Synthesis and Characterization of PVA/CMC Cryogels as Ciprofloxacin Carries

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ABSTRACT

In this thesis, three different types of cryogel systems were prepared by using varying molecular weight of Polyvinly alcohol (PVA), although the molecular weights of PVA were altered, carboxymethyl cellulose (CMC) and ciprofloxacin were remained constant. It was found that due to the high degree of segmental mobility, low molecular weight of PVA was more prone to form PVA – CMC cryogels due to high ability to form hydrogen bondings with carboxymethyl cellulose (CMC).

Swelling results show that low molecular weight PVA had the faster swelling rate and this can explain the fast release of ciprofloxacin within 30 minutes from PVA – CMC cryogels during release studies.

Calibration curve was constructed by measuring the absorbance values of serial dilutions of ciprofloxacin solutions by using 100 ppm ciprofloxacin stock solution.

Release of ciprofloxacin from PVA-CMC cryogel system was best fit to first order release model with R^2 0,9922. It was found that release exponent is lower than 0,45 and it is a good indication of quasi fickian diffusion, which means that ciprofloxacin can release from cryogel system even without requiring swelling by diffusion.

Antimicrobial activity results show that low molecular weight PVA containing PVA – CMC cryogels has 47 mm inhibiton zone when they were freshly used and they have 43 mm inhibition zone by using dried form.

Keywords: Polyvinly alcohol (PVA), carboxymethyl cellulose (CMC), ciprofloxacin, cryogel

ÖZ

Bu tezde üç farklı türde kriyojel sistemi, polivinil alkolun farklı molekül ağırlıkları kullanılarak üretilmiştir. Polivinil alkolun farklı molekül ağırlıkları kullanılmasına rağmen, karboksimetil selülozun ve siprofloksasinin özellikleri sabit tutulmuştur. Düşük molekül ağırlığına sahip PVA polimerleri yüksek zincir hareketlerine sahip olduğu için PVA – CMC kriyojel oluşturmaya daha eğilimlidirler. Düşük molekül ağırlığına sahip PVA polimerlerinin kriyojel oluşturmaya elverişli olmasının başlıca sebebi karboksimetil selülozla hidrojen bağları yapmaya eğilimi olmasından kaynaklanmaktadır.

Şişme grafiğine göre düşük moleküler kütleye sahip PVA dan yapılmış kriyojellerin şişme hızları diğer moleküler ağırlağa sahip PVA'lardan yapılmış kriyojellere göre daha hızlıdır. Bu da salınım çalışmalarında siprofloksasinin neden 30 dakikada hızlıca salındığını açıklamaktadır.

Kalibrasyon grafiği siprofloksasinin 100 ppm stok siprofloksasin solusyonundan yapılan seri seyreltmelerin absorbans değerinin alınmasıyla elde edilir.

PVA – CMC jel sistemlerinden salınan siprofloksasin birinci dereceden salınım modeline uymaktadır ve R^2 değeri 0,9922'dir. Salınım katı n değeri 0.45'ten küçüktür. Bu da Quasi Fickian difüzyon modelini temsil eder. Bunun anlamı ciprofloksasin kriyojel sisteminin şişmesine ihtiyaç duymadan ortama salınabileceğidir.

Antimikrobiyal sonuçlarına göre düşük moleküler ağırlığa sahip PVA içeren PVA – CMC kriyojeller tazeyken kullanıldıklarında 47 mm inhibisyon alanı yaratırken kurutulmuş halde kullanılan jeller 43 m inhibisyon alanı yaratmaktadır.

Anahtar kelimeler: polivinil alkol (PVA), karboksimetil selüloz (CMC) siprofloksasin, kriyojel

DEDICATION

To my family

ACKNOWLEDGMENT

I would like to thank to my supervisor Prof. Dr. Mustafa Gazi and my co-supervisor Asst. Prof. Dr. Mümtaz Güran for their continuous support and great guidence.

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CMC	Carboxymethyl Cellulose
DNA	Deoxyribonucleic Acid
E.Coli	*Escherichia Coli*
FT-IR	Fourier Transform Infrared Spectroscopy
McF	McFarland
MHA	Mueller Hinton Agar
Mwt	Molecular Weight
PVA	Polyvinyl Alcohol
rpm	Rate Per Minute
WHO	World Health Organization

Chapter 1

INTRODUCTION

1.1 Cryogels

Polymeric gels have been used in many different areas of biotechnology. Chromatographic materials, immobilization of molecules and cells, matrices for electrophoresis are some of the examples, which polymeric gels have applications in biotechnology (Bakhshpour et al 2019). Cryogel is derived from the Greek krios, which means ice (Lozinsky 2014). Cryogelation is a process which enable the formation of gel under low temperatures. During cryogelation, solvents will from crystalline , orderred structures and polymer structures will form liquid microphase (Welzel et al 2014). Since solvents were forming crystalline region, all the monomers and initiators will be eluted from these regions and they will be concentrated on the liquid microphase, where polymers are located. These enable the polymerization at the unfrozen liquid microphase. During gel formation, some interactions are required to enable to keep the gel structures intact in aqueous media (Okay and Lozinsky 2014). These interactions can be chemical interactions or physical interactions. Therefore, crosslinker agents may be required during the gel formation (Çetin and Denizli 2015). The main desired feature in gel formation is to obtain gels with high degree of porosity. The formation of porous gel stucture is not always a self forming process, thus, some pore forming chemicals are needed. However, there are two main problems with formation of the pores with pore forming chemicals (Kumar 2016). First, once the gels are formed, pore forming

chemicals should be extracted from the gel structure and this can be achieved washing with solvent. However, using wash solvents creates a problem: trace amount of wash solvent may stuck inside the gel structure. This may affect chemical or physical properties such as the pore capasity of the gels. To eliminate these isssues, researchers are focused on cryogelation. In cryogelation, there is no requirement for the elimination of pore forming chemicals, because of the frozen solvent structure acting like pore forming chemicals and once they defrost, they will be eluted from the structure without requirement for any elution solvent (Chen et al 2016).

1.2 Formation of Cryogels

Cryogel formation is a two step process: freezing of gelation medium followed by thawing at higher temperatures (usually at room temperature).

These two steps can be repeated for several times to increase the porosity of the cryogels. Usually three times repeatition of these steps are favoured. Cryogelation and the pore sizes of cryogels do not depend only on the number of cycle repeated. There are many factors which affect cryogelation such as temperature, time, concentration of polymer, concentration of monomer, type of polymer, molecular weight of polymer, type of solvent and storage duration of cryogel formed (Okay 2014). Careful selection of cryogelation temperature has important role on cryogelation. It should be determined according to the selection of solvent. The cryogelation temperature should be higher than the glass transition temperature of solvent (Gao et al 2013). Cryogelation time is dependent on the type of polymer used, some polymers have limited mobility and as a result they may require longer period of time for the polymerization to happen. Concentration of polymer should be sufficient enough for the cryogel formation. High polymer concentrations indicate

that polymers do not have enough space for the polymerization to happen. Type of polymer play a crucial role in cryogelation. Mobility of the polymer chains can vary depending on the type of polymer. Polymers with mobile segments can easily form cryogels compared to the polymers with rigid, less mobile fragments (Gun'ko 2013). Molecular weights of the polymers also affect the cryogelation. Usually high molecular weight polymers have limited mobility, indicating that they require longer period of time for cryogelation. Solvent selection also affects the cryogels , solvent should form crystalline frozen states in the cryogel structure at the optimal cryogelation temperature (Oztoprak 2014). Storage time of the cryogels may affect the porosity of the cryogel. Longer periods of storage may lead to collapse of pores and decreasing in the degree of porosity.

1.3 Hydrogels

Hydrogels have been discovered in 1960s. Hydrogels has been used in personal care, medicine and engineering. Wide use of hydrogels in biomedical application is due to their bio-friendly nature. Hydrogels consist of hydrophilic polymer networks that retain large amounts of water. Hydrogels have been defined as two or multicomponent systems and the system consist of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Hydrogels can be used for many applications, they can be used for release or adsorption of chemicals in the medium (Hoffman 2012). Hydrogels can be loaded with the desired compounds, so that they can be used for antibacterial, antioxidant, anticancer, antiinflammatory or similar applications. Furthermore, the release capacity of the compounds can be tested. In addition, gels can be used for adsorption of undesired chemicals from the medium , that they can be toxic for ecosystem or living

organisms. Adsorption capacity of the gels can also be tested to find the most effective and efficient gels (Bortolin et al 2012).

1.4 Formation of Hydrogels

Hydrogels may be synthesized with most known chemical ways. These chemical ways include one-step procedures (polymerization and parallel cross-linking of monomers), as well as multiple step procedures (synthesis of polymer molecules having reactive groups and their subsequent cross-linking). Hydrogels may be classified on the basis of their physical and chemical properties. According to the presence of electrical charge hydrogels may be classified in four groups: Nonionic (neutral), Ionic, Amphoteric and Zwitterionic (Hennink and Nostrum 2012). Compared to cryogels, hydrogels generally have an important disadvantage, and it is the requirment for pore forming agents for the gel. Following the formation of porous structure, these pore forming materials should be eliminated from the structure of the gels (Xu et al 2016). Removal of pore forming materials from the gel structure can be very challenging and may require wash buffers and elution with buffers (Wittmann 2015). Using buffer solution to remove the pore forming materials may lead to some of the wash buffer to stuck inside the gels and this may affect the applications of the gels.

1.5 Poly(vinyl) alcohol

Poly(vinyl alcohol) (PVA) is a synthetic polymer with many hydrophilic hydroxyl groups as shown in Figure 1. It is water soluble and has biocompatibility. PVA can be used for gel formation via the help of chemical and physical crosslinking agents (Baker et al 2012). Due to the presence of its hydrogen bond donor/acceptor property, it can form polar-polar interactions by itself or with other polar polymeric structures. PVA containing cryogels with high physical and mechanical strength can be obtained via freeze/thawing method (Hassan and peppas 2000).

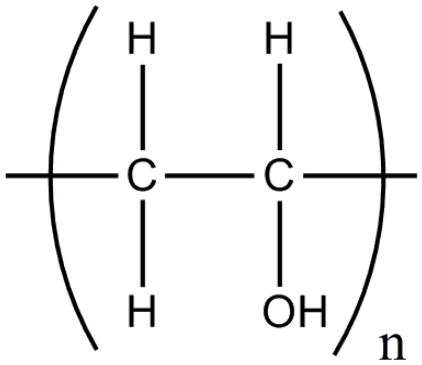


Figure 1 : Chemical Structure of PVA

1.6 Ciprofloxacin

Ciprofloxacin shown in Figure 2 is one of the well known member of fluoroquinolones. Ciprofloxacin is generally effective and it has clinical success against a broad spectrum of organisms such as *Pseudomonas* species, Enterobacteriaceae and staphylococci (Reddy and Navaneetha 2014).

Ciprofloxacin has good penetration capability in most tissues. Therefore ciprofloxacin is usefull in tissue engineering. In a study, ciprofloxacin was impregnated in composite scaffolds of polyvinyl alcohol (PVA) and quaternary bioactive glass (Mabrouk 2013). Microorganisms causes ostemylitis by spreading from the bloodstream or by direct contamination during surgery. To overcome osteomylitis, implantable devices were developed in the presence of microbial agents (Mabrouk 2013). This method improves the safety of the therapeutic strategies. Composite scaffolds containig PVA, bioactive glass and ciprofloxacin is one of the example for this metod.

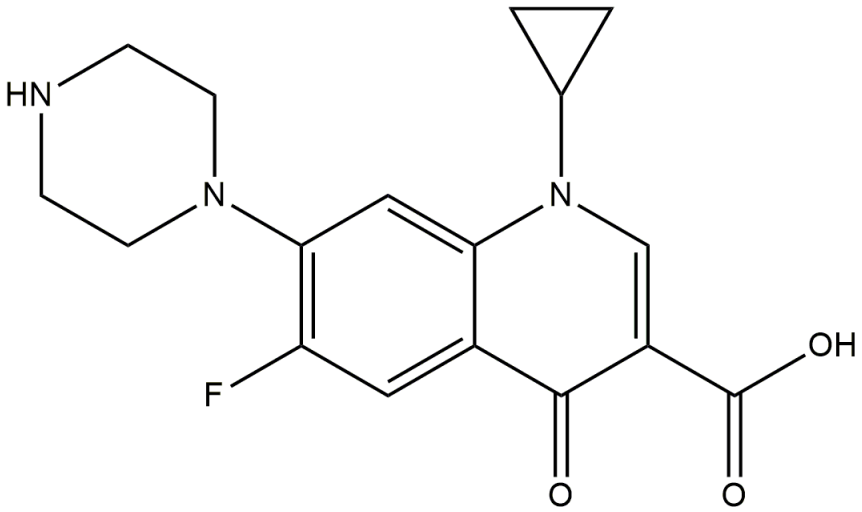


Figure 2: Chemical structure of ciprofloxacin

1.7 Cellulose

Anselme Payen discovered cellulose in 1838. The researcher isolated cellulose from plant matter. Several hundred to many thousands of linear β(1→4) linked D-glucose units link each other to form Cellulose as shown in Figure 3. Cellulose is insoluble in water and most known solvents. Cellulose is chiral and biodegredable. Human can not digest cellulose. Cellulose has been used as a raw material for more than hundred years (Missoum et al 2013). Cellulose is used to make paper.

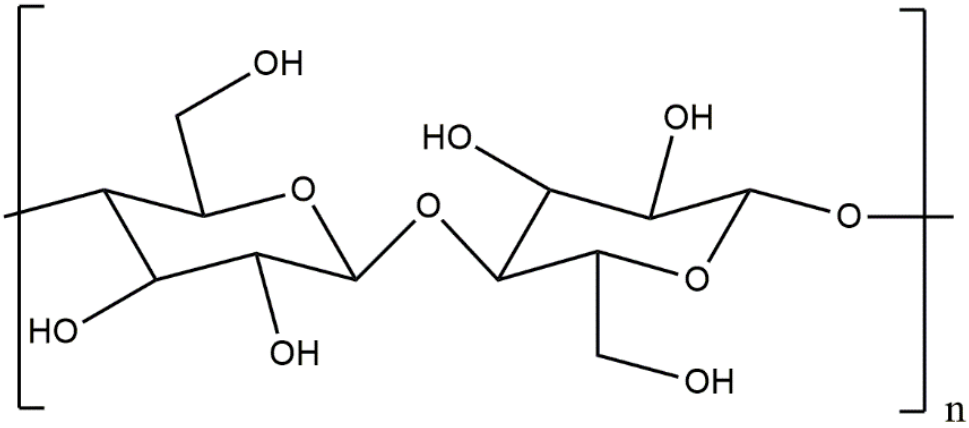


Figure 3: Chemical structure of cellulose

1.8 Derivatives of Cellulose

Carboxymethyl cellulose (CMC) is a cellulose derivative, which is water soluble cellulose ether as shown in Figure 4. CMC is obtained by reacting sodium monochloroacetate with cellulose in alkaline medium (Oun and Rhim 2015). CMC is a bio-friendly substance. At carboxymethylation process polysaccharide is activated with aqueous alkali hydroxide (sodium hydroxide) and converted with monochloroacetic acid or its sodium salt according to the

Williamson ether synthesis yielding the carboxymethyl (CMC) polysaccharide derivative (Khiari et al 2017).

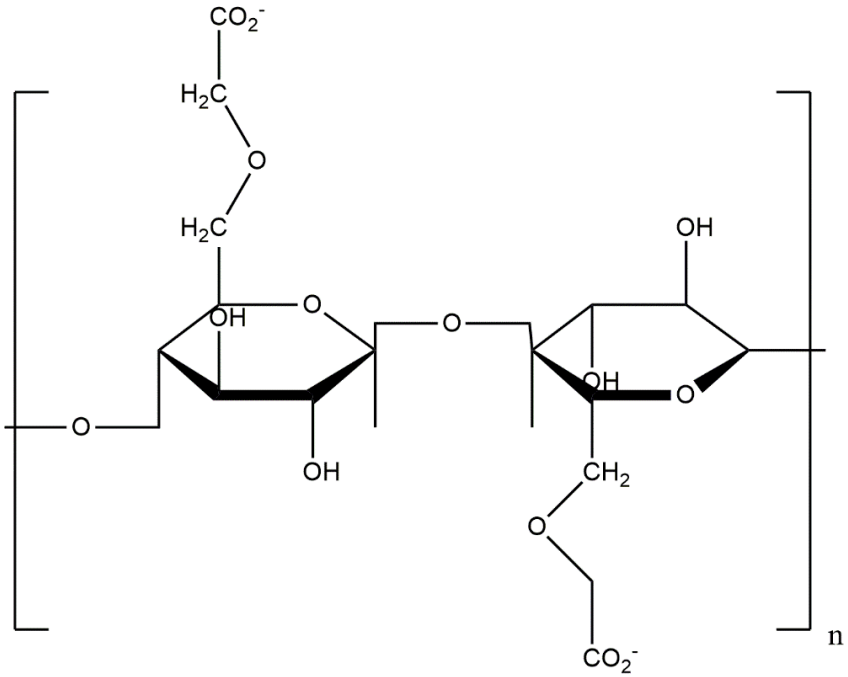


Figure 4: Chemical structure of carboxymethyl cellulose

1.9 Antimicrobial Activity

Struggle against infectious diseases has been one of the important subjects for scientists. Antimicrobial activity has been used for determination of the concentrations of medicines which stop the reproduction of microorganisms. Researchers are working to find the best doses of medicines which have minimal side effects on human and effective on wide spectra of microorganisms.

1.10 Infections

Bacteria, viruses parasites and fungi has pathogenic species which causes infectious diseases. There are a lot of infectious diseases which can be spread from one person to another. Troughout the history, some of infectious diseases have caused world wide deaths. Plaque, also known as Black Death was one of the important pandemics

in history which caused millions of death. *Yersinia pestis* causes plaque and it is carried by rodents .

One of other well known infectious disesases is tuberculosis. *Mycobaterium tuberculosis* bacteria causes Tuberculosis and lungs are the main organs affected by tuberculosis as shown in Figure 5. According to WHO data, tuberculosis is one of the top 10 causes of death worldwide.

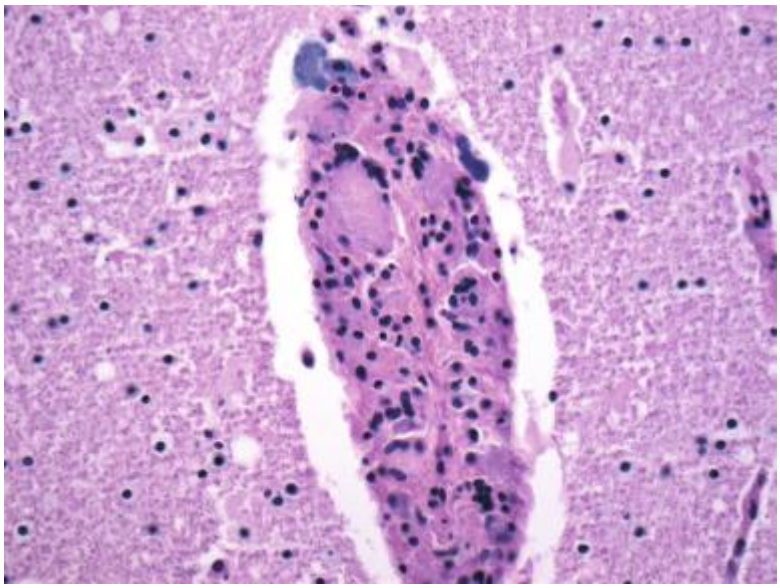


Figure 5: *Mycobacterium tuberculosis*

1.11 Antibacterial Agents

Most of bacteria species reproduce themselves very fast. For example E.coli give fertilization in 20 minutes. Due to these properties it is difficult to contain and eliminate bacteria. They can become resistant against each drug very fast. Scientist have to derive drugs to improve their effects on each bacteria. Therefore, there are a lot of drugs and their derivatives. Different doses of drugs effect on their mortality (Balouiri 2016). Scientists are focusing their studies on drug development in order to

find an optimal drug which has potential effect on wide spectrum of bacteria species and low side effect against human population.

1.12 Aim of the Thesis

The aim of this research is to investigate the antimicrobial activities and the gelation properties of cryogels consisting PVA, CMC and ciprofloxacin.

Chapter 2

EXPERIMENTAL

2.1 Materials

Sodium Carboxymethyl Cellulose (average Mwt 90,000) (Sigma-aldrich) , polyvinyl alcohol: low (30,000-50,000 Mwt), medium (85,000 – 146,000 Mwt), high (146,000 – 186,000 Mwt) (Sigma-aldrich), ciprofloxacin (Sigma-aldrich), distilled water.

2.2 Cryogel Formation

0.5 g carboxymehtyl cellullose was dissolved in 4 ml distilled water (pH~7.4). 0.5 g PVA was dissolved in 4 ml distilled water and 0.02 g ciprofloxacin was dissolved in 2 ml water at room temperature. All of the subtances were mixed. The mixture of solutions were transferred to test tubes and test tubes were placed into the freezer (-22 °C).In the freezer, solvent molecules became crystalline and around these crystalline areas polymers were concentrated and they interact with each other to form hydrogen bonds. Following to freezing, test tubes were transferred from freezer to room temperature for defrosting procedure. During defrosting, melted frozen solvent molecules were removed from the gelation medium. This freeze-thawing process was repeated for three times. Following these cycles, test tubes were cracked opened and the gels were removed from the tubes.

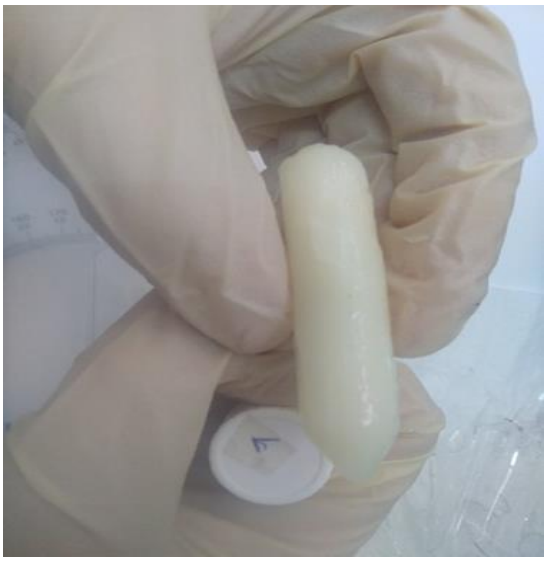


Figure 6: Photo of low Molecular weight PVA-CMC cryogel

2.3 Drying Rate

Drying rates for each type of cryogel were constructed based on the decrease in masses of cryogels. Decrease in masses of cryogels were measured at constant time intervals.

2.4 Percent Swelling

Swelling tests were conducted in phosphate buffer at pH 7.4 and the total mass increase of cryogels were recorded for the calculations of percent swellings.$\% \, Swelling = \frac{W_s - W_d}{W_d} \, X \, 100$ (1)

W_s = Swollen cryogel weight

W_d = Dried cryogel weight

2.5 FTIR

FT-IR results were obtained by using Perkinelmer FT-IR spectrum two (UATR 2) spectrometer by using ATR application. All the samples were used in powder forms.

2.6 Release Studies

Release of ciprofloxacin was studied. Ciprofloxacin loaded PVA – CMC cryogel was transferred into test tube, which contain 8 ml of distilled water at pH 7.4.

2.6.1 Calibration Curve

CT – 2200 Spectrophotometer was used during the construction of calibration curve. Maximum absorbance for ciprofloxacin is about 277 nm. The absorbance values of known concentrations of ciprofloxacin were recorded to conctruct absorbance versus concentrations graph for ciprofloxacin in water.

2.6.2 Percent Ciprofloxacin Released Determination

Percent ciprofloxacin released determined for release kinetic calculations based on the equation below;

$$\% \text{ ciprofloxacin released} = (m/m_0) \times 100 \quad (2)$$

m = amount of ciprofloxacin released (in g)

m_0 = amount of ciprofloxacin loaded in cryogel (in g)

2.6.3 Release Kinetics

For release kinetics four different kinetics models were studied. These release models are zero order, first order, Higuchi and Korsmeyer-peppas release models.

2.6.3.1 Zero Order Model

$$C_t = C_o + k_0 t \quad (3)$$

C_0 = % ciprofloxacin left in cryogels

C_t = % ciprofloxacin released at time "t"

k_0 = zero order release

t = time in minutes

2.6.3.2 First Order Model

$$\ln(100 - C_t) = \ln C_0 - k_1 t \quad (4)$$

C_0 = % ciprofloxacin left in cryogels

C_t = % ciprofloxacin released at time "t"

k_1 = first order relesae constant

t = time in minutes.

2.6.3.3 Higuchi Model

$$m_t/m_0 = k_H t^{1/2} \quad (5)$$

C_t = % ciprofloxacin released at time "t"

k_H = Higuchi release constant

t = time in minutes

M_t = amount of ciprofloxacin released at time "t"

M_0 = Total amount of ciprofloxacin loaded to cryogel.

2.6.3.4 Korsmeyer – Peppas Model

$$\text{Log}\ (C_t / 100\ ) = K_p t^n \quad (6)$$

C_t = % ciprofloxacin released at time "t"

K_p = Korsmeyer – Peppas relase constant

t = time in minutes

n = release exponent

2.7 Antibacterial Activity

Antibacterial activity of prepared cryogels was tested by using *E.coli* ATCC 25922 (Gram-negative). Strain that was kept frozen at -80°C was transfered to a sterile Mueller-Hinton Agar (MHA) by streaking method and incubated at 37°C for 24 h. Then by using sterile saline water, few freshly grown bacteria colonies were transferred into the saline water until bacteria turbidity values reached 0.5 McFarland. Next, a sterile-swap was submerged into just prepared 0.5 McF saline water and then swap was rolled onto a new sterile MHA until all the surface area of the agar was covered with the 0.5 McF saline water. Finally, cryogels to be tested were placed onto these agars seperately enough to allow the observation of the zones more clearly upon formation. Positive control group using a Ciprofloxacin 5 mcg antibiotic disk and negative control group using an empty antibiotic disk was also used for this test.

Chapter 3

RESULTS AND DISCCUSION

3.1 Determination of Optimal Cryogel System

Three different cryogel systems were synthesised by using three different molecular weights of PVA polymer together with carboxymethyl cellulose. Molecular weights of PVA were high Mwt PVA (146,000 – 186,000 Mwt) medium Mwt PVA (85,000 – 146,000 Mwt) and low Mwt PVA (31,000 – 50,000 Mwt). During the synthesis of cryogels, ciprofloxacin was loaded into the cryogels. It was found that low PVA containing cryogel systems has better cryogel forming ability among other types of Mwt of PVA. This is because in low Mwt of PVA polymers, segments of PVA polymers have higher degree of mobility compared to other types of Mwt of PVA. High degree of mobility enables the polymers to form hydrogen bonds between hydroxyl groups of PVA and carbonyl group of CMC. These weak hydrogen bonds inreactions preserve the integrity of the PVA-CMC cryogels. Therefore PVA-CMC cryogel system made up from low Mwt of PVA was preferred to be studied in antibacterial activity test.

3.2 Drying Rate Results

After the synthesis of cryogel systems, drying rate of cryogels were measured at regular time intervals until the masses of cryogels became constant under fumehood at room temperature. It was observed that the cryogel which contain high molecular weight of PVA showed the fastest drying profile compare to others and it got constant at time 540 minutes as given in Figure 7. Low and medium molecular

weight PVA containing cryogel sysytems showed the second highest drying profile and the masses of these cryogel systems become constant at time 1080 minutes. High molecular weight PVA containing cryogels systems showed the highest drying rate because low segmental mobility of the PVA polymer chains. Cryogel systems which contain high molecular weight of PVA did not form properly. Since they did not show any integrity to form proper cryogels, they were more like viscous liquids and these viscous liquids have higher surface area compare to other types of cryogels and that's the reason why they dried earlier.

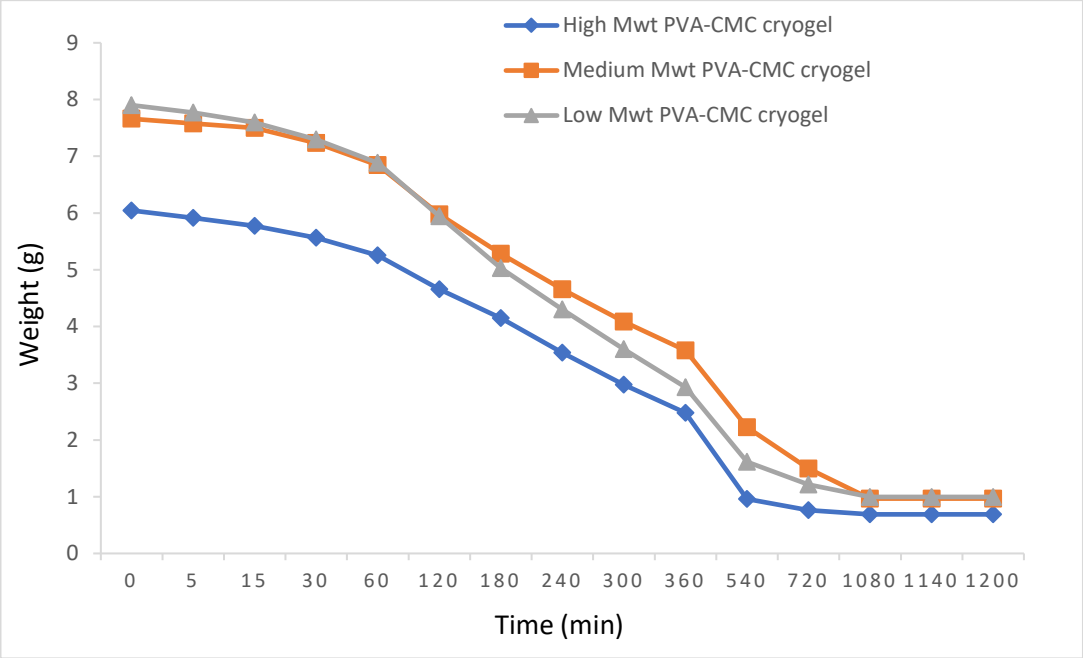


Figure 7: Drying profile of PVA-CMC cryogels

3.3 Percent Swelling Results

Swelling test was conducted in phosphate buffer (at pH 7.4) at room temperature for about 180 minutes as shown in Figure 8. It was observed that low Mwt PVA containing PVA- CMC cryogel system had the fastest percent swelling of 416,6%. Although they had the fastest swelling rate, it did not show highest swelling percent. The highest swelling percent was 478,9% and it belongs to high molecular weight

PVA-CMC cryogel system. Again because of low segmental movements in high molecular weight PVA containing PVA-CMC cryogel systems, the hydrogen bond intereactions between the hydroxl groups of PVA and carbonyl groups of CMC were not sufficient enough, therefore, solvent can easily enter into cryogel systems and causes the cryogel system to swell. Phosphate buffer has positively charged metal ions such as sodium ions and anioic phosphate ions. These ions enable polar-ion inreactions between hydroxyl groups of PVA and these charged ions instead of hydrogen bonding between hydroxyl groups of PVA and corbonyl groups of CMC. This causes easier swelling of PVA-CMC cryogel systems as cryogels swells more solvent molecules will penetrate into cryogel systems and causes further swelling of cryogel systems.

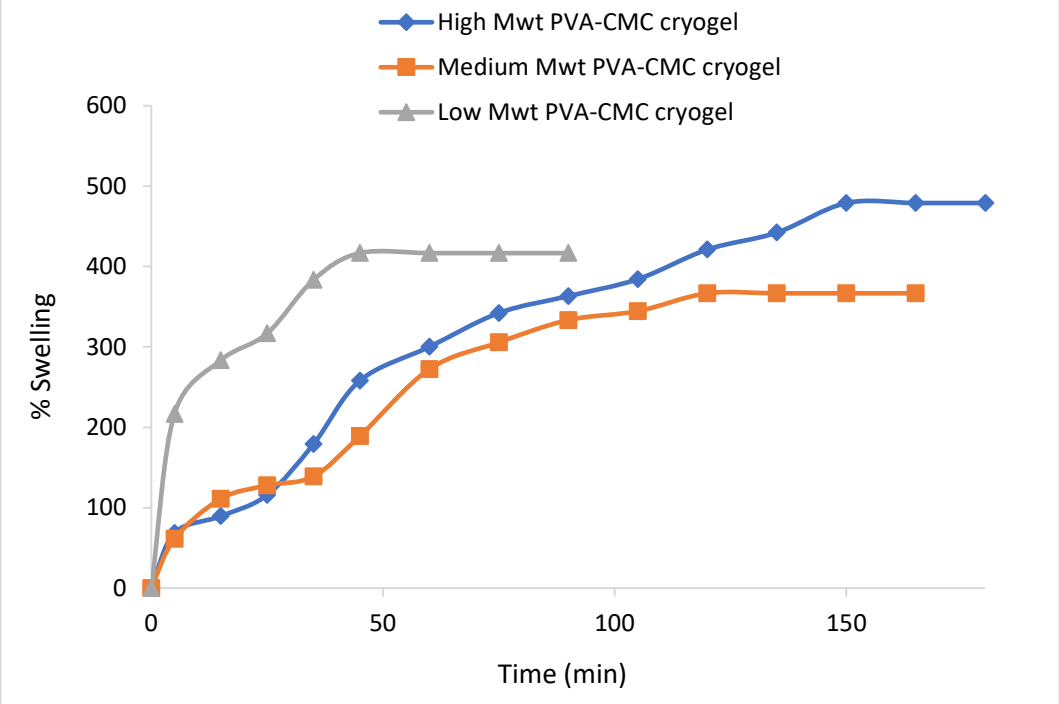


Figure 8: Swelling of PVA-CMC cryogels

3.4 FTIR Results

FTIR spectra of PVA, CMC, Ciprofloxacin and ciprofloxacin loaded cryogel are shown in Figure 9.

FTIR spectrum of PVA: Broad band centered at 3268 cm^{-1} represents hydroxyl group vibrations. Peaks at 2937 and 2907 cm^{-1} represent –CH stretching in PVA. PVA synthesized from hydrolysis of polyvinyl acetate is observed via the presence of the peak at 1557 cm^{-1} , which indicate the presence of carbonyl group of acetate. Carbon – carbon stretchings observed 835 cm^{-1} . O- C bond stretchings were observed at 1086 cm^{-1} .

FTIR spectrum of CMC: Broad band centered at 3260 cm^{-1} represent the presence of –OH stretchings. Carbonyl group of –COONa group was observed at 1590 cm^{-1} due to symetric and asymmetric stretchings of C = O bond. Presence of C – C single bond were observed by the presence of 1410 cm^{-1} and 1320 cm^{-1}. O - C single bond stretchings were observed at 1050 cm^{-1}.

FTIR spectrum of ciprofloxacin: First characteristic peak for ciprofloxacin was observed between 3450 and 3500 cm^{-1} and it represents –OH vibrational stretchings. Peaks at 2620 and 2463 cm^{-1} were observed due to alkene groups and C-H stretchings of aromatic groups. Peak at 1702 cm^{-1} represent the stretchings of carbonyl groups. Peak at 1621 cm^{-1} represent the presence of quinolones. C – O bonds stretchings were detected at sharp peak at 1446 cm^{-1}. Bending vibrations of – OH caused the peak at 1266 cm^{-1} and it strongly indicates the presence of carboxylic acid groups. Peak at 1024 cm^{-1} represents C-F group of ciprofloxacin.

FTIR spectrum of freeze dried ciprofloxacin loaded CMC-PVA cryogel: Cryogels showed similar FT-IR spectrum with CMC. This was because CMC acts like a micelle, which has PVA and ciprofloxacin. 3268 cm^{-1} centered broad peak shows the presence of –OH groups and %T was lower compared to the spectrum of CMC. This was because of extra –OH groups from PVA. Peak at 1590 cm^{-1} represent symetric and asymmetric stretchings of C=O bonds. O-C single bond stretchings were observed at 1054 cm^{-1}.

Overall, FT-IR spectra confirms that PVA and CMC interacts with each other with weak forces such as hydrogen bonds between polar groups and Ciprofloxacin also has hydrogen bond donor –NH group and hydrogen bond acceptor carbonyl groups, so, that ciprpfloxacin can also make weak H bond interactions with the PVA and CMC.

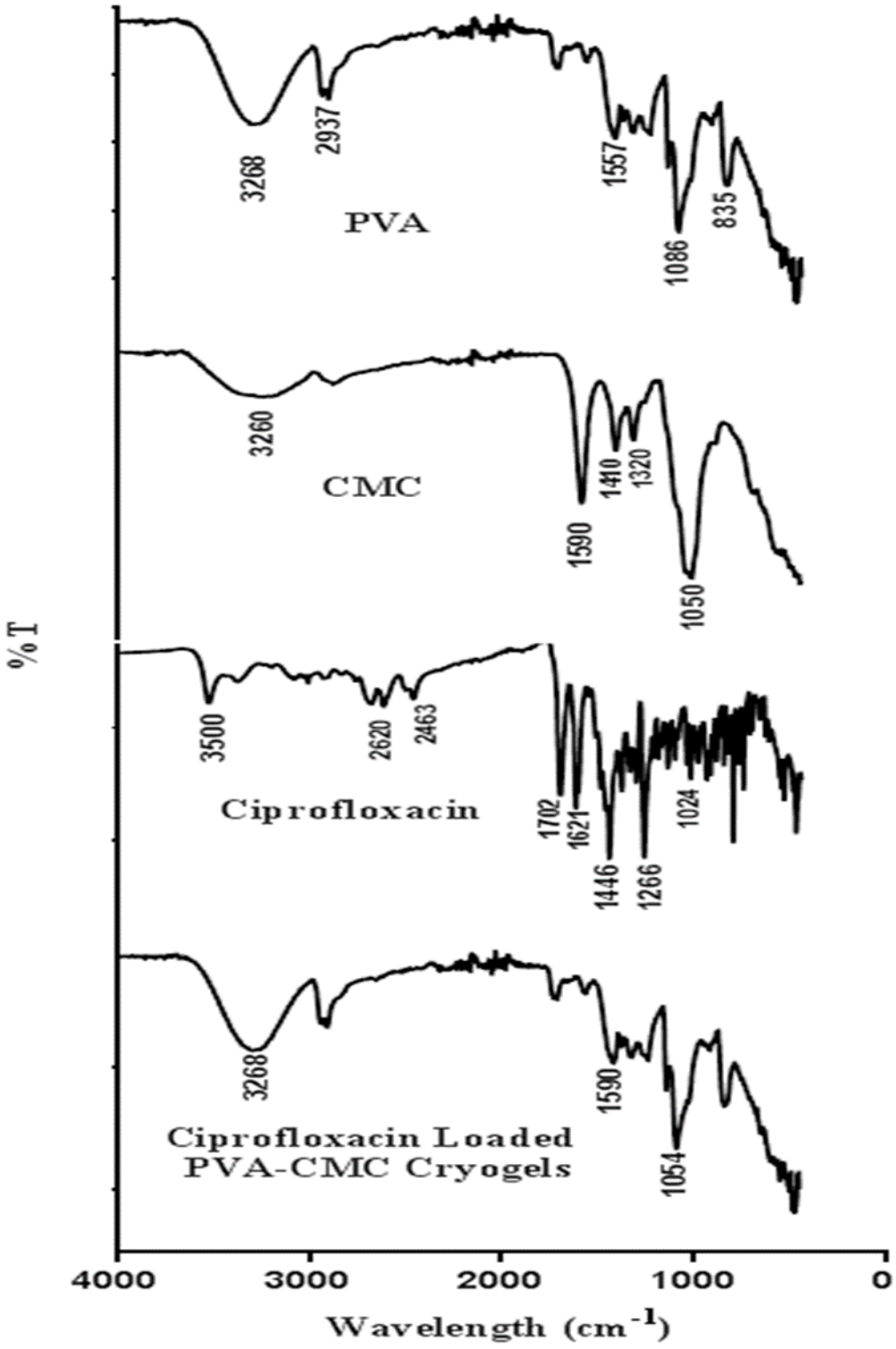


Figure 9: FTIR Spectra of all samples

3.5 Release Studies

Release study was only conducted for low molecular weight PVA containing ciprofloxacin loaded PVA-CMC cryogels. This was because they were the best cryogel system compared to other two types in terms of integrity. Water was chosen as release medium and the pH of distilled water was adjusted to 7.4 to resemble the physiological pH. It was observed that the releasing rate of cirofloxacin from PVA-CMC cryogels was very fast, release rate became constant in first 30 minutes as shown in Figure 10. In concentrations versus time graph, it became constant at 2.42 x 10^{-5} g/ml as given in figure 11.

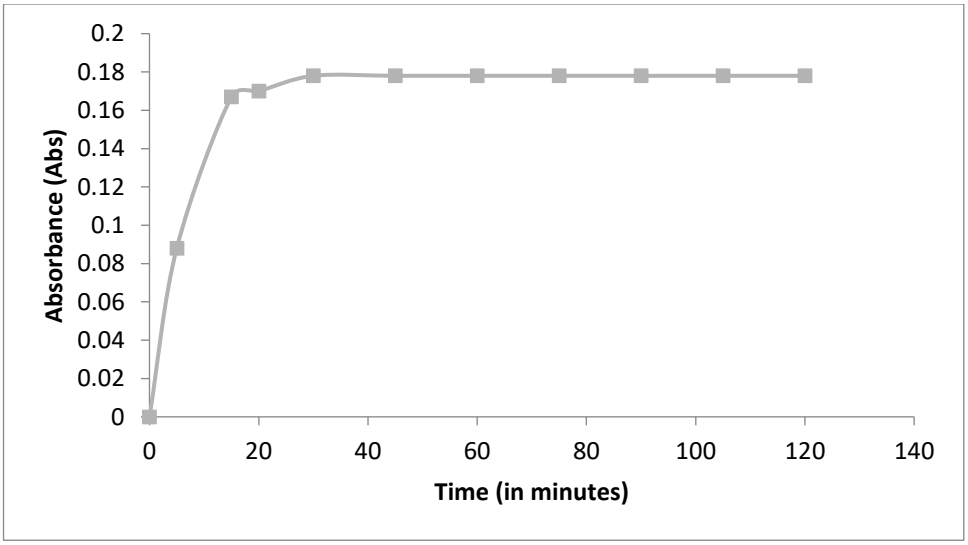


Figure 10: Absorbance versus time graph of PVA-CMC cryogels

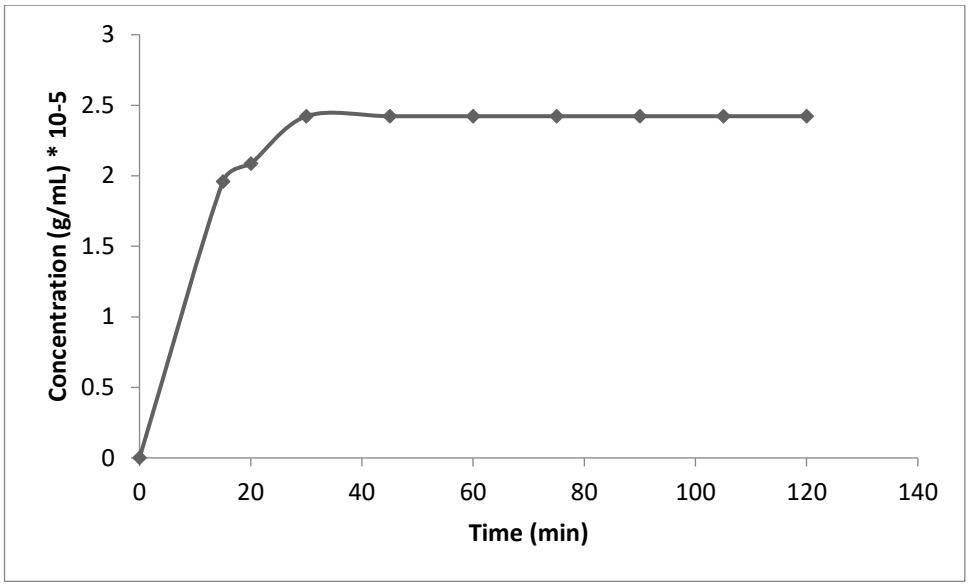


Figure 11: Concentration versus time graph of PVA-CMC cryogels

3.5.1 Calibration Curve

Calibration curve was absorbance versus concentrations graph. The λ_{max} for water was 280 nm UV, therefore, absorbance values were measured at 280 nm. Calibration curve was prepared by using main stock of 0.005 g/ml ciprofloxacin solution and it was serially diluted for several times and the absorbance values of the dilutions of ciprofloxacin were recorded. It was then used to determine the concentration of ciprofloxacin released from cryogel systems. The line equation was y=0.0749x+0,0133 with R^2 value of 0,9922 as shown Figure 12.

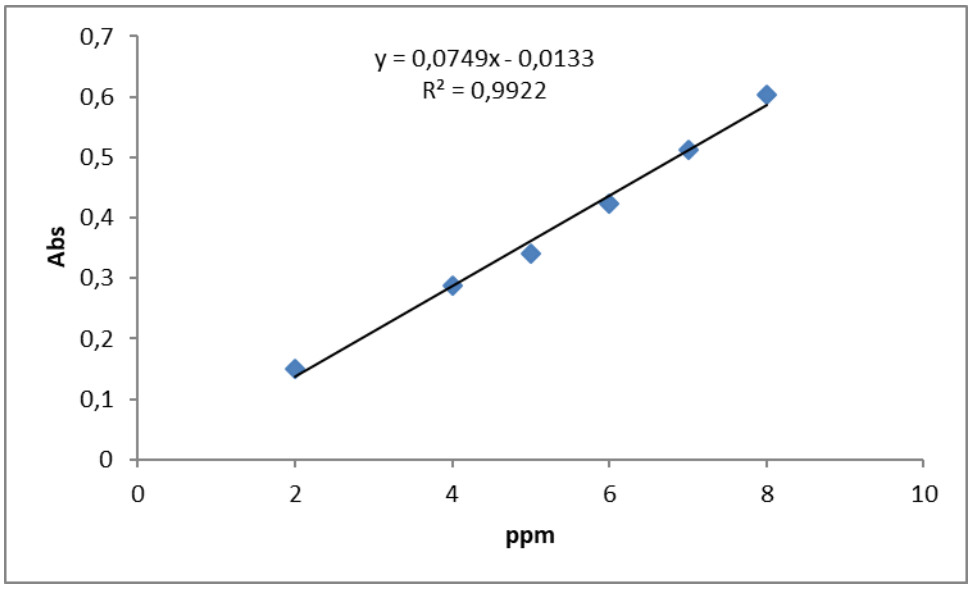


Figure 12: Calibration curve of ciprofloxacin

3.5.2 Release of Ciprofloxacin by time

% ciprofloxacin released versus time graph was constructed. It was found that maximum % ciprofloxacin released was 6,78% at the time 30 minutes as shown in figure 13.

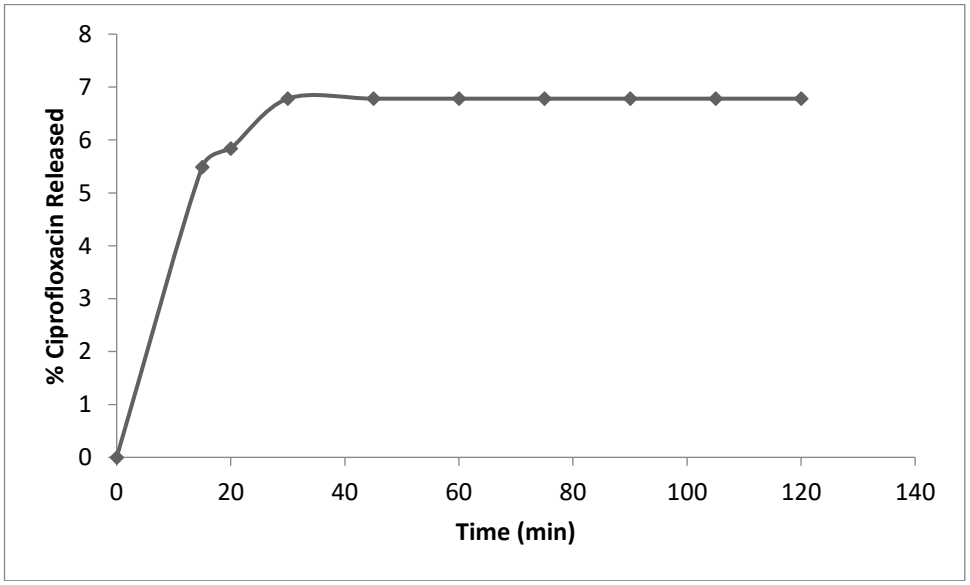


Figure 13: % cumulative release of ciprofloxacin

3.5.3 Release Kinetics Results

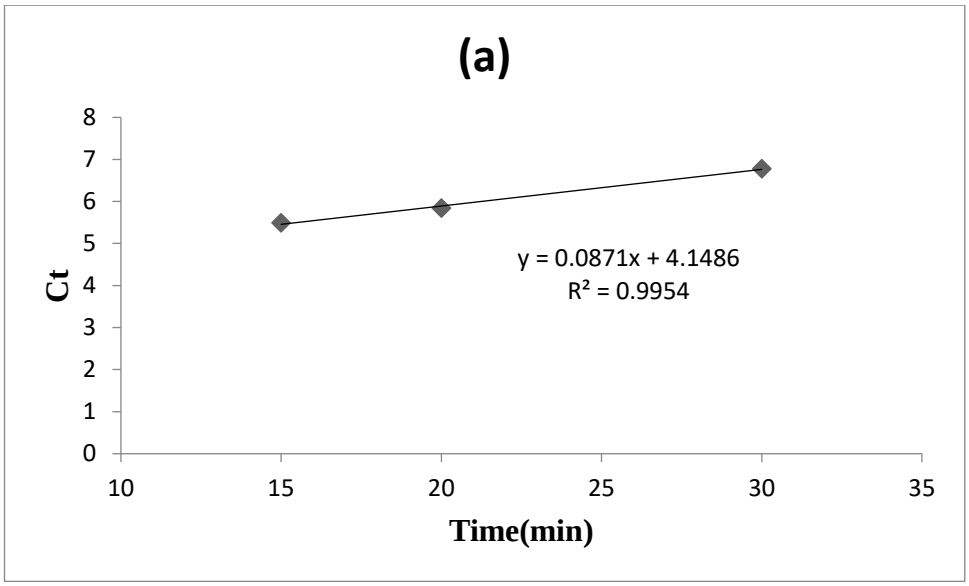
The four different release models of ciprofloxacin from PVA-CMC cryogels were studied. These models were zero order, first order, Higuchi and Korsmeyer-peppas release models. Release kinetic parameters were given in Table 1. In addition, the best fit lines for each release models were given in Figure 14. Aim was to determine R^2 value closest to 1. It was found that the best R^2 value was detected with first order release model indicated that release of ciprofloxacin from PVA-CMC cryogels were sustained and it depends on the concentration of ciprofloxacin in the cryogel system. Release exponent (n) was found as 0,3914 . It indicates the Quasi fickian diffusion based release of ciprofloxacin from cryogels as shown in table 2. It has both fickian and non-fickian diffusion properties. Therefore ciprofloxacin can be released from PVA-CMC cryogel systems even without requiring the cryogel systems to swell through diffusion based release mechanism.

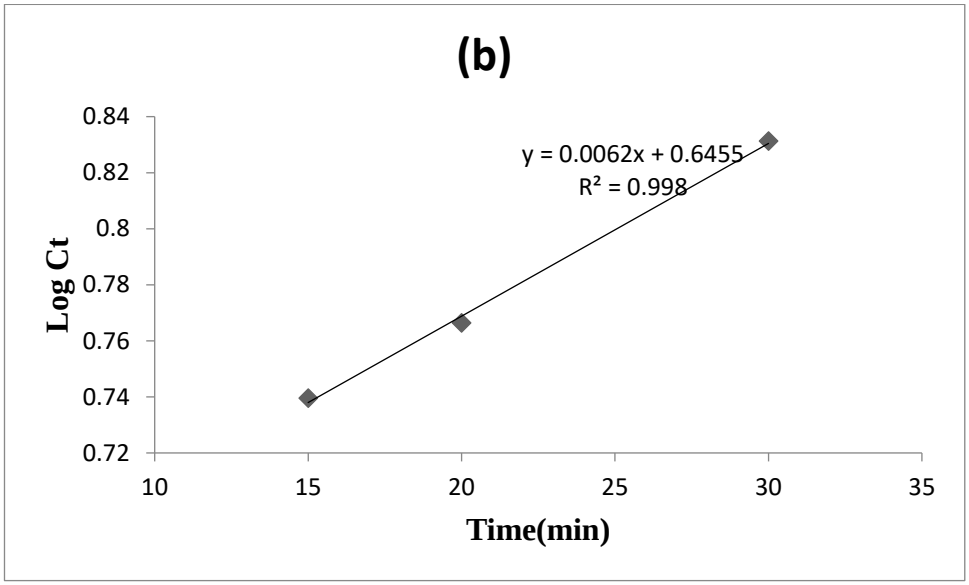
Table 1: Parameters of release models

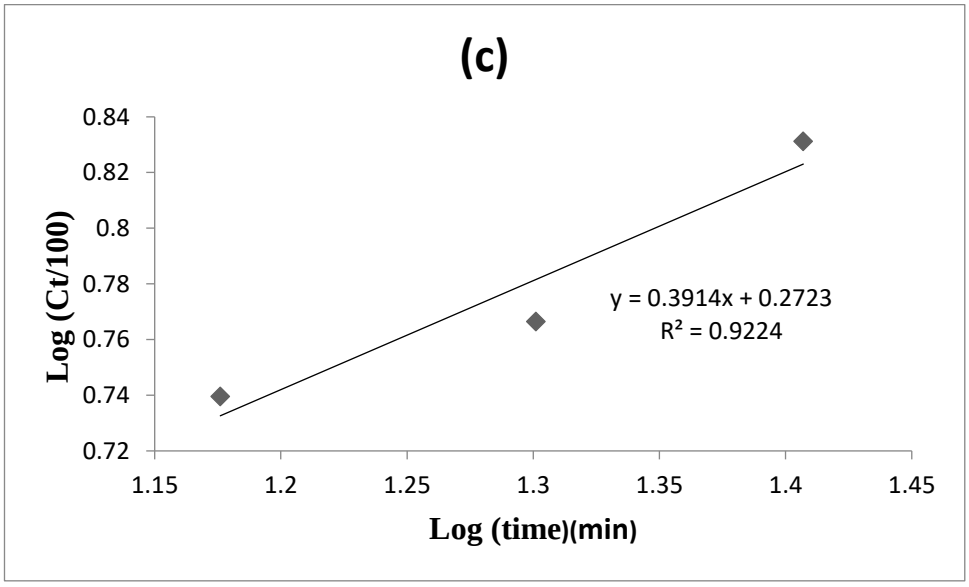
Release Models								
Zero Order		**First Order**		**Higuchi**		**Korsmeyer-peppas**		
R^2	K_0	R^2	K_1	R^2	k_H	R^2	K_P	N
0,9954	0,0871	0,9980	0,0062	0,9873	0,8178	0,9224	1,8011	0,3914

Table 2: Release exponent (n)

Release exponent (n)	**Type of release mechanism**
< 0.45	Quasi Fickian Diffusion
0.5	Fickian Diffusion
0.45<n<0.89	Non-fickian


(a)
Ct
Time(min)
y = 0.0871x + 4.1486
R² = 0.9954
0
1
2
3
4
5
6
7
8
10
15
20
25
30
35


(b)
Log Ct
Time(min)
y = 0.0062x + 0.6455
R² = 0.998
0.72
0.74
0.76
0.78
0.8
0.82
0.84
10
15
20
25
30
35


(c)
Log (Ct/100)
Log (time)(min)
y = 0.3914x + 0.2723
R² = 0.9224
0.72
0.74
0.76
0.78
0.8
0.82
0.84
1.15
1.2
1.25
1.3
1.35
1.4
1.45

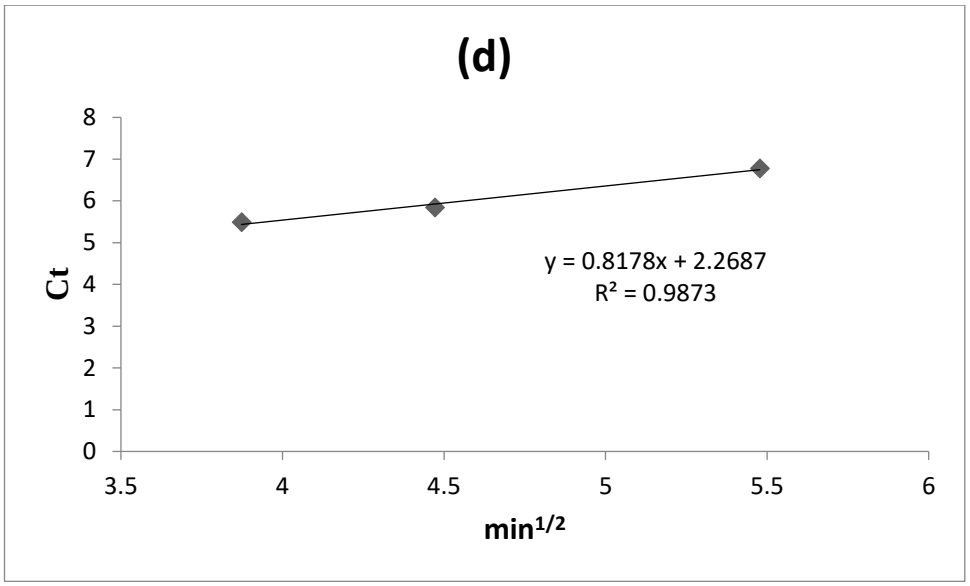


Figure 14: Release Models: a) Zero Order b) First Order c)Korsmeyer Peppas d)Higuchi

3.6 Antimicrobial Activity Results

Inhibition zones for low Mwt PVA containing cryogel dry sample, low Mwt PVA containing cryogel wet sample, medium Mwt PVA containing cryogel wet sample and high Mwt PVA containing wet sample were observed as follows: 43 mm, 47 mm , 53 mm and 50 mm, respectively as given in figures 15 and 16. Ciprofloxacin was used as a positive control agent due to the susceptibility of *E.coli* against this drug. Ciprofloxacin is a member of fluoroquinolone class . It has effect on both Gram negative and Gram positive bacteria. It can interrupt cell cycle by stopping DNA replication via inhibition of some DNA replication enzymes such as type 2 and 4 topoisomerases and DNA gyrases. For the positive control group inhibition zone was 35 mm and there was no inhibition zone formation for the negative control group as expected. It was observed that the inhibition zone of PVA-CMC cryogel loaded with ciprofloxacin covers wider area compare to positive control. This can be related to the amount of ciprofloxacin, in positive control the amount of ciprofloxacin was 5 µg, however, the cryogel systems were loaded with 0,02 g ciprofloxacin.

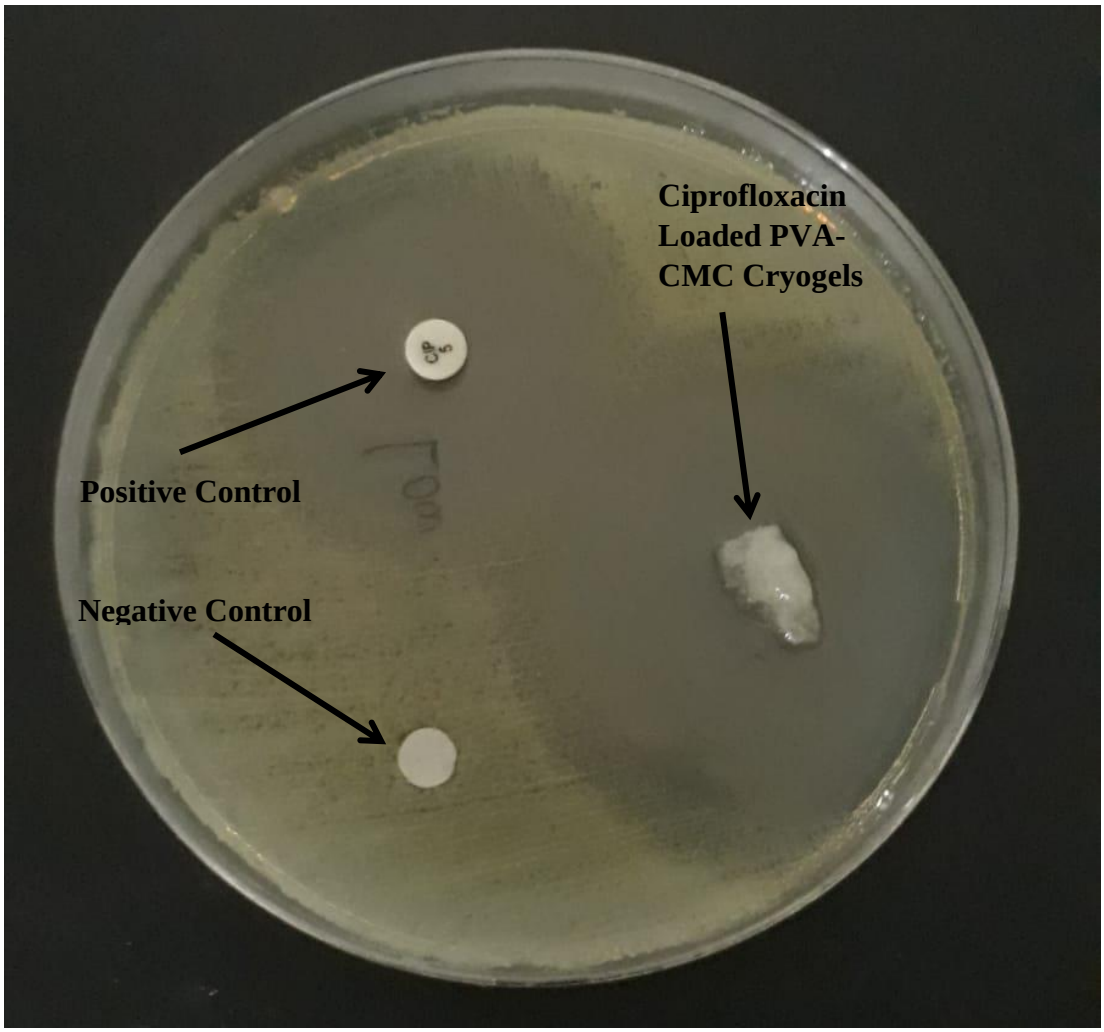


Figure 15: Inhibition zones of positive control, negative control and ciprofloxacin loaded PVA-CMC cryogels.

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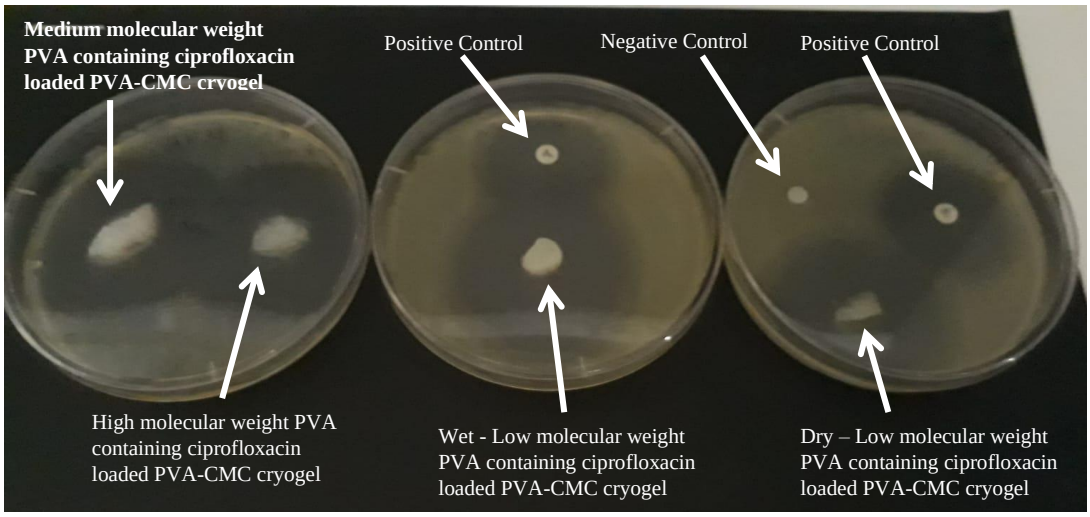

Figure 16: Inhibition zones of loaded PVA-CMC cryogel systems.

Chapter 4

CONCLUSION

Low molecular weight PVA was found as better interms of molecular weights for the sythesis of PVA – CMC cryogels compared to the other molecular weight samples of PVA. In low molecular weight PVA, there are high segmental movements in polymer chains. Therefore, hydroxyl groups of low molecular weight PVA can make hydrogen bondings with carbonyl groups of CMC to form cryogels.

Swelling results show that low molecular weight PVA containing PVA-CMC cryogels had the fastest swelling rate and high molecular weight PVA containig cryogels has the highest swelling percent by 478,9%.

Release studies were done by using low mwt PVA containing cryogel and it was found that this cryogel can release very quickly within 30 minutes and it reaches to 2.42×10^{-5} g/mL in distilled water.

Release kinetic studies show that release of ciprofloxacin from PVA – CMC cryogels best fit to first order release model. This indicates that there is constant release of ciprofloxacin depending on the concentration of ciprofloxacin in the cryogel system.

Antimicrobial activity results shows that ciprofloxacin loaded PVA – CMC cryogels had the better inhibition zone compared to positive control. This can be explained due to high amount of ciprofloxacin in cryogel system compared to positive control.

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