Synthesis and Characterization of PolyHEMA Microparticels for Drug Release

Bahaeddin Emad Fahmi Alkum

Submitted to the
Institute of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
July 2023
Gazimağusa, North Cyprus

Approval of the Institute of Graduate Studies and Research

Prof. Dr. Ali Hakan Ulusoy
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. İzzet Sakallı
Chair, Department of Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. Elvan Yılmaz
Supervisor

Examining Committee

1. Prof. Dr. Mustafa Gazi

2. Prof. Dr. Elvan Yılmaz

3. Assoc. Prof. Dr. Şifa Doğan

ABSTRACT

Poly(HEMA-*co*-EGDMA) microparticles were synthesized by using suspension polymerization at different ratios of the monomer, 2-hydroxy ethyl methacrylate (HEMA) and the crosslinker, ethylene glycol dimethacrylate (EGDMA) at 70 °C for 3 hours with stirring at 350 rpm. The redox couple ammonium persulfate (APS) and N,N,N',N-tetramethylene diamine (TEMED) was used as the initiator. Magnesium oxide (MgO) was used as the stabilizer. Ratios of monomer and crosslinkers were altered to synthesise different microbeads of poly(HEMA-*co*-EGDMA) with different crosslinking ratios. The swelling behaviour of the microparticles was analysed in three different buffer solutions: acetate buffer at pH 2.0, phosphate buffer at pH 7.4 and pH 11 at 37 °C. In addition, loading of flurbiprofen and methylene blue into the microparticles and their release characteristics were investigated. Drug loaded and unloaded samples were analysed by FT-IR spectroscopy and scanning electron microscopy (SEM).

Keywords: Poly(HEMA-*co*-EGDMA), Microbead, Copolymer, Flurbiprofen, Methylene Blue, Controlled Release, HEMA, EGDMA.

ÖZ

Bu çalışmada, süspansiyon polimerizasyon yöntemi kullanılarak poli(HEMA-ko-EGDMA) mikroparçacılar değişik monomer:çapraz bağlayıcı oranları ile sentezlendi. Monomer, 2-hidroksi etil metakrilat (HEMA) ve çapraz bağlayıcı, etilen glikol dimetakrilat (EGDMA) ile redoks başlatıcı çifti amonyum persülfat (APS) ve N,N,N',N-tetrametilen diamin (TEMED) sulu ortamda 350 rmp süratle karıştırılarak 70 °C'ta 3 saatte polimerleştirildi. Magnezyum oksit (MgO) stabilizatör olarak kullanıldı. Mikroparçacıkların şişme davranışları pH 2, pH 7.4 ve pH 11 tampon çözeltilerde 37 °C sıcaklıkta izlendi. Sentezlenen poli(HEMA-ko-EGDMA) mikroparçacıkların flurbiprofen ve metilen mavisi model madde olarak kullanılarak hidrofobik ve hidrofilik ilaçlar için kontrollu salım sistemi olarak davranabilme potansiyelleri incelendi. Sentezlenen örnekler FTIR spektroskopisi ve SEM analizi ile karakterize edildi.

Anahtar Kelimeler: Poly(HEMA-*ko*-EGDMA), Mikro Boncuk, Kopolimer, Flürbiprofen, Metilen Mavisi, Kontrollu Salım, HEMA, EGDMA.

DEDICATION

I dedicate this thesis to my family

ACKNOWLEDGMENTS

First, I would like to thank to my supervisor Prof. Dr. Elvan Yılmaz for supporting and guiding me in this work. I want to thank her for all the help and knowledge she provided to me, and for giving me motivation and positive energy. All thanks and gratitude to my close friend, Mr. Jahed Ozbilenler, for helping me and providing what is necessary to accomplish and finish this work with such good results, also thanks to my best friends in Jordan Eng. Mohammad Qasqas and Mohammad Alkhateeb.

I do not know how to thank my family, especially my dear father, Mr. Emad Alqam, my mother, Ms. Buthaina Alqam, my brother, Eng. Mohammad Emad, and my sisters, Dr. Walaa, and chemist Areej, and finally my thanks to my brother and his family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

APS	Ammonium Peroxydisulfate (APS)
-co-	Copolymer
EGDMA	Ethylene Glycol Dimethylacrylate
FB	Flurbiprofen
HEMA	2-Hydroxyethyl Methacrylate
MgO	Magnesium Oxide
MB	Methylene Blue
μ	Micro Sign
S3F1	Sample 3, Fraction 1
S3F2	Sample 3, Fraction 2
TEMED	N,N, N-Tetramethyl-Ethylenediamine

Chapter 1

INTRODUCTION

Drug delivery systems based on natural and synthetic polymers provide safer and more efficient ways to deliver therapeutic agents into the body at a controlled rate over a period. Controlled drug release allows delivery of smaller amounts of drug compared to traditional drug delivery formulations, thereby minimizing drug side effects. Furthermore, using smart polymer materials, drugs can be delivered to specific locations in a targeted manner. Polymers are advantageous as drug delivery systems because they can be formed into films, fibers, macro, micro or nano-sized particles. Oral, nasal, and drug delivery systems can be developed to overcome drug solubility or stability problems under various physiological conditions. Hydrophilic drugs can be easily encapsulated in hydrogel polymer matrices, whereas encapsulation of hydrophobic drugs is difficult and requires different strategies than encapsulation of hydrophilic drugs. This problem is usually overcome by applying emulsion or suspension polymerization techniques using compatible monomer and drug pairs. Poly(2-hydroxyethyl methacrylate) PolyHEMA is a synthetic polymer that has been successfully tested in many drug delivery systems in the form of hydrogels, microspheres, films or fibers. PolyHEMA can be synthesized by free radical initiation by bulk polymerization, suspension, or emulsion polymerization. It acts as a suitable drug delivery system.

The aim of this work is to investigate the potential of polyHEMA microparticles as drug delivery matrices for a hydrophobic drug, flurbiprofen, and for a hydrophilic drug, methylene blue. Poly(HEMA-*co*-EGDMA) microparticles were prepared by suspension polymerization. Flurbiprofen loading was achieved *in situ* during suspension polymerization of HEMA. Post loading method was used for methylene blue by absorption from solution. The content of this thesis work is limited to investigating the physicochemical principles behind flurbiprofen and methylene blue loading and release in poly(HEMA-*co*-EGDMA) microbeads as model drug/polymer matrix systems. Any further work goes into pharmaceutical and medicinal chemistry fields which is out of scope of this work.

In Chapter 1 of this thesis a brief introduction will be presented on controlled drug release, Suspension polymerization, the method used to prepare the polymeric microparticles. The monomer, HEMA, and the crosslinker EGDMA. Also model drugs flurbiprofen and methylene blue.

In Chapter 2, a literature review will be given covering recent studies on polyHEMA based drug delivery systems and previous work on controlled release systems developed for flurbiprofen and methylene blue.

Chapter 3 will cover the experimental methods used, and in Chapter 4 results obtained will be presented and discussed. Finally, Chapter 5 will summarize the conclusions and future work.

1.1 Controlled Drug Release

Controlled drug release is a method of delivering drugs to the body in a controlled manner. This approach allows the drug to be released at a specific time and location in the body, improving efficacy and reducing side effects [1].

Controlled drug release can be achieved through various methods, such as encapsulation of drugs in polymeric matrices such as hydrogels, microspheres-microparticles (beads) and nanoparticles. These systems can be designed to release drugs at a specific rate and duration, depending on the desired therapeutic effect [1].

One of the most widely used methods of controlled drug release is loading of drugs in polymeric nanoparticles, microparticles (beads). This method allows for targeted delivery of drugs to specific cells or tissues. For example, polymeric nanoparticles containing anti-inflammatory or anti-cancer drugs have been shown to effectively target and kill cancer cells while minimizing toxicity to healthy cells. This method allows for the release of drugs at specific times and locations in the body, resulting in improved efficacy and reduced side effects [2].

Drugs are designed to produce therapeutic effects when administered in appropriate amounts, but excessive amounts can result in toxicity. Knowing the toxic, therapeutic, and ineffective levels of drug release is important for safe and effective drug use. The toxic level of a drug is the maximum amount that can be safely tolerated without producing harmful effects. Toxicity can range from mild symptoms, such as dizziness, to more serious effects, such as organ damage or death. It is important to note that the toxic level of a drug can vary between individuals, based on factors such as age, weight, and genetic variations. The therapeutic level of a drug is the amount that is

required to produce the desired therapeutic effect. This level can vary depending on the type of drug, the condition being treated, and the individual patient. Determining the therapeutic level for a drug typically requires clinical trials, where the safety and efficacy of different doses are evaluated [1]. The ineffective level of a drug is the amount that is too low to produce any therapeutic effect. This can occur if a patient is not taking the recommended dose, if the drug is not absorbed properly, or if the patient has developed tolerance to the drug. Ineffective levels of drug release can lead to a lack of symptom relief and, in some cases, to disease progression [2].

In conclusion, understanding the toxic, therapeutic, and ineffective levels of drug release is essential for safe and effective drug use. These levels can be influenced by factors such as the type of drug, the condition being treated, and the individual patient, and should be carefully monitored by healthcare providers [1,2] (Figure 1).

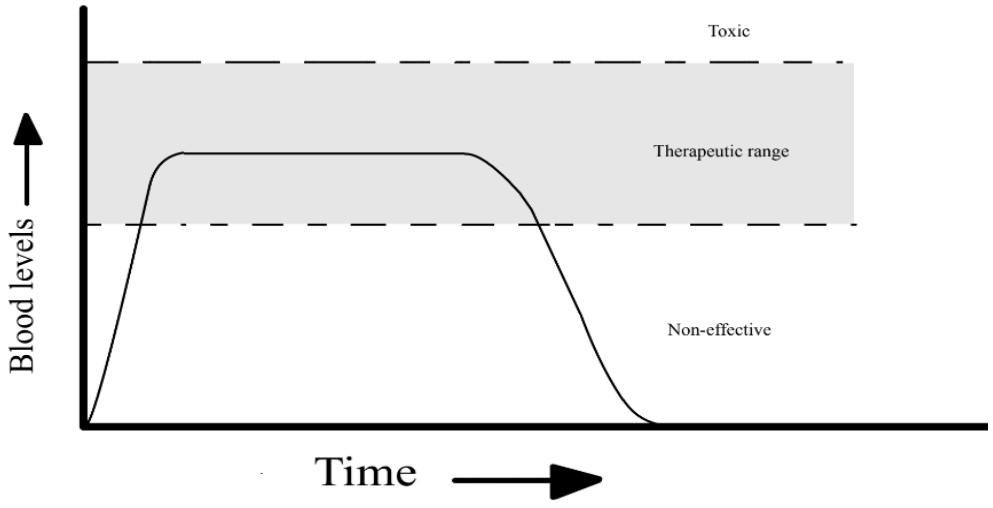


Figure 1: A General Representation of Drug Concentration Versus Time Upon Drug Administration.

1.2 Suspension Polymerization

Suspension polymerization is a process that produces micrometer-sized beads through the polymerization of monomers suspended in a liquid medium like water if the monomer and crosslinker are insoluble in water. In this method, monomers, initiators, and stabilizers are added to a liquid, such as water, and then polymerized to form microparticles (beads). The resulting beads can be utilized for various purposes, including drug delivery, filtration, and separation. One of the benefits of suspension polymerization is its versatility in producing beads of varying sizes, ranging from several micrometers to several millimeters depending on the intended use.

This allows for the creation of beads with specific properties, such as pore size, suitable for different applications. Moreover, suspension polymerization is a simple and cost-effective process, making it ideal for large-scale production. It is easily scalable, making it a popular option for commercial purposes [3].

One of its main limitations is the requirement for a high degree of agitation to keep the monomers suspended, which can be challenging to achieve during large-scale production. Additionally, the beads produced through suspension polymerization are often irregular shape and size, which may pose a problem for certain applications, such as filtration or separation [3].

1.3 2-Hydroxyethyl Methacrylate (HEMA)

2-Hydroxyethyl methacrylate (HEMA) is a hydrophilic monomer used in the synthesis of polymers and copolymers. It is a clear, colourless liquid and commonly used in the production of dental and medical materials, coatings. HEMA is a synthetic monomer with strong mechanical strength and antimicrobial and chemical resistance. By

increasing pH-sensitivity, different ionic monomers can be inserted into it to drastically alter its swelling characteristics and drug release [4].

HEMA is a liquid at room temperature and has a density of 1.05 g/cm^3. It has a low viscosity and is highly polar, making it easily soluble in water and other polar solvents such as ethanol. It has a low boiling point of 100 °C. It is also a versatile monomer that can be used in a wide range of chemical reactions, including esterification, etherification, and amination. It has a high reactivity due to the presence of a highly reactive hydroxyl group and a double bond [4].

One of the most common uses of HEMA is in the production of dental and medical materials. It is used as a component in dental composites and sealants, as well as in the production of contact lenses and other ophthalmic materials. It is also used in the production of coatings and adhesives, due to its low viscosity and high reactivity. HEMA is also used as a monomer during polymerization. It is commonly used in the synthesis of poly HEMA and poly(HEMA-*co*-MMA), which are used in a wide range of applications, including coatings, adhesives, and controlled drug release [4] (Figure 2).

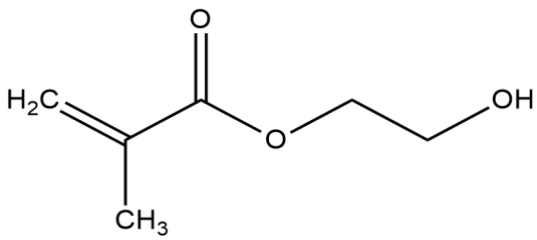


Figure 2: Chemical Structure of HEMA.

1.4 Ethylene Glycol Dimethylacrylate (EGDMA)

Ethylene glycol dimethylacrylate (EGDMA) is a colourless, liquid that is commonly used in the polymerization as crosslinker. It is a reactive diluent used in the manufacturing of coatings, adhesives, and inks. It is also used in the manufacture of dental and medical products, drug production [5].

EGDMA is a liquid at room temperature and has a low viscosity. It is insoluble in water but is soluble in most organic solvents, including alcohols and ketones. It is a crosslinker that forms strong chemical bonds between polymer chains, leading to an increase in the strength and toughness of the polymer. It has a high reactivity ratio and is highly reactive with hydroxyl and carboxyl groups [4,5] (Figure 3).

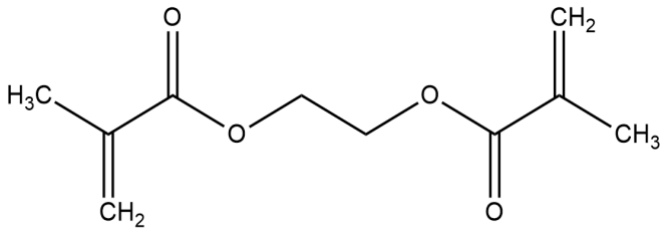


Figure 3: Chemical Structure of EGDMA.

1.5 Poly(2-Hydroxyethyl Methacrylate)

Poly(2-hydroxyethyl Methacrylate) polyHEMA beads (Figure 4) can be produced through the suspension polymerization technique [3,6]. PolyHEMA microparticles are small spherical particles with a smooth surface. They have good stability and are hydrophilic, making them soluble in water and other polar solvents. The resulting microparticles (beads) have good stability in aqueous media and have a uniform size distribution and can be easily separated from the polymerization mixture. PolyHEMA beads have a wide range of applications, including drug delivery, biosensors, and

tissue engineering. They can be functionalized with various substances, such as drugs or biomolecules, to improve their performance in specific applications [6].

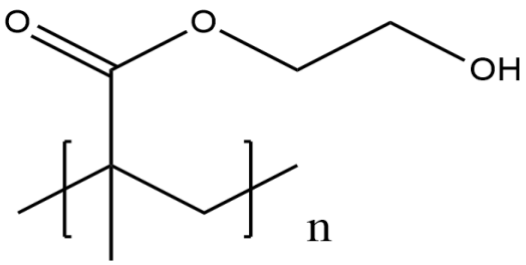


Figure 4: Chemical Structure of PolyHEMA.

1.6 Poly (Ethylene Glycol Dimethylacrylate)

Poly(ethylene glycol dimethacrylate), polyEGDMA, (Figure 5), polymer beads can be produced through suspension polymerization technique similar to polyHEMA. PolyEGDMA beads are small spherical particles with smooth surfaces. They have good stability and are insoluble in water and other solvents. EGDMA is a hydrophobic monomer that can be used for the synthesis of cross-linked polymers. The resulting beads are expected to have good stability and mechanical properties [7].

They have a uniform size distribution and can be easily separated from the polymerization mixture PolyEGDMA beads have a wide range of applications, including drug delivery. They can be functionalized with various substances, such as drugs or biomolecules, to improve their performance in specific applications [7].

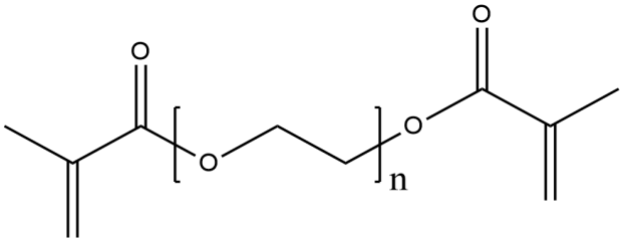


Figure 5: Chemical Structure of PolyEGDMA.

1.7 Poly(HEMA-*co*-EGDMA)

Poly(HEMA-*co*-EGDMA) is a crosslinked copolymer that can be used for a variety of biomedical applications (Figure 6). Poly(HEMA-*co*-EGDMA) finds widespread usage in various fields such as dentistry, optics, biomedicine, and controlled drug release. In the field of dentistry, it is employed as a material for dental adhesives. As a base polymer, it is utilized in the production of soft contact lenses. This copolymer is also a popular choice for various biomedical implants, including stents. Additionally, it serves as a scaffold in tissue engineering applications. Physicochemical properties of poly(HEMA-*co*-EGDMA) can be adjusted by changing the ratio of HEMA to EGDMA [8]. The particle size of poly (HEMA-*co*-EGDMA) can be controlled by adjusting the reaction conditions during polymerization such as temperature and stirring. The average particle size is typically in the range of 75-710 micrometers. The surface of poly(HEMA-*co*-EGDMA) beads is hydrophobic, which makes them useful for encapsulating hydrophobic drugs. The surface can be modified to improve drug adsorption and release. The drug encapsulation efficiency of poly(HEMA-*co*-EGDMA) beads can be improved by optimizing the reaction conditions during polymerization such as temperature, ratio of monomer and crosslinker, and the stirring rate. Efficiency can be influenced by factors such as the size and charge of the drug molecule. Also, the drug release profile from poly(HEMA-*co*-EGDMA) can be controlled by adjusting the composition of the polymer matrix and the porosity of the beads. The release rate can be influenced by factors such as the size and charge of the drug molecule, the composition of the surrounding fluid, and the pH of the release environment [8].

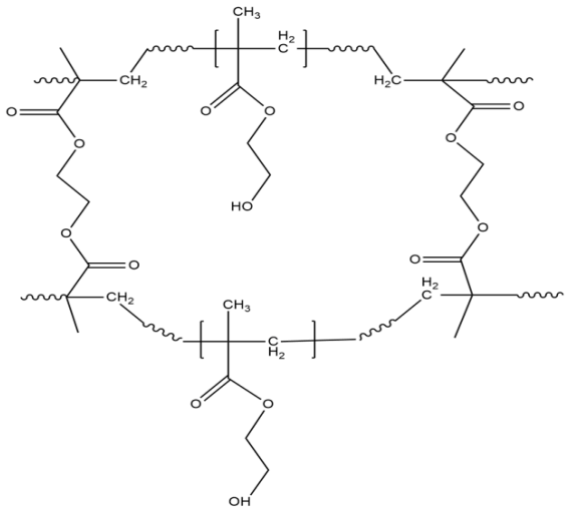


Figure 6: Chemical Structure of Poly(HEMA-*co*-EGDMA).

1.8 Flurbiprofen

Flurbiprofen (Figure 7). Is a type of NSAID (nonsteroidal anti-inflammatory drug) that is used for managing pain and reducing inflammation in conditions like primary dysmenorrhea. In both adolescent and adult females, primary dysmenorrhea (PD) is frequent, underdiagnosed, and undertreated. It is distinguished by painful cramps in the lower abdomen that begin immediately before or at the commencement of menstruation and can linger for three days [9]. It works by suppressing the production of prostaglandins, which contribute to pain and inflammation [9]. When it comes to its properties, flurbiprofen is a white crystalline powder that has high solubility in ethanol and no solubility in water. The solubility of flurbiprofen in water is impacted by pH and temperature, with the drug being more soluble in acidic environments and at elevated temperatures. In ethanol, flurbiprofen is highly soluble, with a solubility of about 150 mg/mL at 25°C. Although the drug is stable in solution, it can be degraded by factors such as light, heat, and strong acids or bases [1,10].

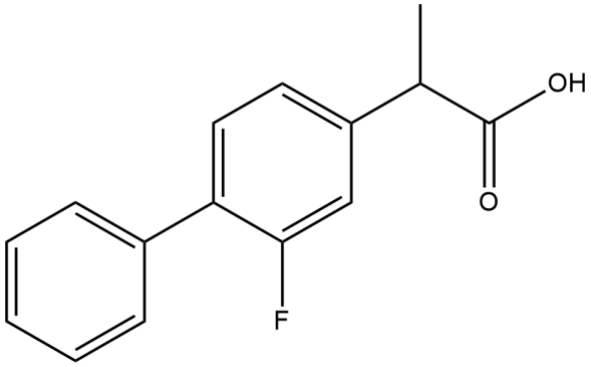


Figure 7: Chemical Structure of Flurbiprofen.

1.9 Methylene Blue (MB)

Methylene blue has the chemical formula $C_{16}H_{18}ClN_3S$ (Figure 8) and is a heterocyclic aromatic organic molecule. It is a thiazine dye that belongs to the phenothiazine derivative family. A common dye that can be used to stain biological tissues is methylene blue because it prefers negatively charged molecules like proteins and nucleic acids. Because it undergoes a reversible oxidation-reduction reaction in the presence of oxidizing or reducing agents, it can also be used as a redox indicator [11].

Methylene blue is colorless when it is reduced, but when it is oxidized, it turns blue. Utilizing methylene blue as an electron acceptor to change non-functional methemoglobin into functional hemoglobinis a method used to treat methemoglobinemia [11].

With a maximum absorbance of about 660 nm, methylene blue has a considerable absorption in the visible spectrum and is soluble in water. It is a helpful tool in analytical and biological applications due to its solubility and absorption qualities.

Methylene blue has a planar and symmetrical structure with a central nitrogen atom that is linked to two aromatic rings thiazine, phenyl and a sulfonate group. The molecule also contains a chloride ion, which is weakly linked to the nitrogen atom. Methylene blue is a medication that has been used for several purposes. This thiazine dye is used frequently to treat methemoglobinemia [12]. A condition where the blood can not carry enough oxygen. Hemoglobin's ferric iron is changed by methylene blue into iron oxide, which improves the efficiency with which oxygen is transported through the blood. Methemoglobinemia is treated with methylene blue, but its potential for neuroprotection has also been studied. Recent research has suggested that it might be helpful in avoiding diseases like stroke, Parkinson's disease, and Zahaimer disease [13]. It is an effective treatment for methemoglobinemia and malaria [11,12,13].

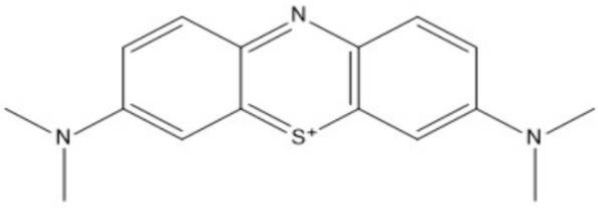


Figure 8: Chemical Structure of Methylene Blue.

Chapter 2

LITERATURE REVIEW

In this part, several examples from the recent literature on polyHEMA based drug delivery systems, flurbiprofen and methylene blue encapsulation/release via polymeric matrices will be presented.

2.1 Release of Ibuprofen Sodium Salt from Porous of Poly (hydroxyethyl methacrylate-*co*-trimethylolpropane trimethacrylate)

In this previous work by Kiery's et.al. [14], crosslinked polymers poly(trimethylolpropane trimethacrylate), copolymer and polymer-drug conjugates based on these materials are described. The resin was created by suspension-emulsion polymerization to take the shape of permanently porous beads.

2.1.1 Encapsulation Process for Ibuprofen into Copolymer

Ibuprofen sodium salt (IBNa) solution was loaded into swollen into polymer matrices to produce a set of polymer-drugs. IBNa was first dissolved in ethanol (EtOH). Then, polymer beads were infused with a fresh alcohol solution. Immediately after the polymer beads in IBNa swelled, they were hermetically sealed and left at 25°C for 3 hours. Finally, the solid product was dried under vacuum for 6 hours at 50°C. The final loading efficiency of the medication was estimated to be 51 mg/g [14].

The ibuprofen salt release studies were carried out in a thermostatic bath at 37 °C with stirring at 280 rpm. By soaking 200 mg of the polymerized drug conjugates in 50 mL of phosphate buffer solution pH 7.4, desorption profiles were obtained. 5 mL of the

release fluid was removed for analysis at predefined intervals, and the dissolution medium was rapidly replaced [14].

2.1.2 Ibuprofen Release Test

Ibuprofen sodium salt release tests for all analyzed conjugates were carried out in the phosphate buffer solution at 37 °C, in order to analyze the effects of various polymer matrices on the drug desorption rate. The (sample 1) which was prepared samples loaded with ibuprofen, is the one from which the ibuprofen salt is leached off the fastest. The % of release is seen simultaneously, and during the first 15 minutes, over 60% of the drug is released. In the case of sample 2, the initial very quick release is greatly reduced. However, only sample 1 has up to 98% of the maximum quantity of salt that may be desorbed, and as the crosslinking component is increased, fewer drugs can be released maximally after 30 hours [14].

2.2 Ca-Alginate – Flurbiprofen Controlled Release System

Sodium alginate solution including flurbiprofen was mixed with calcium chloride solution to prepare flurbiprofen loaded Ca-alginate beads. The optical microscope was used to characterize the prepared flurbiprofen loaded Ca-alginate beads. The swelling % of dried beads were determined in different buffer solutions, phosphate buffer pH 7.4 and acetate buffer pH 2.0. The flurbiprofen release from the produced beads was tested in two releasing mediums, phosphate buffer pH 7.4 and acetate buffer pH 2.0 under 37 °C and shaking at 150 rpm [15].

2.2.1 Preparation of Flurbiprofen-Loaded Ca-Alginate Beads

2 mg of Na-alginate was added in 100 mL of water to prepare Ca-alginate solution including 100 mg of flurbiprofen. Ca-alginate beads were produced by dropping Na-alginate solution in 100 mL solution of 0.2M $CaCl_2$, the solution was placed in a 50 mL glass syringe and placed into a beaker containing 100 mL of $CaCl_2$ until the Ca-

alginate beads loaded FLB spontaneously formed. The leached water was removed before the beads had been washed three times with distilled water and dried in a 40°C oven for 12 hours. However, after 1.5 hours, the swelling% of Ca-alginate beads in phosphate buffer were more than its initial size. Disintegration and dispersion occurred gradually over a period of more than one hour. The profile of loaded-FLB release from alginate beads in water and 0.1 N HCl pH 1.2. The release shows that alginate beads significantly prevent FLB release. After two hours in water and 0.1 M of HCl pH 1.2, only 3.6% and 4.8% of the drug content in the beads were released, respectively [15].

The limited FLB release suggests that alginate beads could act as a reservoir for FLB release prolonging the release time. The release% of FLB from alginate-beads created with varied $CaCl_2$ concentrations was slower than plain drug due to FLB's low solubility and the minimal swelling of alginate in the phosphate buffer. Alginate beads with a larger concentration of Ca^{2+} ions released drugs at a slower rate and to a lesser amount [14]. Alginate beads manufactured in $CaCl_2$, 0.025 M [1:1] ratio have a high release percentage and a high drug loaded. The results demonstrated that when alginate content increased, the release rate increased. The influence of varying D:P ratios on the release% of FLB from produced alginate beads in $CaCl_2$, 0.2 M. Alginate beads created at a [1:0.5] ratio show a high release rate and drug absorption. While alginate beads made at a [1:5] ratio exhibit low drug release and absorption. Consequently, alginate beads made at a [1:1] ratio in $CaCl_2$, 0.2 M were selected as the best type of beads for further research since they had a moderate release % and drug loading [15].

2.3 A Poly (vinyl cinnamate) Film and Poly (2-hydroxyethyl methacrylate) for Controlled Flurbiprofen Release

A crosslinked polymer hybrid known as in poly(vinyl cinnamate-graft-2-hydroxyethyl methacrylate)-v-poly(ethylene glycol dimethacrylate) was created using poly(vinyl cinnamate) (polyVCi), 2-hydroxyethyl methacrylate (HEMA) monomer, and ethylene glycol dimethacrylate (EGDMA) crosslinker by UV initiation. Benzophenone was used as the photoinitiator. The synthesis was improved by altering the concentrations of HEMA, Ph_2CO, EGDMA, and UV irradiation time. PolyVCi is photocrosslinked by photo cyclo addition while the monomer/crosslinker HEMA-EGDMA couple goes through free radical polymerization and crosslinking to produce EGDMA crosslinked polyHEMA. As a result, a simultaneous interpenetrating polymer network is formed. The IPN is made up of a dual network of polyHEMA chains that have been photocrosslinked and EGDMA crosslinked. During network creation, HEMA/EGDMA chains are also grafted onto the polyVCi backbone [1].

2.3.1 Flurbiprofen Loading and Release

PolyVCi was dissolved in THF to obtain a solution with a concentration of 5.0 g/L. Then, this solution was mixed with the polymerization solution, which contained either 20.5 mM or 10.25 mM of FB, 26.5 mM of EGDMA crosslinker, 41.2 mM of HEMA monomer, and 13.7 mM of Ph_2CO, and flurbiprofen with either 10.25 mM or 20.5 mM. The mixture was connected to a bubbling nitrogen gas source and allowed to react for 20 minutes at room temperature after being sealed. They used a Luzchem photoreactor, which included six top and eight side UVA lamps with a 350 nm wavelength and 7670 W.cm-2 of power. Both samples were positioned 15.0 cm from the lamps while a 20 mL homogeneous solution was made, put in glass petri dishes, and exposed to UV irradiation for 8 hours. Loaded films were obtained after UV

exposure and many ethanol washes, and they were then allowed to cure at room temperature. FB2 and FB1 have a concentration of 10.25 mM based on the initial drug concentration, while FB2 was 20.5 mM. So, the encapsulation% of flurbiprofen in polyVCi-*graft*-polyHEMA was 73%, 36.5 mg of drug in FB1 and 43.6%, 43.6 mg of drug [1].

They used 100 mL of ethanol solution at 25°C for loading the film FB1 and FB2, several times, 1 mL of release solution was taken for UV-Vis measurement at 249 nm and directly add fresh ethanol to release solution. They found the absorbance value for each sample after that they corrected to flurbiprofen concentration by using linear equation **y=0.0716x+0.0846, R^2=0.9914** the cumulative drug release for FB1 is two steps. The % release value in the first step was 32% at first 5 hours, after 5 hours to 15 hours no release, then in second step % release value was 100%. FB2 shown 22% of cumulative release first 5 hours in first step of release. The release completed for both samples FB1, FB2 at 35 hours [1].

2.4 Controlled Release of Methylene Blue by LbL Films

A few studies are looking for some technique to produce very thin films with good toughness, as these films are characterized by very low thickness that we can use in many fields. Some studies have shown that the layer-by-layer (LbL) method can be used to form a polymer matrix through which drugs can be delivered. One of the most important applications that attracted the attention of researchers is the use of (LbL) technique in loading and releasing drugs into the body and studying the effect and behaviour of drugs after loading them into these thin films [16].

The researchers carried out an application on the ground by forming (PU/PAA) by layer-by-layer technique and loading the methylene blue MB in this film, where the study was carried out under different conditions, including pH. Some of the results of this study showed that the pH has a major role in the process of PU/PAA films absorption of the drug and thus affects the release process [16]. The LbL films were made on glass slides that had been cleaned for 45 minutes with solution (3:1 v/v mixture of 98% H_2SO_4 and 30% H_2O_2), after that films were washed by deionized water and kept at room temperature to dry [16].

In the first step, glass slides were dipped for one minute in polyurethane solution which was prepared as 1% solution by weight with pH 3.5 to get a positive charge on the surface. After that, glass slides were dipped into two different beakers including deionized water for one minute the glass slide was kept at room temperature to dry. The slides were then dipped in the PAA solution (wt1% pH 3.2) for two minutes The same processes were repeated to get 10 layers of (PU/PAA) [16].

2.4.1 Loading (Absorption)-Release of Methylene Blue in Films

The films coated on a glass slide were dipped in methylene blue solution containing 10 µg/mL of MB and 150 mM sodium chloride, and the loading processes carried at various pH values pH 2.0, pH 7.4 and pH 11. After that, the films were taken out to wash with deionized water to remove any unloaded particles of methylene blue, and then keep the films to dry at room temperature. The amount of MB absorbed was analyzed by visible spectrophotometry at 660 nm [16].

The slides films which were coated with methylene blue were added into solutions with different concentrations of sodium chloride and different value of pH under 20°C

and the samples were placed on a shaker. At regular times, they took one mL of release solution and replaced one mL of fresh salt solution to take the measurement to find the amount of methylene blue was released by using Uv –Vis spectrophotometry at 660 nm. As the results of absorption showed, the amount of methylene blue (loaded methylene blue) was increased when the pH value was changed from pH 3.0 to pH 7.0 in the first 25 minutes. The absorption value reached the highest at pH 5.0 and pH 7.0, 75 % of methylene blue was absorped in pH 5.0 and pH 7.0, however, the absorption % was decreased when the pH increased to 9.0 because the carboxylic group in PAA was converted to COO^- with negative charge, so the attractive forces of electrostatic were promoted positive group in methylene blue, this is a reason for increasing the swelling % to enter the methylene blue particles inside PU\PAA films. The release % of methylene blue in PU\PAA films was increased in sodium chloride pH 5.0 at 140 minutes and no release in water. The release % was changed by changing the pH and concentration of sodium chloride solutions. The release % increased when the pH value changed from pH 5.0 to pH 9.0, so the release % of methylene blue was completed at 130 minutes in pH 9.0 and 100% of release when the pH was 3.0 [16].

2.5 Using Chitosan/Polyacrylic Acid for Controlled Release of Methylene Blue

This article is talking about how researchers use the layer-by-layer films (LbL –films) for different applications in chemistry. They used the LbL technique to prepare thin films to use for biomedical applications [17]. Layer by Layer technique was useful in different research for preparing films which we can use for biomedical applications, for example, drug delivery and controlled release of drugs and studying the behaviour of drugs in different buffer solutions. In previous years, Chitosan (Cts) was used in many different applications of chemistry, for example, analytical chemistry, and

chemistry of polymer nanotechnology. Chitosan is a natural polymer and biodegradable and non-toxic, so in his article, the researcher used chitosan and polyacrylic acid to prepare LbL and loading the methylene blue into these films to study the release of MB under different conditions, pH and concentration of release mediums [17].

2.5.1 Preparing (Cts/PAA) LbL Films

Firstly, they prepared the solution by [3:1v/w mixture of 98% H_2SO_4 and 30% of H_2O_2]. Then the glass slides were dipped in this solution for one hour. After one hour, the glass slides were washed with deionized water and kept to dry at room temperature. The researchers prepared chitosan solution by adding 1mg/mL of chitosan and 1mg/mL of PAA in two different beakers of buffer solution pH 3.2. The slides were dipped in the chitosan solution for two minutes, and then washed with deionized water, they dipped the slides in PAA solution for two minutes, then they washed the slides with ionized water and kept them dry these processes were repeated 10 times to make 10 layers of (Cts/PA) [17].

2.5.2 Loading and Release Test of Methylene Blue

After preparing the films by (LBL) coated on glass slides, they dipped the films in 0.3 mg\mL of methylene blue solution with different sodium chloride con and pH values for 15 minutes. During this process, 0.5 mL of salt solution every 2 minutes to 4 hours, then washed the samples with deionized water and kept dry. After collecting 0.5 mL every 2 minutes, they used the UV-spectrophotometer on 668 nm to find the absorbance values, and the calibration curves of methylene blue were used to calculate the amount of loaded methylene blue [17].

The Cts/PAA films with methylene blue were dipped in the different concentrations of salt solution (10 mM, 20 mM, 50 mM, 100m) and different values of pH (3,2, 5.0, 7.0). 1 mL of salt solution (release medium) was taken to calculate the amount of release MB in solution and 1 mL of salt solution was replaced to release medium. When the ionic strength of the MB solution increased, the amount of loaded methylene blue will decrease. At high salt concentration, due to disruption of the ionic bond between two layers of polymer the layers will be separated from each other. Changes in the concentration of sodium chloride solution affected the loading of methylene blue. The mass of methylene blue was 1 mg/mL in 10 mM NaCl, in pH 7.0 at time zero was 3.0 mg/mL also in 50 mM, 100 mM of sodium chloride solutions were 10 mg/mL of methylene blue absorbed [17].

After 6 minutes, the amount of methylene blue loaded in 10 mM NaCl solution was 2.7 mg\mL and 100 mM of NaCl solution was 2.8 mg/mL. Different values of pH affect the result (amount of methylene blue in Cts/PAA films), higher pH was faster for loading, at first 10 minutes for pH 5.0 to be the films saturated with MB, 8 minutes for pH 7.0 and 2 minutes needed for pH 9.0 to complete absorption of methylene blue [17].

In pH 9.0 the COOH group converted to COO^-. When the pH increases, the PAA was electrostatic interaction between the positive charge of chitosan and the negative charge in methylene blue. For the release amount of MB, the results were different by changing the centration of the release medium and changing the pH values. For example, in 10 mM NaCl, amount of MB released was 0.32 mg/mL, in 50 mM, 100 mM, the amount of drug released was 0.4 mg/mL in the first 2 minutes [17].

After 16 minutes, the amount of methylene blue in 50 mM of the release medium was 0.75 mg/L and 0.6 mg/L in 10 mM of the release medium (NaCl solution) and 0.85 mg/L of MB in 100 mM of the release medium [17].

Chapter 3

EXPERIMENTAL

3.1 Materials

The materials that have been used in this work are shown in Table 1. They were all used as received. Distilled water was used for preparation of solutions.

Table 1: Materials and Their Manufacturing Company.

Material	Manufacture
Ammonium peroxydisulfate (APS)	Sigma-Aldrich, Germany
Ethylene glycol dimethacrylate (EGDMA)	Sigma-Aldrich, Germany
Flurpibrofen	Pharma Modial, North Cyprus
2-Hydroxyethyl methacrylate (HEMA)	Sigma-Aldrich, Germany
Hydrochloric acid	Sigma-Aldrich, Germany
Magnesium oxide (MgO)	Sigma-Aldrich, Germany
Methylene Blue (MB)	Sigma-Aldrich, Germany
N,N,N-Tetramethyl-ethylenediamine	Sigma-Aldrich, Germany
Potassium dihydrogen phosphate	Sigma-Aldrich, Germany
Sodium acetate	Sigma-Aldrich, Germany
Sodium hydroxide	Sigma-Aldrich, Germany

3.2 Preparation of Phosphate Buffer Solutions

3.2.1 Phosphate Buffer Solution (pH 7.4)

It was prepared by mixing 0.1 M 100 mL of potassium dihydrogen phosphate solution with 0.1 M 78.2 mL of sodium hydroxide and diluting it with distilled water with up to 200 mL [18].

3.2.2 Phosphate Buffer Solution (pH 11)

13.6 g of sodium dihydrogen phosphate and 3.12 g of NaOH were added to 1000 mL distilled water to get pH 11 to adjust NaOH and HCl [18].

3.2.3 Acetate Buffer Solution (pH 2.0)

It was prepared by 0.395 g sodium acetate and dissolving it in 800 mL of distilled water. Then 5.72 g glacial acetic acid was added slowly while stirring until the desired pH of 2.0 was reached. The final volume was 1 L with distilled water or use NaOH and HCl to get pH 2.0 [18].

3.3 Preparation of PolyHEMA

The polymerization was carried out in a two neck round bottom flask equipped with a magnetic stirrer reflux condenser and nitrogen gas inlet according to the procedure reported by [3]. HEMA with a volume of 1.39 mL was mixed with 13.59 ml distilled water. 0.183 g of MgO was added to the reaction with a mass of 0.0057 g of APS, finally, the addition of 2 drops of TEMED was required as a redox couple. The reaction was carried out at 70 °C for 3 hours with stirring at 350 rpm. The product was removed from the flask and left to dry at a room temperature of 25 °C.

3.4 Preparation of PolyEGDMA

Homopolymerization of polyEGDMA was carried out in a two-necked round-bottomed flask reactor with a stirrer, a reaction condenser, and a nitrogen gas inlet. The polymer was synthesized using the modified suspension polymerization method.

0.577 mL of EGDMA (ethylene glycol dimethacrylate) monomer and 0.0057 g of APS (ammonium peroxide disulfate) strong oxidizing agent were added to the reaction mixture as the initiator to the reaction medium. Following that, 12.766 mL of distilled water and 0.183 g of magnesium oxide were added to the mixture (MgO). The mixture was then given two drops of N,N,N-tetramethyl ethylenediamine**.** The reaction was carried out at 70 °C for three hours with stirring at 350 rpm. After three hours a white precipitate formed inside the flask, this substance was removed and dried, after that we have to make a separation for this substance to get four fractions > 710 µm (F1), 710-212 µm (F2), 212-150 µm (F3), 150-75 µm (F4).

3.5 Preparation of Copolymer Poly(HEMA-*co*-EGDMA)

Three samples with different ratios of monomer and crosslinker were synthesized by suspension polymerization, sample 1 (S1) [1:0.3] HEAM:EGDMA, sample 2 (S2) [1:0.6] HEMA:EGDMA and sample 3 (S3) [1:0.9] HEMA:EGDMA (Table 2).

Table 2: Ratios of Monomer and Crosslinker.

Sample ID	**HEMA (g)**	**EGDMA (g)**	**APS (g)**	**HEMA:EGDMA Ratio**
S1	1.3505	0.5943	0.0057	[1:0.3]
S2	1.3505	1.1500	0.0057	[1:0.6]
S3	1.3505	1.7829	0.0057	[1:0.9]

Poly(HEMA-*co*-EGDMA) (S3) was prepared by suspension polymerization by adding 1.22 mL of HEMA (hydroxyethyl methacrylate) monomer, 1.731 mL of EGDMA (ethylene glycol dimethylacrylate) crosslinker, and 0.0057 g of APS (ammonium peroxide disulfate) as the catalyst at a ratio of [1:0.9]. After adding 12.22

mL of distilled water and 0.183 g of magnesium oxide (MgO) to the mixture, two drops of N, N, N-tetramethyl ethylenediamine were added to complete the polymerization process. When the copolymer was prepared after 3 hours under 70 °C and stirring at 350 rpm, take it to dry at room temperature 25 °C and separate it to get four fractions with same procedure in polyEGDMA also to prepare (S2) and (S1). We prepared (S1), and (S2) by the same procedure, but we changed the ratio of monomer and crosslinker (Table 2). Then we used sives to separate the copolymer to obtaine different fractions > 710 µm (F1), 710-212 µm (F2), 212-150 µm (F3), 150-75 µm (F4) (Figure 9).

Figure 9: Poly(HEMA-*co*-EGDMA)(S3), A: (F1) >710 µm. B: (F2) 710-212 µm. C: (S3)Before Separation by Sieves.

3.6 Loading of Flurbiprofen in Poly(HEMA-*co*-EGDMA)

A procedure similar to poly(HEMA-*co*-EGDMA) beads was used in preparing flurbiprofen loaded beads. HEMA and EGDMA were mixed in mass ratios of [1:0.3], [1:0.6] and [1:0.9] by adding 1.22 mL of (HEMA (hydroxyethyl methacrylate)) and 1.731 mL of the crosslinker (EGDMA). Then the reaction medium was prepared by adding 0.0057 g of APS, 11.22 mL of distilled water and 0.183 g (MgO) magnesium oxide and 2 drops of (N, N, N-Tetramethyl-ethylenediamine) to the monomer/crosslinker mixture. Then, 15 mg flurbiprofen which was dissolved in 1 mL phosphate buffer pH 7.4 was added and stirred well The synthesis was carried out at

70 °C with stirring at 350 rpm under N_2 gas atmosphere. After three hours, the FLB loaded beads are removed from the flask and kept at room temperature to dry for 24 to 48 hours. The copolymer was separated to four fractions by sieves. The drug-loaded beads were washed in a beaker with 0.2 M HCl to remove any unreacted species of MgO. This washing step was repeated until the filtrate became transparent and clear then filtered, then washed with distilled water and keep the sample at room temperature to dry. The sample was washed with 10 mL of ethanol to remove the surface bound drug. Drug loaded microparticles were kept at room temperature to dry between 24 to 48 hours.

% (Enc %) of the drug in the poly(HEMA-*co*-EGDMA) microparticles was calculated according to equation 1.

$$\boldsymbol{Enc}\% = \left(\frac{\boldsymbol{T.mass - U.mass}}{\boldsymbol{T.mass}}\right) \times \mathbf{100}\% \quad (1)$$

Where:

T. mass: is total amount of drug (mg) , U.mass: is unloaded amount of drug (mg).

3.7 Loading of Methylene Blue in Poly(HEMA-*co*-DEGMA)

Firstly, three solutions of different pH were prepared. 40 mg of methylene blue has been added to 200 mL of phosphate buffer pH 11 with 200 ppm concentration and added 40 mg of methylene blue to 200 mL of phosphate buffer pH 7.4 with 200 ppm of concentration also we prepared acetate buffer pH 2.0 by adding 40 mg of methylene blue to 200 mL of acetate buffer pH 2.0 with 200 ppm of concentration, after that all of solutions were shaken very well until the methylene blue dissolved.

MB was loaded in the microparticles by the absorption method. The second fraction of poly (HEMA-*co*-EGDMA) with [1:0.9] ratio with 710 to 212 µm (S3F2), particle size was used as the polymer matrix, due to the shape of the symmetrical microbeads and their quantity obtained. Three samples of 0.125 g of poly(HEMA-*co*-EGDMA) were taken and placed in the three solutions (dye solutions) at a concentration of 100 ppm, where 25 mL of solutions containing methylene blue, and 25 mL buffer solutions were added to obtain a concentration of 100 ppm, 50 mL inside a flask. After that put them on the shaker at 125 rpm at room temperature, where 1 mL of all three samples was taken every period and 1 ml of the fresh buffer solution was placed in their place for 24 hours to complete the absorption process to determine the amount of methylene blue absorbed by the beads. Then do the filtration for the samples and keep them on a room temperature until 24 hours, after that the three samples were collected and the amount of MB remaining in solution was determined by visible spectrophotometry at a wavelength of 660 nm. Then the percentage of methylene blue that was absorbed by the beads was calculated.

3.8 Swelling Behaviour

SHIMADZU-TW423L model for analytical balance was used to record the increase in masses of the samples. Firstly, a small amount from each sample (approximately 0.30 g) was transferred into 50 mL buffer solutions with various pH values (pH 2.0 and 7.4). Then masse of samples were recorded at regular intervals to monitor the increase in masses of sample (equation 2) after blotting excess water on the surface.

$$\boldsymbol{Swelling}\ \% = \left(\frac{Ws-Wd}{Wd}\right) \times 100\% \qquad (2)$$

Where: W_d is dried weight of sample. W_S is Swollen weight of sample.

3.9 *In Vitro* Drug Release Studies

3.9.1 Release Study for Flurbiprofen

For the release study, the loaded beads was soaked in 50 mL of phosphate buffer pH 7.4 at 37 °C. An aliquot volume of 1.0 mL of solution was taken from the release medium at a given time and the absorbance was measured using a UV-VIS spectrometer at 250 nm. The Uv absorbance taken from the release medium was evaluated according to the flurbiprofen calibration curve using the linear equation **(y = 0.0664 x + 0.0184, R^2 = 0.9935)** (Figure 10), and the concentration of flurbiprofen in the release medium was calculated. The cumulative release% was calculated according to (equation 3).

$$\boldsymbol{Cumulative\ Release}\ \% = \left(\frac{\boldsymbol{Mt}}{\boldsymbol{M\infty}}\right) \times 100\% \qquad (3)$$

Where: $\mathbf{M_t}$ is the amount of drug released from a loaded beads at time t, and M_∞ is the amount of drug released from the loaded beads at infinite time, which is taken as the amount encapsulated. Each time the release medium was replaced with 1.0 mL fresh buffer solution after each draw.

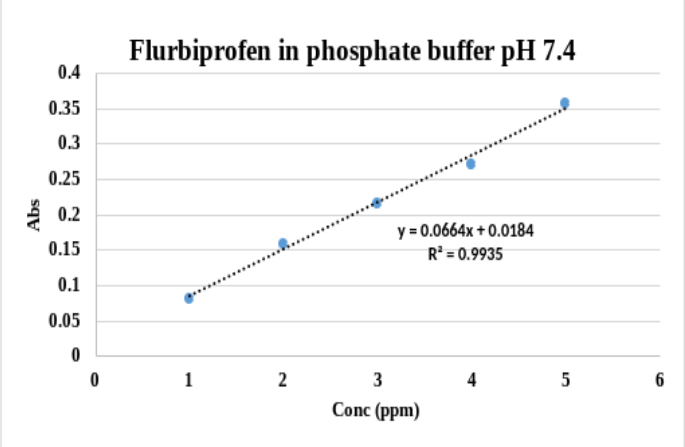


Figure 10: Flurbiprofen Calibration Curve.

3.9.2 Release Study for Methylene Blue

Release study in this case was applied on poly(HEMA-*co*-EGDMA) [1:0.9] ratio with methylene blue which was absorbed in phosphate buffer pH 11. We did the absorption 3 times to get three samples because we need to do release in three different buffer solutions. An amount of 0.125 g of poly(HEMA-*co*-EGDMA) beads loaded with methylene blue was added in 50 mL of phosphate buffer with pH 11 in a beaker. The sample was kept at 37 °C with shaking at 125 rpm. At given time intervals, 1 mL was taken from release solution and replaced with 1 mL of fresh phosphate buffer pH 11. Then at the absorbance of the solution was taken at 660 nm using the CT-2200 UV-Vis spectrophotometer. Release% was calculated according to the methylene blue calibration curve using the linear equation in phosphate buffer pH 11, **Y= 0.1528x + 0.0982.** The same procedure was repeated at pH 7.4 and pH 2.0. The corresponding calibration curves in phosphate buffer at pH 7.4, **Y= 0.1341x + 0.0925** and in acetate buffer at pH 2.0, **Y= 0.1266x + 0.0671** were used to carry out the necessary calculations (Figure 11).

Cumulative release % for MB has been calculated according to equation (3) given above but M_∞ is taken as the amount of drug loaded in the sample.

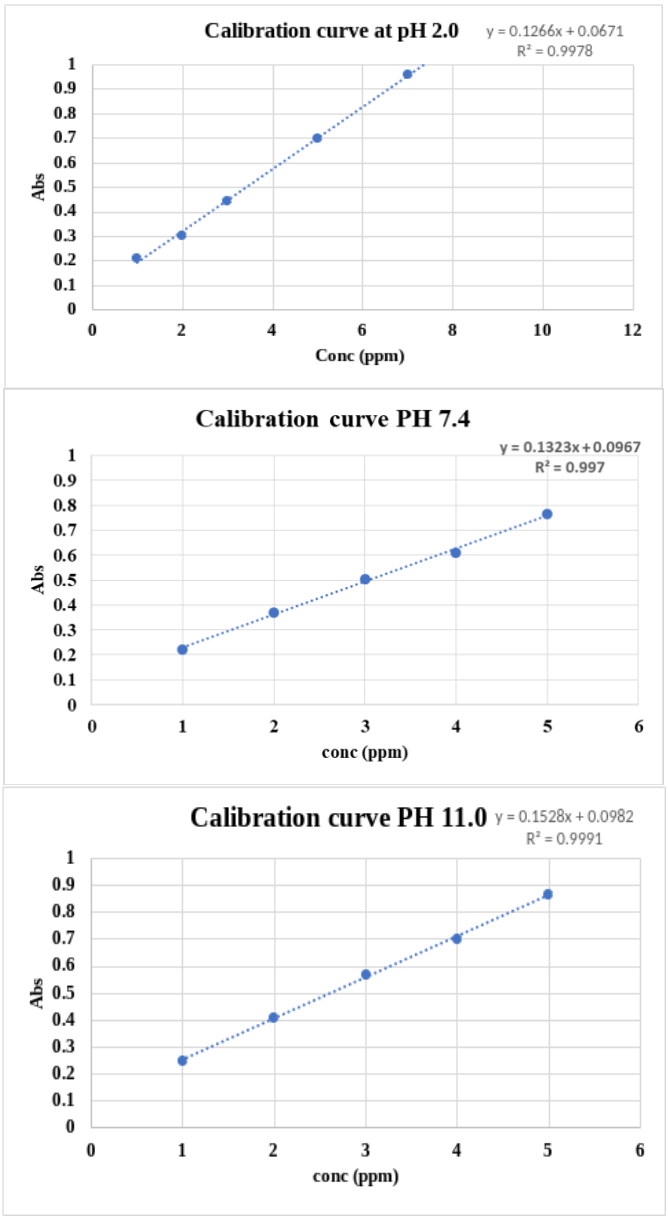


Figure 11: Calibration curves for Methylene Blue.

3.9.3 Drug Release Kinetics of Flurbiprofen and Methylene Blue

Zero-order kinetics: In zero-order kinetics, the rate of drug release is constant over time, meaning that the amount of drug released per unit time is the same throughout the entire release period (equation 4) [1,19].

$$Q_t = Q_0 - K_0 t \quad (4)$$

Where: Q_t is the amount of drug release at time t, K_0 is the apparent dissolution rate constant or zero-order release constant and Q_0 is the initial concentration of drug in the solution.

First-order kinetics: In first-order kinetics, the amount of drug still present in the dose form determines how quickly the drug is released. The rate of drug release gradually decreases as it is released (equation 5) [1,19].

$$\boldsymbol{log\ C} = (\boldsymbol{log\ C_0} - (\frac{\boldsymbol{Kt}}{\boldsymbol{2.303}})) \quad (5)$$

Where: C_0 is the initial concentration of the drug, K is the first order rate constant.

Higuchi kinetics: In Higuchi kinetics, the rate of drug release is proportional to the square root of time. This type of kinetics is often seen in matrix-type drug delivery systems (equation 6) [1,19,20].

$$\boldsymbol{F_t} = (\boldsymbol{Q} - \boldsymbol{K_H}\sqrt{\boldsymbol{t}}) \quad (6)$$

Where: K_H is the Higuchi dissolution constant. Q is the amount of drug released in time t per unit area, f_t is a fraction of drug released at time t.

Korsmeyer- Peppas: A basic "Power law" model of drug release from a polymeric system (equation 7) [1,19,20]**.**

$$\boldsymbol{log}\left(\frac{\boldsymbol{M_t}}{\boldsymbol{M_\infty}}\right) = \boldsymbol{log\ K_p} + \boldsymbol{n\ log\ t} \quad (7)$$

Where: $\frac{M_t}{M_\infty}$ is a fraction of drug released at time t, M_t is the amount of drug released in time t, $M\infty$ is the amount of drug released after time ∞, n is the diffusional exponent or drug release exponent [1,19,20].

Table 3: Drug Release Mechanisms

Exponent (n)	**Drug release Mechanisms**
(n) ≤ 0.45	Fickian diffusion (case I diffusional)
0.45 < n < 0.89	Anomalous (non-fickian) diffusion
(n) = 0.89	Zero- order (Case II transport)
(n) > 0.89	Super case II transport

3.10 Characterizations

3.10.1 Fourier Transform Infrared (FTIR-ATR) Analysis

Chemical structure determination and the investigation of the interactions between poly(HEMA-*co*-EGDMA), poly(HEMA-*co*-EGDMA) with flurbiprofen, poly(HEMA-co-EGDMA) with methylene blue before and after release were carried out using the Perkin Elmer FTIR-ATR spectrophotometer.

3.10.2 Ultraviolet-Visible (Uv-Vis) Spectrometry

A CT-2200 UV-Vis spectrophotometer was used.

3.10.3 Scanning Electron Microscope (SEM) Analysis

The morphology of the surface for each polymer had been investigated by using SEM analysis in HUNITEK University in Ankara.

Chapter 4

RESULTS AND DISCUSSION

4.1 Poly(HEMA-*co*-EGDMA) Microparticle Synthesis

Three samples of poly(HEMA-*co*-EGDMA) beads S1, S2, and S3 were obtained with different concentrations of crosslinker. All these samples were prepared under the same conditions but with different ratios of monomer and crosslinker. The monomer: crosslinker mass ratios are 1:0.3, 1:0.6 and 1:0.9 for S1, S2 and S3 respectively as given in Table 2. The yield of sample 1 (S1) is 52.07% (S2) is 49.98% and (S3) is 94.81% (Figure 12). As the swelling capacity, drug loading efficiency, and drug release kinetics are affected by the particle size distribution of the sample, each sample was fractionated according to size. A given fraction was used in further experiments to maintain a relatively narrower size distribution. After the fractionation process, four different fractions (F) were obtained the full range being 710 μm - 75 μm. The yield of copolymer (S1) is 1.5321 g. The weight of particles of size >710 μm (S1F1) is 0.7889 corresponding to 51.49% of the total product. That of particles (S1F2) with 710-212 μm is 0.5756 g which makes up the of 37.57% of the particles by weight. The mass of the third fraction is 0.1626 g for particle size 212-150 μm (S1F3) equivalent to 10.61%. These values are 0.005 g and 0.326 % for the fourth fraction, 75-150 μm (S1F4). For sample 2 (S2), % of >710 μm (S2F1) is 48.03% of the total product, and 710-212 (S2F2) μm fraction is 29.82%. Also, the % of 212-150 μm (S2F3) is 7.91% and the % of the last fraction 75-150 μm (S2F4) is 1.97%.

On the other hand, the yield of >710 µm (S3F1) is 58.77% of the total product, and the yield of (S3F2) µm is 23.5%. Also, the third fraction 212-150 µm (S3F3) is 4.28% and 1.75% is from 75-150 µm (S3F4) (Figure 13).

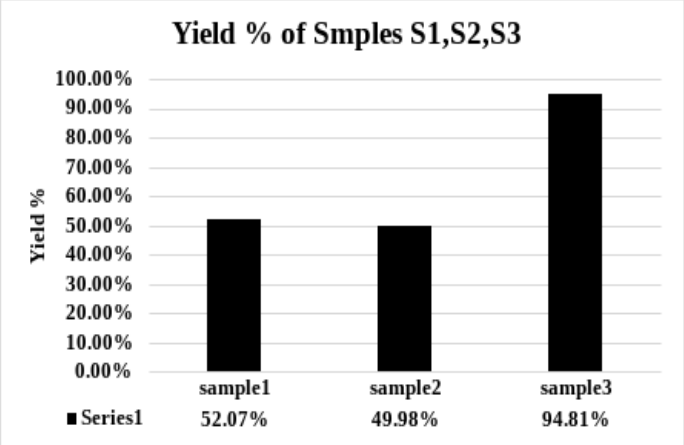


Figure 12: Yield % of Samples S1, S2, and S3.

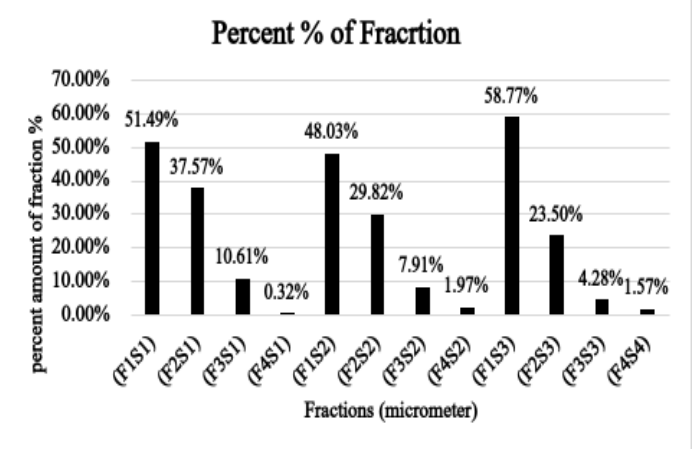


Figure 13: Yield % of Fractions.

4.2 Swelling Behaviour

4.2.1 Swelling Capacity of Poly(HEMA-*co*-EGDMA) Micropartiacles in Acetate Buffer pH 2.0

The swelling test was applied on sample 3 (S3) with F1 and F2. The effect of acetate buffer pH 2.0 on swelling % is given in (Figure 14). S3F1 is >710 μm and (F2) is 710-212 μm. The weight of the copolymer before being placed in the solution was 0.15 ± 0.001g. After 10 minutes, it was observed that the first fraction, S3F1 has a swelling % of 115% ± 53.26 SD and the second fraction S3F2 190% ± 0.511 SD. After 30 minutes, the swelling % of S3F1 is 275% ± 41.625 SD and that of S3F2 is 340% ± 0.632 SD. Then, the swelling rate of S3F1 slows down. At the end of the first hour, % swelling value of S3F1 is 321% ± 27.531 SD and that of S3F2 is 360% ± 2.336 SD. At the end of three hours, the values are S3F1 301% ± 9.383 SD, and S3F2 is 321% ± 0.615 SD. After 12 hours, S3F1, and S3F2 % swelling values increase slightly, S3F2 reaching an equilibrium % swelling value of 374% ± 3.502 SD, by 24 hours. Here, we observe the effect of particle size on the swelling capacity. S3F2, the fraction with smaller particle size demonstrates higher swelling capacity than S3F1. However, S3F2 degrades in solution faster than S3F1 due to the smaller particle size. Both samples degrade in solution over time. Both fractions need 30 days to get a high % of degradation in the phosphate buffer solution (Figure 14).

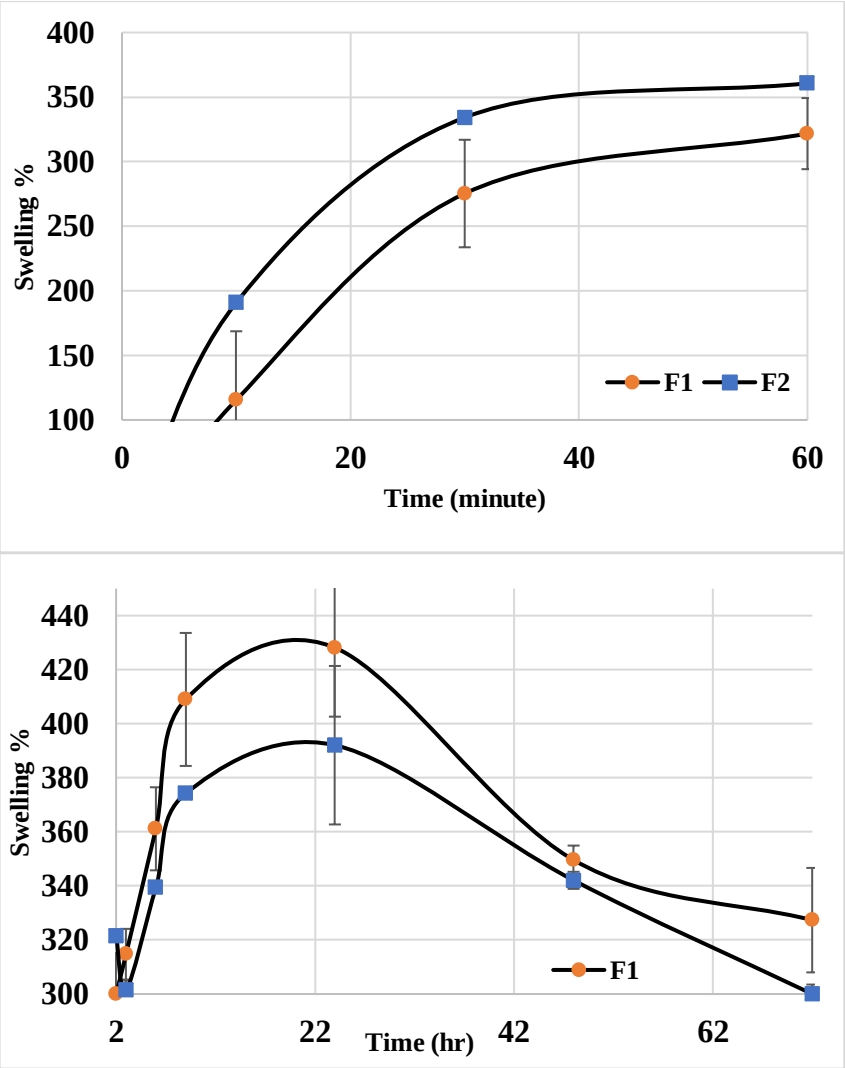


Figure 14: Swelling % of Fractions (S3F1) and (S3F2) in Acetate Buffer pH 2.0.

4.2.2 Swelling Capacity of Poly(HEMA-*co*-EGDMA) in Phosphate Buffer pH 7.4

Referring to (Figure 15), when the polymer was added to phosphate buffer 7.4, we noticed a significant increase in the swelling % in S3F1 to 218% ± 6.599 SD and 279% ± 1.493 SD in S3F2 during first 10 minutes. The swelling % of S3F1 is 308% ± 6.835 SD and swelling % of S3F2 is 466% after 30 minutes. During the first hour, the % of swelling in S3F1 stays constant at 308% ± 18.007 SD but S3F2 increases to 413% ± 54.117 SD. Two hours later, the swelling % of S3F1 significantly decreases to 200% ± 28.425 SD and that of S3F2 to 380%. On the other hand, S3F1 increases again at 6 hours to reach 420% ± 9.475 SD but S3F2 decreases to 346% ± 15.05 SD. After 24

hours both fractions continue to swell more up to S3F1 to 490% ± 11.266 SD and S3F2 to 490% ± 21.260 SD. (Figure 15). These results suggest a swelling deswelling equilibrium behaviour over time.

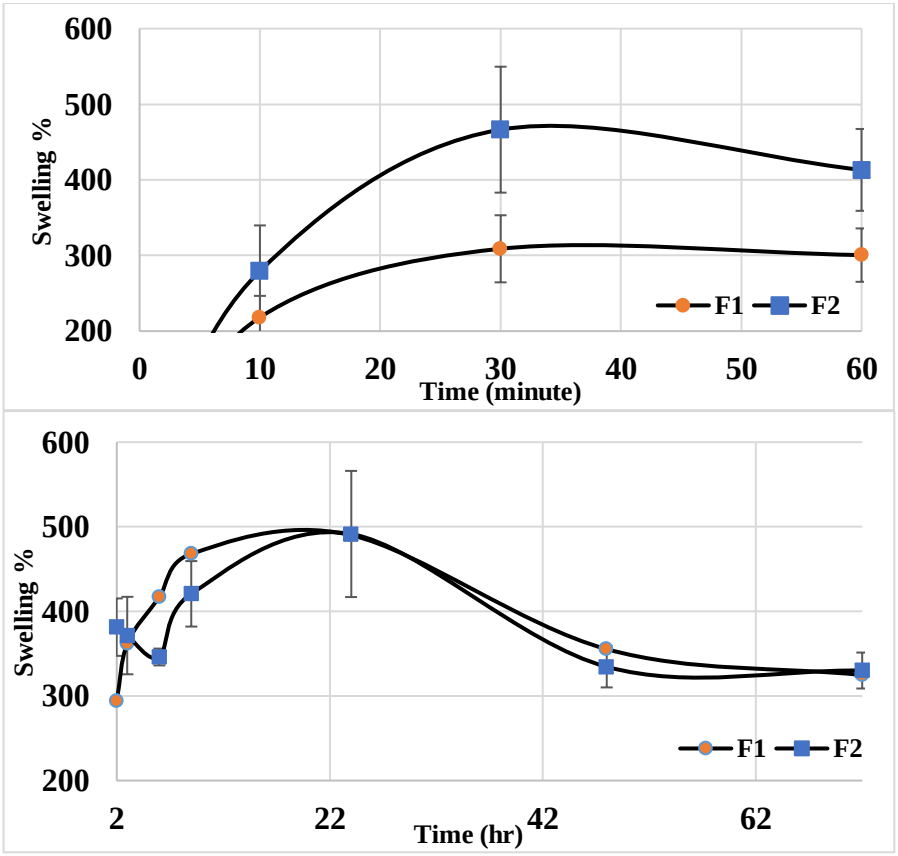


Figure 15: Swelling % of Fractions S3F1 and S3F2 in Phosphate Buffer pH 7.4.

4.2.3 Swelling Capacity of Poly(HEMA-*co*-EGDMA) in Phosphate Buffer pH 11.0

The samples swell rapidly in pH 11 buffer solution S3F1and S3F2 reaching 320% ± 6.599 SD, and in 523% ± 1.4932 SD respectively in 10 minutes. After one hour, the % swelling of S3F1 increases in to 447% ± 3.447 SD and that of S3F2 it reaches 550% ± 5.286 SD. Equilibrium is reached within 48 hours and after 72 hours both begins to decline slightly, to reach on the ninth day in S3F1 to 400% and in S3F2 to 407% (Figure 16).

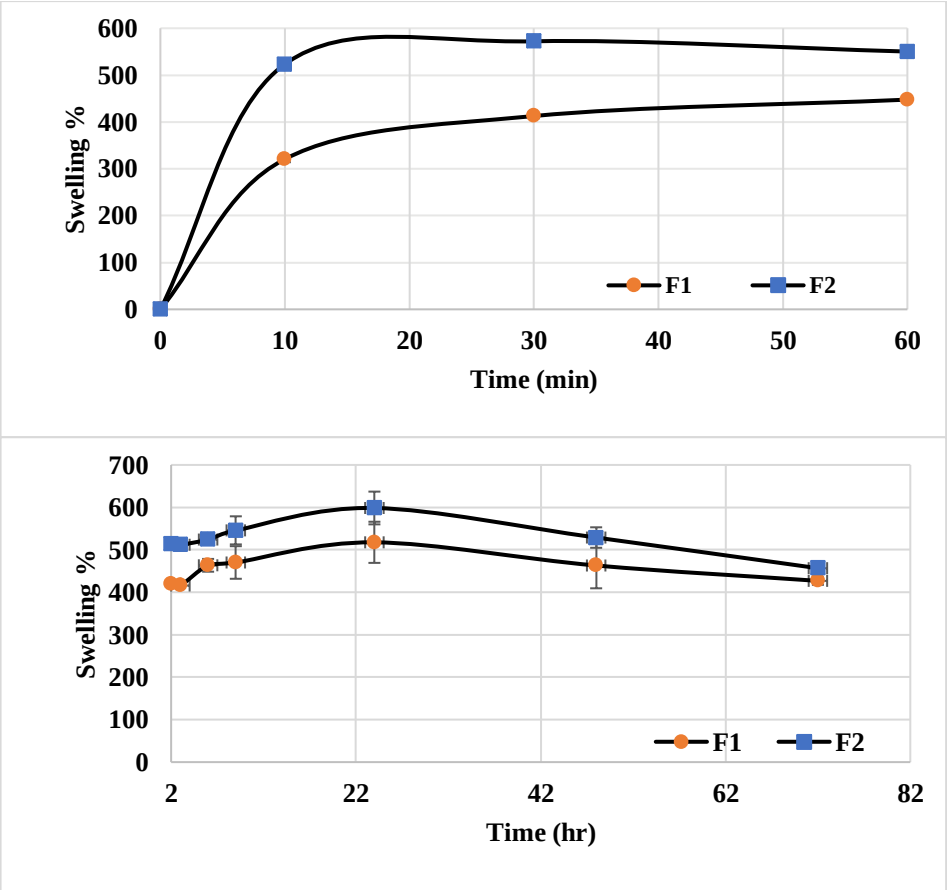


Figure 16: Swelling % of Fractions S3F1 and S3F2 in Phosphate Buffer pH 11.

In short, poly(HEMA-*co*-EGDMA) microparticles swell in aqueous solution and remain intact over a period of time up to 72 hours. The fraction with smaller size exhibits higher swelling % and faster degradation rates at all pH values due to higher surface area. Swelling% values are higher in pH 11 solution due to deprotonation of the -OH group of HEMA in basic media. Repulsive forces in between deprotonated -OH groups cause higher swelling capability of the microparticles. Hence, they are potential drug delivery systems whose drug loading and release capacities can be adjusted according to the pH of the medium and particle size.

4.3 Flurbiprofen Loading and Release

Flurbiprofen was loaded into poly(HEMA-*co*-EGDMA) during suspension polymerization. We obtained three samples, S1, S2 and S3, but we used just one

sample with the first two fractions. S3F1 (>710 μm), S3F2 (710-212 μm). This is due to their average size and consistency of granule size. The amount of flurbiprofen loaded into poly(HEMA-*co*-EGDMA) was 14.5 mg. Accordingly, the encapsulation% of flurbiprofen in S3 is 97%. However, since a fractionation was carried out according to sample size, the amount of drug encapsulated in each sample fraction is an unknown fraction of the total amount 14.5 mg of FB encapsulated. Therefore, the amount encapsulated in each fraction was taken as the mass of the drug released at infinite time (M_∞).

Flurbiprofen release results from S3F2, and S3F1 show, the % release value at first 10 minutes is 57% and 83% for S3F2, increases slightly up to 93% for S3F1 and 97% within 48 hours. After 72 hours, release % is 100% in both of samples (Figure 17). It can be concluded that FB release from the samples exhibits a burst release behaviour due to high particle surface area with high swelling capacity in aqueous solution. Moreover, it is probable that most of the FB sample adhered on the surface of the microparticles rather than being encapsulated in the particles. More evidence is needed by surface analysis to confirm this prediction. It was decided at this point that poly(HEMA-*co*-EGDMA) microparticles were not promising release matrices for FB. Furthermore, there are difficulties in establishing the amount of FB encapsulated in each fraction. FB is a hydrophobic drug and cannot be loaded in the microparticles by the simpler absorption method. The difficulty here is that poly(HEMA-*co*-EGDMA) microparticles do not swell in ethanol or acidic solutions that are solvents for FB. The system needs elaborations that will be out of the scope of a master's thesis. Therefore, further investigations on this system have been postponed for future studies.

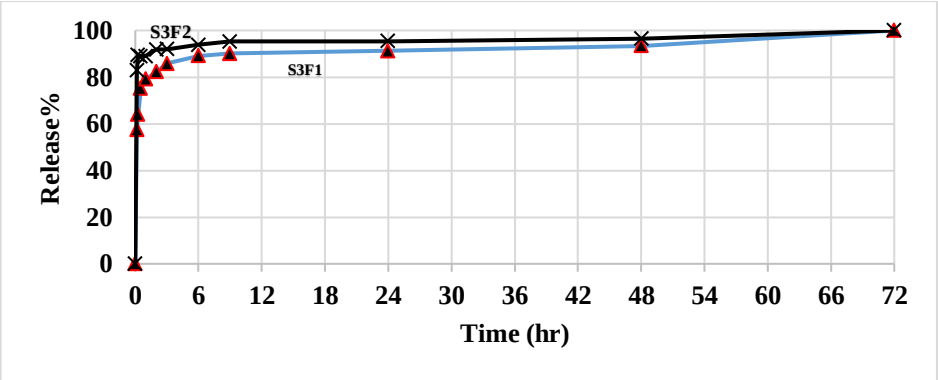


Figure 17: Release % of Flurbiprofen.

4.4 Loading of Methylene Blue in the Microparticles by Absorption in Buffer Solutions

We turned our attention to a hydrophilic model drug, methylene blue, MB, for drug loading and release studies using poly(HEMA-*co*-EGDMA) microparticles as potential matrices. Since MB is a hydrophilic, water soluble compound the absorption method was taken for drug loading. MB was absorbed into the samples at different pH values. The results show that the poly(HEMA-*co*-EGDMA) is more absorbent in pH 11.0, as the total absorption value is 80.04%, which is a better % of absorption (Encapsulation %) compared to pH 7.4, which is 65%, and pH 2.0 68.4%. During the first 10 minutes, the poly(HEMA-*co*-EGDMA) absorbed 0.60 mg of methylene blue, then it began to increase, and the amount of methylene blue absorbed 4.00 mg after 24 hours. In pH 7.4, the total absorbed (Encapsulation) amount of MB is 3.24 mg and 3.4 mg in pH 2.0, so the release test has applied on the sample which is absorbed in dye solution with pH 11 because it has high encapsulation % (Figure 18).

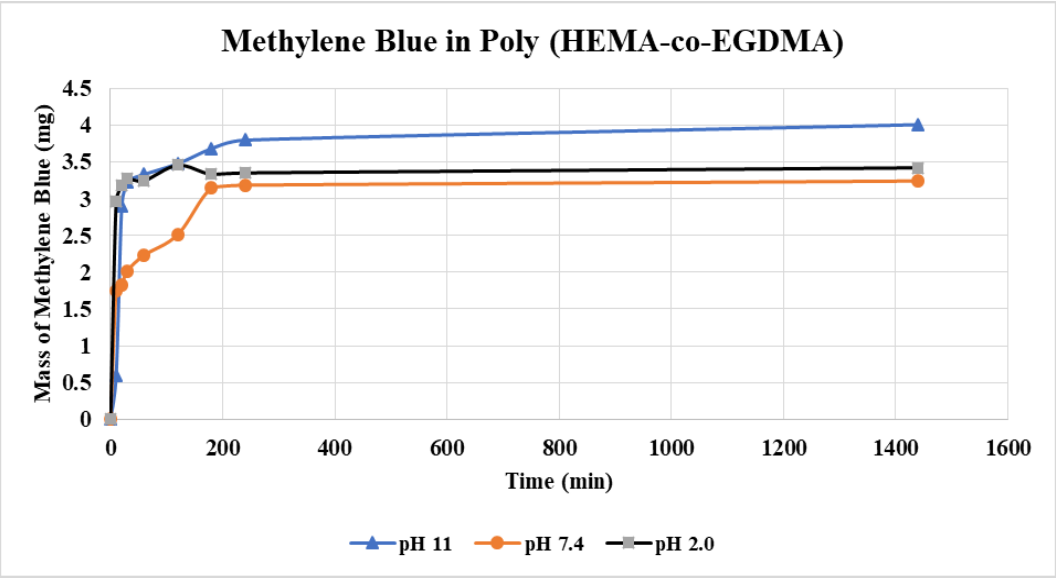


Figure 18: Absorption Values of Methylene Blue.

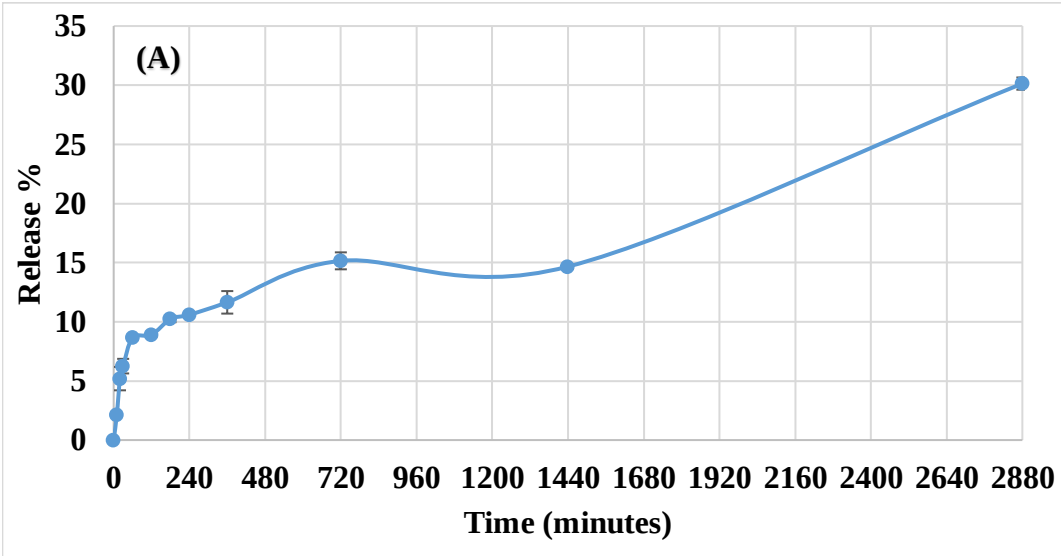
4.5 Release of Methylene Blue

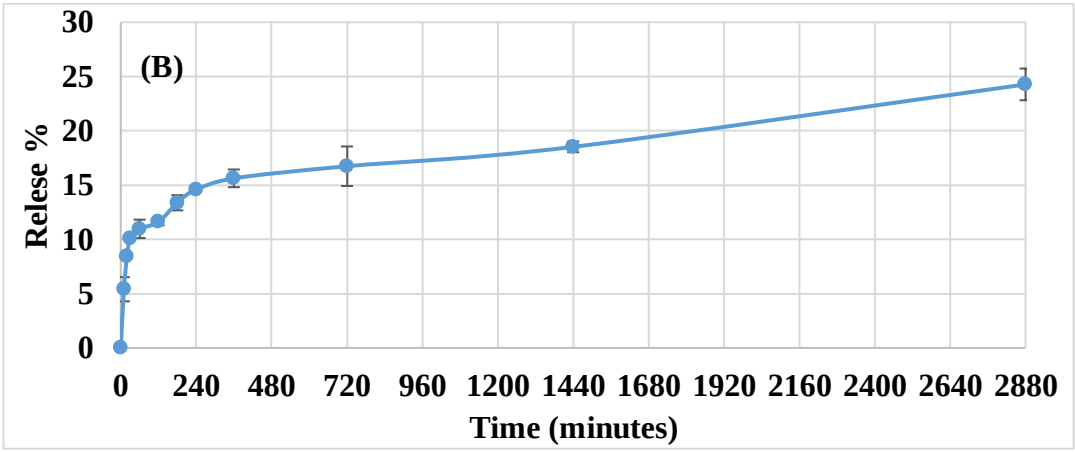
The results of the release test in phosphate buffer pH 11 shown that the release % in the first 10 minutes is 2% ± 0.126 SD, this percentage is good for controlling the release. The release % began to rise gradually and slowly to reach 15% ± 0.716 SD after 12 hours of placing the copolymer pH 11 in phosphate buffer pH 11 (Figure 19). The release % stabilized between 10% ± 0.101 SD to 15% ± 0.182 SD, from 4 hours until 24 hours, then it began to increase to 31% at ± 0.516 SD 48 hours. These results are better than the release value of flurbiprofen because we have been able to adjust the release % until 24 hours (Figure 19).

When the poly(HEMA-*co*-EGDMA) was added in phosphate buffer pH 7.4, the results do not differ too much from the results in pH 11.0, where at first 10 minutes the release % is 5%, ± 1.114 SD after 30 minutes it increases to 9% ± 0.108 SD. It continues to rise slightly and slowly to reach 15.5% ± 0.185 SD after 4 hours, and it continues to rise gradually until 24 hours to reach 19.5% ± 0.494 SD. The % of release value is 25% ± 1.463 SD at 48 hours (Figure 19).

Ten minutes after placing the copolymer in acetate buffer pH 2.0, the release % is 14.5% ± 0.258 SD of methylene blue. It also rises after an hour to reach 15% ± 0.369 SD, and the release % of methylene blue is stable around 19% to 22% ± 0.219 SD up at 12 hours and then reached 25% ± 0.686 SD after 24 hours (Figure 19).

After the issuance of these results, we found that the S3F2 (pH 11.0, pH 7.4 and pH 2.0) in controlling the release of methylene blue are better compared to controlling the release of flurbiprofen (Figure 19). This should be due to successful diffusion of MB into the microparticles because of hydrophilicity of MB. Furthermore, strong intermolecular interactions between MB and poly(HEMA-*co*-EGDMA) microparticles cause retention of a given fraction of MB in the microparticles. It should also be noted that the definition of reference value, M_∞, used in % release calculations for FB and MB are different. In the FB the cumulative mass of the drug released at equilibrium, M_∞, is taken as the reference value according to equation (3), whereas in the MB case this value is the amount loaded in the sample. The reason for this difference is different loading methods applied for FB and MB. FB has been loaded *in situ* during suspension polymerization, and then the samples were fractionated according to size. Hence, it was not possible to calculate the amount of drug loaded in each fraction. MB, on the other hand, was loaded post formation of the microparticles by the absorption method. The amount loaded could be determined by vis spectrophotometry. To be able to make a better comparison % release values for MB were repeated by taking M_∞ as the cumulative mass of the drug released at equilibrium like FB. According to these calculations % release of MB at pH 7.4 at 6 h, corresponds to 68.75%. Under similar conditions FB release is 89.1% (S3F2) and 93.4% (S3F1). These values support the discussion given above.





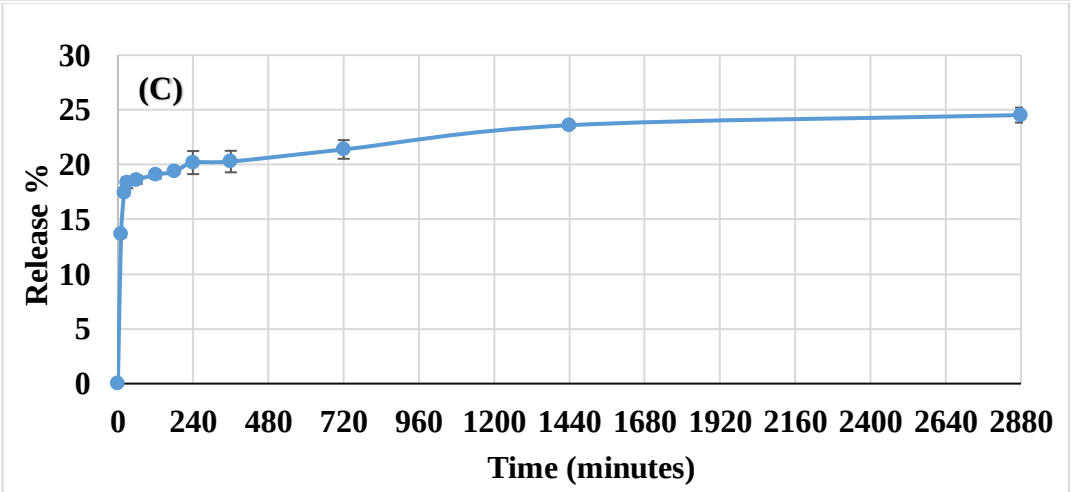


Figure 19: Release % of Methylene Blue in Buffer Solutions(A) pH 11, (B) pH 7.4 and (C) pH 2.0.

To obtain release results for methylene blue three release kinetics models, zero-order release kinetics mode, first-order release kinetics, Higuchi model and Korsmeyer Peppas were tested [20,21]. After we applied all these models on three samples to

release pH 2.0, pH 7.4 and pH 11.0, the best model was found to be Higuchi, because the values of (R^2) were acceptable and all of the R^2 values of each sample were close to each other.

The Higuchi model widely applies to drug release from complex systems in which diffusion is the main release mechanism. The substance's release is proportional to the square root of time, according to this model [1,20,21].

R^2 indicates high values near one, and the results of our samples are as follows pH 2.0 = 0.8889, pH 7.4 = 0.9233, and pH 11 = 0.9413. These results indicate the relationship between the estimated values from the Higuchi model equation and the actual experimental data points. In other words, the Higuchi model appropriately describes the release kinetics for the specific medication and delivery mechanism under consideration (Table 4).

Table 4: Results of Release Kinetics for Methylene Blue.

Kinetics Model		**Release in pH 11**	**Release in pH 7.4**	**Release in pH 2.0**
Zero Order	**(Eq)**	Y=6.8091x+0.3942	Y=0.3057x+0.3597	Y=0.9287x+0.2613
	R^2	0.7248	0.8988	0.9814
	K_0	0.394	0.359	0.2613
First Order	**(Eq)**	Y=0.1898x+0.0213	Y=0.5946x+0.0183	Y=0.3021x+0.0175
	R^2	0.8363	0.5068	0.7832
	K_1	0.437	1.369	0.695
Higuchi Model	**(Eq)**	Y=1.3817x-4.9407	Y=2.8237x-2.658	Y=1.7389x-3.8393
	R^2	0.9413	0.9233	0.8889
	K_H	4.9407	0.9233	3.8393
Korsmeyer- Peppas	**(Eq)**	Y=0.82461x-1.9401	Y=0.4931x-1.0451	Y=0.1527x-0.3617
	K_p	0.0114	0.4348	0.0901
	n	0.8246	0.4931	0.1527
	R^2	0.8414	0.8861	0.7727

4.6 Comparison of Methylene Blue and Flurbiprofen Release with Previous Works

A detailed analysis of previous work on flurbiprofen release and methylene blue release from several different matrices has been presented in Chapter 2, in the literature review section. Here, in Table 5 the results obtained in this study regarding flurbiprofen release are compared to the findings reported in the literature. Studies on flurbiprofen loading and release are scarce in the literature. One recent study carried out in our laboratory reports pulsatile flurbiprofen release from polyVCi/poly(HEMA-*co*-EGDMA) dual network polymer film matrices [1]. The flurbiprofen loading and release behaviour reported in this study is comparable to that reported for ibuprofen sodium salt/poly(HEMA-co- trimethylolpropane trimethacrylate) system where 60% of the drug was released within 15 minutes [14]. Ca-alginate beads did not allow FB release. Only up to 4.8% FB release was observed in that study [15]. It can be understood from this analysis that FB loading and release via polymer beads/microparticles remains a challenge. Either low loading capacities or too fast or too slow release profiles are obtained. This is due to the hydrophobic nature of the drug that is incompatible with hydrophilic polymer matrices. Poly(HEMA-*co*-EGDMA) system reported in this have got the potential to be modified in terms of size distribution, porosity, and degree of crosslinking to optimize FB loading and release through this system. Furthermore, a hybrid system including natural polymers such as alginate and/or chitosan may provide useful outcomes.

Table 5: Comparison of Flurbiprofen Release in Previous Works.

System	Encapsulation %	Release %	Reference
PolyVCi-Poly HEMA-Poly EGDMA	Sample number 1 FB1 73%	Two step release the first step between 0-5h with 25% release. Second step, between 5-35h	[1]
Poly(HEMA-*co*-trimethylolpropane trimethacrylate)	51 mg/g is the amount of drug loaded	During first 15 minutes, 60 % of drug released	[14]
Ca-alginate beads		Up to 4.8% release	[15]
Poly (HEMA-*co*-EGDMA)	97% loaded of flurbiprofen	During first 10 minutes, release 83% is	This work

When results of methylene blue loading and release obtained in this work are compared to those of other workers as summarized in Table 6, it can be deduced that our poly(HEMA-*co*-EGDMA)/ MB system works well for controlled delivery. Up to 31% release is obtained within 48 hours which is a reasonable time period to be considered for a sustained drug delivery system. In other studies given in Table 6, either low loading or very fast release profiles are reported [16-17]. The poly(HEMA-*co*-EGDMA) microparticles can still be further modified to optimize MB release following a similar strategy proposed for FB release given above.

Table 6: Comparison of Methylene Blue Release in Previous Works.

System	Encapsulation %	Release %	References
Poly (HEMA-*co*-EGDMA) with Methylene Blue	80% methylene blue loading	% Release values are 31% in pH 11, 24% in pH 7.4 and 25% in pH 2.0 after 48 hours	This work
(PU/PAA) LbLFilms	Enc % increase by increasing the pH of MB solution. after 1300 min the absorption was 100%	Release % completed 100% after 130 minutes in pH 9.0 and pH 3.0 and completed after 140 min in pH 5	[16]
Chitosan/Polyacrylic Acid	Amount of MB loaded is 2.8 mg/mL in 100 mM NaCl, 2.7 mg/mL in 10 mM NaCl and 3.0 mg/L in 50 mM NaCl	0.60 mM in 10mM NaCl 0.75 mg/L in 50mM NaCl and 0.85 mg/L in 100 mM NaCl 2-18 min	[17]

4.7 FTIR Analysis

FTIR spectra of poly(HEMA-*co*-EGDMA), methylene blue and methylene blue loaded, poly(HEMA-*co*-EGDMA) are presented in (Figure 20). In spectrum of poly(HEMA-*co*-EGDMA), sharp and intense peaks at 1716 cm^{-1} and 1150 cm^{-1} represents the vibrational stretching of carbonyl groups and C-O single bonds in the structure. Vibrations of C-O single bond from the ester functional group detected at 1249 cm^{-1}. This confirms the presence of HEMA and EGDMA in structure of copolymer. Peak at 1452 cm^{-1}corresponds to the C-H bending from the copolymer structure.

In the MB spectrum, strong intense peak at 1595 cm^{-1}indicate the vibrations of C=N. Signals ranges from 1489 cm^{-1} to 1422 cm^{-1} corresponds to the presence of C-H

bending vibrations of MB. Methyl groups in the structure shows bending vibrations at around 1336 cm^{-1}. Presence of C-N and N-N bonds in the structure of MB are represented with 1251 cm^{-1} and 1224 cm^{-1}, respectively. In addition to those bending vibrations of C-N is detected at 1141 cm^{-1}. Two ether moiety via the signal at 1066 cm^{-1}. Two consecutive peaks at 876 cm^{-1} and 816 cm^{-1}belongs to bending vibrations of C-H.

In spectrum of MB loaded into poly(HEMA-co-EGDMA) peaks at 1599 cm^{-1} C=N, 1456 cm^{-1} C=C stretching, 1393 cm^{-1} $–CH_2$ and $–CH_3$ groups, and finally 1338 cm^{-1} (Bending vibrations from $–CH_3$). Confirms the loading of MB to the copolymer sample.

In the flurbiprofen spectrum O-H stretching is observed at 3250 cm^{-1}. Two main characteristic peaks of drug located at 1697 cm^{-1} and 1216 cm^{-1} represent carbonyl group and C-F group respectively. Stretching vibrations of C=C detected via the presence of consecutive three peaks at 1697 cm^{-1}, 1581 cm^{-1} and 1564 cm^{-1}. Bending of C=C is located at 958 cm^{-1} (Figure 21).

In the spectrum of flurbiprofen loaded into copolymer sample, presence of C=O stretching, 1638 cm^{-1} (1592 cm^{-1} and 1558 cm^{-1}), and C=C bending 944 cm^{-1} demonstrates the successful loading of flurbiprofen into poly(HEMA-*co*-EGDMA) microparticles.

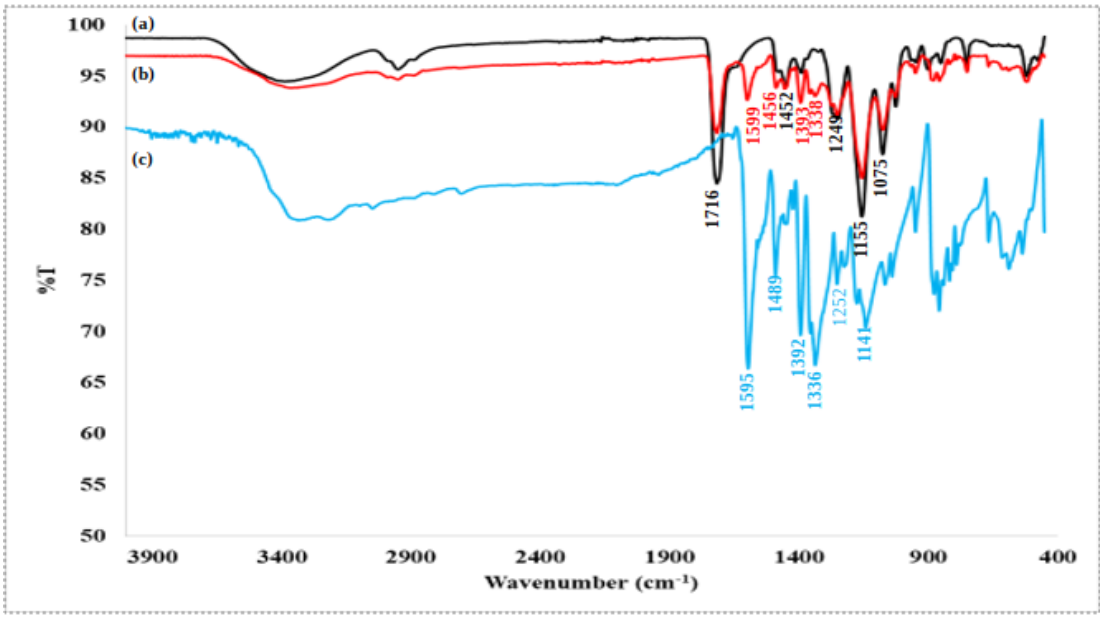


Figure 20: FTIR Spectra of (a) Poly(HEMA-*co*-EGDMA), (b) Methylene blue, (c) Methylene Blue Loaded Poly(HEMA-*co*-EGDMA).

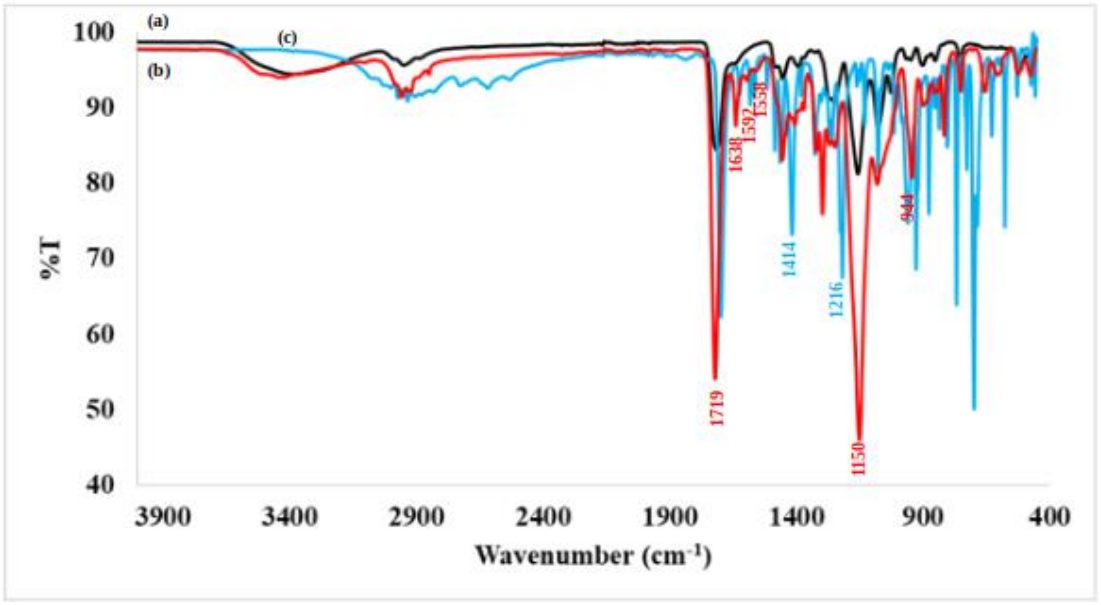


Figure 21: FTIR Spectra of (a) Poly(HEMA-*co*-EGDMA), (b) Flurbiprofen, (c) Flurbiprofen Loaded Poly(HEMA-*co*-EGDMA).

4.8 Scanning Electron Microscope SEM Analysis

The shape and diameter of the poly(HEMA-*co*-EGDMA)/FB(A), poly(HEMA-*co*-EGDMA), also poly(HEMA-*co*-EGDMA) with MB and poly(HEMA-*co*-EGDMA) after release analyzed by using SEM are shown in (Figure 27)(A-D).

The poly(HEMA-*co*-EGDMA)(A) shows regular particles with an average diameter of 0.518 µm. F2, 710-212 µm. While the sample (B) with FMthe average size of microbeads is on the average 0.695 µm diameter. On the other hand, the average diameter of poly(HEMA-*co*-EGDMA) (C) loaded with MB is 0.675 µm and the particles are more agglomerated compared to other samples. After release of methylene blue, average of sample (D) is 0.626 µm with regular shape of particles (Figure 22).

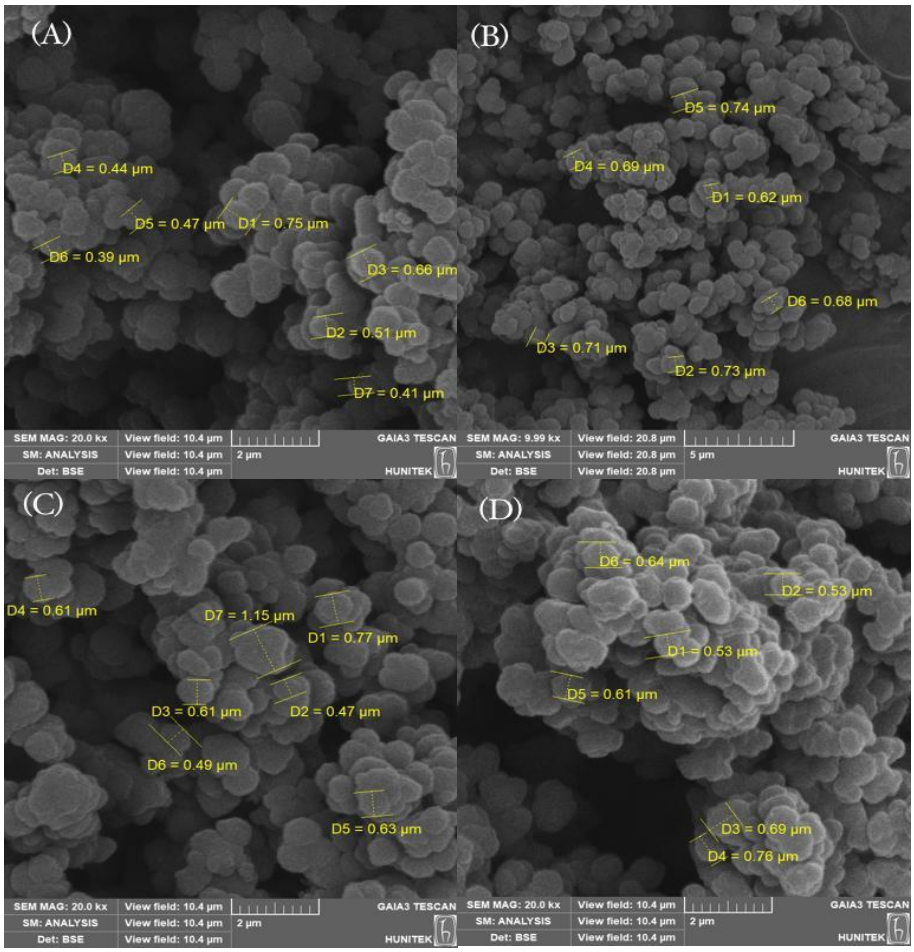


Figure 22: SEM analysis (A) Poly(HEMA-*co*-EGDMA), (B) Poly(HEMA-*co*-EGDMA)/FB, (C) Poly(HEMA-*co*-EGDMA)/MB, (D) Poly(HEMA-*co*-EGDMA) After the Release of MB.

Chapter 5

CONCLUSION

Poly(HEMA-*co*-EGDMA) microparticles can successfully be prepared by suspension polymerization. Although a high loading capacity is obtained for FB, the release profile is not satisfactory since 80% of the drug loaded is released within 10 minutes. This behaviour is attributed to high degree of swelling of the microparticles in aqueous solution. Up to 31% release is obtained within 48 hours for MB loaded in the poly(HEMA-*co*-EGDMA) microparticles synthesized. Even though the microparticles swell to a high degree in aqueous solution, the MB release is limited compared to that of FB. This must be due to stronger intermolecular interactions in between the polymer matric and MB.

It can be concluded that poly(HEMA-*co*-EGDMA) system reported in this study have got the potential to be modified in terms of size distribution, porosity, and degree of crosslinking to optimize both FB and MB loading and release. Furthermore, a hybrid system including natural polymers such as alginate and/or chitosan, or more hydrophobic methacrylates may provide useful outcomes to control drug release by modifying hydrophilicity/hydrophobicity balance of the microparticles.

REFERENCES

1. Mamoon Alokour, Elvan Yilmaz. (2021). A polymer hybrid film based on poly(vinyl cinnamate) and poly(2-hydroxy ethylmethacrylate) for controlled flurbiprofen release. Journal of Polymer Research. 28(4), 2-14 doi: 10.1007/s10965-021-02493-2.24.

2. P. Ferreira, J. F. J. Coelho, J. F. Almeida and M. H. Gi. (2011). Photocrosslinkable Polymers for Biomedical Applications. 3(4), 4-6. doi: 10.5772/18752.

3. Daniel Horak, Frantisek Lennicky, ladimir Rehak and Frantisek Sevek. Porous PolyHEMA Beads Prepared by Suspension Polymerization in Aqueous Medium Journal of Applied Polymer Science., Vol. 49, pp. 2041-2050, 1993.

4. Haroon Rashid, Mahmood Ahmad, Muhammad Usman Minhas, Muhammad Sohail and Muhammad Fakhruddir. Synthesis and Characterization of Poly(hydroxyethyl methacrylate-co-methacrylic acid) Cross Linked Polymeric Network for the Delivery of Analgesic Agent. J.Chem.Soc.Pak., Vol. 37, no. 05, pp. 999, 2015.

5. Celizabeth M. BoazakVaughn, GreeneVaughn, GreeneDebra T and AugusteDebra T Augustehemical. (2015). Structures of HEMA and crosslinkers EGDMA, VM and AM. journal pone. 3(2), 2-3 doi: 10.137.0215895.001

6. Y. Kodama, M. Barsbay, and O. Güven, "Poly(2-hydroxyethyl methacrylate) (PHEMA) grafted polyethylene/polypropylene (PE/PP) nonwoven fabric by y-

initiation: Synthesis, characterization and benefits of RAFT mediation,"Radiat. Phys. Chem., vol. 105, pp. 31-38, 2014.

7. Michał Cegłowski, Joanna Kurczewska, Aleksandra Lusina, Tomasz Nazim and Piotr Ruszkowski. (2015). EGDMA and TRIM-Based Microparticles Imprinted with 5-Fluorouracil for Prolonged Drug Delivery. 14(5), 1027. doi: 10.3390/polym14051027.

8. Nihal Ermis. (2022). Drug Delivery Application of Poly (2-hydroxyethyl methacrylate)/Ethylene glycol Dimethacrylate Composite Hydrogel. Journal of Science. 35(4): 1318-1331. doi: 10.35378/gujs.947687.

9. Rania Itani, Lama Soubra, Samar Karout, Deema Rahme, Lina Karout, and Hani M J Khojah. (2022). Primary Dysmenorrhea: Pathophysiology, Diagnosis, and Treatment Updates. National library of medicine. 43(2): 101–108. doi: 10.4082/kjfm.21.0103.

10. Shubham Tripathi, Arpita Singh, Mayank Tiwari, Sonali Singh, Shubham Tiwari, Abhishek Kumar. (2022). A Review on Topical Drug Delivery System: Proniosomal Powder of Flurbiprofen. International Journal of Cheminformatics Research. 8(2): 26–31p, doi: 10.37628/IJCIR.

11. Santoso a, R. Ediati a, Y. Kusumawati a, H. Bahruji b, D.O. Sulistiono a, D. Prasetyoko. (2020). Review on recent advances of carbon based adsorbent for methylene blue removal from waste water. Materialstoday chemistry. 105 (20): 199-208, doi: org/10.1016/j.mtchem.2019.100233.

12. Pushparajah Mak, Renita S. MDLiebelt and Erica L. MD. (2021). Methylene Blue An Antidote for Methemoglobinemia and Beyond. 37(9): 474-477. doi: 10.1097/PEC.0000000000002526.

13. Hani Atamna, Raj Kumar. (2011). Protective role of methylene blue in Alzheimer's disease via mitochondria and cytochrome oxidase. National Library of Medicine. 21(7): 205-233. doi: 10.3233/JAD-.

14. Agnieszka, KierysAgnieszka, KierysMarta, GrochowiczMarta, Grochowicz. The release of ibuprofen sodium salt from permanently porous poly(hydroxyethyl methacrylate-co-trimethylolpropane trimethacrylate) resins. (2015). Journal of Microporous and Mesoporous Materials, p 133-140. doi: org/10.1016/j.micromeso.2015.06.009.

15. Ahmed A, Abdellatif, Mohamed A, El Hamd. (2016). A Formulation, Optimization and Evaluation of Controlled Released Alginate Beads Loaded-Flurbiprofen. January. Journal of Nanomedicine & Nanotechnology. 17(5): 2-10 doi: 10.4172/2157-7439.1000357.

16. Chunrui DingShimei, XuShimei XuJide, WangJide WangShow, Shun FengShun Feng. (2012). Controlled loading and release of methylene blue from LbL polyurethane/poly(acrylic acid) films .Polymers for Advanced Technologies. 23(9): 1283-1286. doi: 10.1002/pat.2044.

17. Mehmet Şenel, Fatma Ebru Koç. (2019). Controlled release of methylene blue from layer-by-layer assembled chitosan/polyacrylic acid. International Journal of Polymeric Materials. 69(4): 258-262. doi: 10.1080/00914037.2018.1563082.

18. Aakanchha Jain, Richa Jain & Sourabh Jain. (2020). Preparation of Buffer Solution and Measurement of pH. Basic Techniques in Biochemistry, Microbiology and Molecular Biology, pp. 217–219. doi: 10.1007/978-1-4939-9861-6_50.

19. L. Donnelly, J.G. Hardy, S.P. Gorman, D.S. Jones, N.J Irwin, and C.P. McCoy. "Photochemically controlled drug dosing from a polymeric scaffold," Pharm. Accuracy. Vol. 34, no. 7, pp. 1469-1476, 2017.

20. S. Jafari, Mohammad Soleimani, and R. Salehi, "Harmonic Nanotechnology-Based Polymer Drug Delivery Systems for the Treatment of Breast Cancer,". Vol. 68, No. 14, pp. 859–869, 2018.

21. David QuintanarBriza, Nadyr Zorraquín-CornejoAdriana, GanemAdriana ,José Manuel Cornejo and BravoJosé Manuel. "Controlled Release of Model Substances from pH-Sensitive Hydrogels". Journal of the Mexican Chemical Society. vol.52, n.4, pp.272-278. 2008.