

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

DEVELOPING DRUG LOADED HYDROGELS COATED
INTRAOCULAR LENSES USING MICROFLUIDICS FOR OCULAR
DRUG DELIVERY

İsmail BAL

A THESIS OF DOCTORATE
DEPARTMENT OF INTERDISCIPLINARY
NANOSCIENCE AND NANOENGINEERING

ADVISOR: Assoc.Prof. Dr. İsmail KÜÇÜK

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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

OKÜLER İLAÇ DAĞITIMI İÇİN MİKROAKIŞKANLAR
KULLANILARAK İLAÇ YÜKLÜ HİDROJELLERLE KAPLANMIŞ
GÖZ İÇİ LENSLEİN GELİŞTİRİLMESİ

İsmail BAL

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*For my mother, with
deepest gratitude and love*

ABSTRACT

As an alternative to topical treatments of antimicrobial treatment after cataract surgery, the development of supportive platforms using controlled-release intraocular lens (IOL) implants is becoming increasingly important. Controlled drug release systems are in demand because they lower the drug dosage and frequency, minimise side effects due to their targetability and have a favourable impact on patient safety, treatment, and cost because of these features. It has been extensively investigated in recent years owing to their advantages such as keeping the drug at a therapeutic level for a longer period of time and applying it for a long time which is not possible with traditional treatment methods.

Drugs may be delivered to the desired region using liposomal systems, however, their stability might not be enough to maintain drug activity. In this case, systems that combine liposomes into hydrogel structures can be utilized. Alginate (ALG) has been thoroughly researched in many biomedical applications due to its properties including high biocompatibility, low toxicity, mucoadhesiveness and low cost. The performance of controlled drug release systems is directly impacted by hydrogel production processes. Preferring production methods in which the structural properties of hydrogels can be easily controlled is important in the design of these systems. Both the simplicity of hydrogel manufacturing and the ease with which parameters affecting the structural characteristics of the hydrogel can be altered make microfluidic technologies the preferred option. Microfluidic systems allow the production of hydrogel with the desired character and coating form by utilizing the fluid characteristics of low-volume liquids and by controlling the capillary movements of these liquids. Particularly, T-shaped microfluidic junction (TMJ) systems enable the confluence of liquids transported through micron-sized channels, making it possible to create microbubbles with uniform distribution and in desired sizes. These characteristics make it suitable for coating form production as well as hydrogel manufacturing with uniform pore and size distribution and drug-loaded liposomes homogeneously distributed throughout the gel.

In this thesis study, it is aimed to form an ALG hydrogel coating containing homogeneously distributed moxifloxacin-incorporated liposomes using the TMJ method on the IOL implant and to develop antimicrobial treatment after cataract surgery by controlled release of the MXF(1-5% w/w). The hydrogel form and hydrogel-coated IOL implant with the optimal drug release and antimicrobial activity selected for use in antimicrobial treatment after the production and characterization stages. Then, in order to examine MXF drug release behaviour, *in vitro* release studies performed on solely MXF-encapsulated liposomes, MXF-encapsulated liposomes enriched alginate nanocomposite hydrogels and MXF-encapsulated liposomes enriched alginate nanocomposite hydrogel coatings (on IOL).

Keywords: Moxifloxacin, Lecithin, TMJ, Drug Delivery.

ÖZET

Katarakt cerrahisi sonrası antimikrobiyal tedavide topikal tedavilere alternatif olarak, kontrollü salınlı göz içi lens (GİL) implantlarının kullanıldığı destekleyici platformların geliştirilmesi giderek önem kazanmaktadır. Kontrollü ilaç salım sistemleri, ilaç dozajını ve sıklığını düşürmesi, hedeflenebilirliği sayesinde yan etkileri ortadan kaldırması ve bu özellikleriyle hasta güvenliği, tedavi ve maliyet üzerinde olumlu etkiye sahip olması nedeniyle talep görmektedir. Geleneksel tedavi yöntemleriyle mümkün olmayan ilacın daha uzun süre terapötik seviyede tutulması ve uzun süre uygulanması gibi avantajları nedeniyle son yıllarda üzerinde sıkça çalışılmaktadır.

İlaçlar lipozomal sistemler kullanılarak istenen bölgeye iletilebilir, ancak stabiliteleri ilaç aktivitesini sürdürmek için yeterli olmayabilir. Bu durumda lipozomları hidrojel yapılarla birleştiren sistemler kullanılabilir. Aljinat (ALG), yüksek biyouyumluluk, düşük toksisite, mukoadhezyon ve düşük maliyet gibi özellikleri nedeniyle birçok biyomedikal uygulamada kapsamlı bir şekilde araştırılmıştır. Kontrollü ilaç salım sistemlerinin performansı hidrojel yapı üretim süreçlerinden doğrudan etkilenmektedir. Hidrojellerin yapısal özelliklerinin kolaylıkla kontrol edilebildiği üretim yöntemlerinin tercih edilmesi bu sistemlerin tasarımında önemlidir. Hem hidrojel üretiminin basitliği hem de hidrojin yapısal özelliklerini etkileyen parametrelerin değiştirilebilmesinin kolaylığı mikroakışkan teknolojilerini tercih edilen seçenek haline getirmektedir. Mikroakışkan sistemler, düşük hacimli sıvıların akışkan özelliklerinden yararlanarak ve bu sıvıların kılcal hareketlerini kontrol ederek istenen karakterde ve kaplama formunda hidrojel üretimine olanak sağlamaktadır. Özellikle t-şekilli mikroakışkan bağlantı (TMJ) sistemleri, mikron boyutundaki kanallardan taşınan sıvıların bir araya gelmesini sağlayarak homojen dağılımlı ve istenen boyutlarda mikro kabarcıkların oluşturulmasını mümkün kılmaktadır. Bu özellikler, kaplama formu üretiminin yanı sıra tek tip gözenek ve boyut dağılımına ve jel boyunca homojen olarak dağılmış ilaç yüklü lipozomlara sahip hidrojel üretimi için uygun hale getirir.

Bu tez çalışmasında, GİL implantı üzerinde TMJ yöntemi kullanılarak homojen dağılımlı moksifloksasin-içeren lipozomlar içeren ALG hidrojel kaplama oluşturulması ve MXF'nin (%1-5 w/w) kontrollü salınımı ile katarakt cerrahisi sonrası antimikrobiyal tedavi geliştirilmesi amaçlanmıştır. Üretim ve karakterizasyon aşamalarından sonra antimikrobiyal tedavide kullanılmak üzere en uygun ilaç salımı ve antimikrobiyal aktiviteye sahip hidrojel formu ve hidrojel kaplı GİL implantı seçilmiştir. Daha sonra, MXF ilacının salım davranışını incelemek için, yalnızca MXF ile kapsüllenmiş lipozomlar, MXF ile kapsüllenmiş lipozomlarla zenginleştirilmiş alginat nanokompozit hidrojel ve MXF ile kapsüllenmiş lipozomlarla zenginleştirilmiş alginat nanokompozit hidrojel kaplamalar (IOL üzerinde) üzerinde in vitro salım çalışmaları gerçekleştirilmiştir.

Anahtar Kelimeler: Moksifloksasin, Lesitin, T-Şekilli Mikroakışkan Cihaz, İlaç Salımı

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LIST OF SYMBOLS AND ABBREVIATIONS

Γ	: Surface Tension
ζ	: Zeta Potential Value
ALG	: Sodium Alginate
AMD	: Age-related macular degeneration
ASTM	: American Society for Testing and Materials
BAB	: Blood-aqueous barrier
BRB	: Blood-retinal barrier
CA	: Contact Angle
CVS	: Computer vision syndrome
DDS	: Drug Delivery System
DLS	: Dynamic Light Scattering
ECM	: Extracellular matrix
EDS	: Energy-Dispersive X-ray Spectroscopy
EE	: Encapsulation Efficiency
GTÜ	: Gebze Teknik Üniversitesi
IOL	: Intraocular Lens
ISO	: International Organization for Standardization
LEC	: Lens epithelium cells
MIC	: Minimum Inhibitory Concentration
MXF	: Moxifloxacin
PAMAM	: Polyamidoamine
PCO	: Posterior Capsule Opacification
PDI	: Polydispersity Index
PEG40S	: Polyethyleneglycol-40 stearate
PMMA	: Polymethylmethacrylate
SATP	: Standard ambient temperature and pressure conditions
SEM	: Scanning Electron Microscope
TMJ	: T-shaped microfluidic junction device

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1. INTRODUCTION

The human eye, an important part of the sensory nervous system, is a complicated sensory organ that reacts to light and provides vision. From an anatomical perspective, the eye can be classified into two separated segments, including anterior and posterior segments. The anterior segment is made up of the cornea, iris, ciliary body, conjunctiva, lens and aqueous humour, while the posterior segment is made up of the vitreous cavity and three layers: the sclera, choroid and retina [1], [2]. It functions by concentrating light onto the retina, where photoreceptor cells transform it into electrical signals after it has passed through the cornea and lens. The brain then receives these impulses through the optic nerve, enabling the perception of images. Clear and accurate vision is the result of a complex process that includes a number of anatomical components and metabolic interactions [1]. The eye, as one of the sensitive sensory organs, is affected by many diseases that can effect visual acuity and overall eye health. The occurrence of eye diseases depends on causes such as environmental influences, infections, trauma, ageing, or hereditary factors. Diseases occurring in the anterior segment include cataract, dry eye, eye inflammation, glaucoma, etc., while diabetic retinopathy, age-related macular degeneration (AMD), and uveitis are the main posterior segment diseases [3]. **Figure 1.1** exhibit the statistical data on eye diseases affecting the population worldwide[4]. The widespread use of electronic devices, such as computers, tablets, and cell phones, has led to a notable rise in eye-related health issues in recent years, particularly among younger individuals. Long-term usage of these devices has been associated with a number of visual disorders and diseases that are collectively known as computer vision syndrome (CVS) or digital eye strain. It has been verified that the rising incidence of dry eye syndrome is closely linked to the increased use of electronic devices. The frequency of blinking may decrease with prolonged use of electronic devices. Blinking typically aids in distributing tears uniformly across the eye's surface and renews the tear film, both of which are critical for preserving the hydration and health of the eye. Nevertheless, the aging population has significantly contributed to the increasing prevalence of glaucoma and age-related macular degeneration (AMD) [5].

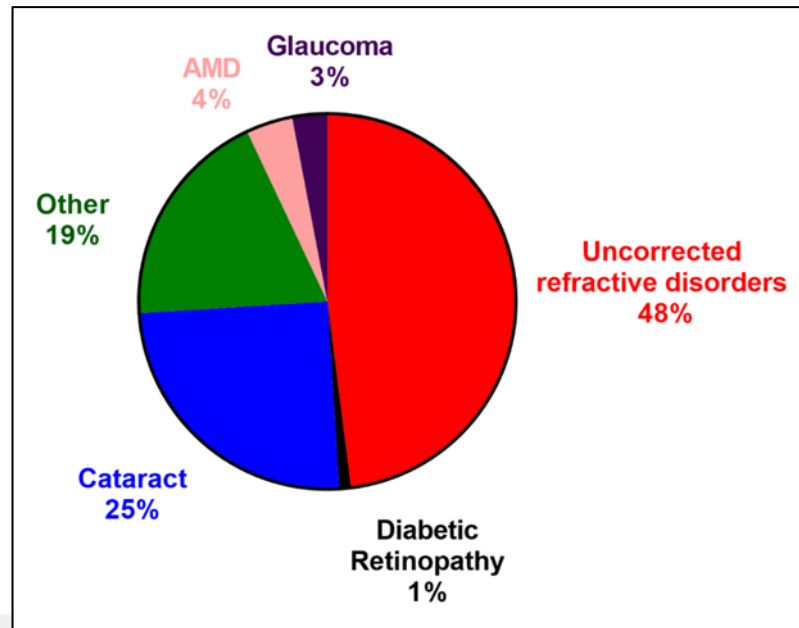


Figure 1.1: Percentage of ophthalmic disease occurrence globally.

Patients' visual clarity and quality of life are directly impacted by eye illnesses. Traditional methods such as intravitreal, intracameral, periocular, topical, subconjunctival, oral, etc. are used to prevent disease-related inflammation, bacterial or viral infection [6]. The treatment strategy may also vary depending on its type and severity. Despite their accessibility, many treatment approaches encounter physiological barriers. While treating of ocular illnesses, the tight junctions of the blood-aqueous barrier (BAB) in the anterior segment and the blood-retinal barrier (BRB) in the posterior segment limit the absorption of the drug from the blood (**Figure 1.2**). Although this issue can be overcome by using high doses of drugs, it may result in various side effects [7].

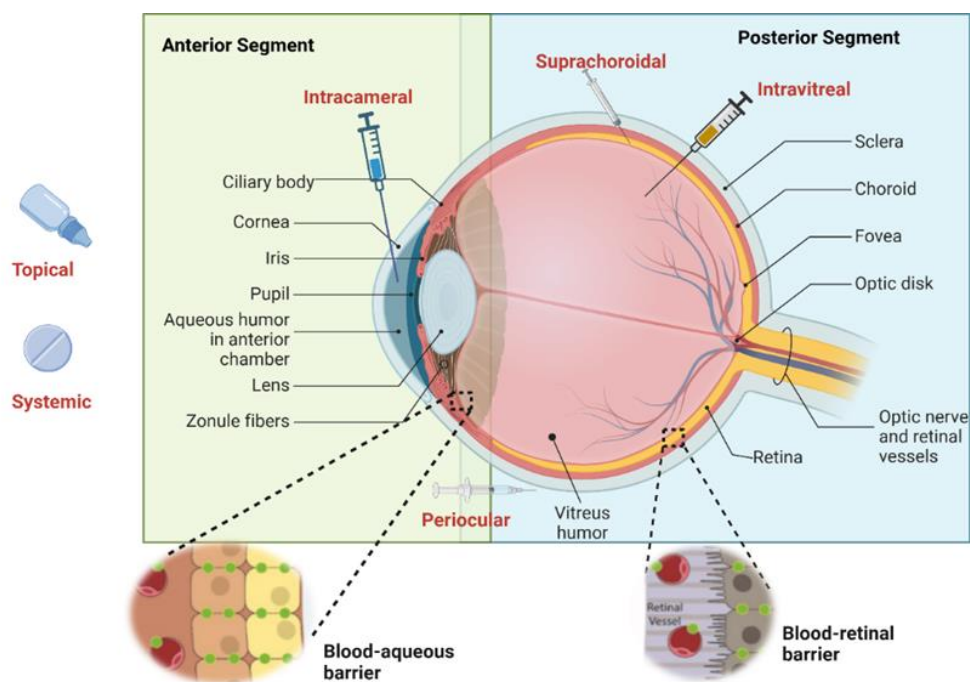


Figure 1.2: Schematic representation of structure of human eye, physiological barriers and drug delivery routes through the eye.

Despite the limitations of ocular barriers, topical application (e.g., eye drops, ointments, gels) has been demonstrated to be the most effective route of ophthalmic drug release [8], [9]. The corneal bioavailability of eye drops, which are preferred for their ease of use and constitute approximately 90% of topical applications, is in the range of 1-5% due to anatomical and physiologic barriers. A considerable portion of the eye drops are instinctively eliminated from the ocular surface by blinking because of the abrupt surge in tear fluid that occurs after applying eye drops. Within 15 to 25 minutes, the applied eye drops are known to be entirely eliminated from the corneal surface [10]. The effectiveness of the remaining eye drops decreases due to the diluting effect of tear fluid. Additionally, the cornea acting as a barrier is another factor that reduces the efficacy of the eye drops [11]. In order to achieve the desired therapeutic effect, the drops must be administered frequently and in high doses. However, this causes sudden fluctuations in drug concentration and reduces patient compliance, especially in the treatment of chronic ophthalmic diseases [12]. In addition, high drug concentration can result in toxic effects [13]. Moreover, it is also more difficult for elderly patients to self-administer eye drops on time to achieve a therapeutic effect.

Therefore, these disadvantages of eye drops have become a significant challenge to be overcome, and controlled drug release systems requires to be developed to prolong drug release and/or enhance the exposure time on the ocular surface. By optimizing the kinetics, timing, and target of drug release, it is possible to provide a more effective and safer treatment. Many disorders, especially those affecting the eyes, can be treated with controlled drug delivery systems because of their sterility and biocompatibility. Rather than relying on frequent and high-dose administration, as in conventional treatments, controlled drug delivery systems provide to keep the drug at therapeutic levels for longer periods of time, reduce possible side effects by targeting the relevant tissues, and increase the bioavailability of the drug by reducing the cost and toxic effect by using smaller amounts. Thus, cost, treatment safety and patient quality of life are positively affected [14].

Globally, there are 94 million blind or visually impaired people, and cataracts are the leading cause of preventable blindness, especially in developing countries [15]. Although the overall prevalence has been declining, it is estimated that more than 10 million people worldwide are blind due to cataracts, while over 35 million individuals suffer from moderate to severe vision impairment [16]. Cataract is characterized by reduced lens transparency and clarity of vision due to clouding of the lens of the eye as a result of structural changes in the lens fibers (**Figure 1.3**) [17]. Premature birth, heredity, chromosomal abnormalities (such as Down syndrome), diabetes, certain metabolic disorders, radiation therapy to the eye area, exposure to sunlight, and altitude can all cause cataracts; however, the primary cause is age-related slowing of metabolic processes [18]. Although the development of cataracts is partially preventable (e.g., quit smoking, protecting the eyes from the direct sunlight, regular ophthalmic examinations, keeping diabetes under control) the only effective treatment method is surgical intervention. Cataract surgery is a common and safe technique that is performed all over the world. It involves removing the opacified lens and implanting an artificial intraocular lens (IOL). Since the implantation of the lens requires an incision of approximately 2-3 mm, it is important that the IOLs used are flexible.

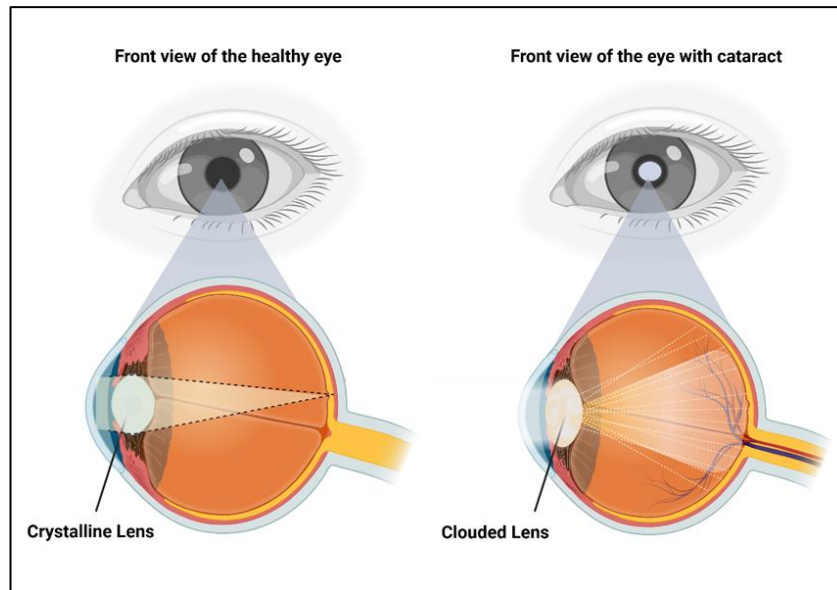


Figure 1.3: Comparison of healthy eye vs. eye with cataract.

1.1. Intraocular Lenses

1.1.1. Historical Background

The use of intraocular lenses (IOLs) as part of cataract surgery dates back to the 18th century. The British ophthalmologist Sir Harold Ridley, known as the inventor of IOL implantation, drew inspiration from clinical observations of fighter pilots who had suffered eye trauma during the Second World War. He pointed out that the plastic Plexiglass acrylic canopy pieces lodged in the fighter pilot's eye during enemy fire remained in the eye for years, were missed even on careful examination, and caused no problems as long as a sharp edge did not contact a sensitive and moving part of the iris. Taking this clue, Ridley and colleagues, in collaboration with the Plastics Division of Imperial Chemical Industries, have developed a clinical-grade artificial lens from a pure form of polymethylmethacrylate (PMMA) [19]. Following these developments, the first IOL implantation was performed in 1949. Since then, cataract surgery has evolved and is now a widely established procedure. Nowadays, cataract surgery is one of the most common frequently performed interventions in the world.

1.1.2 Types of Intraocular Lenses

Intraocular lenses (IOL) are composed of two compartments; 1) optical section, which is responsible for the refraction of the light, and 2) haptic section, ensuring stable fixation to the lens within the capsular bag inside the eye (**Figure 1.4**).

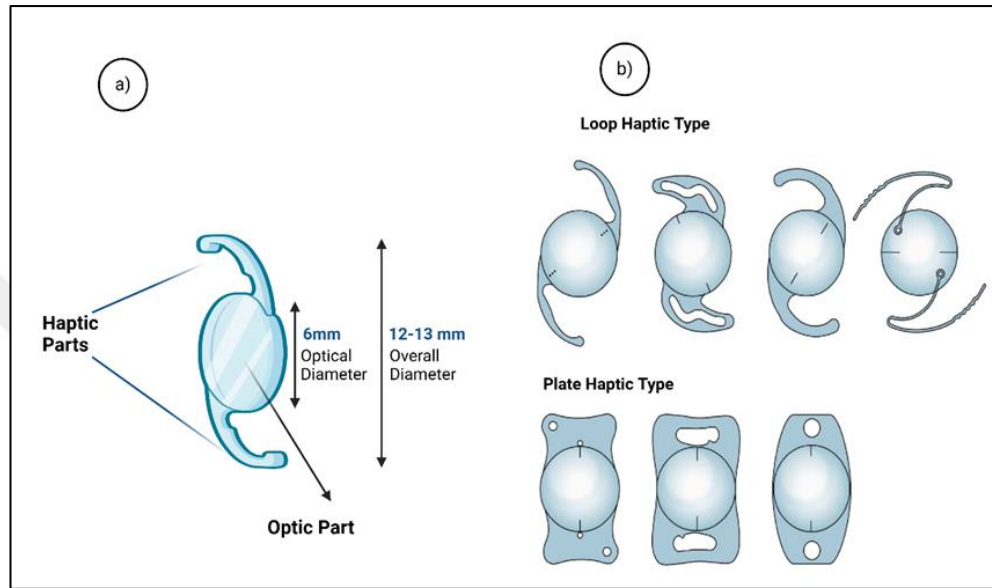


Figure 1.4: a) IOL parts and, b) haptic types for IOLs.

The main disadvantage of PMMA IOLs, which are rigid and non-foldable hydrophobic material despite its high refractive index, is that it requires large incisions during surgery (5-7 mm) and creates a risk of endothelial loss due to adhesion to corneal endothelial cells[20]. These drawbacks of PMMA material have accelerated the search for alternative materials. As explained below, intraocular lenses can be divided into a number of classes based on the sorts of materials they are made of.

1.1.2.1. Rigid Poly(methyl methacrylate) Intraocular Lenses

PMMA is the best-known biomaterial for IOLs. The first IOL was made up of polymethyl methacrylate (PMMA) material, and this polymer material is a rigid, non-folding hydrophobic material. Despite its high refractive index ($n = 1.49$), satisfying bioavailability and good light transmittance, its solid characteristics requires large

incisions in the ocular tissue during surgery and creates a risk of endothelial loss by adhering to the corneal endothelial cells [21], [22].

1.1.2.2. Silicone Intraocular Lenses

Polymers such as silicon-based poly (dimethyl siloxane) and poly (diphenyl siloxane) are used to manufacture hydrophobic, flexible, and soft IOLs. The first foldable silicon IOL was implemented in 1989. Silicone is a synthetic polysiloxane, exhibits notable flexibility and softness due to the free rotation around the Si–O bonds of the main polymer backbone. The Refractive index (n) of hydrophobic silicones is in the range of 1.41-1.46. Silicon-based polymers are thicker in size due to their low refractive indices and therefore require larger incisions during the surgery[23].

Another type of silicone IOLs is Collamer which name is come from the combination of collagen and polymer. They have great biocompatibility with the eye tissues and high-water content which makes them soft material [18].

1.1.2.3. Acrylic Intraocular Lenses

Combining both the adjustable foldability of PMMA and the positive capabilities of silicon with the appropriate refractive index (1.41-1.46), acrylic polymer-based IOLs have been developed for use in cataract surgery as implants [24]. Thanks to the developed acrylic-based foldable IOL implants, the required incision, and postoperative complications can be minimized. Two types of acrylic-based polymer IOLs are available: hydrophobic acrylic and hydrophilic acrylic.

Hydrophobic Acrylic Intraocular Lenses: The polymethacrylic acid esters with hydrophobic acrylic IOL have the ability to absorb lower than 1% water and can remain foldable when they are completely dry. The refractive index (n) of hydrophobic acrylic IOLs ranges from 1.44 to 1.55. Dysphotopsia which is associated with low anterior curvatures and high refractive index is more common in hydrophobic acrylic intraocular lenses compared to other types of acrylic IOLs[25], [26].

Hydrophilic acrylic Intraocular Lenses: Hydrophilic acrylic IOLs have known as a framework that can absorb water in quantities reaching to thousands of times their dry weight. They have excellent biocompatibility due to its hydrophilic surface properties,

with refractive index of 1.43. Additionally, hydrophilic acrylic-based IOLs are preferred because of their easy handling, implantation with an incision less than 2mm, and the low risk of damage in the capsular bag where the IOLs are mounted during implantation. Moreover, all types of IOLs could absorb UV radiation under 400 nm. But some IOLs have a yellow pigment to protect the retina from harmful blue or violet visible light radiation [17], [21].

1.1.3. Post-Operative Concerns: Posterior Capsule Opacification

Although cataract surgery is a widely performed and safe operation, as with any surgical technique, it carries a risk of postoperative complications such as endophthalmitis, ocular inflammation, or posterior capsule opacification (PCO) [27], [28]. Months or even years after IOL implantation, vision impairment may result from lens epithelial cell (LEC) adherence, growth, and proliferation on the posterior portion of the lens capsule and IOL (secondary cataract). LEC growth is influenced by the IOL material, and IOLs with a low contact angle are more biocompatible. Nevertheless, inflammatory cell attachment occurs to some extent even with the most biocompatible IOL material. Due to its high incidence and the relatively high cost of capsulotomy with a Nd: YAG laser, PCO treatment is very expensive. Therefore, IOL designs with sharp edges, low water contact angle, or coated with protein/cell repellent materials have been extensively studied in recent years to prevent PCO [24].

Hydrophobic IOLs show better adhesion to the posterior capsule after implantation due to their adhesive surface properties. This feature reduces the empty space between the capsule and the IOL, preventing the migration of LECs to that area and reducing the PCO incidence [29]. Additionally, IOL optics with sharp posterior edges had a lower incidence of PCO than IOLs with round edges because the edges can act as a mechanical barrier to prevent LEC migration into the posterior capsule [30].

1.1.4. Intraocular Lens-based Drug Delivery Systems

Due to the advantages of IOLs in cataract surgery—such as their permanence within the eye postoperatively and the removal of the need for additional surgical interventions for drug loading—cataract surgery and the postoperative treatment method can be combined into a single procedure. Consequently, IOLs can serve as a

drug delivery platform for antimicrobial treatment after cataract surgery. Towards the late 20th century, research on the utilization of IOLs as drug reservoirs and their potential for long-term controlled drug release became increasingly prevalent[31]. By using IOLs as a drug delivery platform, drugs can be delivered directly into the anterior chamber, providing a higher and more controlled intraocular drug concentration. The drug loaded onto the IOL should not affect the optical transmittance of the lens or its stability within the capsular bag, and should also be biodegradable [32]. In 1985, the first study was assessed to investigate the interaction between IOLs and ophthalmic eye drops. In the following years, IOLs were also shown to have the potential to prevent PCO by inhibiting the proliferation and adhesion of LECs [33]. The frequently used methods utilized for the production of drug-laden IOLs are listed below.

1.1.4.1. Soaking Method

Soaking is the most simple and cost-effective method to obtain drug-laden IOLs but only eligible for the high-affinity drugs. Intraocular lenses can be loaded with the drug by immersing in drug solution or concentrated aqueous solutions, as in contact lenses. Typically, hydrophilic acrylic materials demonstrate hydrophobic acrylic or silicone-based materials in terms of their capacity to absorb and release drugs. The drug load/release kinetics could be affected by drug concentration, molecular weight of the drug, loading duration, IOL material, etc [34]. For example, hydrophilic acrylic lenses coated with gatifloxacin by the immersion method have been reported to have an affinity for fourth-generation fluoroquinolones. In experiments on rabbit eyes, gatifloxacin release was observed for at least 12 hours [35]. In another study, both hydrophobic and hydrophilic IOLs were coated with gefitinib using the immersion method to prevent PCO development. The coatings were reported to delay cell adhesion to the posterior capsule in both types of lenses. Gefitinib was released from the hydrophobic IOL for more than 10 days, according to in vivo drug release experiments [17], [27]. Davis et al. implanted four different antibiotics (moxifloxacin, gatifloxacin, linezolid, and cefuroxime) into hydrophilic acrylic IOLs using the immersion method and then examined the drug concentration in aqueous and vitreous samples. It was reported that drug release from hydrophilic acrylic IOLs occurred for at least 6 hours [36]. Filipe et al performed drug loading by soaking the hydrophilic material of the prototype IOL at 60 °C for four days. The sterilised lenses were

immersed in the drug solution during 30 days. When the lenses' in vivo properties were examined, it was found that the release of moxifloxacin lasted for about two weeks [37].

1.1.4.2. Supercritical Fluid Technology

Supercritical CO₂ (sCO₂) is commonly used as a drug solvent and carrier in the process of supercritical solvent impregnation. Three primary processes are involved in supercritical impregnation: mass transfer from the liquid phase to the polymeric matrix, drug solubilization and network swelling, and CO₂ release, which traps the drug in the matrix. Process parameters, including pressure, temperature, drug concentration, or sCO₂ phase, etc., can be optimized to achieve the desired drug loading and release rate [17]. Hydrogels composed of three chitosan derivatives were impregnated with the anti-inflammatory agent flurbiprofen and the anti-glaucoma medication timolol maleate to develop ophthalmic drug delivery devices[38]. Another study describes norfloxacin impregnation of polymeric IOL material prepared from hydrophilic monomer HEMA and hydrophobic monomer 2-butoxyethyl methacrylate (BEM). Supercritical impregnation resulted in a higher drug loading capacity compared to the traditional soaking technique [24]. Ongkasin et al. impregnated commercially available hydrophobic acrylic IOLs with MTX to prevent PCO formation. The effect of ethanol as a co-solvent at different pressures and for different impregnation times has been studied. Increasing impregnation time led to a higher drug loading as expected; however, those amounts decreased with an increase in pressure. It was reported that drug release was achieved for at least 80 days under all conditions [39].

1.1.4.3. Surface Modification

Surface modification, which includes techniques like layer-by-layer (LbL) deposition, spray coating, spin coating, grafting, and nanoparticle attachment, is one of the strategies used for drug loading into IOLs. Some examples from the literature regarding these methods are given below. Natural polymers, such as alginate and chitosan, were deposited in layers on CI26Y IOL material to evaluate their potential as a controlled drug release platform. The anti-inflammatory drug diclofenac was

released for at least 1 week, and no side effects were observed [40]. Another study investigated the efficacy of antibiotics by modifying the IOL surface. The hydrophilic acrylic surface was modified by plasma-assisted graft copolymerization of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) or [2-(methacryloxy)ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA). Moxifloxacin was found to be effective against *Staphylococcus aureus* and *Staphylococcus epidermidis* up to day 12 [41]. Vieira et al. coated moxifloxacin onto hydrophilic poly[(2-hydroxyethyl methacrylate)-co-(methyl methacrylate)]—a material used for IOLs—using argon plasma-assisted grafting and dipping methods. The study reported that drug release from the prepared system lasted for approximately 10 days [42].

1.1.4.4. Integration of Drug Reservoirs

These systems provide drug release by attaching the drug delivery systems (DDS) to the IOL through either the optic or haptic components. Some examples from literature are given below.

In 1996, Tetz et al. developed a DDS releasing daunorubicin and indomethacin for PCO prevention. Drug-loaded short threads were produced and attached to the IOL surface with fibrin adhesive. While there was no decrease in PCO in the group treated with indomethacin, the formation of PCO dropped by roughly 50% in the group treated with daunorubicin [43]. Garty et al. developed a DDS loaded with NFX using pHEMA and attached it to the haptic part of an IOL. The device maintained the in vitro concentration of the antibiotic above the minimum inhibitory concentration (MIC) for a duration exceeding 14 days. Moreover, in vitro antibacterial activity has been tested and proven against *S. epidermis* [44]. Tan et al. (2016) developed a biodegradable device loaded with moxifloxacin and levofloxacin, which was attached to the periphery of an IOL. The device ensured controlled release of the respective drugs at therapeutic levels a 14-day period [45].

1.2. Polymers and Hydrogels

1.2.1. Polymers

Large molecules or macromolecules known as polymers are made up of numerous repeating subunits that are usually joined to one another by covalent bonds. Because of their many different qualities, both natural and synthetic polymers are employed extensively in daily life. However, because natural polymers are inexpensive, widely accessible, and non-toxic, they are especially beneficial when used in pharmaceutical applications [46]. Compounds, which may have both low and high molecular weights, are synthesized by combining repeating simple units called monomers in a process called polymerization. Monomers are linked together in 3 different ways (linear, branched, or cross-linked) to form the polymer structure[47]. Polymers' enhanced pharmacokinetic qualities have made them a crucial component of drug delivery systems [48]. Because of their better circulation times compared to conventional small drug molecules, they can be directed to specific tissues[49]. The polymers used must be non-toxic, biocompatible, and biodegradable and should also not trigger an immunological reaction[50]. Furthermore, exposure to stabilizers, catalysts, or plasticizers during the manufacturing and preparation of polymers may induce toxic effects. Consequently, auxiliaries that can be harmful should be strictly avoided in all polymer manufacturing operations[51]. Significant advancements have been achieved in the drug delivery systems of hydrogel and liposomes, which use polymers as drug reservoirs [52].

1.2.2. Hydrogels

Three-dimensional networks of cross-linked polymeric chains that easily absorb and hold onto water in the gaps between the chains are known as hydrogels. Because of their high-water content, porosity, and soft consistency, they resemble natural living tissues [53]. Hydrogels can either show chemical stability or degrade, that eventually results in dissolution and disintegration[54].

Hydrogels can be divided into various subgroups based on distinct characteristics. First, they can be classified as natural or synthetic based on their source. Natural polymers are high-molecular-weight compounds derived from natural sources,

including plants, microorganisms, and animals. Because of their affordability, accessibility, capacity for different chemical changes, biodegradability, and biocompatibility, they have a wide range of uses, especially in the food, cosmetics, and pharmaceutical industries. In addition, the biological properties of natural polymers, such as cell recognition and interaction, enzymatic degradability, resemblance to the extracellular matrix (ECM), and chemical flexibility, make them the preferred materials for drug delivery. The similarity of natural polymers to ECM components allows them to avoid the immunological reactions and toxicity commonly associated with synthetic polymers[50].

One reason natural polymers have gathered significant attention is their ease of acquisition from abundant and renewable natural resources. Polysaccharides, as natural polymers, are frequently used in hydrogel design for biomedical applications by forming hydrophilic hydrogels due to their high biocompatibility and biodegradability. Alginate, cellulose, chitosan, dextran, heparin, hyaluronic acid, and pullulan are the most commonly used polysaccharides in hydrogel development, especially in drug delivery studies[55]. Alginate is the one of them that is of importance to this study and will be covered in more detail in the sections that follow.

Depending on the cross-linking method, hydrogels can be categorized into two groups: chemical and physical (**Figure 1.5**). While physically cross-linked hydrogels are created by hydrophobic, ionic, or non-covalent interactions like hydrogen bonding, which make the physical networks more transient, chemically cross-linked hydrogels display permanent interactions brought about by covalent bonds between polymer chains[56]. The reversible properties of physically cross-linked hydrogels usually make them susceptible to changes in temperature, ionic strength, crosslinking initiators, and polymer composition. Physically cross-linked hydrogels usually have weak mechanical qualities, which makes their blood circulation less stable. Chemical cross-linking, which creates covalent links between polymer chains, improves the stability and integrity of cross-linked networks. This provides better gel stability compared to physically cross-linked hydrogels by preventing dissolution of polymer chains in aqueous media [57].

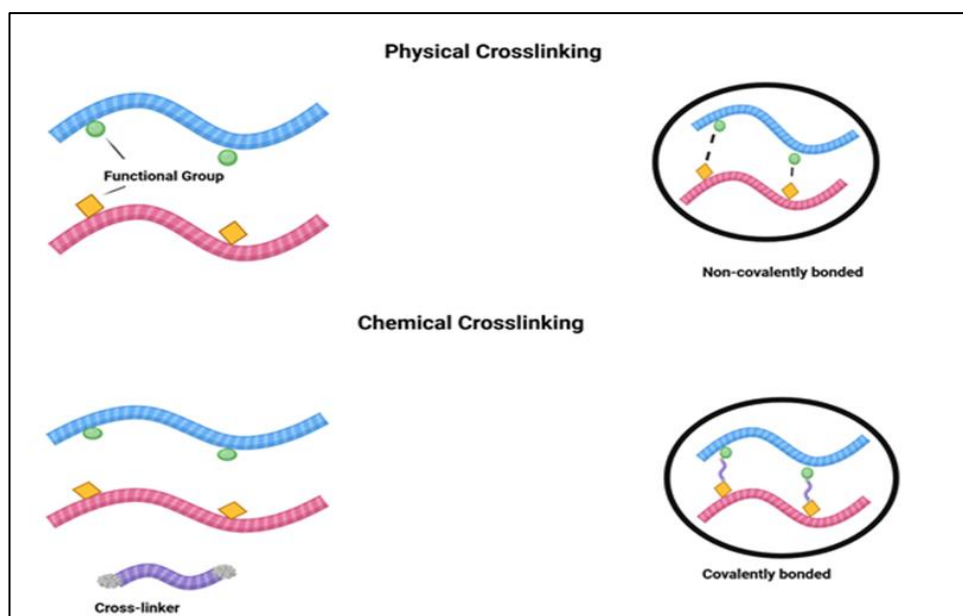


Figure 1.5: Physical and Chemical Crosslinking Mechanism.

1.1.2.1. Hydrogel-based Drug Delivery in Ocular Route

Eye diseases and visual impairments are widespread globally, and with the continuous advancements in technology and medical science, novel treatment methods are being developed on a daily basis. The location of the affected tissue and the degree of invasiveness determine the current treatments for eye diseases; nevertheless, each approach has pros and cons of its own. However, the anatomical structure of the eye and its physiological barriers prevent therapeutic agents from reaching the targeted sites in effective doses, prolonging treatment durations or rendering treatment ineffective[20]. To address this challenge, additional methods, including colloids and polymeric implants as well as hydrogels capable of sustained drug release, are frequently employed. Through the following drug delivery mechanisms, hydrogels can increase the therapeutic efficacy of ophthalmic medications in the treatment of ocular diseases that cannot be accomplished by traditional methods: prolonging the drug's residence time at the application site; promoting sustained release of the drug at the target site; and enabling the co-administration of multiple drugs [58]. For example, customized eye drops have been developed to increase the residence time on the corneal surface, which is the biggest challenge associated with topical applications. A cationic nanoemulsion containing chitosan and poliximin B has been successfully

developed for the treatment of ocular tuberculosis. In vitro mucoadhesion tests showed that it enhances its efficacy by increasing its retention time on the ocular surface through electrostatic interaction with the mucin layer [59]. For post-cataract surgery treatment, norfloxacin was loaded into pHEMA hydrogels and surface-modified with octadecyl isocyanate to create a rate-limiting barrier to control drug release. The controlled drug release system was tested and found to exhibit an antibacterial effect [44]. In another study, chitosan nanoparticles containing the antiproliferative drug doxorubicin were prepared by ionic gelation and designed as one of the multilayer polyelectrolyte layers on the IOL surface. The polyelectrolyte layers' adhesion, proliferation, and migration of lens epithelial cells were significantly inhibited in in vitro cell culture assays, and the development of PCO was significantly inhibited in an in vivo rabbit model [60].

Although cross-linking of hydrogels enhances mechanical integrity and reduces dissolution rates, drug release is not notably prolonged by simple cross-linking. These macroscopic hydrogels, composed of randomly interlinked polymer chains, feature large pores that result in the quick release of encapsulated drug molecules. Composite hydrogel systems in which drug-incorporated nanocarriers are integrated into the hydrogel matrix have been widely investigated. Composite systems composed of polymers and liposomes have been shown to be advantageous over liposomes or polymers alone due to their behavior, such as improving the viscosity and stability of liposomes, prolonging drug half-life, and maintaining the bioactivity of the drugs [61].

1.3. Nanoparticles in Ocular Drug Delivery

One of the main objectives of treating many diseases is to get therapeutic substances to the intended location. Controlled drug delivery systems can be used to overcome the drawbacks of traditional delivery techniques, including decreased efficacy, poor biodistribution, and inadequate selectivity. Drugs are delivered to the site of action by being incorporated into nanocarriers, which raises the drug concentration in the target tissues. This method reduces the possibility of adverse effects and permits the use of lower pharmacological dosages [62]. Nanoparticles have the potential to improve the stability and solubility of encapsulated therapeutics, promote transport across membranes, and prolong their circulation time within the target area, thereby improving both safety and efficacy [63].

Numerous nanoparticle-based systems have been created and employed as cutting-edge platforms for ocular drug administration, including nanomicelles, nanoparticles, liposomes, niosomes, dendrimers, nanosuspensions, nanoemulsions, and nanocrystals [64].

1.3.1. Nanomicelles

Nanomicelles (**Figure 1.6**) are structures most commonly used to formulate clear aqueous solutions and are generally formed using amphiphilic molecules. Due to its high drug entrapment capacity and ease of formation, it has increasingly been applied in ocular tissues [64]. Nanomicelles are appropriate for self-assembling into structures with hydrophobic cores and hydrophilic shells. The tear film, corneal and conjunctival barriers, and nasolacrimal drainages are among the biological barriers and drainages in the eye that this arrangement helps shield the encapsulated drug from.

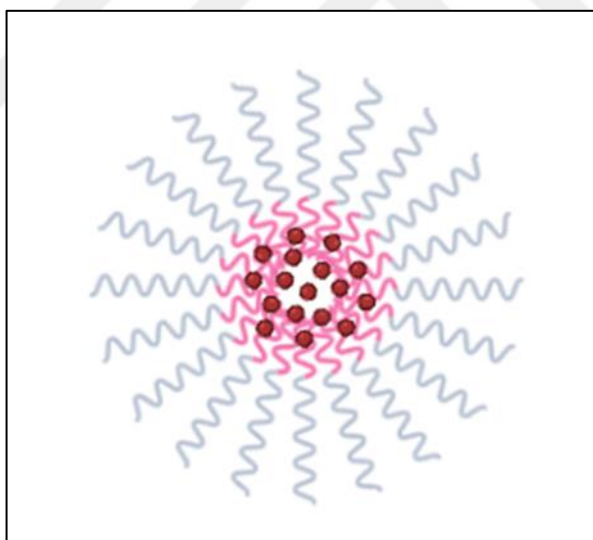


Figure 1.6: Drug Loaded Nanomicelle.

Chitosan oligosaccharide-valylvaline-stearic acid (CSO-VV-SA) nanomicelles were produced for topical ocular drug delivery. The resulting nanomicelles exhibited no cytotoxic effects on human corneal and conjunctival epithelial cells. An in vivo pre-corneal retention study showed that dexamethasone released from the nanomicelles could be detected in rabbit tears for more than 3 hours [65].

1.3.2. Dendrimers

A "dendrimer" stands for any polymer consisting of units that repeat and branch repeatedly (**Figure 1.7**). Branched and star-shaped polymeric systems are used as carriers for ocular drug delivery. Drug release behavior is directly impacted by the selection of functional groups, molecular weight, and surface charge. These systems' extremely branching nature makes it easier to incorporate both hydrophilic and hydrophobic medications [66]. Dendrimers can be used in topical or injectable formulations as ocular drug delivery.

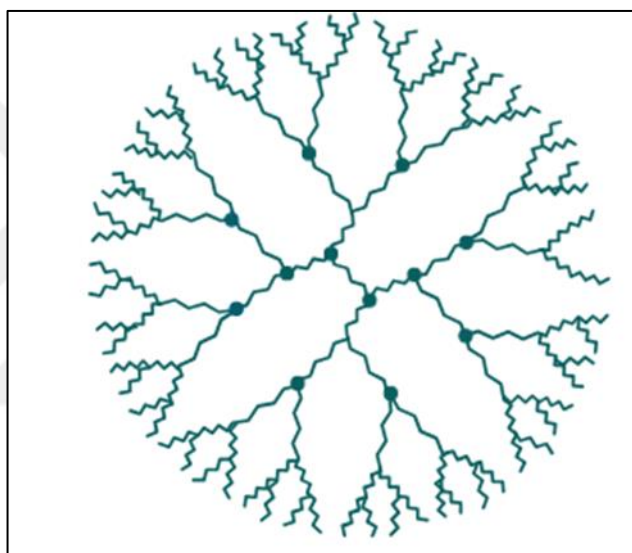


Figure 1.7: Structure of dendrimers.

Commercially available polyamidoamine (PAMAM) dendrimers serve as a prominent example of dendrimer-based applications in ocular drug delivery. These PAMAM dendrimers are widely recognized for their ability to enhance corneal residence time [67].

1.3.3. Nanocrystals

These systems mainly consist of the active ingredient, with only a small amount of excipients, such as stabilizers or surfactants. Their adhesive properties increase the residence time of the drug in the pericorneal area, which may result in increased

bioavailability of the drug. In comparison to polymer nanoparticles, nanocrystals require fewer processing steps for their production[68]. The duration of the drug's residence in the eye and, as a result, its bioavailability were enhanced by a cationic nanocrystal formulation including dexamethasone acetate nanocrystals and polymyxin B, which were obtained in the size range of roughly 200-250 nm (**Figure 1.8**). Stability testing at different temperatures demonstrated that the chemical content remained virtually unchanged for 6 months. In addition, no in vitro cytotoxicity was observed in the fibroblast cell culture evaluation. These results are regarded as a promising alternative as they show better performance compared to existing methods [69].

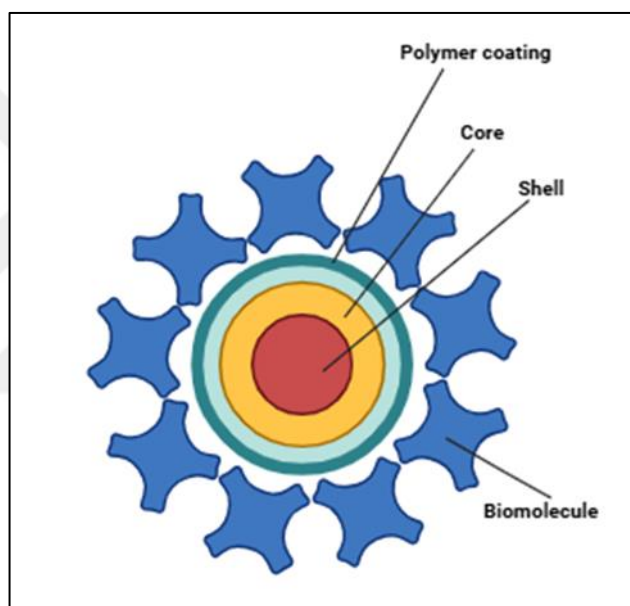


Figure 1.8: Structure of nanocrystals.

1.3.4. Niosomes

In aqueous media, niosomes are formed between amphiphilic non-ionic components in bilayer sacs. Biodegradable, biocompatible, and non-toxic, niosomes have the ability to encapsulate both hydrophilic and hydrophobic drugs (**Figure 1.9**). Niosomes are utilized in drug delivery systems designed with the strategy of increasing drug permeability through the cornea[70]. They improve patient compliance due to their water-based nature in comparison with oily dosage formulations. Additionally, the

bioavailability of the drug can be enhanced by providing oral, parenteral, and topical routes to the targeted area. Doxycycline HCl-loaded niosomes for ocular delivery have been formulated and thoroughly characterized. In vitro release studies demonstrated sustained drug release, while a Draize test confirmed that the formulation was well-tolerated by the ocular tissue. These findings collectively indicate that niosomes could serve as a promising alternative for ocular drug delivery[71].

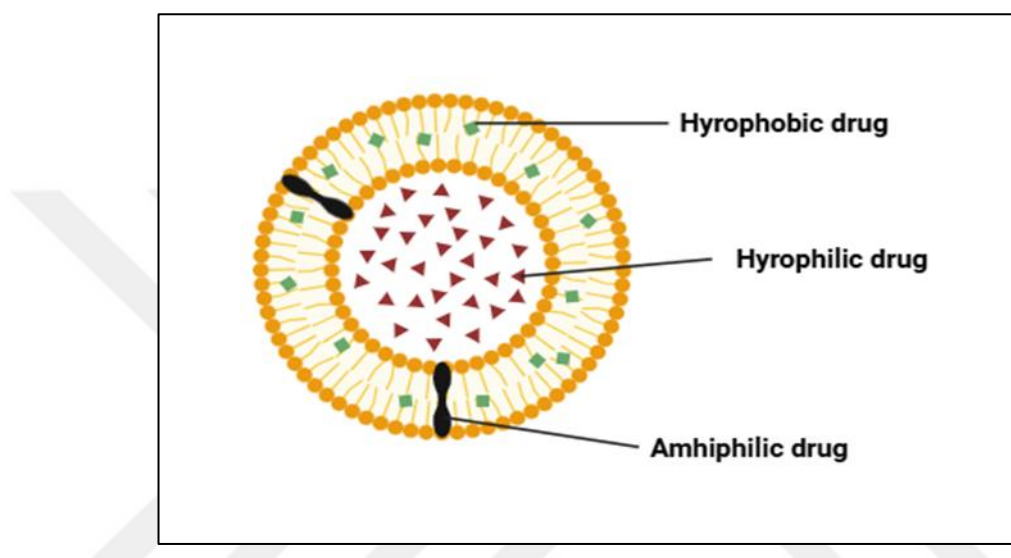


Figure 1.9: Structure of niosomes.

1.3.5. Liposomes

The use of liposomal nanoparticles in medication delivery has been thoroughly investigated in a number of studies carried out throughout the last ten years. Liposomes are tiny vesicles that usually have an aqueous core encircled by one or more concentric lipid bilayers. They range in size from 10 nm to 1 μm (**Figure 1.10**). Due to their unique characteristics, they have the ability of encapsulating both hydrophilic and hydrophobic therapeutic agent [72]. When considered as drug delivery vehicles, liposomes have been shown to enhance the cellular uptake, physicochemical properties, and bioavailability of drug molecules because of the high surface area/volume ratio. Additionally, they offer several advantages, including the stabilization of both hydrophilic and poorly water-soluble drugs [73]. The encapsulation of drug molecules in liposomes provides protection against enzymatic

degradation during circulation in physiological media, thereby increasing their stability. Additionally, liposomes have become attractive carriers in ocular drug delivery applications because of their excellent biocompatibility, membrane-like structure, and capacity to encapsulate hydrophilic and hydrophobic drugs [74]. Several studies have shown that adding mucoadhesive polymers to liposomes improves the drug's residence time in the precorneal region and the drug molecules' absorption into the corneal tissues, especially when treating anterior segment diseases, even though liposomes can be used to treat both anterior and posterior segment diseases.

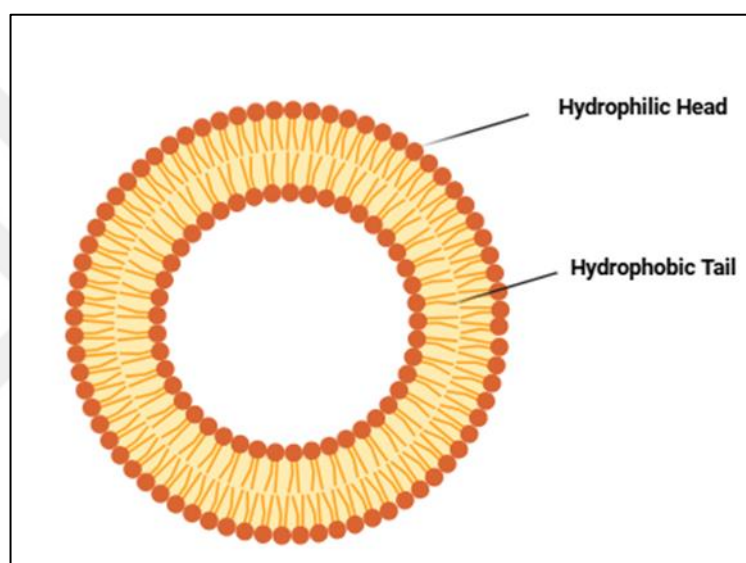


Figure 1.10: Scheme of a liposome.

The in vivo stability of pharmaceutical compounds in the body is extremely poor. The encapsulation of drugs in liposomes provides protection against enzymatic hydrolysis in physiological environments and thus improves their stability. Liposomes represent highly successful systems for delivering drugs to target tissues. However, their intrinsic instability may limit their therapeutic potential. To overcome this issue the incorporation of drug-loaded liposomes into polymeric hydrogel systems has been shown to further improve drug stabilization.

Nanocomposite hydrogel systems, in which drug-encapsulated liposomes are incorporated into polymeric hydrogel systems, have attracted considerable research interest in recent years.

1.4. Nanocomposite Hydrogel Systems

In recent years, interest in combining nanoscale procedures with other disciplines to produce enhanced materials has grown significantly. Nanocomposite hydrogels represent outstanding example of integrating biomaterial science and nanotechnology.

The release of drug molecules encapsulated in liposomes from the hydrogel matrix depends on the stability of the liposomes during their dispersion in the semi-solid formulation[75]. Liposomes are nanoparticle systems that are successful in delivering drugs to the target site; however, their stability may be insufficient to maintain drug efficacy. To address this issue, nanocomposite hydrogel systems obtained by combining liposomes with hydrogel systems can be employed. Composite hydrogel systems containing polymer and liposomes have been shown to facilitate more prolonged drug release than polymer or liposome forms alone. Composite hydrogel systems improve liposome viscosity, stability, and half-life of liposomes while also preserving drug bioactivity [61]. The presence of liposomes has been shown to increase diffusion resistance, thereby enabling a longer drug release compared to hydrogels without liposomes [76].

The hydrogel structure of the nanocomposite preserves the liposomes' structural integrity and activity, allowing for therapeutically effective and prolonged drug release to the target region.

1.4.1. Hydrogel Production Methods

It is commonly recognized that the manufacturing process has a direct impact on the drug release performance of hydrogel structures produced using various production techniques and utilized in controlled drug delivery systems. Therefore, it is important to prioritise fabrication methods that allow precise control of the structural properties of hydrogels in drug delivery systems [77].

In general, the synthesis of hydrogels is characterised by three fundamental steps: a monomer, an initiator and a crosslinking agent. To control the heat of polymerization and the properties of the final hydrogels, diluents like water or other aqueous solutions can be added to the formulation.

1.4.1.1. Bulk Polymerization

Bulk polymerization is a simple technique involving only monomer and initiators typically dissolved in the monomer. The heat generated during polymerisation significantly increases the viscosity of the reaction. Bulk polymerisation of the monomers results in the formation of a glassy, transparent hydrogel characterized by high rigidity and a homogeneous structure[78]. Despite being the most straightforward and the most preferred technique, the inability to control the character of the polymerisation is a factor limiting its use.

1.4.1.2. Solution Polymerization and Crosslinking

After ionic or neutral monomers are mixed with a multifunctional crosslinking agent, polymerization is thermally initiated by UV irradiation or a redox initiator system. When the water content during polymerization surpasses the water content of system's equilibrium swelling capacity, phase separation occurs and resulting in formation of heterogeneous hydrogel structures. The presence of numerous chemical components in the reaction environment complicates process optimization. And also requires additional steps including the removal of residual chemicals during polymer production and purification [78], [79].

1.4.1.3. Suspension Polymerization or Inverse-suspension Polymerization

A homogeneous medium for reactions is generated when monomers and initiators are evenly distributed throughout the hydrocarbon phase. Dispersion polymerization offers significant advantages, allowing products to be obtained as powders or microspheres. The polymerization is known as "inverse suspension" since the water-in-oil (w/o) technique is employed rather than the more popular oil-in-water (o/w) process. The viscosity of the monomer solution, agitation speed, rotor design, and dispersant type are some of the variables that affect particle size and form [80]. The application of the system is limited due to difficulties in optimizing and poor reproducibility.

1.4.1.4. 3D Printing

The American Society for Testing and Materials (ASTM) and the International Organization for Standardization (ISO) have officially defined three-dimensional (3D) printing as a manufacturing process that produces parts by successively depositing materials, usually layer by layer. The most used techniques can be classified as material jetting, material extrusion and vat photopolymerization [81].

Using the material jetting technique, droplets of raw materials like wax and photopolymer resin are selectively deposited. This technique provides remarkable control over the deposition pattern and makes it easier to build heterogeneous cell formations with precisely defined positions. Therefore, it typically results in higher print resolution (50 μm) and faster print rates [82]. In addition to necessitating hydrogel inks with low viscosity and lower cell densities, this method may also be associated with challenges such as inhomogeneous droplet formation, inaccurate deposition placement, and decreased cell viability resulting from exposure to heat or electrical forces [83].

Material extrusion is a fabrication technique in which material is selectively deposited through a nozzle or orifice under controlled conditions. Although it is characterized by its simplicity and cost-effectiveness, its applicability to liquids with high viscosity is limited despite shear thinning behavior. Moreover, the risk of nozzle clogging can make it challenging to achieve consistent and reliable printing outcomes. This approach facilitates the utilization of hydrogel inks with high cell densities; however, to achieve higher volumetric output, it typically provides at lower printing speeds and resolutions (100 μm) mainly because of reducing nozzle diameters [81].

Vat photopolymerization uses light-activated polymerization to selectively cure liquid photopolymers that are contained in a vat. Light-assisted 3D printing offers significant benefits over nozzle-based methods in terms of printing resolution and speed (20 - 200 μm). Various viscosity spectra are permissible, provided that the hydrogel inks are capable of photo crosslinking. Ensuring that the materials are transparent to the wavelength of the applied light source is essential for achieving sufficient light penetration depth, a crucial parameter that determines the structural integrity and overall quality of the printed product [81].

1.4.1.5. Conventional Method

The procedure involves dropping separated the prepared polymer solution and crosslinking agent onto the substrate via a micropipette, enabling controlled hydrogel formation through localized crosslinking. Compared to other methods, it stands out due to its low cost, the ease with which its physical and chemical properties can be controlled, its high efficiency, and its reproducibility.

1.4.1.6. Microfluidics

Microfluidic systems represent an interdisciplinary area that integrates engineering, nanotechnology, and biotechnology. These systems offer several advantages, including minimal sample volume requirements, high sensitivity, simple setups, reproducibility, cost-effectiveness and high efficiency[84]. Because they allow for highly exact control over the flow rate, microfluidic devices hold promise for producing hydrogels by producing microparticles with perfect size, shape, and mechanical characteristics [85]. Furthermore, microfluidic methods allow for the creation of micron-sized polymeric structures that may then be gelled and hydrogelled for use in drug delivery systems and cell culture platforms [86], [87]. Using multiphase systems comprising a continuous gas phase, a liquid dispersed phase, and a surfactant, this technique creates microbubbles. Shear stress and interfacial tension between two immiscible phases are the main factors that drive droplet formation. By reducing the interfacial tension between the phases, the surfactant prevents droplets from aggregating together [85], [88]. Microliter volumes of liquid or gas become entrapped within each other and adopt a spherical shape in order to compensate for interfacial instability. The laminar flow characteristics of the hydrodynamic flow in the microfluidic system eliminate the physicomachanical and chemical variability typically seen in large-scale systems, enabling controlled and reproducible flow generation. This allows for the generation of uniformly sized microbubbles, provided that the parameters within the microfluidic system remain constant[89]. Following microbubble formation, hydrogel synthesis is typically achieved by introducing a crosslinking agent outside the microfluidic channel, which induces polymer network stabilization. The final size and shape of the resulting microbubbles are influenced by the channel diameters, the flow regime, and the viscosity of the respective phases.

Meanwhile, the size of the droplets can be modulated by adjusting the flow rates and the volumetric ratios of the two immiscible phases[90]. **Figure 1. 11** depicts various microfluidic devices capable of generating microfluidic junction devices capable of generating microbubbles with high monodispersity. Microfluidic systems enable the production of uniformly distributed bubbles, allowing porous hydrogel structures suitable for use in controlled drug delivery applications[91]. The microbubbles formed via the microfluidic system allow for a consistent release profile and enhanced content uniformity in the controlled release of pharmaceutical agents.

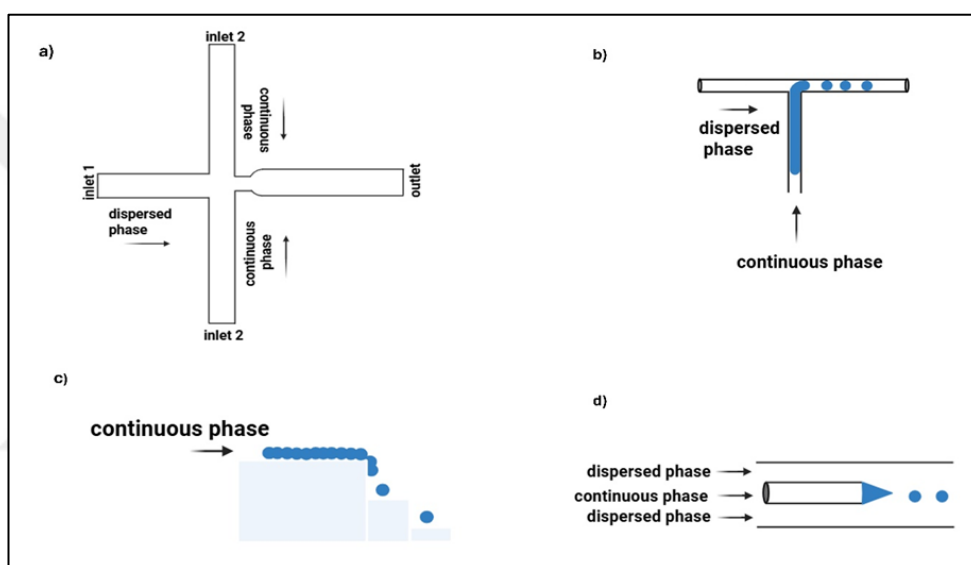


Figure 1.11: Illustrations of the several microfluidic devices **a)** flow (stream) focusing, **b)** T-junction, **c)** terrace-like and, **d)** co-flowing stream.

1.5. Drug Delivery Profiles and Mechanism of Drug Release

A drug delivery system is characterized as a technological approach that enables the attainment of optimal therapeutic efficacy by allowing precise control over drug transport and release within the body [92]. Drug delivery systems are designed to increase the effectiveness of medicinal drugs at the site of action, which lowers the frequency of doses, minimizes negative side effects, and prolongs the duration of drug release.

Three main types of extended drug release systems have been developed to facilitate a better understanding of the kinetics associated with long-term drug release system

(Figure 1.12). A plot of plasma drug concentration as a function of time is commonly used to illustrate a drug's release profile. Drug release kinetics can be defined as zero-order kinetics when a constant amount of drug is eliminated per unit time, and the release rate remains independent of the drug concentration. Zero-order drug delivery systems are made to release the medication consistently, providing long-term, sustained drug levels within the therapeutic window [93].

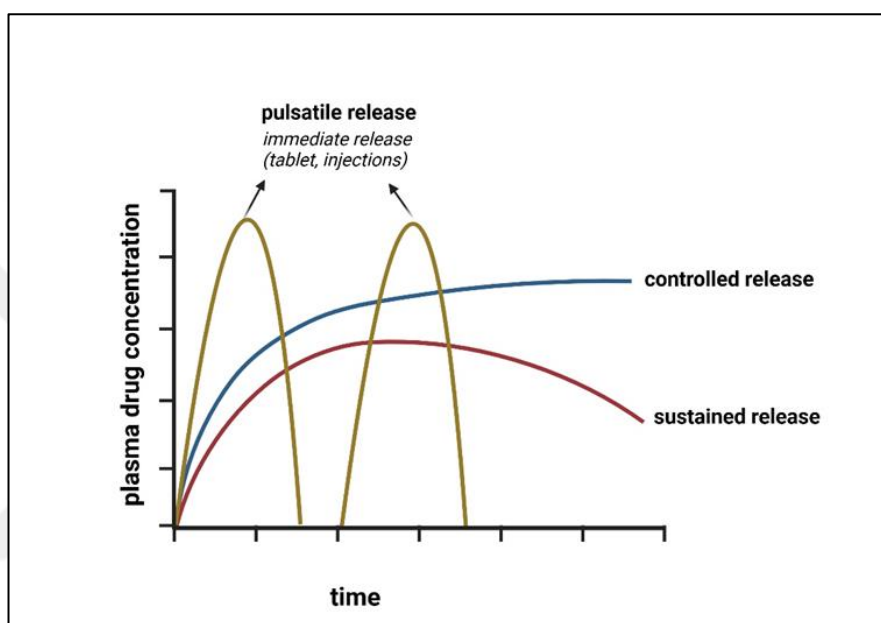


Figure 1.12: Graphical representation of drug release kinetics.

2. PURPOSE OF THE STUDY

The aim of the thesis is to produce MXF-encapsulated liposome-enriched alginate hydrogels using the T-shaped microfluidic technique to coat the hydrogels with uniform pore and size distribution on IOL haptics and to design them as an alternative drug delivery platform to conventional antimicrobial treatment after cataract surgery. Furthermore, characterization of the resulting coating, determination of its antimicrobial activity, and drug release behaviour will be carried out to evaluate its potential as an alternative method to conventional antimicrobial treatment after surgery. Sodium alginate polymer was preferred due to its biocompatibility, toxicity, cost-effectiveness, compatibility with MXF drug, and liposomal structures. It is seen that various combinations of alginate and MXF are utilized in the treatment of infection and wound healing, but there is no study in the literature on the production of MXF-encapsulated liposome-enriched alginate hydrogels by the microfluidic method, coating them on the IOL haptics as a layer and then using them for antimicrobial treatment.

The drug release mechanism is integrated with the cataract surgery process, eliminating the need for a second operation to deliver the drug to the eye. By adjusting variables like drug concentration and microfluidic technique parameters that impact microbubble formation, the amount of drug to be loaded onto the IOL haptics may be regulated.

By controlled release of the drug, uniform ALG hydrogels containing homogeneously dispersed liposomal MXF drug were coated on the IOL implant by the T-shaped microfluidic method, and an alternative drug delivery platform for postoperative antimicrobial treatment was designed.

The main aim of this thesis study is to entrap the antibiotic drug MXF into the natural liposomal nanoparticle obtained from soy lecithin, to produce ALG hydrogels with uniform pores and distribution consisting of homogeneously dispersed MXF-encapsulated liposome nanoparticles using a T-shaped microfluidic junction device, to coat the hydrogels on IOL haptics, and then to characterize all products and to determine their antimicrobial activity and drug release behavior.

To this end, the flowing outputs are targeted:

- Optimization of ALG polymer solution concentrations containing MXF-encapsulated liposome nanoparticles that can flow through micron-sized channels in the T-shaped microfluidic system to obtain ALG hydrogel structures with uniform pore and size distribution,
- Incorporation of MXF-encapsulated liposomal nanoparticles into the hydrogel coating matrix for coating on IOLs via T-shaped microfluidic system,
- Obtaining ALG hydrogels containing MXF-encapsulated liposome nanoparticles from micron-sized microbubbles with uniform pore and size distribution using T-shaped microfluidic method and coating both on a substrate and on IOL haptic,
- Evaluation of the resulting coatings' structural, thermal, and chemical characteristics and obtain MXF-encapsulated liposome-enriched ALG hydrogels for therapeutic drug release over the desired duration

3. EXPERIMENTS

3.1.Introduction

This chapter discusses the production of porous film structures using the T-shaped microfluidic junction device and micropipetting technique. Information regarding the materials and methods employed in their characterization is also provided.

3.2.Materials

The encapsulated drug, moxifloxacin hydrochloride, was acquired from Sanovel Pharmaceuticals Company, Turkey. L- α - Phosphatidylcholine (from soybean, Type IV-S) used for liposome manufacturing. Additionally, polyethyleneglycol-40 stearate (PEG-40S) were purchased from Sigma-Aldrich, USA. In addition, powder sodium alginate was used for hydrogel coating layer, and cross-linking agent calcium chloride dihydrate were purchased from Sigma-Aldrich, USA. Inert gas nitrogen (N_2) was provided from Linde Gas (99.9% purity, Kocaeli, Turkey). Distilled water was filtered using a Millipore device (Arium 611UV, Sartorius, Germany).

3.2.1.Moxifloxacin Hydrochloride

Moxifloxacin hydrochloride (MXF) ophthalmic solution, a broad-spectrum antibiotic belonging to the 8-methoxyfluoroquinolone class, exhibits average to outstanding efficacy against clinically relevant gram-negative ocular infections and improved effectiveness against Streptococci and Staphylococci (**Figure 3.1**) [94].

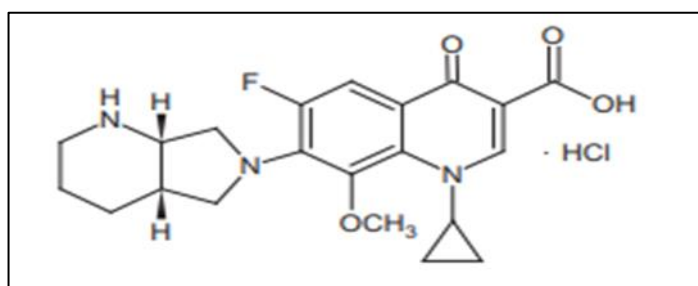


Figure 3.1: Structural Formulation of Moxifloxacin Hydrochloride [94].

MXF, a fourth-generation synthetic fluoroquinolone antibacterial agent, differs from previous fluoroquinolones such as levofloxacin and ciprofloxacin, in having higher activity against Gram -(+) bacteria and anaerobes. Moxifloxacin is commonly used for the treatment and prevention of bacterial conjunctivitis and other external eye infections because of its advantageous pharmacokinetic characteristics and wide antibacterial coverage[95].

3.2.2 Soy Lecithin

"The term 'lecithin' covers a heterogeneous mixture of lipids, including triglycerides, phospholipids - such as phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine - and glycolipids (**Figure 3.2**). In biochemistry field, the term lecithin refers to pristine phosphatidylcholine, a phospholipid derived from the phosphate fraction of various sources, particularly soybeans, sunflower seeds, rice beans and rape seed [96].

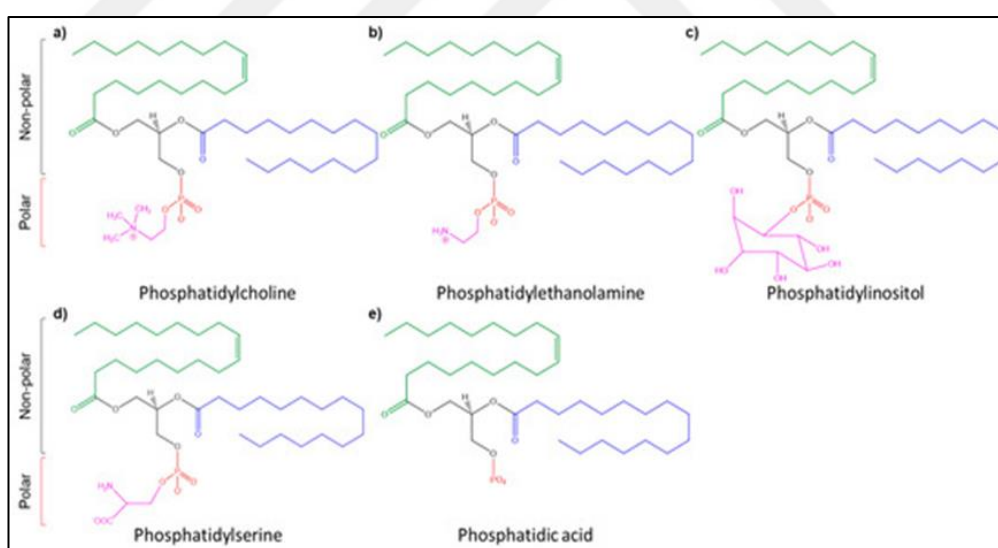


Figure 3.2: Chemical formulation of different phospholipid-derived lecithin [96].

Because of its many uses, lecithin is utilized extensively in the culinary, pharmaceutical, and cosmetic sectors. Moreover, lecithin is a phosphatide found in all living tissues and is a major component of nervous tissue or brain substance. Also

found in lung surfactant and amniotic fluid [97]. Lecithins derived from natural sources (i.e. soybeans, eggs, etc.) are widely utilized in encapsulation and controlled release studies due to their self-assembly behavior, tunable physicochemical properties, and high drug-loading capacity [98]. Their low toxicity and excellent biocompatibility also make them ideal carriers for the delivery of therapeutic agents. Lecithin is a phosphatide found in all living tissues and is a major component of nervous tissue or brain substance. Also found in lung surfactant and amniotic fluid. Lecithin from sources other than soybeans for liposomal formation has several drawbacks, even though natural phospholipids can also be derived from animal sources such egg yolk, milk, and marine sources [99]. Because they include more polyunsaturated fatty acids, soybean lecithin and other plant-derived lecithins are more stable than lipids generated from cows and eggs [100], [101].

Moreover, soybean lecithin is typically regarded as more economical, safer, and more stable to manufacture compared to animal-derived alternatives.

3.2.3. Alginate

Alginates (ALG) are an anionic polymer containing 1,4-linked β -D mannuronic acid (M) and α -L-glucuronic acid (G), sourced from brown algae (**Figure 3.3**). The M and G contents vary according to the extraction source of the alginates[102].

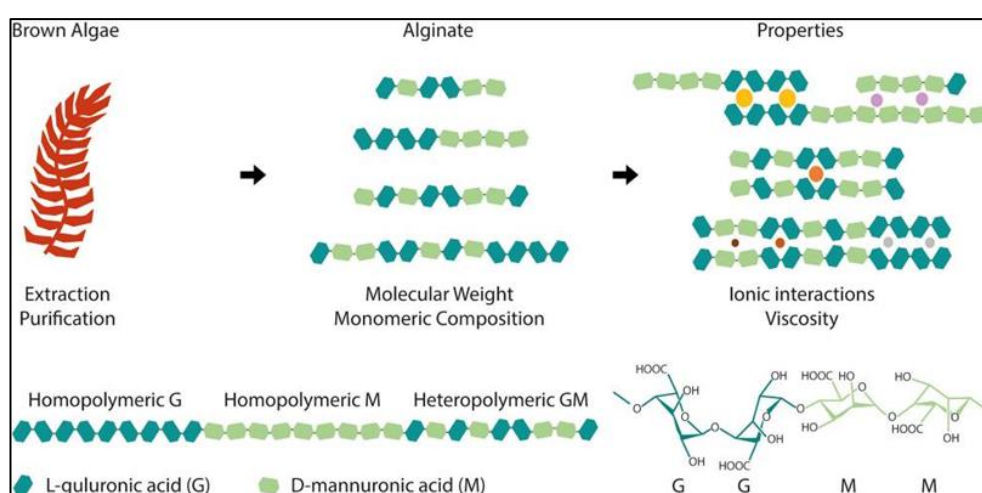


Figure 3.3: Alginate Structure [102].

Because of their strong mucoadhesive qualities, low toxicity, excellent biocompatibility, induced gelation capabilities, and relative affordability, alginates have been extensively studied and used in the great majority of biomedical applications [103]. Regarding biomedical applications, an attractive feature of alginate is its ability to form polymeric three-dimensional hydrogel networks characterized by high water retention. These structures undergo rapid gelation through interaction with multivalent cations (Ca^{2+} , Sr^{2+} , Ba^{2+} , etc.) under physiologically mild and biocompatible conditions [104]. During gel formation, ion exchange occurs between divalent cations and alginate, and the cations bind to the G-blocks of the alginate, forming a 3D network described as an 'egg-box' model. Alginate hydrogels have favorable swelling properties due to the presence of hydrophilic functional groups and form an intercellular matrix-like structure for cell growth.

Due to its capacity to form hydrogels, alginate is widely used as a stabilizer, thickener, gelling agent and emulsifier in various industrial and biomedical applications [105]. The characteristic properties of alginate hydrogels, such as mechanical strength and porosity, depend on several factors including the G/M ratio, the type of ionic cross-linking agent (divalent cations), the concentration and the viscosity of the alginate solution. Alginates with a high G-content tend to form stiffer, more rigid hydrogels, whereas those with a high M-content typically yield softer, more elastic hydrogel networks [106]. The molecular weight of alginate varies between 32,000 to 400,000 g/mol. As the molecular weight of the alginate rises during gel manufacture, it becomes more viscous [107]. The resultant alginate hydrogel matrices serve as the foundation for controlled drug release by forming chemical or physical barriers that prevent drug molecules from diffusing. The tunable nature of these properties of alginate hydrogels enables to achieve drug release with the desired duration and therapeutic efficiency.

3.2.4. Polyoxyethylene-40 stearate

Polyoxyethylene-40 stearate (PEG-40S) is a well-known non-toxic, biocompatible, biodegradable, and non-ionic surfactant that is frequently used in food and medicine applications. PEG-40S is an 18-carbon synthetic polyethylene glycol lipid that is derived from stearic acid [108], [109].

Depending on the degree of adsorption, surfactant molecules reduce the interfacial tension between the liquid and the solid as well as the dynamic surface tension of the liquid at interfaces. The dynamic interfacial tension effect becomes more pronounced at low surfactant concentrations, while the use of surfactants in solutions with a T-shaped device geometry tends to reduce the microbubble diameter [110]. In addition, PEG40S is employed to enhance the stability of the microbubbles.

3.2.5. Calcium Chloride

Calcium chloride was used as a cross-linking agent to obtain cross-linked hydrogel networks. Among divalent cations, calcium chloride (Ca^{2+}) is widely employed for its ability to induce rapid gelation. Its prevalence in biomedical and industrial applications is largely related to its biocompatibility, accessibility, and low cost [111].

3.3. Preparation Liposomal Nanoparticles with /without Moxifloxacin

In this study, the probe sonication method was used to form MXF-encapsulated liposome nanoparticles (Nanografi Nano Teknoloji, Turkey). MXF and SL were mixed at two different ratios (SL:MXF:1:1 and SL:MXF:2:1) to produce the encapsulated liposome nanoparticles.

Initially, 10 mg MXF was added into 10 ml DI water and sonicated to obtain blank liposome nanoparticles for control. Two formulations were prepared by dissolving 10 mg of MXF with either 10 mg or 20 mg of soy lecithin added in DI water with 10 mL, corresponding to SL:MXF ratios of 1:1 and 2:1, respectively. The resulting mixtures were encapsulated using ultrasonic force (**Figure 3.4**).

The processing parameters for probe sonication in the preparation of liposomal nanoparticles were optimised by varying the sonication time in 2-minute intervals from 2 to 10 minutes, and by applying different amplitude settings (20%, 40% and 60%) (**Table 3.1**).

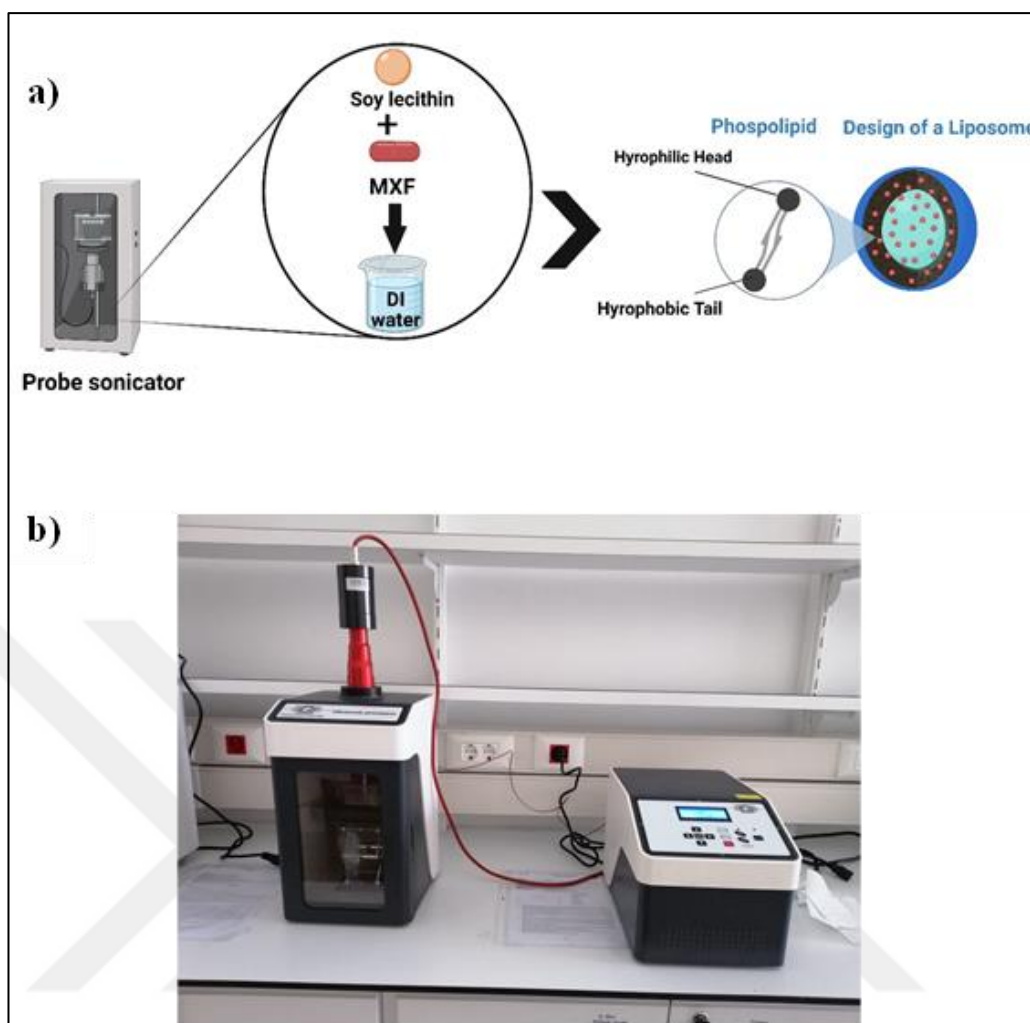


Figure 3.4: **a)** Schematization of liposome nanoparticle production; **b)** Probe Sonication device.

Table 3.1: Liposome formulations prepared with different parameters and designated labels.

Amplitude (%)	Sonication Time (Min)				
	2	4	6	8	10
20	L1	L2	L3	L4	L5
40	L6	L7	L8	L9	L10
60	L11	L12	L13	L14	L15

The optimisation of sonication parameters, including time and amplitude, prevented excessive heating and maintained the stability of the drug. Furthermore, to maintain the temperature under control, the beaker with the solution was placed in a glass

container and surrounded by ice cubes. To increase the cooling effect, a cool pack was positioned beneath the glass container (**Figure 3.5**).

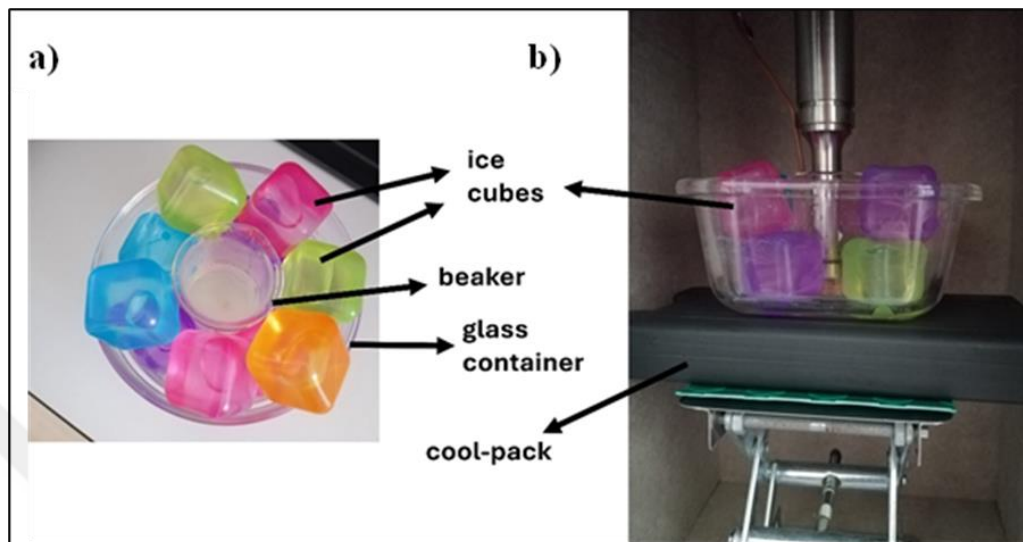


Figure 3.5: Placing beaker into the probe sonicator; **a)** top view, **b)** opposite view.



Figure 3.6: Lyophilizer device (freeze-drier).

Following synthesis process, the liposome nanoparticles were freeze-dried (at $-55\text{ }^{\circ}\text{C}$, 1 mbar, Christ, Alpha 1-2 LD Plus) for further characterization (**Figure 3.6**). **Figure**

3.7 illustrates the liposome nanoparticle production with different concentrations via probe sonicator with varying amplitude and sonication time parameters.

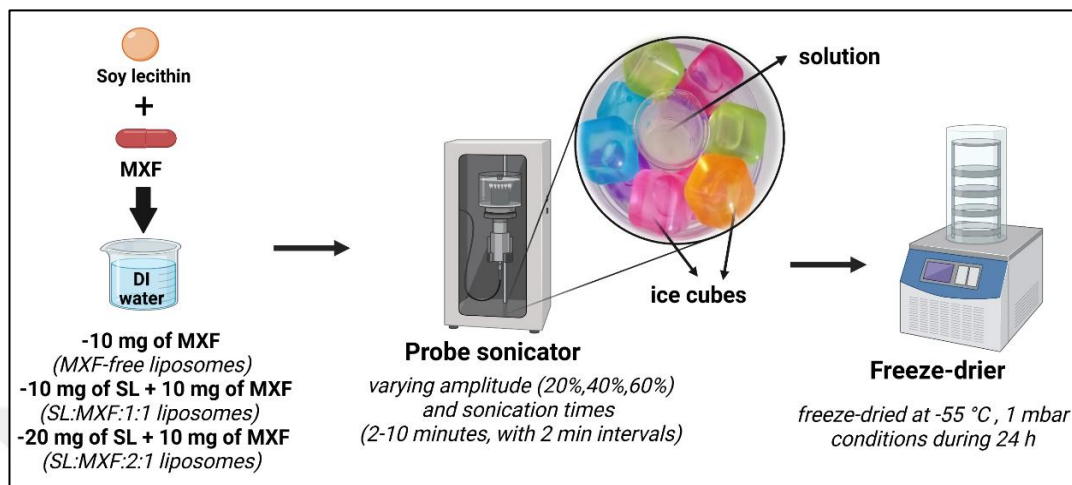


Figure 3.7: Schematic representaion of liposome nanoparticle production workflow.

3.3.1. Investigation of Moxifloxacin-free and Moxifloxacin-Encapsulated Liposome Nanoparticles

Prior to their incorporation into the hydrogel system, analyses of MXF-free and MXF-encapsulated liposome nanoparticles were performed. The steps involved in the characterization of the relevant liposomes are detailed below.

3.3.1.1. Dynamic Light Scattering

When the sample is exposed to a laser beam, variations in the intensity of the scattered light at a specified scattering angle (θ) are detected using a high-speed photon detector. By measuring the spontaneous changes in scattered light intensity from a suspension or solution, the particle size can be determined. The mean size of liposomes formulated at SL:MXF:1:1 and SL:MXF:2:1 ratios was measured using a dynamic light scattering (DLS) device (Zetasizer, Nano series, Malvern Instruments Limited), (**Figure 3.8a**) based on the production parameters listed in **Table 3.1**.



Figure 3.8: a) Zetasizer and, b) measuring cells (quartz cuvette) used for determining particle size.

3.3.1.2. Zeta Potential Measurement

The zeta potential is used to investigate the interactions between colloidal electrolytes. The fundamental principle is that particles with oppositely charged surfaces are attracted, while charged particles are repelled. Each particle moves through the solution together with the ions that are closest to it. The zeta potential is the potential at the shear surface between this unit and the surrounding medium. The surface charges of liposomes formulated at SL:MXF:1:1 and SL:MXF:2:1 ratios was measured using Zetasizer, based on the production parameters listed in Table 3.1. The zeta-potential values of liposomes formulated at SL:MXF:1:1 and SL:MXF:2:1 ratios was measured using Zetasizer device (Nano series, Malvern Instruments Limited), (**Figure 3.8a**), but with different software and measuring cells (**Figure 3.9**).



Figure 3.9: Folded capillary zeta cell.

3.3.1.3. Encapsulation Efficiency

Encapsulation efficiency (EE) indicates the proportion of the drug entrapped within the liposomal formulation. The concentration of MXF in DI water was determined at 290 nm using a UV–VIS spectrophotometer, and analysed quantitatively by comparison with the calibration curve. The encapsulation efficiencies of liposomes were calculated using equation 3.1.

$$EE(\%) = \left[\frac{\text{Total amount of determined MXF in the liposome}}{\text{Initial amount of MXF loading}} \right] \times 100 \quad (3.1)$$

3.3.1.4. Fourier Transform Infrared Spectroscopy

The basic idea is that, depending on the bond type and the molecular environment, light is absorbed at specific frequencies by chemical bonds between various elements. Fourier Transform Infrared Spectroscopy (FTIR) analysis (PerkinElmer Spectrum One

series FT-IR instrument, Version 5.0.1) (**Figure 3.10**) was conducted to assess the chemical structure of starting materials and for all liposomes (**Table 3.1**).



Figure 3.10: Fourier Transform Infrared Spectrometer.

3.3.1.5. Scanning Electron Microscopy

Scanning Electron Microscope (SEM) (**Figure 3.11a**), equipped with an electron gun located at the top of device, typically produce images with using focused beam of electrons. The basic working principle involves the emission of electrons from a metal and accelerated them a strong electric field (**Figure 3.11b**). Before starting analysis, all samples were coated with gold to prevent charging and make them electrically conductive.

In our experiments, SEM (PHILIPS XL 30 SFEG, with at an accelerating voltage of 5 kV) was used to characterize the surface morphology of materials and liposomes.

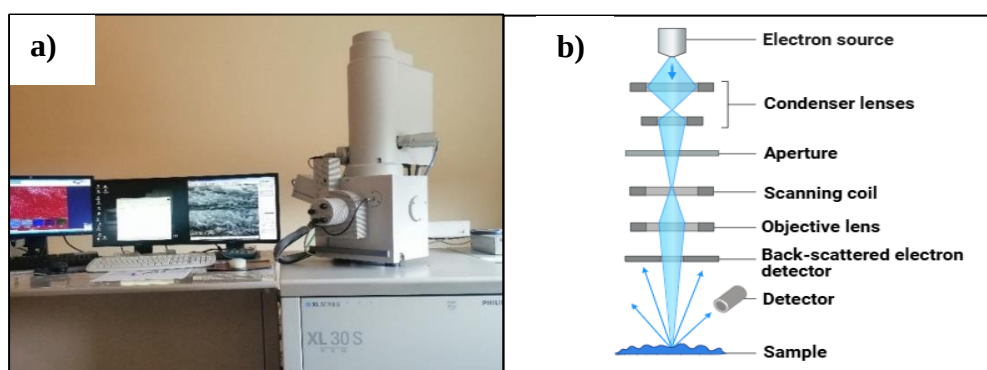


Figure 3.11: a) Scanning electron microscope and, b) working diagram of scanning electron microscope.

3.3.1.6. Contact Angle Measurement

The contact angle is a parameter used to quantify the wetting behaviour of solid-liquid interaction. The interaction between a liquid and an ideal solid surface is often used to calculate the contact angle. Atomic-level smoothness, chemical homogeneity, chemical inertness, and resistance to liquid deformation are characteristics of an ideal solid surface. High surface energy, which suggests hydrophilic properties and high wettability, is shown by a low contact angle (**Figure 3.12**).

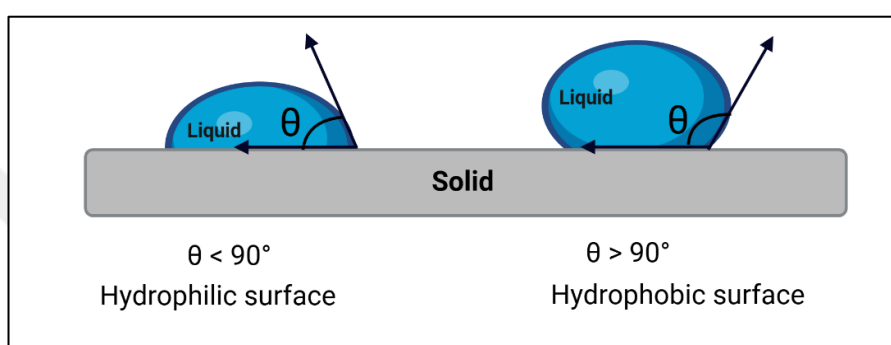


Figure 3.12: A drop of water on an ideal solid substrate.

In our experiments, in order to assess the hydrophobicity of the samples, contact angle measurements were conducted using optical tensiometer and Attension Theta Contact Angle Meter (**Figure 3.13**).



Figure 3.13: Illustration of Contact Angle Meter.

3.3.1.7. Fluorescence/Polarized Microscopy Imaging

Fluorescent/polarized microscopy observations were conducted to track the encapsulated drugs to provide an enhanced understanding of liposomes. The particle size distribution and captured MXF-encapsulated liposome nanoparticles are shown below. Moreover, particle size distribution analysis was also performed with using Image J software. Fluorescence/polarized microscopy analysis were conducted to confirm particle size and drug encapsulation with using Leica CTR600 (**Figure 3.14**).



Figure 3.14: Fluorescent/Polarized Microscope.

3.4.Preparation of Polymer and Crosslinking Solutions

Polymeric solution were prepared by dissolving pharmaceutical-grade powder sodium alginate (%0.5-5 w/v) in deionized water by stirring with a magnetic stirrer. Then, the surfactant polyethene glycol stearate (PEG40S) (0,25%) was added to the sodium alginate solution and stirred for about 1 hour to dissolve completely. Lastly, MXF-encapsulated liposomes (0.1–0.5% w/v) were added to the solution and stired until a

homogeneous solution was obtained. CaCl_2 crosslinker solution for gelling process prepared with deionised water at 1-2% (w/v) concentration (**Figure 3.15**). Table 3.2 shows the prepared polymeric solutions at different concentrations.

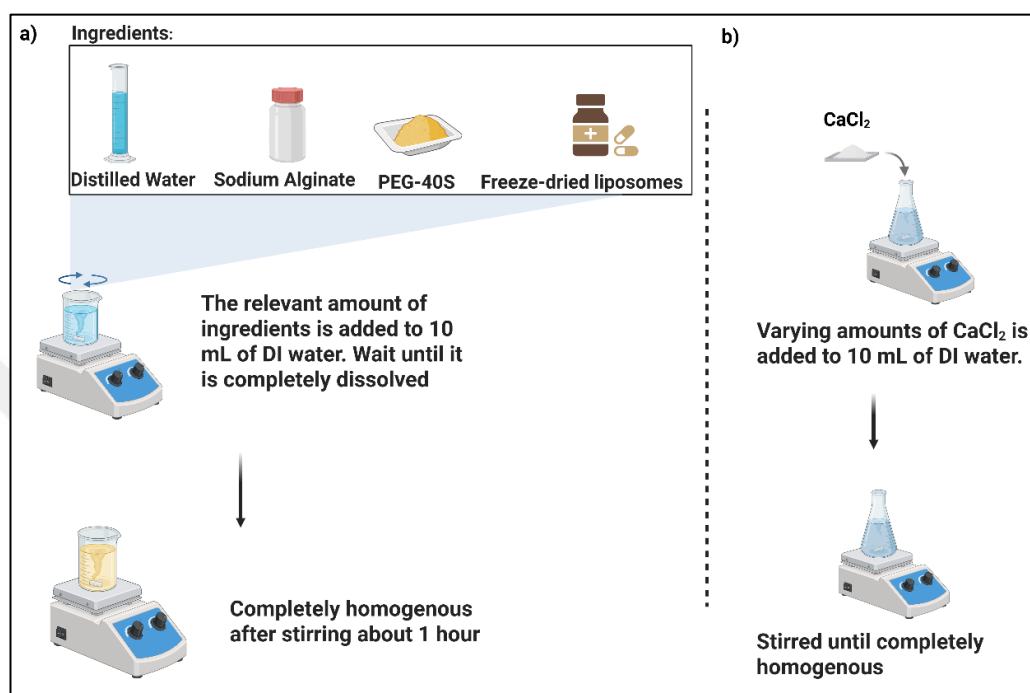


Figure 3.15: Workflows of **a)** polymer solution and, **b)** crosslinking solution preparations.

Table 3.2: Polymer solution concentrations for hydrogel production

Solution	Alginate Concentration (%w/v)	PEG40S Concentration (%w/v)	MXF-encapsulated liposome concentration (w/v)
F1	1	0,25	1
F2	1	0,50	1
F3	1	0,75	1
F4	2	0,25	1
F5	2	0,50	1
F6	2	0,75	1

3.5. Characterization of Solutions

3.5.1. Density

The density (mass per unit volume, kg.m^{-3}) of liquids can be determined by using a conical-shaped device called pycnometer (a 25 ml standard bottle). Pycnometer bottles are generally constructed from glass and are equipped with a ground glass stopper featuring a capillary tube designed to release air bubbles (**Figure 3.16**). It uses a precision balance to measure the density of a substance in relation to a working liquid, commonly water or mercury.



Figure 3.16: Schematic illustration of pycnometer.

Weighing the empty flask is the first step in the process. After that, it is weighed once with the reference liquid and once more with the testing liquid. Using the formula below, these weights are used to determine the liquid's relative density

$$(W_2 - W_0)/\rho \quad (3.2)$$

where W_2 is the mass of pycnometer with liquid only and W_0 is the mass of pycnometer only, while stands for ρ the density.

Under standard ambient temperature and pressure (SATP) conditions, three consecutive measurements were conducted, and the average values were taken to determine the densities. The densities of alginate polymer solutions (given in Table 3.2) were investigated with a pycnometer (Pycnometer Density Bottle Meter, ISOLAB, Germany).

3.5.2. Viscosity

Viscosity can be described as the resistance to flow or internal friction that a fluid exhibits when a force is applied to it. Higher viscosity fluids, like syrup, deform less readily than lower viscosity fluids, like water. This is a fluid-specific feature.

The viscosity of alginate polymer solutions (given in Table 3.2) were measured at 23°C by tuning fork method (**Figure 3.17**). The procedure involves immersing the plates in a 40 mL dispersion cuvette (model SV-10, Vibro Viscometer, A&D, Japan). All the measurements were triplicate.



Figure 3.17: Experimental setup for measuring viscosity.

3.5.3.Surface Tension

Surface tension, a unique interfacial phenomenon that allows a liquid to behave like an elastic material, is caused by the cohesive attraction between molecules at the liquid's surface. It is denoted by γ and the unit is N/m.

The surface tension of the 1% (w/v) alginate polymer solutions were measured with using contact angle device (Theta Contact Angle Meter, Attension, Biolin Scientific, Sweden), offering a sensitivity of ± 0.1 mN/m (**Figure 3.18**). The device's needle and the pendant drop method were used to measure surface tension at 25 °C. Based on the drop profile at the point of detachment, the surface tension was evaluated.

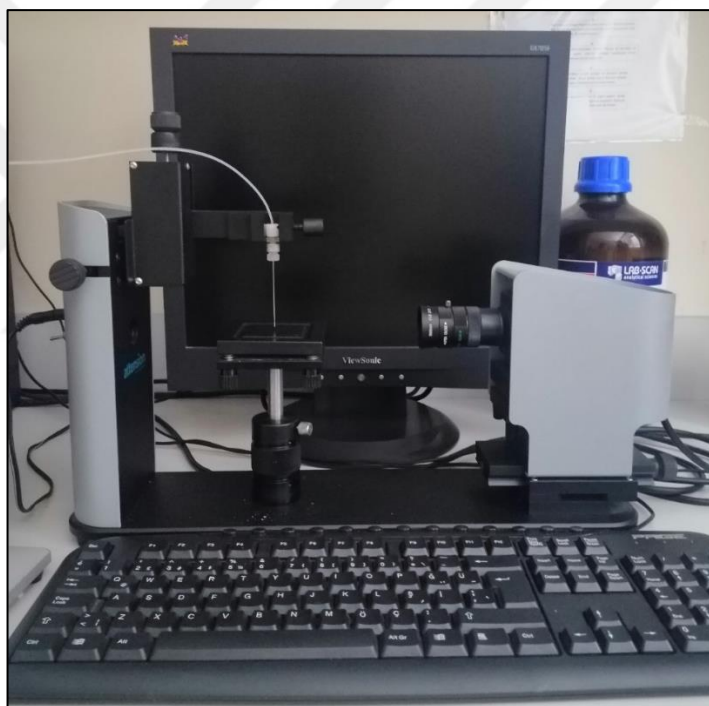


Figure 3.18: Contact Angle Meter

3.6.Preparation of Moxifloxacin Encapsulated Liposome Enriched Alginate Hydrogel Coatings

The probe sonication approach was used to sonicate soy lecithin and moxifloxacin in order to encapsulate the drug through liposome nanoparticles. The final solutions were lyophilized for make them suitable to characterization. The MXF-encapsulated

liposomes were then added to the alginate polymer solution and coated onto polymeric substrates and IOL haptics using two different methods: the TMJ device approach and the micropipetting technique. To confirm whether the hydrogel coating layers were properly produced, the resulting hydrogel coating constructions were characterized.

3.6.1. Conventional Method

The MXF-free and MXF-encapsulated, liposome-enriched alginate nanocomposite hydrogel solution is dropped onto suitable substrate material and IOL haptics in the traditional micropipetting method for developing alginate-based hydrogel coatings (**Figure 3.19**). Cross-linking was then done using a 1-2% (w/v) calcium chloride solution. The final nanocomposite hydrogel products were lyophilized with a freeze-drier (at -55°C , 1 mbar, Christ, Alpha 1-2 LD Plus, Germany).

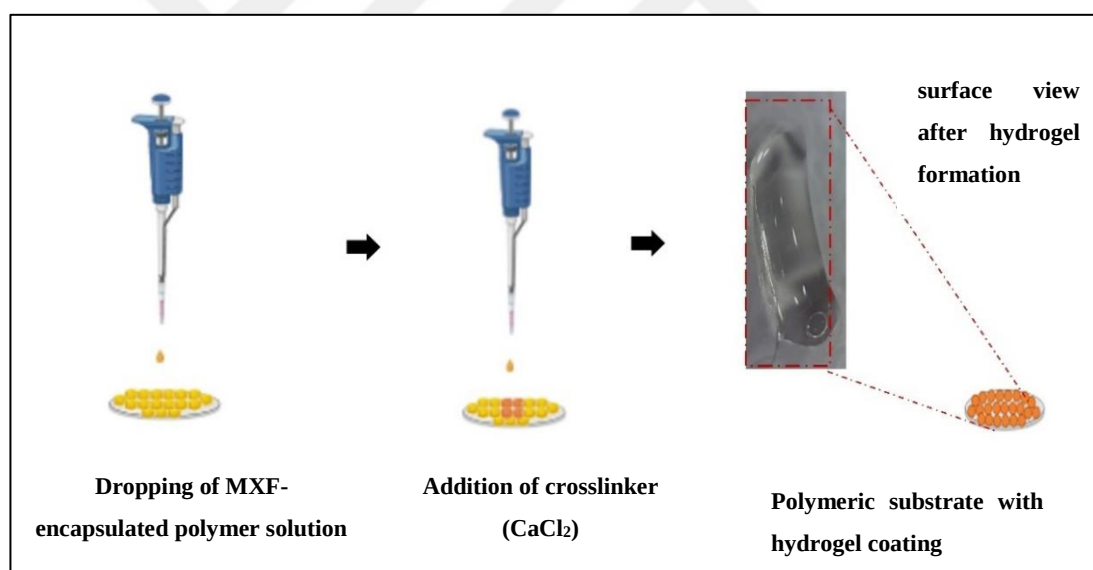


Figure 3.189: Hyrogel production with micropipetting technique.

3.6.2. T-shaped Microfluidic Junction Device

A transparent polymer, polymethyl methacrylate (PMMA) was employed for construction of T-shaped microfluidic junction device system by the CNC (Computer Numerical Control). Thin Teflon capillaries with a 200 μm inner diameter, a 15 cm inlet, and a 5 cm outlet line and anti-leakage apparatus were attached to the TMJ

system (**Figure 3.20**). Monodisperse microbubbles are produced by attaching the inert gas, nitrogen (N₂), feeding capillary vertically and controlling it with a digital flow meter. First, high-precision syringes (BD) with a 10 mL capacity attached to the syringe pump were horizontally positioned to attach the polymer solution to the system (IPS Series, Inovenso Pump Systems, Istanbul, Türkiye). The polymer solution was prepared by dissolving sodium alginate at concentrations ranging from 0.5 to 5% (w/v), polyethylene glycol (PEG) at 0.25% (w/v), and lyophilized MXF-loaded liposomes at concentrations between 0.1 and 0.5% (w/v) in 10 mL of deionized water.. After that, the mixture was vigorously stirred until a homogeneous solution was obtained. The polymeric solution added to thin Teflon tubes at various liquid flow rates, ranging from 50 – 300 $\mu\text{L}/\text{min}$. Concurrently, high-purity nitrogen gas introduced to the system at variable pressure ranges between 0.3 and 0.8 bar, which will be managed by a flow meter[112].

The formation of microbubbles at the junction of the channels arises from the different interfacial tensions of the liquid and gas phases introduced into the system through different channels. At the interface where the two phases meet, these changes in interfacial tension assist in the formation and stability of microbubbles. Microbubbles accumulated in the outflow channel without mixing with each other were dripped onto the IOL haptic and polymeric substrates at a distance of approximately 1 cm and deposited as a single, uniform layer. Cross-linking was then performed using a 1-2% (w/v) calcium chloride solution. The samples were kept at a temperature of +4 °C for one hour and rinsed three times with distilled water to remove any residual calcium chloride ions. To facilitate degassing of the hydrogel, it was placed in an oven at 30 mbar pressure during one hour. Promoting the development of uniform porous structures connected by internal channels is the aim of the TMJ device method. The final products of hydrogel coatings were lyophilized.

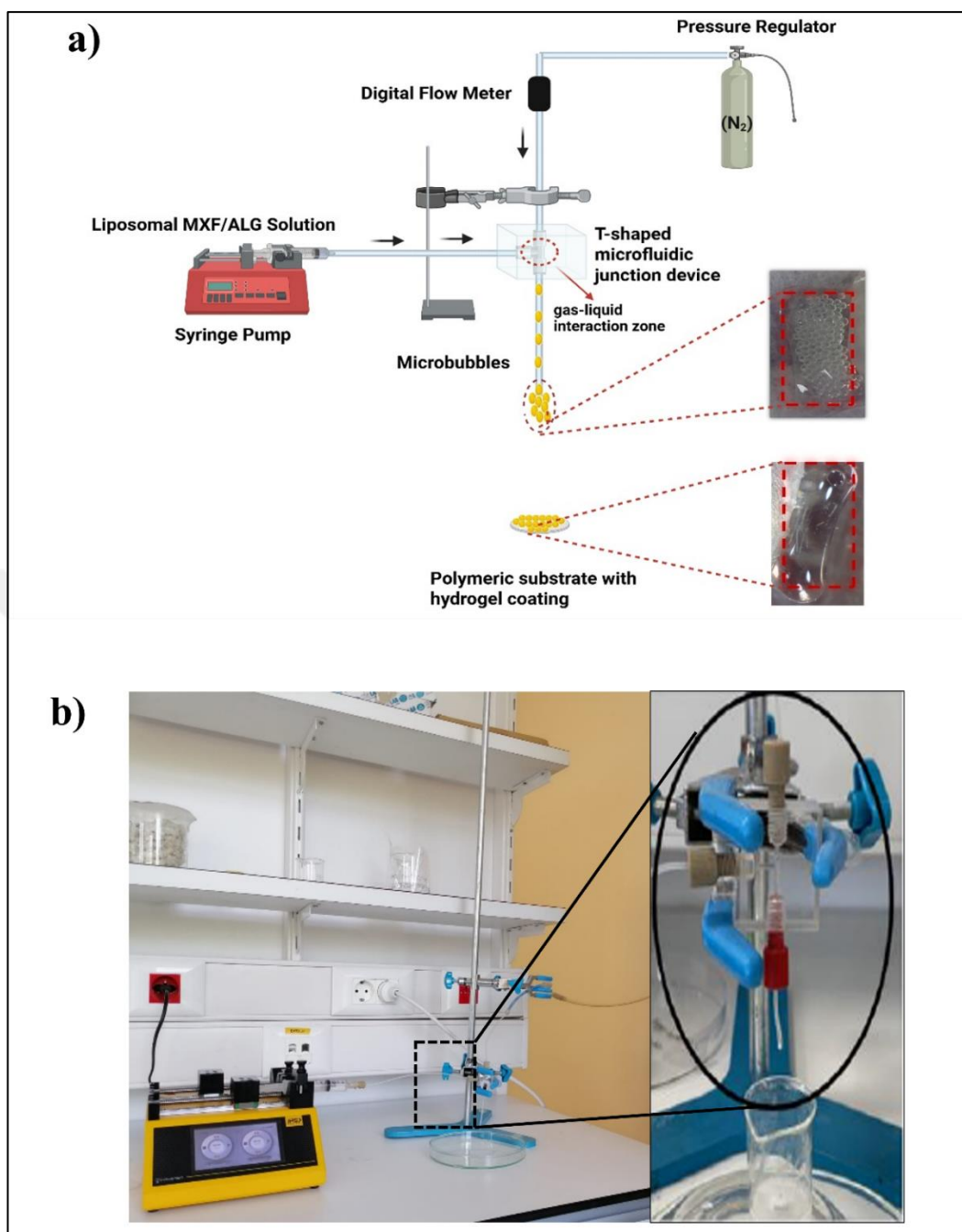


Figure 3.20: a) Schematic drawing showing the design and operating principle of the TMJ device, **b)** image of the system assembled for experimental use.

3.7. Characterization of Microbubbles and Nanocomposite Hydrogel Structures

3.7.1. Optical Microscopy Investigation

An optical microscope (Zeiss Imager M1m, Germany) was extensively used for determining morphological structures of microbubbles produced by TMJ device (**Figure 3.21**). Polymer solutions of different concentrations were deposited on glass slides under varying parameters, such as flow rate and gas pressure. Optical microscope images taken at 5× and 10× magnifications were then examined using ImageJ software to examine the effect of the varying parameters on bubble size.



Figure 3.21: Image of the optical microscope employed for microbubble imaging.

3.7.2. Determining the Pore Size and Pore Size Distribution of Microbubbles

The diameters and size distribution of the microbubbles produced by the TMJ device were determined from images captured using an optical microscope (Zeiss Imager

M1m,Germany). Micrographs were taken at different magnifications, and the diameters of the microbubbles were measured using the ImageJ software. The polydispersity index of microbubbles (PDI) was calculated using equation 3.3.

$$PDI(\%) = \sigma / d_{ave} \times 100 \quad (3.3)$$

PDI: polydispersity index

σ : standard deviation of the mean microbubble diameter

d_{ave} : mean microbubble diameter

3.7.3. Fourier Transform Infrared Spectroscopy Analysis

The interaction between the chemical compounds in the MXF-encapsulated liposome-enriched alginate nanocomposite hydrogels was analysed by using FTIR instrument with a scanning range of 4000 - 400 cm^{-1} .

3.7.4. Scanning Electron Microscopy

The morphology of the MXF-encapsulated liposome-enriched alginate nanocomposite hydrogels was examined by SEM to investigate their morphological features. To ensure an accurate assessment, an average of five images were taken for every sample at various magnifications. Energy Dispersive Spectroscopy (EDS) microanalysis was used to further examine the elemental composition by measuring the energy and intensity distributions of the X-ray signals produced by a focussed electron beam on the sample. The elemental mapping of the resulting samples was determined using the EDS to identify the spatial distribution of significant elements within the hydrogel coatings.

3.7.5. Swelling Ratio

The swelling behavior of alginate nanocomposite hydrogels enriched with either MXF-free or MXF-encapsulated liposomes was evaluated using standard gravimetric techniques. The preweighted, dried nanocomposites (W_d) were then submerged in 10

mL of deionised water at room temperature for 120 minutes. At designated time intervals, the samples were removed and carefully blotted with filter paper to remove any residual surface moisture. The swollen weight (W_s) was then calculated by reweighing them. All measurements were conducted in triplicate to ensure accuracy. Next, the following formula was used to determine the swelling index of nanocomposites:

$$\text{Swelling Index (\%)} = \frac{W_s - W_d}{W_d} \times 100 \quad (3.4)$$

3.7.6. Mechanical Properties

The mechanical characteristics of MXF-free and MXF-encapsulated liposome-enriched hydrogels were examined using an mechanical universal tester (Instron 3382, Universal Testing Systems, USA) at ambient temperature (**Figure 3.22**). All hydrogel sample were adjusted to measure 12 mm of length, 4 mm of width, and 1.2 mm of thickness, and were then attached to crossheads using thin rods. Mechanical testing was performed in position control mode while arranging 0.1 mm/s to crosshead speed. The mechanical characteristics of the samples were then determined by converting the force-displacement data into stress-strain data. All measurements were conducted in triplicate to secure accuracy, subsequently, the average and standard deviation were computed for each set of measurements.



Figure 3.192: Mechanical Universal Tester.

3.7.7. In vitro Drug Release Performance

Both MXF-encapsulated liposomes and MXF-encapsulated liposome-enriched alginate nanocomposite hydrogels were produced by i) micropipetting and ii) TMJ device were placed in separate beakers containing 10 mL of DI water. Then, the beakers were submerged in a water bath at 37 ± 0.5 °C to mimic the physiological conditions. Aluminum foil is used to prevent possible evaporation and to preserve the water bath (**Figure 3.23**). At predetermined times, aliquots of 2 mL were taken from the beakers and immediately replaced with the equal volume (2 mL) of the fresh DI water to maintain the equilibrium conditions. Collected samples were filtered to remove any residues and analyzed via UV spectrophotometer at λ_{max} of 290 nm for the quantitative analysis (**Figure 3.24**).

The absorbance values reflected the concentration of moxifloxacin in each sample. The total cumulative drug concentration released over time was calculated using the mean values of the triplicate experiments. The cumulative concentration of MXF released at each time point was determined using the obtained absorbance values and a standard calibration curve. This approach enabled the preparation of a release profile for the MXF-encapsulated liposomes and the liposome-enriched alginate nanocomposite hydrogels.

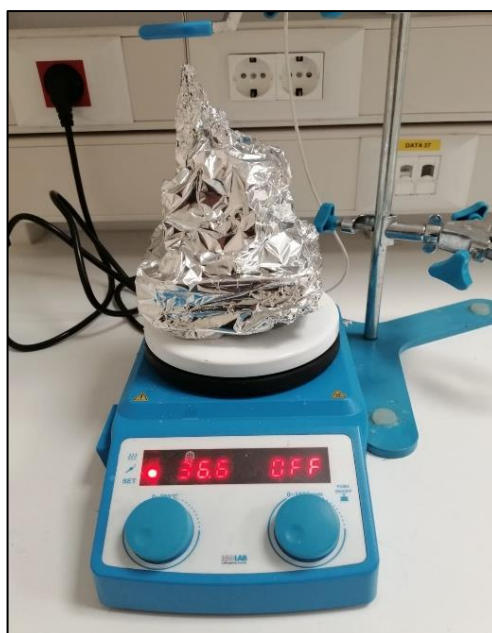


Figure 3.203: Experimental setup for drug release studies.

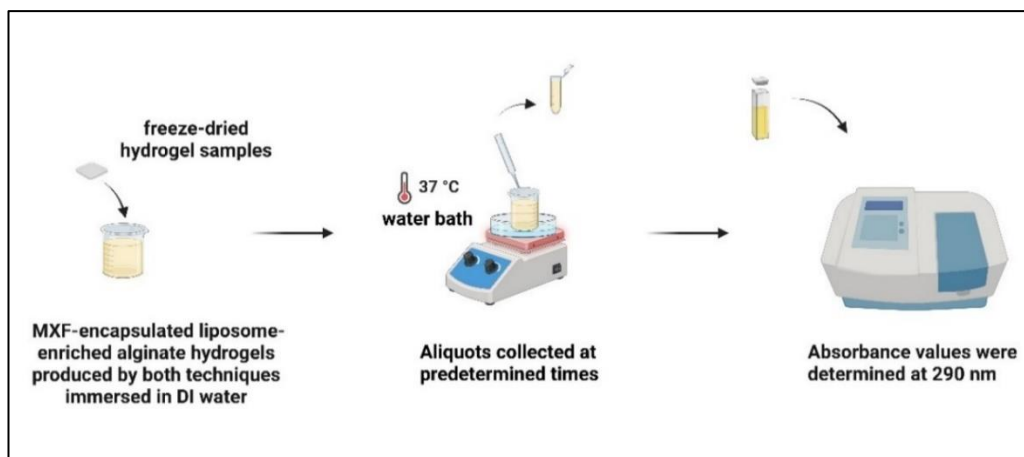


Figure 3.24: Schematical abstract for drug release studies procedure.

3.7.7.1. Evaluating Drug Release Profiles

Drug release behaviours for all formulations were examined to understand the dissolution profile, employing several kinetic models such as first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell model.

The first-order kinetic model is particularly helpful for formulations that incorporate water-soluble medicines in porous matrices because it depicts drug release from pharmaceutical systems where the release rate is concentration-dependent. The drug release behaviour in the first-order model can be shown by the following equation:

$$\log C = \log C_0 - \frac{K_1 t}{2.303} \quad (3.5)$$

where K_1 is the first-order rate constant (time^{-1}), C_0 is the first concentration of the drug, and C is the percent of drug remaining at time t .

Higuchi model, drug diffusion only takes place in one dimension and the initial drug concentration in the matrix is significantly higher than the drug's solubility. According to the Higuchi model can be described with using the equation 3.6:

$$\frac{M_t}{M_\infty} = K_h t^{1/2} \quad (3.6)$$

where M_t/M_∞ corresponds to the release percentage of the drug measured at each time interval (t), M_t denotes the quantity of drug released at a given time t, M_∞ is the amount of drug released after time ∞ , and K_h stands for the Higuchi release constant.

The equation for Korsmeyer–Peppas model is given below:

$$M_t/M_\infty = K_{kp} t^n \quad (3.7)$$

$$\log(M_t/M_\infty) = \log K_{kp} + n \log t \quad (3.8)$$

Here, M_t/M_∞ is a proportion of drug released at time t, M_t represents the amount of drug released as a function of time t, M_∞ is the measured release of the drug at time t ∞ , n is the drug release exponent, and K_{kp} is the Korsmeyer release rate constant[113].

The Hixson–Crowell model can be defined as follows:

$$W_0^{1/3} - W^{1/3} = k_4 t \quad (3.9)$$

where W_0 is the initial amount of substance, W is the amount of undissolved substance at t, k_4 is stands for the specific release constant, where t is the time [114].

4. RESULTS

This section first provides characterisation results on both MXF-free and MXF-loaded liposomes. This is followed by an evaluation of the polymer solution used for hydrogel preparation. Finally, it presents the results of the characterisation of the MXF-encapsulated liposome enriched alginate nanocomposite hydrogels produced using the micropipetting technique and TMJ device method. A comparative analysis of these resultant constructs is also provided.

4.1 Freeze-Dried Liposome Characterization

This section shows the analysis results for MXF-free liposomes, as well as SL:MXF:1:1 and SL:MXF:2:1 liposomes. **Figure 4.1** depicts free-dried liposomes. Dynamic light scattering (DLS), polydispersity index (PDI), encapsulation efficiency (EE) and zeta-potential (ζ) values for all liposome groups are given as follows:



Figure 4.1: Freeze-dried liposome nanoparticles.

4.1.1. Dynamic Light Scattering

Figure 4.2 represents the results for MXF-free liposome nanoparticles produced by probe sonication with various amplitude and duration. The particle size ranges from 151 to 200.9 nm.

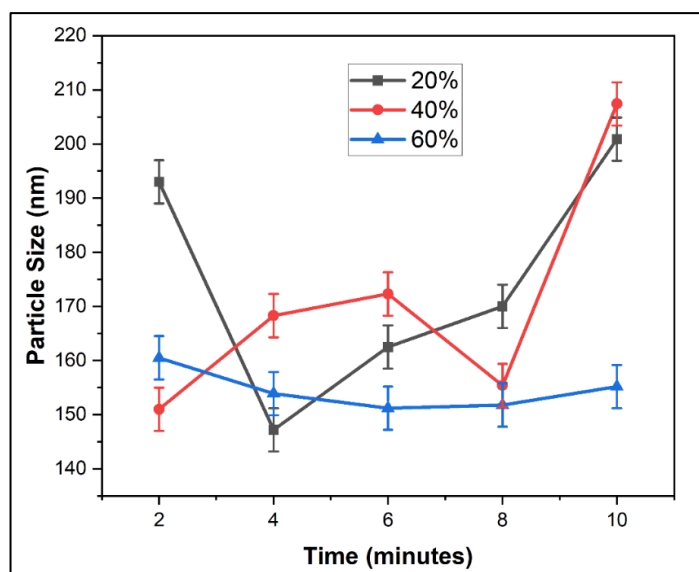


Figure 4.2: Particle size values for MXF-free liposomes.

Figure 4.3 represents the results for liposome nanoparticles formulated in SL:MXF:1:1 ratio produced by probe sonication. Particle size values of liposome nanoparticles ranges from 127 nm to 224 nm for fifteen liposomes. According to the literature, desired particle size for drug delivery applications has been reported between 50 nm and 200 nm in size[115]. In general, it can be concluded that increasing the sonication time reduces the particle size while sonication time increased from 2 minutes to 10 minutes. The results show that the particle sizes of the liposome nanoparticles obtained using the probe sonicator are appropriate for use in ocular drug delivery treatments.

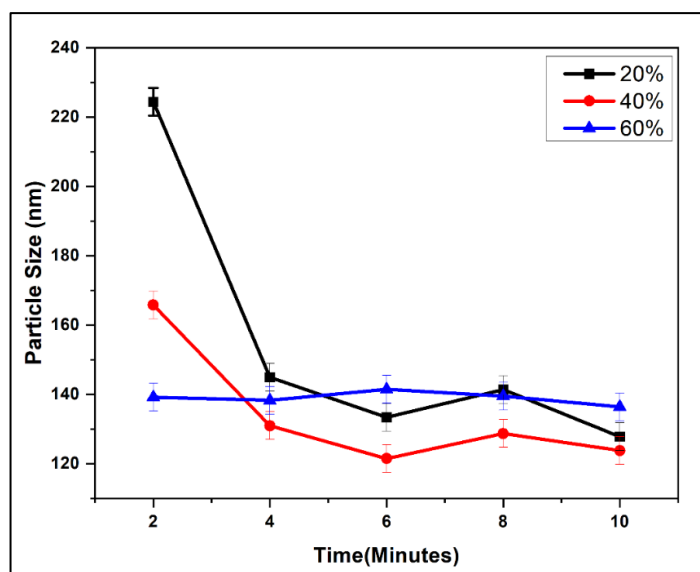


Figure 4.3: Particle size values for liposome nanoparticles formulated in SL:MXF:1:1 ratio.

Figure 4.4 represents the results for liposome nanoparticles formulated in SL:MXF:2:1 ratio produced by probe sonication. Particle size values of liposome nanoparticles ranges from 150 nm to 186 nm for fifteen liposomes. A decrease in particle size was observed as the sonication time varied from 2 minutes to 10 minutes, provided that the amplitude value remained constant. The results show that the particle sizes of the liposome nanoparticles obtained using the probe sonicator are appropriate for use in ocular drug delivery treatments.

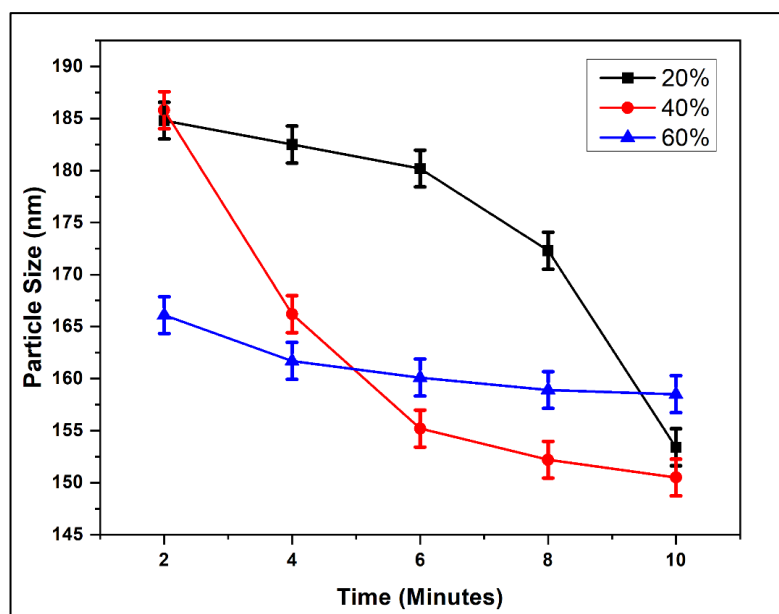


Figure 4.4: Particle size values for liposome nanoparticles formulated in SL:MXF:2:1 ratio.

4.1.2. Polydispersity Index

The PDI values belong to MXF-free, as well as SL:MXF:1:1 and SL:MXF:2:1 liposome nanoparticles are given below.

Table 4.1 shows PDI values of MXF-free liposome nanoparticles produced by probe sonicator. PDI values between 0 and 1 indicate that the size distribution of liposome nanoparticles is within the range accepted in the literature. This suggests that the drug-free liposome nanoparticles produced as the control group have suitable properties.

Table 4.1: PDI values for MXF-free liposome nanoparticles.

Amplitude (%)	Sonication Time (mins)				
	2	4	6	8	10
20 %	0.382 ± 0.028	0.235 ± 0.007	0.193 ± 0.014	0.256 ± 0.025	0.288 ± 0.022
40 %	0.179 ± 0.018	0.227 ± 0.007	0.327 ± 0.027	0.223 ± 0.016	0.310 ± 0.036
60 %	0.222 ± 0.016	0.247 ± 0.021	0.220 ± 0.019	0.229 ± 0.009	0.231 ± 0.015

Table 4.2 shows the PDI values for fifteen liposome nanoparticles formulated in SL:MXF:1:1 ratio. As can be seen from the table, the PDI values of the related liposome nanoparticles vary between 0 and 1. The PDI value for liposome nanoparticles is an important factor that affects the product's bulk properties, stability, performance, and appearance[116]. The particle sizes falling within the specified value range means uniformity among the particles. A narrow size distribution is indicated by the values being near 0. As a result, every liposome nanoparticle that is created might be able to contain a comparable quantity of drug.

Table 4.2: PDI values for liposome nanoparticles formulated in SL:MXF:1:1.

Amplitude (%)	Sonication Time (mins)				
	2	4	6	8	10
20 %	0.532 ± 0.030	0.466 ± 0.063	0.419 ± 0.002	0.393 ± 0.009	0.414 ± 0.026
40 %	0.413 ± 0.015	0.296 ± 0.015	0.368 ± 0.047	0.386 ± 0.035	0.470 ± 0.012
60 %	0.446 ± 0.020	0.414 ± 0.028	0.322 ± 0.030	0.532 ± 0.015	0.419 ± 0.018

Table 4.3 shows the PDI values for fifteen liposome nanoparticles formulated in SL:MXF:2:1 ratio. The PDI values of the related liposomes vary between 0 and 1 similar to SL:MXF:1:1 ratio liposome nanoparticles. Although the PDI values of liposome nanoparticles formulated in SL:MXF:1:1 ratio is closer to 1 compared to those of SL:MXF:2:1 liposomes, they show a narrow distribution since they are in the value range between 0 and 1.

Table 4.3: PDI values for liposome nanoparticles formulated in SL:MXF:2:1.

Amplitude (%)	Sonication Time (mins)				
	2	4	6	8	10
20 %	0.367 ± 0.028	0.363 ± 0.021	0.379 ± 0.038	0.358 ± 0.052	0.473 ± 0.031
40 %	0.367 ± 0.048	0.462 ± 0.007	0.345 ± 0.018	0.360 ± 0.041	0.426 ± 0.029
60 %	0.420 ± 0.012	0.372 ± 0.021	0.409 ± 0.027	0.327 ± 0.052	0.412 ± 0.007

4.1.3. Zeta-Potential (ζ) Values

Figure 4.5 represents the results for MXF-free liposome nanoparticles produced by probe sonication with various amplitude and duration. The zeta potential values of MXF-free liposome nanoparticles ranging from -37.2 to -56.6 mV, indicating good colloidal stability.

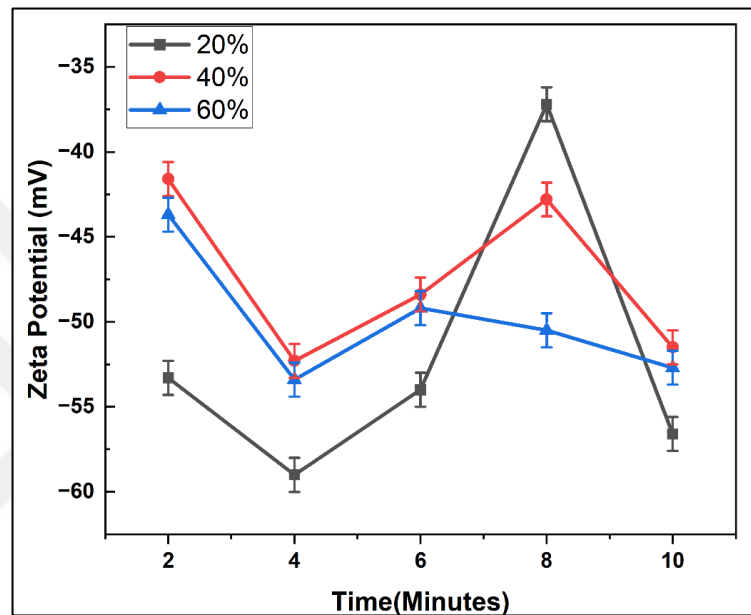


Figure 4.5: Zeta potential values for MXF-values for MXF-free liposome nanoparticles.

Figure 4.6 shows the zeta potential results for liposome nanoparticles formulated in SL:MXF:1:1 ratio produced by probe sonication. Zeta potential values that are considered bigger than +30 mV or lower than -30 mV are regarded as highly stable due to the repulsive force, creates physical colloidal stability (against coalescence)[117]. The surface charge values for the 15 various liposome nanoparticle formulations change from -21,9 mV to -32,8mV. The zeta potentials of liposome nanoparticles formulated in the SL:MXF:1:1 ratio does not seem to have sufficient repulsive force to ensure colloidal stability.

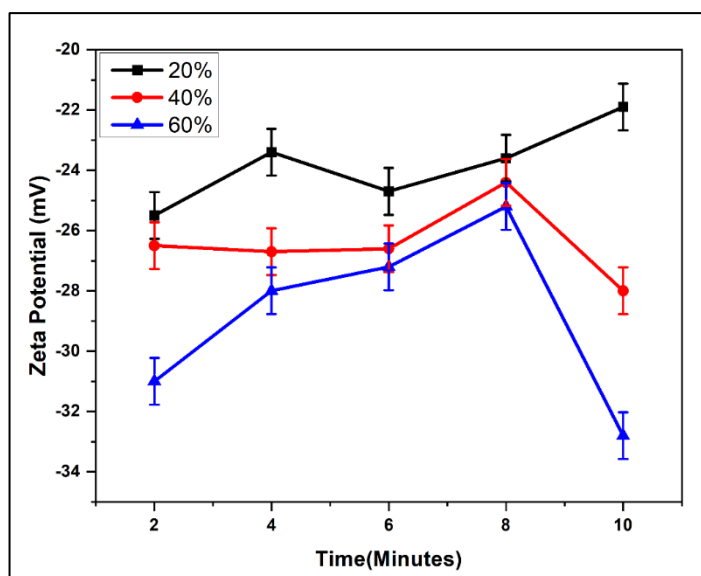


Figure 4.6: Zeta-potential values of liposome nanoparticles formulated in SL:MXF:1:1 ratio.

Figure 4.7 shows the zeta potential results for liposome nanoparticles formulated in SL:MXF:2:1 ratio produced by probe sonication. The surface charge values for the 15 various liposome nanoparticle formulations vary from -35.5 mV to -30 mV (exception of sample L3 was -27 mV). This feature presented that all liposome nanoparticles produced consistent with literature and have good stability.

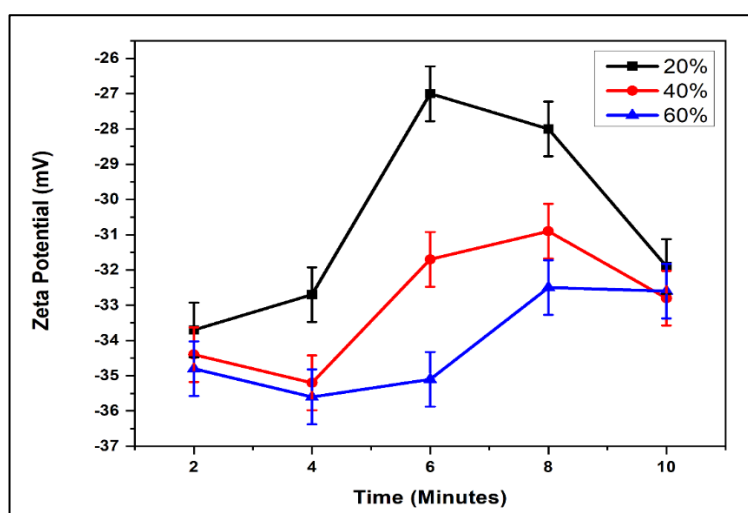


Figure 4.7: Zeta-potential values of liposome nanoparticles formulated in SL:MXF:2:1 ratio.

The phosphate groups in lecithin cause a negative potential. This explains why MXF-free liposome nanoparticles have a natural negative surface charge. In the absence of the drug, the surface remains more free and negative. The partially cationic groups found in MXF interact with lecithin, resulting in a slight surface neutralisation. SL:MXF:1:1 liposome nanoparticles contain less lecithin, so the negative charge from phospholipids is less dominant. In addition, SL:MXF:2:1 liposome nanoparticles contain more phosphate groups, resulting in a more pronounced negative charge on the surface. Furthermore, the high lecithin concentration at the 2:1 ratio contributes to the system's electrostatic stability by maintaining the zeta potential above -30 mV despite the presence of MXF (except L3).

4.1.4. Encapsulation Efficiency

Figure 4.8 shows EE values for liposome nanoparticles formulated in SL:MXF:1:1 ratio achieved. EE values of the moxifloxacin-encapsulated liposome nanoparticles vary from 53,56% to 69,43% for fifteen various liposomes. The encapsulation efficiency can be enhanced by using organic solvents like ethanol and chloroform[118]. The nanocomposite hydrogel coating obtained on the IOL haptic will be inserted directly into the eye. Considering the sensitive structure of the eye, only deionized (DI) water was preferred as the solvent.

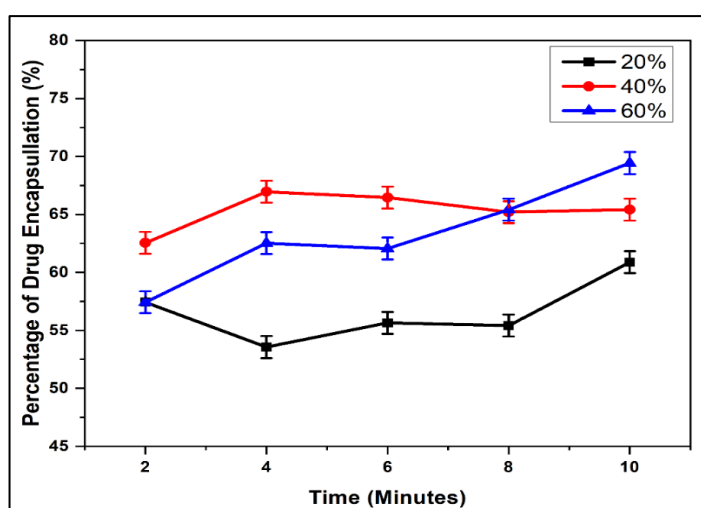


Figure 4.8: Encapsulation efficiency values for liposome nanoparticles formulated in SL:MXF:1:1 ratio.

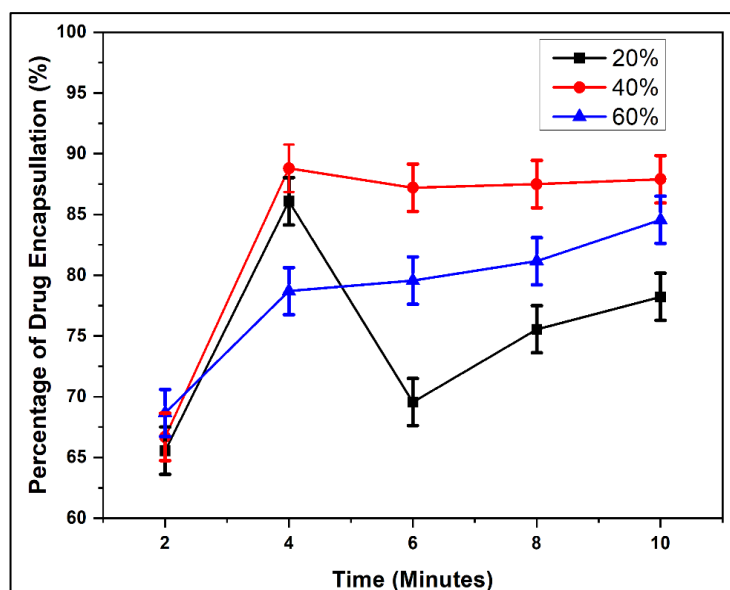


Figure 4.9: Encapsulation efficiency values for liposome nanoparticles formulated in SL:MXF:2:1 ratio.

Figure 4.9 shows EE values for liposome nanoparticles formulated in SL:MXF:2:1 ratio achieved. EE values of the moxifloxacin-encapsulated liposome nanoparticles vary from 65.55 % to 88.8% for the fifteen various liposome nanoparticle formulations achieved indicating good encapsulation efficiency [119].

SL:MXF:2:1 ratio liposome nanoparticles have a higher concentration of phospholipids. This results in the formation of more lipid bilayers and, consequently, more drug encapsulation. SL:MXF:1:1 liposome nanoparticles have a lower lecithin concentration, meaning some of the drug may remain outside the liposome without being encapsulated. If the amount of drug per liposome is excessive, the excess remains outside, leading to a decrease in encapsulation efficiency.

4.1.5 Scanning Electron Microscopy Images

For a comprehensive and accurate comparison, SEM images of the pure states of the materials used in hydrogel preparation are also presented in this section. Accordingly, the SEM image of pure MXF is shown in **Figure 4.10**, and that of pure SL is presented in **Figure 4.11**.

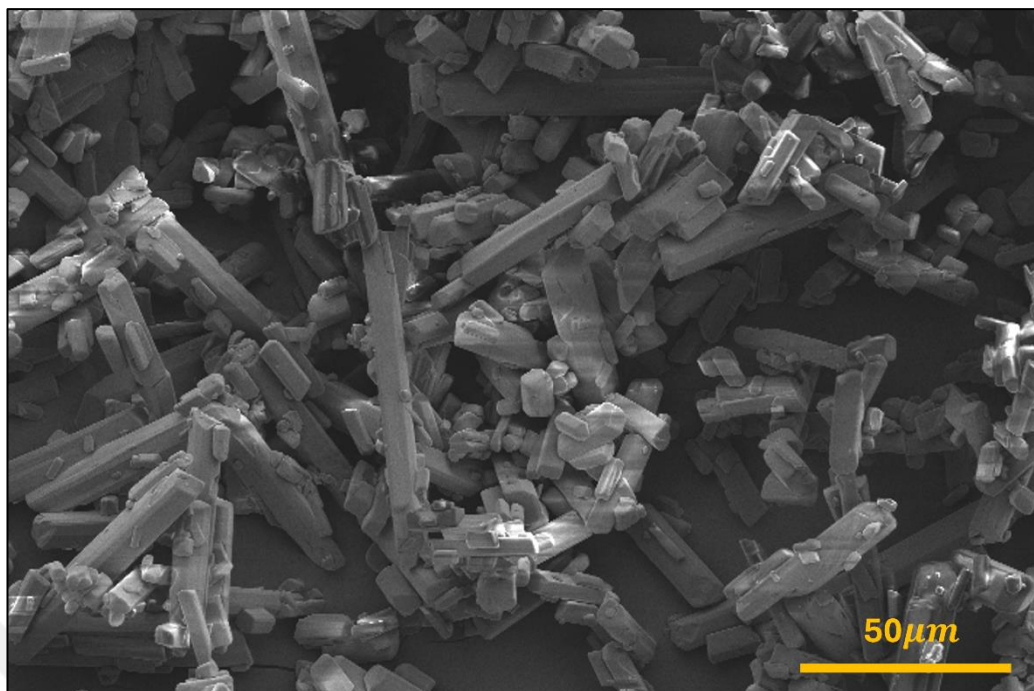


Figure 4.10: SEM view of pure moxifloxacin hydrochloride.

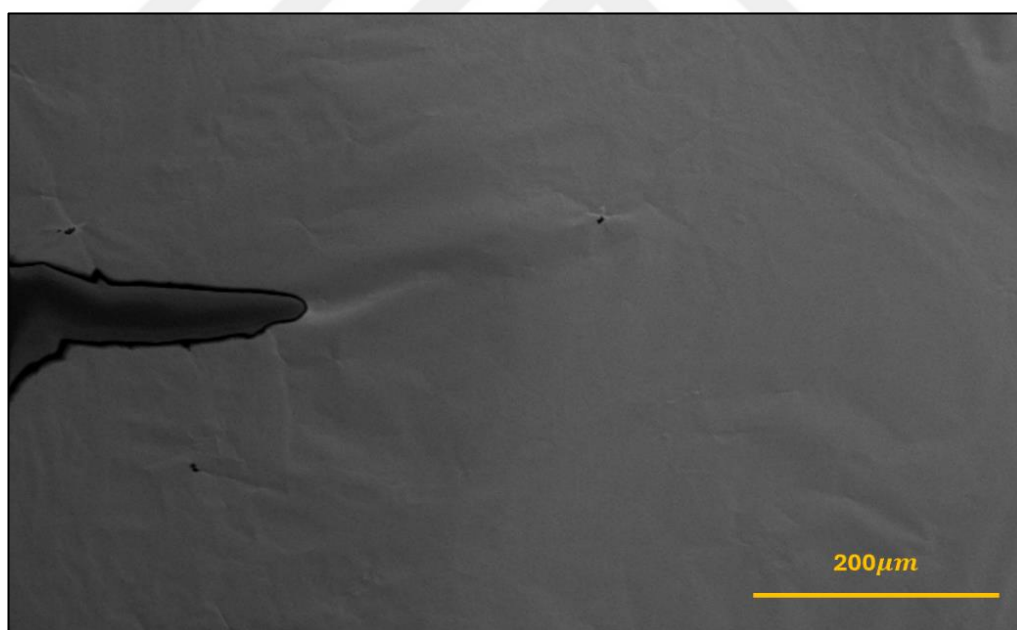


Figure 4.11: SEM micrograph of pure polyoxyethylene 40 streate.

Figure 4.12 depicts the morphological characteristics of MXF-free liposome nanoparticle via utilizing SEM micrograph and reveals an irregular and amorphous structure. Rather than having a distinct shape, it is better described as irregular clumps

intertwined with each other. The curvy and wavy layers suggest presence of overlapping phospholipid bilayers.

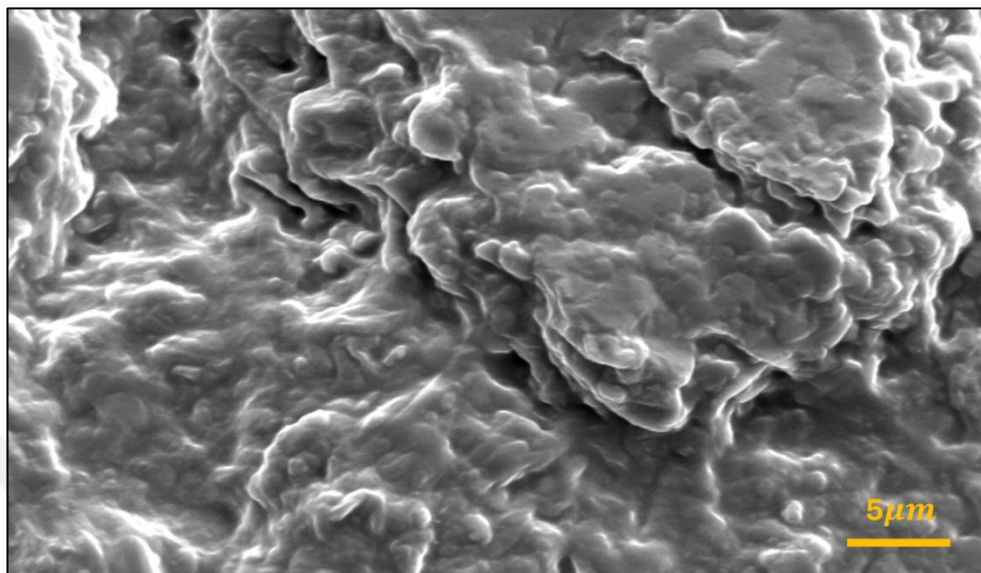


Figure 4.12: SEM image of MXF-free liposome nanoparticle.

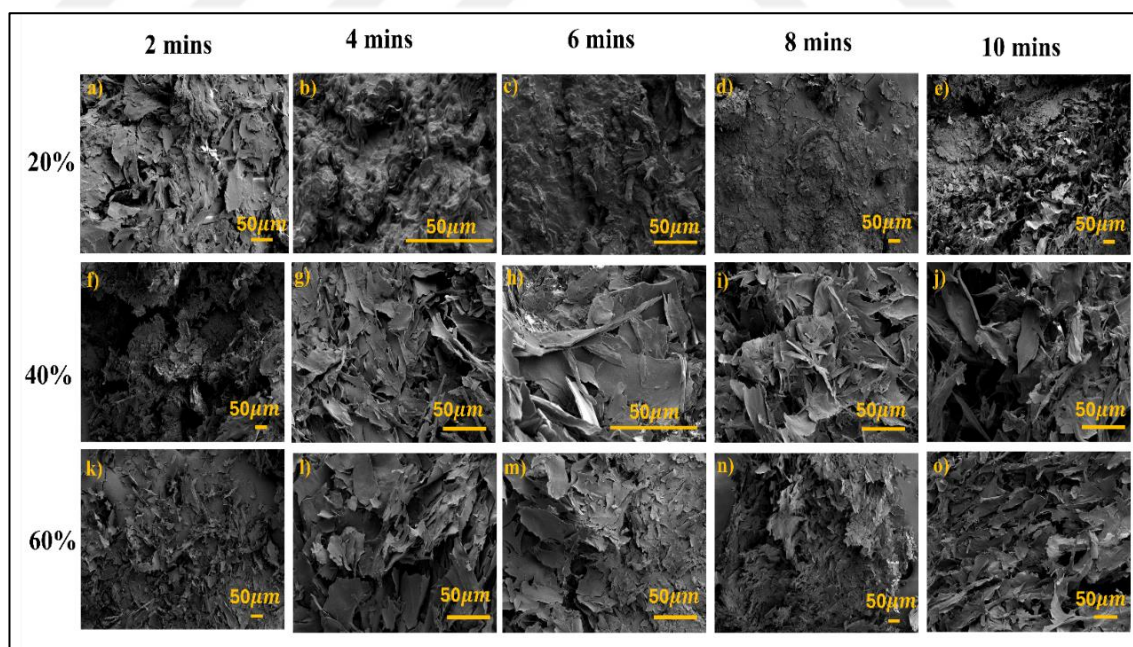


Figure4.13: SEM micrographs of liposome nanoparticles formulated in SL:MXF:1:1 ratio. The scale bar represents 50 μm.

Figure 4.13 shows the surface morphology and three-dimensional nature of liposome nanoparticles formulated in SL:MXF:1:1 ratio produced by probe sonication technique. SEM analysis revealed that liposome nanoparticles have a non-spherical shape with unclearly defined boundaries. Although liposomes are generally recognized as spherical, liposome nanoparticles formulated in SL:MXF:1:1 ratio are usually in the form of paper tears, lamellar-like structures, or agglomerations.

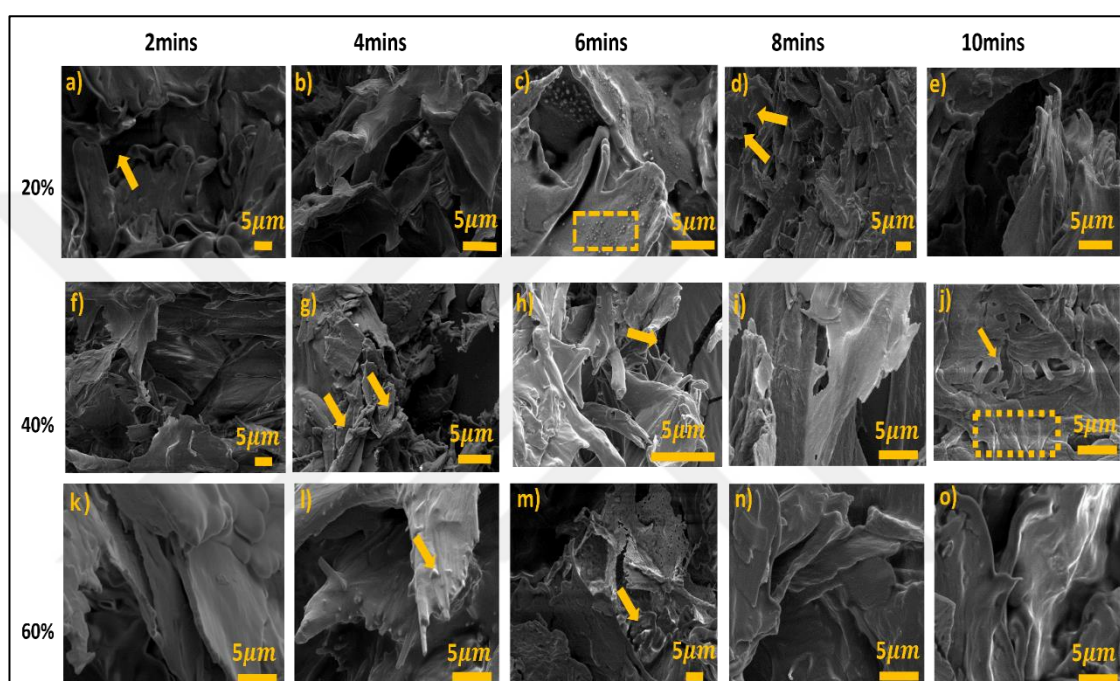


Figure 4.14: SEM micrographs of liposome nanoparticles formulated in SL:MXF:2:1 ratio. The scale bar represents 5 μm .

SEM micrographs for liposome nanoparticles formulated in SL:MXF:2:1 ratio are depicted in **Figure 4.14**. Individual particles are apparent in the light grey areas of the SEM images, which show the presence of tiny circular structures. Areas where buildings are relatively more common are highlighted using arrows and dashed squares. It's obvious that the moxifloxacin drug is enclosed in tiny granular particles rather than a spherical form. The literature indicates that liposomes are typically recognized as spherical structures. However, it has been noted that spherical liposomes can be generated through various methods, including inkjet printing, rotary evaporation, and thin film hydration techniques that use organic solvents like methanol or chloroform [120]. Since the study focuses on the natural production, only DI water

was used as the solvent. The absence of spherical morphology in the liposomes can be attributed to the solvent selection.

4.1.6. Fourier-Transform Infrared Spectroscopy Analysis

Figure 4.15 shows the FTIR spectra belongs to selected liposome nanoparticles among formulated in SL:MXF:1:1 ratio. **Figure 4.16** shows the FTIR spectra belongs to selected liposome nanoparticles among formulated in SL:MXF:2:1 ratio. Regarding the FTIR spectra for liposome nanoparticles, relevant wavenumbers of MXF and soy lecithin were observed for all of them. Considering the results of encapsulation efficiency, zeta potential, SEM, etc. and other characterizations, L7 was identified as the optimal candidate to be used as the reference liposome formulation. To evaluate the effect of parameter such as sonication time and amplitude, L12 and L14 were selected to ensure a reliable comparison, using L7 as a reference. In pure MXF spectra, the stretching vibrations C=O at 1735 cm⁻¹, N-H at 2964 cm⁻¹, and O-H at 3327 cm⁻¹ displayed characteristic peaks. For the O-H and C=O stretching frequencies, soy lecithin exhibited peaks at 3671 cm⁻¹ and 1703 cm⁻¹, respectively. The FTIR spectra of the produced liposome nanoparticles showed not any noticeable changes, with the principal absorption bands of lecithin and moxifloxacin hydrochloride being recognized. This implies that there is no significant potential for chemical affinity between the drugs and the lipid components.

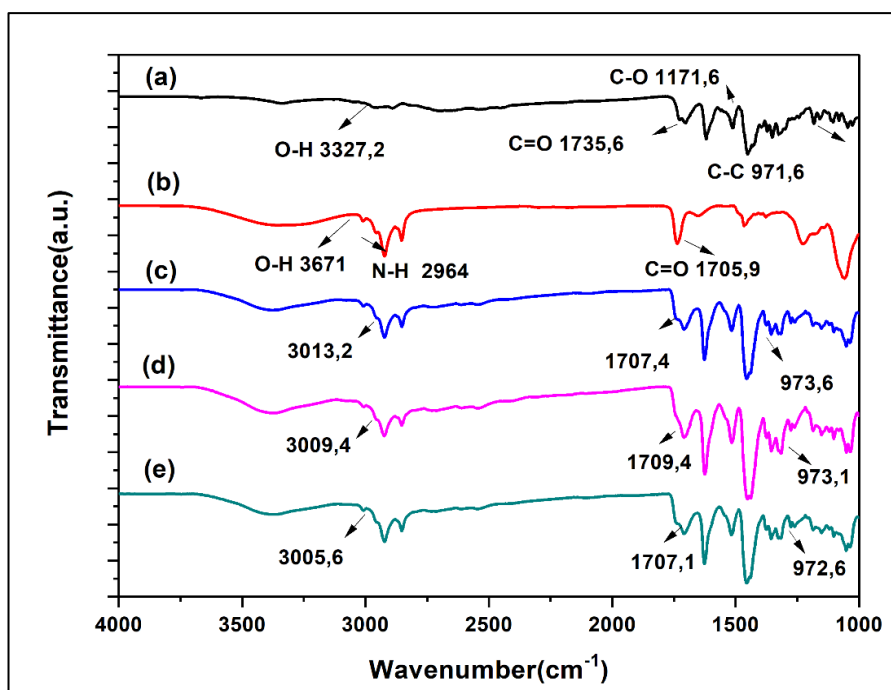


Figure 4.15: FTIR spectra for **a)** pure MXF, **b)** pure soy lecithin, **c)** SL:MXF:1:1 liposomes (L7), **d)** SL:MXF:1:1 liposomes (L12), **e)** SL:MXF:1:1 liposomes (L14).

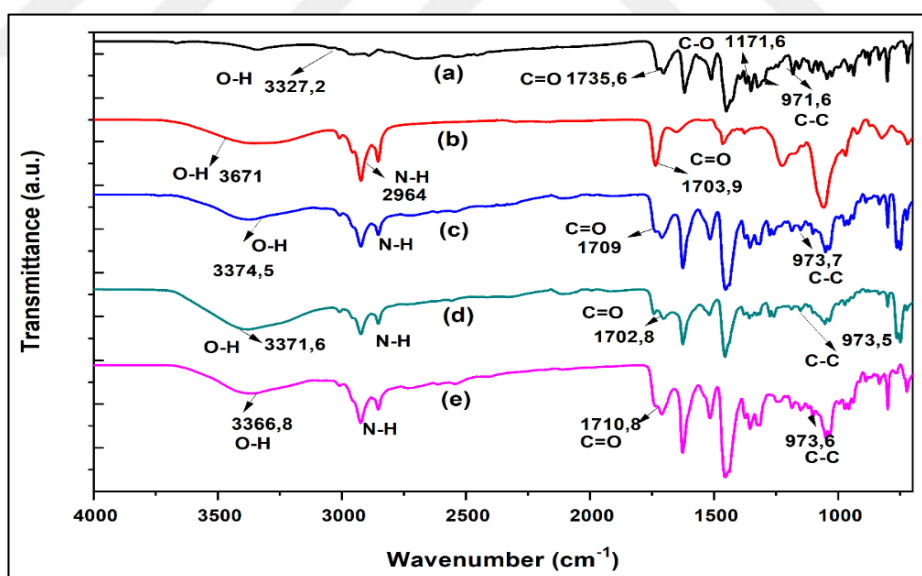


Figure 4.16: FTIR spectrum for **a)** pure MXF, **b)** pure soy lecithin, **c)** SL:MXF:2:1 liposomes L7, **d)** SL:MXF:2:1 liposomes (L12) liposomes, **e)** SL:MXF:2:1 liposomes (L14).

4.1.7. Contact Angle Measurements

Contact Angle Measurements were made for liposome nanoparticles formulated in SL:MXF:1:1 ratio and SL:MXF:2:1 ratio liposome nanoparticle as well.

Table 4.4 and **Table 4.5** show contact angle measurements for liposome nanoparticles formulated in SL:MXF:1:1 ratio and SL:MXF:2:1 ratio, respectively. The measurements of contact angles were less than 90 degrees, indicating hydrophilicity. It is likely that the phosphate groups of lecithin and possible free hydroxyl groups create a hydrophilic character on the surface. When hydrophilic drugs are added to hydrogel matrices, the usual outcome is a burst release, which produces a rapid drug release profile. Therefore, encapsulation of hydrophilic drugs within liposome nanoparticles enables controlled drug release[121].

Table 4.4: Contact Angle Measurements for liposome nanoparticles formulated in SL: MXF:1:1 ratio.

Amplitude (%)	Sonication Time (mins)				
	2	4	6	8	10
20 %	$\leq 5^\circ$	$\leq 5^\circ$	$= 12^\circ \pm 1$	$\leq 5^\circ$	$= 9^\circ \pm 1$
40 %	$\leq 5^\circ$	$= 11^\circ \pm 1$	$= 16^\circ \pm 1$	$\leq 5^\circ$	$= 7^\circ \pm 1$
60 %	$\leq 5^\circ$	$= 8^\circ \pm 1$	$= 9^\circ \pm 1$	$= 6^\circ \pm 1$	$= 7^\circ \pm 1$

Table 4.5: Contact Angle Measurements for liposome nanoparticles formulated in SL: MXF:2:1 ratio.

Amplitude (%)	Sonication Time (mins)				
	2	4	6	8	10
20 %	$\leq 7^\circ$	$= 5^\circ \pm 1$	$= 11^\circ \pm 1$	$\leq 5^\circ$	$= 9^\circ \pm 1$
40 %	$= 7^\circ \pm 1$	$= 12^\circ$	$= 16^\circ \pm 1$	$\leq 5^\circ$	$= 7^\circ \pm 1$
60 %	$\leq 5^\circ$	$= 8^\circ$	$= 10^\circ \pm 1$	$= 8^\circ \pm 1$	$= 6^\circ$

4.1.8. Fluorescent/Polarized Microscopy Analysis

Fluorescent/polarized microscopy observations were conducted to track the encapsulated drugs to provide an enhanced understanding of liposome nanoparticles. The particle size distribution and captured MXF-encapsulated liposome nanoparticles are shown below. Moreover, particle size distribution analysis was also performed with using Image J software.

Figure 4.17 shows the fluorescence microscopy image of liposome nanoparticles formulated SL:MXF:1:1. Image J software was utilized to evaluate the mean particle size which resulted in $196 \pm 0,284$ nm.

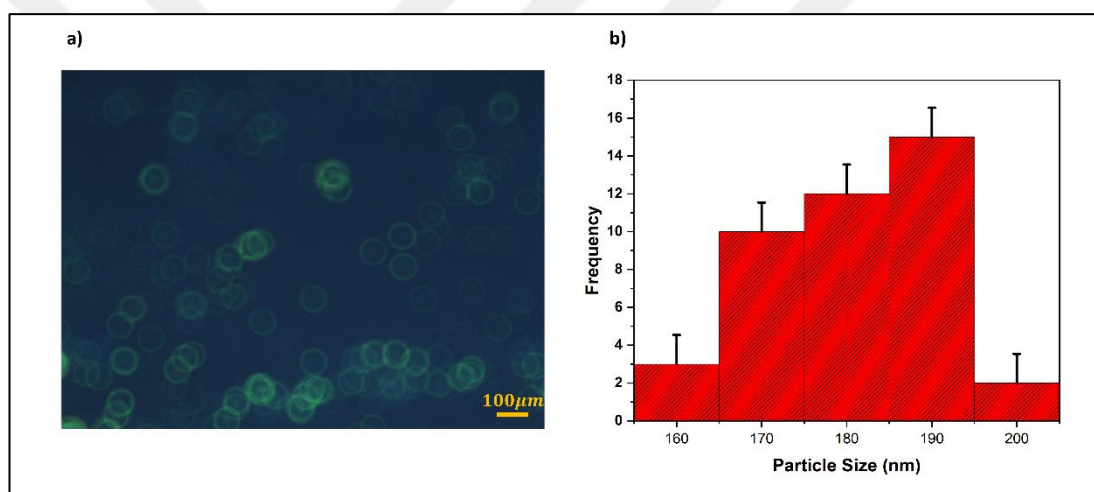


Figure 4.17: a) fluorescence microscope image, b) size distribution of liposome L7 (SL:MXF:1:1).

Figure 4.18 shows the fluorescent/polarized microscopy images for liposome nanoparticles formulated in SL:MXF:2:1 ratio. Non-capsulated(free) particles are also visible. The average particle size, which was determined to be $174 \pm 0,566$ nm, was thought to be consistent with the DLS results. Fluorescence microscopy made it easier to image and identify moxifloxacin (MXF) because of its luminous characteristics. The various layers of the liposome structure were also made visible by this method. Notably, the multivesicular vesicles contained smaller internal vesicles arranged in a non-concentric manner [122]. The fluorescent signal emitted by liposome

nanoparticles confirms that MXF has been encapsulated successfully. The absence of any aggregation supports the physical stability of the system.

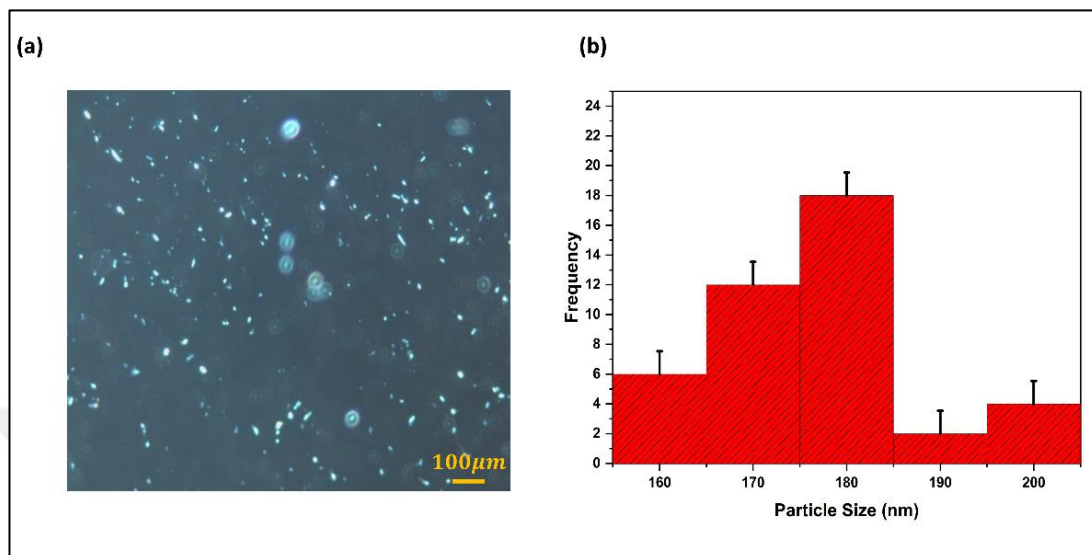


Figure 4.18: a) microscope image b) size distribution of the liposome L7 (SL:MXF:2:1).

After evaluating all the analytical data for liposomes, the liposome nanoparticle formulated in SL:MXF:2:1 ratio with labelled L7 was chosen for incorporation into the alginate hydrogel coating solution.

4.2. Characterization of Polymer Solution

The rheological properties of fluids used in microfluidic systems affect microbubble formation and size. All formulations were mixed until completely homogeneous to ensure accurate and precise measurements.

Characterization studies were conducted with various concentrations of ingredients. The most important properties of the fluid are viscosity and surface tension. **Table 4.6** shows some values for solutions used to produce microbubbles.

Table 4.6: Parameters for polymer solutions.

Formulation Code	Alginate Concentration (%w/v)	PEG40S Concentration (%w/v)	MXF-encapsulated liposome concentration (%w/v)	Density (g/cm^3)	Surface Tension ($mN.m^{-1}$)	Viscosity ($Pa.s$)
F1	1	0,25	1	1.01 ± 0.02	57.20 ± 0.36	11.10 ± 0.02
F2	1	0,50	1	1.01 ± 0.28	62.37 ± 0.27	14.01 ± 0.03
F3	1	0,75	1	1.02 ± 0.52	61.78 ± 0.22	16.20 ± 0.01
F4	2	0,25	1	1.04 ± 0.01	62.17 ± 0.54	19.10 ± 0.02
F5	2	0,50	1	1.15 ± 0.01	61.74 ± 0.29	20.10 ± 0.02
F6	2	0,75	1	1.20 ± 0.04	61.73 ± 0.26	23.20 ± 0.02

4.3 Production and Characterization of Moxifloxacin-Encapsulated Liposomes Enriched Alginate Hydrogel Nanocomposites

This section describes the production of hydrogels using the TMJ device method and micropipetting technique, and the characterisation of the resulting nanocomposite hydrogels. The L7 liposome has been selected from among the freeze-dried liposome nanoparticles whose production and characterisation have been completed.

4.3.1. Production of Moxifloxacin-Encapsulated Liposomes Enriched Hydrogel Nanocomposites by Micropipetting Technique

This technique involves the controlled dispensing of pre-formulated polymer solutions onto selected substrates via micropipette, and then a cross-linking agent is introduced to induce gelation. The coatings were successfully deposited to both a polymeric substrate and the haptic part of an IOL, enabling evaluation of coating behavior on different surfaces. During the coating process, the optic zone of the IOL was masked with Parafilm to prevent potential damage or unintended coating, thereby preserving its transparency (**Figure 4.19**).

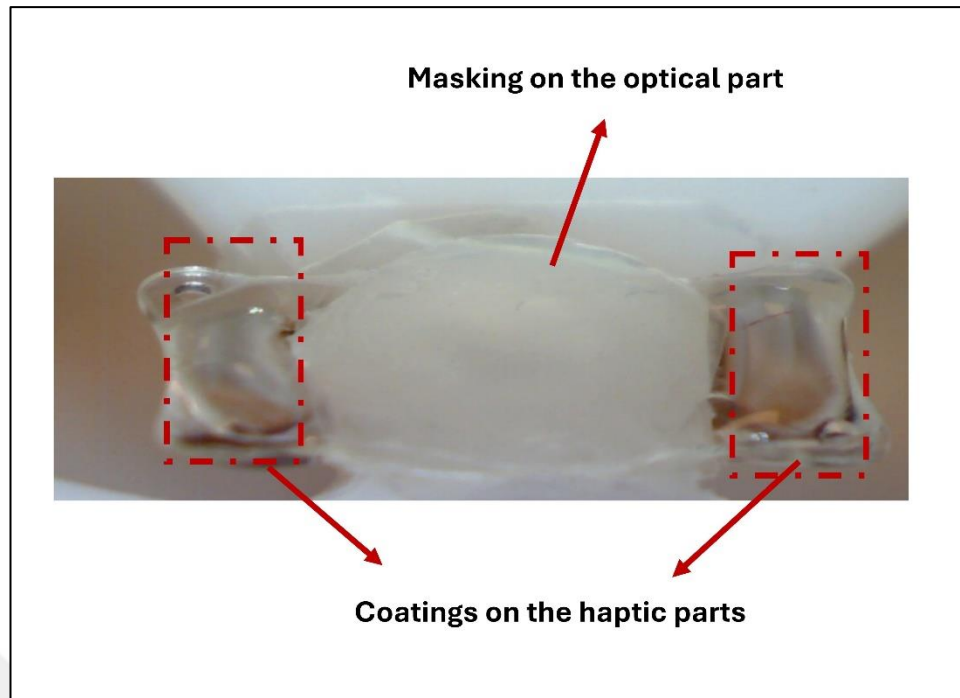


Figure 4.19: Hydrogel production on IOL haptics by micropipetting technique.

4.3.2. Production of Moxifloxacin-Encapsulated Liposomes Enriched Hydrogel Nanocomposites by T-shaped Microfluidic Junction Device

4.3.2.1. Formation of Microbubbles by T-shaped Microfluidic Junction Device

MXF-encapsulated liposome-enriched alginate hydrogel coatings were obtained using the T-shaped microfluidic junction device, which initially facilitated a constant and uniform flow of bubbles. When nitrogen (N_2), an inert gas, is injected at various pressures, the alginate-based polymer solution maintains a steady flow rate, causing necking in the output channel. Different shear stresses are produced by the interaction of the continuous phase (gas) and the non-mixing dispersion phase (liquid), which is followed by the creation of microbubbles (**Figure 4.20**). The produced bubbles are collected on polymeric substrates or IOL haptics after flowing down via the exit channel.

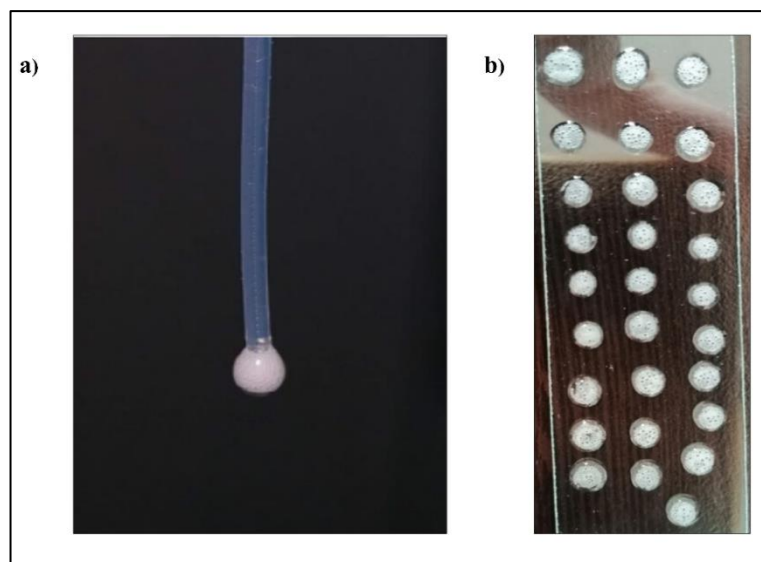


Figure 4.20: a) Cluster of microbubbles at the exit channel of TMJ device, **b)** collected microbubbles on a glass slide.

The effects of the process parameters on the size, and size distribution ratio of the microbubbles were investigated. **Figure 4.21** shows the optical microscope images of liposome nanoparticles formulated in SL:MXF:1:1 ratio (Formulation code F1). The impact of flow rate and gas pressure on microbubble size and its size distribution were examined.

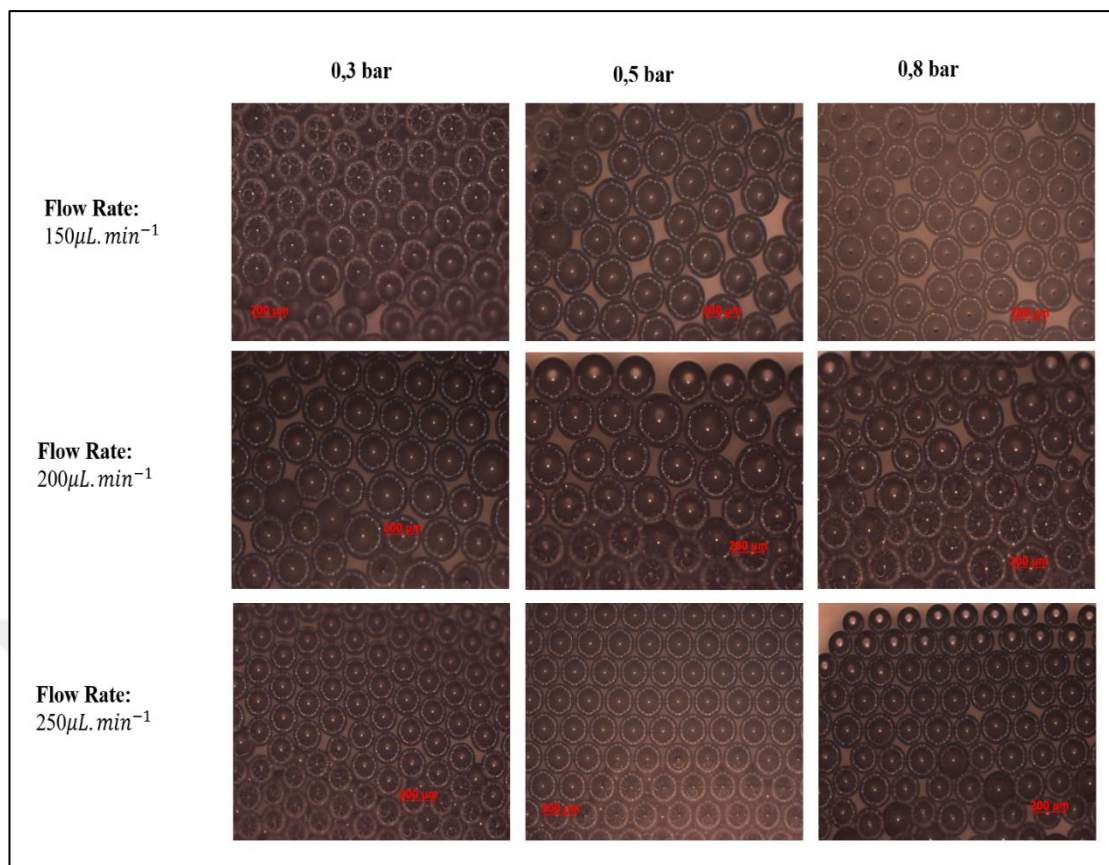


Figure 4.21: Optical microscope images of liposome nanoparticles formulated in SL:MXF:1:1 ratio under various gas pressure and flow rates.

The microbubble size distribution at various gas pressures of 0.3, 0.5, and 0.8 bar, with corresponding flow rates of 150, 200, and 250 $\mu\text{L}.\text{min}^{-1}$ illustrated in **Figure 4.22**. Under constant gas flow rate, increasing gas inlet pressure resulted in an increase in the average microbubble diameter as expected. For liposome nanoparticles formulated in SL:MXF:1:1 ratio, the PDI values varied between 6.5% and 10.3%, while the lowest PDI value was observed in microbubbles produced under conditions 0.5 bar gas pressure and 250 $\mu\text{L}.\text{min}^{-1}$ flow rate. Although the increase in gas pressure leads to an increase in microbubble size, a similar trend was not observed for the PDI values. Increasing the liquid phase flow rate under constant pressure resulted in a decrease in the average microbubble diameter. As expected, this is the result of an increase in the ratio (Q_l/Q_d) of increasing liquid phase flow rate (Q_l) to dispersed phase flow rate (Q_d). When the flow rate was increased from 150 to 250 at constant gas pressure, the PDI values initially increased and then decreased, but no significant changes were

observed. This is thought to be the result of an irregularity in the flow regime and may be due to the inability of the flow rate to balance the gas pressure.

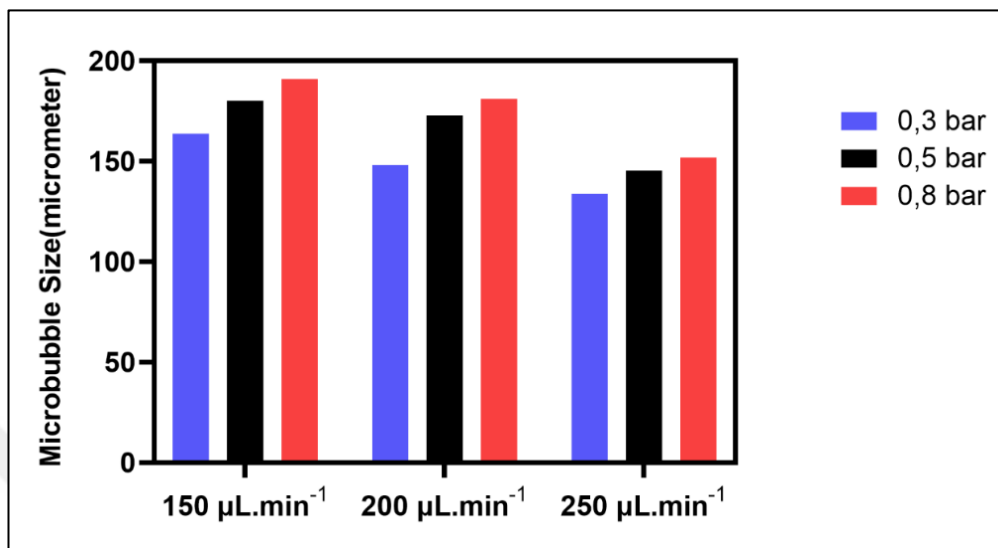


Figure 4.22: Particle size distribution of liposome nanoparticles formulated in SL:MXF:1:1 ratio under various gas pressure and flow rates.

Figure 4.23 shows the optical microscope images of liposome nanoparticles formulated in SL:MXF:2:1 ratio (Formulation code F1). The impact of flow rate and gas pressure on microbubble size and its size distribution were examined.

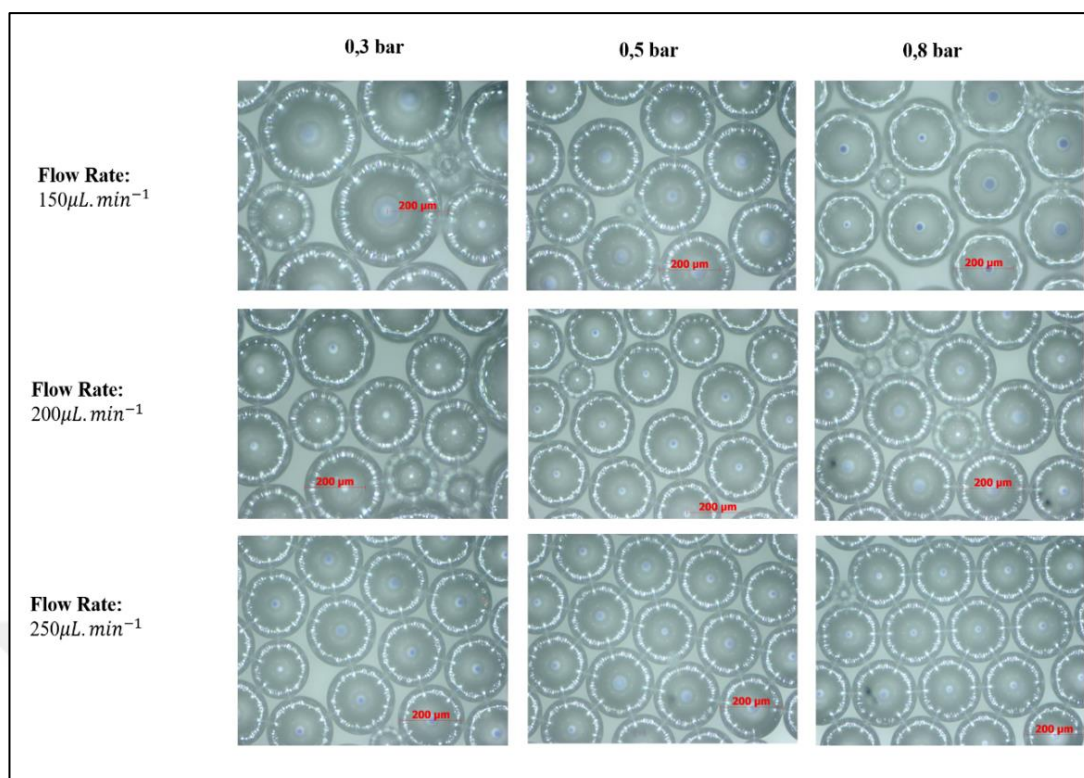


Figure 4.23. Optical microscope images of microbubbles using F1 solution under various gas pressure and flow rates.

The microbubble size distribution at various gas pressures of 0.3, 0.5, and 0.8 bar, with corresponding flow rates of 150, 200, and 250 $\mu\text{L} \cdot \text{min}^{-1}$ illustrated in **Figure 4.24**. When compared to liposome nanoparticles formulated in a SL:MXF:1:1 ratio, similar behavior was observed in response to changes in gas flow rate and pressure. As expected, increasing gas flow rate under constant gas inlet pressure resulted in an decrease in the average microbubble diameter.

PDI values ranged from 2,48 % to 6,32%, while the lowest PDI value was observed in microbubbles produced under conditions 0.3 bar gas pressure and 250 $\mu\text{L} \cdot \text{min}^{-1}$ flow rate for liposome nanoparticles formulated in SL:MXF:2:1 ratio. Lower PDI values were observed compared to liposome nanoparticles formulated in the SL:MXF:1:1 ratio. This can be attributed to a more homogeneous distribution, which resulted in the lower PDI. The increase in the encapsulation efficiency as the lecithin concentration increased may have contributed to this more uniform distribution. The lower amount of remaining unencapsulated soy lecithin and moxifloxacin is thought to have led to a lower PDI value.

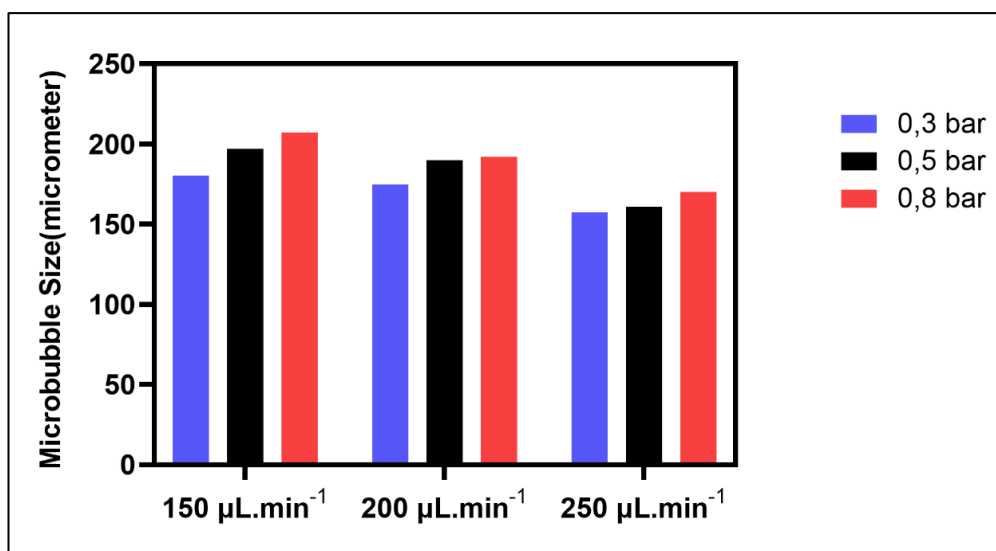


Figure 4.24: Particle size distribution of liposome nanoparticles formulated in SL:MXF:2:1 ratio under various gas pressure and flow rates

The relationship between flow rate and microbubble size exhibits the same behavior with liposome nanoparticles formulated in SL:MXF:1:1 ratio. Increasing the liquid phase flow rate under constant pressure resulted in a decrease in the average microbubble diameter as can be seen in **Figure 4.24**.

Increasing the concentration of soy lecithin also affected the PDI values. Compared to liposome nanoparticles formulated in SL:MXF:1:1 ratio, the PDI values were nearly halved. For instance, while the PDI value for the SL:MXF:1:1 ratio prepared under conditions of 0.3 bar pressure and a 150 $\mu\text{L}.\text{min}^{-1}$ flow rate was 8.3%, it was reduced to 4.12% for the equivalent in a 2:1 ratio.

To investigate the effect of alginate concentration on microbubble formation, bubbles were produced at varying gas pressures and flow rates using F4 solution. Representative images of the resulting microbubbles are presented in **Figure 4.25**. Microbubble sizes and size distributions were quantitatively analyzed using optical microscope images.

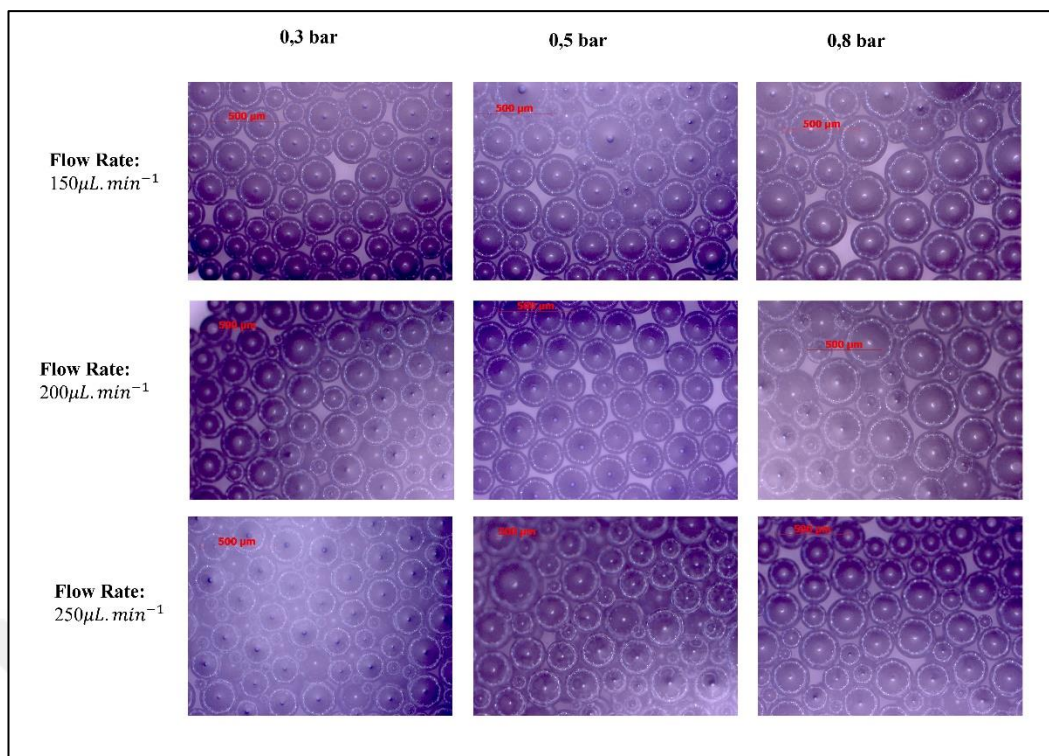


Figure 4.25: Optical microscope images of microbubbles using F4 solution under various gas pressure and flow rates.

As observed in the examples above, increasing flow rate under constant pressure resulted in a decrease in microbubble size. Similarly, under conditions of constant pressure and constant flow rate, increasing alginate concentration caused a decrease in bubble size. As the alginate concentration increased, the bubble diameter decreased and the PDI values increased. The microbubble size ranged from 156.2 to 204.1 μm , and the PDI value varied from 2.6% to 6.5%.

The decrease in microbubble diameter with increasing polymer concentration can be explained by an increase in the solution's viscosity. Higher viscosity reduces the shear stress and requires higher gas pressure for microbubble formation. When the flow rate and gas pressure are constant, an increase in viscosity leads to an increase in gas–liquid interfacial tension, resulting in the formation of smaller microbubbles[123], [124].

The effect of surfactant concentration on microbubble formation is demonstrated by microbubbles obtained using F2 solution, as shown in **Figure 4.26**.

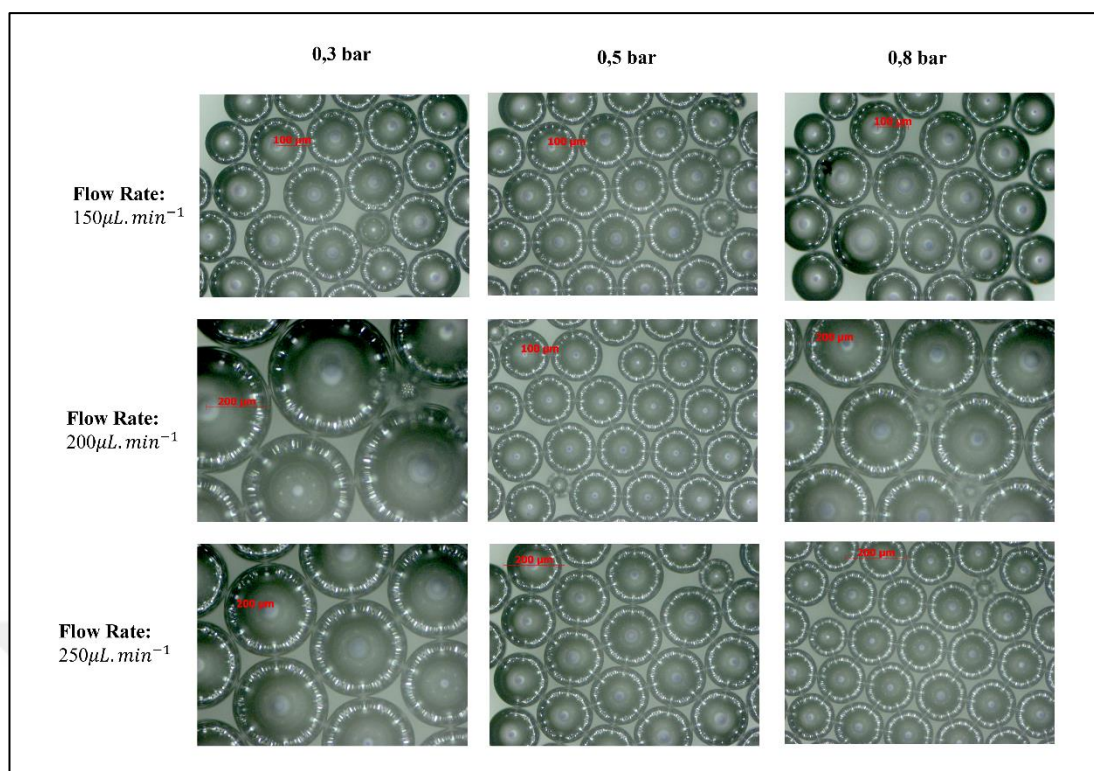


Figure 4.26: Optical microscope images of microbubbles using F2 solution under various gas pressure and flow rates.

The decrease in microbubble size observed with increasing flow rate at a constant pressure also occurred at an increased PEG concentration. Under conditions of constant gas pressure and flow rate, an increase in PEG concentration caused a slight increase in microbubble size. The microbubble size ranged from 165.1 to 210.1 nm while PDI values vary between 1.74-3.12 %. Increasing the concentration of PEG40S increased the viscosity of the solution and caused a slight increase in surface tension. These findings suggest that the concentration of surfactant does not have a significant effect beyond a certain level. However, higher surfactant concentrations led to a reduction in PDI values, indicating enhanced size uniformity of the microbubbles.

4.3.2.2 Production of Moxifloxacin-Encapsulated Liposome Enriched Hydrogel Nanocomposites by T-shaped Microfluidic Junction Device Method

The formation of the hydrogel was completed by the addition of a cross-linking agent following the deposition of microbubbles, as described in section 4.3.2.1, on a suitable polymeric substrate or IOL haptic (**Figure 4.27**).

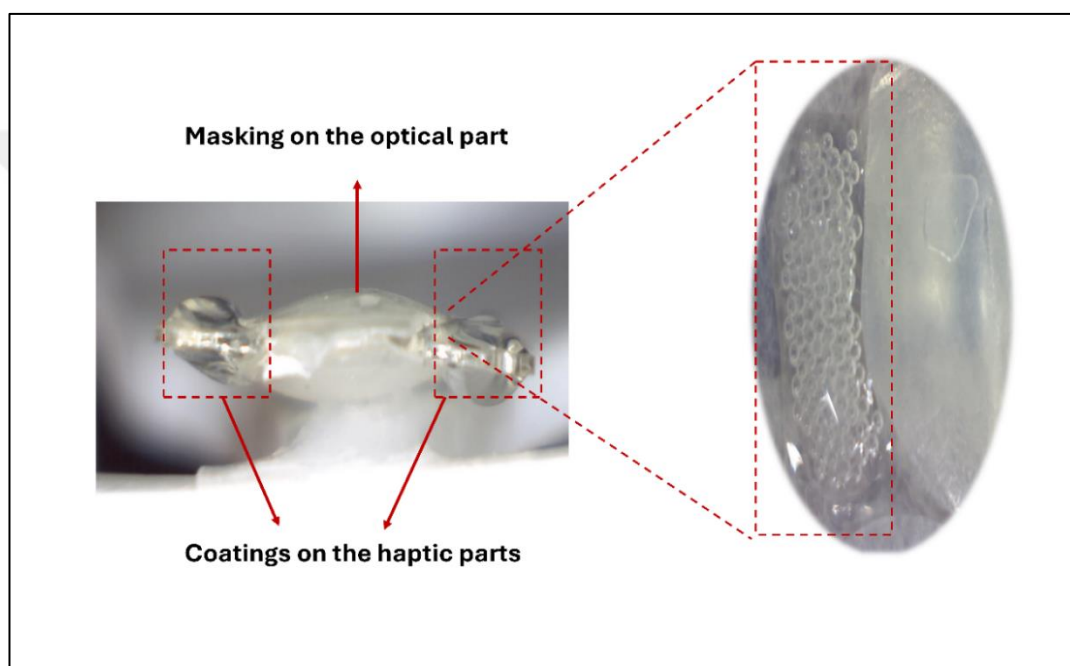


Figure 4.27: Hydrogel production on IOL haptics by TMJ method.

4.3.3 Fourier Transform Infrared Spectroscopy Method

FTIR spectroscopy was used to compare the infrared spectra of the starting materials and the final products in order to obtain insight into potential changes in the chemical structure of the hydrogel films.

As previously described, the drug-loaded liposome nanoparticles were incorporated into the alginate hydrogel system. To make the comparison clearer, FTIR data for the pure components, the drug-loaded liposome nanoparticles and the hydrogels

containing the drug-loaded liposome nanoparticles, produced using two different methods, were analyzed and compared (**Figure 4.28**).

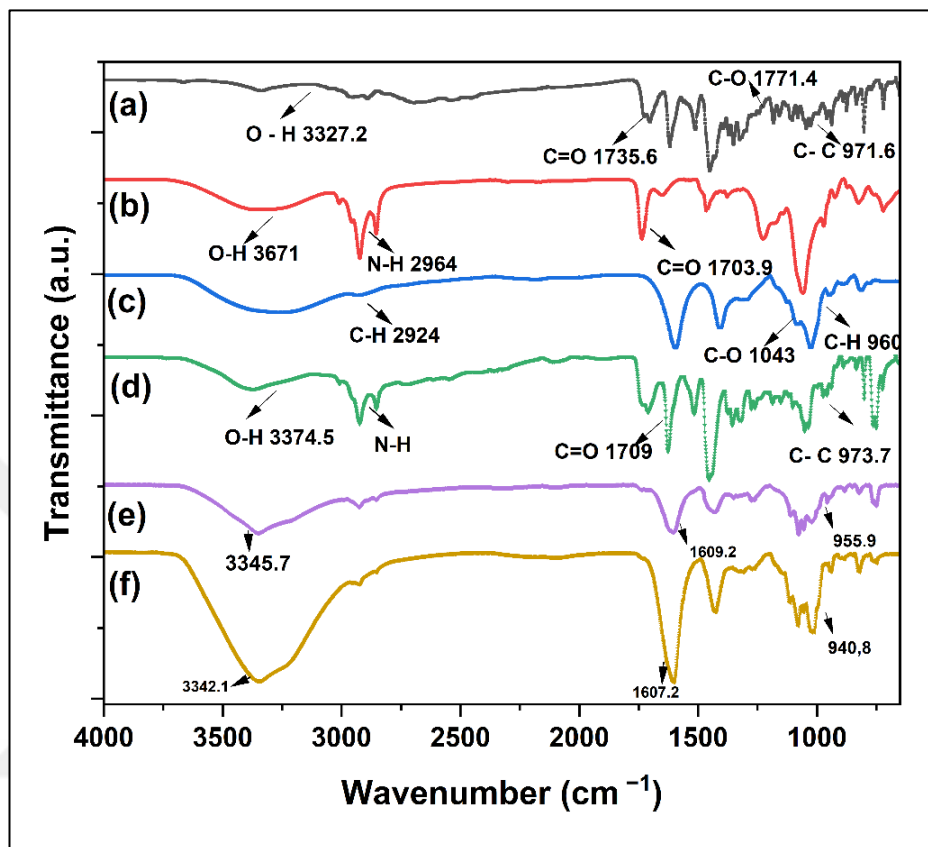


Figure 4.28: FTIR spectrum for **a)** moxifloxacin, **b)** soy lecithin, **c)** alginate, **d)** L7 liposomes formulated with SL:MXF:2:1, and the nanocomposite hydrogel coatings via **e)** the TMJ and **f)** the micropipetting.

The well-performing incorporation of the drug into the hydrogel matrix was indicated by the FTIR spectrum of the MXF-loaded liposome nanoparticle integrated hydrogels made using both micropipette and microfluidic methods. These hydrogels showed the corresponding absorption peaks exhibited by MXF, which were attributed to C=O stretching. An $N(CH_3)_3$ group bond was detected at 960 cm^{-1} and $P=O$ at 1231 cm^{-1} in the corresponding absorption from the soy lecithin [125], [126].

Notably, when comparing the spectra of the composite hydrogels to those of the pure components, small variations in peak intensities and wavenumber locations were observed. These spectral changes could indicate weak physical interactions, such

hydrogen bonds, between the polar groups of alginate or the phospholipid components of the liposome nanoparticles and the functional groups of MXF, such as the hydroxyl and carbonyl groups. MXF and the carrier matrix did not form a strong covalent bonding, as seen by the lack of new peaks or major spectral alterations. This suggests that the drug retains its structural integrity within the delivery system. The results therefore support the hypothesis that MXF is physically entrapped within the hydrogel network instead of chemically bound, which is advantageous for controlled release applications.

4.3.4 Scanning Electron Microscope Analysis for Hydrogel Nanocomposites

To provide a better understanding, SEM images of the raw materials used in hydrogel formation have been included alongside SEM images of nanocomposite hydrogels. **Figure 4.29** and **Figure 4.30** shows SEM micrographs of pure alginate and Polyoxyethylene-40 stearate, respectively.

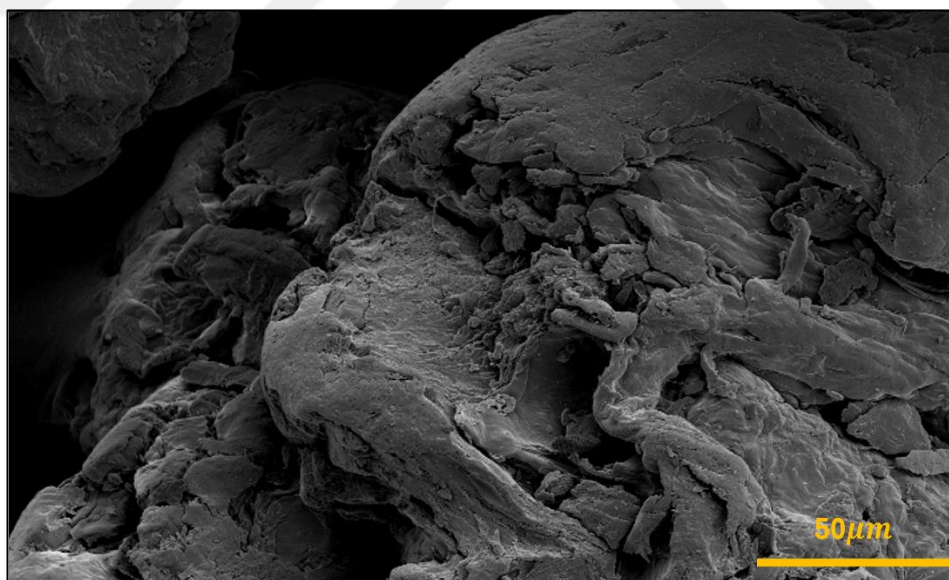


Figure 4.29: SEM view of pure alginate.

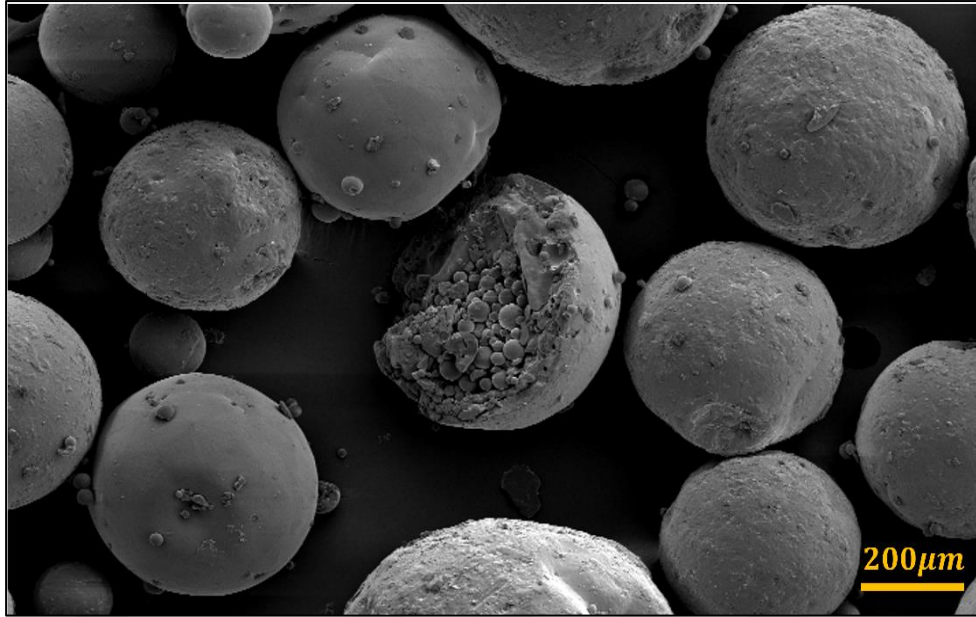


Figure 4.30: SEM view of raw polyoxyethylene-40 stearate.

The microstructural differences of hydrogels produced using different parameters and methods were investigated. **Figure 4.31** show the SEM micrographs produced via micropipetting technique by using F1 and F4 formulations and varying crosslinker concentrations (1-2%) for L7 liposome with the ratio of SL:MXF:2:1. The hydrogels produced with low alginate and cross-linking agent concentrations (%1) tend to exhibit a loosely packed, irregular, and sagging morphology. A higher cross-linking density contributes to forming of a more homogenous hydrogel matrix that is more uniform and structurally stable. Because of the higher polymer density, a more compact hydrogel structure is formed at higher alginate concentrations. Furthermore, increasing the cross-linking agent concentration improves structural integrity and produces a more resilient matrix that doesn't appear to deformation or collapse.

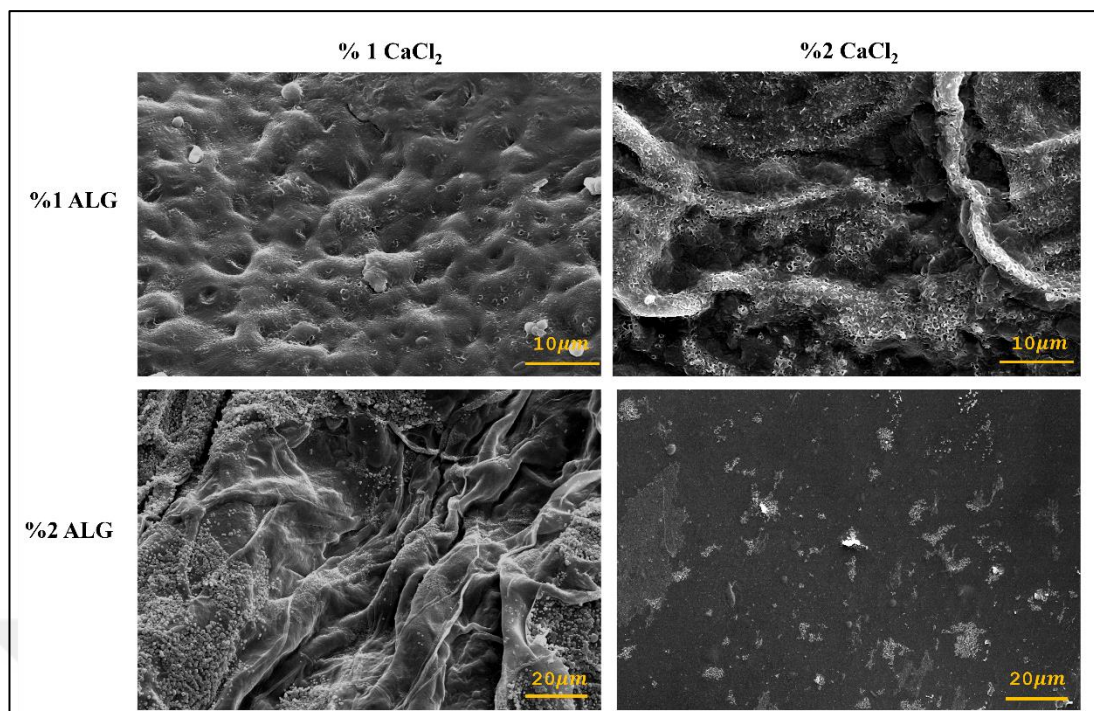


Figure 4.31: The SEM micrographs belong to hydrogel structures formulated using L7 liposome (SL:MXF:2:1) with F1 and F4 solutions and varying concentrations of cross-linking agent, produced via micropipetting. The scale bar represents to 10 μm for the upper images and 20 μm for the lower images.

Figure 4.32 show the SEM micrographs produced via TMJ technique by using F1 and F4 formulations and varying crosslinker concentrations (1-2%) for L7 liposome with the ratio of SL:MXF:2:1. All micrographs showed the formation of porous hydrogels, a key feature of the microfluidic technique. Although the porous structure has been preserved, the increased cross-link density has strengthened the structural integrity and resulted in a more homogeneous distribution.

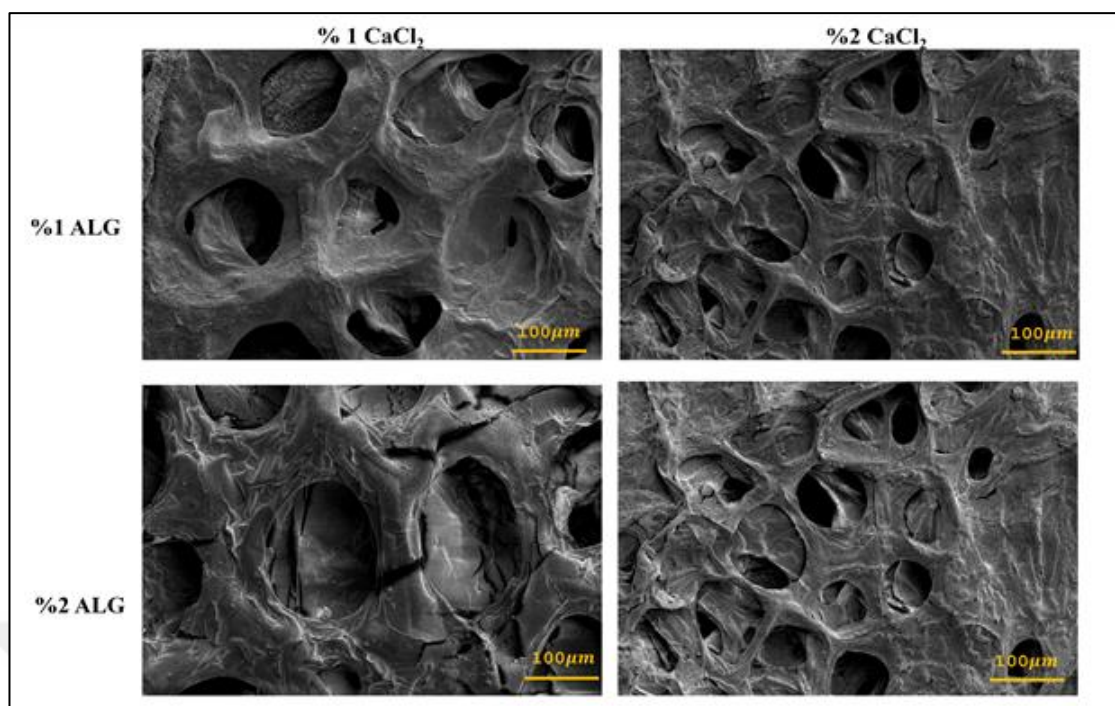


Figure 4.32: The SEM micrographs belong to hydrogel structures formulated using L7 liposome (SL:MXF:2:1) with F1 and F4 solutions and varying concentrations of cross-linking agent, produced via TMJ. The Scale bar represents to 100 μm .

Figure 4.33 shows a comparison of the morphological structures of hydrogel matrices, with/without drug loading, which were fabricated using different production methods. Notably, the significant effect of the production method is porous nature of hydrogel coating provided by TMJ method. This difference plays a key role explaining the characteristic difference in drug release behaviour. The presence of a porous structure increases the surface area, enabling sustained drug release.

Figure 4.33 thus depicts the surface morphologies of alginate-based nanocomposite hydrogels, both MXF-free and, MXF-loaded, enriched with liposome nanoparticles, and manufactured using different fabrication techniques: the TMJ device method and the micropipetting technique. This comparison demonstrates how production method affects structural characteristics like surface topology and porosity.

Additionally, hydrogels produced by the micropipetting technique had a distinct layered texture. The TMJ device approach, on the other hand, made it easier for a monodisperse and highly porous network structure to emerge, suggesting a more consistent and interconnected design.

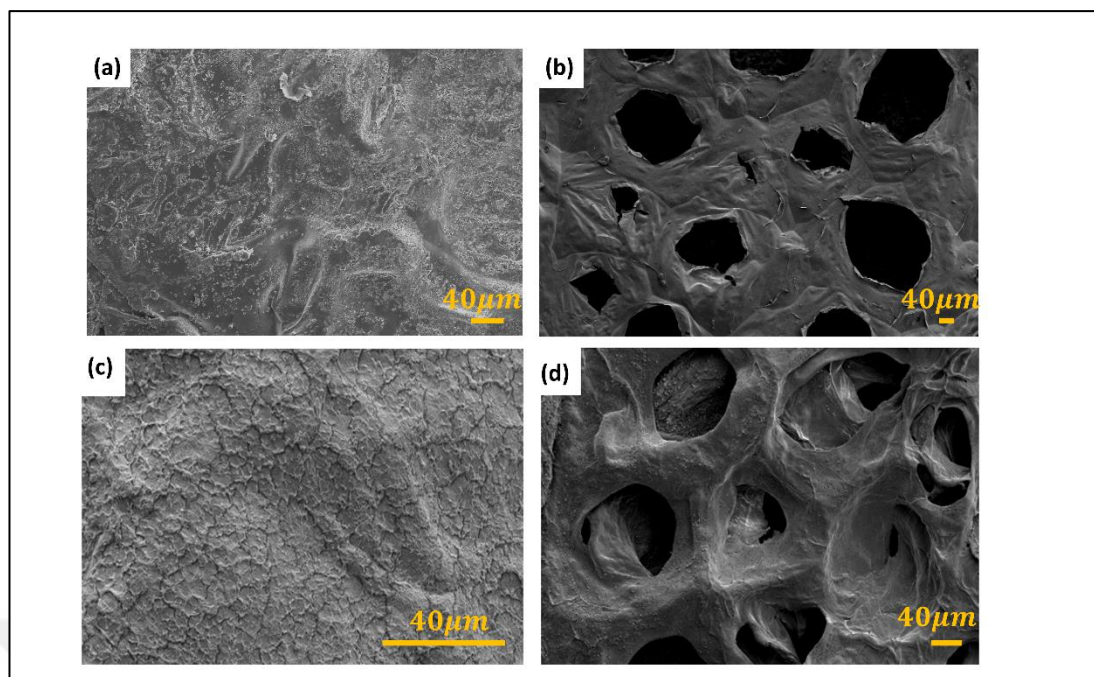


Figure 4.33: SEM micrographs of the nanocomposite hydrogel coating do not containing MXF via using **a)** the micropipetting and **b)** the TMJ device technique; nanocomposite hydrogels produced by **c)** the micropipetting technique and **d)** the TMJ device.

As illustrated in **Figure 4.33 c,d**, the small scattered particles observed on the surface are likely MXF-encapsulated liposome nanoparticles. The presence of crystal structures within the surface morphology can be attributed to the MXF drug, which may indicate partial crystallisation or aggregation during production.

Figure 4.34 shows the EDS result of the alginate nanocomposite hydrogel enriched with MXF-encapsulated liposome nanoparticles. The Energy-Dispersive X-ray Spectroscopy (EDS) detector counts the emitted X-rays to determine the relative concentration of the element of interest at every spot on the sample surface by separating its characteristic X-ray peaks. Liposomal MXF can be found both inside and outside the porous hydrogel structure. The presence of a fluorescence concentration of 0.40% and a phosphate content of 0.02% indicates that the liposome nanoparticles and MXF have both been successfully incorporated into the hydrogel matrix.

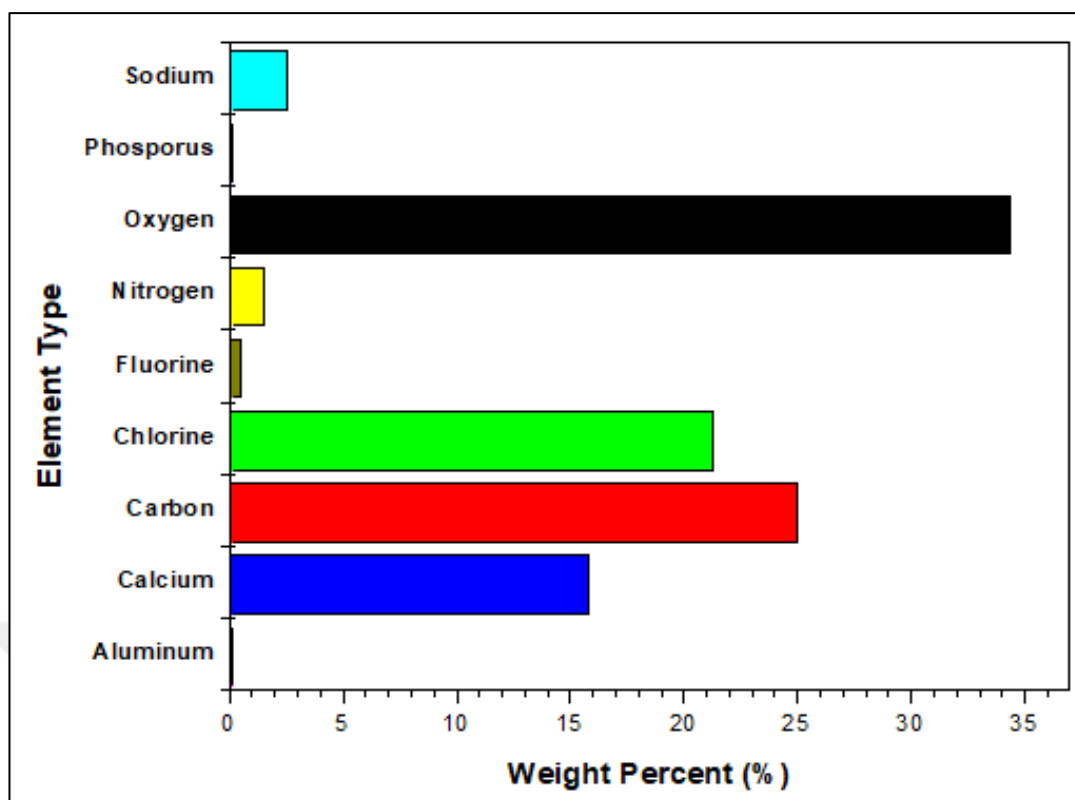


Figure 4.34: EDS analysis of the alginate nanocomposite hydrogel coatings.

4.3.5. Contact Angle Analysis for Hydrogel Nanocomposites

In addition to the contact angle measurements of the liposome nanoparticles described in Section 4.1.7, contact angle measurements were also performed on the produced hydrogels. To enable a meaningful comparison, the contact angles of pristine sodium alginate and of the MXF-encapsulated liposome nanoparticle and MXF-encapsulated liposome-enriched alginate nanocomposite hydrogel were compared. Contact angle measurements finished in ' $<5^\circ$ ', ' $=12^\circ$ ', and ' $<5^\circ$ ', respectively (**Figure 4.35**). As previously mentioned, a contact angle of less than 90 degrees reflects hydrophilicity. The addition of hydrophilic drugs in hydrogels often ends with burst release, an undesirable phenomenon as it means that most of the drug is released all at once. Therefore, encapsulating hydrophilic drugs in liposomes or including drug-loaded liposomes in hydrogel systems has proven to be an effective strategy to achieve controlled drug release [121]. Moreover, drug delivery systems can readily interact with body fluids thanks to their hydrophilic surface characteristics. At the same time, by improving the solubility and diffusion of drugs the water absorption and swelling

behavior of hydrophilic structures create a favorable environment for controlled release.

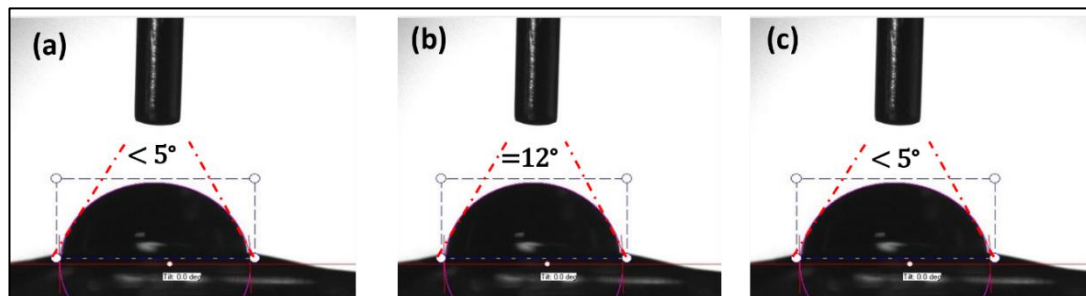


Figure 4.35 Contact angle values of **a)** pure alginate, **b)** liposome with labelled L7, and **c)** nanocomposite hydrogels.

4.3.6 Swelling Profiles of Hydrogel Nanocomposites

Figure 4.36 illustrates the changes in swelling behaviour of hydrogels produced using two different methods, under varying concentrations of polymer and cross-linker. **Figure 4.36 a)** represents hydrogels produced by micropipetting and, **b)** TMJ device. The effect of the hydrogel production method on swelling behaviour is clearly visible.

As shown in **Figures 4.36a** and **Figure 4.36b**, an increase in cross-linker concentration results in a decrease in swelling ratio when the alginate concentrations remain constant. This can be explained by the denser cross-linked network, which restricts the hydrogel's ability to absorb water by reducing the interstitial spaces between polymer chains.

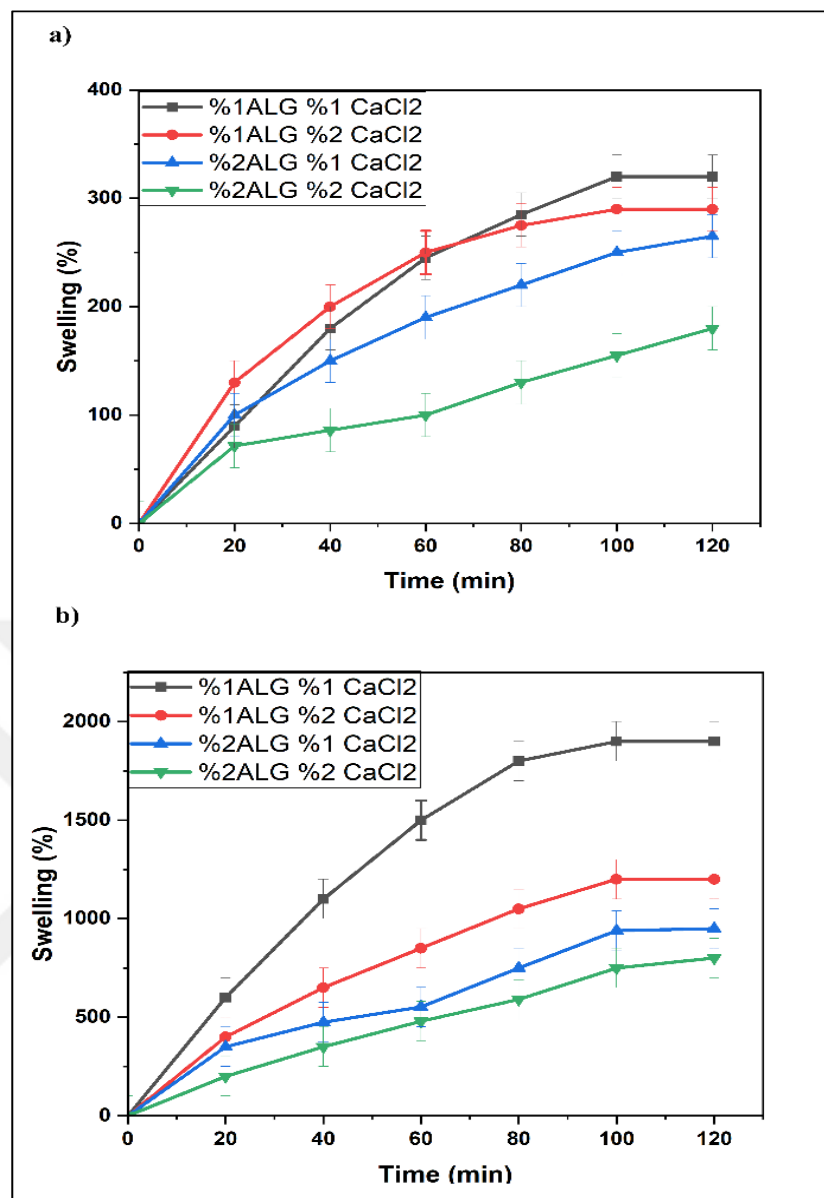


Figure 4.36: Comparison of swelling ratio of nanocomposite hydrogels produced via **a)** micropipetting and, **b)** TMJ device technique with varying polymer and crosslinker concentrations.

Figure 4.37 depicts the percent swelling of MXF-free and MXF-encapsulated liposome-enriched alginate nanocomposites obtained by micropipetting and TMJ methods. Swelling ratio is a characteristic property describing the water absorption capacity of the hydrogel. The swelling ratio also affects the release behavior of encapsulated drug molecules incorporated into the hydrogel. Three primary steps can be used to characterize the swelling behavior of nanocomposite hydrogels. Water molecules first adhere to the hydrogel's surface. These molecules join the crosslinked

polymer network through diffusion, breaking hydrogen bonds and impairing intermolecular interactions, which leads to the expansion of the polymer chains. Ultimately, porous domains emerge as the network structure gets more hydrated, which promotes additional water absorption and increases the swelling capacity. **Figure 4.37** provides a schematic illustration of this multistep swelling mechanism, emphasizing the water's gradual interaction with the hydrogel matrix at each step.

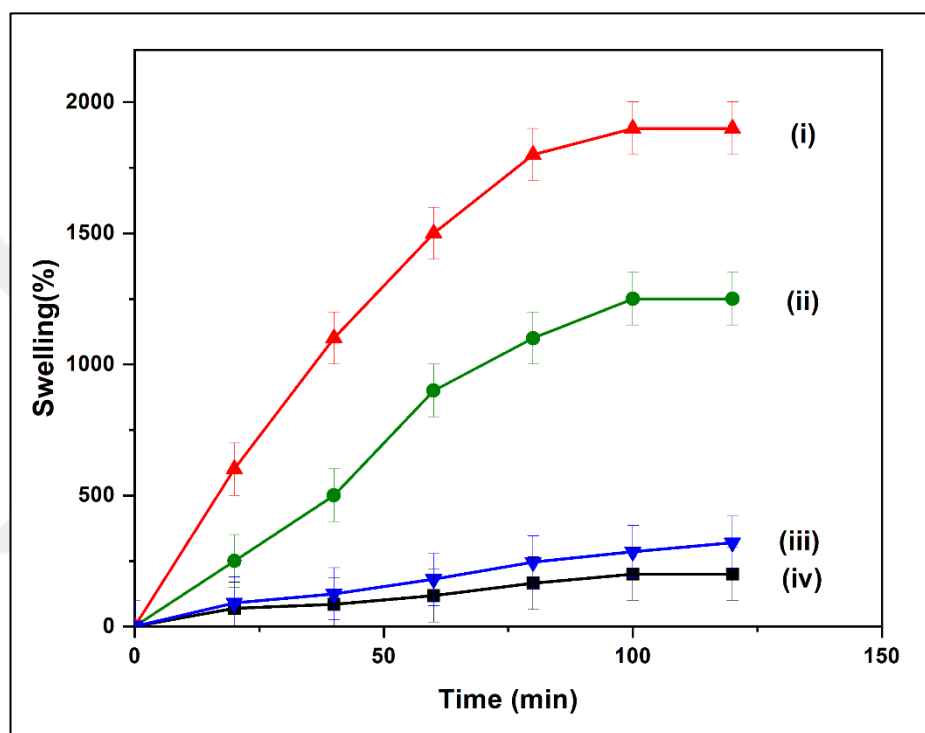


Figure 4.37: The swelling ratio results of **i)** nanocomposite hydrogel via microfluidic method, **ii)** pure alginate via TMJ, **iii)** nanocomposite hydrogel via micropipetting technique, **iv)** pure alginate micropipetting technique.

4.3.7 Mechanical Properties of Hydrogel Nanocomposites

Figure 4.38 illustrates the mechanical properties of the liposome-enriched alginate nanocomposite hydrogels produced using the TMJ and micropipetting methods, with and without MXF encapsulation. The tensile strength and Young's modulus values of the structures with and without MXF were then compared.

The existence of microbubble structure is assumed to be the cause of the lower tensile strength seen in hydrogels produced by TMJ. Microbubbles decrease intermolecular interactions and act as stress concentrators during mechanical loading, creating structural discontinuities in the hydrogel matrix. This decreases the tensile strength, both by reducing the effective cross-sectional area bearing the load and by disrupting the consistent alignment of polymer chains. However, both pure alginate and alginate-based nanocomposite hydrogels showed a discernible improvement in tensile strength upon the addition of MXF-encapsulated liposome nanoparticles. This improvement is probably the result of liposomes' capacity to engage in or promote further crosslinking within the polymer matrix, which strengthens the network's cohesiveness and adds structural reinforcement that increases the material's resistance to tensile deformation.

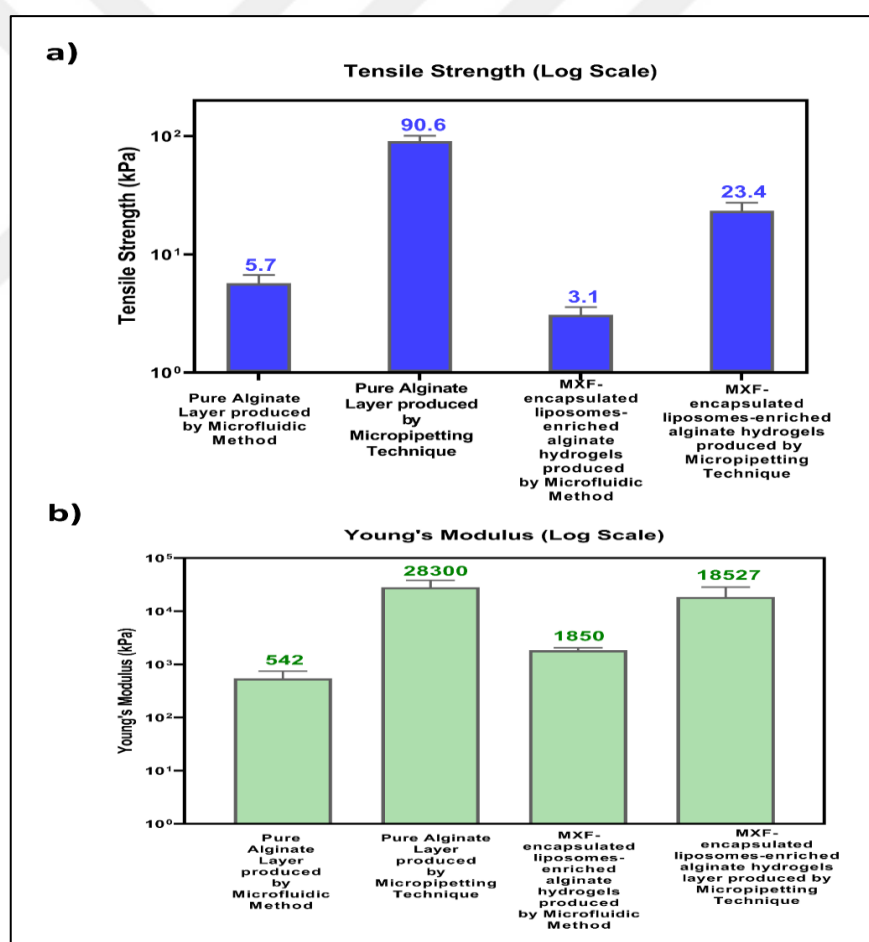


Figure 4.38: Mechanical properties of MXF-free and MXF-encapsulated liposome-enriched alginate nanocomposite hydrogels, **a)** Tensile strength and **b)** Young's modulus.

The Young's modulus values shown in **Figure 4.38 b)** are in line with the tensile strength findings. Hydrogels produced using the TMJ device exhibit greater elasticity as they contain microbubbles that can be compressed and expanded, enabling the material to deform more easily and enhancing its overall flexibility.

4.3.8. In Vitro Drug Release Analysis of the Alginate based Nanocomposite Hydrogels

Before proceeding with the drug release studies, a calibration curve was created using different concentrations of MXF. The resulting calibration curve is shown in **Figure 4.39** and is expressed by the following equation:

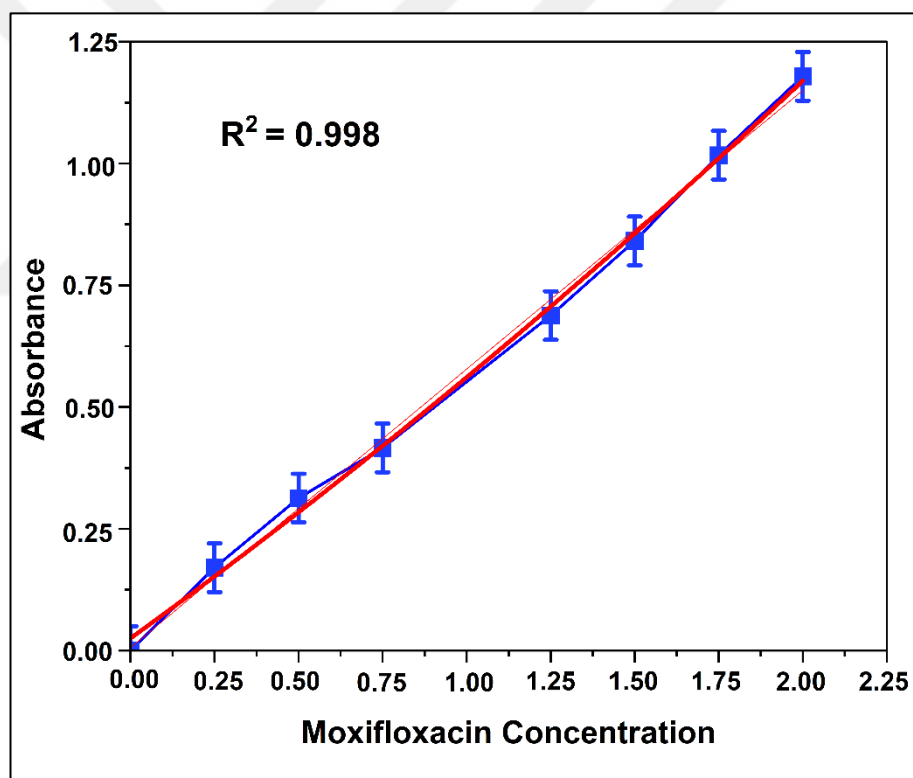


Figure 4.39: a) Calibration curve of the drug MXF at different concentrations at ambient temperature.

After dissolution tests, drug release profiles of MXF-free and MXF-encapsulated liposome enriched alginate hydrogel nanocomposites produced by micropipetting and TMJ device methods were compared (**Figure 4.40**).

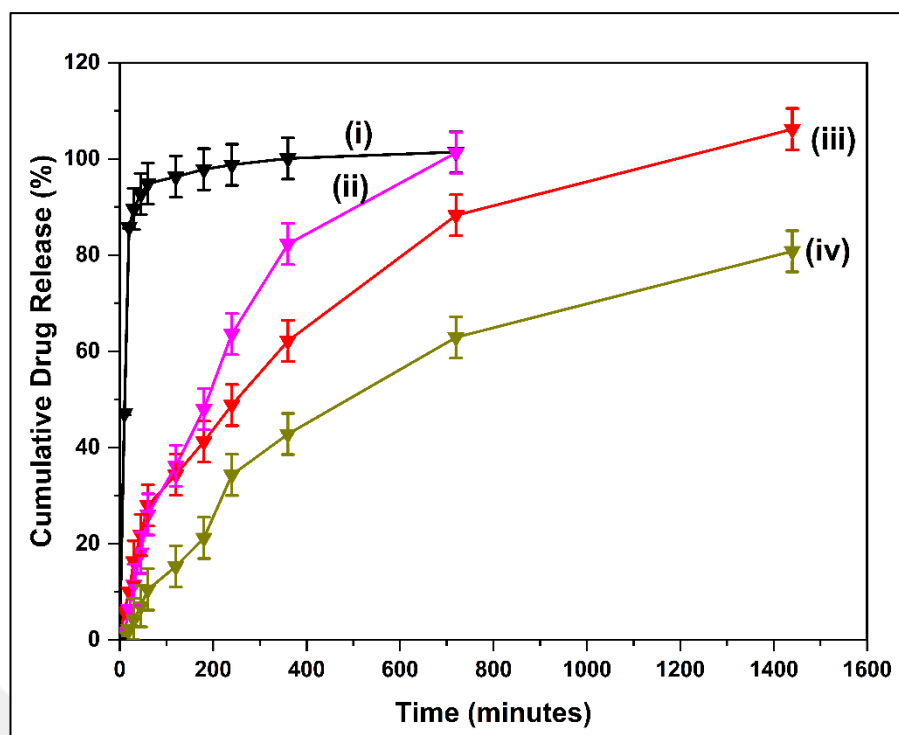


Figure 4.40: Comparison of in vitro cumulative drug release behavior (i) drug-encapsulated liposome (micropipetting), (ii) drug-encapsulated liposome (TMJ), (iii) nanocomposite hydrogels (micropipetting technique), and (iv) nanocomposite hydrogels (TMJ).

As expected, hydrogels manufactured via the TMJ device technique exhibited a slower and more controlled drug release profile compared to those produced by micropipetting. The liposome formulation obtained via micropipetting exhibited 'burst release', whereby a large proportion of the drug was released immediately. However, the equivalent formulation produced using the TMJ device method exhibited a relatively longer drug release. This result provides an answer to the question of how manufacturing techniques can change drug release behaviour.

Alginate nanocomposite hydrogels produced with the TMJ device showed a better sustained release profile, according to a comparative study of drug release behavior. Drug release was considerably altered by adding MXF-encapsulated liposome nanoparticles to the hydrogel matrix. Because the liposome nanoparticles were stable when distributed in the hydrogel, the release of the full drug content was delayed in comparison to formulations that solely contained MXF-encapsulated liposome nanoparticles. By incorporating MXF-encapsulated liposome nanoparticles into the

hydrogel network, the cross-linked chains in the hydrogel act as a barrier and drug diffusion is restricted. When water molecules penetrate the hydrogel, polymer diffusion occurs, enabling the homogenous distribution of drug-loaded liposome nanoparticles throughout the polymeric network. The water molecules then interact with the encapsulated drug molecules, dissolving them. It is the amount of dissolved drug molecules that determines the amount of drug released from the hydrogel.

Drug release profiles were examined using different dissolution models including first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell. Two different release patterns were observed in the first 60 min and 120–1440 min in the monitoring of whole drug release. The presence of gel swelling and diffusion erosion in the first hour suggests that the Korsmeyer model was followed. The R^2 value shows that the MXF liposome nanoparticle formulation produced by the micropipetting method complies with first-order release kinetics. In contrast, the MXF liposomal formulation prepared using the TMJ method followed the Korsmeyer–Peppas model, indicating that release is governed by diffusion. When the nanocomposite hydrogels were compared, it is evident that the hydrogel produced by the micropipetting method followed the Hixson–Crowell model, whereas the hydrogel produced by the TMJ device obeyed first-order kinetics.

Figure 4.41 illustrates the release behaviour of nanocomposite hydrogels that were produced as scaffolds and then deposited as coatings on IOLs via using the TMJ technique. The IOL base acts as a carrier for the hydrogel, and the drug release behaviour is relatively slower compared to the scaffold structure. This can be explained by the coating form having a lower surface exposed to the surrounding aqueous medium.

Table 4.7. Drug release kinetics for hydrogel coatings produced by the micropipetting method and the TMJ device technique for (a) the first 60 min and (b) between 120 and 1440 min

a)				
Formulation	R ² Value Observed by Applying Different Dissolution Models			
	First-Order	Higuchi	Korsmeyer–Peppas	Hixson–Crowell
MXF-encapsulated liposome nanoparticle (micropipetting method)	0.981	0.878	0.943 n = 0.294	0.977
MXF-encapsulated liposome nanoparticle (microfluidic method)	0.996	0.915	0.997 n = 0.864	0.995
Nanocomposite hydrogel (micropipetting method)	0.960	0.754	0.996 n = 1.282	0.966
Nanocomposite hydrogels (microfluidic method)	0.959	0.754	0.998 n = 1.370	0.962
b)				
Formulation	R ² Value Observed by Applying Different Dissolution Models			
	First Order	Higuchi	Korsmeyer–Peppas	Hixson–Crowell
MXF-encapsulated liposome nanoparticle (micropipetting method)	0.676	–262.951	0.974	–135.750
MXF-encapsulated liposome nanoparticle (microfluidic method)	0.976	0.961	0.978 n = 0.441	0.967
Nanocomposite hydrogel (micropipetting method)	0.965	0.944	0.945 n = 0.515	0.994
Nanocomposite hydrogels (microfluidic method)	0.974	0.949	0.958 n = 0.556	0.924

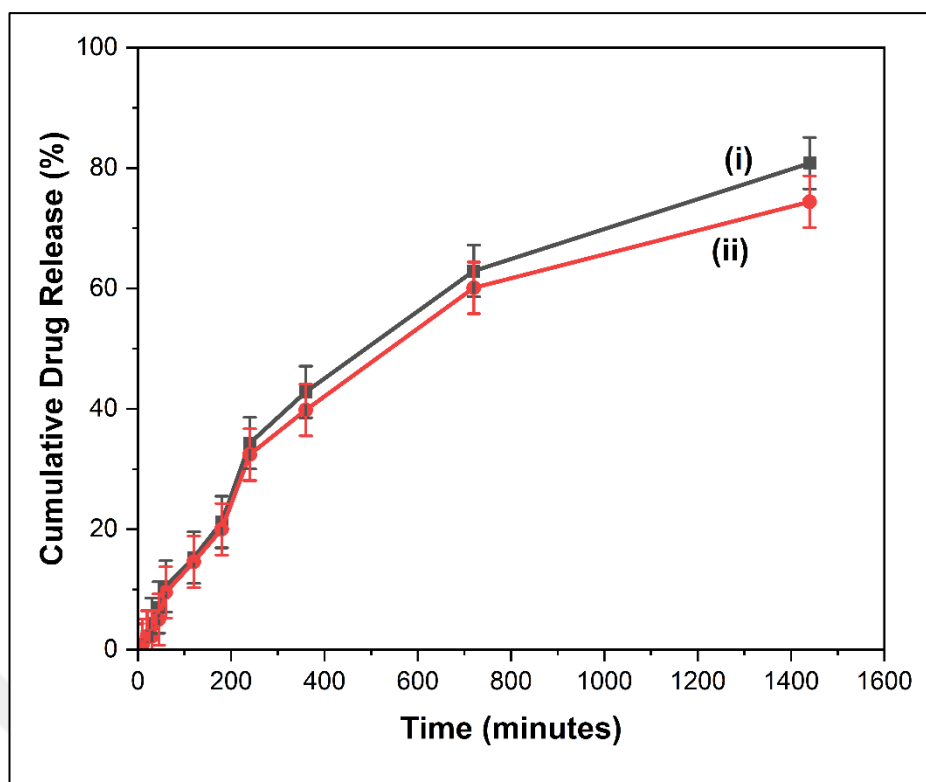


Figure 4.41: Comparison of drug release behaviour of **i)** MXF-encapsulated liposome enriched alginate nanocomposite hydrogel and, **ii)** MXF-encapsulated liposome enriched alginate nanocomposite hydrogel coating on IOL.

5.CONCLUSIONS

This study aimed to prepare and evaluate drug-loaded liposome nanoparticles, as well as their physical incorporation into alginate hydrogels, for use in ocular applications. For this purpose, MXF drug was first encapsulated into SL using a probe sonicator. SL and MXF were mixed at two different ratios (1:1 and 2:1) to obtain liposome nanoparticles. By applying various amplitudes and sonication times for each ratio, 15 different liposome formulations were produced (30 in total). MXF-free, without drug, liposome nanoparticles were also produced as control samples. All liposome formulations were then freeze-dried and prepared for characterization. The average particle size and PDI values were determined for three groups of liposome nanoparticles using the DLS technique. The average particle sizes for MXF-free, SL:MXF:1:1, and SL:MXF:2:1 liposome nanoparticles were calculated as 176.7 ± 0.024 , 162.5 ± 0.034 and 155 ± 0.041 , respectively. The results indicated that all values were within the desirable and acceptable range of 50–200 nm for use in ocular drug delivery applications.

The PDI values, a key parameter for the stability, bulk characteristics, and performance of liposome nanoparticles, were determined for the MXF-free, the SL:MXF 1:1 ratio, and the SL:MXF 2:1 ratio liposome nanoparticles. For all formulations, PDI values ranging between 0 and 1—particularly those closer to zero—indicate a narrow size distribution, suggesting that the particles are uniformly distributed. This uniformity implies that all liposome nanoparticles are likely to encapsulate nearly equal amounts of drug. The narrow size distribution of liposome nanoparticles offers several advantages, including controllable and predictable drug release, high repeatability, a stable formulation, and more effective transport to the target.

The zeta potential values of all liposome nanoparticle formulations were determined to unveil their surface charge and stability. The zeta potential values for MXF-free liposome nanoparticles ranged between -37.2 and -56.6 mV. The high negative zeta potential values of MXF-free liposome nanoparticles consisting solely of SL can be attributed to the presence of negatively charged phosphate groups in lecithin. The zeta potential values for SL:MXF:1:1 liposome nanoparticles ranged between -21.9 and -32.8 mV and for SL:MXF:2:1 between -30 and -35.5 mV. The cationic amine groups

contained in MXF interacted with lecithin, slightly neutralizing the surface SL:MXF:2:1 liposome nanoparticles contain more phosphate groups, which causes the negative charge to become dominant. More negative charge means a more stable system.

The EE values for SL:MXF:1:1 ratio liposome nanoparticles range between 53.56 – 69.43 % while those for SL:MXF:2:1 ratio liposome nanoparticles range between 65.55 – 88.80%. The higher phospholipid content for SL:MXF:2:1 ratio liposome nanoparticles resulted in better EE. The structure of liposome nanoparticles increases the area surrounding the drug, which improves efficiencies.

SEM analysis was performed on freeze-dried liposome nanoparticles. Formulations prepared at a SL:MXF:1:1 ratio had an irregular, torn-paper-like structure, whereas those prepared at a SL:MXF:2:1 ratio exhibited a more uniform, granular structure. The morphological difference between these two groups of liposome nanoparticles can be attributed to their EE. A more uniform surface and consistent particle size may also support degree of monodispersity.

FTIR analysis was performed to investigate any potential chemical interactions between the drug and the lipid components. The primary absorption bands of MXF and SL were found to be preserved in the liposomal formulation, indicating no significant chemical interaction between them.

The hydrophobicity of liposome nanoparticles was determined by measuring contact angle. The CA value for all MXF-encapsulated constructs was less than 90 degrees, indicating hydrophilicity. The hydrophilic nature of the surface is due to the phosphate groups of lecithin and the presence of free hydroxyl groups. This contributes to easier dispersion in aqueous surroundings and increased biocompatibility with body fluids.

Both encapsulated and non-encapsulated liposomal nanoparticles were visualized using fluorescence microscopy. The presence of a fluorescent signal emitted from the liposomal nanoparticles indicates that the drug MXF was successfully encapsulated. Additionally, this analysis shows that the particle sizes correspond to the DLS results, indicating a homogeneous distribution without agglomeration.

Following an overall evaluation of the aforementioned analyses, the SL:MXF:2:1 ratio demonstrated superior performance compared to the SL:MXF:1:1 ratio in liposome nanoparticles. Using different amplitudes and sonication times revealed that the L7

liposome had optimal characterization results, including higher EE, favorable particle size (DLS), and stable zeta potential, within the SL:MXF:2:1 ratio liposome nanoparticles. Therefore, L7 was selected as the reference liposome. Thus, optimization of the liposome for incorporation in the hydrogel system was achieved.

Drug-encapsulated liposome nanoparticles were incorporated into hydrogels using two distinct approaches: micropipetting technique and the TMJ device method. The hydrogels produced by the TMJ device approach were thought to provide more controlled and prolonged drug release, and it was aimed to produce structures with uniform pore and size distribution. The prolonged treatment duration due to the low bioavailability of frequently used topical eye drops has led to the search for alternative solutions. For this reason, the approach of delivering drugs to the site of action by incorporating them into hydrogels made from biocompatible polymers was adopted. Incorporating drugs into liposome nanoparticles protects them against enzymatic degradation. However, this method may not ensure sufficient stability for controlled and sustained release. In this case, incorporating drug-encapsulated liposome nanoparticles in a hydrogel network increases their stability and improves drug bioavailability. Hydrogels are suitable for ophthalmic drug delivery systems due to their high water uptake capacity, controllable surface properties and biocompatible natures. The semi-solid nature of hydrogels provides flexible application convenience and helps maintain enzyme stability. Such stability is difficult to achieve in solid systems, whereas denaturation occurs in liquid systems. Considering that the systems in the body have a semi-solid formulation, the use of hydrogels is a kind of mimicking the natural system. Alginate polymer was chosen due to its biocompatibility, ease of availability, mucoadhesive properties, and its ability to readily undergo gelation in the presence of multivalent cations. By employing the probe sonication technique, MXF was successfully encapsulated in liposome nanoparticles and incorporated into alginate hydrogel coating structures for deposition on polymeric substrates. Embedding MXF-encapsulated liposome nanoparticles into the hydrogel system prolonged the release profile of the total drug content. Additionally, the presence of porous structures in hydrogels produced using the TMJ device method enables controllable and prolonged drug release. Drug release characteristics are directly impacted by structural characteristics. The drug release behavior was affected directly

by the encapsulation of MXF in liposome nanoparticles and their dispersion in an alginate hydrogel matrix.



6. FUTURE WORKS

Based on the findings of this research, several directions for future work are proposed as follows:

In this study, care was taken to avoid using water-based organic solvents when forming liposome nanoparticles. The use of solvents that are easily compatible with the body may facilitate liposome nanoparticle production.

An optical microscope or high-speed camera can be integrated into the microfluidic device. Although the droplets obtained remain stable for some time, integrating an optical microscope or high-speed camera into the device will facilitate the capture of high-quality images.

If a natural polymer other than alginate is to be used, its compatibility with microfluidic systems should be carefully evaluated. Polymers with low solubility or high viscosity may hinder the formation of uniform and continuous droplets.

The drying parameters and conditions of hydrogels containing drug-loaded liposome nanoparticles can be examined. This is because the preferred drying method directly affects drug release.

Investigating the antimicrobial kinetics of the obtained hydrogels, such as ocular irritation, adhesion and corneal permeation, brings us a step closer to applying them to the human body.

A systematic design of the device or method that eliminates the difficulties encountered in coating is required due to the very small surface area of intraocular lens haptics. This will enable more consistent and repeatable coatings.

To facilitate the commercialization of drug-loaded hydrogel-coated intraocular lenses (IOLs), comprehensive studies on maintaining optimal storage conditions throughout the entire period from formulation to end-use are essential.

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PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

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