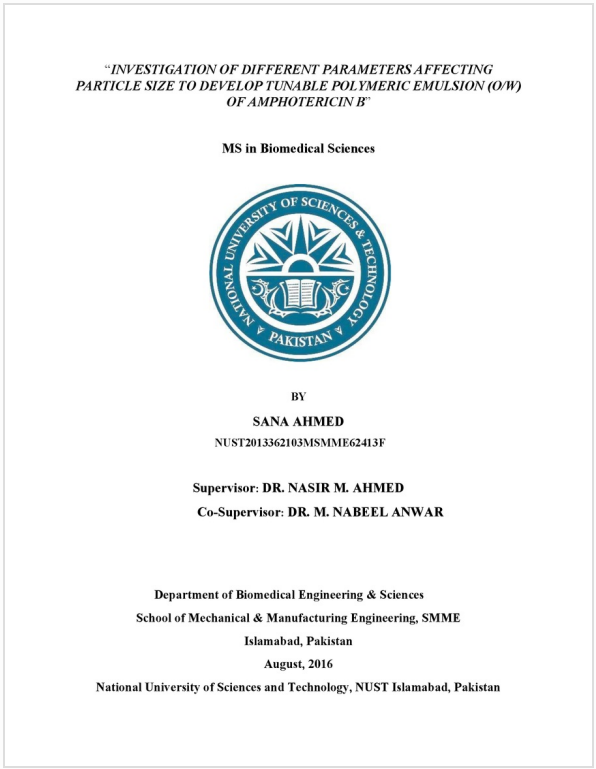

"INVESTIGATION OF DIFFERENT PARAMETERS AFFECTING PARTICLE SIZE TO DEVELOP TUNABLE POLYMERIC EMULSION (O/W) OF AMPHOTERICIN B"

MS in Biomedical Sciences

BY

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