

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

**HIGH-THROUGHPUT PRODUCTION OF DRUG-LOADED
MICROPARTICLES USING A MICROFABRICATED NOZZLE ARRAY**



Ph.D. THESIS

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Department of Molecular Biology-Genetics and Biotechnology

Molecular Biology-Genetics and Biotechnology Programme

JULY 2025

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

**MİKROFABRİKASYONLU NOZUL DİZİSİ KULLANILARAK İLAÇ YÜKLÜ
MİKROPARTİKÜLLERİN YÜKSEK KAPASİTELİ ÜRETİMİ**

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To my family,



FOREWORD

This thesis stems from my deep interest in controlled drug delivery and microparticle production technologies, as well as my strong motivation to contribute to this rapidly growing field. This work is not merely an academic research project but also a reflection of my scientific curiosity, determination, and learning journey. I hope that the findings and approaches presented here will inspire future research and make meaningful contributions to advancing drug delivery technologies.

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July 2025

Süleyman ÇELİK



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ABBREVIATIONS

BOE	: Buffered oxide etch
CDDS	: Controlled drug derlivery systems
CHL	: Chloramphenicol
DCM	: Dichloromethane
DMC	: Dimethyl carbonate
EDS	: Energy-dispersive X-ray spectroscopy
FDA	: Food and Drug Administration
FDA	: U.S. Food and Drug Administration
FESEM	: Field emission scanning electron microscopy
FIB	: Focused ion beam
FTIR	: Fourier-transform infrared spectroscopy
KOH	: Potassium hydroxide
LbL	: Layer-by-layer
MPS	: Microparticle production system
MPs	: Polymeric microparticles
MW	: Molecular weight
NPs	: Polymeric nanoparticles
OM	: Optical microscope
PC	: Polycarbonate
PDMS	: Poly(dimethyl siloxane)
PEG	: Polyethylene glycol
PGA	: Poly(glycolic acid)
PLA	: Poly(lactic acid)
PLGA	: Poly(D, L-lactide-co-glycolide)
PMMA	: Poly(methyl methacrylate)
PVA	: Polyvinyl alcohol
SEM	: Scanning electron microscope
W/O	: Water in oil
W/O/W	: Water in oil in water



SYMBOLS

μm : Micrometer

nm : Nanometer

kHz : Kilohertz

t : Time

°C : Celsius

Cl : Chlorine





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HIGH-THROUGHPUT PRODUCTION OF DRUG-LOADED MICROPARTICLES USING A MICROFABRICATED NOZZLE ARRAY

SUMMARY

Controlled drug delivery systems have emerged as a promising solution to the limitations associated with conventional therapeutic approaches. Traditional drug administration often leads to fluctuating plasma concentrations, resulting in reduced efficacy or undesirable side effects. In contrast, polymer-based microsphere systems allow for a more controlled, sustained release of active agents, improving therapeutic outcomes and patient compliance. Among these, biodegradable and biocompatible polymeric microparticles stand out for their ability to encapsulate and gradually release pharmaceuticals in a site-specific and time-regulated manner.

In this context, the present study introduces a novel Microparticle Production System (MPS), specifically designed to generate microparticles with high throughput, controlled size distribution, and consistent morphology. The system operates by integrating a microfabricated nozzle array and a piezoelectric transducer, enabling the atomization of polymer solutions into uniform microdroplets. These droplets are subsequently solidified via a drying process, forming solid microparticles. This system offers significant advantages in process control, scalability, and particle uniformity compared to conventional methods, making it particularly suitable for sensitive pharmaceutical applications.

The polymer selected for microparticle formation was poly(D, L-lactide-co-glycolide) (PLGA), a widely used material in drug delivery systems due to its biodegradable nature and tunable degradation profile. PLGA enables encapsulation of both hydrophilic and hydrophobic drugs and allows for controlled drug release over extended periods. In this study, PLGA was dissolved in two different organic solvents—dichloromethane (DCM) and dimethyl carbonate (DMC)—to investigate the impact of solvent volatility on particle morphology. The different evaporation rates of these solvents led to distinct outcomes in particle structure. DCM, being more volatile, induced rapid solvent removal, resulting in porous surfaces ideal for drug diffusion. On the other hand, DMC allowed for a slower evaporation process, producing smoother but smaller size-uniform particles.

Microparticles produced via the MPS system displayed a mean diameter of 8.9 ± 1.7 μm , reflecting excellent size uniformity across batches. The system was operated at a resonant frequency of 46.7 kHz, a setting optimized to ensure consistent atomization and droplet formation. Scanning electron microscopy (SEM) revealed differences in surface texture based on solvent choice. At the same time, focused ion beam (FIB) sectioning provided insights into the particles' internal lamellar and porous architecture. These analyses confirmed that the solvent evaporation dynamics strongly influenced both the surface and internal structures, thereby affecting the particles' mechanical integrity and drug release behavior.

To evaluate the drug loading and release performance of the MPS system, chloramphenicol (CHL) was used as a model active pharmaceutical ingredient. CHL

was first dissolved in ethanol and subsequently mixed with the PLGA-DCM solution to form a uniform polymer-drug mixture. This solution was then processed through the MPS system to yield drug-loaded microparticles. The resulting particles exhibited a loading efficiency of 16.2% and encapsulation efficiency of 38.7%, indicating the system's capability to successfully incorporate and retain drug molecules within the polymer matrix.

The presence of CHL within the microparticles was confirmed through energy-dispersive X-ray spectroscopy (EDS), which detected chlorine, a unique element found in CHL but absent in PLGA, in the particle composition. Fourier-transform infrared spectroscopy (FTIR) explored potential interactions between the polymer and the drug. While certain functional groups indicated minor physical interactions, no substantial changes in the PLGA backbone were observed, suggesting that CHL was physically incorporated without altering the polymer's chemical structure.

In vitro drug release studies were conducted over 100 hours in phosphate-buffered saline (PBS) at 37°C. The release profile followed a characteristic triphasic pattern: an initial burst phase due to the rapid dissolution of surface-bound drug, a slower sustained release phase governed by diffusion through the polymer matrix, and a final accelerated phase corresponding to the degradation of the PLGA structure. This triphasic behavior is highly desirable in controlled release systems, where early therapeutic concentrations followed by prolonged drug presence are often needed.

To assess the biological functionality of the drug-loaded particles, *Escherichia coli* (*E. coli*) cultures were exposed to four experimental groups: free CHL (positive control), plain PLGA particles, CHL-loaded PLGA particles, and a negative control containing only *E. coli*. Viability assays demonstrated that while the plain PLGA had no impact on bacterial growth, CHL-loaded PLGA microparticles significantly inhibited bacterial proliferation, particularly after 8 hours, coinciding with the time drug concentration became therapeutically effective. The free CHL group showed immediate inhibition, but the sustained activity offered by the CHL-loaded particles highlighted the controlled release functionality of the system.

The MPS system presents numerous practical benefits compared to conventional microparticle fabrication methods such as emulsion evaporation, spray drying, and microfluidics. Traditional techniques often struggle with size heterogeneity, drug loss, or low production rates. Moreover, heat-based methods like spray drying can degrade thermosensitive drugs. In contrast, the MPS system enables precise control over droplet size, does not require elevated temperatures, and offers scalability for industrial production. Its compatibility with various polymer and drug combinations further enhances its applicability.

The implications of this work extend beyond the scope of the current study. The MPS system is a viable platform for controlled antibiotic delivery and has potential applications in cancer therapy, vaccine formulation, gene delivery, and localized treatment of chronic conditions. Its ability to produce structurally stable, chemically inert, and biologically effective microparticles makes it suitable for systemic and localized drug delivery approaches. Furthermore, its modularity allows for future customization, such as the incorporation of targeting ligands or stimuli-responsive release mechanisms.

In summary, the study introduces a reliable, scalable, and efficient system for producing drug-loaded PLGA microparticles with highly desirable characteristics for controlled release applications. The integration of microfabrication and piezoelectric

atomization technologies enables the fine-tuning of particle size and morphology, while maintaining a high throughput. The system's success in encapsulating chloramphenicol and sustaining its release over an extended period demonstrates its potential in delivering pharmaceutical agents effectively and safely. With further optimization and adaptation to various therapeutic agents, the MPS platform may be a foundational technology in developing next-generation drug delivery systems.





MİKROFABRİKASYONLU NOZUL DİZİSİ KULLANILARAK İLAÇ YÜKLÜ MİKROPARTİKÜLLERİN YÜKSEK KAPASİTELİ ÜRETİMİ

ÖZET

Kontrollü ilaç salım sistemleri, modern tedavi yaklaşımlarında etkinlik, güvenlik ve hasta uyumu açısından ciddi avantajlar sunan yenilikçi teknolojilerdir. Geleneksel ilaç uygulamalarının çoğu, kandaki ilaç konsantrasyonunun hızlı iniş çıkışlarına sebep olmakta; bu durum terapötik etkilerin azalmasına veya toksik dozlara ulaşılmasına yol açabilmektedir. Bu nedenle, etkin maddenin hedef bölgeye zaman kontrollü şekilde taşınmasını sağlayan polimer bazlı mikropartiküller, farmasötik araştırmaların merkezinde yer almaktadır. Özellikle biyobozunur ve biyouyumlu özellik gösteren polimerler ile sentezlenen mikropartiküller, ilacın kontrollü salımını mümkün kılarak, hem farmakokinetik hem de farmakodinamik parametreleri optimize etme imkânı sunmaktadır.

Bu bağlamda, bu çalışmada tanıtılan Mikropartikül Üretim Sistemi (MPS), yüksek verimli, homojen yapıda ve kontrollü boyut dağılımına sahip mikropartiküllerin üretilmesine olanak tanıyan, mikroüretim teknolojilerine dayalı bir platformdur. Sistem, mikroışlenmiş püskürtme nozulu dizisinin piezoelektrik bir transdüserle entegre edilmesiyle çalışmakta, bu yapı sayesinde polimer çözeltisi mikrodamlacıklar hâlinde atomize edilerek parçacık oluşumu sağlanmaktadır. Oluşan damlacıklar, sistemin tasarlanan hava akışı ve kurutma düzenekleriyle katı partiküllere dönüşmekte, böylece geleneksel yöntemlere kıyasla daha tutarlı ve ölçeklenebilir bir üretim süreci gerçekleştirilmiş olmaktadır.

Çalışmada taşıyıcı polimer olarak, yaygın biçimde ilaç taşıma sistemlerinde tercih edilen Poli(D,L-laktid-ko-glikolid) (PLGA) kullanılmıştır. PLGA'nın parçalanabilirliği, salım süresini kontrol etme imkânı sunmakta; yüzey özellikleri, yükleme kapasitesi ve biyolojik bozunma profili sayesinde farklı farmasötik formlarda kullanılabilir. PLGA, burada farklı çözücülerle çözündürülerek püskürtme sistemine aktarılmış, bu çözücülerin buharlaşma hızlarının mikropartikül morfolojisi üzerindeki etkileri kapsamlı biçimde incelenmiştir.

DCM (diklorometan) ve DMC (dimetil karbonat) çözücülerıyla hazırlanan PLGA çözeltileri karşılaştırıldığında, çözücü seçiminin partikülün hem yüzey morfolojisini hem de iç yapısını doğrudan etkilediği belirlenmiştir. DCM'nin yüksek uçuculuğu sayesinde hızlı buharlaşma gerçekleşmiş ve bu durum partikül yüzeyinde belirgin gözenekliliğe yol açmıştır. Bu yapı, kontrollü salım açısından avantajlı bir mimari sunmaktadır. DMC ile hazırlanan partiküller ise daha düz yüzeyli ve gözeneksiz bir yapı sergilemiş, ancak partikül boyutları daha geniş bir dağılıma yayılmıştır. Bu durum, buharlaşma hızının partikül oluşumu sırasında damlacıkların yeniden koalesans (birleşme) eğilimini etkilediğini göstermektedir.

Geliştirilen sistemle üretilen partiküllerin ortalama boyutu $8.9 \pm 1.7 \mu\text{m}$ olarak ölçülmüş ve boyut homojenliği oldukça yüksek bulunmuştur. Bu değer, partikül temelli salım sistemlerinde istenen hedef aralıklar içerisinde yer almakta ve hedeflenebilir biyolojik sistemler için elverişli bir profil çizmektedir. Üretim sürecinde

sistemin rezonans frekansı 46.7 kHz olarak belirlenmiş ve atomizasyon işlemi bu frekans üzerinden gerçekleştirilmiştir. Üretilen mikropartiküller, taramalı elektron mikroskobu (SEM) ile analiz edilerek yüzey yapısı değerlendirilmiş; ayrıca odaklanmış iyon ışını (FIB) kesiti alınarak iç yapıları detaylı biçimde incelenmiştir.

Model ilaç olarak kullanılan kloramfenikol (CHL), geniş spektrumlu bir antibakteriyel ajandır ve sistemin ilaç yükleme ve salım performansını değerlendirmek amacıyla PLGA matrisine entegre edilmiştir. CHL, etanol içinde çözülerek DCM bazlı PLGA çözeltisine karıştırılmış, ardından MPS sistemi ile mikropartiküllere dönüştürülmüştür. Üretilen CHL-yüklü partiküllerde yükleme verimi %16.2, kapsülleme verimi ise %38.7 olarak hesaplanmıştır. Bu değerler, sistemin biyolojik ajanları taşıma kapasitesinin makul düzeyde olduğunu göstermektedir.

CHL'nin mikropartiküllere başarıyla yüklendiği, yapılan EDS (enerji dağılımı spektroskopisi) analizleriyle doğrulanmıştır. PLGA yapısında doğal olarak bulunmayan klor (Cl) elementinin CHL-yüklü partiküllerde tespit edilmesi, yüklemenin başarıyla gerçekleştiğinin kanıtı olarak sunulmuştur. Ayrıca FTIR spektroskopisi ile yapılan değerlendirmelerde, CHL'nin bazı işlevsel gruplarının PLGA matrisiyle fiziksel düzeyde etkileşime girdiği ancak yapısal bütünlüğü bozmadığı gözlemlenmiştir. Bu durum, ilaç-polimer etkileşiminin kimyasal bağlanma yerine fiziksel bütünleşmeye dayandığını, dolayısıyla ilaç stabilitesinin korunarak salım profilinin yönlendirilebileceğini göstermektedir.

CHL yüklü mikropartiküllerin in vitro salım profili, PBS içerisinde 100 saat boyunca takip edilmiştir. Salım eğrisi, üç fazlı klasik bir ilaç salım davranışı göstermiştir. İlk fazda, partikül yüzeyine yakın konumlanmış ilaçların hızlı çözünmesiyle gerçekleşen bir "burst" salım evresi gözlenmiştir. Bunu, partikül matrisinin içinden ilacın yavaş ve sürekli difüzyonu ile gerçekleşen ikinci faz izlemiştir. Son fazda ise PLGA polimer matrisinin su ile etkileşime girerek hidrolize olması sonucu matrisin bozulmasıyla birlikte, kalan ilacın hızla dışarı salındığı bir dönem meydana gelmiştir. Bu üçlü salım modeli, PLGA'nın parçalanabilir yapısının kinetiğe olan etkisini net biçimde ortaya koymaktadır.

Mikropartiküllerin biyolojik etkisi ise *Escherichia coli* (*E. coli*) bakterisi kullanılarak değerlendirilmiştir. Deney grupları arasında serbest CHL, sadece PLGA, CHL yüklü PLGA ve negatif kontrol (yalnızca bakteri) olmak üzere dört farklı koşul oluşturulmuştur. Bu deneysel düzende, CHL yüklü PLGA partiküllerinin bakteriyel büyümeyi baskıladığı gözlenmiş, özellikle 8 saat sonrasında ilaç salımının yeterli düzeye ulaşmasıyla birlikte bakteriyel çoğalmanın neredeyse tamamen inhibe edildiği belirlenmiştir. Serbest CHL'nin daha erken etki gösterdiği görülmüş olsa da, CHL yüklü partiküllerin daha uzun süreli bir antibakteriyel etkinlik sağladığı dikkat çekmektedir. Bu, sistemin sürdürülebilir ve kontrollü salım özelliklerini biyolojik bağlamda da doğrulamaktadır.

MPS sistemi, klasik üretim yöntemleriyle karşılaştırıldığında hem teknik hem de operasyonel açıdan birçok avantaj sunmaktadır. Özellikle spray drying gibi ısıya duyarlı ilaçlar açısından sakıncalı olabilecek yöntemlerin yerine, düşük sıcaklıkta çalışan ve yüksek hassasiyet sunan bir alternatif sağlamaktadır. Emülsiyon bazlı tekniklerde yaşanan boyut kontrolsüzlüğü, fazlar arası geçişlerde yaşanan dengesizlikler gibi problemler bu sistemde gözlenmemektedir. Ayrıca mikroakışkan çip tabanlı yöntemlere kıyasla daha yüksek verim ve operasyon kolaylığı sunmaktadır.

Sonuç olarak, geliştirilen MPS sistemi, ilaç yüklü mikropartikül üretiminde hem akademik araştırmalarda hem de farmasötik endüstride kullanılabilecek potansiyele sahip, ölçeklenebilir ve etkin bir teknolojik platformdur. Sistem, yüksek frekanslı atomizasyon mekanizması sayesinde kontrollü boyutlarda mikropartiküller üretmekte; çözücü seçimi, partikül morfolojisi ve ilaç yükleme-salım özellikleri üzerinde doğrudan etki sağlamaktadır. Bu yönüyle, özellikle hedefe yönelik tedaviler, uzun süreli salım sistemleri, aşı taşıyıcı yapılar ve lokal tedavi yaklaşımları gibi birçok alanda uygulanabilirliği mümkündür. Gelecekte, farklı polimer kombinasyonları, çoklu ilaç yükleme stratejileri ve biyomoleküllerin (DNA, RNA, protein vb.) taşınması gibi alanlara uyarlanarak sistemin fonksiyonel kapsamı daha da genişletilebilir.





1. INTRODUCTION

Recent advances in nanobiotechnology have introduced innovative materials to enhance controlled drug release; however, the demand for delivery systems with high efficiency and adjustable release profiles persists (Bai, Smith, Wang, Butterworth, & Tirella, 2022; Kalaydina, 2018). Among various strategies, the use of biodegradable polymeric microspheres has emerged as one of the most effective approaches for achieving sustained and controlled drug release. These microspheres provide significant flexibility by allowing drugs to be encapsulated within small carriers, thus enabling precise control over the duration and rate of release (Kapoor et al., 2015).

Poly(lactic-co-glycolic acid) (PLGA) is a highly researched biodegradable polymer. It can encapsulate various active agents, including small hydrophilic and hydrophobic molecules, as well as large biomolecules like proteins and DNA (Danhier et al., 2012; Jain, 2000; Su et al., 2021). Due to its excellent biocompatibility and its degradation products—lactic acid and glycolic acid, which are naturally metabolized in the body—PLGA is particularly appealing for biomedical uses (Danhier et al., 2012; Makadia & Siegel, 2011; Su et al., 2021). The release of drugs from PLGA microspheres generally takes place through a combination of polymer degradation and erosion of the matrix (Anderson & Shive, 1997; Freiberg & Zhu, 2004; Giteau, Venier-Julienne, Aubert-Pouëssel, & Benoit, 2008; Makadia & Siegel, 2011).

PLGA has received approval from international regulatory agencies such as the FDA and EMA for use in humans through various administration routes, including oral, injectable, and implantable systems, often without the need for surgical intervention (Danhier et al., 2012; Freiberg & Zhu, 2004; Giteau et al., 2008; Makadia & Siegel, 2011). This regulatory approval has opened the door for its widespread use across diverse medical applications. Nevertheless, several challenges still impede the broader implementation of PLGA-based delivery systems. These include low drug loading efficiencies, difficulties in fine-tuning release profiles, and stability issues during storage or after administration (Sheffey, Siew, Tanner, & Eniola-Adefeso, 2022; Su et al., 2021; Varde & Pack, 2004; Vashi, 2024). Overcoming these obstacles is essential

for improving the performance and reliability of controlled release technologies. Traditional drug administration methods, primarily based on repeated dosing, often cause significant fluctuations in plasma drug concentrations. These fluctuations can lead to delayed therapeutic responses or toxic side effects (Croitoru et al., 2024; Sheehan, Reilly, Fu, & Alphas, 2012). Additionally, conventional formulations typically distribute the drug non-specifically throughout the body, raising the risk of adverse systemic effects (D. Liu, Yang, Xiong, & Gu, 2016; Rana, Adhikary, Singh, Das, & Bhatnagar, 2023). To address these limitations, research efforts have increasingly focused on developing controlled drug delivery systems. These systems, typically made up of polymeric matrices loaded with active pharmaceutical ingredients, aim to localize the drug at the target site while maintaining therapeutic concentrations over extended periods (González, Ferrandez-Montero, & Domínguez-Robles, 2023). A key advantage of these platforms is their potential to minimize side effects, especially in orally administered drugs where systemic exposure can lead to undesirable interactions. Controlled systems also provide precise control over dosing and release kinetics, ultimately enhancing therapeutic efficacy (A. Pillai, Bhande, & Pardhi, 2023).

The kinetics of drug release from these systems are influenced by several factors, including the physicochemical properties of the polymer matrix (such as composition, molecular weight, crystallinity, porosity, and size), the characteristics of the encapsulated drug (such as diffusion coefficient and stability), and the physiological conditions at the site of action (such as pH and fluid dynamics) (Abbasnezhad et al., 2021; Dash, Murthy, Nath, & Chowdhury, 2010; Paarakh, Jose, Setty, & Peterchristoper, 2018). Despite extensive research, a comprehensive understanding of how these variables interact to affect drug release remains incomplete. Therefore, a deeper exploration of release mechanisms is essential for the rational design of optimized and patient-specific drug delivery platforms.

The advancement of drug delivery technologies has fundamentally reshaped modern pharmaceutical and biomedical sciences. As the demand for enhanced therapeutic efficacy, reduced toxicity, and improved patient compliance grows, the limitations of conventional dosing methods have become increasingly apparent. Issues such as frequent administration, unstable plasma drug levels, and inadequate targeting compromise both treatment outcomes and patient comfort, especially for drugs with

short half-lives or narrow therapeutic windows. In response, polymer-based controlled drug delivery systems have garnered considerable attention. Biodegradable polymeric microparticles, particularly those made from PLGA, stand out for their ability to encapsulate various drugs, protect them from degradation, and facilitate programmable release profiles (D. Liu et al., 2016; Su et al., 2021). Their versatility positions them as promising candidates for applications ranging from cancer treatment to chronic disease management and vaccine delivery.

PLGA's predictable degradation behavior, biocompatibility, and FDA approval contribute to its popularity in microparticle fabrication. By manipulating factors such as copolymer ratio, molecular weight, porosity, and surface area, it is possible to fine-tune the drug release properties of PLGA microparticles (D. Liu et al., 2016; Su et al., 2021). Nevertheless, the performance of these systems strongly depends on the uniformity, morphology, and size distribution of the particles, which are largely determined by the production method.

Despite the significant interest, conventional production techniques present ongoing challenges, including emulsion-solvent evaporation, spray drying, double emulsion (W/O/W), and microfluidic methods. These methods often yield particles with wide size distributions, low encapsulation efficiencies, and scalability issues (Lim et al., 2022; Su et al., 2021). Furthermore, the use of harsh solvents or high processing temperatures may negatively impact drug stability and microparticle quality, complicating the transition from research to industrial application (Otte, Soh, & Park, 2023).

To overcome these limitations, this thesis introduces and evaluates a novel Microparticle Production System (MPS) designed to enable the high-throughput fabrication of PLGA microparticles with precise control over size, morphology, and reproducibility. The MPS integrates a microfabricated nozzle array with a piezoelectric transducer, facilitating the ultrasonic atomization of polymer-drug solutions into uniform microdroplets, which are subsequently dried in flight to form stable microparticles. By carefully modulating parameters such as solvent properties, nozzle dimensions, and ultrasound frequency, this system offers improved control over particle formation.

In this thesis, PLGA was dissolved in two different solvents: dichloromethane (DCM) and dimethyl carbonate (DMC) to investigate the influence of solvent evaporation dynamics on microparticle morphology and structure. DCM, characterized by its low boiling point and high volatility, was compared with DMC, an environmentally safer alternative that has lower vapor pressure and a higher boiling point. This comparison aims to elucidate the impact of solvent properties on critical particle attributes such as porosity, internal architecture, and drug encapsulation efficiency.

Chloramphenicol (CHL), a broad-spectrum antibiotic, was chosen as a model drug to evaluate the encapsulation and functional performance of the fabricated microparticles. The microparticles were characterized using a comprehensive set of techniques, including scanning electron microscopy (SEM) to assess surface morphology, focused ion beam-scanning electron microscopy (FIB-SEM) for internal structure analysis, Fourier-transform infrared spectroscopy (FTIR) to investigate chemical interactions, and energy-dispersive X-ray spectroscopy (EDS) for studies on drug distribution.

Additionally, *in vitro* release studies were conducted in phosphate-buffered saline (PBS) to monitor the release kinetics of CHL over 100 hours, capturing essential phases of diffusion and degradation. The antimicrobial activity of the CHL-loaded microparticles was also evaluated using *Escherichia coli* (*E. coli*) as a model organism, verifying the biological efficacy of the system under controlled conditions.

Overall, this thesis aims to demonstrate the technical feasibility and therapeutic potential of the MPS platform as an alternative to conventional microparticle production methods. The proposed system not only offers scalable and tunable fabrication of polymeric drug carriers but also provides the potential for seamless translation to industrial-scale manufacturing, thereby advancing the field of controlled drug delivery technologies.

1.1 Aim and Scope of Thesis

The primary aim of this thesis is to develop and evaluate a novel microparticle production system designed to overcome the fundamental limitations of conventional microparticle fabrication methods used in controlled drug delivery applications. Traditional techniques, such as emulsion-solvent evaporation, spray drying, and

microfluidics, often suffer from drawbacks including broad particle size distributions, poor scalability, low encapsulation efficiency, and harsh processing conditions that may compromise drug stability. In response to these challenges, this thesis introduces a microfabrication-based approach that integrates a piezoelectric transducer with a microstructured nozzle array to achieve high-throughput, uniform, and tunable production of PLGA-based microparticles.

The scope of the thesis includes both the engineering aspects of system development and the pharmaceutical performance of the produced particles. Specifically, the research investigates how solvent selection affects the morphology and internal structure of microparticles, as these factors critically influence drug loading efficiency, release kinetics, and biological functionality. By using poly(D, L-lactide-co-glycolide) (PLGA) as the carrier polymer and chloramphenicol (CHL) as the model drug, the study allows for a comprehensive evaluation of the MPS platform for producing microparticles suitable for antibiotic delivery applications.

Characterization techniques such as scanning electron microscopy (SEM), focused ion beam (FIB) sectioning, Fourier-transform infrared spectroscopy (FTIR), and energy-dispersive X-ray spectroscopy (EDS) are used to evaluate particle size, surface morphology, internal structure, and chemical composition. Additionally, drug loading capacity and encapsulation efficiency are measured, and in vitro drug release studies are performed over a period of 100 hours to observe the release behavior of CHL from the PLGA microparticles. The antimicrobial efficacy of the drug-loaded microparticles is also assessed against *Escherichia coli* to validate the therapeutic potential of the produced microparticles.

This research ultimately aims to demonstrate the scientific and practical feasibility of the MPS platform as a scalable, repeatable, and low-temperature alternative to traditional methods. By providing fine control over particle formation and structural properties, the proposed system has the potential to advance the production of drug-loaded microparticles not only for antibiotics but also for a wide variety of therapeutic agents. The findings of this thesis are expected to contribute to the ongoing development of next generation drug delivery systems that combine precision engineering with pharmaceutical efficacy.

1.2 Literature Review

1.2.1 Controlled drug delivery systems

Maintaining drug plasma concentration within a defined therapeutic window in pharmacotherapy is critical for achieving optimal therapeutic outcomes (Buclin et al., 2020; Xiao, Xu, Qian, & Xu, 2024). This window, bounded by the minimum effective concentration and the minimum toxic concentration, ensures that the drug is present at levels high enough to be effective but not so high as to cause toxicity. Traditional drug administration methods, particularly those requiring frequent dosing, often lead to significant fluctuations in plasma drug levels. These fluctuations can cause the drug to fall below therapeutic levels or approach toxic concentrations, thereby reducing efficacy and increasing the risk of adverse effects (Johannessen Landmark, Johannessen, & Patsalos, 2020; Langer, 2001; Ohdo, 2003).

To overcome these challenges, controlled drug delivery systems (CDDS) have been developed to deliver active pharmaceutical ingredients (APIs) at predetermined rates for extended periods. These systems ensure a more stable plasma concentration, keeping drug levels within the therapeutic window, thereby reducing the frequency of administration and minimizing side effects (Albertini et al., 2015; Ramazani et al., 2015).

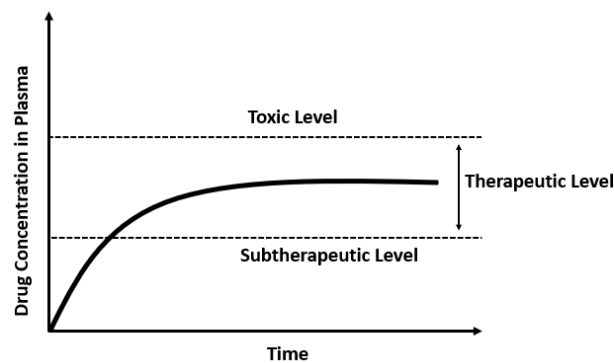


Figure 1.1: A schematic representation of drug plasma concentration over time for controlled drug delivery systems, highlighting the therapeutic window.

The provided figure (Figure 1.1) illustrates the typical plasma concentration time profile of a controlled drug release formulation. The vertical axis represents drug concentration of plasma, while the horizontal axis shows time. Following drug administration, the graph shows an initial rise in plasma drug levels, which gradually plateaus into a steady state concentration. This plateau remains within the therapeutic

window, defined by the dashed lines indicating subtherapeutic levels and toxic levels. Unlike the pronounced peaks and troughs often observed with conventional formulations, this profile demonstrates how controlled drug release maintains plasma concentrations within the therapeutic range, minimizing fluctuations and enhancing therapeutic efficacy.

Controlled release systems are widely used in medicine, pharmacy, agriculture, and environmental science, with medical and pharmaceutical applications being the most developed and prevalent (Langer & Peppas, 2003; Park, 2014). Their development has been supported by advances in polymer technologies, which have improved the stability, biocompatibility, and functionality of drug delivery platforms (Albertini et al., 2015; Anderson & Shive, 1997; Danhier et al., 2012; Langer & Peppas, 2003). The main objectives of CDDS include minimizing drug level fluctuations, improving patient compliance, and enhancing the efficiency and safety of therapy by targeting drug delivery to specific tissues (Visscher et al., 1985).

Microparticles and nanoparticles are commonly used carriers in controlled drug delivery systems (CDDS) (Kumari, Yadav, & Yadav, 2010). These systems encapsulate drugs in various forms, including microspheres, microcapsules, or microsphere structures, utilizing natural, modified natural, or synthetic polymers (Anderson & Shive, 1997). Microspheres, in particular, are matrix-based systems in which the drug is evenly dispersed throughout the polymer. In contrast, microcapsules feature a core-shell structure where the drug resides in a cavity surrounded by a polymer membrane (Berkland, King, Cox, Kim, & Pack, 2002). This structural difference significantly affects the drug release profile.

Among the most widely used polymers in CDDS is poly(lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible copolymer approved by the U.S. Food and Drug Administration (FDA). PLGA's tunable degradation rate, metabolic safety, and capability to encapsulate both hydrophilic and hydrophobic drugs make it a preferred material for sustained release formulations (Anderson & Shive, 1997; Makadia & Siegel, 2011; Visscher et al., 1985). PLGA-based microparticles can maintain drug concentrations within the therapeutic range for periods extending from days to several months, thereby enhancing therapeutic efficacy, reducing adverse effects, and improving patient adherence (Marquette et al., 2014; Wan et al., 2013; L. Wang et al., 2013).

Microparticles, defined as solid particles ranging from 1 to 1000 μm , serve as versatile carriers capable of encapsulating a wide array of therapeutic agents, including antibiotics, anticancer drugs, local anesthetics, proteins, antigens, and nucleic acid-based therapeutics such as antisense oligonucleotides and siRNA (Ahmed & Bodmeier, 2009; Alcalá-Alcalá, Urbán-Morlán, Aguilar-Rosas, & Quintanar-Guerrero, 2013; De Temmerman et al., 2012; Giteau et al., 2008; Huang, Li, Hong, & Yeh, 2013; Jain, 2000; Prémey et al., 2012). Their release characteristics can be tailored by adjusting parameters like particle size, polymer molecular weight, surface morphology, and the lactide to glycolide ratio (Do et al., 2015; Fredenberg, Wahlgren, Reslow, & Axelsson, 2011; Jain, 2000; Makadia & Siegel, 2011). Additionally, these systems are compatible with various routes of administration, including intra articular, parenteral, ocular, periodontal, oral, and pulmonary pathways (Cilurzo, Selmin, Minghetti, & Montanari, 2008; Danhier et al., 2012; He et al., 2006; Mountziaris et al., 2012; Mundargi et al., 2007; Stevanovic & Uskokovic, 2009).

The benefits of microparticle based CDDS include improved drug stability, extended drug action, reduced dosing frequency, enhanced bioavailability, and decreased toxicity. These factors contribute to better therapeutic outcomes and patient compliance (Freiberg & Zhu, 2004; Varde & Pack, 2004; Wischke & Schwendeman, 2008). In addition to pharmaceutical applications, microparticles also play vital roles in biomedical engineering, such as cell delivery, tissue engineering scaffolds, and 3D bioprinting applications (Anderson & Shive, 1997; Jain, 2000; Sarker et al., 2014; Su et al., 2021; Y. S. Zhang & Khademhosseini, 2017).

Although their advantages, production of microparticles presents certain challenges. Conventional methods, such as single or double emulsion solvent evaporation, spray drying, and microfluidic approaches, can lead to heterogeneous particle sizes, low encapsulation efficiencies, and potential degradation of sensitive compounds due to harsh processing conditions (Kandasamy & Naveen, 2022; Makadia & Siegel, 2011). To address these limitations, existing research focuses on improving fabrication techniques and exploring alternative, more sustainable materials and methods (Patil et al., 2023).

In conclusion, controlled drug delivery systems have altered modern therapeutics by providing sustained, localized, and programmable drug release profiles. These systems address the limitations of conventional drug administration and continuously change

with advances in polymer science, nanotechnology, and fabrication methods. Consequently, they hold great promise for precision medicine and the effective treatment of a wide range of diseases (Langer & Peppas, 2003; Park, 2014; Juergen Siepmann & Siepmann, 2012).

1.2.2 Drug release mechanisms and profiles

Controlled drug release systems are advanced biotechnological platforms that deliver therapeutic agents in a regulated routine, optimizing both the rate and duration of release. By preserving therapeutic drug concentrations over extended periods, these systems increase the efficacy and safety of pharmacological treatments. Among the materials used for controlled release, poly(lactic-co-glycolic acid) (PLGA) stands out because of its biocompatibility, biodegradability, and its ability to degrade without leaving toxic residues in the body (Kumari et al., 2010).

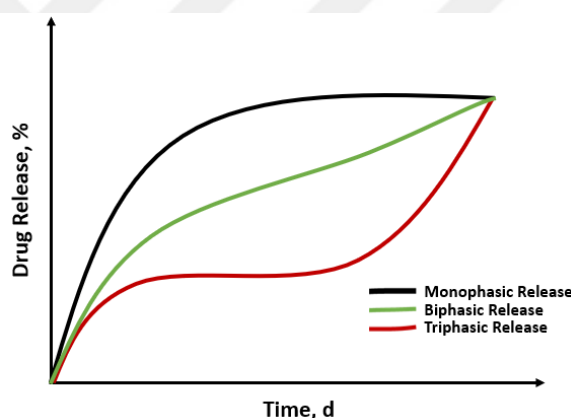


Figure 1.2: Schematic representation of three characteristic release profiles obtained from PLGA MP.

Depending on formulation parameters, fabrication techniques, and drug properties, drug release from PLGA microparticles typically exhibits monophasic, biphasic, or triphasic profiles (Figure 1.2). Although monophasic profiles, characterized by a continuous and uniform release, are rarely observed, biphasic and triphasic patterns are much more common (Fredenberg et al., 2011; Y. Xu, Kim, Saylor, & Koo, 2017). In biphasic release, an initial burst phase is followed by a slower, sustained release. However, the triphasic release profile is the most frequently encountered and consists of three distinct stages: (I) an initial burst release of surface-associated drug molecules, (II) a diffusion-controlled sustained release phase through the polymer matrix, and (III) an accelerated release phase linked to the onset of polymer degradation and

erosion (Fredenberg et al., 2011; Nair & Laurencin, 2007; Y. Xu et al., 2017). Microparticles produced by solvent extraction/evaporation methods often display a triphasic profile, starting with a sharp initial burst, followed by a lag phase with minimal release, and culminating in rapid drug liberation due to polymer matrix breakdown (Pagels & Prud'Homme, 2015; J. Wang, Wang, & Schwendeman, 2002; Y.-Y. Yang, Chung, & Ng, 2001).

Drug release from PLGA microparticles typically follows a triphasic release pattern, which consists of an initial burst release, a diffusion-controlled sustained release phase, and a final accelerated release associated with polymer degradation (Fredenberg et al., 2011; Nair & Laurencin, 2007). Each of these stages is governed by distinct physicochemical processes, and understanding these mechanisms is critical for optimizing therapeutic outcomes and designing effective delivery systems.

The initial burst release phase is characterized by the rapid release of drug molecules located near or at the particle surface immediately after administration. This phenomenon is mainly attributed to the weak physical entrapment of the drug in the outer regions of the microparticle and the presence of surface pores. While burst release can effectively achieve therapeutic drug levels quickly, excessive initial release may lead to undesired systemic toxicity. Factors influencing burst release include particle size, surface porosity, drug distribution, and the selection of organic solvent during fabrication (Allison, 2008; Angelova & Hunkeler, 1999; Sah, 1999; Silva, Cardoso, e Silva, Freitas, & Sousa, 2015).

After the initial burst, the drug release enters the diffusion controlled phase, through which the encapsulated drug slowly diffuses through the polymer matrix and any aqueous-filled pores that develop over time. The diffusion rate is influenced by the polymer's hydrophilicity, porosity, and degradation rate, along with the physicochemical properties of the drug itself, including its molecular weight and solubility. This phase is essential for maintaining sustained therapeutic drug levels over extended periods, reducing the need for repeated dosing, and improving patient compliance. The final phase involves an accelerated release due to polymer degradation, also known as bulk erosion. As PLGA hydrolytically degrades into lactic and glycolic acid monomers, the polymer matrix loses its structural integrity, causing the collapse of the microparticle framework and the rapid release of any remaining drug. The degradation rate of PLGA, and consequently the timing of this release phase,

can be accurately adjusted by modifying the polymer's molecular weight, the lactide to glycolide ratio, and the microparticle's internal architecture (Lu, Garcia, & Mikos, 1999; Makadia & Siegel, 2011; Nair & Laurencin, 2007; J Siepmann & Göpferich, 2001).

The mechanisms governing drug release from PLGA systems are complex and typically involve one or more dominant processes, such as diffusion, erosion, swelling, osmosis, and stimuli-responsive behaviors (Göpferich, 1996). In diffusion-controlled systems, the active pharmaceutical ingredient (API) is either encapsulated within a reservoir type device or embedded in a polymeric matrix. The driving force for drug release in these systems is the concentration gradient between the system's interior and the surrounding medium. These systems typically rely on Fick's law of diffusion, and the release rate is strongly influenced by the drug's diffusivity, the polymer matrix's nature, and its porosity (Lee & Yeo, 2015). Some diffusion based systems exhibit an initial burst release phase, particularly when no diffusion limiting barrier exists around the matrix, which may be advantageous or disadvantageous depending on the therapeutic application.

Osmotic controlled drug delivery systems utilizes a semi permeable polymer membrane that surrounds the drug core. Water from the external environment enters the system due to differences in osmotic pressure, dissolving the drug and enabling its release through a pre formed osmotic hole. This mechanism has been shown to deliver zero order release kinetics under optimized conditions. For instance, osmotic systems have been successfully applied in the design of oral controlled release tablets, which enable prolonged therapeutic effects with minimal fluctuations in plasma drug concentration (Venkateswarlu et al., 2019; Verma, Krishna, & Garg, 2002).

In swelling-controlled release systems, a hydrophilic polymer matrix absorbs water upon contact with biological fluids, which leads to volumetric expansion and subsequent drug release. The release kinetics in such systems depend on the rate of water uptake and the relaxation of polymer chains, which together modulate drug diffusion. These mechanisms are often utilized in hydrogels and matrix-based oral dosage forms. The interplay between the swelling front and the erosion front is a crucial determinant of the release profile (Peppas, Bures, Leobandung, & Ichikawa, 2000).

Degradation controlled release systems are extensively used in modern drug delivery applications, particularly with biodegradable polymers like PLGA. In these systems, the polymer matrix undergoes hydrolytic or enzymatic degradation, resulting in the gradual release of the encapsulated drug. The degradation rate can be adjusted by changing polymer composition, molecular weight, and crystallinity. These systems are especially beneficial for long term drug delivery, as they eliminate the need for medical removal of the carrier matrix. Recent research has emphasized the significance of microstructural properties and polymer drug interactions in influencing the release behavior of biodegradable systems (D'Souza & DeLuca, 2006; Fredenberg et al., 2011; Fu, Pack, Klibanov, & Langer, 2000; Zolnik & Burgess, 2008).

Stimuli responsive drug delivery systems represent a newer class of mechanisms in which the release of the drug is triggered by specific internal (e.g., pH, enzyme levels, redox potential) or external stimuli (e.g., temperature, light, magnetic field). These systems provide spatial and temporal control over drug release, which is crucial in targeted cancer therapies and regenerative medicine. For instance, recent developments in pH sensitive micelles and nanoparticles have facilitated the selective release of chemotherapeutic agents in the acidic microenvironment of tumors, thereby reducing systemic toxicity (Bae & Park, 2011; Balamuralidhara et al., 2011; D. Liu et al., 2016; X. Zhang et al., 2015).

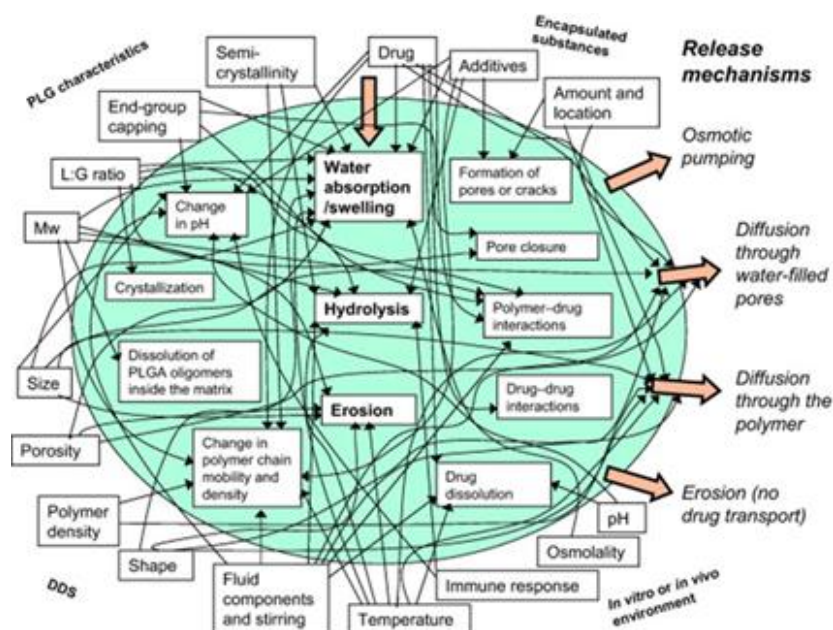


Figure 1.3: Controlled drug release systems: mechanisms and parameters (Fredenberg et al., 2011).

Fredenberg et al. (2011) conducted an extensive study on the mechanisms that control drug release from controlled-release systems, focusing specifically on diffusion, erosion, and swelling as the primary mechanisms of release. Their research presents a clear and organized depiction of how these mechanisms function within polymer-based delivery systems. Moreover, the study emphasizes the significant impact of various factors, including polymer characteristics such as molecular weight, crystallinity, and porosity, along with external environmental conditions like pH, temperature, and enzymatic activity, on the kinetics of drug release. The visual schema introduced in the study effectively illustrates the intricate relationship between formulation elements and release behavior, providing crucial insights for designing and optimizing controlled drug delivery systems (Fredenberg et al., 2011).

Despite significant progress, a complete understanding of how these mechanisms jointly influence drug release kinetics remains challenging. This complexity stems from the interaction of various factors, such as the physicochemical characteristics of the drug, the structural features of the polymer matrix (like composition, molecular weight, crystallinity, and porosity), along with the environmental conditions at the delivery site (including pH and fluid dynamics). Gaining deeper understanding of these elements is essential for the informed design of next-generation drug delivery systems that can offer personalized therapies based on each patient's needs.

1.2.3 Polymeric microparticles and PLGA

Polymeric microparticles have gained significant attention in recent decades as essential components in the development of advanced drug delivery systems. Their ability to encapsulate a wide range of therapeutic agents, from small-molecule drugs to peptides, proteins, and nucleic acids, has established them as a foundational technology in pharmaceutical sciences. These systems are primarily designed to control the spatial and temporal release of active pharmaceutical ingredients (APIs), thereby enhancing therapeutic efficacy, reducing systemic side effects, and improving patient compliance (Makadia & Siegel, 2011).

The effectiveness of polymeric microparticles in controlled drug delivery stems from their versatile physicochemical properties, including customizable size, morphology, porosity, surface chemistry, and biodegradation profiles. This tunability allows for the creation of carriers tailored to specific disease targets, administration routes, and pharmacokinetic requirements (Danhier et al., 2012).

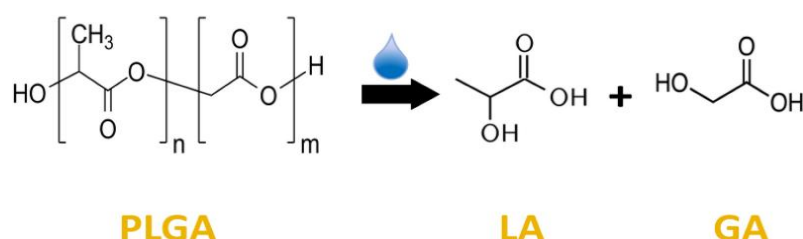


Figure 1.4: The chemical structure of PLGA polymer consists of hydrolytically cleaved lactic acid (LA) and glycolic acid (GA) monomers (Rapier, Shea, & Lee, 2021).

Among the various classes of polymers used for microparticle fabrication, biodegradable polymers stand out for their ability to break down into nontoxic metabolites and their compatibility with biological systems. Particularly, poly(lactic-co-glycolic acid) (PLGA) has emerged as the gold standard for microparticle-based drug delivery. PLGA is a synthetic, aliphatic copolymer made of lactic acid and glycolic acid monomers, which are metabolized through natural pathways in the human body, specifically the Krebs cycle, and then excreted as carbon dioxide and water (Anderson & Shive, 1997). The biodegradability and biocompatibility of PLGA have garnered approval from major regulatory agencies like the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), resulting in its widespread use in both parenteral and localized drug delivery platforms.

One of the most striking features of PLGA is its customizable degradation kinetics, which can be modulated by altering the molar ratio of lactic acid to glycolic acid, the polymer molecular weight, and the terminal group chemistry (Jain, 2000). For example, increasing the lactic acid content or the molecular weight typically results in slower degradation, while a higher glycolic acid content accelerates hydrolysis. Additionally, PLGA can be functionalized or blended with other polymers, surfactants, or inorganic materials to further enhance its mechanical strength, hydrophilicity, and drug release behavior. The hydrolytic degradation of PLGA occurs through bulk erosion, where water penetrates the polymer matrix, leading to random chain scission and a gradual decrease in molecular weight before mass loss (Fredenberg et al., 2011).

This degradation mechanism allows for the design of drug release profiles that range from hours to several months, making PLGA microparticles suitable for both acute and chronic treatments.

PLGA microparticles can be synthesized using various methods, each having unique advantages and drawbacks. The emulsion solvent evaporation technique is the most frequently utilized approach due to its scalability, simplicity and applicability to a wide range of drug types. In this process, PLGA is dissolved in an organic solvent, along with the drug, and then emulsified in an aqueous phase containing stabilizers such as polyvinyl alcohol (PVA). Solid microparticles form upon evaporating the solvent. Other new fabrication techniques, including spray drying, microfluidics, electrospraying, and membrane emulsification, allow for better control over particle size, uniformity, and internal structure (X. Li et al., 2024). Particularly, microfluidic and MEMS based nozzle technologies have created new opportunities for generating uniform, structurally advanced microparticles, thus addressing the constraints of traditional batch methods (Çelik, Çelik, Koşar, & Kılıç, 2025).

The clinical significance of PLGA microparticles is evident from various commercial products. For example, Lupron Depot®, which treats prostate cancer and endometriosis, provides leuprolide acetate over several months via PLGA microparticles. Likewise, Risperdal Consta® serves as an extended release formulation of risperidone for schizophrenia treatment. These instances not only illustrate the practical use of PLGA based microparticles but also their ability to improve therapeutic results through controlled release strategies (Zolnik & Burgess, 2008).

In spite of several advantages, PLGA based microparticle systems encounter specific challenges. The initial burst release, resulting from drug accumulation at the particle surface, can cause suboptimal pharmacokinetics or local toxicity. Furthermore, acidic degradation products may lead to local inflammation or compromise the stability of acid-sensitive biomolecules. These challenges have led to the investigation of strategies like co-polymerization with neutralizing agents, surface modification, and the incorporation of pH buffers to improve formulation stability and biocompatibility (Kumari et al., 2010).

Polymeric microparticles are present a mature yet continually evolving class of drug delivery systems. With their unique combination of biocompatibility, biodegradability, and customizable properties, PLGA microparticles play a vital role in developing controlled release systems that tackle intricate therapeutic issues. As the drug development landscape increasingly prioritizes precision, sustainability, and patient-focused results, enhancing PLGA microparticles through innovations in materials science and microengineering will continue to lead pharmaceutical advancements.

1.2.4 Conventional fabrication techniques and limitations

In the past few decades, several conventional fabrication methods have been developed and improved for producing polymeric microparticles, particularly for controlled drug delivery applications. These techniques are typically categorized based on the characteristics of the solvent systems and the emulsification types used. Frequently employed methods include phase separation, single and double emulsification, microfluidic emulsification, lithographic patterning, electrodynamic spraying, and mechanical fragmentation.

The production of drug loaded microparticles is a core element of designing controlled drug delivery systems (DDS), enabling sustained and regulated release of therapeutic agents over extended periods. This controlled release not only boosts therapeutic effectiveness but also decreases the administration frequency and limits systemic side effects. While traditional fabrication methods are widely used and studied in pharmaceutical and biomedical fields, challenges remain, particularly concerning scalability, inter-batch consistency, and accurate modulation of drug release rates. These challenges highlight the continuous need for innovation and refinement in microparticle fabrication technologies to address the growing requirements of advanced drug delivery systems.

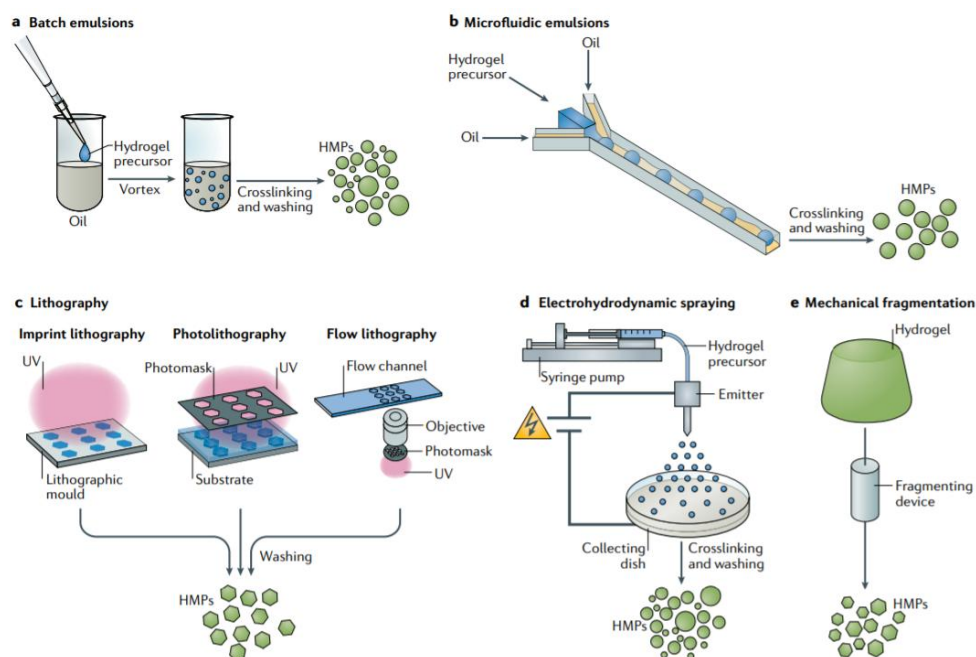


Figure 1.5: Conventional microparticle producing methods (Daly, Riley, Segura, & Burdick, 2020).

Figure 1.5 shows that microparticles can typically be produced using five different methods: phase separation, emulsification, microfluidic emulsification, lithography, electrodynamic spraying, and mechanical fragmentation (Daly et al., 2020). Apart from microfluidic and lithographic techniques, it's usually challenging to create uniform and size controllable microparticles. However, the other methods might be more applicable for industrial scale production. In the meantime controlling particle size is vital in many applications, microfluidic technology is often favored. However, its inherently low throughput makes it unsuitable for large scale producing. Although lithography based techniques offer flexibility, their complexity and high cost make them less ideal for mass production.

Traditional methods for producing PLGA-based microparticles primarily include single or double emulsion solvent evaporation, spray drying, and microfluidics. While these techniques offer several advantages, they also present unique challenges that may affect the quality, reproducibility, and scalability of microparticles designed for drug delivery applications.

Single emulsion solvent evaporation method (O/W) technique is widely utilized for encapsulating hydrophobic drugs, including steroids and anti inflammatory agents. Initially, the hydrophobic drug is dissolved in a volatile organic solvents together with a polymer like PLGA. This organic phase is then emulsified into an aqueous solution

containing a surfactant, typically poly(vinyl alcohol) (PVA). Following solvent evaporation, microparticles form and are subsequently collected. The size of the particles and their encapsulation efficiency can be adjusted by modifying process parameters such as polymer concentration, emulsifier concentration, and stirring speed (Jain, 2000; Wischke & Schwendeman, 2008).

Double emulsion solvent evaporation method (W/O/W) approach is ideal for encapsulating water soluble drugs and delicate biological compounds, like proteins, peptides, and nucleic acids. The process starts by emulsifying an aqueous drug solution into a polymer-containing organic solvent to create a water-in-oil (W/O) emulsion. This initial emulsion is then re-emulsified in a second aqueous phase to produce a water-in-oil-in-water (W/O/W) emulsion. The organic solvent is subsequently eliminated through evaporation, resulting in drug loaded microparticles. While this method facilitates the encapsulation of hydrophilic molecules, it frequently faces challenges with low encapsulation efficiency due to drug leakage that occurs during the emulsification and solvent removal processes (Bilati, Allémann, & Doelker, 2005; Jain, 2000; Sinha & Trehan, 2003).

Spray drying involves atomizing a polymer drug solution or suspension into a heated chamber through a nozzle system. As the solvent quickly evaporates, solid microparticles are formed. This method provides high throughput and is ideal for large scale industrial production. However, the process's high temperatures and shear forces possibly will damage sensitive molecules, and maintaining uniform particle size and morphology can also present difficulties (Bodmeier & McGinity, 1987).

Phase separation (coacervation) technique separates a polymer rich phase from a polymer lean phase by introducing a non solvent or altering environmental factors like temperature or pH. The drug is embedded within the polymer rich phase, which subsequently precipitates to create microparticles. While phase separation can achieve high encapsulation efficiencies, it frequently necessitates toxic solvents and does not provide precise control over particle morphology (Gharanjig & Farzi, 2023; Wei et al., 2024).

Microfluidic systems provide precise control over particle size and uniformity through the generation of droplets via micro-scale channels. These techniques enable the production of highly uniform microparticles with adjustable sizes and structures,

making them especially effective for encapsulating multiple drugs or achieving controlled release profiles. Nevertheless, the throughput of microfluidic systems tends to be low, which restricts their scalability for industrial use (Amoyav & Benny, 2019; Teh, Lin, Hung, & Lee, 2008).

Additional techniques comprise electrospraying, mechanical fragmentation, and lithography methods. Electrospraying produces particles with narrow size distributions; however, it faces challenges like low production rates and possible electrical hazards. Mechanical fragmentation entails reducing bulk materials into smaller particles, usually leading to polydispersity. Lithography based techniques can create microparticles with precise shapes but are complicated and costly, constraining their practical use.

The traditional methods for microparticle production have lots of significant drawbacks:

- Polydispersity: Emulsion based methods, such as single and double emulsions, often result in a wide particle size distribution. This heterogeneity can lead to variable drug release profiles, reduced therapeutic efficacy, and inconsistent pharmacokinetics (Freiberg & Zhu, 2004).
- Drug instability and poor encapsulation efficiency: Especially for hydrophilic and sensitive compounds, the high shear forces and interfacial stresses present during emulsification may cause drug denaturation or leakage into the external aqueous phase, resulting in reduced encapsulation efficiency (Bilati et al., 2005).
- Use of toxic solvents: Many conventional methods depend on volatile organic solvents such as dichloromethane and chloroform, which raise safety and environmental concerns. Residual solvents in the final product can be toxic and require additional purification steps to comply with regulatory standards (Mirmoghaddam, Kaykhani, & Yahyavi, 2015).
- Scalability issues: While spray drying is scalable, it often lacks precise control over particle size and internal structure. In contrast, microfluidic methods offer excellent control over particle characteristics but are constrained by low production rates, rendering them unsuitable for large-scale manufacturing.

- Variability between batches and inconsistent reproducibility: Conventional techniques frequently experience fluctuations in process parameters, resulting in differing particle characteristics across various batches. This inconsistency can obstruct clinical application and regulatory clearance (Makadia & Siegel, 2011).

The rate of drug release is closely tied to microparticle size. Smaller microparticles, having a greater surface area relative to their volume, generally release their drug content more quickly. As a result, the early stage of drug release is primarily influenced by these smaller particles, leading to a burst release effect. Over time, as larger particles increasingly contribute, the release rate tends to decrease. This highlights the vital role of creating monodisperse microparticles in controlled drug delivery systems. Maintaining uniform particle size not only promotes consistent pharmacokinetic profiles but also improves the predictability of therapeutic outcomes.

In addition, traditional fabrication techniques come with further limitations. Emulsion based methods frequently lead to wide particle size distributions, poor encapsulation efficiencies, and involve complicated emulsification processes that are difficult to scale (L. Wang et al., 2013; Zeigerson, 2007). Although spray drying is appropriate for large scale production, it exposes formulations to elevated temperatures, which can harm thermosensitive drugs (Ameri & Maa, 2006). Microfluidic systems offer accurate size control and uniformity but are generally constrained by low throughput and demand advanced fabrication and operation (J. Wu, Yadavali, Lee, & Issadore, 2021).

Other techniques, including coacervation and phase separation, are used for encapsulating proteins and peptides. However, these approaches frequently depend on organic solvents and require precise control of pH and temperature to ensure drug stability, which introduces additional complexity to the production process.

Additionally, many conventional methods involve toxic solvents and labor intensive protocols, failing to provide acceptable control over the internal structure of microparticles. The internal structure plays a crucial role in drug release profiles, underscoring the need for better fabrication methods.

Despite significant advancements, traditional fabrication techniques for PLGA-based microparticles are still inadequate for fully addressing the needs of controlled drug delivery applications. A clear demand exists for innovative fabrication technologies that provide high-throughput, monodisperse particle production with adjustable structures, while ensuring drug stability and facilitating industrial scalability.

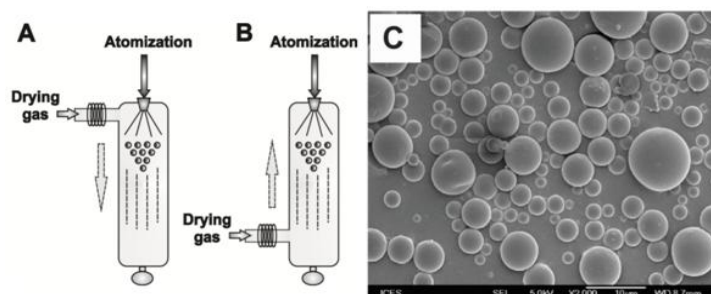


Figure 1.6: Atomization-based microparticle production system (A, B) and produced polydisperse microparticles (Sosnik & Seremeta, 2015).

The research by Seremeta et al. shows that the polymeric microparticles created with the spray drying technique were not monodisperse, leading to different drug release profiles (Sosnik & Seremeta, 2015). The variation in particle sizes can lead to an initial burst of drug release from smaller particles, followed by an extended release from larger ones. This variability highlights the necessity of obtaining uniform particle sizes to guarantee consistent and predictable therapeutic results.

Recent progress in microfabrication, microfluidics, and MEMS technology has facilitated the creation of cutting-edge microparticle fabrication systems that address the constraints of traditional techniques. These advanced systems provide improved control over particle size, shape, and internal structure, allowing for the design of advanced drug delivery platforms customized for particular therapeutic uses (Zafar et al., 2024).

This thesis presents the development of an advanced microparticle production system that integrates microfluidic and microporous membrane technologies. The innovative platform is designed for the simultaneous operation of multiple units, enabling high throughput production of polymeric microparticles with as similar in size as possible size distributions, customizable internal structures, and scalable outputs suitable for industrial use. Microfabrication methods were utilized to create microporous array structures, facilitating the atomization of polymer drug solutions into monodisperse microdroplets through acoustic and ultrasonic wave application.

One of the system's key features is the solidification of droplets during time of flight, controlled by solvent evaporation and the airborne trajectory duration. This process leads to the creation of solid microparticles with distinct morphologies. By carefully adjusting crucial parameters like waveform type, outlet pressure, and pore diameter, fine control was achieved over the resulting microparticles' size, surface properties, and structural integrity.

This innovative production method effectively overcomes several intrinsic drawbacks of traditional particle fabrication techniques, especially in terms of scalability, structural consistency, encapsulation efficacy, and process repeatability. Additionally, the system created in this research offers a strong and scalable solution for producing advanced drug delivery vehicles on an industrial scale, greatly enhancing production efficiency while maintaining precise control over the properties of the particles. As a result, it shows great potential in fulfilling the changing requirements of contemporary controlled drug delivery technologies.

This innovative manufacturing technology is projected to greatly improve production efficiency while ensuring accurate control over particle morphology and size. As a result, the suggested approach is expected to provide a distinctive and scalable solution for the industrial scale creation of polymeric drug delivery systems.

1.2.5 Advances in microfabrication for microparticle production

Atomization, which involves converting a liquid into an aerosol, is a widely used technique valued for its versatility and efficiency across many fields. Its applications include spray drying (Carné-Sánchez, Imaz, Cano-Sarabia, & MasPOCH, 2013), mass spectrometry, spray coating (Awais et al., 2013), drug delivery (de Heij, van der Schoot, Bo, Hess, & de Rooij, 2000), advanced manufacturing (Sharma & Jackson, 2021a, 2021b), and dry powder formulation (Foerster, Gengenbach, Woo, & Selomulya, 2016). Typically, traditional atomization methods utilize compressed air or pressure to create droplets. Extensive research has been conducted on the mechanisms, flow patterns, and spray structures of air pressure atomizers, yielding valuable insights into their functionality and performance across different applications (Qiao, Qian, Zhu, Shao, & Wang, 2021; P.-K. Wu, Kirkendall, Fuller, & Nejad, 1998; X. M. Yang, Chen, Li, Liu, & Zhao, 2011).

In addition to air pressure-based systems, ultrasonic atomization has gained significant attention, particularly from high-frequency ultrasound transducers operating in the fundamental vibration mode. These devices utilize membrane oscillations to break liquid films into droplets through cavitation. The ultrasonic vibrations generate micro shock waves and surface tension waves beneath the liquid film, causing droplets to detach from the liquid and resulting in a broad distribution of aerosol droplet sizes. The dynamics of ultrasonic atomizers have been extensively studied both experimentally and numerically, with research focusing on their efficiency and the impact of various parameters on droplet size distribution (Deepu, Peng, & Moghaddam, 2018; Y. Li & Umemura, 2014; R. Pillai, Borg, & Reese, 2018; W.-N. Wang et al., 2008; Y. Zhang, Yuan, & Wang, 2021).

However, despite the extensive research and application of these atomization methods, certain limitations persist. These limitations include high energy consumption, complicated setup requirements, low selectivity for various liquids, and limited control over droplet size distribution. Consequently, atomizers frequently produce non-uniform and larger droplets, which can negatively affect their efficacy in certain applications. Therefore, the continued development of more energy-efficient, precise, and versatile atomization techniques is vital for addressing these issues and enhancing the performance of atomization-based systems across multiple fields.

In contrast, advanced MicroParticle Systems (MPS) effectively address these limitations. Utilizing MEMS based micronozzle arrays, the system ensures more accurate droplet generation while enhancing uniformity and efficiency. These innovations tackle the challenges of traditional atomization methods, providing improved control over droplet size distribution and increased adaptability to different liquids. The successful outcomes achieved with these cutting edge systems demonstrate their potential for more efficient and customized applications in drug delivery and various other fields, representing a significant progression beyond conventional atomization techniques.

In response to these challenges, recent research has concentrated on microfabrication methods that integrate microscale engineering with precise control of particle generation. Techniques like piezoelectric actuation, ultrasonic atomization, and inkjet-based droplet formation have demonstrated potential for creating monodisperse microparticles with consistent characteristics (Teh et al., 2008; Q. Xu et al., 2009).

Combining microfabricated nozzle arrays with ultrasonic transducers provides an efficient solution for scalable, low-temperature atomization of polymer-drug solutions. In this setting, piezoelectric vibrations cause quick and even droplet breakup, resulting in fine droplets that turn into microparticles when the solvent evaporates. These systems enable control of droplet size by adjusting operational parameters like excitation frequency, nozzle design, and solution viscosity (Maa, Nguyen, Sweeney, Shire, & Hsu, 1999).

This method is particularly effective for creating biodegradable microparticles because it minimizes the requirement for high thermal energy and lowers the risks associated with solvent toxicity. Nonetheless, the use of this approach for producing PLGA microparticles intended for drug delivery is still restricted, highlighting the need for additional research and refinement.

1.2.6 Solvent selection and its role in particle morphology

The choice of solvent is a critical factor in determining microparticle morphology, especially concerning surface porosity, internal lamellar structure, and particle integrity. Solvent characteristics such as volatility, solubility in aqueous phases, and evaporation rate directly impact the dynamics of droplet solidification and polymer precipitation (Freiberg & Zhu, 2004). For example, dichloromethane (DCM) is frequently utilized as a solvent in PLGA processing because of its high volatility and effective polymer solubility. It evaporates quickly, often resulting in porous structures that can improve drug release. On the other hand, dimethyl carbonate (DMC) has a higher boiling point and evaporates at a slower pace, which generally leads to smoother and denser particle surfaces. Comparative research indicates that slower solvent removal can decrease internal porosity but may also influence drug diffusion and release kinetics (Crotts & Park, 1998).

The selection of organic solvents plays a crucial role in the safety and physicochemical characteristics of PLGA microparticles. Recent developments promote the adoption of green solvents like ethyl acetate, dimethyl carbonate, and supercritical CO₂ to create safer formulations (Hines & Kaplan, 2013; Mehrabani, Vatanpour, & Koyuncu, 2022). Porogen agents like sodium chloride (NaCl), ammonium bicarbonate, and pluronics are essential for controlling particle porosity, thereby influencing drug release profiles.

These porogens can be categorized by their operational mechanisms: osmotic agents facilitate pore creation through osmotic pressure, gas-foaming agents produce pores by releasing CO₂, and extractable porogens are eliminated after processing to form voids (Dastidar, Saha, & Chowdhury, 2018; Tang et al., 2012).

1.2.7 Chloramphenicol as a model drug

Chloramphenicol (CHL) is a broad spectrum antibiotic that prevents bacterial protein synthesis by attaching to the 50S ribosomal subunit (Davies & Davies, 2010). It is frequently utilized as a representative drug in formulation research because of its well established antimicrobial properties, UV-Vis detectability, and moderate solubility in organic solvents. CHL has been encapsulated in different polymeric carriers to assess drug loading efficiency, release profiles, and retention of bioactivity (Mandal et al., 2009). In this context, CHL serves as a dependable tool for evaluating the encapsulation and release behavior of PLGA microparticles made by innovative fabrication systems. Additionally, its antibacterial properties against *E. coli* provide a simple platform for assessing the biological functionality of drug delivery formulations in vitro.

1.2.8 Summary, research gap, and novelty

Microparticle-based drug delivery systems offer significant advantages by enabling controlled and sustained release of therapeutic agents, thereby improving efficacy, patient compliance, and safety. Among the materials used, PLGA is widely preferred due to its biocompatibility, biodegradability, and established clinical use. Nevertheless, traditional fabrication techniques such as solvent evaporation, spray drying, and coacervation face persistent challenges, including size heterogeneity, limited reproducibility, and difficulties in scalability. These shortcomings hinder the translation of laboratory-scale findings into clinically relevant and industrially viable drug delivery formulations.

Emerging microfabrication techniques provide a promising alternative by offering precision and structural control; however, their application to PLGA-based microparticle production for drug delivery remains largely underexplored. Moreover, there is a limited systematic understanding of how solvent properties influence the internal morphology and release behavior of microparticles produced through ultrasonic atomization.

To address these challenges, this thesis proposes the development and evaluation of a high-throughput microparticle production system (MPS) that integrates a microfabricated nozzle array with a piezoelectric transducer. The research plan involves designing and optimizing nozzle arrays using silicon-based lithography and etching, integrating them with a piezoelectric actuator for stable atomization, and assembling the MPS with a customized collection unit. PLGA-based microparticles will then be produced under controlled conditions using solvents with different volatilities (DCM and DMC) to examine their influence on particle morphology, porosity, and lamellar structure. Comprehensive characterization will be performed using SEM, FIB-SEM, FTIR, and EDS analyses.

Chloramphenicol (CHL) will serve as a model drug to investigate encapsulation efficiency, release kinetics, and antimicrobial activity. These studies will not only demonstrate the therapeutic potential of drug-loaded PLGA microparticles but also provide fundamental insights into the relationship between solvent-driven structural features and drug release performance.

The novelty of this thesis lies in presenting a scalable, reproducible, and structurally tunable production method that overcomes the limitations of conventional techniques. By systematically evaluating solvent effects, optimizing microfabrication-based atomization, and validating antimicrobial efficacy, the research aims to contribute a versatile platform positioned at the intersection of microfabrication, pharmaceutical engineering, and biomedical sciences. Ultimately, this work provides a pathway toward next-generation drug delivery systems with improved precision, stability, and clinical applicability.

2. MATERIALS AND METHODS

2.1 Materials

Double-sided polished silicon wafers, oriented at (100) with a thickness of 525 μm and a 4-inch diameter, were acquired from Si-Mat Silicon Materials in Kaufering, Germany. Positive photoresist AZ5214E and AZ726 MIF developer were sourced from Microchemicals GmbH in Ulm, Germany. High-purity quartz plates, measuring 5 inches in diameter with a thickness of 0.09 inches, were obtained from Hoya Corporation in Tokyo, Japan, for photomask fabrication.

Potassium hydroxide (KOH, $\geq 85\%$ purity), buffered oxide etch (BOE, 6:1 NH_4F), and piranha solution components ($\text{H}_2\text{SO}_4\text{:O}_2$, 3:1) were sourced from Sigma-Aldrich in St. Louis, MO, USA. The semiconductor-grade silicon nitride (Si_3N_3) precursors for plasma-enhanced chemical vapor deposition (PECVD) were acquired from Linde AG, based in Munich, Germany.

Poly(D, L-lactide-co-glycolide) (PLGA, $(\text{C}_4\text{H}_4\text{O}_4)_m(\text{C}_6\text{H}_8\text{O}_4)_n$), chloramphenicol (CHL, $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$), and solvents such as dichloromethane (DCM, CH_2Cl_2) and dimethyl carbonate (DMC, $\text{C}_3\text{H}_6\text{O}_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and utilized without further purification. The ultrasonic transducer (HS-R8351505, Guangdong, CN) was obtained from He Shuai Company. Conductive epoxy adhesive (EPO-TEK® H20E, MA, USA) was sourced from EPOXY TECHNOLOGY, INC., while insulating epoxy (Araldite AV 138 M-1 HV/Hardener HV998-1, Basel, CH) was acquired from Huntsman Advanced Materials.

To fabricate microparticles, poly(D, L-lactide-co-glycolide) (PLGA) (Resomer RG503H, Evonik Industries, Germany), which has an 85:15 lactide to glycolide ratio, was selected. PLGA was preferred due to its beneficial characteristics, including biodegradability, biocompatibility, and its widespread use in controlled drug delivery systems. Chloramphenicol (CHL), a broad spectrum antibiotic recognized for its effective antimicrobial properties, served as the model drug to assess the drug encapsulation and release capabilities of the microparticles.

To prepare the PLGA solutions, two different organic solvents were used: dichloromethane (DCM) and dimethyl carbonate (DMC). DCM is favored in microparticle production due to its high volatility and fast evaporation rate. DMC was chosen for comparison, as it has lower volatility and is regarded as more environmentally friendly. Ethanol (EtOH) served as a cosolvent for dissolving CHL. All chemicals were of analytical grade and used without further purification. Phosphate-buffered saline (PBS, pH 7.4) served as the release medium in the in vitro studies.

Design and Operation of the Microparticle Production System (MPS)

2.1.1 Wafer preparation and photomask design

The Microparticle Production System (MPS) development started with the choice of a 4-inch, double-side polished, (100)-oriented silicon wafer, measuring 525 μm in thickness. This particular wafer orientation was selected due to its crystalline anisotropy, which ensures controlled etching profiles and predictable angle formations (54.7° compared to the wafer surface) during anisotropic wet etching. Before processing, the wafers were cleaned following a standard RCA cleaning procedure to eliminate organic contaminants, native oxide, and ionic impurities.

An optical photomask was created with LayoutEditor software to establish microporous structures distributed across the wafer surface. The first design featured a 4×4 array of micropores, while later iterations incorporated 5×5 and 7×7 arrays.

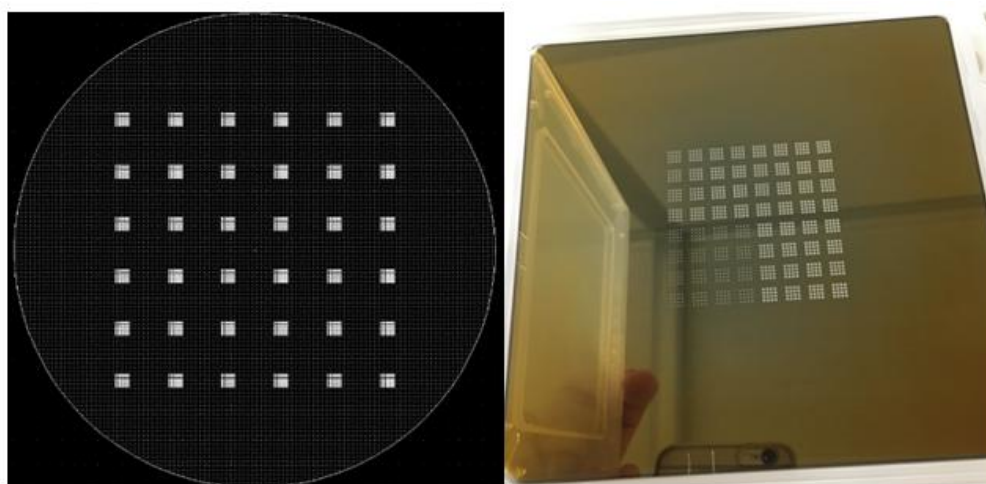


Figure 2.1: Optical mask design (A) and the fabricated optical quartz mask (B).

The photomask was created on a high-precision quartz plate through an electron beam lithography system (Vistec EBPG 5200), achieving a resolution of 0.1 μm .

It underwent development and chrome etching, adhering to the manufacturer's protocol, followed by a thorough cleaning process to ensure optical clarity and stability throughout the photolithography process. The completed photomask was examined with an optical microscopy system to confirm pattern fidelity and dimensional accuracy.

2.1.2 Silicon nitride deposition and photolithography

After cleaning the wafer, a 400 nm layer of silicon nitride (Si_3N_4) was uniformly deposited on both sides of the silicon wafer using a Plasma-Enhanced Chemical Vapor Deposition (PECVD) system (Oxford PlasmaLab System 100). The deposition parameters were optimized as follows: 650 mTorr chamber pressure, 20 W RF power, 300°C substrate temperature, SiH_4 , NH_3 , and N_2 gases flow rates are optimized as 20 sccm. The deposition rate was about 10 nm/min, requiring 40 minutes in total to reach the target thickness of 400 nm. This Si_3N_4 layer acted as a hard mask due to its exceptional resistance to potassium hydroxide (KOH) etching, exhibiting an etch selectivity of approximately 1:1000 (Si_3N_4) in a KOH solution.

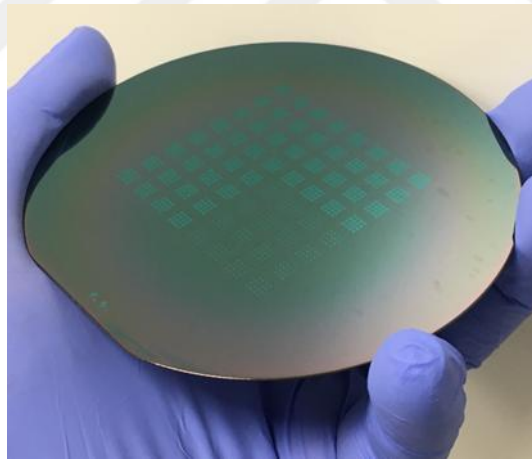


Figure 2.2: Silicon wafer after optical patterning.

Before photolithography, the wafers were baked at 150°C for 30 minutes to eliminate any remaining moisture, then cooled to room temperature. Next, the top surface of the wafer was coated with AZ5214E positive optical photoresist through a spin-coating process. This procedure involved dispensing 5 mL of photoresist in the center of the wafer, spreading it at 500 rpm for 5 seconds, and finally spinning it at 4000 rpm for 45 seconds to attain a consistent thickness of roughly 1.4 μm . The coated wafers underwent a soft bake on a hotplate at 110°C for 60 seconds to evaporate the solvent and enhance adhesion.

The photolithography process utilized an optical lithography system (Midas MDA-60MS Mask Aligner) operating at a wavelength of 365 nm (i-line) and an intensity of 20 mW/cm². The wafers were precisely aligned with the photomask and exposed for 8 seconds, achieving an exposure dose of 160 mJ/cm². After exposure, the wafers were developed in AZ726 MIF developer for 60 seconds with gentle agitation, thoroughly rinsed with deionized water, and dried using nitrogen gas. The quality of the pattern transfer was evaluated through optical microscopy, and the dimensions of the patterns were measured using a profilometer (KLA-TENCOR P6) to confirm the accurate replication of the photomask features.

2.1.3 Hard mask patterning and silicon etching

Following successful photolithography, the silicon nitride hard mask was patterned using a Deep Reactive Ion Etching (DRIE) system (Oxford PlasmaLab System 100 ICP 300). The etching occurred in two stages: first, a short oxygen plasma treatment (50 W, 20 sccm O₂, 30 seconds) to eliminate any organic residues, and then the main etching operation using SF₆/C₄F₈ chemistry.

The key etching parameters included a chamber pressure of 10 mTorr, ICP power of 1500 W, RF power of 100 W, SF₆ flow rate of 50 sccm, C₄F₈ flow rate of 10 sccm, along with helium backside cooling at 10 Torr. The etching duration was carefully timed to ensure full removal of the Si₃N₄ layer in the exposed areas while minimizing over-etching. Typically, the etch rate for Si₃N₄ under these circumstances was around 80 nm/min, leading to an etch time of 5 minutes to eliminate the 400 nm thick Si₃N₄ layer.

After completing the DRIE process, the remaining photoresist was removed using a combination of oxygen plasma treatment (300 W, 50 sccm O₂, 10 minutes) and a piranha solution (H₂SO₄:O₂, 3:1) applied at 80°C for 10 minutes. The wafers were rinsed thoroughly with deionized water and dried with nitrogen gas. The patterned hard mask was examined using optical microscopy and scanning electron microscopy (SEM) to ensure that the Si₃N₄ layer was fully removed from the exposed areas and to confirm the fidelity of the pattern.

The patterned wafer was diced into manageable chips, typically measuring 10 mm × 10 mm, using a precision dicing saw (Disco DAD320) equipped with a 50 μm-thick diamond blade. The cutting speed was set at 4 mm/s, while the spindle operated at

30,000 rpm. This dicing procedure occurred before wet etching to prevent wafer handling issues and improve yield, as the final etched structures would be mechanically fragile. Afterwards, the diced chips were cleaned with isopropyl alcohol and deionized water to eliminate any dicing debris, and then dried with nitrogen gas.

2.1.4 Anisotropic wet etching and structure formation

The anisotropic wet etching process utilized a 40% (w/v) KOH solution, kept at $80 \pm 1^\circ\text{C}$ in a temperature-controlled bath. The setup featured a reflux condenser to reduce evaporation and preserve solution concentration, along with a magnetic stirrer running at 100 rpm to maintain uniform etching conditions. This process took advantage of the selective etching properties of silicon's crystallographic planes, where the etch rate for (100) planes is considerably greater than for (111) planes, with a ratio of about 100:1. Consequently, this anisotropic behavior leads to the creation of V-shaped structures, characterized by sidewalls that form at a 54.7° angle to the wafer surface.

During the etching process, hydrogen gas was visibly released, indicating the progress of the reaction according to the equation (2.1) (Seidel, Csepregi, Heuberger, & Baumgärtel, 1990):

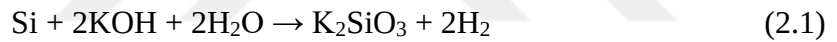


Figure 2.3: Wet Etching of micronozzle structures on the silicon wafer using KOH.

The etching process was regularly observed with optical microscopy to monitor progress and evaluate dimensional changes. Around six hours into the etching, the window openings on the silicon wafer extended through to the other side, creating vertical V-shaped profiles (Figure 2.5). The precise etching duration differed based on the wafer's thickness and the specific dimensions of the pattern, with larger openings taking less time to etch completely.

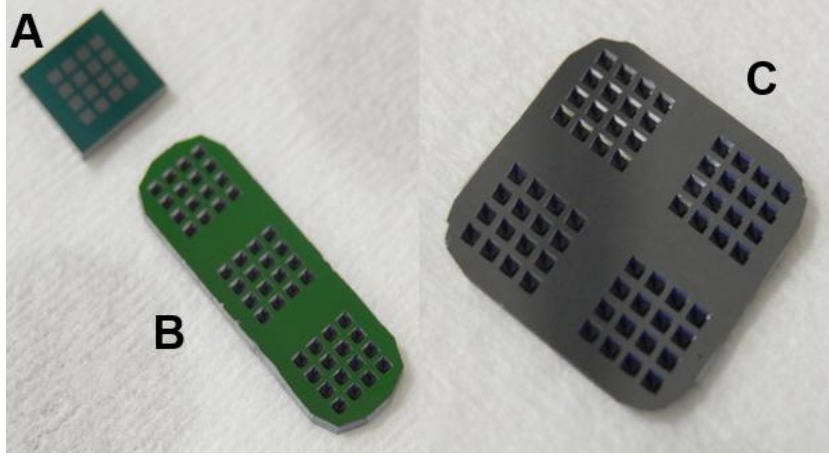


Figure 2.4: Microporous structures formed after wet etching: (A) Patterned hard mask on the substrate, (B) Wet etching performed using KOH, and (C) Final structure after removal of the hard mask.

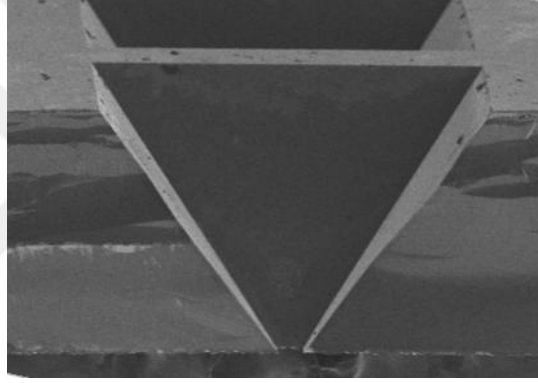


Figure 2.5: V-grooves on the Si (100) wafer crosssection.

The anisotropic etching characteristics of (100)-oriented silicon caused the initially set surface window size (typically $700 \times 700 \mu\text{m}$) to decrease as etching proceeded. This change resulted from lateral etching occurring at the edges of the top window (well-known as undercutting), leading to the final dimensions of the openings at the bottom of the wafer being influenced by both the wafer thickness and the etching duration. The relationship between the width of the top opening (W_1), the width of the bottom opening (W_2), and the wafer thickness (t) can be approximated by with equation (2.2) (Kovacs, Maluf, & Petersen, 2002):

$$W_2 = W_1 - 2t/\tan(54.7^\circ) \quad (2.2)$$

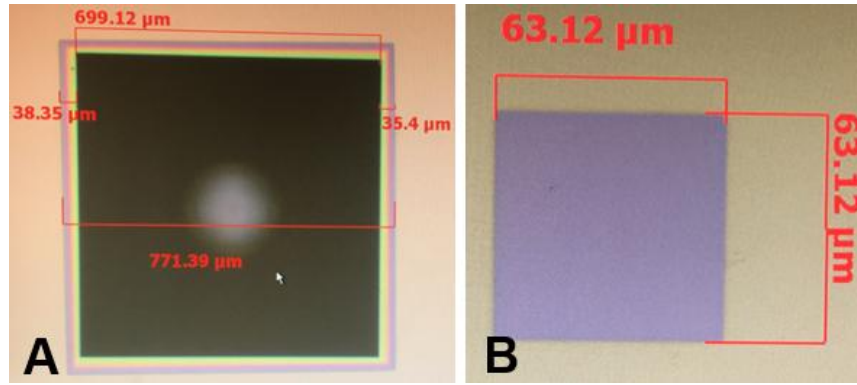


Figure 2.6: Optical microscope images of the microporous structures after wet etching from the (A) Front and (B) Back sides.

Once the etching process concluded, the chips underwent a thorough rinse with deionized water and were dried with nitrogen gas. The residual silicon nitride hard mask was eliminated using a buffered oxide etch (BOE, 6:1 NH_4F) for 15 minutes, followed by another rinse with deionized water and drying with nitrogen gas. The finished microporous structures were analyzed using optical microscopy and scanning electron microscopy (SEM) to evaluate pore dimensions, sidewall angles, and the overall quality of the structure.

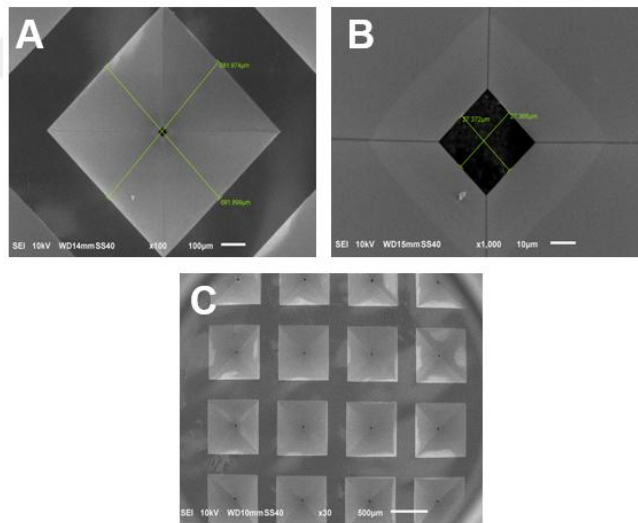


Figure 2.7: SEM images showcasing the microporous structures after wet etching: (A) Top opening, (B) Bottom opening, and (C) Overall view of the structures.

2.1.5 Design optimization and iterative development

Initial experimental trials using the 4×4 pore arrays (16 pores per chip) experienced challenges with microparticle merging during ejection, attributed to the close proximity of adjacent nozzles. Analysis conducted with a high-speed camera (Phantom v1610, Vision Research, NJ, USA) at 10,000 frames per second revealed that particles

merged while in flight after being ejected from the pores. This merging effect was quantitatively evaluated by measuring the distance between droplets and tracking the occurrence of coalescence events in relation to their distance from the pore exit.

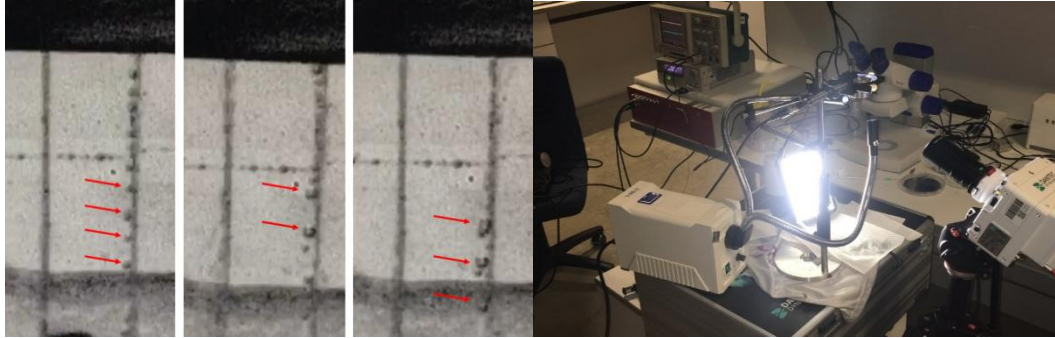


Figure 2.8: Coalescence of microparticles in the liquid phase after ejection from the micronozzle, captured using a high-speed camera (left), along with the experimental setup used for imaging (right). Red arrows show the ejected microparticles.

To resolve these concerns, a structured design optimization process was carried out across multiple iterations:

- **First iteration:** The number of pores was increased from 4×4 to 5×5 (25 pores per chip) to provide greater spatial separation between adjacent pores. The spacing between the pores was enlarged from 150 μm to 200 μm , while the pore diameter remained constant at 28 μm .
- **Second iteration:** Nozzle-like protrusions were added around each pore to spatially isolate the emerging droplets and to direct the fluid jets apart. These protrusions extended 50 μm from the silicon surface and featured a tapered profile, which reduced flow resistance while enhancing directional control.
- **Third iteration:** The design underwent further enhancement, increasing the pore array to 7×7 (49 pores per chip) and fine-tuning the nozzle orifices to $22 \pm 0.5 \mu\text{m}$ to ensure effective droplet breakup dynamics. Additionally, the pore spacing was modified to 250 μm to support the larger number of pores and to prevent jet merging.

Each design iteration was fabricated using the same microfabrication processes described earlier, with suitable adjustments to the photomask design and etching parameters.

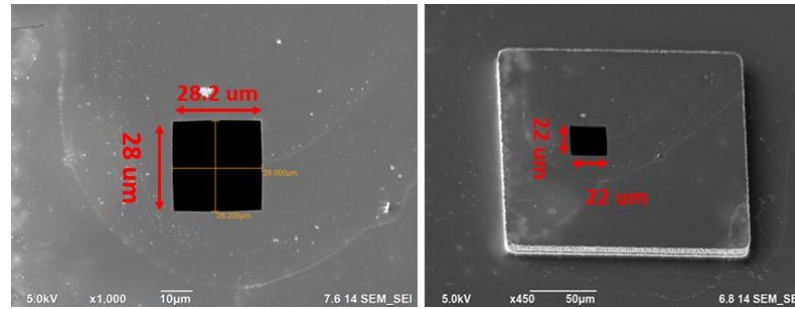


Figure 2.9: Comparison of two designs: a structure with a microporous (left) and one with nozzle like protrusions (right).

2.1.6 Assembly of the microparticle production head

The microparticle production head was constructed using a layer-by-layer method, combining the microfabricated silicon chips with the actuation and fluid delivery systems. The assembly involved these steps:

- **Metal disc preparation:** A 316 stainless steel disc (30 mm diameter, 1 mm thickness) was precisely machined to include a central hole (10 mm diameter) for the silicon chip. The disc's surface was polished to a mirror finish ($R_a < 0.2 \mu\text{m}$) and thoroughly cleaned with isopropyl alcohol and acetone to guarantee optimal bonding.
- **Silicon chip bonding:** The microfabricated silicon chip with the micropore array was attached to the metal disc using Araldite AV 138 M-1 HV/Hardener HV998-1 epoxy adhesive. To prepare the adhesive, the resin and hardener were mixed in a 4:1 weight ratio and degassed under vacuum to eliminate air bubbles. A thin layer of adhesive was spread on the metal disc, and the silicon chip was accurately positioned over the central hole. The assembly underwent curing at 80°C for 2 hours under consistent pressure to ensure effective bonding and prevent leakage.
- **Preparation of PZT-8 piezoelectric transducer:** A PZT-8 piezoelectric transducer disk, measuring 35 mm in diameter and 5 mm in thickness, was created by adhering conductive electrodes to both sides with EPO-TEK H20E conductive epoxy. This epoxy was cured at 120°C for 15 minutes as per the instructions provided by the manufacturer. The electrodes were then connected to insulated copper wires using a soldering iron, and the joints were protected with a thin layer of non-conductive epoxy to avoid short circuits.

- **Bonding the piezoelectric transducer:** The prepared piezoelectric transducer was affixed to the rear of the metal disc (the side opposite the silicon chip) with a thin layer of Araldite epoxy. This assembly was cured at 80°C for 2 hours under consistent pressure to ensure effective bonding while maintaining the piezoelectric properties.
- **Reservoir attachment:** A cylindrical reservoir made of stainless steel 316 (inner diameter: 18 mm, height: 30 mm, wall thickness: 10 mm) was created featuring a threaded cap and O-ring seal for convenient filling and sealing. The piezoelectric transducer was bonded to the reservoir using Araldite epoxy, ensuring that the bonding area was restricted to the perimeter to preserve the system's acoustic properties. The assembly underwent curing at 80°C for 2 hours.
- **Housing integration:** The built components were incorporated into a custom-designed casing that offered mechanical support and electrical connections. This housing was machined from aluminum and anodized to resist corrosion.

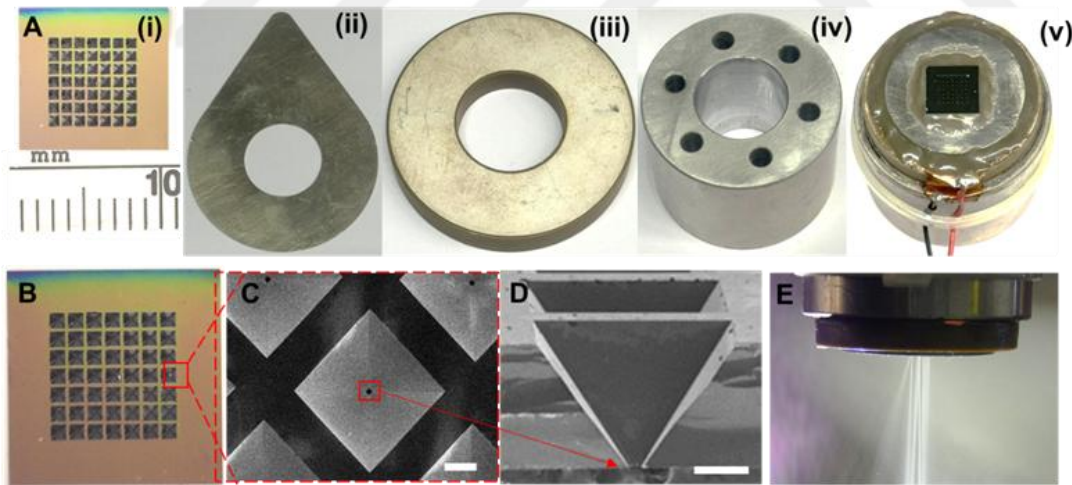


Figure 2.10: Assembly of the components of the microparticle production head; (A) Images of the components include: (i) microfabricated spray nozzle array, (ii) metal disc, (iii) ultrasonic (piezoelectric) transducer, (iv) solution reservoir, and (v) the assembled microparticle production head. (B) A close-up of the microfabricated spray nozzle array. (C-D) SEM image showing a single nozzle (shown with scale bars of 200 μm). (E) Image depicting an operational microparticle production system (Çelik et al., 2025).

The completely assembled microparticle production head was evaluated for mechanical integrity, electrical functionality, and fluidic performance prior to its integration with the electronic control system.

2.1.7 Electronic control system design and implementation

The electronic control system was designed to provide precise control over the frequency, amplitude, and waveform characteristics of the piezoelectric actuator. The system included the following components:

- **Signal generation unit:** An Agilent 33220A function generator facilitated the production of the primary waveform, offering precise frequency control (resolution: 1 mHz) and waveform shaping features. The signal generator was set to emit a sinusoidal waveform, with frequencies adjustable from 40 to 50 kHz, aligning with the resonance frequency of the piezoelectric actuator.
- **Amplification unit:** A high-voltage amplifier (Nanomagnetics Instruments) was custom-designed to boost the low-voltage signal (usually 5 Vpp) from the function generator to the necessary high-voltage levels (50-100 Vpp) for the piezoelectric actuator.
- **Signal monitoring:** A Tektronix TDS 3034B digital oscilloscope, with a bandwidth of 300 MHz and a sampling rate of 2.5 GS/s, enabled real-time monitoring of both input and output signals. This facilitated accurate adjustments of waveform parameters and verification of amplifier performance.

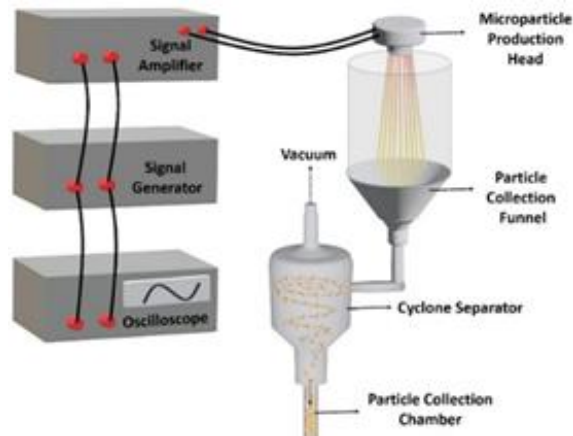


Figure 2.11: Design and components of the micro-jet nozzle-based microparticle production system (Çelik et al., 2025).



Figure 2.12: General view of the newly designed micro particle system (MPS).

The electronic control system was analyzed to find the optimal operational parameters for the piezoelectric actuator. Resonance characterization involved sweeping the frequency from 10 kHz to 80 kHz in increments of 0.1 kHz, while recording the electrical impedance and mechanical response of the system. The primary resonance frequency was determined to be 46.7 ± 0.5 kHz, which was chosen as the operational frequency. At this frequency, the amplification system output 65 V_{pp} signals to the piezo disk, ensuring maximum atomization efficiency.

2.1.8 Theory and operational principles of MPS fluid dynamics

The microparticle production system's fluid dynamics are grounded in acoustic atomization principles. High-frequency mechanical oscillations create standing waves on the liquid surface, which ultimately break into droplets once the amplitude surpasses a critical threshold. The fluid flow through the micropores can be described by the Hagen–Poiseuille equation (2.3) (Squires & Quake, 2005):

$$Q = \pi r^4 \Delta P / 8 \mu L \quad (2.3)$$

In equation (2.3), Q represents the volumetric flow rate in cubic meters per second (m^3/s), reflecting the amount of fluid moving through a pore over time. The parameter r denotes the radius of the pore, measured in meters (m), and is crucial for defining how fluid flows through the pore structure. The dynamic viscosity of the fluid, denoted by μ and measured in pascal-seconds ($\text{Pa}\cdot\text{s}$), indicates the internal resistance the fluid

encounters while flowing. The pressure difference across the pore, represented by ΔP and expressed in pascals (Pa), acts as the driving force for the fluid's movement. Lastly, L indicates the effective length of the pore, also measured in meters (m), which affects the resistance the fluid faces along its path.

The pressure difference (ΔP) is the combination of static pressure (from fluid column height) and dynamic pressure (from piezoelectric actuation) calculated with equation (2.4) (Cengel & Cimbala, 2013):

$$\Delta P = \rho gh + P_{dyn} \quad (2.4)$$

In equation (2.4), ρ stands for fluid density, quantified in kilograms per cubic meter (kg/m^3), indicating the mass of fluid per unit volume. The symbol g represents gravitational acceleration, typically valued at 9.81 meters per second squared (m/s^2), playing a role in the hydrostatic pressure of the fluid. The variable h denotes the height of the fluid column in meters (m), which directly impacts the pressure the fluid exerts under gravity. Furthermore, P_{dyn} indicates the dynamic pressure component produced by piezoelectric actuation, measured in pascals (Pa), accounting for the pressure variations caused by mechanical oscillations. Together, these parameters influence the total pressure on the fluid system and are essential for analyzing fluid dynamics in environments affected by both gravitational and actuated forces.

The dynamic pressure component correlates directly with the vibration amplitude (A) and frequency (f) of the piezoelectric actuator (2.5), (Cengel & Cimbala, 2013):

$$P_{dyn} = \rho A f^2 (2\pi d) \quad (2.5)$$

In equation (2.4), ρ denotes fluid density measured in kilograms per cubic meter (kg/m^3), indicating the mass of fluid within a unit volume. The term A refers to the vibration amplitude expressed in meters (m), representing the maximum degree of oscillatory motion. The variable f indicates the vibration frequency in hertz (Hz), detailing the number of oscillations that happen each second. Finally, d signifies the effective displacement of the piezoelectric actuator in meters (m), illustrating the range of the actuator's movement during operation. Collectively, these parameters quantify the dynamic pressure contribution that arises from high-frequency piezoelectric vibrations, which play a crucial role in microfluidic and droplet generation systems.

The process of droplet formation adheres to the Rayleigh-Plateau instability mechanism, occurring when a liquid jet breaks into droplets once its length surpasses its circumference multiplied by π . The estimated diameter of the resulting droplets (D_d) can be calculated as follows:

$$D_d = 1.89D_j \quad (2.6)$$

Where D_j represents the jet diameter, which is roughly equal to the pore diameter under the studied conditions.

In the optimized system featuring a pore diameter of 22 μm , the expected average droplet diameter is roughly 41.6 μm . Nevertheless, as the solvent evaporates during particle formation, the resulting microparticle diameter is considerably smaller and depends directly on the polymer concentration in the solution.

The theoretical framework describing the fluid dynamics of the MPS was validated through direct experimental characterization of the produced microparticles. Scanning electron microscopy (SEM) was employed to accurately measure the surface morphology and size distribution of the particles generated under controlled operating conditions. The SEM images revealed that the microparticles exhibited a highly uniform spherical geometry, with an average diameter of $8.9 \pm 1.7 \mu\text{m}$, which is consistent with the theoretical predictions after accounting for solvent evaporation and polymer solidification effects. The correlation between the predicted droplet size ($\sim 41.6 \mu\text{m}$, based on the 22 μm pore diameter and Rayleigh-Plateau instability theory) and the final microparticle dimensions further confirmed the role of solvent volatility and polymer concentration in reducing droplet size during drying. These results provided strong empirical support for the fluid dynamic model, demonstrating that the interplay of acoustic atomization, Hagen–Poiseuille flow, and Rayleigh-Plateau instability can reliably explain the droplet-to-particle transition observed in the MPS. Thus, the SEM-based measurements served as a critical validation tool, bridging theoretical predictions with experimental outcomes.

2.1.9 Particle collection system design and fabrication

Microparticles produced through micronozzle-based atomization systems find extensive applications in pharmaceuticals, cosmetics, food technology, and materials science, thanks to their small size, uniform distribution, and adjustable properties (Y. Liu, Yang, Hui, Ranaweera, & Zhao, 2022; Q. Xu et al., 2009). However, their

collection is often complicated by high dispersion velocities and tiny diameters, making efficient separation and collection methods essential. Cyclone separators, in particular, are preferred for their simplicity, high efficiency, low operational costs, and minimal maintenance needs (Elsayed & Lacor, 2011).

Cyclone separators utilize centrifugal force to separate solid particles from a gas stream. During standard operation, particle-rich air is introduced tangentially into the cyclone chamber's upper cylindrical section. As the air spirals down along the inner wall, centrifugal forces push the denser particles toward the outer walls. These particles then descend into a conical section and gather in the collection chamber due to gravity, while the cleansed air exits through a centrally positioned outlet at the top (Leith, 1972). The separation's effectiveness can be explained using Stokes' law along with equations for centrifugal force, which consider particle size, density, and flow dynamics (Chen & Shi, 2007).

In this thesis, a specially designed cyclone separator was used to gather PLGA microparticles generated through ultrasonic atomization via a micronozzle array. During its operation, liquid droplets on the micron scale, which contained the polymer and drug, were drawn into the cyclone chamber under negative pressure. Upon entering, the organic solvent (such as dichloromethane or dimethyl carbonate) quickly evaporated, resulting in solid microparticles. These particles, measuring between 5 to 20 μm , spiraled downward through the cyclone's cylindrical structure. When particles struck the inner walls, their momentum decreased, aiding their gravitational settling into the collection chamber. This system enabled efficient recovery of dried microparticles without clogging, and it was compatible with solvents, making it crucial for consistent downstream applications in drug delivery.

The geometry of the cyclone, airflow rate, and particle size are crucial factors affecting separation efficiency. Optimized systems can achieve over 90% collection efficiency for particles in the sub-20 μm range, making them particularly suitable for lab-scale and industrial microparticle production setups (P. Zhang, Chen, Duan, & Wang, 2019). Thus, the use of cyclone separators offers a scalable and effective solution for capturing microparticles generated in continuous production environments.

The cyclone separator consisted of the following components:

- **Inlet funnel:** A conical funnel at the top of the separator with a diameter of 160 mm, tapering to 20 mm over a length of 200 mm. The funnel is positioned directly below the microparticle production head to capture the generated microparticles.
- **Cyclone body:** A cylindrical body with a diameter of 70 mm and a height of 100 mm features a tangential inlet port for the particle-laden airstream. The tangential inlet creates a swirling flow pattern, generating centrifugal forces that push the particles toward the walls.
- **Conical section:** A conical section features a top diameter of 50 mm, tapering to 15 mm over a length of 60 mm. This conical shape accelerates the swirling flow, enhancing the centrifugal effect and directing the particles toward the collection chamber.
- **Collection chamber:** A cylindrical chamber measuring 20 mm in diameter and 60 mm in height at the base of the conical section is designed to collect the separated particles.
- **Central outlet pipe:** A cylindrical pipe with a diameter of 15 mm extends from the top of the cyclone body to approximately one-third of its height. This pipe allows the cleaned air to exit while preventing particle re-entrainment.

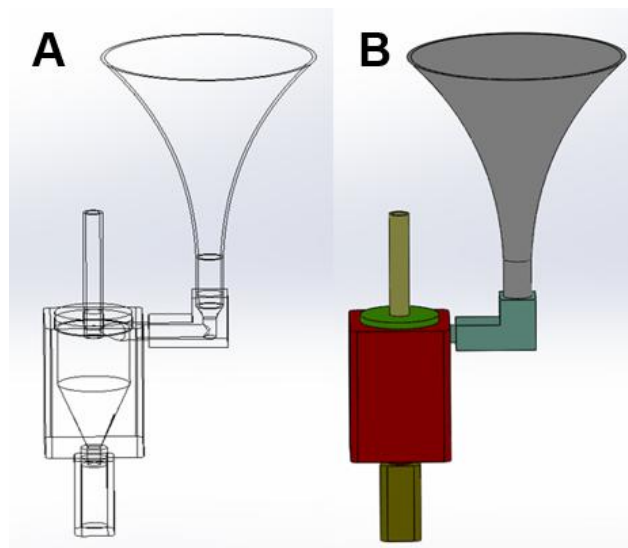


Figure 2.13: Illustration of the cyclone separator, featuring its wireframe design (A) alongside the solid model (B) depiction.

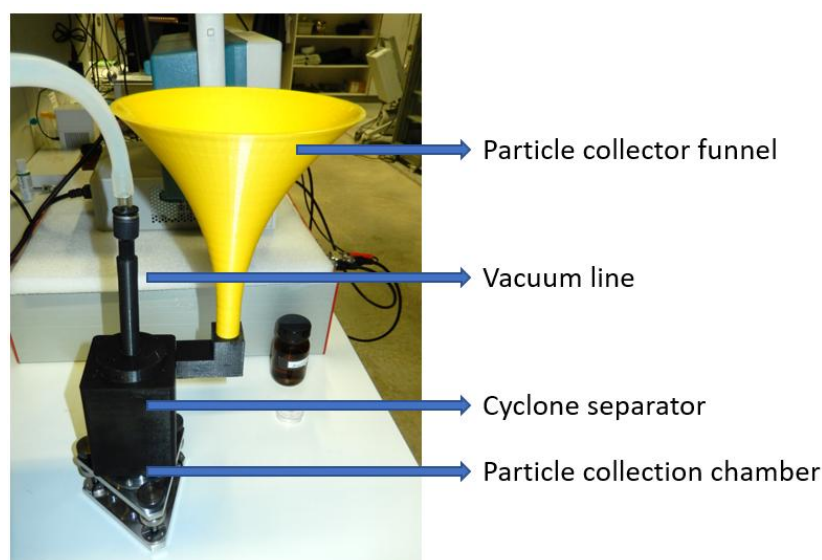


Figure 2.14: The cyclone separator created with a 3D printer and its separate parts.

The cyclone separator was manufactured using a high-resolution 3D printer (Bambu Lab X1 Carbon, Shenzhen, China) using ABS filaments. To improve the performance and durability of the cyclone separator, the entire inner surface was coated with a thin layer of polydimethylsiloxane (PDMS), which was prepared by mixing Sylgard 184 base and curing agent in a 10:1 ratio. The PDMS coating served two essential purposes:

- Protection from solvent degradation, since the organic solvents used in the polymer solutions could potentially degrade the 3D-printed ABS material over time.
- Reduce wall adhesion effects and surface roughness to ensure high recovery efficiency by preventing particles from sticking to the walls.

The PDMS coating was applied using a two-step process. First, the inner surfaces of the separator were cleaned with isopropyl alcohol to remove dust and particles, enhancing adhesion. Then, the PDMS mixture was introduced and allowed to flow over all surfaces while the separator was rotated to ensure complete coverage. The coated separator was then cured at 60 °C for 4 hours to complete the cross-linking process.

The cyclone separator's collection efficiency was assessed using model particles of known size and concentration. The system achieved collection efficiencies of 92-95% for particles within the 5-20 μm size range, which corresponded with the target size range for the produced microparticles.

2.2 Preparation of Drug-Loaded PLGA Solutions

The performance of the advanced system was evaluated using PLGA with an 85:15 lactide ratio (catalog number 430471, Sigma-Aldrich) for microparticle production. Two distinct polymer solutions were formulated to create PLGA microparticle solutions using the organic solvents DCM and DMC, which are commonly employed to dissolve PLGA. Briefly, 700 mg of PLGA was weighed and transferred into two clean, dry 250 ml glass beakers. Subsequently, 100 ml of DCM (catalog number 34856, Sigma-Aldrich) was added to one beaker, and 100 ml of DMC (catalog number 517127, Sigma-Aldrich) was added to the other using graduated cylinders. Each mixture was continuously stirred with a magnetic stirrer at room temperature overnight until the PLGA was completely dissolved in both solvents. The resulting 7 mg/ml PLGA solutions in DCM and DMC were stored separately in tightly sealed glass containers to prevent solvent evaporation until further use. Drug loading studies were performed exclusively with PLGA solutions prepared in DCM. For the production of drug-loaded PLGA microparticles, chloramphenicol (CHL) (catalog number C0378, Sigma-Aldrich) was incorporated into the previously described PLGA solution in DCM. In this process, 35 mg of CHL was weighed, dissolved in 1 mL of ethanol (catalog number 1.00974, Sigma-Aldrich), and mixed into a 9 mL PLGA solution in DCM, achieving a final concentration of 3.5 mg of CHL/mL. Continuous stirring was maintained to ensure uniform distribution of the drug within the polymer.

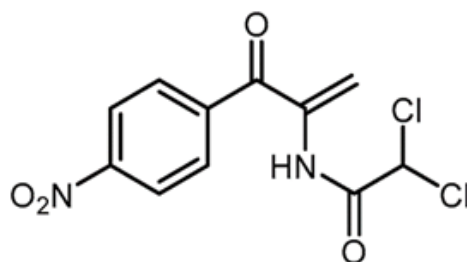


Figure 2.15: Chemical structure of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) (Tevyashova, 2021).

To prepare the drug-loaded microparticles, PLGA was first dissolved in DCM or DMC to create a 5% (w/v) polymer solution. In parallel, CHL was dissolved in ethanol and added dropwise to the PLGA solution while continuously stirring to ensure homogeneity. The final solution was degassed under mild vacuum to remove air bubbles before being introduced into the MPS.

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2.3 Characterization of Microparticles

2.3.1 Morphological analysis via SEM

The morphological and elemental characterization of drug-loaded PLGA microparticles was achieved using advanced imaging and spectroscopy techniques to thoroughly evaluate particle shape, surface texture, internal architecture, and chemical uniformity. These properties play a crucial role in influencing drug encapsulation efficiency, release kinetics, and ultimately, the performance of the drug delivery system (Bilati et al., 2005; Makadia & Siegel, 2011; J Siepmann & Göpferich, 2001).

High-resolution morphological examinations were conducted using a dual-beam JEOL JIB-4601 MultiBeam FIB-SEM system that integrates scanning electron microscopy (SEM) with focused ion beam (FIB) capabilities. Before imaging, the microparticles were mounted on aluminum stubs with double-sided carbon tape and sputter-coated with a thin conductive film of gold-palladium (Au-Pd) using a Ted Pella 108 Auto

Sputter Coater. This coating, approximately 10 nm thick, mitigated surface charging effects and enhanced image resolution under the electron beam, which is especially critical for non-conductive polymeric samples (Kohl & Reimer, 2008).

Imaging took place in a high vacuum environment with an accelerating voltage set at 5 kV. SEM images were captured at different magnifications, and particle size distributions were determined using ImageJ software, where more than 100 particles per sample were analyzed.

The SEM images revealed that most of the particles exhibited a spherical morphology with smooth to slightly wrinkled surfaces, depending on the formulation conditions, such as solvent systems and emulsification parameters. In some formulations, collapsed or dimpled surfaces were observed, possibly due to rapid solvent evaporation during the solidification phase.

2.3.2 Internal structure analysis via FIB-SEM

A thorough understanding of the internal microarchitecture of drug-loaded microparticles is crucial for assessing their functional performance in controlled drug release applications. Internal morphology, including porosity, pore interconnectivity, and the presence of core-shell or multi-phase structures, significantly influences drug encapsulation efficiency, degradation kinetics, and release mechanisms (Makadia & Siegel, 2011; J Siepmann & Göpferich, 2001).

The FIB milling procedure began with selecting individual microparticles on the SEM stage. A protective carbon (C) layer was then deposited over the area of interest using ion-beam-assisted deposition. This carbon capping effectively prevented redeposition artifacts and maintained surface integrity during the trenching process. Following this, gallium ions were aimed at the sample to ablate material in a controlled manner, exposing cross-sections that are perpendicular to the particle surface. Imaging of the revealed interior was conducted with the SEM column at accelerating voltages between 5 and 10 kV, fine-tuned to optimize both resolution and material contrast.

FIB cross sections revealed a complex internal structure featuring interconnected porous networks, promoting sustained drug release by easing diffusion pathways (Makadia & Siegel, 2011). The FIB-SEM analysis showed a wide variety of internal morphologies among different formulations. Particularly notable was a sponge like porous network present throughout the microparticle core, which suggests phase

separation occurred during solvent evaporation. These pores, which ranged in size from sub-micron to several microns, appeared interconnected in some particles, indicating that the solvent system and emulsification processes played a crucial role in porogen formation (Jain, 2000; Zolnik & Burgess, 2007).

In certain samples, a distinct core-shell morphology was identified, characterized by a denser outer polymer layer encapsulating a more porous inner core. This architecture is particularly beneficial for achieving delayed or biphasic drug release profiles.

FIB cross-sections indicated that microparticles made with more volatile organic solvents showed greater internal porosity. This is likely a result of swift phase inversion and solvent evaporation during the solidification of the particles. Conversely, systems with slower evaporation rates produced particles with smoother, more uniform interiors. These findings align with theoretical predictions about solvent-polymer interactions in PLGA matrices and underscore how process parameters influence internal structure.

2.3.3 Elemental analysis via EDS

To enhance the morphological analysis with compositional data, energy-dispersive X-ray spectroscopy (EDS) was utilized through an Oxford X-Max detector integrated into the SEM system. EDS analyses were performed on both the outer surface and the cross-sectioned interiors of the microparticles. The elemental maps and point spectra yielded qualitative and semi-quantitative data regarding the distribution of essential elements (C, O, and leftover elements from surfactants or drug molecules), confirming the chemical integrity of the microparticles and the consistent dispersion of the encapsulated therapeutic agents.

In chloramphenicol loaded microparticles, the presence of chlorine (Cl) peaks in the EDS spectra provided qualitative confirmation of successful drug incorporation, as chlorine is absent in PLGA but serves as a crucial component in CHL.

Elemental mapping using EDS revealed a consistent distribution of carbon and oxygen across the matrix, showing no signs of phase separation or elemental clustering, which indicates a stable and reproducible formulation process. Additionally, the distribution of elements internally was evaluated through point EDS analysis on the exposed cross-sections.

The resulting spectra demonstrated a uniform distribution of carbon and oxygen, with no observable segregation of residual solvents or drug crystallization, further supporting the formulation's homogeneity.

The SEM-FIB-EDS analyses collectively offered vital insights into the structural and compositional characteristics of the fabricated microparticles. These findings established a crucial foundation for linking morphological features with the in vitro release profiles and for understanding the structure-function relationships inherent to PLGA-based drug delivery systems.

2.3.4 Spectroscopic analysis via FTIR

Fourier-transform infrared spectroscopy (FTIR) was employed to evaluate the chemical structure and molecular interactions within both drug-loaded and blank PLGA microparticles. This analytical technique is widely utilized in pharmaceutical research to identify functional groups, detect potential interactions between drug and polymer, and verify the successful encapsulation of active pharmaceutical ingredients (Das & Chaudhury, 2011; Makadia & Siegel, 2011; Patel, Patel, Amin, & Patel, 2005).

The FTIR analysis was performed using a Shimadzu IRAffinity-1S spectrophotometer, which provides high sensitivity and a wide spectral range, making it suitable for the thorough evaluation of organic and polymeric materials. Spectra were collected in the range of 4000–500 cm^{-1} using attenuated total reflectance (ATR) mode.

For spectral acquisition, microparticle samples were finely ground with spectroscopic-grade potassium bromide (KBr) at an approximate ratio of 1:100 (w/w) and compressed into translucent discs under vacuum pressure using a hydraulic pellet press. The FTIR spectra were recorded in the range of 4000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} , and averaged over 32 scans to improve the signal-to-noise ratio. All measurements were conducted at room temperature under a dry air purge to minimize atmospheric interference from water vapor and carbon dioxide.

The FTIR spectra of blank PLGA microparticles exhibited characteristic absorption bands that corresponded to the chemical structure of PLGA. The strong bands at 1755 cm^{-1} and 1085 cm^{-1} were attributed to the C=O stretching of the ester groups and the C–O–C stretching vibrations of the aliphatic polyester backbone, respectively.

Additionally, the CH₂ asymmetric and symmetric stretching modes appeared at 2995 cm⁻¹ and 2945 cm⁻¹, confirming the polymer identity (Makadia & Siegel, 2011).

In contrast, the spectra of drug-loaded PLGA microparticles revealed additional peaks and intensity shifts, indicating successful drug incorporation and possible intermolecular interactions. Depending on the chemical structure of the encapsulated drug, new peaks were observed in the fingerprint region (1500–500 cm⁻¹), corresponding to aromatic or heterocyclic ring vibrations, as well as amide or hydroxyl functional groups. Notably, slight shifts in the ester carbonyl peak position (from 1755 cm⁻¹ to approximately 1748 cm⁻¹) and broadening of the OH stretching band (typically near 3400 cm⁻¹) were observed, suggesting weak hydrogen bonding between the drug and the PLGA matrix (M. Li, Rouaud, & Poncelet, 2008; Makadia & Siegel, 2011).

These spectral differences between blank and drug-loaded formulations provided qualitative confirmation of drug encapsulation and supported the absence of significant chemical degradation or incompatibility. No new bands corresponding to degradation products or polymer-drug reaction products were detected, indicating that the formulation process did not affect the primary chemical structure of the components.

In summary, FTIR analysis proved to be a non-destructive and trustworthy technique for confirming the presence and integrity of both the polymeric carrier and the enclosed drug. Additionally, it facilitated the detection of possible drug–polymer interactions that could affect drug release characteristics and stability as time progresses.

2.4 Drug Loading and Encapsulation Efficiency

To measure the quantity of CHL encapsulated in PLGA microparticles, a specified mass of dried drug-loaded particles was first dissolved in DCM, then extracted with ethanol. To determine the encapsulation efficiency (EE) and loading efficiency (LE), 750 µg of drug-loaded PLGA microparticles were dissolved in 1 mL of DCM. The drug concentration in the resulting solution was measured using a validated spectrophotometric method at a wavelength of 278 nm (Thermo Scientific NanoDrop 2000c, USA) 23.

The encapsulation efficiency (EE %) and loading efficiency (LE %) are calculated using the equations provided in the following (2.7) and (2.7) (Knap, Kwiecień, Reczyńska-Kolman, & Pamuła, 2023):

$$EE (\%) = \frac{\text{weight of encapsulated drug in microparticles}}{\text{initial weight of drug}} \times 100 \quad (2.7)$$

$$LE (\%) = \frac{\text{weight of drug in microparticles}}{\text{weight of microparticles}} \times 100 \quad (2.8)$$

Each analysis was performed three times to guarantee accuracy and consistency.

2.5 In-Vitro Drug Release Study

In vitro drug release studies were performed in phosphate-buffered saline (PBS, pH 7.4) at 37°C to mimic physiological conditions. An accurately weighed amount of CHL-loaded microparticles was suspended in 10 mL of PBS and placed in a shaker incubator at 100 rpm. At predetermined time intervals (0, 1, 2, 4, 8, 24, 48, 72, and 100 hours), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS to maintain sink conditions. The CHL concentration in each sample was measured using UV-Vis spectrophotometry at 278 nm. Cumulative drug release profiles were plotted to assess release kinetics.

2.6 Antimicrobial Activity Assay

The antimicrobial efficacy of the CHL-loaded microparticles was evaluated against *Escherichia coli* (*E. coli* K12 strain). Bacterial cultures were established to examine the effects of plain and drug-loaded microparticles on bacterial growth.

Initially, *E. coli* samples were inoculated onto solid agar media at a concentration of 1.5% (w/v) and incubated at 37°C for 24 hours to obtain single colonies. Individual colonies from each plate were then transferred into 3 mL of liquid Luria-Bertani (LB) medium and incubated overnight at 37°C in a shaking incubator to ensure optimal bacterial growth.

After overnight incubation, each culture was separated into four groups with three replicates to assess different treatment conditions:

- **Negative control (*E. coli* only):** No extra substances (i.e., microparticles, antibiotics) are included, enabling the assessment of baseline bacterial growth.
- **Plain PLGA + *E. coli*:** Treated with 7 mg/mL plain PLGA microparticles to investigate the effect of plain PLGA microparticles and their degradation products on bacterial growth.
- **Drug-loaded PLGA + *E. coli*:** Treated with 7 mg/mL PLGA microparticles loaded with 16.2% CHL, this group was designed to study the combined effects of PLGA microparticles and released CHL on bacterial growth, as PLGA degradation could facilitate sustained antibiotic release.
- **Positive control (Drug only):** Treated with 1.14 mg/mL CHL to evaluate the direct impact of the antibiotic, serving as a positive control to compare the effectiveness of CHL-loaded microparticles with free CHL.

Before use, the drug-loaded microparticles were washed three times with 1X PBS buffer to eliminate any non-encapsulated drugs from the surface, ensuring that only the encapsulated drug would be released into the medium.

Optical density measurements at 600 nm (OD₆₀₀) were recorded at regular intervals to monitor bacterial growth. The decrease in OD values in the groups treated with CHL-loaded particles was interpreted as a measure of drug release and antibacterial effectiveness.

2.7 Statistical Analysis

Statistical analyses were conducted in this study to ensure the robustness and reliability of the experimental findings. All quantitative data are expressed as mean $\pm$ standard deviation (SD), providing a transparent representation of measurement uncertainties and natural variability. To enhance reproducibility and minimize random error, each experiment was performed in triplicate ($n = 3$), unless otherwise specified. The data were analyzed using both one-way and two-way ANOVA after verification of normality assumptions, with Tukey's post-hoc test applied for detailed group comparisons. Statistical significance was set at $p < 0.05$, while more stringent

thresholds ($p < 0.01$, $p < 0.001$, and $p < 0.0001$) were employed to validate critical differences across experimental groups. This rigorous statistical framework not only strengthened the reproducibility of the results but also minimized the likelihood of false-positive interpretations. By systematically addressing uncertainties, integrating replicate measurements, and validating outcomes through established statistical methodologies, the study ensured that the reported performance of the MPS system and the therapeutic efficacy of the drug-loaded microparticles can be regarded as both scientifically credible and reproducible.



3. RESULTS AND SUGGESTIONS

3.1 Morphological Characterization of PLGA Microparticles

PLGA microparticles produced with the Microparticle Production System (MPS) were carefully examined to better understand their shape and surface properties. For this purpose, scanning electron microscopy (SEM) was used, as it provides highly detailed images that allow the evaluation of particle morphology at the micro- and nanoscale. The observations clearly showed that the MPS was able to generate microparticles with a uniform spherical structure, regardless of the type of solvent used during the fabrication process. This consistent outcome highlights the reliability and precision of the system compared to many traditional production methods, which often result in irregular or polydisperse particles.

3.1.1 External morphology and surface characteristics

SEM images demonstrated that microparticles fabricated using dichloromethane (DCM) exhibited clearly defined spherical shapes with significant surface porosity (Figure 3.1A). These particles displayed visible pores and minor indentations across their surfaces, likely due to the rapid evaporation of DCM during the droplet solidification phase. The presence of these surface pores increases the surface area, which may significantly influence the initial burst release behavior observed in drug release studies.

In contrast, microparticles produced using dimethyl carbonate (DMC) exhibited significantly smoother outer surfaces with minimal visible porosity (Figure 3.1B). This morphological difference is due to the slower evaporation rate of DMC, which facilitated more uniform polymer precipitation during particle formation, resulting in a denser and more compact outer structure with fewer surface irregularities.

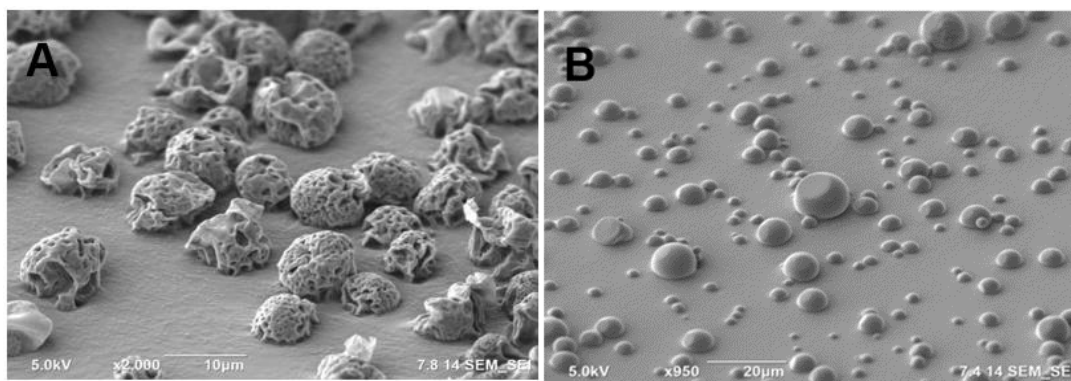


Figure 3.1: SEM images of PLGA microparticles: (A) particles fabricated using DCM as the solvent, exhibiting a porous surface morphology; (B) particles produced with DMC as the solvent, showing a smooth and dense surface texture.

3.1.2 Particle size distribution analysis

Quantitative analysis of SEM micrographs showed that both DCM and DMC formulations produced microparticles with controlled size distributions, each exhibiting unique characteristics. The microparticles derived from DCM displayed a mean diameter of $8.9 \pm 1.7 \mu\text{m}$ and a polydispersity index (PDI) of 0.19, reflecting a relatively narrow size distribution. The consistency of these particles indicates effective droplet control during atomization.

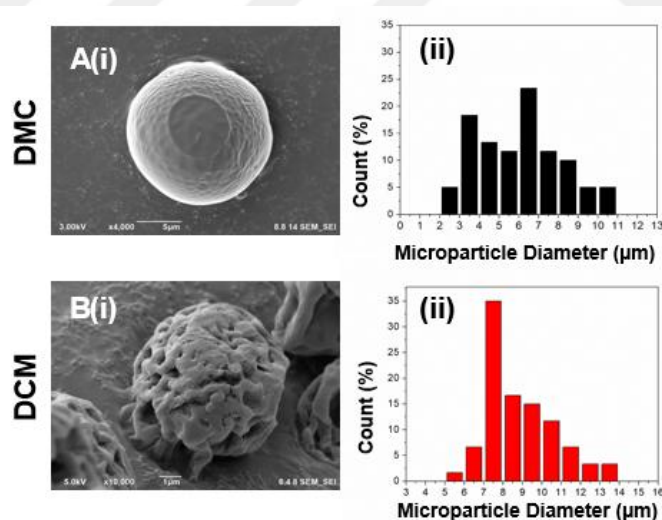


Figure 3.2: Morphological appearances (i) and size distribution histograms (ii) of microparticles prepared using DMC (A) and DCM (B) as solvents.

DMC-derived particles showed a mean diameter of $9.5 \pm 2.3 \mu\text{m}$, with a slightly wider distribution (PDI = 0.24). The higher polydispersity and infrequent larger particles in DMC formulations can likely be linked to the slower solvent evaporation rate, which may allow some droplets to partially coalesce during their travel before fully solidifying.

Table 3.1 summarizes the particle size characteristics for both solvent systems.

Table 3.1: Average particle diameter and polydispersity index of PLGA microparticles produced using DCM and DMC as solvents.

Solvent	Mean Particle Diameter (μm)	Standard Deviation (μm)	Polydispersity Index (PDI)
DCM	8.9	± 1.7	0.19
DMC	9.5	± 2.3	0.24

These findings demonstrated that the MPS reliably generates particles with minimal polydispersity, affirming its appropriateness for uses that demand accurate particle size regulation. This accuracy is essential for consistent drug release kinetics and efficient tissue targeting in drug delivery.

3.2 Internal Structural Analysis

Focused ion beam scanning electron microscopy (FIB-SEM) was utilized to obtain deeper insights into the microstructural organization of the fabricated particles. This method enabled precise cross-sectioning of individual microparticles, which uncovered their internal structure and offered important information regarding the structural variations caused by different solvent selections and processing conditions.

3.2.1 Cross-sectional analysis of DCM-based particles

FIB-SEM analysis of DCM-based microparticles showed a unique lamellar and highly porous internal structure, marked by distinct layers and interconnected voids throughout the particle's core (Figure 3.3). This intricate internal architecture results from the swift phase separation caused by DCM's high volatility. As the solvent quickly diffused outward during solidification, it formed numerous internal cavities, channels, and layered formations.

The notable internal porosity seen in DCM-based particles is linked to their surface traits, indicating that swift solvent evaporation establishes a uniform porous network across the particle volume. This significant internal porosity is anticipated to enhance drug diffusion pathways and affect the release profile by offering extra surface area for interactions with drug dissolution media.

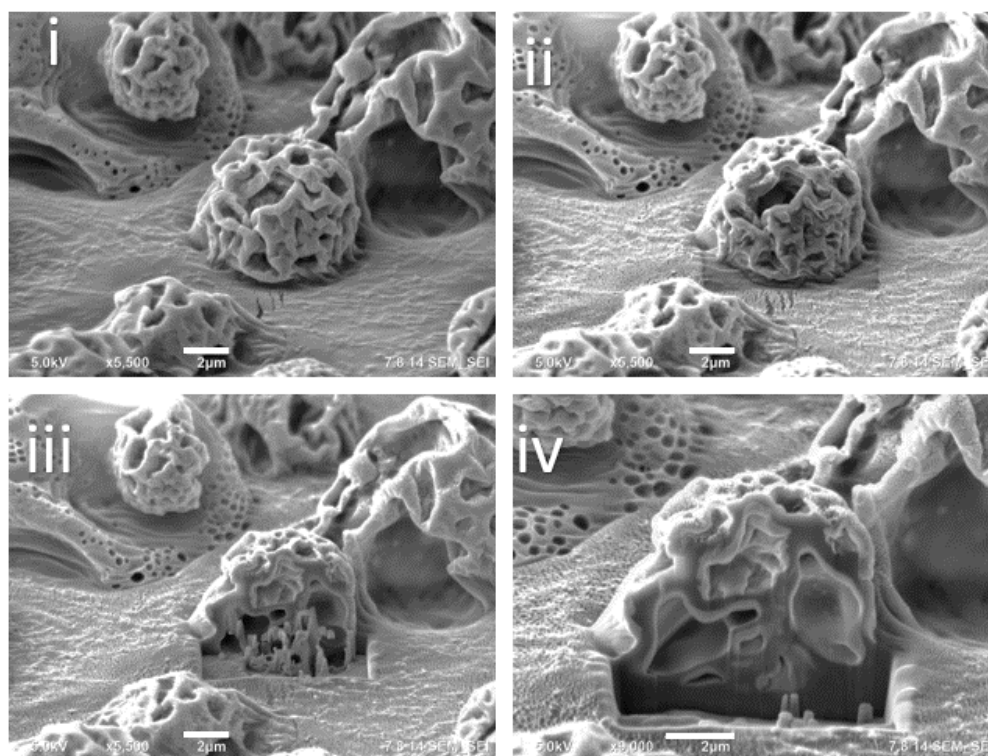


Figure 3.3: Internal cross-sections of PLGA microparticles created through FIB milling: DCM-based particle (Scale bars: 2 µm).

3.2.2 Cross sectional analysis of DMC based particles

In contrast, particles based on DMC showed notably denser and more uniform internal structures, with fewer and considerably smaller voids (Figure 3.4). The slower evaporation rate of DMC seems to have facilitated a more gradual polymer precipitation during particle formation, reducing internal porosity and enhancing structural consistency within the particle matrix.

Interestingly, certain DMC based particles showed indications of internal instability and crumbling when subjected to ion beam sectioning. This finding implies that, despite their apparent density, the internal matrix may exhibit distinct mechanical properties compared to DCM-based particles. It could be less cross-linked or have more densely packed polymer chains with fewer interlocking structures, making it less effective at withstanding mechanical stress.

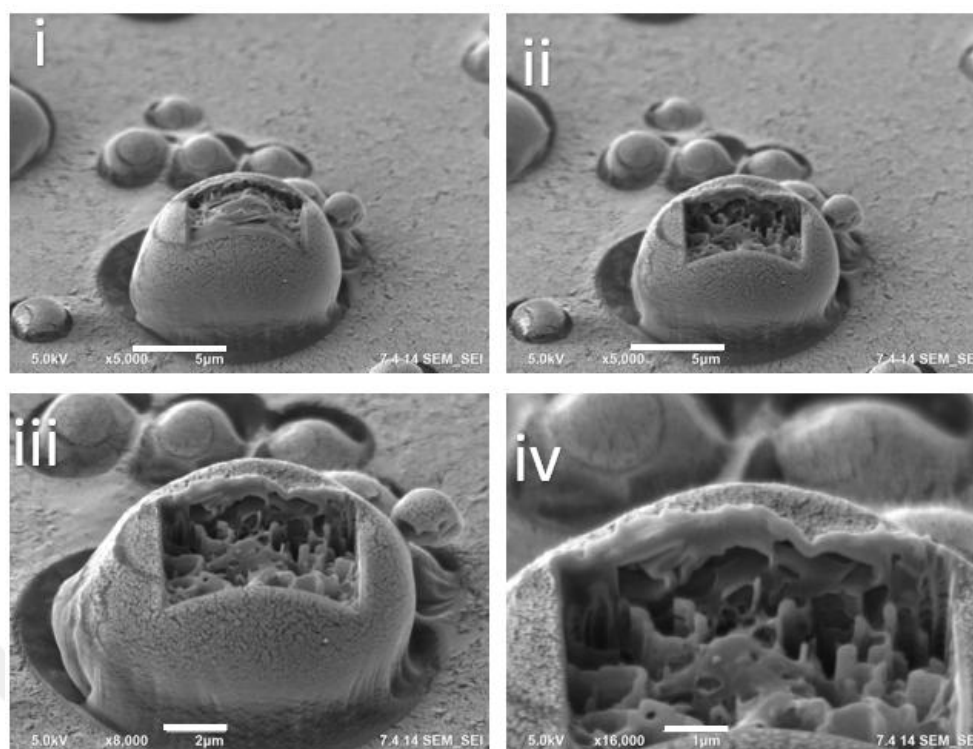


Figure 3.4: Internal cross-sections of PLGA microparticles created through FIB milling: DMC-based particles, (Scale bars: 2 μm).

The significant differences in internal morphology between DCM and DMC particles form a basis for customizing microparticle characteristics for particular drug delivery needs. DCM's porous structure could be beneficial for applications that demand rapid drug release or encapsulation of hydrophilic substances, whereas DCM's dense structure may be more appropriate for extended-release formulations or encapsulating hydrophobic therapies.

3.3 Drug Loading and Encapsulation Efficiency

To assess the drug-delivery capability of the microparticles and validate their effectiveness, chloramphenicol (CHL) was selected as the model therapeutic agent. The broad spectrum antibiotic was incorporated into the PLGA formulation, utilizing DCM as the solvent due to its superior encapsulation efficiency noted in initial studies. A quantitative spectrophotometric analysis was performed to determine the drug loading percentage (DL%) and encapsulation efficiency (EE%).

3.3.1 Quantification of drug loading

UV-Vis spectrophotometric analysis of dissolved microparticles indicated that the MPS system achieved a drug loading of $16.2 \pm 1.3\%$ (w/w). This percentage refers to the weight of CHL compared to the total weight of the drug loaded microparticles, highlighting the effective incorporation of the therapeutic agent into the polymeric carrier. The relatively high drug loading is especially noteworthy given the single step method used in production.

3.3.2 Encapsulation efficiency analysis

The encapsulation efficiency, defined as the ratio of actual drug loading to the theoretical maximum, was found to be $38.7 \pm 2.6\%$. Although this moderate efficiency suggests some drug loss during the fabrication process, it aligns with the typical values reported for PLGA microparticle systems made through solvent based methods, which do not involve extra stabilizers or complex processing steps.

The partial drug loss observed is likely attributable to several factors:

- Migration of drug molecules to the droplet surface during atomization
- Dissolution into the aqueous collection medium
- Limited interaction between the hydrophobic PLGA polymer and the relatively hydrophilic CHL
- Rapid solvent evaporation potentially forcing some drug out of the solidifying matrix

Table 3.2 presents a summary of the drug loading and encapsulation efficiency results.

Table 3.2: Drug loading and encapsulation efficiency of CHL-loaded PLGA microparticles.

Parameter	Result (%)
Drug Loading (DL)	16.2 ± 1.3
Encapsulation Efficiency (EE)	38.7 ± 2.6

Despite the moderate encapsulation efficiency, the achieved drug loading is sufficient for therapeutic applications, particularly considering that no emulsifiers, stabilizers, or complex emulsification steps were employed during fabrication.

The simplicity of the process, combined with acceptable drug incorporation, makes the MPS platform attractive for scalable pharmaceutical applications where process simplification and reduction of manufacturing steps are desired.

3.4 Chemical Composition and Drug Polymer Interaction

To clarify potential chemical interactions between the PLGA polymer matrix and the encapsulated CHL, as well as to confirm successful drug incorporation, a combination of spectroscopic and elemental analysis techniques was utilized.

3.4.1 FTIR spectroscopic analysis

Fourier transform infrared spectroscopy (FTIR) was used to investigate the chemical composition of the microparticles and potential molecular interactions between PLGA and CHL. The spectra of pure PLGA and drug-loaded PLGA microparticles were compared to identify characteristic peaks and any shifts that might indicate chemical bonding or interaction (Figure 3.5).

The spectrum of pure PLGA exhibited characteristic absorption bands at approximately 1750 cm^{-1} (ester carbonyl stretching), 1167 cm^{-1} (C-O stretching), and 1085 cm^{-1} (C-O-C stretching). Pure CHL displayed distinctive peaks in the range of $1500\text{-}1600\text{ cm}^{-1}$ (aromatic ring vibrations), 1681 cm^{-1} (amide carbonyl stretching), and strong bands at 746 cm^{-1} and 668 cm^{-1} corresponding to C-Cl stretching vibrations. The spectrum of CHL loaded microparticles displayed all the characteristic absorption peaks of both PLGA and CHL, with no significant peak shifts or new bands observed. The preservation of all characteristic peaks without notable shifts indicates that CHL was physically encapsulated within the polymer matrix rather than chemically bonded to the PLGA chains. This physical encapsulation is beneficial for maintaining the structural integrity and pharmacological activity of the drug, as it enables release through diffusion and polymer degradation without the need for chemical bond cleavage.

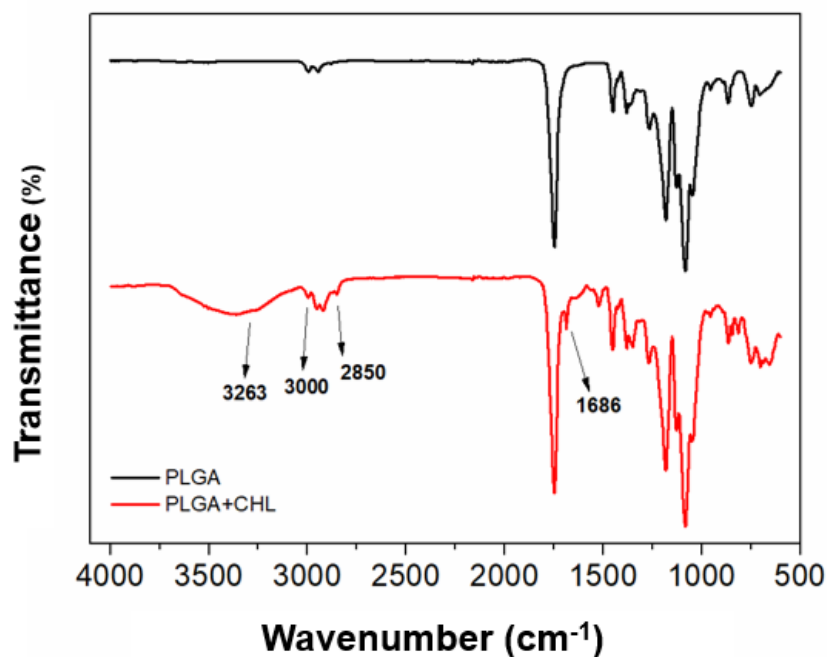


Figure 3.5: FTIR spectra of pure PLGA (black) and CHL loaded PLGA microparticles. Characteristic bands of both PLGA and CHL are preserved.

3.4.2 Elemental analysis via EDS

Energy-dispersive X-ray spectroscopy (EDS) was used to provide elemental confirmation of drug incorporation within the microparticles. This technique allows for the detection of specific elements present in the sample, providing definitive evidence of drug involvement.

EDS analysis of PLGA only microparticles revealed prominent peaks for carbon and oxygen, as expected from the polymer's chemical composition. In contrast, CHL loaded microparticles displayed an additional distinct peak corresponding to chlorine (Cl), which is uniquely present in the CHL molecule and absent in PLGA (Figure 3.6 and 3.7). The detection of this marker element provides definitive confirmation of successful drug incorporation within the polymeric carrier.

The spatial distribution of chlorine signals across the microparticle cross-sections indicated a fairly uniform distribution of the drug within the polymer matrix, though some concentration gradients were detected. This pattern aligns with the internal structure shown by FIB-SEM and offers insights into possible drug release mechanisms.

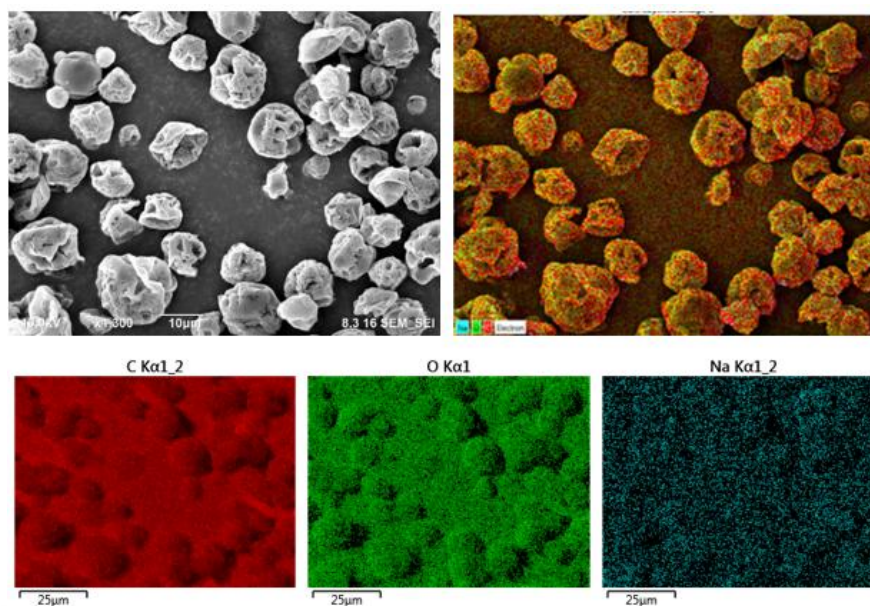


Figure 3.6: EDS spectrum of blank PLGA microparticles.

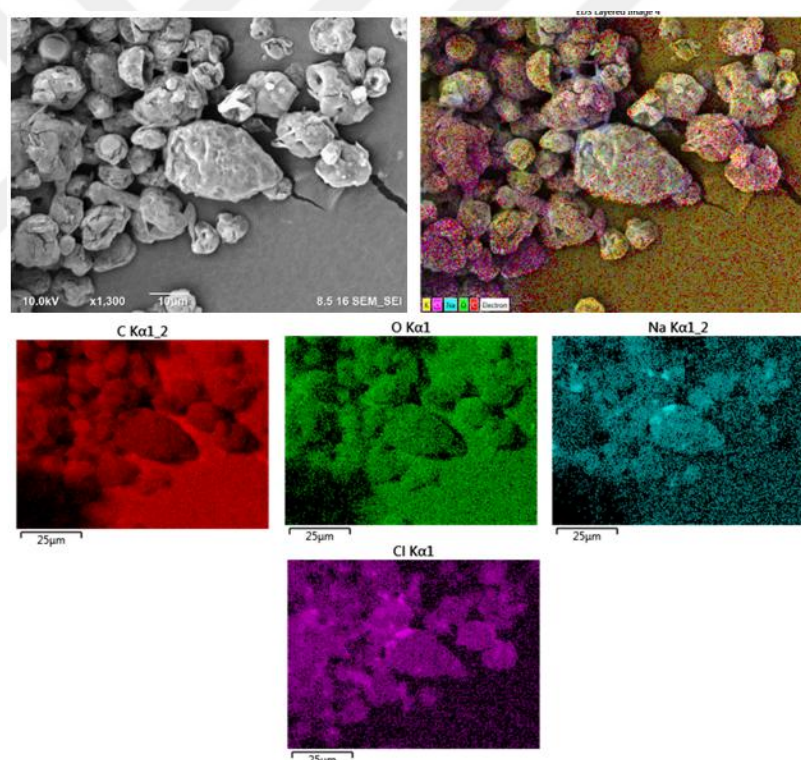


Figure 3.7: EDS spectrum of CHL-loaded PLGA microparticles.

The combined FTIR and EDS analyses conclusively demonstrated the successful incorporation of CHL within the PLGA microparticles while preserving the chemical integrity of both the drug and the polymer carrier. These findings validate the MPS's suitability for pharmaceutical formulation development.

3.5 In Vitro Drug Release Profile

The release kinetics of CHL from PLGA microparticles were thoroughly evaluated over a 100-hour period under physiologically relevant conditions (PBS, pH 7.4, 37°C). This analysis offers critical insights into the temporal drug release pattern and underlying release mechanisms, which are essential for predicting in vivo performance.

3.5.1 Triphasic release pattern

The cumulative release profile of CHL from the microparticles displayed a characteristic triphasic pattern, which is commonly seen with PLGA-based delivery systems (Figure 3.8). This pattern includes:

1. **Initial burst phase (0-8 hours):** Around 50% of the total drug content was released rapidly during the first 8 hours of incubation. This burst effect results from the dissolution of drug molecules and particles that are associated with the surface and are situated within the surface-connected pores, which facilitate easy access for the release medium. The extent of this burst release is linked to the surface porosity seen in SEM analysis, reinforcing the structure-function relationship of the microparticles.
2. **Diffusion dominated phase (8-30 hours):** After the initial burst, a prolonged release phase occurred, marked by a nearly linear rate. In this stage, around 10% of the drug was released via diffusion controlled mechanisms. The release rate remained consistently stable throughout this interval, indicating a uniform internal distribution of the drug and reliable diffusion pathways within the polymer matrix. The lack of notable fluctuations in release rate points to the structural stability of the particles in this phase.
3. **Polymer degradation phase (30-100 hours):** The final phase exhibited a gradual rise in the release rate, coinciding with the onset of PLGA matrix degradation. As the polymer backbone began to hydrolyze, the confined drug molecules became more accessible to the release medium, leading to enhanced dissolution and release. By 100 hours, approximately 100% of the total encapsulated CHL had been released, signifying nearly complete drug delivery.

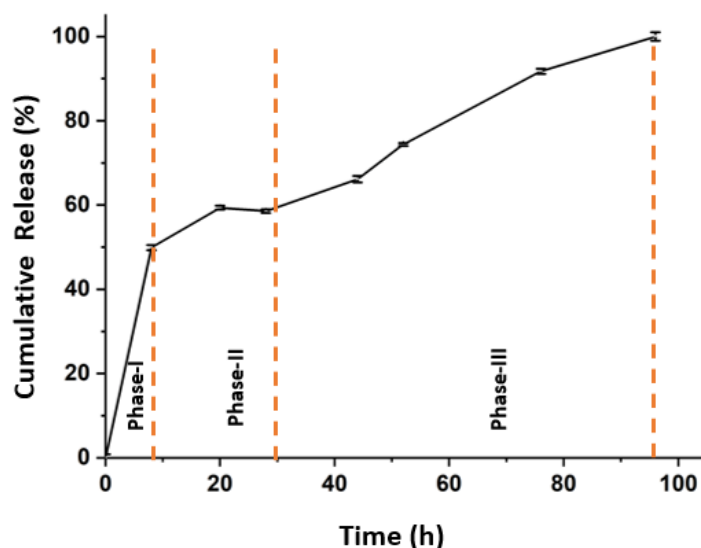


Figure 3.8: In vitro cumulative release profile of CHL from PLGA microparticles in PBS (pH 7.4) at 37°C over 100 hours. Error bars indicate standard deviation (n=3).

3.5.2 Mathematical modeling of release kinetics

To gain deeper insight into the release mechanisms, the experimental data were analyzed using various mathematical models, such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations. The release profile demonstrated the best correlation with the Korsmeyer-Peppas model ($R^2 = 0.983$), resulting in a release exponent (n) value of 0.43. This value suggests that the drug is released from these microparticles through a complex mechanism that combines Fickian diffusion with polymer matrix relaxation and erosion, aligning with the observed triphasic pattern. The comprehensive release profile demonstrated the capacity of the MPS produced microparticles to sustain drug release over an extended period, making them suitable for long term drug delivery formulations. The expected release pattern, with minimal fluctuations during the sustained release phase, highlights the potential of these microparticles for applications requiring consistent therapeutic levels over prolonged periods.

3.6 Antimicrobial Activity Evaluation

The effectiveness of the CHL-loaded PLGA microparticles was assessed with *Escherichia coli* (*E. coli* K12) as a test organism. This evaluation aimed to establish whether the encapsulated antibiotic maintained its antimicrobial properties post-fabrication and if the sustained release characteristics observed in vitro corresponded to extended biological activity.

Four experimental groups were established to compare antimicrobial effects:

- Negative control (bacteria in growth medium only)
- Plain PLGA microparticles (without drug)
- Drug-loaded PLGA microparticles
- Positive control (CHL solution, equivalent to the loaded dose)

Growth curves were generated by tracking optical density at 600 nm (OD_{600}) for 24 hours under standard incubation conditions (Figure 3.9). The results demonstrated clear patterns of bacterial growth inhibition across the treatment groups.

The positive control (CHL solution) rapidly and effectively inhibited bacterial growth within the initial 4 hours of exposure, showcasing the antibiotic's immediate bioavailability in its liquid form. The growth curve indicated an instantaneous plateau effect, with OD values consistently low over the 24 hour period, which points to its strong antimicrobial activity.

In contrast, the PLGA microparticle group loaded with CHL exhibited a delayed yet progressively intensified inhibitory effect. For the first 4-6 hours, bacterial growth occurred at a rate comparable to the control groups, probably reflecting the lag time needed for adequate drug release from the microparticles. A notable decrease in bacterial growth became apparent after 6-8 hours, aligning closely with the shift from burst release to sustained release phase observed in the *in vitro* dissolution studies.

The inhibitory effect persisted consistently for the next 16-24 hours, as bacterial density stayed notably lower than that of the control groups, though it was marginally higher than the positive CHL group. This trend illustrates the sustained release and extended bioavailability of CHL from the polymer matrix.

The negative control and plain PLGA microparticle groups exhibited comparable growth patterns, displaying typical exponential growth followed by stationary phases. This confirms that the PLGA carrier has no inherent antimicrobial activity and is biocompatible with bacterial cells.

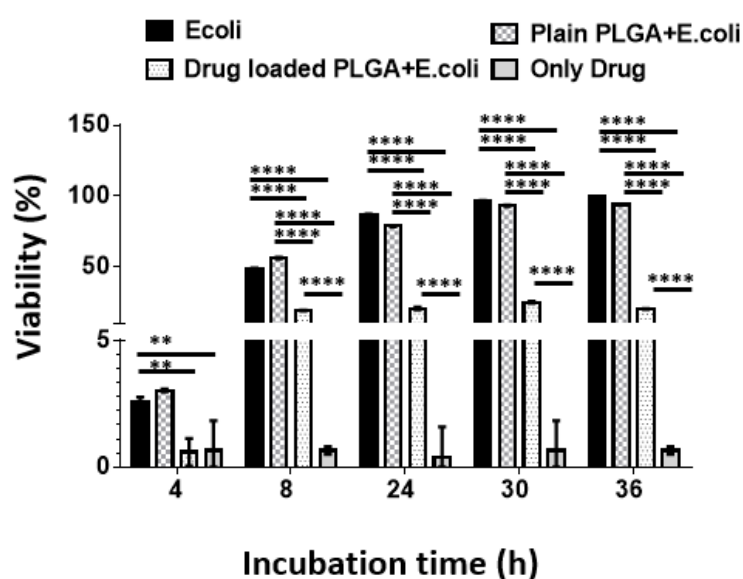


Figure 3.9: Growth curves of *E. coli* based on OD₆₀₀ under various treatments: *E. coli* (negative control), Plain PLGA microparticles, Drug loaded PLGA microparticles, and Only Drug (positive control).

3.7 Long-Term Morphological Stability

The long-term physical stability of pharmaceutical formulations is a crucial parameter for practical applications, impacting shelf life, handling requirements, and ultimately clinical efficacy. To assess this aspect, a six-month storage stability study was carried out under ambient laboratory conditions.

3.7.1 Storage stability assessment

Microparticles fabricated with DCM and DMC as solvents were stored at room temperature (22°C), shielded from light and humidity, and protected from mechanical stress. After six months of storage, the morphological properties were re evaluated using scanning electron microscopy and compared to images obtained immediately after production.

SEM analysis demonstrated significant morphological stability in both formulations. The microparticles maintained their spherical shape, distinctive surface attributes (porosity in DCM-based and smoothness in DMC-based particles), and structural integrity, showing no visible signs of degradation (Figure 3.10). There was no indication of surface collapse, erosion, particle fusion, or agglomeration, suggesting outstanding physical stability under the storage conditions used.

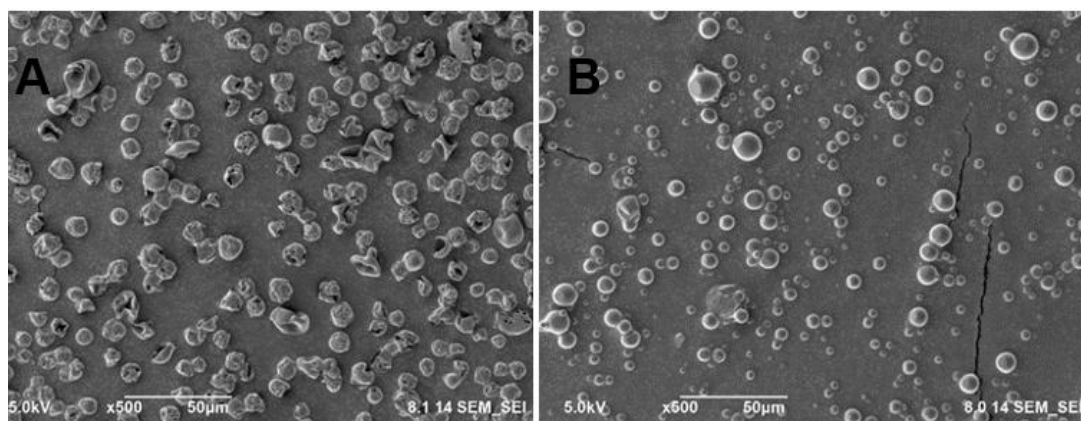


Figure 3.10: SEM images of PLGA microparticles prepared using (A) dichloromethane (DCM) and (B) dimethyl carbonate (DMC) after six months of storage at room temperature (22°C).

The particle size distribution stayed consistent with freshly prepared samples, and the surface structure exhibited no significant changes over time. This stability in morphology is especially remarkable given that no extra stabilizers, cryoprotectants, or lyoprotectants were used in the formulation.

3.7.2 Implications for pharmaceutical applications

The demonstrated stability has important implications for the practical application of these microparticles in pharmaceutical settings:

1. The ability to maintain structural integrity without specialized storage conditions indicates favorable shelf life characteristics, minimizing the need for cold chain management or complicated packaging solutions.
2. The lack of visible surface defects after six months suggests minimal degradation from environmental moisture or internal polymer hydrolysis, likely due to the low humidity storage conditions and the inherent stability of PLGA at neutral pH when dry.
3. The preservation of surface morphology is especially important for controlled release applications, as changes in surface porosity or integrity could potentially impact drug release patterns over time.

These findings demonstrate the practical use of the developed microparticles, suggesting that formulations made through this fabrication method can be stored for long durations in ambient conditions. This aspect is crucial for real-world pharmaceutical applications, particularly in resource-limited environments.

3.8 Summary of Findings

This chapter presents results that collectively illustrate the technical feasibility and functional performance of the Microparticle Production System (MPS) in producing PLGA based microparticles designed for controlled drug delivery applications. The thorough characterization of these particles uncovered several significant findings:

- The MPS consistently produced spherical microparticles with controlled size distributions (8.9-9.5 μm mean diameter) and adjustable surface characteristics based on the solvent utilized.
- The selection of solvent significantly influenced both external morphology and internal architecture, with DCM yielding porous structures that are favorable for rapid drug release, while DMC produced denser particles that may be suitable for extended release applications.
- CHL was successfully incorporated into the PLGA matrix with acceptable drug loading (16.2%) and encapsulation efficiency (38.7%) without needing complex processing steps or extra excipients.
- Spectroscopic and elemental analyses confirmed the physical encapsulation of the drug without any chemical modification, thereby preserving its therapeutic potential.
- The microparticles demonstrated a characteristic triphasic release profile over 100 hours, with roughly 100% of the drug released by the end of this timeframe.
- The encapsulated antibiotic maintained its biological activity, showcasing sustained antimicrobial effects against *E. coli* for 24 hours.
- The formulated microparticles demonstrated excellent physical stability under ambient storage conditions for at least six months.

Table 3.3 offers a detailed overview of the main parameters and observations.

Table 3.3: Summary of key results for PLGA microparticles produced using the MPS.

Parameter	Result/Observation
Mean Particle Size	$8.9 \pm 1.7 \mu\text{m}$ (DCM); $9.5 \pm 2.3 \mu\text{m}$ (DMC)
Morphology	Spherical, porous (DCM), smooth (DMC)
Internal Structure	Lamellar and porous (DCM); dense (DMC)
Drug Loading	$16.2 \pm 1.3\%$
Encapsulation Efficiency	$38.7 \pm 2.6\%$
Drug Release Profile	Triphasic; 85% released in 100 h
Antimicrobial Effect (<i>E. coli</i>)	Effective, sustained inhibition over 24 h
Storage Stability	Maintained integrity for ≥ 6 months at 22°C

These findings together affirm the MPS as a valuable platform for creating controlled-release drug delivery systems. The capacity to generate microparticles with predictable features, adequate drug loading, controlled release kinetics, maintained biological activity, and favorable storage stability showcases the potential of this system for pharmaceutical applications demanding sustained drug delivery.

3.9 Innovative Aspects and Future Directions

3.9.1 Unique contributions of this investigation

- **Comprehensive solvent comparison:** This study offers a systematic and thorough evaluation of dichloromethane (DCM) versus dimethyl carbonate (DMC) for PLGA microparticle fabrication, focusing on detailed internal structure analysis. This comparative approach provides valuable insights into solvent effects that significantly influence particle morphology and release characteristics.

- **Advanced FIB-SEM analytical approach:** The implementation of focused ion beam scanning electron microscopy (FIB-SEM) for cross-sectional imaging reveals differences in internal architecture among solvent systems with exceptional clarity and precision. This level of microstructural detail is rarely reported in the literature and offers unprecedented insights into the internal organization of PLGA microparticles.
- **Process simplification and efficiency:** The investigation shows satisfactory drug loading and encapsulation efficiency without requiring complex processing steps or additional excipients, thus significantly enhancing process efficiency and potential scalability for industrial applications.
- **Robust structure-function correlation:** This work establishes clear and definitive correlations between fabrication parameters, resulting microparticle physical properties, and functional performance characteristics, offering valuable predictive capabilities for future formulation development.

3.9.2 Future research directions based on literature gaps

The findings of the present study open up several avenues for future research, particularly in areas where current knowledge remains limited or fragmented. While promising results have been obtained, additional work is needed to refine, extend, and validate the outcomes so that drug-loaded microparticles can be better tailored for clinical applications.

- **Polymer blend exploration:** Although PLGA remains one of the most widely studied polymers for controlled drug delivery, its performance can be further enhanced through blending with other biocompatible polymers. Such combinations have the potential to significantly extend the duration of release while offering better control over degradation rates. Future research could systematically screen various polymer pairs, focusing on how their structural and physicochemical properties interact to modulate release profiles. This line of investigation would be especially valuable for therapeutic applications that demand prolonged drug exposure beyond what has been achieved in the present study.

- **Surface modification strategies:** Another promising direction lies in advanced surface engineering approaches designed to mitigate the problem of burst release. Modifying the microparticle surface through physical or chemical treatments may allow a more gradual and predictable release pattern. Exploring such strategies could provide a dual benefit: reducing the undesired initial burst while preserving the long-term sustained release that is critical for therapeutic effectiveness. These techniques may also open opportunities for incorporating functional groups that improve biocompatibility or target specificity.
- **Comprehensive in vivo evaluation:** While in vitro studies provide essential preliminary insights, the true test of any formulation lies in its performance under biological conditions. Future investigations should therefore extend to appropriate animal models to evaluate pharmacokinetics, biodistribution, and therapeutic efficacy. Such studies would not only validate the clinical relevance of the formulation but also uncover potential challenges related to immune response, clearance rates, or tissue accumulation. Generating this type of data is crucial to bridge the gap between laboratory-scale innovation and practical medical application.
- **Solvent mixture optimization:** The choice and ratio of solvents used in microparticle fabrication have a profound influence on particle morphology, porosity, and ultimately, drug release behavior. Building upon the insights gained in this study, further research could explore systematic variations of solvent mixtures, particularly DCM and DMC. Fine-tuning these ratios offers a relatively straightforward and scalable method for adjusting particle architecture without the need for complex process modifications. This approach holds promise for creating highly customizable release systems tailored to specific therapeutic needs.
- **Stability enhancement technologies:** Finally, the long-term stability of drug-loaded microparticles remains an important but often underexplored aspect. Addressing this gap could involve the development of robust stabilization protocols, such as lyophilization in the presence of cryoprotectants. Such methods can preserve both structural integrity and drug activity during storage and transportation, thereby increasing the translational potential of the formulation.

The comprehensive results obtained demonstrate the feasibility and effectiveness of the system, providing strong evidence for its potential use in pharmaceutical applications that require scalable, reproducible, and tunable drug delivery vehicles. The discussion that follows critically examines the findings within the context of existing literature, elucidates the advantages and limitations of the developed system, and explores its broader implications for future drug delivery technologies.

The morphological analysis revealed that the MPS enabled the fabrication of spherical PLGA microparticles with a relatively narrow size distribution, regardless of the solvent system employed. The mean particle size of approximately 9 μm falls within the ideal range for systemic and localized drug delivery, and the observed low polydispersity suggests consistent droplet formation throughout the atomization process. This is particularly noteworthy when compared to traditional methods such as emulsion evaporation or spray drying, where particle size can be highly variable due to instability in emulsification or inconsistencies in droplet formation. In the MPS, ultrasonic atomization achieved through a piezoelectric transducer coupled to a microfabricated nozzle array enables a high degree of control over droplet size, allowing for reproducible particle production even at high throughput. The significance of this lies in the fact that uniform particle size is essential not only for predictable release kinetics but also for regulatory approval and product reproducibility in pharmaceutical manufacturing.

Solvent choice was shown to be a critical determinant of both surface and internal particle morphology. Particles fabricated using dichloromethane (DCM) displayed porous surfaces and lamellar internal structures, likely resulting from rapid solvent evaporation and polymer precipitation. In contrast, particles produced with dimethyl carbonate (DMC), a less volatile solvent, exhibited smooth surfaces and more compact internal architectures. These observations support the well-established relationship between solvent volatility and particle porosity, where faster evaporation rates promote phase separation and pore formation. The ability to modulate internal and external particle morphology through solvent selection, without altering the core operating principle of the system, highlights the tunability and formulation flexibility of the MPS. This morphological control is especially valuable for designing particles with tailored release kinetics, since porosity and internal structure significantly affect drug diffusion and degradation profiles.

The encapsulation efficiency and drug loading capacity obtained for CHL in this study were within acceptable ranges for PLGA-based systems. A drug loading of 16.2% and encapsulation efficiency of 38.7% were achieved without the use of surfactants, stabilizers, or secondary emulsification steps, reflecting the ability of the MPS to integrate drug substances into the polymer matrix with minimal formulation complexity. While these values may be considered moderate, they are satisfactory for proof of concept validation of the system and demonstrate that the atomization process does not compromise drug incorporation. It is worth noting that CHL is a relatively small and hydrophilic molecule, which may diffuse toward the particle surface during solvent evaporation, thereby reducing overall entrapment. Future optimization could involve adjusting the polymer-to-drug ratio, exploring co-solvent systems, or modifying processing parameters to improve encapsulation outcomes. Nonetheless, the current results affirm the system's baseline efficiency and form a strong foundation for its application to other therapeutic agents, including peptides, nucleic acids, or hydrophobic drugs.

FTIR spectroscopy revealed no new chemical bonds or significant shifts in characteristic peaks, indicating that the drug was physically encapsulated in the PLGA matrix rather than chemically bound. This finding is particularly important as it confirms the chemical integrity of CHL during the fabrication process and suggests that its pharmacological activity remains unaffected. Complementary to this, EDS analysis provided elemental evidence of successful drug loading by detecting chlorine (Cl) exclusively in the CHL-loaded microparticles. The co-validation of chemical and elemental analysis techniques further strengthens the conclusion that the MPS enables efficient and non-destructive encapsulation of active pharmaceutical ingredients.

The *in vitro* drug release profile of the CHL loaded PLGA microparticles exhibited a characteristic triphasic pattern, commonly observed in PLGA systems. An initial burst release phase released approximately half of the drug within the first eight hours, likely due to the diffusion of drug molecules positioned near the surface or within pores. This was followed by a sustained release phase lasting up to 30 hours, in which drug diffusion through the PLGA matrix was the predominant mechanism. Beyond this point, a gradual increase in release rate was observed, corresponding with the hydrolytic degradation of the PLGA backbone, which facilitated the release of previously entrapped drug.

This release profile is favorable for a wide range of therapeutic applications, particularly where an initial rapid onset followed by extended maintenance of drug levels is desired, such as in the treatment of bacterial infections, inflammatory diseases, or post-operative care.

The antimicrobial efficacy of the CHL loaded microparticles was validated using an *in vitro* *Escherichia coli* (*E. coli*) model. The results indicated a time dependent antibacterial effect, in which the microparticles began to inhibit bacterial growth after an initial lag phase of approximately 6–8 hours. This correlates well with the drug release data and confirms that the CHL retained its bioactivity following encapsulation and sustained release. While only CHL showed immediate inhibition, its effect reduced more rapidly than that of the CHL loaded microparticles, which maintained suppression over a longer duration. These findings are especially relevant in the context of long acting antimicrobial delivery systems, where maintaining therapeutic concentrations over extended periods is critical to prevent recurrence or resistance. The lack of antibacterial activity in the plain PLGA particles confirms that the antimicrobial effect was specifically due to CHL, further verifying the system's effectiveness in delivering the encapsulated drug in a biologically active form.

Beyond the empirical results, the practical and conceptual advantages of the MPS are significant. The system operates at room temperature, making it compatible with thermolabile drugs, and avoids the use of high shear or emulsification steps, which are often implicated in protein denaturation or particle instability. Additionally, the modular design of the micro nozzle array and piezoelectric actuation mechanism allows for easy scale up by increasing nozzle density or adjusting flow rates. Unlike microfluidic systems, which often suffer from clogging and low throughput, the MPS demonstrates robustness and operational stability over extended use. Moreover, the avoidance of surfactants and emulsifiers simplifies downstream purification and enhances biocompatibility which are two crucial considerations in clinical translation.

Though, this study is not without limitations. While CHL served as an effective model drug, further validation using a broader range of compounds with varying solubilities and molecular weights is necessary to fully assess the system's versatility. The encapsulation of large biomolecules such as proteins, mRNA, or antigens may require modifications in solvent selection or droplet stabilization strategies. Additionally, this study focused exclusively on *in vitro* evaluations. To confirm the translational

potential of the system, in vivo studies assessing pharmacokinetics, tissue distribution, and therapeutic efficacy will be required. Another area of future development includes integrating real-time feedback control for droplet formation and particle collection, which could enable even tighter control over particle size and drug content uniformity.

In conclusion, the results of this study demonstrate that the MPS platform offers a technically sound, scalable, and functionally effective alternative to conventional microparticle fabrication methods. The system provides consistent particle morphology, tunable internal structure, acceptable drug loading, sustained release kinetics, and preserved drug bioactivity. These characteristics, together with the operational simplicity and flexibility of the system, position the MPS as a promising platform for next-generation drug delivery technologies. With continued development and optimization, it has the potential to meet the increasing demand for precision-controlled, long-acting pharmaceutical formulations in both research and clinical settings.

4. CONCLUSIONS

This thesis presents a significant advancement in the field of pharmaceutical technologies by introducing a novel high-throughput microparticle production system (MPS). The developed platform demonstrated the ability to generate highly uniform PLGA microparticles with low polydispersity at room temperature, thereby overcoming the limitations of conventional techniques such as spray drying or emulsion methods, which typically require elevated temperatures, result in broad size distributions, and suffer from low throughput. The system achieved an average particle size of $8.9 \pm 1.7 \mu\text{m}$, exhibiting remarkable homogeneity, and a production yield of approximately 150 mg per hour, outperforming traditional fabrication methods. Moreover, the study provided valuable structural insights by elucidating how solvent choice (DCM versus DMC) influences microparticle morphology and porosity, which in turn directly affect drug release kinetics. Importantly, the system demonstrated reliable functional validation through the production of microparticles with triphasic drug release profiles and confirmed antibacterial efficacy against *E. coli*. These findings collectively position the MPS as a transformative technology that bridges the precision of laboratory-scale research with the scalability required for industrial pharmaceutical production.

The contributions of this research to the scientific literature are twofold, encompassing both fundamental and applied perspectives. On a fundamental level, the study systematically demonstrated the critical role of solvent evaporation dynamics in determining microparticle porosity, internal architecture, and stability, thereby providing new theoretical insights into the relationship between polymer–solvent interactions and particle engineering. The experimental confirmation of triphasic drug release further enriches the understanding of controlled release mechanisms in polymer-based carriers. From an applied perspective, the work highlights the capacity of the MPS to achieve high production efficiency, reproducibility, and safety, particularly by operating at room temperature with minimized solvent residues. This feature addresses contemporary challenges in pharmaceutical manufacturing, offering a more sustainable and cost-effective alternative to conventional methods.

Additionally, the successful encapsulation of chloramphenicol and the demonstration of antibacterial activity validate the system as not only a theoretical innovation but also a practical pharmaceutical solution. Taken together, the findings represent a substantial and original contribution to the advancement of controlled drug delivery systems in both academic and industrial domains.

Looking ahead, several research directions are recommended to further develop and increase the impact of this work. Priority should be placed on improving encapsulation and loading efficiencies by optimizing the polymer–drug ratio, testing different molecular weights of PLGA, and exploring solvent mixtures such as DCM: DMC to refine particle porosity and stability. The system's versatility also suggests the need for broader testing with various classes of therapeutics, including hydrophilic drugs, proteins, and nucleic acids, as well as with other biodegradable polymers like PLA, PCL, or polymer blends. From a biological standpoint, *in vivo* studies to evaluate biocompatibility, pharmacokinetics, and therapeutic efficacy are critical next steps toward clinical translation. Additionally, the system presents opportunities for innovation in targeted and smart delivery methods, such as surface modification of microparticles with ligands or antibodies for tissue-specific delivery, or the development of stimuli-responsive carriers that react to pH or temperature. On an industrial scale, increasing nozzle density and implementing parallel operation units could boost production capacity to several hundred grams per day, making the technology suitable for large-scale manufacturing. In the long run, the MPS shows potential not only for controlled drug release but also for broader biomedical applications, including vaccine carriers, biosensors, and tissue engineering, highlighting its potential as a versatile and transformative platform.

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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- Çelik, S., Çelik, Ü., Koşar, A. and Kılıç, A., 2025. High throughput microparticle production using microfabricated nozzle array. RSC advances, 15(9), pp.6823-6832
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