MITOMYCIN C RELEASE FROM MOLECULARLY IMPRINTED MICROCRYOGELS

MOLEKÜLER BASKILANMIŞ MİKROKRİYOJELLERDEN MİTOMİSİN C SALIMI

Soheil ZABIHI IMECHEH

PROF. Dr. ADİL DENİZLİ

Supervisor

Prof. Dr. Handan YAVUZ ALAGÖZ

Co- Supervisor

Submitted to

Graduate School of Science and Engineering Hacettepe University as a Partial Fulfillment to the Requirements for the Award of the Degree of Doctor of Philosophy in Chemistry.

2024

For Whom My Heart Beats With Loving Them

(My Family)

ABSTRACT

MITOMYCIN C RELEASE FROM MOLECULARLY IMPRINTED MICROCRYOGELS

Soheil ZABIHI IMECHEH

Doctor of Philosophy, Department of Chemistry

Supervisor: Prof. Dr. Adil DENİZLİ

Co- Supervisor: Prof. Dr. Handan YAVUZ ALAGÖZ

August 2024, 120 pages

Molecularly imprinted polymers (MIPs) offer the self-organization of functional monomer/s around target molecules, leading formation of area for the target molecules with the high selectivity. Here, microscale cryogels as injectable forms of [poly (hydroxyethyl methacrylate)] p(HEMA) was developed as novel drug delivery materials using molecular imprinting technology for control the released of Mitomycin C (MMC) as an anti-cancer drug. The MMC imprinted microcryogels were prepared using a micro stencil array chip with microwells of 200 μm diameter and 500 μm thickness and then characterized by Scanning electron microscope (SEM) and swelling degree properties. Furthermore, the non-imprinted microcryogels were prepared to show the imprinted

effect of the drug released performance of MMC. The findings showed that the drug imprinted microcryogels could be cost-friendly, and injectable for control release of anti-cancer drugs.

Keywords: Injectable Microcryogels, Molecularly Imprinted Polymers, Anticancer drugs, Drug controlled release, Drug Delivery, Mitomycin C.

ÖZET

MOLEKÜLER BASKILANMIŞ MİKROKRİYOJELLERDEN MİTOMİSİN C SALIMI

Soheil ZABIHI IMECHEH

Doktora, Kimya Bölümü

Tez Danışmanı: Prof. Dr. Adil Denizli

Eş Danışman: Prof. Dr. Handan YAVUZ ALAGÖZ

Ağustos 2024, 120 sayfa

Kontrollü ilaç salınımının amacı ilacın vücudun en uygun bölgesine terapötik doz ile salımını sağlamaktır. Bunun yanında ilacın yan etkilerini azaltmak, hastaya en uygun doz şekline verilmesini sağlamak ve ilacın farmakolojik etkisini uzun süre tutmaktır. Moleküler baskılama işlemi, kalıp molekül ile çevresindeki fonksiyonel monomer ve çapraz bağlayıcının üç boyutlu etkileşimlerine dayalı bir teknolojidir. Kriyojeller, kısmen donmuş monomer veya polimer çözeltileriyle hazırlanan üç boyutlu jel matriksleridir. Makrogözenekli yapıları sayesinde hızlı şişme kinetiği ile ilaç salım sistemlerinde kullanılabilirler. Mitomisin C (MMC) çok geniş spektrumlu bir antibiyotiktir. Antiproliferatif özelliğinden dolayı göğüs, prostat kanserlerinde kullanılır. Mesane yüzey kanseri tedavisi için ilk tercih edilen ilaçtır. Oftalmolojide, glokom hastalıklarında ve trabulektomi hastalıklarında yardımcı ajan olarak kullanılmaktadır. Bu çalışmada MMC'nin kontrollü salımı için MMC baskılanmış mikrokriyojeller hazırlanmış ve in-vitro ortamda ilaç salımı incelenmiş. Fonksiyonel monomer özellikli tanıma bölgeleri

oluşturmak için MMC ile kompleksi oluşturulmuş. Mikrokriyojellerin yüzey yapısı ve spesifik yüzey alanları SEM ve BET yöntemleri ile belirlenmiştir. Ortam pH'si, ortam sıcaklığı, çapraz bağlama ajanı miktarı ve ilaç miktarı gibi parametreler değiştirilerek çevre şartları ve polimer yapısının mikrokriyojellerden ilaç salım miktarına etkisini incelenmiştir. Bu çalışmalarla, tezde uygulanan kontrollü salınım tekniğiyle, ilacın veriminin korunması için gereken miktarda uygulanması sağlanmış, aşırı ya da az ilaç kullanımının önüne geçilmesi önlenmiş olmuştur. Ayrıca biyoteknolojik yaklaşım mikrokriyojellerin makrogözenekleri ile yüksek senekleri sayesinde mevcut yerli ya da yabancı kaynaklı üretilen kontrollü salım sistemlerine avantaj sağlayacaktır. Mitomisin C baskılanmış kriyojel membranlardan, baskılanmamış kriyojel membranlara göre, Mitomisin C salımı çok daha fazla miktarlarda ve daha uzun sürede gerçekleşmiştir. Mitomisin C baskılanmış ve baskılanmamış membranların ilaç salımı, güç yasası olarak da ifade edilen, Fick eşitliği kullanılarak matematiksel olarak analiz edilmiştir. Elde edilen ilaç salım mekanizmasını belirleyen 'n' salım üsteli, tüm salım çalışmalarında 0.5 ile 1 arasında bulunmuştur. Bu değerler arasında kalan 'n' değeri güç yasasına göre, salımın non-fickian kanuna uyduğunu göstermektedir. Güç yasasından elde edilen ve difüzyon katsayısı olarak ifade edilen 'k' değeri, kriyojel yapısındaki monomer çapraz bağlayıcı oranının ve kalıp molekül miktarlarının artmasıyla artmıştır. Baskılanmış ve baskılanmamış kriyojel membranların Mitomisin C salımı için yapılan matematiksel analiz sonucu baskılanmamış kriyojel membranlar için elde edilen 'k' değerinin daha büyük olduğu bulunmuştur. Bu sonuç, Mitomisin C baskılanmamış kriyojel membranlardan deneysel olarak elde edilen verilere göre daha hızlı ilaç salımının gerçekleştiğini desteklemektedir.

Anahtar Kelimeler: Enjekte Edilebilir Mikrokriyojeller, Moleküler Baskılı Polimerler, Kanser önleyici ilaçlar, İlaç kontrollü salım, İlaç Taşıma, Mitomisin C.

ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my research supervisor and head of biochemistry at Hacettepe University Prof. Dr. Adil Denizli for giving me the opportunity to do research and providing invaluable guidance throughout this research. His dynamism, vision, sincerity, and motivation have deeply inspired me. He has taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under his guidance. I am extremely grateful for what he has offered me. I would also like to thank him for his friendship, empathy, and great sense of humor. I would like to say thanks to my cheerful advisor Prof. Dr. Handan Yavuz Alagöz. I gained an indispensable experience during the time that I have worked with her. I would like to thank deeply to each member of BIOREG research group to make the laboratory as a second home for me, I have a lot of amazing memory with you that I'll never forget. I feel very happy, lucky, and private person, because of your friendships. I would like to thank to my friends; Dr. Monireh Bakhshipour, Dr. Semra Akgönüllü, Merve Çalışır, Aykut Arif Topçu and Jamileh Salimi thanks a lot for everything.

I am extending my heartfelt thanks to my father that passed away and my mother may God give her more life, and my wife for supporting for their acceptance and patience during the discussion I had with him on research work and thesis preparation. I am extremely grateful to my parents for their love, prayers, caring and sacrifices for educating and preparing me for my future. I would like to thank God, who has given me his blessing and power to pursue my dreams. I could have never achieved this without the faith I have in you. Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Soheil ZABIHI IMECHEH

Ankara-Turkey

CONTENTS

FIGURE LEGENDS

TABLE LEGENDS

SYMBOLS AND ABBREVIATIONS

Symbols

k	Diffusion coefficient
n	Release mechanism

Abbreviations

APS	Amonyum Persulfate
MAH	N-Methacryloyl-(L)-Histidine
MBAAm	N, N'-Methylene Bisacrylamide
MIP	Molecular Imprinting Polimer
HEMA	2-hydroxyethylmethacrylate
SEM	Scanning Electron Microscope
TEMED	N, N, N', N'-tetramethylene
MIPs	Molecularly imprinted polymers
MMC	Mitomycin C
DDSs	Drug Delivery Systems
SR	Sustained Release
PEG	Polyethylene Glycohol
TDD	Targeted Drug Delivery
NGs	Nanogels
ATRP	Atom Transfer Radical Polymerization
NMRP	Nitroxide-mediated Radical Polymerization
EGDMA	Ethylene glycol dimethylacrylate
PEO	Polyethylene Oxide
POE	Polyoxy Ethylene
PVA	Polyvinyl Alcohol
pHEMA	Poly-2-hydroxyethyl Methacrylate
PVP	Polyvinil Pyrolidon
PMAAm	Polymethacrylamide
PVME	Polyvinylmethylether
MTT	Microculture Tetrazolium Assay
NIP	Unimprinted P(HEMA-MATrp) Microcryogel

1. INTRODUCTION

Current pharmaceutical delivery technology has only been around for 60 years and from this period, various drug carriers have been developed. The first generation of drug carriers between 1950 and 1980 were highly successful in generating for oral and transdermal drug release studies, but the second generation of drug carries (1980-2010) did not show the same performances for clinical trials as compared to the first generation of drug carrier platforms.

Earliest trials of developing the drug carries were based on physicochemical issues, whereas the further generation battled biological obstacles. Though their physicochemical properties can be adjusted, controlled drug delivery systems are unable to cross biological barriers.

Beginning in 2010, third-generation drug delivery systems sought to address both physical and biological constraints, such as pharmaceuticals' poor aqua dispersibility, the ability to regulate the drug release kinetics of the chosen medication, and—most importantly—the distribution of a drug to target a specific body area (such as an organ or tissue) rather than the entire body. Furthermore, the reactivity of body against the formulations could be minimized, so, the forthcoming success of The methods for administering drugs is depended regarding whether the novel drug release approaches can tackle with some limitations imposed by the human body and whether the development process [1].

Nowadays, polymeric drug delivery technologies and the recent advances of the drug carriers have progressed significantly and the formulation or the technology Polymeric medication delivery refers to the process by which a medicinal substance enters the body more easily. Biodegradable and bio-reducible polymers are an excellent candidate for a variety of innovative medication delivery methods. The field's development is necessitated by the prospects of research for practical applications [2].

Within a predetermined time, frame, the medicine was released from the polymer framework. In recent years, researchers, biochemists, and pharmaceutical companies have become interested in smart medication delivery systems. Protein therapeutics were also aided by drug delivery method and the target of controlled drug release studies have been to improve medication treatment effectiveness and safety.

The goal of this procedure is to get the medicine into the body in a more convenient therapeutic amount, in addition to prevent drug adverse effects, maintain the pharmacological action of drug co-administration, and offer a low dose to the patient for an extended period. The benefit of medication delivery is that it reduces drug side effects, reduces the minimal dose given to the patient, and prolongs the medicine's pharmacological benefits.

Nowadays, MIT opens a new avenue for drug release studies [3] and MIT enables to create the specific recognition sides in a polymer matrix [4]. Prior to the distinct identification cavities forming, the functional monomers as well as the target molecule are pre-arranged into the polymer solution afterwards, the polymerization has occurred with the use of the proper crosslinker, and the initiator agents. After polymerization, the target molecule is removed from the polymer matrix using the appropriate solvent in order to create the precise binding cavities that the target molecule is complementary to. [4]. These plastic antibody analogues are highly selective against the target molecules, affordable, and robust materials as compared with the natural antibodies; so, these tailor-made antibodies have taken the attention of various application fields as well [5-9].

In the 1950s, Streptomyces caespitosus or Streptomyces lavandulae containg aziridine were used to produce MMC and its aziridine, a powerful DNA crosslinker, [10] binding to DNA in tumor cells forms crosslinks within a double helix; so, MMC could be a potential for anticancer impact by preventing DNA replication.

Cancer is a serious disease affecting human population and this global health problem occurs through a sequence of subsequent gene modifications that are influenced by substances, bacteria, viruses, and radiation. [11]. When the normal cells become cancerous, they start to grow and divide uncontrollably leading to tumor formation; then, the cancer cells begin to infiltrate the nearby tissues and disperse throughout the physique. [12].

In recent years, surgery, radiotherapy, and chemotherapy have been used in various types of cancer treatments; but chemotherapy aims to cure cancer It is a successful treatment for inoperable tumors and has the fewest negative effects on the host cells. [12-24].

Cryogels made of materials with that connect macropores have been made in partially frozen environments. [25] moreover during the polymerization, such assets could be easily fabricated with the different forms adore the cartridge itself form [26], the disc format [27], microcryogel forms [28-33] by adjusting the polymerization conditions. So, these elastic materials have potentially used in affinity chromatography, tissue engineering, and drug release studies.

In this doctoral thesis, that p(HEMA)-derived injectable microcryogels They were manufactured using APS and TEMED Pairs of ions in a semi-frozen process by radical free polymerization. Before the formation of tailor-made MMC receptors, MAH as the functional monomer and Cu^{+2} ions were chelated, then, MMC and the functional monomer-Cu^{+2} complex was pre-organized at room temperature. Following that, the MMC-monomer/ion complex and HEMA were pre-arranged, the polymer solution containing APS and TEMED was added into the 200 μm diameter and 500 μm thickness microwells on the microstencil array chip, and the polymerization took place for 12 hours in semi-frozen conditions. Once, the microplate was thawed the p(HEMA) at the surrounding temperature and the based microcryogels were collected. Thereafter, the proper solvent (1.0 M NaCI) was used to extract MMC from the microcryogels to create the tailor-made MMC receptors.

After the creation of MMC receptors, molecularly imprinted MMC microcryogels (mMMC) was outlined includes testing for oedema and SEM. Following About the characterization studies, the MMC released performance and the cytotoxicity of mMMC durg carrier were investigated in the aqueous solution and against the mouse fibroblast cell line L929, respectively.

2. GENERAL INFORMATION

2.1. Drug Delivery History

Only 65 years ago, current medication delivery technology was invented, and several medication delivery devices were created during this time. The first production from 1950 to 1980 was effective in developing a wide range of sustained release orally and topically preparations for medicinal applications. The second production from 1980 to 2010, has much less success in creating clinical treatments owing to the in great part to challenges of nature to exist handled. The initial cohort of drug carrier systems were capable of coping with the physicochemical issues, whereas the second-generation drug delivery systems was not enough to success thanks to some problems of the biological obstacles. Sustained release systems of the third generation were started in 2010 and aimed to overcome some limitations of biological and physicochemical problems such as poor solubility of pharmaceuticals, manage the drug release kinetics, and the others. This third-generation drug carriers basically aimed to delivery of the medication to the body's designated target [34] and minimize their drug reactivity during the in-vivo studies. The comparison of the first, second, and the third generation of drug carriers They appeared in the following table. 2.1. [35]

Table 2.1. The evolution of medication methods of delivery since 1950 to the on hand together with the advancements that will be needed in the future. [35]

First-hand Knowledge	The second phase	Third-Generation
Fundamentals of release with control	efficient approaches to delivery	Approaches of modified administration
Oral delivery: twice daily or once daily	Zero-order distribution: Zero-order against first-order	Drug delivery with poor solubility and non-toxic excipients

administration into the epidermis: Every day or every week	Administration of protein and peptide molecules Using polymers that biodegrade for a long-term depot and delivery into the lungs	Distribution of protein and peptide molecules Delivery for more than six months Manage the release kinetics with non-invasive administration.
Drug release mechanisms: ion exchange, osmosis, diffusion, and dissolution	Hydrogels and intelligent polymeric Environmentally variable and controlling (exclusively in vitro)	Hydrogels and intelligent polymeric The sensitivity and specificity of signals Rapid reaction kinetics (operating in vivo)
	Nanoparticles that Delivery aimed at tumors Delivery of genes	Personalized medication administration Non-toxic to cells other than target cells Crossing blood-brain barrier
Effective management of the physical features of drug delivery mechanisms	unable to go past biological obstacles	Barriers relating to biochemistry and biology must be addressed.

In recent years, scientists in the pharmaceutical and related industries have created increasingly complex and effective medicines. These medications are distinguished by their capacity to release their bioactive ingredients at the appropriate time, location, and safe concentration, i.e., control toxicity. Most of these drugs are mostly composed of protein and/or DNA and scientists have been developing the drug carrier platforms that are biocompatible, biofunctional, and/or biomimetic to address these issues. Additionally,

similar methods-controlling the timing, location, and concentration of active component release will be used to medications with high levels of toxicity and typically limited therapeutic windows. These factors render conventional approaches to medication administration inefficient.

2.2. Drug Delivery Systems (DDSs)

DDSs aim to make the drug delivery more impressive abovementioned, and the medication is the most crucial component in any formulation.

immediately as an updated medicine has been being created, Usually, it is prepared in the least dose form that may be used to effectively treat the illness. Distinct medications require different formulizations due to their physicochemical and biological features. It is now feasible to better manage the pharmacokinetic, pharmacokinetic properties, toxicology, immunology, and effectiveness of medications by designing a range of DDSs.

The main purpose of the drug carrier (Figure 2.1.) [36] is to keep the drug level in the constant level and avoid the high-level doses of a drug during the initial pass of the metabolism because some medications are more effective in its therapeutic range, but, if its concentration is higher than the therapeutic range, the drug could be hazardous or show any therapeutic effect. According to this angle of view, the modest improvement despite the prescription drug design is essential for the distribution of medication to cellular objectives.

Recently, several approaches have been conducted to reduce medicine dosage, improve the sustain release, and minimize the side or adverse effects of a drug; hence, the new concepts play a crucial role in understanding drug pharmacokinetics, pharmacodynamics, nonspecific toxicity, immunogenicity, and effectiveness in this sense, DDSs integrate the multidisciplinary fields e.g., polymer, molecular biology, pharmaceutics.

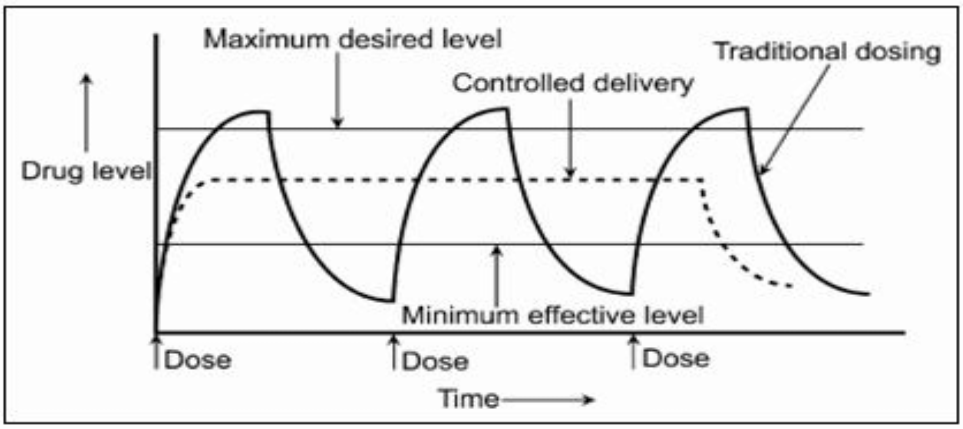


Figure 2.1. A Drug Release System and its Control of the Drug Release Behavior. [36]

Nowadays, some of the examples of drug carriers such as liquid polymers, micelles, low-density lipoprotein, lipid membranes, and microspheres made of insoluble or biodegradable biopolymers are favorable candidates for DDSs and furthermore, the transporters may be made to deteriorate gradually, react to stimuli (similar temperature and pH levels), or may also be specifically targeted (for example, by adding to antigens onto a particular target region). Targeting is the capacity to guide a drug-loaded mechanism to a certain location. For drug release, there are two key processes to consider:

Passive

Active targeting

When compared to systemic administrations of the comparable medicine, two key parameters abovementioned offer to decrease the non-specific drug distribution as a prescribed drug, regulate or regulate the substance released kinetics, minimize the adverse consequences of a drug, may maximize the effectiveness of therapy. Moreover, their slow released of a drug prevents the unwanted side or the adverse effects of the drug during the fast released.

The following are the key benefits of DDSs,

The therapeutic value of a medicine always maintained in the plasma.

The medicine was administered locally, which decreased the negative side effects.

Drugs having a limited half-life are protected against degradation.

Controlled medication release, as opposed to injection, resulting in a lesser risk of rapid overload and pain in the patient.

The patient's complaint about continual drug use is decreased via controlled medication discharge.

2.2.1 Medication Method of Delivery Kinds Involve:

Polymeric systems

Non-polymeric systems or Pumping systems

2.2.1.1. Polymeric Systems

The polymers include frequently utilized in the administration of pharmaceuticals since for themselves interface or volume factors and have been employed throughout medicine preparations as well as the delivery of drugs. These drug carriers might take how grafts are created for regulated medication transportation. The Polymers employed throughout colloid medication delivery carriers with microscopic particles have a strong benefit in the delivery of drugs due to their improved dosage form and release characteristics. Polymeric drug carriers are widely accessible and have well-established biochemistry thanks to the non-toxicity, biodegradability, and biocompatibility; for instance, some nanoparticle-based carriers can transcend the divide between the brain and the blood and as a result, they are particularly useful for targeted medication delivery. Furthermore, the surface modified polymers including silica-based polymers with antibodies, the specific biomarkers, and biomolecules can change the physical characteristics of polymers; so, the polymer conjugates e.g. Lupron Depot, Zoladex, or In Caspar PEG the intron could effectively applied for the rehabilitation, of various types of malignancy diseases. DDs are being used to ensure patient compliance through regulated drug delivery [37].

The polymers' features are as follows:

Biocompatible

Nonpoisonous

Not aggressive

Fundamentals of dissolution and permeability

Made a choice

No recyclable

Acceptable mechanical attributes

Effortless manufacture

Cost savings

Three types of polymers can be explored.

Polymers that are hydrophilic

Polymers that are hydrophobic

Polymers that are biodegradable

Hydrophilic polymers (hydrogels) fabricated with natural or synthetic derivatives are the most significant compounds utilized in medication delivery (Figure 2.2). [38]. These hydrogels are rendered unable to dissolve with water via forming primarily as well as second-hand linkages, causing the framework to absorb swelling as well as liquid. Those properties the hydrogel material, highlight related remarkable biologic compatibility. Hydrogels are substances perhaps fabricated with the variety of shapes, including foam, film, sheet, and blocks. By altering the preparation conditions, hydrogels of various architectures may be produced. The table (2.2) shows the widely studied polymers to be used in preparation of hydrogels.

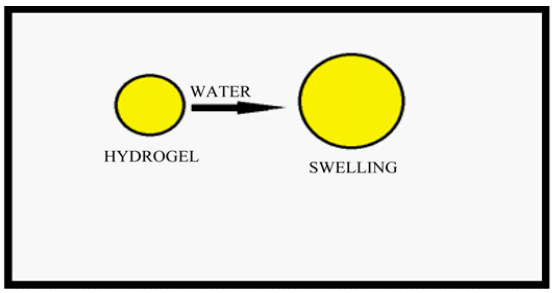


Figure 2.2. Hydrogel Based DDs [38].

Table 2.2. Manufactured Polymers With Hydrophilic Qualities.

Polymers	**Abbreviations**	**Molecules of Monomers**
Polyethylene oxide	PEO	-H2CH2O-
Polyethylene glycohol	PEG	$-CH_2-CH_2-O-$
Polyoxy ethylene	POE	HO-(CH2CH2O) n-H
Polyvinyl alcohol	PVA	$-CH_2-COHH_2-$
Poly-2-hydroxyethyl methacrylate	PHEMA	$CH_2-CCH_3-CO-OH_2CH_2-OH$
Poly-vinil Pyrolidon	PVP	N-vinylpyrrolidone
Poly-methacrylamide	PmAAm	$CH_2-CCH_3-CONH_2$
Poly-vinylmethylether	PvME	$CH_2-CH-OCH_3$

In biomedical applications, hydrogels, which containing frequently employed and their benefits are discussed more that follows.

- Water reduces the glass transition temperatures, resulting in the development of hydrogels and elastomeric forms, which are commonly employed in implantation procedures.
- The swollen nature of hydrogels facilitates the elimination of initiators, solvents, and other contaminants prior to their application in biological environments.
- The low surface tension among the gel and the water-based medium causes, small molecules can readily permeate using the hydrogel material.

Drug release from polymer systems can occur through four broad pathways, which are categorised as follows:

1. Diffusion control system

2. A system-controlled chemical

3. Systems using the solvent

4. Timed-release the systems

2.3.1. Diffusion-Controlled Systems:

Diffusion plays a crucial role in DDs and the physical and chemical events, such as water diffusion, drug dissolving, drug diffusion, polymer swelling, polymer dissolving, and/or polymer degradation, frequently influence the total release rate.

Diffusion controlled systems are classified into two types:

1. Systems of reservoirs

2. Structures in matrices

2.3.1.1 Systems of Reservoirs

Within reservoir structures, when the medication is encased within one polymer cell and maintained in a saturated state within the core, its release tends to adhere to zero-order kinetics until the drug is almost fully exhausted. Drug release occurs in these systems via membrane diffusion Figure (2.3) [39]. As a result, the rate at which drugs diffuse is determined through the kind and the membrane's depth. Films, cylinders, spheres, and other geometric shapes can be used to create these systems.

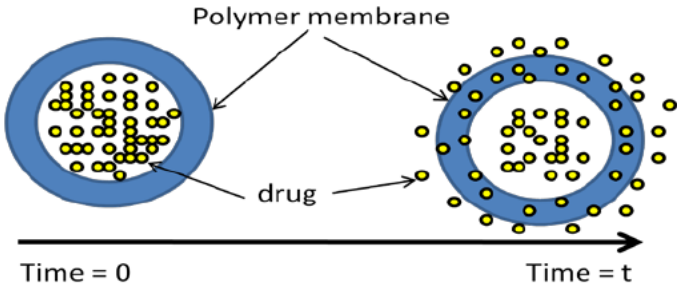


Figure 2.3: Diffusion-Controlled Reservoir Delivery System ideal [39].

2.3.1.2 Matrix Systems:

They are the types of regulated drug delivery systems that release the medication continually to use both dissolving controlled and diffusing controlled techniques. To control the release of medications with varying solubility properties, the pharmaceuticals are distributed in hydrogel hydrophobic compounds, an insoluble matrix of stiff non hydrogel hydrophobic materials, or plastic materials. [40].

A well-combined mixture of one or more medications and a gelling agent, like hydrophilic polymers, is called a matrix. In pharmaceutical technology, matrix tablets used for sustained release (SR) are crucial for drug delivery systems (DDS). They eliminate the need for complex manufacturing techniques like coating and palletization. The rate of medication release from these dosage forms was mainly regulated by a specific type or amount of polymer utilized within their formulation. In generally the hydrophilic polymer matrix is commonly utilized to create an SR dosage form [41].

Systems with matrices include frequently utilized in extended-release studies and release regulates and increases the duration that the dissolving or distributed medication. The sustained release approach can produce therapeutically efficacious concentrations throughout the circulating system for an prolonged duration, which enhanced patient adherence. [42].

The dissolved drug in the polymer is achieved as a homogenous dispersion in matrix systems, which may be manufactured in two distinct configurations. Figure (2.4) [43]. Matrix systems are easier to prepare than reservoir systems and may be monitored in a variety of ways. Matrix systems are inexpensive to manufacture.

The following characteristics influence the speed at which drugs leave rigid structures:

Medication solution within the polymer

Coefficients of diffusion and distribution

Geometry of the system

Concentration

Porosity

Tortuosity

When compared to the reservoir system, drug release from the matrix system is far more difficult. The matrix and reservoir systems may be used to deliver desired dosages of low and high molecular weight compounds.

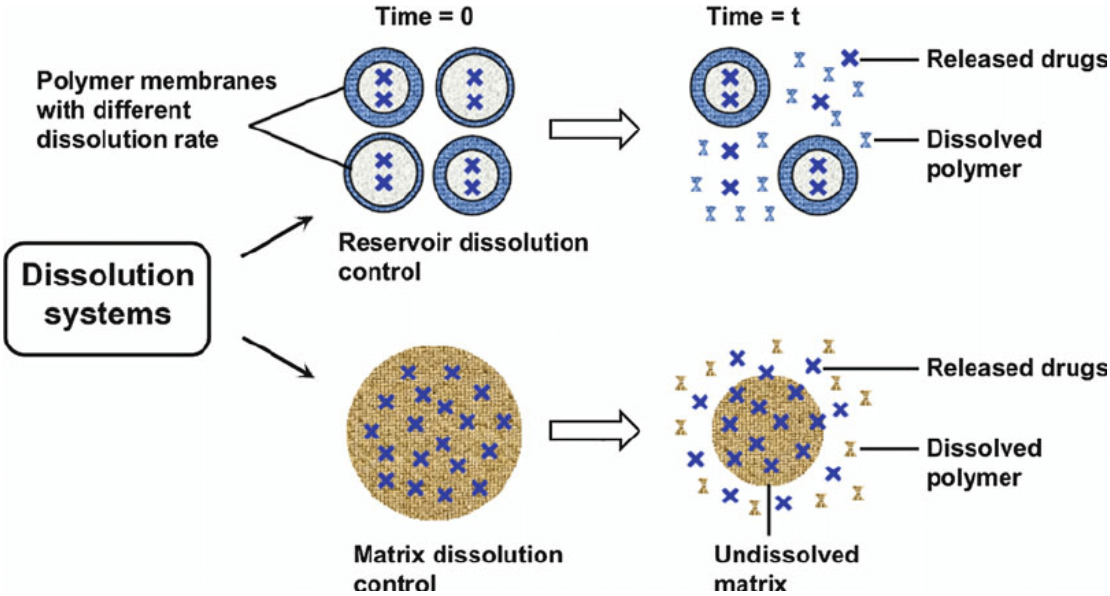


Figure 2.4: Dissolution Controlled Release Systems Diagram. [43].

2.3.2. Chemically Controlled Systems:

Chemically controlled technologies are one of these innovative medication delivery technologies. These rely on a chemical process to liberate the medicine from the polymer in which it is encased. Because the polymers are bio erodible, the technology will disintegrate and will not have to be withdrawn from of the body after usage. To assure the safety of these polymers, the procedure by which they travel thru the body must be rigorously thoroughly tested.

DDS are rapidly becoming developed due to the numerous therapeutic benefits, one of these benefits is their capacity to administer the effective medication at a steady rate across time, with no delayed beginning of action and no interference from intestinal physiology or other external factors.

The accuracy that the rate of medication distribution is known reduces the risk of negative effects. It also helps improve compliance owing to longer dose intervals. Finally, because of all these characteristics, it produces a better therapeutic impact.

This system may be divided into two categories. Pendant chain systems and biodegradable systems.

2.3.2.1. Biodegradable Systems:

The medicine is disseminated in the polymer in biodegradable systems, as it is in matrix systems. However, when the medication diffuses, the polymer phase dissolves. This degradation can occur because of enzyme catalysis, acid or base hydrolysis, or oxidation. Biodegradable systems can make use of matrix and reservoir systems. After drug administration, the polymeric membrane covering the drug in the reservoir system separates. Biodegradable polymer-based drug delivery systems provide the advantage of dissolving the matrix after the drug is released, ensuring no residual material remains in the tissue. This benefit must be considered against the possible negative of decaying polymers adding complexity to the design of usable materials.

2.3.2.2. Pendant Chain Systems:

The medication is not physically integrated into the polymer by this process. Instead, it is connected to its backbone by a reactive chemical link that rapidly breaks in the presence of water or certain enzymes. The medication is released because of the bond's hydrolysis. The backbone of a polymer is known as the pendent chain. When a medicine is water-soluble, it able to delivered to particular organs or cells for gradual restricted releases.

An insoluble pendent chain, on the other hand, serves more as a drug reservoir. The geometry of the device and the water-solubility of the polymer backbone can both influence how quickly the chemical link dissolves to release the medicine. Figure (2.5). [44].

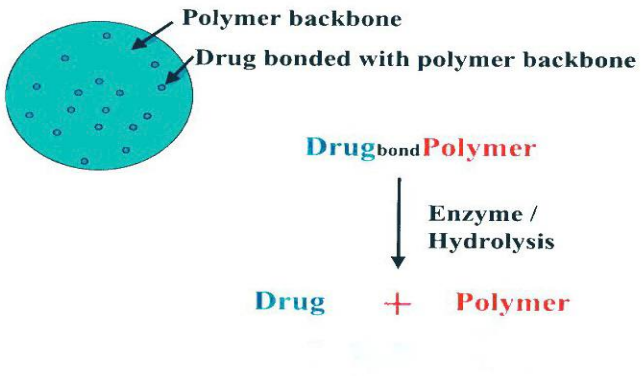


Figure 2.5: Ideal Pendant Chain Delivery System. [44].

2.3.2.3. Swelling Controlled Systems:

Swelling is one the key parameter for the swelling controlled systems and firstly, the medication dissolves and permeates the exterior absorbing media and then passes through the polymeric carrier like hydrogel. However, the swelling rate of the polymeric material could be controlled the use of the crosslinker and the degree of its amount. Furthermore, the diffusion other molecules like imprisoned pharmaceuticals could be affected the polymeric materials rate of swelling and how well it releases drugs. Figure (2.6). [45].

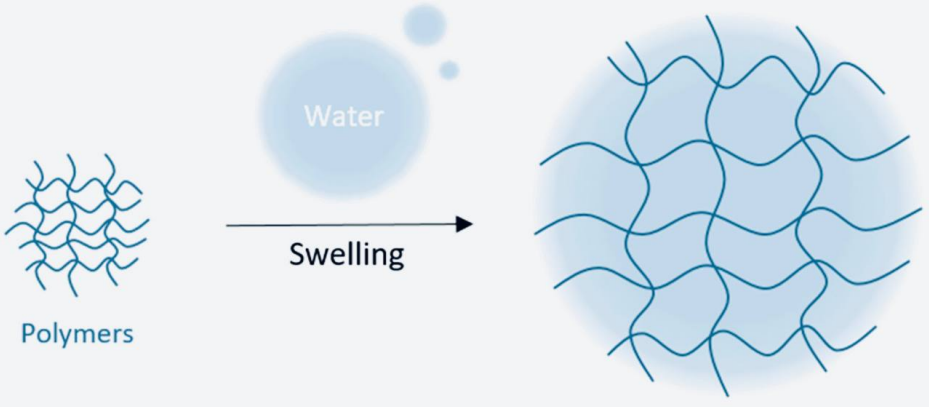


Figure 2.6: Swelling-Controlled Diffusion Mechanism. [45].

2.3.2.4. Modulated-Release Systems

The external conditions for instance, pH conditions, the ionic strength, the electromagnetic radiation, UV light, and temperature factors play a key role in controlling the modulated-release systems.

2.3.2.5. Pump Systems

Pump systems are often bigger and costlier than polymeric systems and the medicine is delivered directly to the patient's blood, which is the system's most significant advantage.

2.4. Systems for Regulated Drug Delivery

Delivery methods for drugs (DDS) are engineered to release active pharmaceutical agents in a manner that achieves the intended therapeutic outcome while overcoming some of the drawbacks associated with traditional drug delivery methods, such as limited bioavailability and fluctuating drug levels in the bloodstream. DDS facilitates controlled release, thereby ensuring that the therapeutic substance is delivered efficiently. Conventional methods, including tablets, capsules, syrups, and ointments, often exhibit issues like low their capacity to maintain sustained release may be hampered by bioavailability and irregular plasma drug levels. In the absence of a reliable delivery mechanism, the overall efficacy of the treatment may be compromised. Additionally, for optimal therapeutic effect and safety, it is essential that the medication is administered at a regulated rate and accurately targeted. To address these limitations, systems for the controlled distribution of drugs have been created. In the last two decades, these systems have evolved significantly, progressing from larger-scale formulations to advanced nanoscale and intelligent targeted delivery technologies.

2.5. Drug Delivery Methods

Drugs can be ingested in various categories of ways for example, nasal, oral, ocular, sublingual, vaginal, transdermal, pulmonary, and buccal (Figure 2.7). [46]. The "beginning point" – the site where the medicine is delivered – is used to categorize these routes. Each option offers a unique set of benefits and drawbacks.

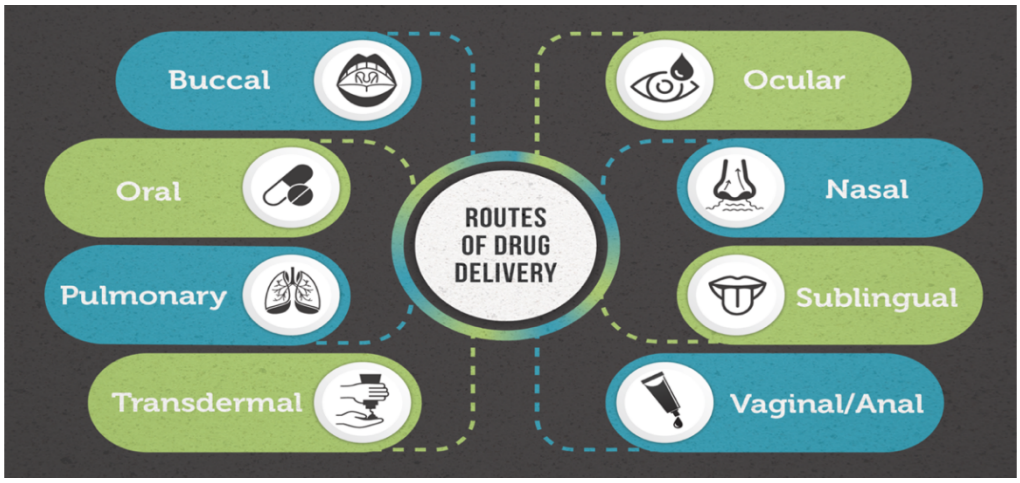


Figure 2.7: Different Routes of Drug Delivery. [46].

2.5.1 Enteral Route of Medication

Oral administration remains which was most popular and practical technique for administering medication due to its cost-effectiveness. The digestive system is the main location where drugs are absorbed, where the amount of drug absorbed through the intestinal mucosa determines its bioavailability. It is important to consider the first-pass effect for orally administered drugs. This phenomenon involves a significant reduction in the drug's concentration prior to it reaching the bloodstream, frequently because of the liver's metabolism [47].

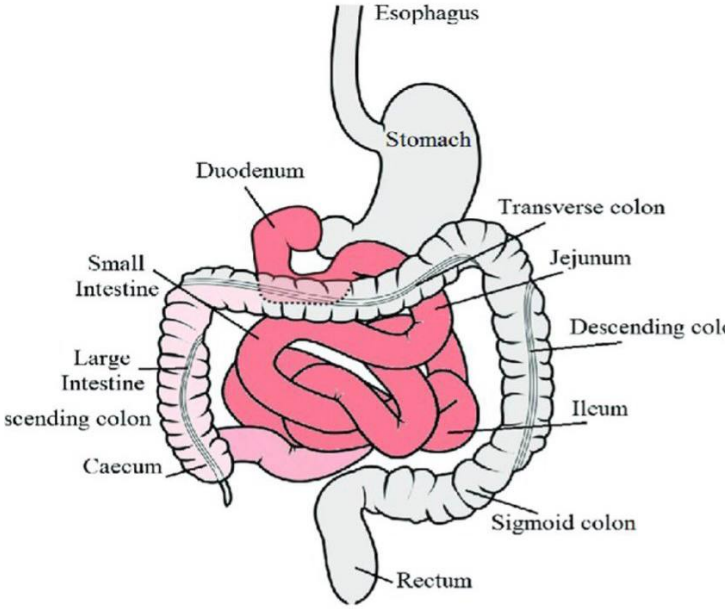


Figure 2.8: Schematic illustration of the Digestive System with the main areas for Medication absorption highlighted in red. [48].

A second method of administering medicine via the enteral route the lingual or sublingual method offers the benefit of avoiding the initial effect. A further type of enteral route for administering medication the lingual or sublingual route offers the benefit of not having the first-pass impact. When a medicine is injected sublingually or buccally, it passively diffuses via the bloodstream in the oral cavity, avoiding the liver vasculature and entering the superior vena cava. Mucosal tissues are much less permeability and has a lower capacity of medication absorption as contrasted to lingual region, which has a high permeability membrane and quick access to the deeper capillary.

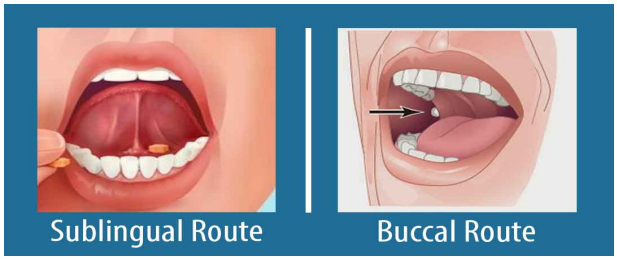


Figure 2.9: lingual or Sublingual Approach. [49].

Another intrathecal method for drug administration is the rectal route, which facilitates rapid and efficient absorption of the drug through the richly vascularized rectal epithelium. When drugs are administered intravenously, they largely avoid first-pass metabolism and are transported passively, similar to lingual and sublingual routes. Approximately half of the medication administered rectally reaches the liver directly.

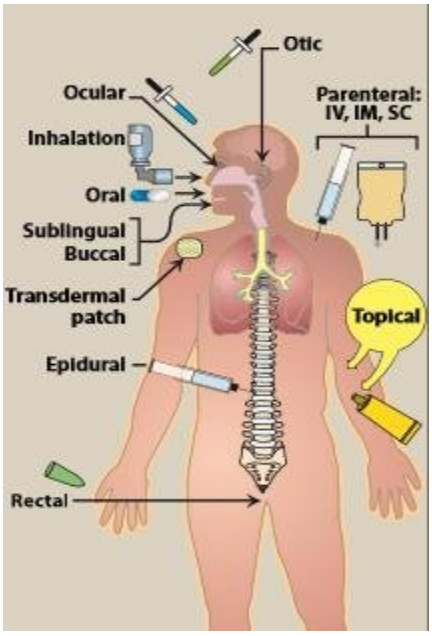


Figure 2.10: Typical medication administration routes. IM stands for intramuscular, SC for subcutaneous. [50].

2.5.2 The Parenteral Medication Route

Injection via intravenous means is the most widely utilized parenteral method for administering medication, offering the advantage of bypassing the liver's first-pass metabolism. Peripheral veins are often selected for drug delivery due to their easy access and superficial location. The upper extremities are generally preferred for intravenous administration because they experience fewer instances of thrombophlebitis and thrombosis compared to the lower limbs. Common sites include the metacarpal veins on the back of the hand and the median basilic or cephalic veins in the arm. In the lower extremities, the dorsal venous plexus of the foot can also be utilized.

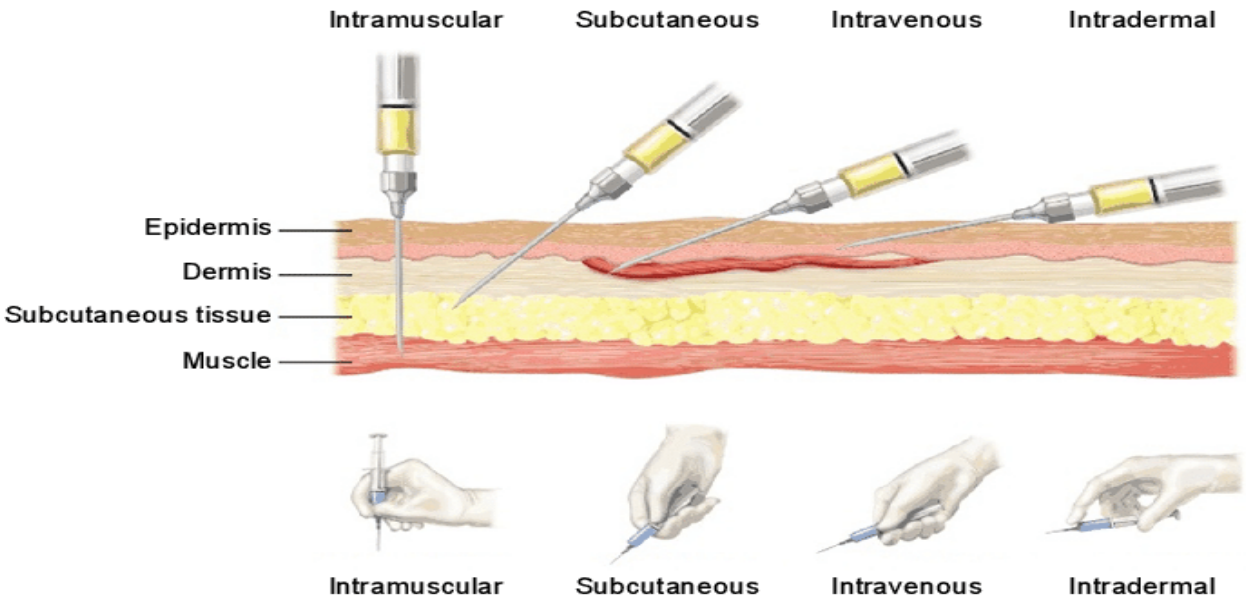


Figure 2.11: Structure of the skin, subcutaneous layers, and typical methods for administering parenteral drugs. [51].

Intramuscular injections can be administered in several muscle sites, including the deltoid, dorsogluteal, ventrogluteal, rectus femoris, and vastus lateralis muscles. While the dorsogluteal site, located in the upper outer quadrant of the buttock, is commonly used for intramuscular injections, it poses a risk of injury to the superior gluteal artery and sciatic nerve. In contrast, the ventrogluteal site, which targets the gluteus medius muscle, is preferred due to its reduced risk of these complications and is therefore recommended.

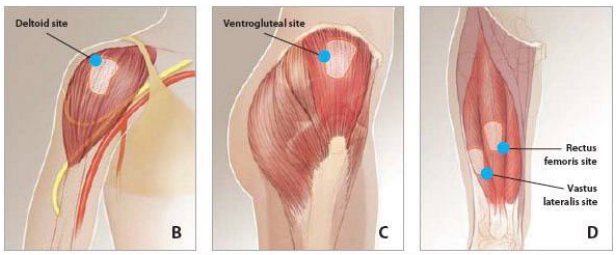


Figure 2.12: Deltoid, ventrogluteal, rectus femoris, and vastus lateralis sites are possible intramuscular injection sites. [52].

Another method of delivering medicine via the parental route is by subcutaneously, which is administered to the cutis layer of the skin, which is located immediately underneath the

dermal and epidermal layers. Due to the lack of blood arteries in subcutaneous tissue, medicines are absorbed slowly and continuously. The front part of the thigh, upper back, outer area of the upper arm, belly, and the upper portion of the buttock below the hip bone are just a few locations where subcutaneous medicine can be injected.

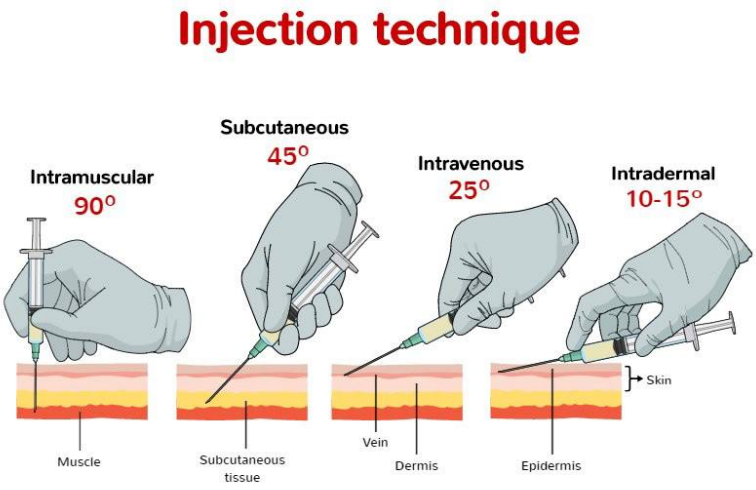


Figure 2.13: Parenteral Route of Drug Administration. [53].

2.5.3 Nasal Drug Delivery System

The general accessibility of medications only available through intravenous infusion has indeed been extended to intranasal drug delivery. The huge surface area, permeable endothelium barrier, higher overall blood circulation, prevention of the first metabolic, and easy availability are responsible for this. Numerous chemical, peptides, and protein medications as well as others have been orally administered for treatment response in recent times. After nasal delivery, drugs are quickly removed from the nasal passage, leading to quick systemically drug absorption. Here, many strategies are addressed for lengthening the duration that medication formulations spend in the nasal passage, which enhances intranasal medication absorption. The research emphasizes the value and benefits of nasal medication delivery devices that feature bioadhesive qualities. Both oral and peroral delivery of bioadhesive systems—or, more accurately, mucoadhesive systems—has previously been created. The nasal mucosa is a perfect location for bioadhesive medication delivery devices. The impact of microspheres and other

bioadhesive drug delivery methods on nasal medication absorption are covered in this article. It has been proven that drug delivery systems such microspheres, liposomes, and gels have high bioadhesive properties and quickly expand when in contact with the nasal mucosa.

These drug delivery techniques can protect the medication from enzymatic degradation in nasal secretions and control the rate at which the drug is removed from the nasal cavity. Detailed understanding of these systems' mechanisms and effectiveness is crucial for developing advanced and efficient treatments for peptide drugs and other medications that would typically require parenteral administration. Consequently, bioadhesive drug delivery systems can enhance both the bioavailability and retention time of orally administered drugs. Although most research to date has focused on the use of microspheres, liposomes, and gels for delivering macromolecules like insulin and growth hormone, the underlying principles could potentially be applied to other therapeutic agents. It is important to emphasize that maximizing the contact time between the formulation and the nasal mucosa can lead to successful absorption of many medications. [54].

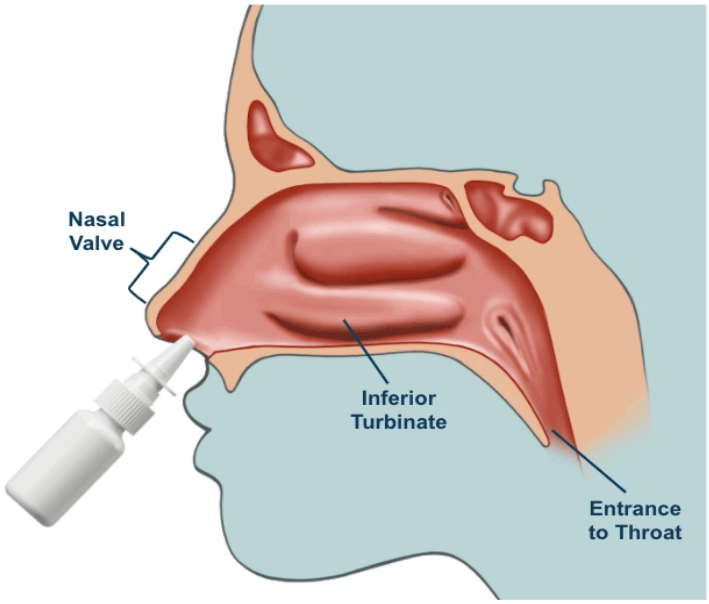


Figure 2.14: Intranasal Drug Delivery.[55].

2.5.4 Buccal Drug Delivery

Mucosa preparations were created to facilitate improved systemic delivery and longer localized treatment. Although bypassing first-pass metabolism, the mucous membrane poses a significant barrier to drug absorption, especially for biologics such as proteins and oligonucleotides. This challenge has prompted recent advances in genomics and proteomics. Formulations that can adhere to the buccal mucosa are preferred since the buccal route is frequently employed for prolonged medication administration. To maintain a formulation during buccal drug administration, bioadhesive polymers are generally hydrophilic macromolecules with plenty of hydrogen bonding groups. In contrast to location ligands like lectins, more recent second-generation bioadhesives have been created. These include modified or novel polymers that enable improved adherence and/or drug administration.

A broad variety of formulations (tablet, patch, liquids, and semisolids) have been created during the past 20 years for buccal medication administration, but only a small number have made it to market. At present, this delivery method is limited to a small range of lipophilic molecules that can easily traverse the buccal mucosa. However, if the current barriers to buccal drug administration are addressed, this route could become crucial for delivering a broader range of active agents in the future. Developing new or enhanced techniques may be necessary to address challenges related to patient acceptance and the effective systemic distribution of larger molecules, such as proteins, oligonucleotides, and polysaccharides, through this method. [56].

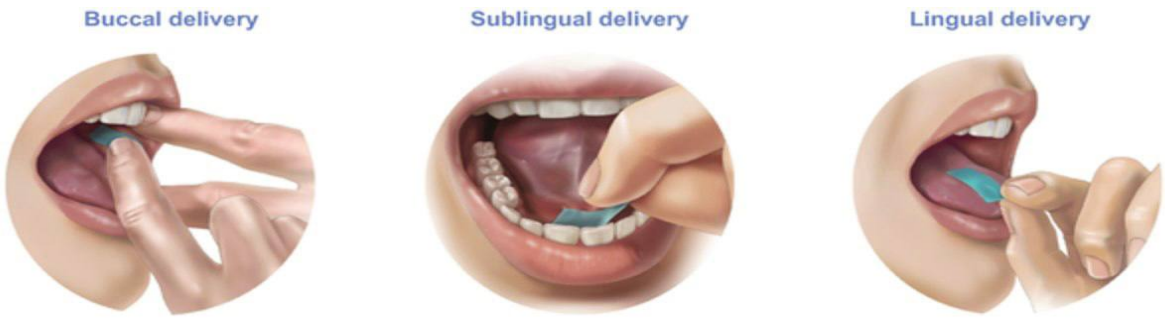


Figure 2.15: Buccal Drug Delivery System. [57].

2.5.5 Ocular Drug Delivery

Ocular drug administration poses significant challenges due to the unique anatomy of the human eye, presenting difficulties for pharmaceutical scientists and drug delivery experts. Delivering drugs effectively, especially to the posterior chamber, is complicated by a combination of static barriers (including layers such as the ocular surface, conjunctiva, and retina, with barriers like the blood-aqueous and blood-retinal barriers), dynamic barriers (such as choroidal and conjunctival blood flow, lymphatic clearance, and tear dilution), and secretions. Recent advancements have led to the identification of influx transporters in various ocular tissues and the development of targeted transporter-based drug delivery systems. Furthermore, extensive research has been conducted on both static and dynamic barriers using colloidal delivery systems like nanoparticles, nanomicelles, liposomes, and microemulsions [58]. To sustain drug levels at the target site, innovative methods such as fibrin sealant-based techniques and bioadhesive gels have been introduced. Future enhancements in ocular drug delivery may come from the development of noninvasive, sustained release devices and exploring the potential of topical applications for delivering drugs to the posterior segment. Recent progress in ophthalmic drug delivery has shown promise in overcoming the challenges posed by various anterior and posterior segment disorders. [59].

Figure 2.16: Ocular Drug Delivery System. [60].

2.5.6 Oral Drug Delivery

Oral drug usage is a way of ingesting chemicals via mouth. Orally, a wide range of medicines and pharmaceuticals are administered. One example of an oral medication form is:

Capsules.

Tablets.

Lozenges.

Liquids suitable for drinking.

The most usual technique is to take medications orally, which includes several biological processes.

When a medicine is administered orally, as a tablet or liquid, it is absorbed by the stomach and gut lining and processed by the liver.

The PH and metabolic enzymes in a person's gastrointestinal system have a considerable impact on oral absorption.

Because of these unique circumstances, it is difficult to anticipate how a person would react to various medications. [61].

Oral administration remains the most commonly used method for delivering medication, favored for its non-invasive nature, high patient compliance, and ease of use. Several factors affect the absorption of orally administered drugs, such as stability, mucosal permeability, and solubility in the digestive system. Addressing these challenges involves exploring the physicochemical, biochemical, metabolic, and biological factors that impact overall drug bioavailability. To enhance oral absorption, various pharmaceutical technologies and delivery systems, such as nanocarriers, micelles, cyclodextrins, and lipid-based carriers, have been examined. This review will go over the physiological and pharmacological challenges affecting oral drug bioavailability, along with both traditional and novel drug delivery methods. It will also address issues related to pediatric formulations and developmental considerations.

Oral medication is the most widely used medication delivery technique because of its practicality, preference of patients, affordability, and simplicity of large-scale

manufacturing. Approximately 60% of commercially available small drug compounds are administered orally. Currently, approximately 90% of the global market for pharmaceuticals meant for human consumption is made up of oral formulations. Oral pharmaceutical products make up roughly 84% of the top-selling drugs, with a $35 billion market value with a 10% yearly growth rate [62].

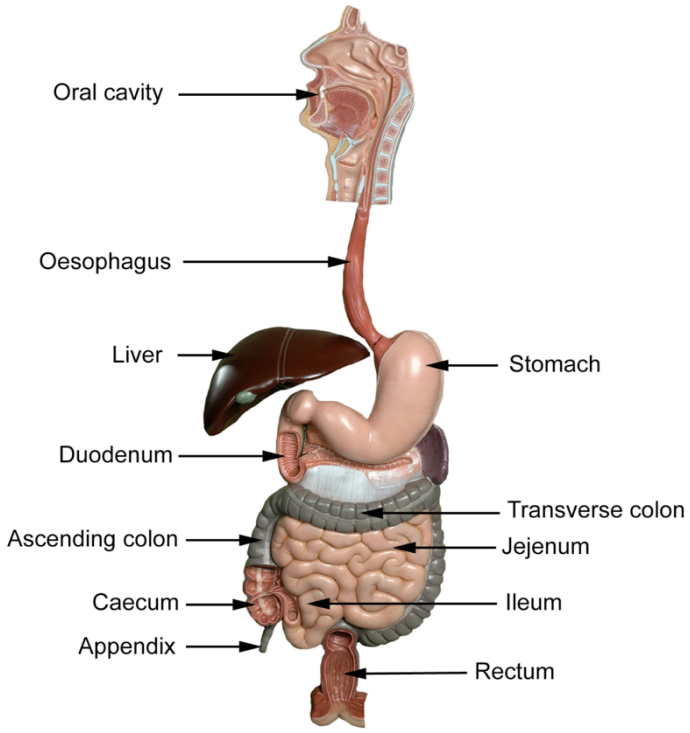


Figure 2.17: Oral Drug Delivery System. [63].

Oral formulations are generally preferred by patients over alternative parenteral methods, including injections administered intravenously, subcutaneously, and intramuscularly, along with inhalation treatments for ailments including asthma [64]. Additionally, oral medications have the ability to target particular regions of the gastrointestinal (GI) tract, making it possible to treat a variety of pathological conditions locally, including infections, inflammations, bowel disorders, gastric and colorectal cancers, gastro-duodenal ulcers, and gastro-esophageal reflux disease. Although these benefits, the creation of oral dosage forms involves multiple issues, which are primarily due to the physical features of medications, such as low soluble in water and permeability.

Additionally, pharmaceutical absorption might be hindered by poor biological and chemical safety, as well as physiological obstacles such as pH, efflux transporter, and metabolic enzymes. Furthermore, certain medicines might induce skin discomfort and nausea [65]. Several research has been conducted over the last four decades to better understand the mechanisms of medication administration and transportation, gastrointestinal passage, the microclimate of the Gastrointestinal system, and drug stability in GI fluids [66].

2.5.7 Pulmonary Drug Delivery

The effectiveness of a treatment largely depends on the methods used for drug delivery and maintaining the drug at an optimal concentration; deviations from this concentration can either be harmful or ineffective. The slow progress in treating severe illnesses has highlighted the expanding requirement for a multimodal strategy to deliver therapeutic drugs to particular tissue locations. Innovations in managing pharmacokinetics, pharmacodynamics, immunogenicity, and biorecognition possess the capacity to improve therapy results and medication efficacy. These advanced drug delivery systems depend on multidisciplinary domains like molecular biology, bioconjugate chemistry, pharmaceutical technology, and polymer science. Existing and emerging drug delivery and targeting systems are designed to minimize drug degradation, reduce adverse side effects, and improve bioavailability. For over two decades, the promise of nanotechnology has been acknowledged by researchers, leading to significant progress in the targeting and delivery of drugs. Recent developments in drug delivery techniques are helping to mitigate unwanted toxicities and improve therapeutic effectiveness [67].

Due to the lung's ability to absorb medicines both for systemic and local distribution, respiratory medication delivery has sparked great interest in pharmaceutical research. The massive surface area and extremely porous air-to-blood barrier of the respiratory system make it a very receptive location for administration of medication, particularly for the local, fast, and effective treatment of conditions like chronic obstructive pulmonary disorder and asthmatic. However, the creation of inhaled medications is complicated because three requirements must be satisfied to accomplish targeted deposition.

The aerosol formulation for inhalation must be designed so that the medication can be effectively deposited throughout the respiratory tract, including the deep lung areas.

The delivery device and formulation should generate an aerosol cloud with a sizable percentage of precisely sized particles.

The deposition of the drug should provide both functional and therapeutic benefits.

A comprehensive understanding of these factors and their interactions helps to optimize the performance and effectiveness of pulmonary drug delivery systems. However, much of the existing research has concentrated on particles within the 1-5 µm range, which are known to deposit effectively in the lung. Particles larger than 5 µm are often swallowed after hitting the oropharynx. Assuming a continuum of particle behavior, finer particles take longer to deposit, with most particles smaller than 1 µm expected to be exhaled. Currently, particles smaller than 1 µm are not considered significant for effective drug delivery [68].

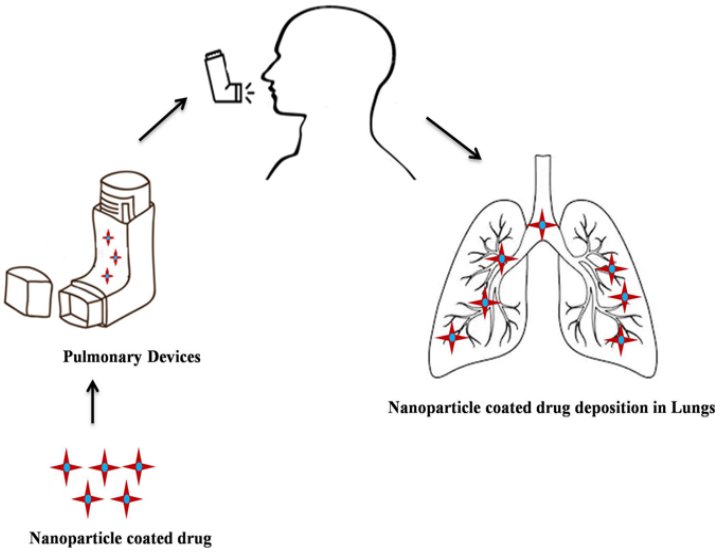


Figure 2.18: Pulmonary Drug Delivery. [69].

2.5.8 Sublingual Drug Delivery

Sublingual distribution involves the medicine being given along the bottom of the mouth via the mucosal membrane linings. The sublingual route is the most researched since it is

considerably more permeable and results in quick absorption of medicines with good acceptance. Sublingual medications are made in two forms: tablets and capsules.

The tablets are meant to dissolve quickly, while the capsules are typically made of soft gelatin that envelopes a liquid medicine. [70].

2.5.9 Drug Distribution by Transdermal

Generally speaking, transdermal drug delivery permits continuous dosage, similar to intravenous infusion, for medications with short half-lives. Transdermal drug delivery, on the other hand, is non-invasive and does not require hospitalization. With a surface area of roughly 2 m^2, the epidermis is the largest organ in the human body and has an intricate layered structure. The dermis and epidermis are the two tissue layers that make it up. [71].

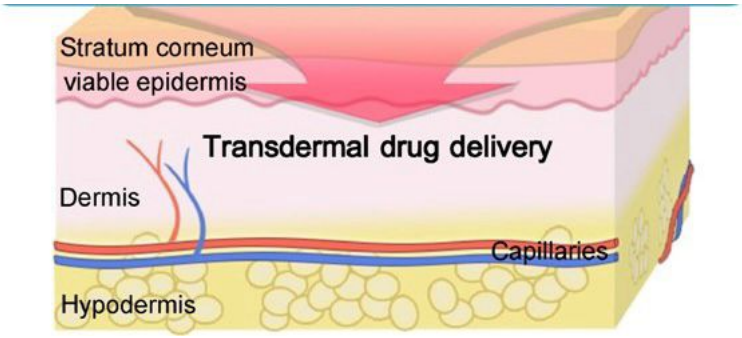


Figure 2.19: Pathways for Transdermal Delivery. [72].

2.5.10 Vaginal/Anal Drug Delivery

Even though the although parenteral, nasal, ocular, rectal, and vaginal medication administration are not as common as oral drug delivery, they may be used for particular purposes. Some justifications for administering medications via the rectal or vaginal route are as follows:

• The oral route is not feasible for the patient. If the patient suffers from a gastrointestinal ailment, this could be the situation, is queasy, or is recovering from surgery (in situations

where the patient might not be cognizant and cannot swallow a dose form). Furthermore, rectal medication may be more beneficial than oral treatment for some patient groups, such as the very young, the very old, or the mentally disturbed.

• The proposed medication is not suitable for oral administration. This may be the case when the medication is inadequately stable at the PHs to which it is exposed in the gastrointestinal system, or when the drug is sensitive to enzymatic destruction in the gastrointestinal tract or during the first pass through the liver following absorption. Additionally, medications having an unpleasant taste might be supplied to the patient rectally. Certain medicines that are likely to be abused, such as those used in suicide, have also been studied for formulation into suppositories.

• When treating the rectum or vagina requires targeted therapy.

Aside from these obvious benefits, the rectal and vaginal routes have numerous disadvantages. Patients often detest these pathways because to their anogenital and sexual connections, hence alternate routes are recommended. Depending on culture and tradition, certain nations, such as the United Kingdom and the United States, have significant emotions of aversion to rectal administration of medications, whereas continental and eastern Europe have perfect acceptance. More reasonable considerations include delayed and often inadequate medication absorption, as well as significant inter- and intra-subject variability. Furthermore, proctitis has been recorded with long-term rectal delivery. There are further issues with large-scale suppository manufacture and achieving a sufficient shelf-life.

As a result, the rectum and vagina are not first-choice pathways. As a result, these modes of drug administration are among the least preferred, resulting in a market size of fewer than 1% of the entire pharmaceuticals industry. [73].

2.6. Targeted Drug Delivery (TDD)

Because of its capacity to improve the transport of medications and genes to tumor locations while protecting them from the outside world, transdermal drug delivery, or TDD, is quickly becoming an essential part of cancer treatment. Three-dimensional hydrophilic polymer networks known as stimulus-responsive nanogels (NGs) have the ability to alter their structural characteristics in reaction to outside stimuli. These nanogels

are produced via self-assembly or covalent bonding mechanisms. Since NGs are stable, simple to synthesize, allow for exact control over particle size, and are easily functionalized, they have been thoroughly investigated as cutting-edge drug delivery systems for a variety of anticancer medications and genes. They have the ability to control particle sizes ranging from 5 to 400 nm and modify the conditions of polymerization accordingly [74].

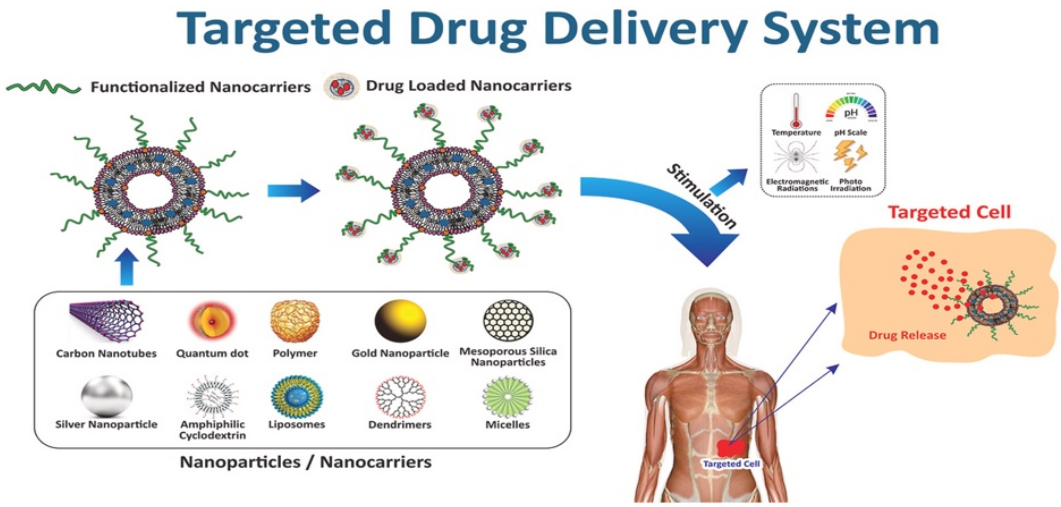


Figure 2.20: Targeted Drug Delivery (TDD) System. [75].

2.7. Cancer

Some cancer medicines induce cells to cease growing, a condition known as programmed cell death. This criterion applies to novel chemical compounds being investigated for use in chemotherapy. It is also desired to create new formulations with improved efficacy for anticancer medications that are already on the market. One of the most significant aspects of anti-cancer medication formulation is the development of appropriate drug delivery mechanisms.

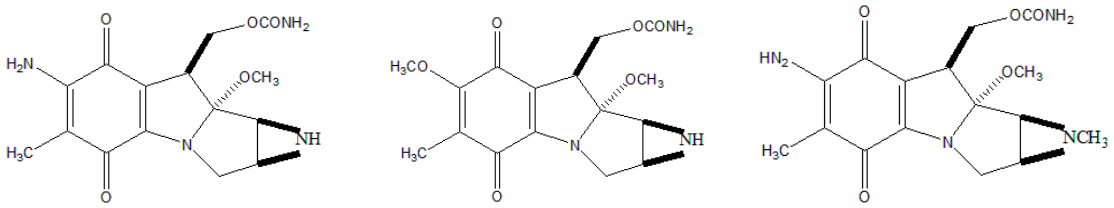
Anti-cancer medications are always very toxic and have major adverse effects. One strategy to limit side effects is to get the medications to the cancer site without allowing them to seep into other areas. This necessitates that drug delivery methods enable safe

encapsulation of the medications prior to reaching the cancer site without leakage, while still being able to release the pharmaceuticals once inside the cancer tissues.

Therefore, the drug delivery systems need to make sure that the drugs are securely encapsulated before they reach the cancer site and then disintegrate once inside the cancer tissues. Controlled release materials are required to meet these standards.

2.8. Mitomycin C

A class of extremely cytotoxic antibiotics known as mitomycin's is generated by Streptomyces cultures. The initial members of this antibiotic class were identified in 1956, with mitomycin C (MMC) being isolated two years later. Streptomyces caespitosus broth is the source of mitomycin C, an antibiotic and anti-neoplastic drug shown in Figure 2.21 (C15H18N4O5, molecular weight 334.33) [76]. Since its FDA approval in 1974, MMC has been used to treat gastric and pancreatic cancers in conjunction with other anticancer medications. It is also still used in chemotherapy for head and neck, cervical, stomach, pancreatic, and colon cancers. MMC is the usual treatment for superficial bladder malignancies when taken alone [77].



Mitomycin C Mitomycin A porfiromycin

Figure 2.21: Anti- Tumor Antibiotic Mitomycin Family Structure. [76].

2.8.1. Mitomycin C Activation Mechanisms

The mechanism of MMC reductive activation has been elucidated through studies on its interactions with oxidoreductase enzymes and chemical reductants. This process involves

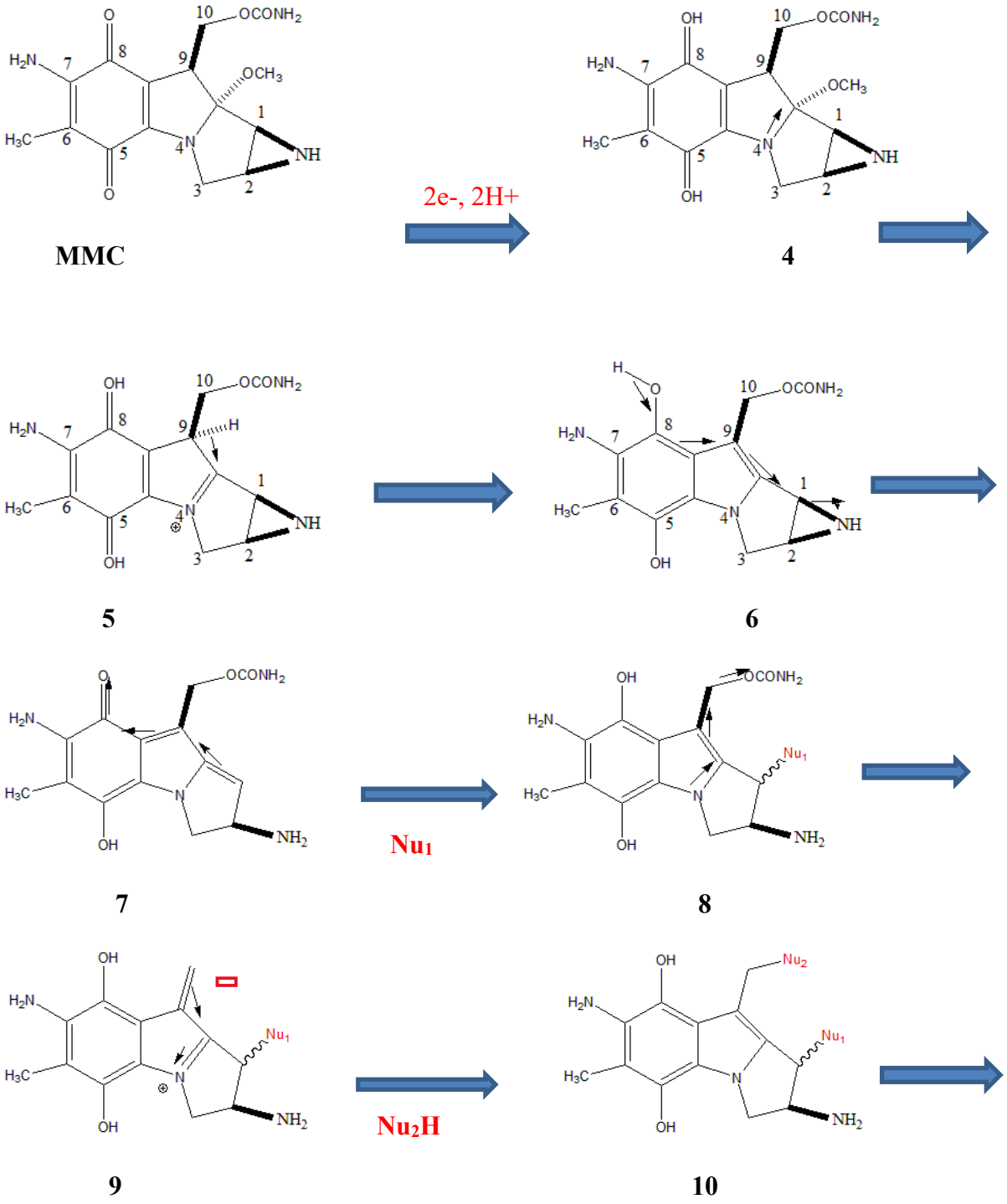
a complex series of events that convert the inert C1 and C10 locations into carbons that are electrophilic (see Figure 2.22) [78, 79].

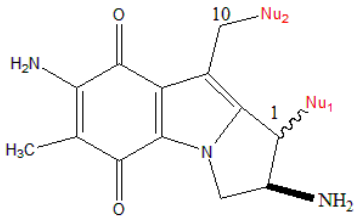
The first stage of MMC's reductive activation, which turns it into a bis-alkylating agent, is the reduction of the quinone ring to its hydroquinone form. After this reduction, hydroquinone 4 is produced. This compound helps to liberate the vinylogous amide conjugation at N4 and removes methanol from the hemiaminal group, which forms the iminium intermediate 5. Pyrrole 6 is created when this intermediate isomerises. Quinonemethide 7 is formed when the aziridine ring opens intramolecularly due to the formation of the C1, C9 double bond. Quinonemethide 7 serves as a substrate for nucleophilic addition at C1 [80].

After that, the monosubstituted intermediate 8's carbamoyloxy group is eliminated to create 9, which then experiences a second nucleophilic addition at C10 to make the reduced form of the bis-alkylated derivative, 10. Ultimately, the bis-substituted mitosene 11 is created by oxidising the hydroquinone ring. The observation of 1,2-cis and 1,2-trans substituted mitosenes after nucleophilic addition to activated MMC implies that an SN1 mechanism is responsible for nucleophilic addition at C1. The structure 12 (see Figure 2.23) of the indoloquinone that is present in the derivatives generated during the reductive activation of MMC is referred to as "mitosene". Since its initial use in the literature on mitomycins, the term "mitosene" has been widely used by researchers to refer to a common structural characteristic shared by compounds resulting from the chemical breakdown of different mitomycins. The previously described method is sometimes called "bifunctional reductive activation" to distinguish it from an alternate reductive activation pathway that only activates C1 for nucleophilic addition while leaving the C10 carbamoyloxy group intact. The alternate pathway, referred to as "monofunctional activation," is the creation of compound 13 (see Figure 2.24) [81] through an autocatalytic interaction between reduced aziridinomitosene 6 and unreduced MMC. After interaction with nucleophiles, the aziridinomitosene 13 acts as a monoalkylating agent at C1, yielding mitosene derivatives with the general structure 15.

A mixture of cis and trans C1-substituted mitosenes is produced during this activation process, which is comparable to the bifunctional activation mechanism. This suggests that an SN1 mechanism is responsible for alkylation at C1. The first stage is the opening of the aziridine ring by an acid catalyst, which results in the creation of intermediate 14,

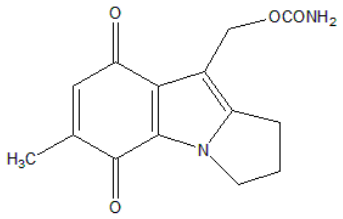
which has a carbocation at C1. The two isomeric mitosenes are formed in the second stage when this intermediate combines with a nucleophile at C1 (refer to Figure 2.25) [82].





11

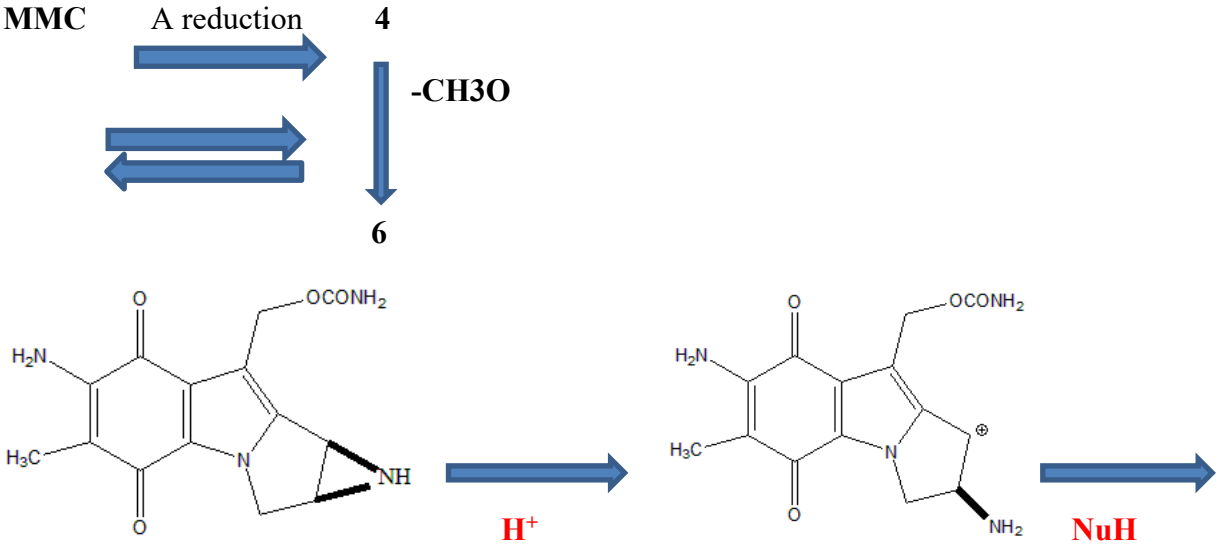
Figure 2.22: The Mechanism of reductive activation of mitomycin C and its reactions as a dual-purpose alkylating agent. [78–79].



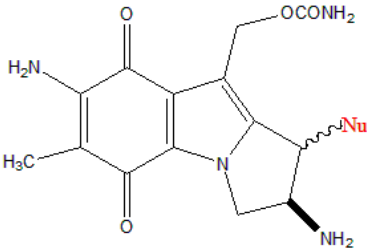
12

Mitosene.

Figure 2.23. The Mitosene Core's Structure [80].



13 **14**



15

Figure 2.24: The Mechanism of MMC's Monofunctional Alkylating Agent Reaction and Autocatalytic Reductive Activation. [81].

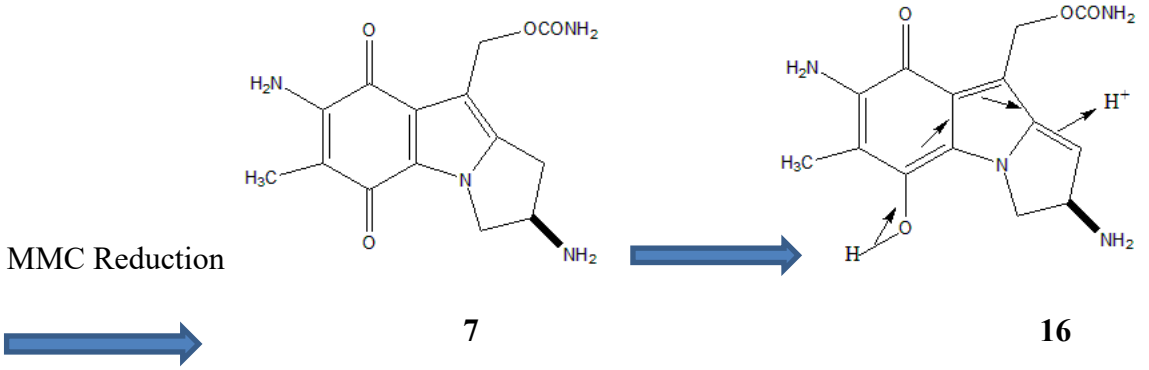


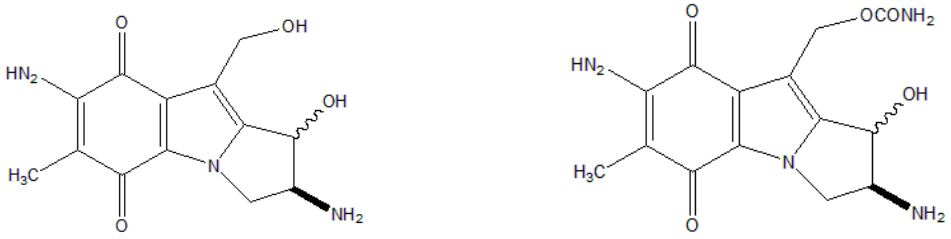
Figure 2.25: MMC Conversion to the Primary Metabolite that MMC in Tumour Tissue produces, 2,7-diaminomitosene. [82].

MMC doesn't have to be reduced in order to be transformed into an alkylating substance by acidic activation. This activation occurs under acidic conditions with a pH below 5, which limits its significance in the drug's in vivo action. The process involves the acid-catalyzed elimination of methanol from MMC, resulting in the formation of aziridinomitosene 13. This compound then undergoes alkylation at C1, similar to the autocatalytic activation mechanism described earlier (see Figure 2.24) [81].

The degradation of MMC yields the C1-reduced metabolite 2,7-diaminomitosene 16 (2,7-DAM), which confuses the activation methods of MMC (see Figure 2.25). The most significant in vivo product of MMC, this metabolite serves as a monoalkylating agent by

connecting nucleophiles at C10 during oxidative activation. Similar to the bifunctional activation approach described in step 8, 2,7-DAM is formed through a process that diverges after intermediate 7, at which point C1 exhibits dual reactivity as the electrophile and a nucleophile (see Figure 2.25) [82].

Apart from 2,7-DAM, 10-decarbamoyl-1-hydroxymitosenes 17 (Figure 2.22) are additionally metabolites generated by MMC in tumor tissues in vivo, which are the consequence of the bifunctional reductive activation shown at step 6. These three metabolites (16, cis-17, and trans-17) and 1-hydroxymitosenes 18 are produced by the autocatalytic process of enzymatic reduction of MMC in cell-free systems (for more information, to Figure 2.26) [83].



17 **18**

Figure 2.26. Following the hydrolysis of simplistically reactivated MMC, a compound of C1-substituted mitosenes has been generated [83].

2.9. Imprinting Biological Macromolecules and Assemblies at the Molecular Level.

The creation and production of novel materials designed to detect proteins and other biological entities, including cells and viruses, hold significant theoretical and practical importance. These materials have potential applications in various areas such as biopharmaceutical purification, drug delivery, diagnostics, and sensor technology. Currently, many of these functions are fulfilled by naturally produced recognition substances, such as enzymes, receptors, and antibody. In bioseparation processes, the cost of recovering products, such as enzymes or hormones, constitutes a major part of the production expenses. Enzyme-substrate relationships, hormone-receptor binding, and binding of antigens to antibodies are examples of highly selective interactions that are usually accomplished with exceptional accuracy. However, these methods can be

prohibitively expensive and often involve fragile systems. This has driven the need for more cost-effective, durable, and reusable alternatives to these expensive and delicate recognition agents.

Using a method known as "molecular imprinting," polymer with particular functional groups are created that mimic the physical arrangement of target molecules, has primarily been applied to small organic compounds. In this technology, the target molecule interacts with functional monomers through various interactions such as covalent bonds, noncovalent forces, or metal ion coordination. This interaction forms a complex, which is then polymerized to create a binding site tailored to recognize the target molecule. Once the target molecule is removed from the polymer, a recognition cavity with complementary functionality and shape remains, as illustrated in Figure 2.27 [84].

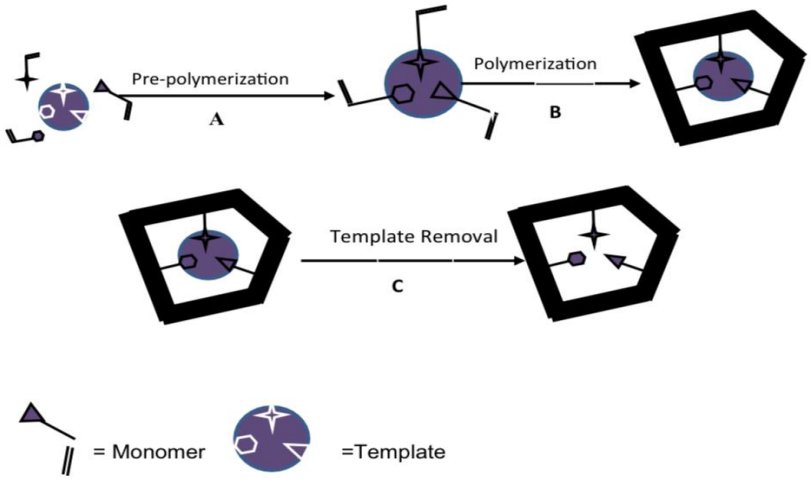


Figure:2.27 The molecular imprinting approach is demonstrated by the following steps: A) pre-polymerization complex of template and function monomer; B) polymerization process utilizing cross linkers; and C) template removal with extract solvents. [84].

Notably, little investigation has been carried out on the creation of imprinted polymers for the detection of protein and other biological agents. Crafting imprinted polymers for biopolymers presents greater challenges compared to small molecules due to several factors. The complexity of protein and cell surfaces, which have numerous competing

binding sites, is one major limitation. Additionally, factors such as temperature sensitivity, pH levels, solvent types, biocompatibility, and the large size of these molecules must be considered while creating ways of molecular imprinting on multiple substrates. This highlights the need for innovative chemistry to create imprinted polymers capable of selectively recognizing and binding biological macromolecules and assemblies.

Regardless of these difficulties, advancements have been achieved in developing imprinted polymers for protein detection. An early example provided by Mosbach and his team involved creating a thin polymer layer on porous silica beads specifically designed to recognize the transferrin glycoprotein. Two unique carbohydrate moieties, both with branches that culminate in sialic acid groups, are present in transferrin. Accepted for its capacity to bind covalently to cis-diol groups (such as those in carbohydrates found in glycoproteins), boric acid, was used to create the binding site for the polymer [85, 86]. N— [2-hydroxy-3— (tripropoxy silyl) propoxy] propyl-3-amino-benzene boronic acid (I), a boronate methicone active monomer, was produced by the researchers and equilibrated with transferrin. After adding a variety of organic silanes, the mixture underwent polymerisation.

Transferrin, which and BSA (bovine serum albumin) were used as templates to create polysiloxanes, while protein was absent from a control polymer. Relative retention, which is determined by dividing the elution volume of BSA by the elution volume of transferrin, is the measure of the polymers' selectivity for transferrin measured by chromatographic investigations. The transferrin-imprinted polymer showed a higher affinity for transferrin than BSA, as shown by its relative retention of 2.16, which was higher than 1.22 for both of the control and BSA-imprinted polymers.Arnold and colleagues suggested a unique and flexible method for producing molecularly imprinted matrices for protein recognition and binding [87]. They produced complementary binding among matrix-bound metallic chelates and coordinating ligands on protein surfaces by taking advantage of metal coordination contact.

This approach was justified by the theory that, because protein surfaces are complicated, effective and universal recognition would depend on the carefully chosen arrangement of as few different functional groups as is practical. Therefore, when proteins come into contact with a receptor, the insertion of strong and distinctive binding reactions is

essential for protein differentiation. The majority of these criteria have been thought to be met via metal coordination action. Histidines, which are surface-exposed metal coordinated residues, exhibit a strong affinity for a variety of metal ions, such Cu2+, Zn2+, Hg2+, and others. Immobilised metal affinity chromatography, which is used for purging proteins, was made practicable by the affinity which metal chelates exhibit for proteins [88].

Venton and associates investigated the creation of imprinted polysiloxanes for protein recognition by developing functional sol-gel matrices using protein-based templates [89]. Their approach suggested that during the polymerization process, Silanol monomers' organic functional side chains could interact using the protein surfaces' corresponding groups of functionalities, resulting in the formation of specific binding sites for proteins within the polymer matrices. They used 3-aminopropyl triethoxysilane as the functional monomer and tetraethyl orthosilicate as the cross-linker, with urease and BSA as the template proteins for the sol-gel synthesis. After forming the matrices, pronase digestion was employed to remove the template proteins.

The silica matrices created using urease as the template exhibited a higher binding affinity for urease compared to non-templated proteins like BSA. However, when hemoglobin and myoglobin were used as templates, the resulting polymers failed to differentiate between the template proteins and non-templates. This underscores the necessity for designing matrices with highly specialized binding interactions to achieve effective protein recognition.

2.9.1. Factors that Influence the Molecular Imprinting Process.

The molecular imprinting structure depends on a number of variables like the temperature, solvent, initiator, active monomer crosslinker, behavior of initiation, and template chemical structure and amount. Recognition cavity incorporated into polymeric structure governs binding characterization, stability, selectivity, and binding kinetics. It is critical to adjust these parameters to achieve high recognition capacity with good selectivity. Metal ions, antibiotics, medicines, carbohydrates, amino acids, peptides, proteins, and even cells have all been imprinted using molecular imprinting procedures. [90-94].

The type of template will dictate which functional monomers are used. Functional groups in the target should be able to interact with monomers to provide a clearly defined orientation between functional monomers and the template molecule. Additionally, it should maintain its stability during the polymerization process. [95]. Good fidelity for the recognition cavity and little conformational change after rebinding to the binding site are required for high selectivity, which depends on the stability of the template. For an effective imprinting procedure, the chemical structure of functional monomers is also a crucial consideration because in order to keep the target's conformational state intact, they need to bind with the template molecule in the right orientation [96]. The most widely used functional monomers include styrene, acrylamide, methyl methacrylate, trifluoromethacrylic acid, 4 vinylbenzoic acid, acrylic acid, methacrylic acid, and methacrylic acid. The best monomer for molecular imprinting is selected based on the strength and chemical composition of the chemical bonds among the monomer and reference molecules.The cross linker plays a crucial role in the capacity of molecular imprinting polymers to recognize one another by giving the polymer networks the stiffness required to sustain the active site. Ethylene glycol dimethacrylate, divinylbenzene, N, N-ethylenebismethacrylamide, and trimethylolpropane trimethacrylate are the most used cross linkers. To prevent non-specific adsorption, cross linkers should ideally not interact with template molecules [97]. A common method for starting the polymerization of molecular imprinting polymer synthesis is the creation of free-radical.

2.10. Techniques for Polymerization.

The polymerization procedure is mostly carried out using four approaches. Based on the results, these strategies are divided into two types. Let's take a closer look at four prevalent polymerization procedures.

2.10.1 Heterogeneous Techniques

The term "heterogeneous procedures" refers to two distinct approaches, namely bulk polymerization, and solution polymerization. Figure 2.28. [98].

The monomer is taken as a liquid in the bulk polymerization process, and an initiator is dissolved in it. The chain transferring agent is then allowed to dissolve in the solution. This solution is then exposed to light and agitated to ensure optimum mass and heat transmission. This technique produces a wide molecule with a heavy distribution of mass. Furthermore, as the process improves, the mixing gets increasingly complex. Bulk polymerization yields the purest form of polymer.

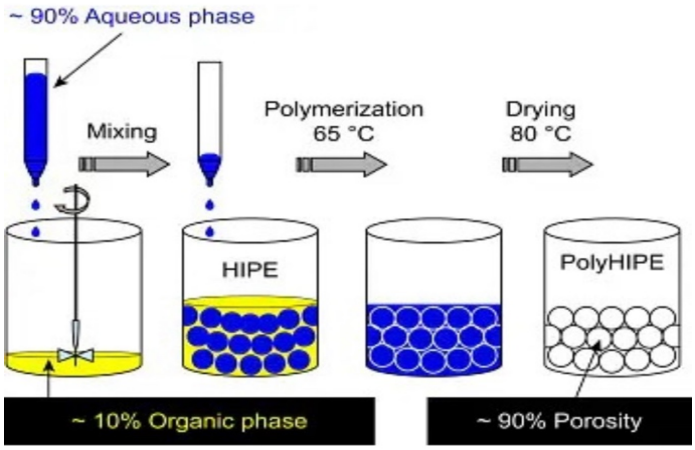


Figure:2.28: Heterogeneous Techniques. [98].

2.10.2 Homogeneous Techniques:

Solutions polymerization is the second commonly used method for homogeneous polymerization. In this procedure, both the chain agent and the monomer are simultaneously dissolved in a solvent. Anywhere it is employed, the free radical initiator dissolves in the solvent. Wherever the polymers are expected to be utilized as a solution, this technique is advised. Homogeneous polymerization is split into two distinct processes, namely suspension polymerization and emulsified polymerization, like heterogeneous polymerization. Figure 2.29

While dissolving with the chain agent, bulk polymerization was found to be releasing too much heat. It occasionally detonated due to overheating, which made this risky. Suspension polymerization was created to address this flaw in bulk polymerization. In this instance, the monomer was dispersed in water as tiny droplets, which was stabilized and kept from coalescing by employing appropriate water-soluble protective colloids and

surface agents. Heat is transferred in the water as the monomer droplets form. But keep in mind that this method only functions in liquids, hence it is only appropriate for monomers that are not soluble in water.

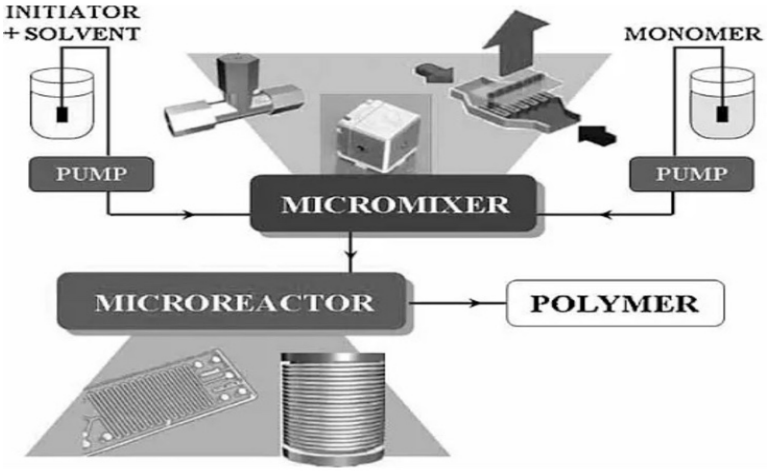


Figure 2.29: Homogeneous Techniques. [99].

Beyond all of this, though, emulsion polymerization is the most well-liked and often employed method of polymerization. In this method, the monomer is emulsified by surface agents, buffers, and protective colloids after being disseminated in an aqueous phase as tiny droplets. The agents' reduction of surface tension makes the environment favorable for the formation of micelles by surfactants. The monomer is emulsified in a hydrocarbon phase that exists inside the micelles.

2.10.3 Free Radical Polymerization Technique.

Vinylic monomers are most commonly polymerized by free radical polymerization. A monomer attaches to the terminal free radical reactive site, also referred to as the "active center," in this process of chain reactions, allowing the polymer chain to expand. The active core moves to the end of the newly widened chain upon incorporation of the monomer. Because of its adaptability to a variety of polymerizable groups, such as styrene, vinylic, acrylic, and methacrylic derivatives, this method is preferred. It is also

resistant to different solvents, small contaminants, and the presence of multiple functional groups in the monomers. Nonetheless, the inherent limitations of conventional free radical polymerization are embedded within the process itself. It is particularly challenging to add specified end groups, manage molar masses and polydispersity's, or create unique macromolecular designs like block copolymers. New free radical polymerization techniques, sometimes referred to as "controlled" free radical polymerization, have recently been discovered in attempt to get around these restrictions [100].

These procedures often include the use of chemicals that reversibly radicals which spread into inactive species. As a result, the polymer chains often develop concurrently rather than sequentially. Chain growth and monomer consumption proceed at a similar pace as a result.

i) Nitroxide-mediated Radical Polymerization (NMRP), ii) Atom Move Radical polymerizations (ATRP) [101,102], which is catalyzed by Transition The metal Complexes, and iii) more recently, (iii) polymerizations via Reversible Addition Fragmentation Chain Switch (RAFT) [103-105] or Macromolecular Design via Interchange Xanthates are employed as chain-transferring agents in this scenario. These techniques have garnered the most attention. The final of these approaches is based on degenerative chain transfer, in contrast, the first two work via permanently preventing the development of polymer radical.

2.11. Type of the Molecular Imprinting.

Covalent bonds, noncovalent bonds, and metal ion coordination are the driving factors for the interaction between template and functional monomer. These interactions are used to construct molecular imprinting procedures, which are described further below.

2.11.1 Covalent Imprinting

The covalent imprinting process copolymerizes the template molecule with useful monomers. Following polymerization, a covalent connection is chemically broken, creating a permanent cavity with good chemical orientation. Due to the creation of

persistent covalent connections, precise fit recognition sites are created, which reduce non-specific interactions and prevent template molecule leakage. The main drawback is the potential harm to the imprinted sites caused by using strong chemicals to chemically split the template molecules. Covalent bonds result in a powerful contact between the template and the monomer, but they also have delayed rebinding kinetics, which is a problem for quick separation and purification procedures and minimal template removal. [106].

2.11.2. Non-Covalent Imprinting

In the non-covalent imprinting method, molecular imprinting polymers are created by reorganizing template and functional monomers through secondary interactions like Coulombic interactions, van der Waals forces, and hydrogen bonds prior to polymerization. This approach's major advantage is its versatility, allowing it to be applied to a broad spectrum of template molecules. It is also known for its simplicity and adaptability. However, using an excess of functional monomers can lead to poor template-monomer complexation and the formation of heterogeneous binding sites. Although water is commonly used as a solvent in molecular imprinting due to the limited solubility of many biomolecules in organic solvents, the weak nature of hydrogen bonds in this environment can diminish the system's recognition capability [107-108].

2.11.3. Molecular Imprinting Mediated by Metal Ions

Because metal ion coordination is stable as well as precise, it is very useful for molecular identification. Metal chelating monomers are grouped around a metal ion that combines with a template molecule during the imprinted process. Usually, this metal ion is the transitional metal. This metal ion acts as an intermediary, helping to align the functional monomer and template molecule to form an imprint with high specificity and accuracy. Additionally, because metal ion interactions are generally stronger than hydrogen bonds, metal ion coordination remains more stable in aqueous environments. For instance, the binding energy of the combination of the histidine's imidazole residue and Cu+2 is 4.8 kcal/mol, while the binding energy of a normal hydrogen bonding contact is greater than 1 kcal/mol. [109].

Additionally, the rapid binding process of metal ion coordination allows for quick changes in binding strength by selecting the right metal ion for a particular template molecule. Additionally, a new metal ion might be used to improve selectivity, or a molecular imprinting polymer could be used for a different purpose [110]. To produce highly specific molecular imprinting polymers in aqueous media, metal ion coordination technique offers a significant potential.

2.12. Cryogels

Cryotropic gelation produces cryogels, which are macroporous polymeric gels. Biotechnology and biology are becoming more interested in macroporous polymeric materials. The synthesis involves key steps such as moderate freezing, maintaining the frozen state, and later thawing solutions or colloidal dispersions with monomer or polymer precursors. During the moderately frozen state (where the temperature is only a few tens of degrees below the solvent's freezing point), cryotropic gelation and associated chemical reactions occur in the liquid microphase. Cryotropic gelation can happen in both aqueous and organic media if the cooling conditions and solvent facilitate crystallization. Figure 2.30 depicts the typical cryotropic gelation strategy. [111- 112].

Cryogels have a tissue-like elasticity and can withstand significant deformations without breaking, which is one of their main advantages over other macroporous materials with holes of comparable size. Cryogels can develop when appropriate monomers are polymerized or polycondensed in frozen solutions under the influence of chemical or radiation initiators. Cryogel production in solvent polymer cross linking agent systems is another potential. Non-covalent physical gels may be created by simply chilling a solvent polymeric system if it exhibits a fast rate of material consolidation upon cooling, an upper critical solution temperature, and a polymer concentration over the critical point. [113].

Wide holes in cryogels typically link to one another because, when the initial solution of the gel-forming precursors freezes, each solvent crystal develops until it comes into touch with the facet of the next crystal. Pore size can vary by hundreds of micrometers to tenths of a micrometer. Thus, it becomes feasible to create convective masses of soluble materials and particles that are the size of complete cells. Cryogels have a wide range of potential uses in biotechnological and biomedical applications, including collagen

materials for medical use and carriers for immobilizing enzymes, microbial cells, and antibodies. [114].

A new class of biomaterials called polymer cyrogels has lately gained interest as viable scaffolds for tissues in regenerative medicine. The term "cyrogel" is made up of two words: "gel," which describes a colloid semi-soft material, and "cryo," which is derived from the Greek letter KPIδ (cyros), which indicates cold or ice. Nowadays, the word "cyrogel" has more than three distinct meanings in scientific literature and pertains to three different families of chemicals. (2) Sol gel technique is used to generate polymeric and inorganic cyrogels, that are then freeze dried. (1) During cryoprecipitation blood plasma therapy, a gelatinous precipitate forms after freezing to 4°C. This has led to the synthesis of a variety of inorganic oxides, polymers, and cyrogels, such as: (1) single and mixed inorganic oxides; (2) synthesised and neutral polymer cyrogels; and (3) cyrogels synthesized in a cooling solvent, typically water [115].

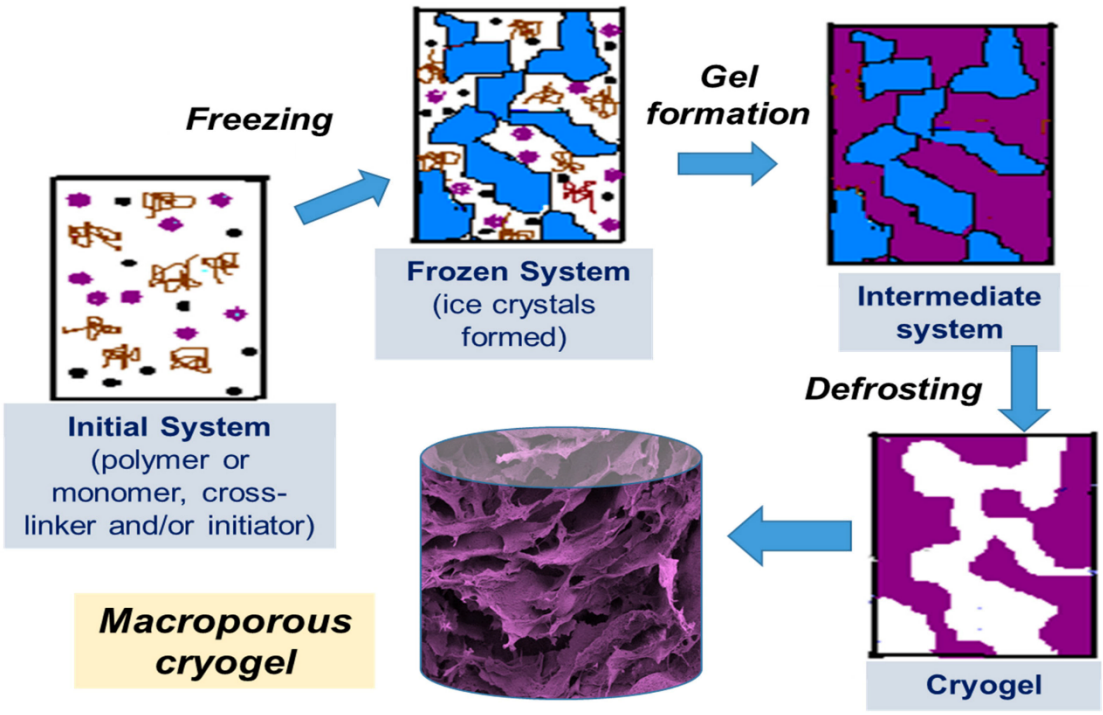


Figure 2.30. An Example of how to Make a Cryogel [111].

2.12.1. The Cryogels in Biological Chromatography

The first Cryogels were discovered fifty years ago, but it took until roughly 15 years ago for their potential in bio separation to be understood. In particular, the fields of biotechnology, biomedicine, and pharmaceutics pay close attention to macro porous cryogels. Advantages when working with viscous media like blood, plasma, and plant or animal tissue extract come from their vast interconnected holes (Figure 2.31). [116]. Cryogels offer both adsorption and elution a very short residence time, minimal pressure drop, and short diffusion route. The purification of physiologically important proteins is greatly facilitated by these characteristics of cryogels. Biopolymers may be separated using cryogels without any restrictions on diffusion. Comparing Cryogels to traditional polymeric beads, the latter perform better when it comes to separating Natural sources such as polysaccharides, proteins, and nucleic acids. Cryogels can immobilize various ligands to facilitate the separation or removal of various compounds. Cryogels have already been synthesized in a variety of forms during the past ten years, and several ligands, on them, such as transition- metal ions, tryptophan, histidine, concanavalin A, protein A, and triazine dyes. [117–128] Utilizing these ligand-immobilized cryogels, several macromolecules such as IgG, lysozyme, albumin, DNA, cytochrome c, cholesterol, and lectins can be separated. Through their great porosity and physicochemical properties, cryogels are ideal supports for immobilizing cells. Cryogels made of poly (vinyl alcohol) and polyacrylamide can be used to capture or adsorb cells. Cryogels provide a helpful three-dimensional framework for the growth and formation of cells.

Cell affinity is greatly influenced by the surface qualities of supplementary resources utilized, particularly within tissue engineering. Cryogels are very desirable materials for cell affinity because they are hydrophilic and porous [129-130]. Additionally, cell affinity chromatography makes use of cryogels. They work well for separating cells, including B and T lymphocytes. Proximity interactions based on antigens are frequently employed to separate cells from crude extract because of their high specificity. Isolation of a particular cell type is made possible by the antibody's affinity for the cell surface antigen [131-138]. Cryogels have recently been employed in systems for the controlling delivery of medicine. They are effective delivery mechanisms for the medication 5-fluorouracil.

2.13. Control of Administering Medication and Molecular Imprinting Method

It is now feasible to generate a material with distinct physical and chemical characteristics thanks to the ongoing development of molecular imprinting technology in several ways for various applications in numerous fields. The development of molecular imprinting as a useful technique for analytical applications to extract certain compounds from the complex environment has recently taken place. Pollutant elimination is another use for molecular imprinting. Additionally, the creation and use of molecular imprinting drug delivery systems is growing along with the number of research and patents.

In recent years, molecular imprinting has emerged as one of the most popular techniques for administering controlled substances. In the early stages of controlled release's development, molecular imprinting has been used in pharmacology. For theophylline and diapeza, Vtakis et al. created non-covalent molecular imprinting devices, but they did not employ them in controlled drug delivery systems [139]. The easy manufacture of drugs via molecular imprinting has been employed by many organizations [140]. The benefits of molecular imprinting in medication delivery include:

1. MIP enables therapeutic medicines' delayed release by serving as reservoirs.

2. Increase the capacity for loading.

3. The quantity of crosslinker and exchange the crosslinker are advantageous.

4. Therapeutic release may take place depending on the environment.

5. Enantioselective eutomer is released or finished loading.

6. Longer release was induced by the functional monomer's affinity for the template molecule [141].

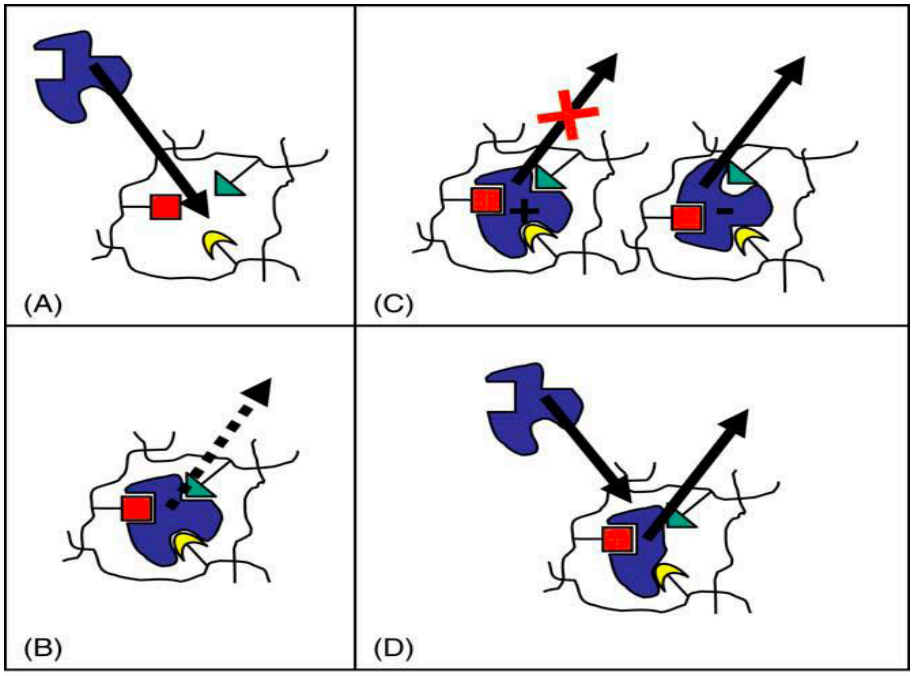


Figure 2.31. Molecularly Imprinted Drug Delivery Systems. [116].

A. **Drug loading step:** The binding sites in the molecular imprinting give high loading capacity for suppressing medicines.

B. **Extended Release:** Because of their strong affinities to molecular imprinting, imprinting drug molecules release slowly.

C. **Enantioselective release:** In the polymer, non-imprinting enantiomers were released more quickly than imprinting enantiomers.

D. **Sensitive impact release:** A medicine with low affinity is getting increasingly high affinity when it binds to the exterior and includes a warning.

Asthma is treated with theophylline, which has a 30–110 m therapeutic window. Highly toxic if greater than 110 m Theophylline release was accomplished in research by loading a tiny quantity of medication at pH 7 [142].

2.14. Consequences of Cross-Linkers and Functional Monomers

In their study, Alvarez Lorenzo et al. altered the rates at which the template molecule (timolol), the cross-linking monomer (MAA), and the drug release monomer (EGDMA) released their pharmaceuticals [143]. According to the cross-linker and monomer ratio, excessive template molecules were employed during the synthesis of molecular imprinting, which resulted in a fast drug release. There was no significant drug-drug binding for small amounts of functional monomers. High affinity binding sites for template molecules were created because of increasing the template molecule amount to delay release [144]. Cross-linkers are often utilized in the polymerization process at a 10:80 ratios. The three-dimensional binding site structure was conditioned by the cross-linker. Utilizing the appropriate quantity of cross-linker High rate causes the hydrophobic characteristic to grow and prevents conformational change. Due to tissue friction (such as that caused by intraocular implants), the high rate of cross-linker may cause injury [145]. For this reason, molecular imprinting-based regulated drug delivery systems did not employ high and low cross linker ratios.

3. EXPERIMENTAL

3.1 Materials and Methods

2-hydroxyetyhl methacrylate (HEMA), ammonium persulfate (APS), N, N′-methylenebis(acrylamide) (MBAAm), N, N, N', N'-tetramethyl ethylene diamine (TEMED) was bought from Sigma Chemical Co. (St. Louis, MO, USA). N-methacryloyl-(l)-histidine methyl ester (MAH) monomer was obtained from NanoReg (Ankara, Turkey). Mitomycin C (MMC) was purchased from Kyowa, Hakko Kogyo Co Ltd. (Tokyo, Japan). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany).

3.2. Preparation of MIP-µcryogel

In the first stage, amino acid-based MAH to be used as functional monomer was synthesized according to the procedure in previous studies [29]. Then a pre-complex was formed with metal ions of MAH and Cu (II) at 1: 1 molar ratio. 1.0 mmol MMC was added to this pre-complex formation. The final complex was then dissolved in 1 mL of 10 mM phosphate buffer (PB) (pH 7.4) and the resulting solution was incubated at 25 ° C for 30 minutes. A certain amount of MAH-Cu (II)-MMC complex and ÇHEMA were added to a beaker. In deionized water, 0.283 g of MBAAm was dissolved, then mixed with the previous solution and the total water volume was fixed at 12 mL. After adding 25 mg of APS and 20 µL of TEMED, the mixture was transferred to a microstencil array chip with microwells of 200 µm diameter and 500 µm thickness. The mixture was cryotropic gelled for 24 hours at -14 °C by clamping the microstencil array between two glass plates. Next, the microstencil array chip was placed into a freeze-dryer (Christ Freeze Dryer - Alpha 1–2 LD, USA) and treated at -56 °C and 0.0010 mbar for 2 h. Finally, MIP-µcryogels were collected. The same procedure was applied for non-imprinted microcryogels, but MMC was not added to its gel-forming mixture for prepare the non-imprinted microcryogels (NIP-µcryogel). Here, MAH and Cu (II) was added together for obtain pre-complex formation. Then, HEMA were added to a beaker and 0.283 g of MBAAm solution was mixed with the previous solution. In the end, 25 mg of

APS, 20 µL of TEMED were added and transferred to a microstencil array chip with same diameter and thickness. Additionally, PHEMA microcryogels (PHEMA-µcryogel) were prepared the same above method, without using MAH-Cu (II) and MMC molecules.

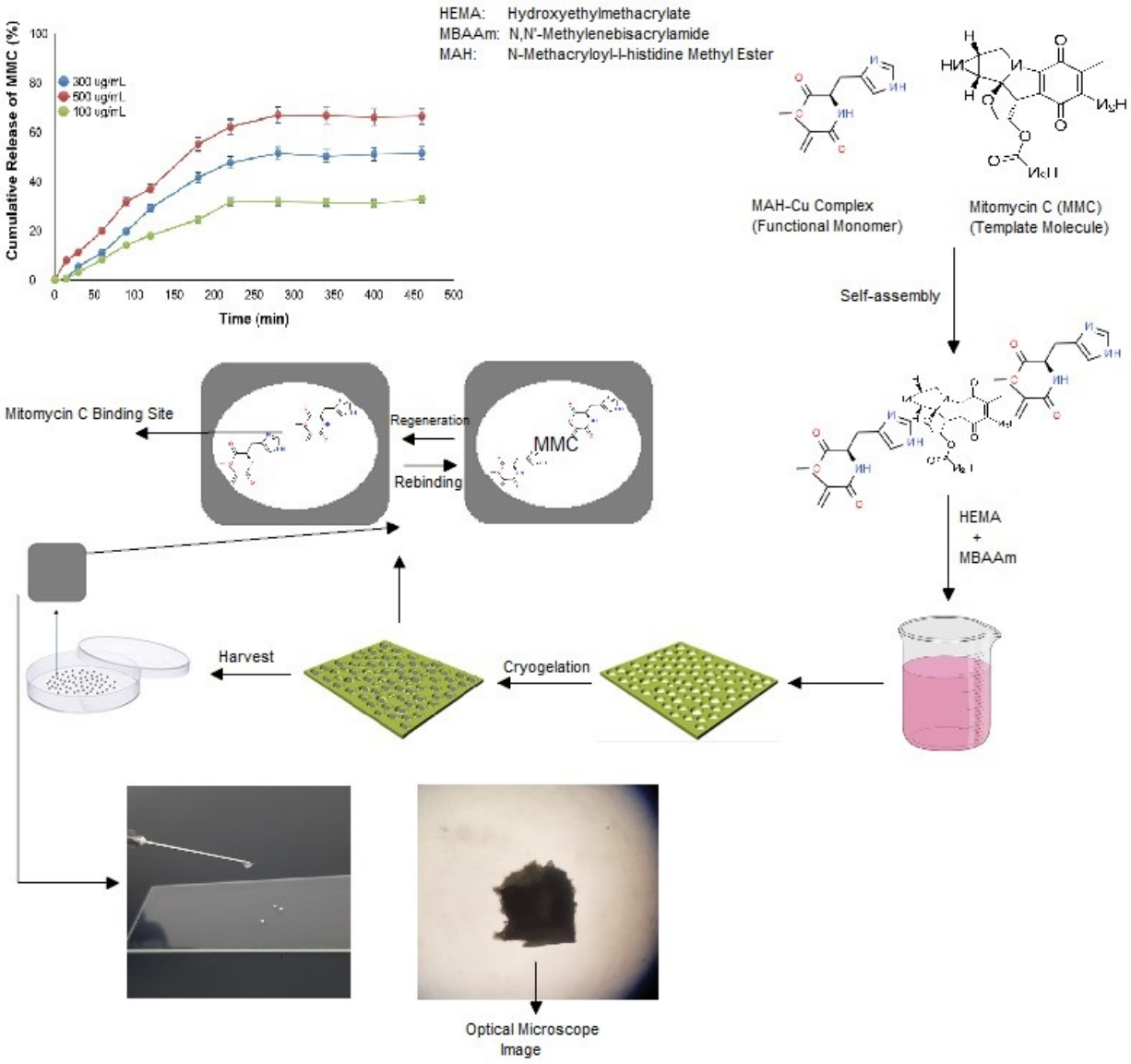


Figure 3.1. The Schematic Preparation of Injectable µcryogel

3.3. Characterization Experiments of µcryogel

The surface morphologies of injectable µcryogel were examined by SEM (GAIA3, Tescan, Czech Republic) and before SEM analysis, the surfaces of dried injectable

µcryogel were coated with gold (50 nm) and their images were taken with different magnifications.

Thermo Scientific Nicolet iS10 FTIR spectrometer was used to study the IR spectrum of pHEMA and pMIPs in the range of 4000–400 cm^{-1} wave numbers.

Before calculating the swelling behavior of µcryogel, the dried µcryogel were weighted (Wo, g) and immersed in 10 mL DW at room temperature for 2 h, after wiped with a filter paper and weighted (Ws, g) again. Afterwards, their swelling degrees were calculated with equation 1.

$$\text{Swelling degree\%} = [(W_s - W_o) / W_o)] \times 100 \quad (1)$$

3.4. Releasing Performance of the µcryogel

The MIP-µcryogels were first dried and then used in MMC release studies conducted in 3 ml of PBS buffer at pH 7.4 and 37°C. At specific time points, samples of the release medium were withdrawn, and an equal volume of fresh buffer was added. The leakage of Cu (II) from the injectable µcryogel was analyzed using a graphite furnace atomic absorption spectrometer (Analyst 800/Perkin-Elmer, Shelton, CT). Data on medium and drug transport were collected from three replicates, and mean values were reported. Various MMC concentrations were tested in the MIP-µcryogels, with MMC concentration quantified at 365 nm using a UV/Vis spectrophotometer (Shimadzu, Model 1601, Tokyo, Japan). Additionally, the influence of pH on MMC release was examined across different pH values to assess its impact on release percentage.

3.5. In vitro Cytotoxicity Tests for MIP, NIP and pHEMA Based µcryogel

To evaluate the in vitro cytotoxicity of the developed drug carriers, the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay was employed [21], using the L929 mouse fibroblast cell line. Prior to conducting the cytotoxicity assays, the cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 10% L-glutamine. These cultures were maintained in a

humidified incubator set at 37 °C with an atmosphere of 95% air and 5% CO2. The cell culture medium was refreshed every three days to ensure optimal growth conditions.

Following the cell cultivation phase, L929 cells were harvested and prepared at a density of 300 cells/mL. These cells were then plated into 96-well plates, and the sterilized drug carriers were introduced into the wells. The plates were then incubated at 37 °C for a period of 72 hours. After this incubation period, the original cell culture medium was removed, and the extract media from the drug carriers were introduced into the wells. This new media was incubated at 37 °C for an additional 24 hours.

Subsequently, 100 µL of cell culture medium containing 10% MTT solution was added to each well. The plates were then incubated at 37 °C for 4 hours. Following this incubation, the plates were allowed to stand at room temperature for 30 minutes in the dark. The cell viability was then assessed using an automated enzyme-linked immunosorbent assay (ELISA) at a wavelength of 570 nm. Control wells were maintained with regular cell medium throughout the experiment.

For evaluating the viability of pMIPs, the MTT assay was performed on the MDA-MB-231 breast cancer cell line. Cells were cultured in DMEM/F12 medium, which included 10% fetal bovine serum and 1% penicillin/streptomycin, at a temperature of 37 °C. After cultivation, the cells were plated into 96-well plates and incubated under a 5% CO2 atmosphere for 12 and 24 hours. Post-incubation, the cell media was replaced with PBS buffer, and the drug carriers, including pMIPs, pNIPs, and pHEMA as a control material, were added to the wells. Absorbance readings were then taken using a spectrophotometer microplate reader (1QuantTM, Biotech Instruments Inc., USA) at 570 nm to determine the cytotoxicity of the different materials.

4. RESULTS AND DISCUSSION

4.1. Characterization Studies of µcryogel

The optic images of MIP-µcryogel were shown in Figure 4.1. Their dry forms are round-shapes and like a piece of chalk (A). When MIP-µcryogels were swollen up in water, they could easily pass through the needle without the pressure (B).

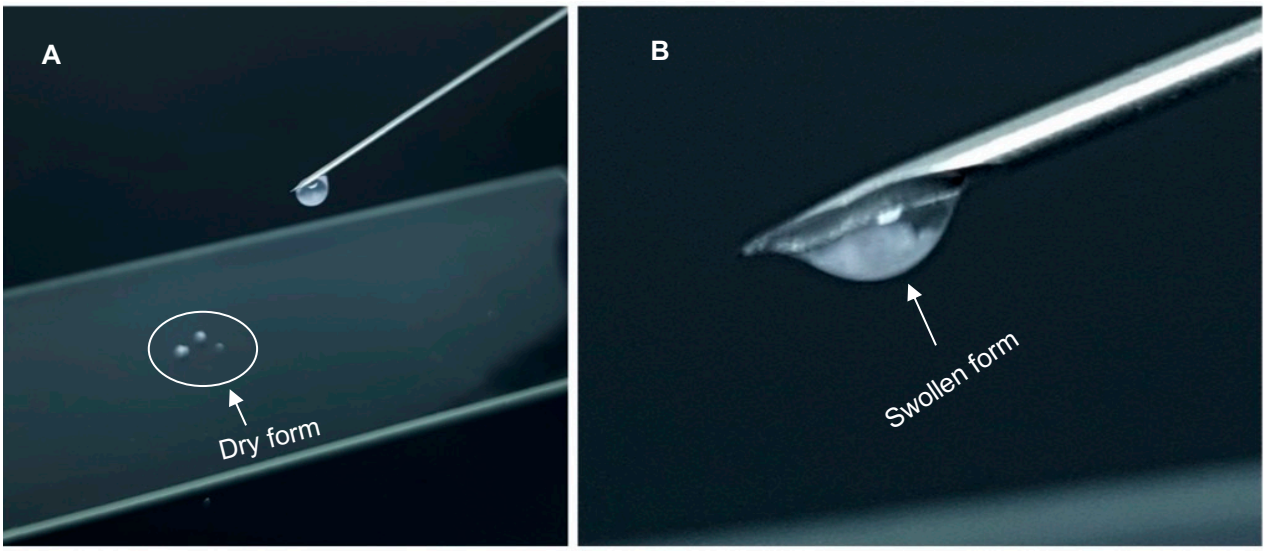


Figure 4.1. Optical images of MIP-µcryogel.

4.2. FTIR Results of pHEMA and pMIPs

In Figure The FTIR results of pHEMA and pMIPs were in accordance with the previous studies [163-165] and according to the FTIR results, the functional monomer, MAH, were successfully jointed into the polymer chain. The -OH strecthing, aliphatic -CH strecthing, carbonly C=O strecthing, and C-O strecthing bands of p(HEMA) were observed at 3269 cm^{-1}, 2943 cm^{-1}, 1718 cm^{-1}, and 1153 cm^{-1}, respectively. The peaks around 1648 cm^{-1} and 1530 cm^{-1} as described decribed amide I (C=O) streching and amide II (C=O) strecthing, respectively and these peaks that were stemmed from the crosslinker are the characteristic peaks of p(HEMA). The ester bond around at 1444 cm^{-1}, C-N bond from crosslinker at 1389 cm^{-1}, the ether bonds at 1235 cm^{-1}, and at 1074 cm^{-1} were observed. Our results were in accordance with the previous studies .

The same characteristic peaks of p(HEMA) were also found on pMIPs and the incorporation of MAH monomer into the polymer chain increase the intensity of amide I band at 1634 cm^{-1} and amide II band at 1527 cm^{-1} of the prepared microcryogel.

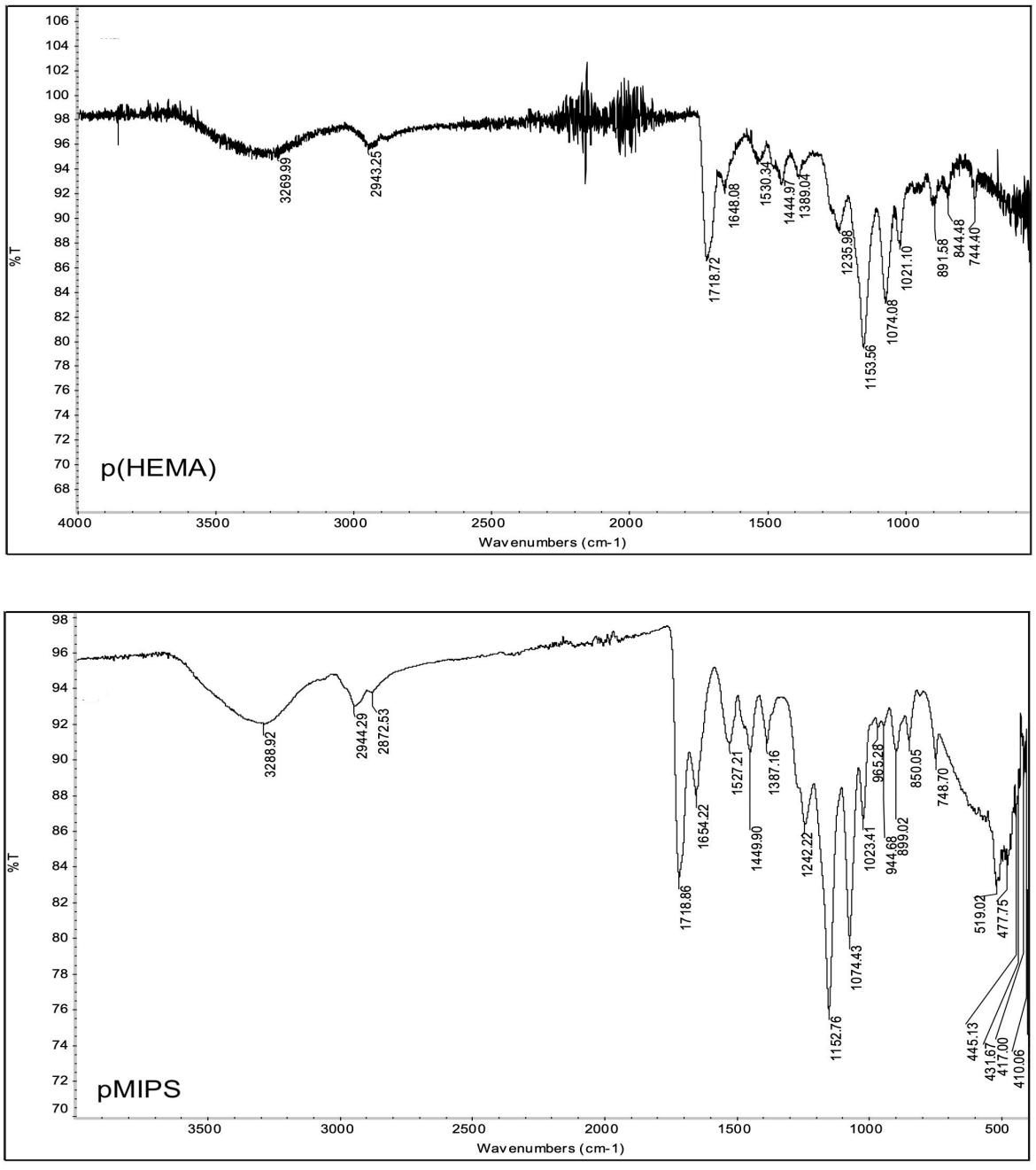


Figure 4.2. The FTIR Results of pHEMA and pMIPs:

4.3. SEM Results of pHEMA and pMIPs

The scanning electron microscopy (SEM) images of both pMIPs and pNIPs are displayed in Figure 4.3, providing a comprehensive view of their morphological characteristics. Panels A, B, and C of Figure 4.3 present images of pMIPs, while panels D, E, and F show pNIPs. The SEM images in Figure 4.3A and D reveal that both types of microcryogels exhibit a substantial, bulky form. The surfaces of these cryogels are notably rough and exhibit a sponge-like morphology. This texture is highlighted in Figures 4.3 C and D, where the porous structure and rough surface of the microcryogels are clearly visible.

Additionally, the Fourier-transform infrared (FTIR) spectroscopy results for pHEMA and pMIPs align well with previously published studies [154–156]. These FTIR results confirm that the functional monomer, methacrylic acid (MAH), has been successfully integrated into the polymer chain, indicating successful polymerization and functionalization.

The swelling behavior of pMIPs was evaluated at various pH levels, ranging from 4 to 7.4. The swelling percentages recorded were 24% at pH 4, 36% at pH 5, 38% at pH 6, and 39% at pH 7.4. This data illustrates a clear trend: the swelling ratio of pMIPs increases with rising pH. In acidic environments, the MAH monomer's NH_3^+ groups and undissociated carboxyl groups (–COOH) interact with the –OH groups of p(HEMA) through hydrogen bonding. These interactions facilitate the formation of crystalline structures within the gel, which in turn leads to a reduction in the swelling ratio under acidic conditions.

In summary, the SEM images provide visual evidence of the bulkiness and rough, sponge-like surface texture of both pMIPs and pNIPs. The FTIR analysis confirms the successful integration of MAH into the polymer chains, while the swelling studies reveal that the degree of swelling in pMIPs is influenced by pH, with increased pH leading to higher swelling percentages and the formation of crystalline structures in acidic conditions.

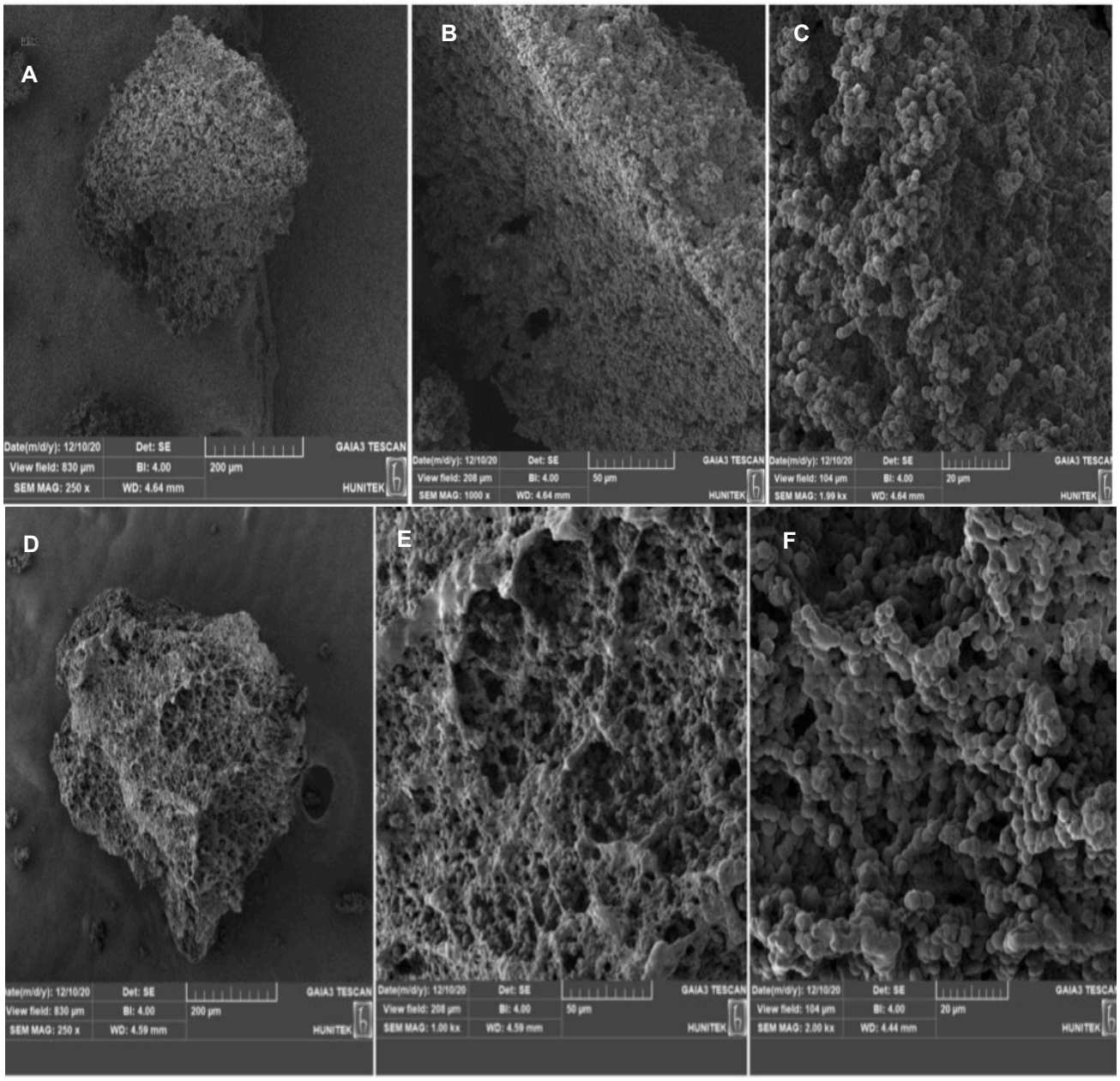


Figure 4.3. SEM images of MIP (A, B and C) and NIP (D, E, and F) µcryogels.

In addition, the behavior of pMIPs in varying pH environments reveals significant insights into their swelling characteristics. In acidic conditions, the –OH groups of the p(HEMA) polymer engage in strong hydrogen bonding interactions with the polymer chain. This interaction leads to a reduction in the swelling degree, as these bonds effectively restrict the expansion of the polymer matrix [157].

Conversely, in alkaline media, the carboxyl groups present in the MAH monomer tend to ionize, converting into carboxylate ions. This ionization reduces the ability of the

carboxyl groups to form hydrogen bonds with the –OH groups of p(HEMA). Consequently, this results in a decrease in the formation of crystalline structures within the cryogel, which contributes to an increased swelling degree of the pMIPs [157].

Moreover, an increase in the pH level of the medium leads to a reduction in the concentration of hydrogen ions (H+). This reduction weakens the hydrogen bonds within the polymer chain, resulting in a relaxation of the polymer chains in the matrix. As the hydrogen bonds become less robust, the polymer network becomes more amenable to the diffusion of water molecules. This influx of water into the polymer network further enhances the swelling degree of the pMIPs [158].

4.4. PH Effects on the MMC Releasing Performance

The release characteristics of pMIPs were evaluated over a pH range of 4.0 to 7.4, and the performance in terms of MMC release was found to be notably effective. The experimental results, which are detailed in Figure 4.4, reveal several important trends.

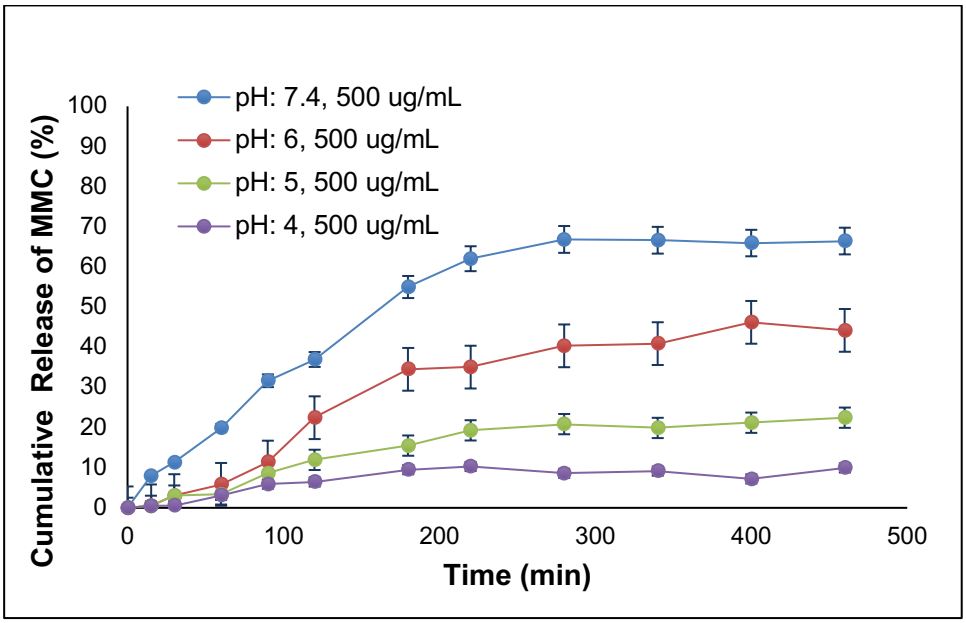


Figure 4.4. The pH effect on MMC releasing of MIP-µcryogel (MMC concentration: 500 µg/mL, shaken rate: 120 rpm, T: 25 °C).

According to the data presented in Figure 4.4, the release of MMC from pMIPs increases gradually with rising pH values. This trend indicates that pMIPs exhibit enhanced release capabilities in more basic environments. Despite this general trend, the cumulative release of MMC at physiological pH (around 7.4) remained relatively stable and reached approximately 70%. This steady release at physiological pH could be attributed to several factors. One possible explanation is that the acidic conditions at lower pH values might negatively impact the stability of MMC, thus reducing its release efficiency.

Furthermore, the observed release pattern might also be influenced by the electrostatic interactions between the amino acid-based functional monomer and MMC. As the pH changes, the repulsive forces between similarly charged groups on the monomer and MMC can affect the release dynamics. Specifically, in acidic conditions, the increased repulsion due to the presence of similarly charged groups could hinder the release of MMC, resulting in decreased performance.

Overall, the pH of the medium plays a critical role in modulating the release behavior of MMC from pMIPs, with a noticeable increase in release as pH becomes more basic and a more controlled release observed at physiological pH.

4.5. Carrier Effect on MMC Releasing Performances

The release profiles of MMC from pMIPs, pNIPs, and pHEMA are depicted in Figure 4.5, highlighting significant differences in their performance. According to the figure, the release duration of MMC from pMIPs was notably extended, lasting nearly 500 minutes. In contrast, MMC release from both pNIPs and pHEMA occurred much more quickly, with the initial burst release observed within a brief period of less than 100 minutes.

This difference in release behavior can be attributed to the differences in the design and composition of the drug carriers. In the case of pHEMA and pNIPs, the absence of specifically designed MMC receptors during their fabrication resulted in a lack of tailored interactions with the drug. As a result, MMC was released rapidly from these carriers due to the absence of mechanisms to control or slow down the release process.

On the other hand, the pNIPs drug carrier exhibited a superior release performance compared to the control pHEMA carrier. This improved performance can be explained by the inclusion of Cu (II) ions in the pNIPs fabrication process. The Cu (II) ions were

likely coordinated with the nitrogen (N) and oxygen (O) atoms of the MMC molecules through coordinate covalent bonds. This interaction contributed to a more gradual release of MMC, thereby extending the release duration. In brief, the release characteristics of MMC from these different drug carriers demonstrate that pMIPs provided the longest release time, while pNIPs, although better than pHEMA, still exhibited a more rapid initial release due to the presence of Cu (II) ions, which facilitated extended MMC release through coordinate bonding interactions.

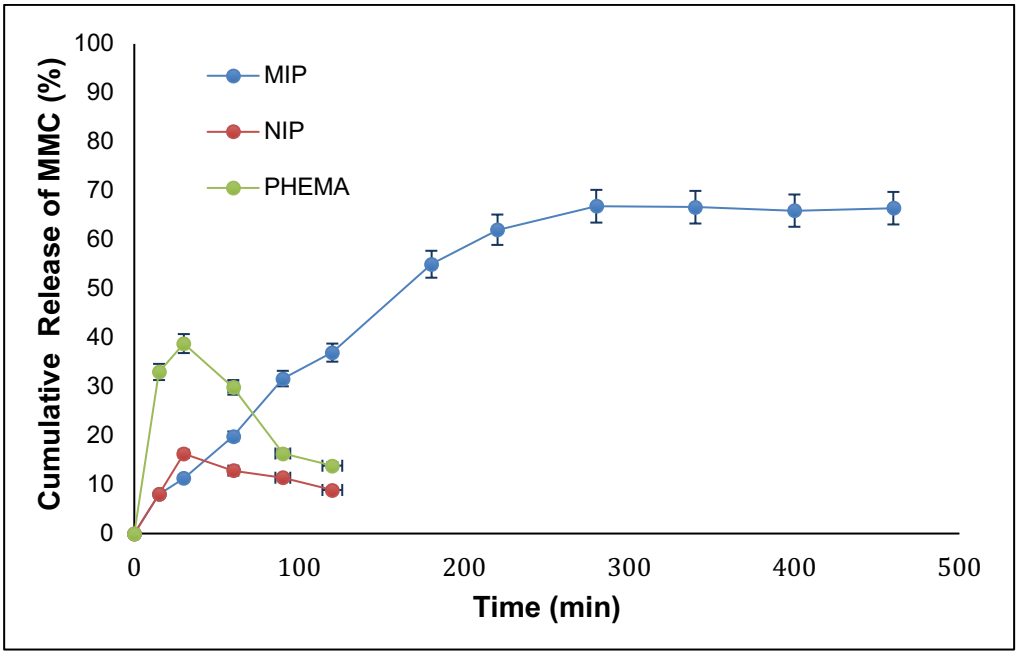


Figure 4.5. The releasing behaviors of MIP, NIP and PHEMA-µcryogels (pH 7.4, MMC concentration 500 µg/mL, shaken rate; 120 rpm and T: 25 °C).

The data illustrated in Figure 4.5 reveals significant variations in the release profiles of MMC from different µcryogel carriers. Specifically, the MIP-µcryogel demonstrated a considerably prolonged release duration, extending up to approximately 500 minutes, compared to the other µcryogels. This extended-release period contrasts sharply with the behavior observed for NIP and PHEMA drug carriers, where the MMC was released rapidly, with an initial burst occurring in less than 100 minutes.

The release profile of MMC from pMIPs was assessed for various MMC concentrations, with the findings depicted in Figure 4.6. This figure illustrates that as the concentration of MMC increased, the total amount of MMC released also rose accordingly. Notably, the release profiles for different MMC concentrations plateaued after approximately 200 minutes, indicating that the release process reached a steady state for each concentration level.

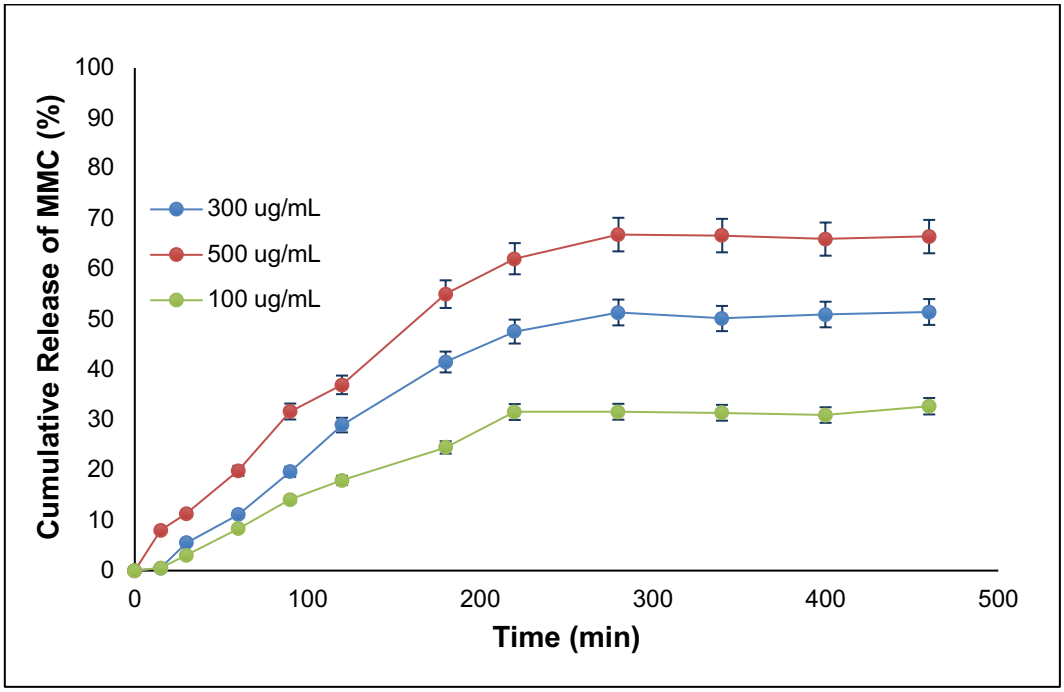


Figure 4.6. The demonstration of different amounts of MMC loading effect (100, 300, 500 µg/mL)

The effect of the imprinting amount on the release was observed by trying different amounts of MMC. In Figure 4.6, the release rate of 100, 300, 500 µg/mL MIP-µcryogels were presented. According to the results, as the amount of MMC increased, the releasing rates are also mounted.

4.6. Kinetic Studies

Developing effective drug delivery systems hinges significantly on the design and optimization of drug carriers. As carriers play a central role in ensuring the efficient delivery of therapeutic agents, the design of these carriers is a crucial aspect of enhancing

the overall effectiveness of drug delivery systems. To achieve this, mathematical modeling has emerged as a highly valuable tool in the design and evaluation of various carriers. These models have demonstrated their utility in providing insights into the functional performance of drug delivery systems.

In addition to carrier design, understanding the mechanisms of drug release and its kinetics is essential. Various physical models are employed to investigate and elucidate the different stages and detailed mechanisms involved in the release of drugs from carriers. This understanding is critical for optimizing drug release profiles and ensuring that therapeutic agents are delivered in a controlled and effective manner.

Among the models used for this purpose, the Korsmeyer–Peppas model and the Higuchi model are commonly applied to analyze the mechanism and kinetics of in vitro drug release. The Korsmeyer–Peppas model (Eq. 2) and the Higuchi model (Eq. 3) are particularly useful for this analysis. These models provide valuable equations and frameworks for interpreting the release behavior of drugs from carriers, helping researchers to refine and optimize drug delivery systems.

By employing these mathematical models, researchers can gain a deeper understanding of the drug release processes, allowing for the development of more effective and efficient drug delivery systems. This approach ensures that the design of drug carriers is not only based on empirical observations but is also supported by robust theoretical frameworks, ultimately leading to advancements in drug delivery technology.

$$M^t / M^\infty = k_p t^n \quad (2)$$

$$M^t / M^\infty = k_H \, t^{1/2} \quad (3)$$

where M^t/M^∞ is the fraction of drug released to time t, k_H represents the Higuchi rate constant.

The kinetic constant k_p plays a crucial role in describing the drug release mechanism, as it is intricately linked to the shape of the tablets. Specifically, this constant serves as an indicator of the structural and geometric properties of the tablets, reflecting how their

physical form influences the release dynamics. Another significant parameter in these equations is the exponent n, which denotes the diffusional exponent of the release mechanism. The shape of the tablet has a direct impact on its diffusional properties, making this exponent dependent on the tablet's geometry. For cylindrical tablets, the value of n varies depending on the type of release mechanism: it is 0.45 for Fickian release, falls between 0.45 and 0.89 for non-Fickian release, equals 0.89 for Case II release, and exceeds 0.89 for super Case II type release [159–161].

In addition to the Korsmeyer–Peppas model, other mathematical models are also employed to elucidate the drug release mechanisms. These include the zero-order, first-order, and Peppas–Sahlin models. The zero-order model is particularly relevant when the drug release is governed by the degradation of the matrix rather than diffusion. The equation for the zero-order model reflects the rate of drug release rather than the cumulative release. In this context, the "order" in the model's equation refers to the rate of release rather than the order of the cumulative amount released.

Understanding these models and constants is essential for characterizing the drug release profile from various carriers. By examining the influence of tablet shape and employing different mathematical models, researchers can better comprehend the release mechanisms and optimize drug delivery systems accordingly.

$$M^t/M^\infty = k_0 t \quad (4)$$

For drug release that exhibits exponential characteristics, the First-order model is an appropriate tool for analyzing and understanding this behavior. This model takes into consideration both the dissolution rate of the drug and the geometry of the matrix. The relationship is expressed through the following equation, which provides insights into how these factors influence the release dynamics.

$$M^t/M^\infty = 1\text{-}\exp(-k_1 t) \quad (5)$$

When drug release is governed by mechanisms of diffusion and relaxation, the Peppas–Sahlin model is an appropriate choice to describe the delivery process. This model, which

can be expressed through the following equation, effectively represents how these controlling factors influence the release of the drug.

$$M^t / M^\infty = k_d t^m + k_r t^2 m \quad (6)$$

The constants k_d and k_r represent the rates of diffusion and relaxation, respectively, while mmm denotes the exponent related to purely Fickian diffusion [161, 162]. Table 4.1 provides the correlation coefficients R^2 and the exponents for MMC delivery, showcasing the fit of the data to various models. Based on the value of n, it is evident that MMC release follows a non-Fickian diffusion pattern in this research and shared in Table 4.2.

Table 4.1. The Releasing Performances of Some Drug Carriers for MMC.

Drug Carrier	**Mitomycin C Dosage**	**Release Rate**	**Refrences**
Gelatin scaffold	30 mg	45% in 45 days	[146]
Chitosan/β-glycerophosphate gels	2 mg	11% to 52% in 30 min	[147]
Cellulose based thermo-gel	0.2-0.4 (µg/ 100 µl	7.45% to 24.69% in 3-14 days	[148]
Magnetic nanoparticles	2-8 mg	54% to 92.5% in 30 h	[149]
Polybutylcyanoacrylate nanoparticles	1% (w/v)	-	[150]
Polycarbonate magnetic microspheres	4 mg	78% in 7days	[151]
Fluorescent carbon dots	0.3 mg	-	[152]

Mesoporous silica nanoparticles	6 mg	32% in 24 h and 40% in 72 h	[153]
Fe_3O_4-CS/GP gels	0.1 mg	75.33% in 2 h	[154]
MIP-µcryogels	500 µg/mL	70% in 460 min	In this study

Table 4.2: Release Kinetics of Different Amounts of MMC From pMIPs.

Zero	**Concentration of MMC**		**K_0**	**R^2**
	100 µg/mL		0.145	0.99
	300 µg/mL		0.203	0.96
	500 µg/mL		0.238	0.97
First	**Concentration of MMC**		**− k/2.303**	**R^2**
	100 µg/mL		0.007	*0.71*
	300 µg/mL		0.003	*0.83*
	500 µg/mL		0.004	*0.90*
Korsmeyer–Peppas	**Concentration of MMC**	**n**	**k_p**	**R^2**
	100 µg/mL	0.97	0.71	0.96
	300 µg/mL	0.93	0.65	0.98
	500 µg/mL	0.91	0.62	0.97
Higuchi	**Concentration of MMC**		**k_H ($mn^{-0.5}$)**	**R^2**

	100 μg/mL		2.5	0.95
	300 μg/mL		3.3	0.90
	500 μg/mL		4.7	0.97
Peppas–Sahlin	**Concentration of MMC**	**k_d**	**k_r**	**R^2**
	100 μg/mL	1.04	0.0001	0.98
	300 μg/mL	2.61	0.0011	0.96
	500 μg/mL	3.23	0.0021	0.95

4.7. MTT Assay Results for pMIPs, pNIPs and pHEMA

The cytotoxicity of the drug carriers was evaluated using the mouse fibroblast cell line L929. After administration of the carriers, the viability of the cells was assessed at 12 and 24 hours. For this assessment, the pMIPs, NIPs, and pHEMA were each loaded with 50 μg/mL MMC. The viability measurements obtained were 95.01 ± 0.29% for pMIPs, 94.21 ± 0.43% for NIPs, and 99.06 ± 1.04% for pHEMA. These results suggest that pMIPs exhibit minimal cytotoxicity and could be promising candidates for use in drug delivery applications.

To determine cell viability, the MTT assay was employed. This widely used method measures cell viability by assessing the activity of mitochondrial dehydrogenases. The principle behind the MTT assay involves the conversion of the tetrazolium salt to formazan, which is directly related to mitochondrial function. The amount of formazan produced correlates with the number of viable cells, and changes in this amount reflect variations in cell viability. As illustrated in Figure 4.7, the cell viability results for NIPs and pHEMA (used as a control polymer) were comparable at 24 and 48 hours. In contrast, pMIPs demonstrated a significant impact on cell viability, affecting 75–80% of MDA MB 231 breast cancer cells. This suggests that pMIPs may possess noteworthy cytotoxic effects against this particular cancer cell line.

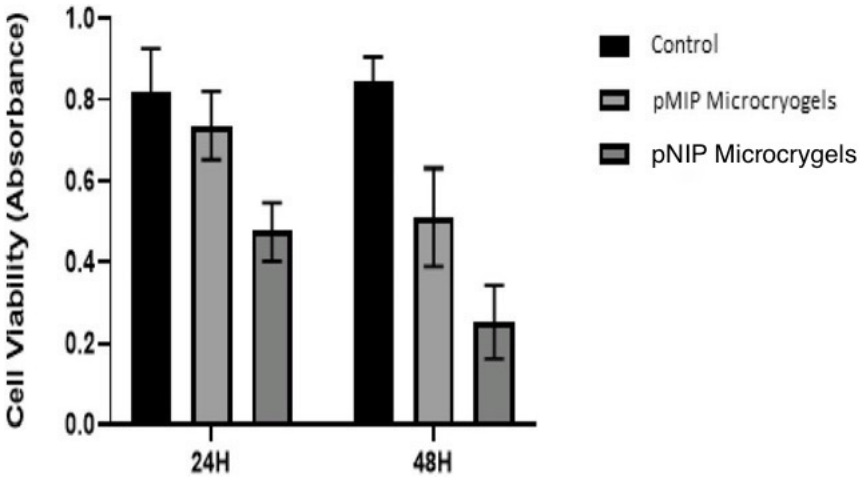


Figure 4.7. Cell Viability Results of pMIP, pNIP, pHEMA (control) Microcryogels

5. CONCLUSION

The chemotherapeutic agent MMC, renowned for its effectiveness in treating various types of cancer, presents notable challenges due to its limited half-life and pronounced side effects associated with systemic administration. These issues impede its full therapeutic potential and highlight the need for innovative delivery solutions. To address these limitations, the development of a drug carrier system that can precisely control the release of MMC is essential. Such a system would significantly enhance MMC's therapeutic efficacy by extending its release duration and mitigating its systemic side effects. By optimizing the controlled release mechanisms, it is possible to deliver MMC in a more targeted and sustained manner, thereby improving patient outcomes and safety.

In this study, our objective was to develop a microscale drug carrier designed to improve the release performance of MMC. We employed molecular imprinting technology to create specific receptors for MMC, enabling better control and enhancement of its release in aqueous environments. The results of our experiments indicated that the custom-designed MMC receptors substantially improved the release performance of MMC from the injectable cryogels. Additionally, the microscale drug carrier demonstrated negligible cytotoxicity and performed well in cell viability tests. These findings suggest that the injectable drug carrier holds promise for future applications in drug delivery research.

In this research, we have prepared injectable μcryogels imprinted with the low-cost anti-cancer drug MMC and assessed their potential for sustained drug delivery applications. The μcryogels exhibited a high drug loading efficiency, ranging from 80% to 82%, and were able to continuously release MMC over an extended period, up to 460 min. The favorable characteristics of these μcryogels, including their release kinetics and interconnected macroporous structures, make them suitable for controlled release applications in anti-cancer treatments. Notably, the μcryogels demonstrated a non-Fickian diffusion type, indicating a complex release mechanism that supports their effectiveness in drug delivery systems.

6.REFERENCES

[1]. Davar, N.; Ghosh, S. Oral controlled release-based products for life cycle management. In: Wen, H.; Park, K., editors. Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice. New York, NY: John Wiley & Sons; 2010. p. 305-320.

[2]. Anderson JM, Kim SW. Advances in Drug Delivery Systems (3), Book Review. J Pharm Sci. 1989; 78:608–609.

[3]. Spivak, A.S., Optimization, evaluation and characterization of molecularly imprinted polymers, Advanced Drug Delivery Reviews, 1780,1779-1794, 2005.

[4]. T.P. Rao, S. Daniel, J.M. Gladis, Trends in Anal. Chem. 23 (2004) 28.

[5]. A. Ersöz, A. Denizli, A. Özcan, R. Say, Biosens. Bioelectron. 20 (2005) 2197.

[6]. Pappioannou, EH, Liakopoulou-Kyriakides, M, Papi, RM and Kyriakidis, DA. Artificial receptor for peptide recognition in protic media: The role of metal ion coordination. Materials Science and Engineering B: 152, 28-32, 2008.

[7]. Oral, E., Peppas, N.A., Hydrophilic molecularly imprinted poly(hydroxyethyl-methacrylate) polymers, Journal of Biomedical Material Research, 78A, 205-210, 2006.

[8]. Plieva, F.M.; Galaev, I.Y.; Mattiasson, B. Macroporous gels prepared at subzero temperatures as novel materials for chromatography of particulate-containing fluids and cell culture applications. J Sep. Sci. 2007, 30, 1657–1671. [CrossRef] [PubMed] 3. Mikhalovsky, S.V.

[9]. Savina, I.N.; Dainiak, M.; Ivanov, A.E.; Galaev, I.Y. 5.03-Biomaterials/Cryogels, Comprehensive Biotechnology, 2nd ed.; Moo-Young, M., Ed.; Academic Press: Burlington, MA, USA, 2011; pp. 11–22.

[10]. Plieva, F.M., Karlsson, M., Aguilar, M.R., Gomez, D., Mikhalovsky, S., Galaev, Y.U., Pore structure in supermacroporous polyacrylamide based cryogels, Soft Matterials, 1, 303-309, 2005.

[11]. S.H. Hassanpour, M. Dehghani, Review of cancer from perspective of molecular, J. Cancer Research and Practise, 4 (2017) 127-129.

[12]. S. Chowdhury, F. Yusof, W. W. Salim, N. Sulaiman, M. O. Faruck, an overview of drug delivery vehicles for cancer treatment: Nanocarriers and nanoparticles including photovoltaic nanoparticles. Journal of Photochemistry and Photobiology B: Biology, 164 (2016) 151-159.

[13]. A. Alam, U. Farooq, R. Singh, V.P. Dubey, S. Kumar, et al. Chemotherapy Treatment and Strategy Schemes: A Review. Open Acc J of Toxicol. 2(5) (2018) 555600.

[14]. P.S. Knoepfler, Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. (2009) 27 (5): 1050-1056.

[15]. J. Verweij, H.M. Pinedo, Mitomycin C mechanism of action, usefulness, and limitations. Anti-Cancer Drug (1) (1990) 1-15.

[16]. E.A. Marquez, A. Katsanos, V.P. Kozobolis, A.G.P. Konstas, M.A. Teus, A critical overview of the biological effects of mitomycin c application on the cornea following refractive surgery, Adv. Ther. 36 (2019) 786-797.

[17]. H. Yang, M. Wang, Y. Huang, Q. Qiao, C. Zhao, M. Zhao, In vitro and in vivo evaluation of a novel mitomycin nanomicelle delivery system, RSC Adv., 9 (2019) 14708-14717.

[18]. M. Kotmakchiev, G. Kantarcı, V. Cetintas, et al., Cytotoxicity of a novel oil/water microemulsion system loaded with mitomycin C in in vitro lung cancer models, Drug Dev. Res., 73(4) (2012) 185–195.

[19]. P. Lulinski, molecularly imprinted polymers-based drug delivery devices: a way to application in modern pharmacotherapy, a review, Materials Science and Engineering C, 76 (2017) 1344-1353.

[20]. M. Bakhshpour, H. Yavuz, A. Denizli, Controlled release of mitomycin C from PHEMAH–Cu (II) cryogel membranes. Artificial Cells, Nanomedicine, and Biotechnology, 46 (2018) 946-954.

[21]. P. Öncel, K. Çetin, A. A. Topçu, H. Yavuz, A. Denizli, Molecularly imprinted cryogel membranes for mitomycin C delivery, Journal of Biomaterials Science, Polymer Edition, 28(6) (2017) 519-531.

[22]. N. Sanadgol, J. Wackerling, Developments of smart drug-delivery systems Based on Magnetic Molecularly Imprinted Polymers for Targeted Cancer Therapy: A Short Review, Pharmaceutics 12 (2020) 831.

[23]. D. Juric, N.A. Rohner, H.A. von Recum, Molecular Imprinting of Cyclodextrin Supramolecular Hydrogels Improves Drug Loading and Delivery, Macromol. Biosc. 19 (2019) 1-9.

[24]. J. Bai, Y. Zhang, L. Chen, H. Yan, C. Zang, L. Liu, X. Xu, Synthesis, and characterization of paclitaxel-imprinted microparticles for T controlled release of an anticancer drug, Materials Science & Engineering C. (2018) 92: 338-348.

[25]. M. Bakhspour, A.A. Topcu, N. Bereli, H. Alkan, A. Denizli, Poly (Hydroxyethyl Methacrylate) Immunoaffinity Cryogel Column for the Purification of Human Immunoglobulin M, Gels. 6 (4) (2020) 1-13.

[26]. E. Özgür, N. Bereli, D. Türkmen, S. Ünal, A. Denizli, PHEMA cryogel for in-vitro removal of anti-dsDNA antibodies from SLE plasma, Materials Science and Engineering C. (2011) 31: 915-920.

[27]. Y. Saylan, A. Denizli Supermacroporous Composite Cryogels in Biomedical Applications. Gels. 5(2) (2019) 20.

[28]. P. Villard et al., Autoclavable and Injectable Cryogels for Biomedical Applications, Adv. Healthcare Mater. 8 (2019) 1900679.

[29]. K. Cetin, A. Denizli, Immunoaffinity microcryogels for purification of transferrin, Journal of Chromatograhy B. 1114-115 (2019) 5-12.

[30]. M. Bakhshpour, N. Idil, I. Perçin, A. Denizli Biomedical Applications of Polymeric Cryogels. Applied Sciences. 9(3) (2019) 553.

[31]. D. Xing, W. Liu, B. Wang, J. J. Li, Y. Zhao, H. Li, A. Liu, Y. Du, J. Lin, Intra-articular Injection of Cell-laden 3D Microcryogels Empower Low-dose Cell Therapy for Osteoarthritis in a Rat Model, Cell Transplantation. 29 (2020) 1-12.

[32]. Y.P. Fang, P.Y. Hu, Y.B. Huang, Diminishing the side effect of mitomycin C by using pH-sensitive liposomes: in vitro characterization and in vivo pharmacokinetics, Drug Design, Development and Therapy. 12 (2018) 159-169.

[33]. W.J. Underberg, H. Lingeman, Aspects of the chemical stability of mitomycin and porfiromycin in acidic solution, J Pharm Sci. 72(5) (1983) 549-553.

[34]. Lee, PI.; Li, J-X. Evolution of oral controlled release dosage forms. In: Wen, H.; Park, K., editors. Oral Controlled Release Formulation Design and Drug Delivery. Hoboken, NJ: John Wiley & Sons, Inc.; 2010. p. 21-31.

[35]. Park K. Controlled drug delivery systems: past forward and future back. Journal Of Controlled Release 2014; 190:3-8

[36]. Ref: Versypt, A.N.F., 2012. Modeling of controlled-release drug delivery from autocatalytically degrading polymer microspheres. University of Illinois at Urbana-Champaign.

[37]. Schmaljohann, D. (2006) Thermo and pH Responsive Polymers in Drug Delivery. Advanced Drug Delivery Reviews, 58, 1655-1670.

[39]. Srivastava, A., Yadav, T., Sharma, S., Nayak, A., Kumari, A.A. and Mishra, N., 2015. Polymers in drug delivery. Journal of Biosciences and Medicines, 4(1), pp.69-84.,

[40]. ME Aulton. “Pharmaceutics” The Science of dosage form design, 2nd ed., Churchill Livingstone, 2002.

[41]. Joseph R Robinson, Vincent H Lee. Controlled drug delivery, 2nd ed., Marcel Dekker, 1987; 4-15.

[42]. Altaf AS, Friend DR, MASRx and COSRx Sustained-release technology in modified release drug delivery technology, Marcell Dekker Inc., New York, 2003.

[43]. Huynh, C.T. and Lee, D.S., 2014. Controlled release. Encyclopedia of polymeric nanomaterials, 2014, pp.1-12.

[44]. Jerbic, I.S., 2018. Biodegradable synthetic polymers and their application in advanced drug delivery systems (DDS). Nano Tech Appl, 1(1), pp.1-9.

[45]. Van Gheluwe, L., Chourpa, I., Gaigne, C. and Munnier, E., 2021. Polymer-based smart drug delivery systems for skin application and demonstration of stimuli-responsiveness. Polymers, 13(8), p.1285.

[46]. Lansdowne, L.E., Published: December 4, 2020

[47]. Prasad, V., De Jesús, K., and Mailankody, S. (2017). The high price of anticancer drugs: origins, implications, barriers, solutions. Nat. Rev. Clin. Oncol. 14 (6), 381. doi:10.1038/nrclinonc.2017.31.

[48]. Alqahtani, M.S., Kazi, M., Alsenaidy, M.A. and Ahmad, M.Z., 2021. Advances in oral drug delivery. Frontiers in pharmacology, 12, p.618411.

[49]. Arfijanto, M.V., Hadi, U. and Yoes Prijatna Dachlan, Y., 2020. Procalcitonin, IL-1ß, HSP10 and Resolvin D2 Mechanism as Sepsis Biomarkers in Sepsis Model. International Journal of Pharmaceutical Research, 12(4), pp.1388-1394.

[50]. Tallarida, R.J., Raffa, R.B. and McGonigle, P., 2012. Principles in general pharmacology. Springer Science & Business Media.

[51]. Ahmed, T.A. and Aljaeid, B.M., 2016. Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug design, development and therapy, pp.483-507.

[52]. Hopkins, U. and Arias, C.Y., 2013. Large-volume IM injections: a review of best practices. Oncology Nurse Advisor, 4(1), pp.32-37.

[53]. Hedaya, M.A., 2012. Basic pharmacokinetics. CRC Press.

[54]. Kissel T, Werner U. Nasal delivery of peptides: an in vitro cell culture model for the investigation of transport and metabolism in human nasal epithelium. J Control Rel 1998; 53: 195–203.

[55]. Pires, A., Fortuna, A., Alves, G. and Falcão, A., 2009. Intranasal drug delivery: how, why and what for? Journal of Pharmacy & Pharmaceutical Sciences, 12(3), pp.288-311.

[56]. BETZ SF, BAXTER SM, FETROW JS: Function first: a powerful approach to post genomic drug discovery. Drug Discov. Today (2002) 7:8765–871.

[57]. Verma, S., Kaul, M., Rawat, A. and Saini, S., 2011. An overview on buccal drug delivery system. International journal of pharmaceutical sciences and research, 2(6), p.1303.

[58]. R Gaudana, HK Ananthula, A Parenky, AK Mitra - The AAPS journal, 2010 – Springer.

[59]. Ananthula HK, Vaishya RD, Barot M, Mitra AK. Duane's Ophthalmology. In: Tasman W, Jaeger EA, editors. Bioavailability. Philadelphia: Lippincott Williams & Wilkins; 2009.

[60]. Patel, A., Cholkar, K., Agrahari, V. and Mitra, A.K., 2013. Ocular drug delivery systems: An overview. World journal of pharmacology, 2(2), p.47.

[61]. InformedHealth.org [Internet]. (2017, August 10). Using medication: Oral medications. Cologne, Germany: Institute for Quality and Efficiency in Healthcare (IQWiG).

[62]. Prasad, V., De Jesús, K., and Mailankody, S. (2017). The high price of anticancer drugs: origins, implications, barriers, solutions. Nat. Rev. Clin. Oncol. 14 (6),381. doi:10.1038/nrclinonc.2017.31.

[63]. Vertzoni, M., Augustijns, P., Grimm, M., Koziolek, M., Lemmens, G., Parrott, N., Pentafragka, C., Reppas, C., Rubbens, J., Van Den Abeele, J. and Vanuytsel, T., 2019. Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: An UNGAP review. European journal of pharmaceutical sciences, 134, pp.153-175.

[64]. Khan, K., and Cohen, J. (2008). The impact of medication regimen factors on adherence to chronic treatment: a review of literature. J. Behav. Med. 31 (3),213–224.

[65]. Rubbens, J., Veiga, R., Brouwers, J., and Augustijns, P. (2018). Exploring gastric drug absorption in fasted and fed state rats. Int. J. Pharmaceut. 548 (1),636–641. doi: 10.1016/j.ijpharm.2018.07.017.

[66]. Daugherty, A. L., and Mrsny, R. J. (1999). Transcellular uptake mechanisms of the intestinal epithelial barrier Part one. Pharm. Sci. Technol. Today. 4 (4), 144–151.doi:10.1016/s1461-5347(99)00142.

[67]. De Kruijf, W.; Ehrhardt, C. Inhalation delivery of complex drugs-the next steps. Curr. Opin. Pharmacol. 2017, 36, 52–57.

[68]. Demoly P, Hagedoorn P, de Boer A, Frijlink H, “The clinical relevance of dry powder inhaler performance for drug delivery”. Resp Med, 2014, Vol 108, pp 1195-1203.

[69]. Chenthamara, D., Subramaniam, S., Ramakrishnan, S.G., Krishnaswamy, S., Essa, M.M., Lin, F.H. and Qoronfleh, M.W., 2019. Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials research*, *23*(1), pp.1-29.

[70]. Ahuja, A., Khar, R.K., Ali, J., 1997. Mucoadhesive drug delivery systems. Drug Dev. Ind. Pharm. 23, 489–515.

[71]. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol 2008;26(11):1261e8.

[72]. Jiang, T., Xu, G., Chen, G., Zheng, Y., He, B. and Gu, Z., 2020. Progress in transdermal drug delivery systems for cancer therapy. Nano Research, 13, pp.1810-1824.

[73]. Mazen M. El-Hammadi, José L. Arias, in Theory and Applications of Nonparenteral Nanomedicines, 2021.

[74]. Muller, R; Keck, C (2004). "Challenges and solutions for the delivery of biotech drugs – a review of drug nanocrystal technology and lipid nanoparticles". Journal of Biotechnology. 113 (1–3): 151–170.

[75]. Galm, U.; Hager, M. H.; Van Lanen, S. G.; Ju, J.; Thorson, J. S.; Shen, B. Chem. Rev. 2005, 105, 739- 758.

[76]. Shah, A., Aftab, S., Nisar, J., Ashiq, M.N. and Iftikhar, F.J., 2021. Nanocarriers for targeted drug delivery. Journal of Drug Delivery Science and Technology, 62, p.102426.

[77]. Wakaki, S., Marumo, H., Tomioka, K., Shimizu, G., Kato, E., Kamada, H., Kudo, S., Fujimoto, Y., Isolation of new fractions of antitumor mitomycins, Antibiot. Chemother. 8, 228 240, 1958.

[78]. Horák, D., Babic, M., Macková, H., Benes, M.J., Stambergova A. and Scouten, Preparation and properties of magnetic nano and microsized particles for biological and environmental separations, Journal of Separation Science, 30(11), 1751 72, 2007.

[79]. Benes, M.J., Stambergova A. and Scouten, W.H., In: Moleculor interaction in bioseparations, Trinidad and Tobago NGO, 313 319, 1993.

[80]. Zheng, Z., Touve, M., Barnes, J., Reich, N. and Zhang, L., 2014. Synthesis-Enabled Probing of Mitosene Structural Space Leads to Improved IC50 over Mitomycin C. Angewandte Chemie, 126(35), pp.9456-9459.

[81]. Paz, M.M., 2013. Reductive activation of mitomycins A and C by vitamin C. Bioorganic chemistry, 48, pp.1-7.

[82]. Chirrey, L., Cummings, J., Halbert, G.W. and Smyth, J.F., 1995. Conversion of mitomycin C to 2, 7-diaminomitosene and 10-decarbamoyl 2, 7-diaminomitosene in tumour tissue in vivo. Cancer chemotherapy and pharmacology, 35, pp.318-322.

[83]. Paz, M.M. and Pritsos, C.A., 2012. The molecular toxicology of mitomycin C. In Advances in Molecular Toxicology (Vol. 6, pp. 243-299). Elsevier.

[84]. Lamaoui, A., Mani, V., Durmus, C., Salama, K.N. and Amine, A., 2023. Molecularly imprinted polymers: A closer look at the template removal and analyte binding. Biosensors and Bioelectronics, p.115774.

[85]. Horák, D., Babic, M., Macková, H., Benes, M.J., Stambergova A. and Scouten, Preparation and properties of magnetic nano and microsized particles for biological and environmental separations, Journal of Separation Science, 30(11), 1751 72, 2007.

[86]. Benes, M.J., Stambergova A. and Scouten, W.H., In: Molecular interaction in bioseparations, Trinidad and Tobago NGO, 313 319, 1993.

[87]. Mallik, S., Johnson R.D., and Arnold, P.H., J., Am. From 3D to 2D: A Review of the Molecular Imprinting of Proteins, Chemical Society Reviews, 116, 8909, 1994.

[88]. Arnold, F.H., Metal-affinity separations: a new dimension in protein processing. Biotechnology, 9, 151, 1991.

[89]. Lozinsky, V.I., Russian Chemical Bulletin, Polymeric cryogels as a new family of macroporous and supermacroporous materials for biotechnological purposes. International Edition, 57, 1015-1032, 2008.

[90]. Borje Sellergren., Direct Drug Determination by Selective Sample Enrichment on an Imprinted Polymer., Analytical. Chemistry. 66, 1678-1582, 1994.

[91]. Eiichi Toorisaka, Kazuya Uezu, Masahiro Goto, Shintaro Furusaki., A molecularly imprinted polymer that shows enzymatic activity., Biochemical Engineering Journal, 14, 85–91, 2003.

[92]. Daniel M. Hawkins, Derek Stevenson, Subrayal M. Reddy., Investigation of protein imprinting in hydrogel-based molecularly imprinted polymers (HydroMIPs)., Analytica Chimica Acta, 542, 61–65, 2005.

[93]. Mickael Doué, Emmanuelle Bichon, Gaud Dervilly-Pinel, Valérie Pichon, Florence Chapuis-Hugon, Eric Lesellier, Caroline West, Fabrice Monteau, Bruno Le Bize., Molecularly Imprinted polymer applied to the selective isolation of urinary steroid hormones: An efficient tool in the control of natural steroid hormones abuse in cattle., Journal of Chromatography A, 1270, 51–61, 2012.

[94]. N. Farhanah. Ab. Halim, M. Noor. Ahmad, A. Y. Md. Shakaffa f and N. Derama., Grafting Amino-acid Molecular Imprinted Polymer on Carbon Nanotube for Sensing., Procedia Engineering, 53, 64 –70, 2013.

[95]. Z.G. Peng, K. Hidajat, M.S. Uddin., Adsorption and desorption of lysozyme on nano-sized magnetic particles and its conformational changes., Colloids and Surfaces B: Biointerfaces, 35, 169–174, 2004.

[96]. Kazuyoshi Yano, Takeshi Nakagiri, Toshifumi Takeuchi, Jun Matsui, Kazunori Ikebukuro, Isao Karube., Stereoselective recognition of dipeptide derivatives in molecularly imprinted polymers which incorporate an L-valine derivative as a novel functional monomer., Analytica Chimica Acta, 357, 91±98, 1997.

[97]. Hsin-Yi Lin, John Rick, Tse-Chuan Chou., Optimizing the formulation of a myoglobin molecularly imprinted thin-film polymer—formed using a micro-contact imprinting method., Biosensors and Bioelectronics, 22, 3293–3301, 2007.

[98]. Thickett, S.C. and Teo, G.H., 2019. Recent advances in colloidal nanocomposite design via heterogeneous polymerization techniques. Polymer Chemistry, 10(23), pp.2906-2924.

[99]. Brinson, H.F. and Brinson, L.C., 2008. Polymer engineering science and viscoelasticity. An introduction, pp.99-157.

[100]. Fischer, H., The Persistent Radical Effect: A Principle for Selective Radical Reactions and Living Radical Polymerizations, Chemistry Review, 101, 3581, 2001.

[101]. Georges, M.K., Veregin, R.P.N., Kazmaier, P.M., Hamer, G.K., Macromolecules, Effects of Initiator Homolysis Rate Constant on Kinetics and Chain Length Distribution in Living Free-Radical Polymerization, Chemistry Review, 26, 2987, 1993.

[102]. Matejtschuk, P., Thiophilic affinity chromatography and related methods, Methods in Molecular Biology, 244, 195-204, 2004.

[103]. Matyjaszweski, K.; “Advances in Controlled/Living Radical Polymerization”, Oxford University Press, Portland, 224, 2003.

[104]. Kamigaito, M., Ando, T., Sawamoto, M., Metal-Catalyzed Living Radical

Polymerization, Chemical Reviews, 101, 3689, 2001.

[105]. Le, T.P.T., Moad, G., Rizzardo, E., Thang, S., International. Pat. Appl., 980, 1478, 115-390, 1998.

[106]. Shiomi, T, Matsui, M, Mizukami, F and Sakaguchi, K. A method for the molecular imprinting of hemoglobin on silica surfaces using silanes. Biomaterials, 26, 5564-5571, 2005.

[107]. Hwang, C-C and Lee, W-C. Chromatographic characteristics of cholesterolimprinted polymers prepared by covalent and non-covalent imprinting methods. Journal of Chromatography A. 962, 69-78, 2002.

[108]. Liu, J-q and Wulff, G. Functional Mimicry of Carboxypeptidase A by a Combination of Transition State Stabilization and a Defined Orientation of Catalytic Moieties in Molecularly Imprinted Polymers. Journal of the American Chemical Society. 130, 8044-8054, 2008.

[109]. Dhal, PK and Arnold, FH. Metal-coordination interactions in the templatemediated synthesis of substrate-selective polymers: recognition of bis(imidazole) substrates by copper (II) iminodiacetate containing polymers. Macromolecules. Tetrahedron Lett, 25, 7051-7059, 1992.

[110]. Huang, J, Hu, Y, Hu, Y and Li, G. Disposable terbium (III) salicylate complex imprinted membrane using solid phase surface fluorescence method for fast separation and detection of salicylic acid in pharmaceuticals and human urine. Talanta. 107, 49-54, 2013.

[111]. Savina, I.N., Zoughaib, M. and Yergeshov, A.A., 2021. Design and assessment of biodegradable macroporous cryogels as advanced tissue engineering and drug carrying materials. Gels, 7(3), p.79.

[112]. Lozinsky, V.I., 2002. Cryogels on the basis of natural and synthetic polymers: preparation, properties and application. Russian Chemical Reviews, 71(6), pp.489-511.

[113]. Plieva, F.M., Galaev, L.Yu., Noppe, W., Mattiasson, B., Saccharomyces cerevisiae immobilization in polyacrylamide hydrogel obtained at low temperature. Trends in microbiology, Vol-16, 543-551, 2008.

[114]. Lozinsky, V.I., Galaev, I.Y., Plieva, F.M., Savina, I.N., Jungvid, H., Mattiasson, B., Polymeric cryogels as promising materials of biotechnological interest. Trends in Biotechnology, 21, 10, 2003.

[115]. Savina, I.N., Cnudde, V., Hollander, S.D., Hoorebeke, L.V., Mattiasson, B., I.Y. Galaev, F.D. Prez, Polymeric cryogels as promising materials. Soft Matter, 3, 1179-1184, 2007.

[116]. Cunliffe, D., Kirby, A. and Alexander, C., 2005. Molecularly imprinted drug delivery systems. *Advanced drug delivery reviews*, *57*(12), pp.1836-1853.

[117]. Lozinsky, V.I., Fatima, Y. G., Plieva, M., Savina, I.N, Jungvid, H., Mattiasson, B., Polymeric cryogels as promising materials of biotechnological interest, Trends in Biotechnology, 21(10), 445-451, 2003.

[118]. Alkan H, Bereli H, Baysal Z, Denizli A. Antibody purification with protein A attached supermacroporous poly (hydroxyethyl methacrylate) cryogel. Biochemical Engineering Journal, 45, 201-208, 2009.

[119]. Gun'ko VM, Savina IN, Mikhalovsky SV. Cryogels: morphological, structural and adsorption characterisation. Advances in Colloids and Interface Science, 187,1-46, 2013.

[120]. Babaç C., Yavuz H., Galaev I. Y., Pişkin E., Denizli A., Binding of antibodies to concanavalin A-modified monolithic cryogel. Reactive and Functional Polymers, 66, 1263–1271, 2006.

[121]. Arvidsson P., Plieva F. M., Savina I. N., Lozinsky V. I., Fexby S., Bülow L., Galaev IY., Matiasson B., Chromatography of microbial cells using continuous supermacroporous affinity and ion-exchange columns. Journal of Chromatography A, 977, 27–38, 2002.

[122]. Henderson, T. M. A., Ladewig, K., Haylock, D. N., Mclean, K. M., O'Connor, A. J., Cryogels for biomedical applications, Journal of Material Chemistry B., 1, 2682–2695, 2013.

[123]. Reichelt S., Abe C., Hainich S., Knolle W., Decker U., Prager A., Robert Konieczny. Electron-beam derived polymeric cryogels. Soft Matterials, 9, 2484–2492, 2013.

[124]. Bereli N., Şener G., Altıntaş E. B., Yavuz H., Denizli A. Poly (glycidyl methacrylate) beads embedded cryogels for pseudo-spesific affinity depletion of albümin and Immunoglobulin G, Materials Science and Engineering C, 30, 323–329, 2010.

[125]. Perçin I., Sağlar E., Yavuz H., Aksöz E., Denizli A. Poly (Hydroxyethyl methacrylate) based affinity cryogel for plasmid DNA purification. International Journal of Biological Macromolecules, 48, 577–582, 2011.

[126]. Özgür E., Bereli N., Türkmen D., Ünal S., Denizli A. PHEMA Cryogel for in-vitro removal of anti-dsDNA antibodies from SLE plasma, Materials Science and Engineering C, 31, 915–920, 2011.

[127]. Bereli N., Şener G., Yavuz H., Denizli A. Oriented immobilized anti-LDL antibody carrying poly (Hydroxyethyl methacrylate) cryogel for cholesterol removal from human plasma, Materials Science and Engineering C, 31, 1078–1083, 2011.

[128]. Çimen D., Denizli, A. Immobilized metal affinity monolithic cryogels for cytocrom c purification. Colloids Surf B: Biointerfaces, 93, 29–35, 2012.

[129]. Lozinsky, V.I. and Plieva, F.M. Poly (vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments. Enzyme Microbial Technology, 23, 227–242, 1998.

[130]. Lusta K. A, Immobilization of E. coli cells in macroporous polyacrylamide cryogels. Applied Biochemistry and Microbiology, 24, 418–426, 1988.

[131]. Kumar A, Srivastava A. Cell separation using cryogel-based affinity chromatography. Nature Protocols, 5, 1737-1747, 2010.

[132]. Kumar A., Plieva F.M., Galaev I.Y., Mattiasson B. Affinity fractionationof lymphocytes using a monolithic cryogel. Journal of Immunology Methods, 283,185–194, 2003.

[133]. Dainiak M.B., Galaev I.Y., Mattiasson B. Macroporous monolithic hydrogels in a 96-minicolumn plate format for cell surface-analysis and integrated binding/quantification of cells. Enzyme Microbial Technology, 40, 688–695, 2007.

[134]. Dainiak M.B., Plieva F.M., Galaev I.Y., Hatti-Kaul R., Mattiasson B. Cell chromatography: separation of different microbial cells using IMAC supermacroporous monolithic columns. Biotechnology Progress, 21, 644–649, 2005.

[135]. Dainiak M.B., Galaev I.Y., Mattiasson B., Affinity cryogel monoliths for screening for optimal separation conditions and chromatographic separation of cells. Journal of Chromatography A, 1123, 145–150, 2006.

[136]. Ahlqvist J., Kumar A. Sundstrom H. Ledung E. Hornsten EG. Enfors SO, Matiasson B. Affinity binding of inclusion bodies on supermacroporous monolithic cryogels using labeling with specific antibodies. Journal of Biotechnology, 122, 216–225, 2006.

[137]. Teilum A. Hansson MJ., Dainiak MB, Mansson R., Surve S. Elmer E. Onnerfjord P, Matiasson G. Binding mitochondria to cryogel monoliths allows detection of proteins specifically released following permeability transition. Analytical Biochemistry, 348, 209–221, 2006.

[138]. Williams S.L., Eccleston M.E., Slater, N.K.H. Affinity capture of a biotinylated retrovirus on macroporous monolithic adsorbents: towards a rapid single-step purification process. Biotechnology and Bioengineering, 89, 783–787, 2005.

[139]. Vlatakis, G., Andersson, L. I., Muller, R., Mosbach, K., Drug assay using antibody mimics made by molecular imprinting, Nature, 361, 645-647, 1993.

[140]. Wang, X.N., Recum. H, A., Affinity-Based Drug Delivery, Macromolecular Bioscience, 11, 321-332, 2011.

[141]. David, R.K., Peppas, N.A., Mimicking biological delivery through feedback-controlled drug release systems based on molecular imprinting, AIChE Journal, 55, 1311–1324, 2009.

[142]. Norell, M.C., Andersson, H, S., Nicholls I. A., Theophylline molecularly imprinted polymer dissociation kinetics: a novel sustained release drug dosage mechanism, Journal of Molecular Recognition, 11, 98, 1998.

[143]. Alvarez-Lorenzo, C., Concheiro, A., Molecularly Imprinted for Drug Delivery, Journal Chromatography B, 804, 231-245, 2004.

[144]. Wulf, G., Molecular Imprinting in Cross-linked Materials with the Aid of Molecular Templates –A Way towards Artificial Antibodies. Angewandte Chemie International Edition in English, 34, 1812-1232, 1995.

[145]. Akgöl, S., Türkmen, D., Denizli, A., Cu (II)-incorporated, histidine-containing, magnetic-metal-complexing beads as specific sorbents for the metal chelate affinity of albumin. Journal of Applied Polymer Scince, 93, 2669-2677, 2004.

[146]. J. Zhu, F. Yang, F. He, X. Tian, S. Tang, X. Chen, A tubular gelatin scaffold capable of the time-dependent controlled release of epidermal growth factor and mitomycin C, Colloids and Surfaces B: Biointerfaces. 135 (2015), 416-424.

[147]. O.M. Kolawole, W.M. Lau, V.V. Khutoryanskiy, Chitosan/β-glycerophosphate in situ gelling mucoadhesive systems for intravesical delivery of mitomycin-C, International Journal of Pharmaceutics: X, 1(2019), 100007.

[148]. T. Sultana, H. Van Hai, M. Park, S. Lee, B. Lee, Controlled release of Mitomycin C from modified cellulose based thermo-gel prevents post-operative de Novo peritoneal adhesion, Carbohydrate Polymers, 229 (2020) 115552.

[149]. D. Türkmen, N. Bereli, M.E. Çorman, H. Shaikh, S. Akgöl, S., A. Denizli, Molecular imprinted magnetic nanoparticles for controlled delivery of mitomycin C, Artificial Cells, Nanomedicine, and Biotechnology, 42(5) (2013) 316-322.

[150]. Y. Xi-xiao, C. Jan-hai, L. Shi-ting, G. Dan, Z. Xv-xin, Polybutylcyanoacrylate nanoparticles as a carrier for mitomycin C in rabbits bearing VX2-liver tumor, Regulatory Toxicology and Pharmacology, 46(3) (2006) 211-217.

[151]. B. Hu, Y. Tu, G. Yan, R. Zhuo, S.E. Bottle, Y. Wu, C. Fan, Duan, Y, Polycarbonate microspheres containing mitomycin C and magnetic powders as potential hepatic carcinoma therapeutics, Colloids and Surfaces B: Biointerfaces, 84(2) (2011) 550-555.

[152]. Wu, M., Wang, S., Wang, Y., Zhang, F. and Shao, T., 2020. Targeted delivery of mitomycin C-loaded and LDL-conjugated mesoporous silica nanoparticles for inhibiting the proliferation of pterygium subconjunctival fibroblasts. Experimental Eye Research, 197, p.108124.

[153]. Sun, X., Sun, P., Li, B., Liu, Y., Wang, M., Suo, N., Yang, M., Zhang, D. and Jin, X., 2016. A new drug delivery system for mitomycin C to improve intravesical instillation. Materials & Design, 110, pp.849-857.

[154]. Attieh DM, Chaib H, Armutcu C, Uzun L, Elkak A, Denizli A (2013) Immunoglobulin G purification from bovine serum with pseudo-specific macroporous cryogels. Sep Purif Technol 118:816–822.

[155]. Derazshamshir A, Baydemir G, Yılmaz F, Bereli N, Denizli A (2016) Preparation of cryogel columns for depletion of hemoglobin from human blood. Artif Cell Nanomed 44(3):792–799.

[156]. Andac M, Tamahkar E, Denizli A (2021) Molecularly imprinted smart cryogels for selective nickel recognition in aqueous solutions. J Appl Polym Sci 138: e49746.

[157]. Bajpai AK, Saini R (2005) Preparation and characterization of biocompatible spongy cryogels of poly (vinyl alcohol) –gelatin and study of water sorption behaviour. Poly Int 54:1233–1242.

[158]. Chaturvedi A, Bajpai AK, Bajpai J, Singh SK (2016) Evaluation of poly (vinyl alcohol) based cryogel–zinc oxide nanocomposites for possible applications as wound dressing materials. Mater Sci Eng C 65:408–418.

[159]. Higuchi T (1963) Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 52:1145–1149.

[160]. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA (1983) Mechanisms of solute release from porous ydrophilic polymers. Int J Pharm 15:25–35.

[161]. Vergnaud JM (1993) Liquid transport-controlled release processes in polymeric materials: applica- tions to oral dosage forms. Int J Pharm 90:89–94.

[162]. Tamahkar E, Bakhshpour M, Denizli A (2019) Molecularly imprinted composite bacterial cellulose nanofibers for antibiotic release. J Biomater Sci Polym Ed 30(6):450–461.

[163]. Attieh DM, Chaib H, Armutcu C, Uzun L, Elkak A, Denizli A (2013) Immunoglobulin G purification from bovine serum with pseudo-specific macroporous cryogels. Sep Purif.Technol. 118, 816-822.

[164]. Derazshamshir A, Baydemir G, Yılmaz F, Bereli N, Denizli A (2016) Preparation of cryogel columns for depletion of hemoglobin from human blood. Artif Cell Nanomed 44(3) 792-799.

[165]. Andac M, Tamahkar E, Denizli A (2021) Molecularly imprinted smart cryogels for selective nickel recognition in aqueous solutions. J Appl Polym Sci 138, e49746.

APPENDIX 2- Publications Derived from Thesis

Preparation of molecular imprinted injectable polymeric micro cryogels for control release of mitomycin C