Synthesis and Characterization of Poly[2-(diethylamino ethyl methacrylate)] Hydrogels

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ABSTRACT

Poly[2-(diethylamino ethyl methacrylate)] (PDEAEM) and its ethylene glycol dimethacrylate (EGDMA) cross-linked hydrogels were synthesized by free radical polymerization. Chemical structure was characterized by FTIR spectroscopy. The morphology of the homopolymer and of the hydrogels was investigated by SEM analysis. Swelling properties of the EGDMA crosslinked hydrogels of PDEAEM were followed under acidic and neutral conditions. The results revealed weakly pH responsive swelling behaviour with equilibrium swelling capacity values of 50% in acidic medium and 30% in distilled water. The drug loading and delivery properties of the homopolymer and its hydrogels were investigated using ciprofloxacin HCl as the model compound. EGDMA crosslinked hydrogels of PDEAEM exhibited 35% CFX loading capacity at pH=4 in 72 h. A percent cumulative drug release value of 60 was obtained with the hydrogel that (DEAEM/EGDMA = 1:7.5 by mole) within 2 hours in acidic solution.

Keywords: 2-(diethylamino ethyl methacrylate), ethylene glycol dimethacrylate, hydrogels, ciprofloxacin-HCl, potassium persulfate, free radical polymerization.

ÖZ

Bu çalışmada poli[2-(dietilamino etil metakrilat)] (PDEAEM) homopolimer ve onun etilen glikol dimetakrilat (EGDMA) ile çapraz bağlanmış hidrojellerinin sentezi ve karakterizasyonu incelenmiştir. Polimerler serbest radikal polimerleşmesi yöntemi ile çözelti içinde azot ortamında sentezlenmiştir. Ürünlerin kimyasal yapısı FTIR spektroskopisi ile, morfolojisi ise SEM analizi yöntemi ile belirlenmiştir. Ürünlerin şişme davranışları asidik ortamda ve saf suda takip edilmiştir. Asidik ortamda %50, saf suda ise %30 oranında ağırlıkça şişme saptanmıştır. Bu durumda hidrojellerin zayıf da olsa pH'a bağlı şişme davranışı gösterdikleri anlaşılmıştır. Homopolimerin ve hidrojellerin ilaç hapsetme ve ilaç salım özellikleri ciprofloxacin HCl antibiyotik ilaç model alınarak araştırılmıştır. EGDMA ile çapraz bağlanmış PDEAEM hidrojellerin pH=4 çözelti içinde 72 saat sonunda %35 CFX yüklenme kapasitesine sahip olduğu ve (DEAEM/EGDMA = 1:7.5 mol oranı) hidrojel örneğinin hapsedilen ilacın %60'ını asitli ortamda 2 saat içinde saldığı belirlenmiştir.

Anahtar Kelimeler: 2-(dietilamino etil metakrilat), etilen glikol dimetakrilat, hidrojel, siprofloksasin-HCl, potassium persülfat, serbest radikal polimerleşme.

…… to the Glory of God

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INTRODUCTION

The importance of biomaterials in medical advancements cannot be over emphasized. Polymers with interesting features such as stimuli-responsiveness especially in terms of temperature, ionic strength and pH sensitivity amongst others have interesting and vital roles in the field of nanomedicine, biotechnology, biomedicine and gene/drug delivery (Liu et al., 2008). These polymers are best described as smart polymers.

Poly[2-(diethylamino ethyl methacrylate)] PDEAEM is pH sensitive. It was found to have a pKa of about 7.3 (Liu et al., 2015). PDEAEM is a weak polybasic polymer. In aqueous solution, it has the ability to form cationic polyelectrolyte. It binds efficiently with DNAs carrying negative charges (Gan et al., 2003). It has the molecular formula $(C_{10}H_{19}O_2)_n$. PDEAEM is a polymer with high potentials in nanomedicine, biotechnology, biomedicine and gene/drug delivery systems. The molecular structure of the monomer 2-(diethylamino ethyl methacrylate) (DEAEM) is given in Figure 1.1.

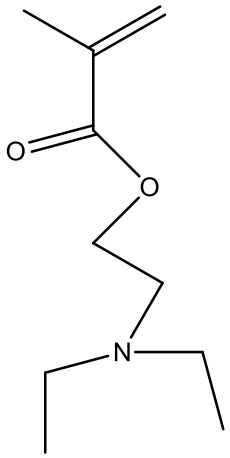

Figure 1.1: 2-(diethylamino ethyl methacrylate)

1.1 Synthesis

2-(diethylamino ethyl methacrylate) is the monomer of PDEAEM and it has been studied extensively. It can be polymerized readily by free radical polymerization (Marek et al., 2010). The molecular structure of the polymer PDEAEM is given in Figure 1.2.

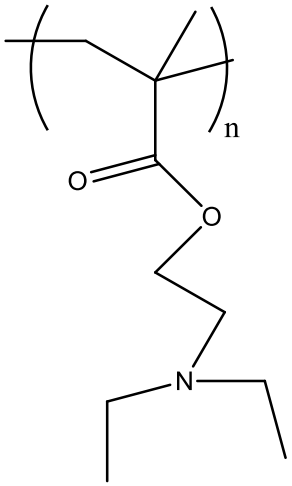

Figure 1.2: Poly[2-(diethylamino ethyl methacrylate)]

PDEAEM and its hydrogels through grafting or copolymerization have been synthesized by various ways in the past. They have been synthesized through methods such as anionic polymerization, reversible addition fragmentation chain

transfer polymerization, atom transfer radical polymerization, in addition to free radical polymerization.

According to Anderson et. al., 2002, the homopolymer of PDEAEM was synthesized by anionic polymerization in tetra hydrofuran as the solvent and potassium t-butoxide as initiator. The advantages of this method are very narrow molar distribution controlled by monomer initiator ratio, about 100% monomer conversion and fast polymerization.

Controlled living polymerization of DEAEM by atom transfer radical polymerization was reported by Gan et. al., 2003. In this method, methanol was used as solvent, CuCl as catalyst, p – TsCl (p-toluene sulfonyl Chloride) and 1,1,4,7,10,10 – Hexamethyltriethylenetetraamine as ligand. The main advantage here in this method is, very narrow polydispersity index (PDI) block with well-defined polymer.

RAFT (Reversible addition fragmentation chain transfer) polymerization was reported by Feng et. al., 2014 using DMP (2- dodecylsulfonylthiocarbonylsufanyl - 2 - methyl propanoic acid) as chain transfer agent, 1,4 - dioxane - solvent and the initiator used was asoboisisobutyronitrile (AIBN). Polymer was carried out at 70ºC for 18hours which resulted in 69% monomer conversion. The advantages of this method are narrow PDI control over most of PDEAEM by simply adjusting monomer/chain transfer agent (CTA) ratio, a better control over molecular weight and PDI by increasing polymerization temperature from 60ºC – 70ºC.

Table 1.1 gives a summary of the polymerization of DEAEM through the various techniques mentioned above.

Table 1.1: Summary of polymerization techniques for PDEAEM

	RAFT	**ANIONIC**	**ATRP**	**GTP**
Solvent	Dioxane	THF	Methanol	THF
Initiator	AIBN	Kt-Butoxide	p-TsCl	MTS
Ligand/Chain transfer agent	-/DMP		HMTETA	
Catalyst			CuCl	TBABB
Temperature	70ºC	At room temp for 20mins & at 50ºC for 20mins	60ºC	Room Temperature
Reaction time	18h	40mins	3.5h	1h
% Conversion	69	About 100	82	More than 98
PDI (Mw/Mn)	1.14	1.12	1.07	1.05
Advantage	Better control over molecular weight and PDI	A very narrow PDI. About 100% monomer conversion. Fast polymerization	A very narrow PDI block with well-defined polymer structure	A very narrow PDI. High yield. Controlled molecular weight

1.2 Properties of PDEAEM

Based on the several characterizations of PDEAEM, it has been discovered that:

1. PDEAEM has a Tg of about 20ºC

2. PDEAEM is a weak cationic polybase with pKa of about 7.3. This makes the polymer pH sensitive. At pH above its pKa, it is hydrophobic because its tertiary

amino groups are deprotonated. On the other hand, the polymer becomes a cationic hydrophilic polymer at pH below its pKa, due to protonation of its tertiary amines.

3. PDEAEM is biodegradable.

4. The polymer is cytotoxic but this effect is drastically reduced by crosslinking or copolymerization with cross-linkers such as PEG as seen in a number of its hydrogels. Hence its issue of biocompatibility is resolved.

5. PDEAEM has a good charge density

6. The polymer has an excellent DNA binding ability.

7. PDEAEM is elastic and gummy(sticky)

1.3 Hydrogels

Hydrogels can be described as three-dimensional system structure resulting from natural and/or synthetic polymers with the ability to absorb and retain substantial amount of water (Gulrez and Al-Assaf, 2011). Hydrogel is defined as "a water-swollen and crosslinked polymeric network produced by simple reaction of one or more monomers". In another view, hydrogel is also defined as "a polymeric material that exhibits the ability to swell and retain significant amount of water within its structure without dissolving in the water" (Ahmed, 2015). The structure of hydrogel is created by the hydrophilic groups available in a polymeric system through hydration in an aqueous medium or environment (Gulrez and Al-Assaf, 2011). Hydrogels are also called gels and the names can be used interchangeably in context. Figure 1.3 shows a diagrammatic representation of a prototype hydrogel preparation (Ahmed, 2015).

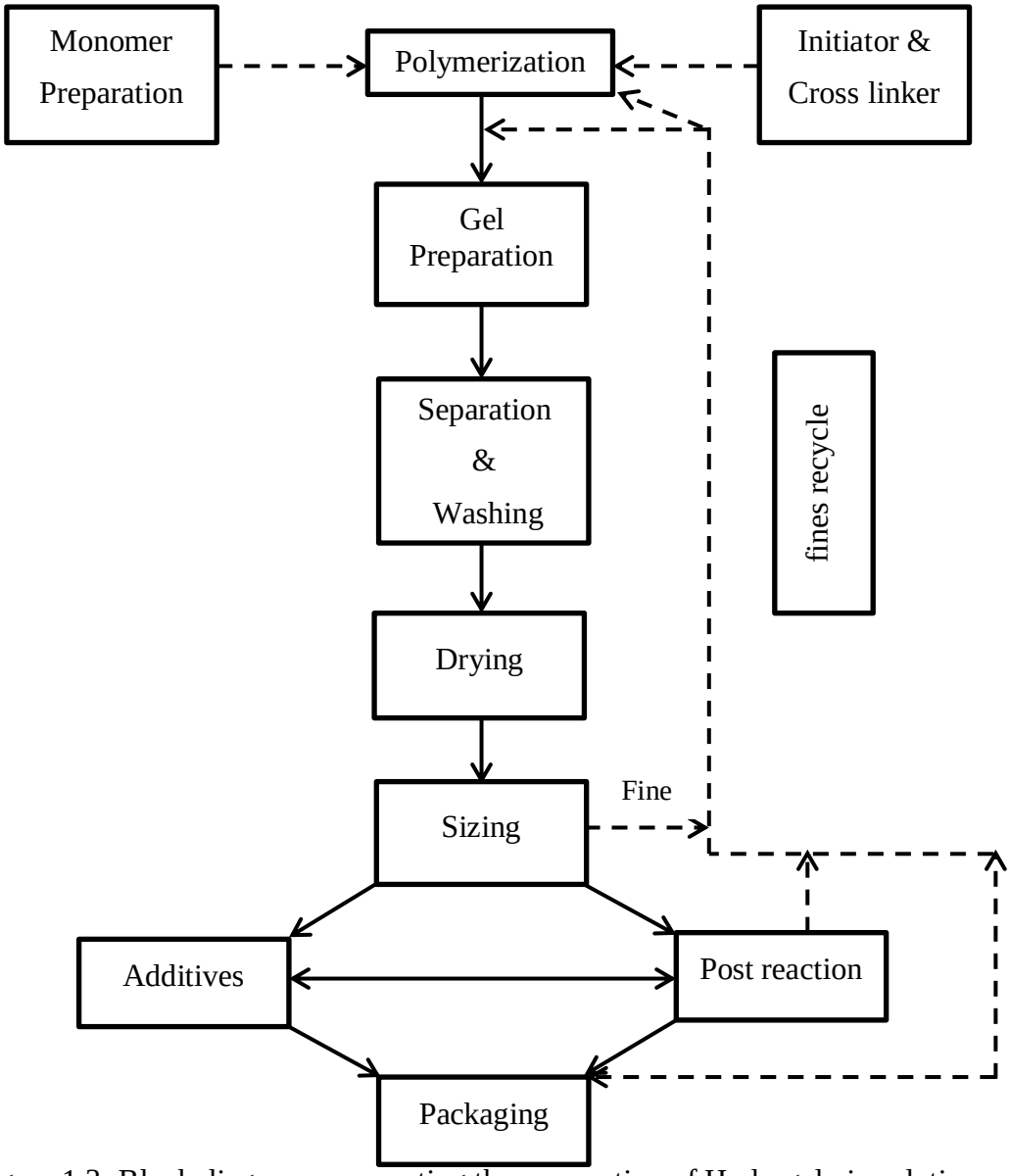

Figure 1.3: Block diagram representing the preparation of Hydrogel via solution polymerization method/crosslinking method

Hydrogels can be classified as follows:

1. Source: Hydrogels can be of natural origin or synthetic origin

2. Composition: Hydrogels can be homopolymeric, copolymeric or multipolymeric. Homopolymeric hydrogels in their networks have only one type of monomer. Copolymeric hydrogels have two or more different species of monomers in their networks. The arrangements of the monomers in these networks may be block or random configuration. Multipolymeric hydrogels on the other hand are otherwise

known as IPNs (Interpenetrating Polymeric Hydrogels). These set of hydrogels have two independently crosslinked polymers either synthetic and/or natural, in their networks. The composition can also be a non crosslinked polymer and a crosslinked polymer.

3. Configuration: Hydrogels can also be crystalline, semi crystalline or amorphous in their configuration

4. Crosslinking type: The type of crosslinking in the hydrogel network can be permanent which is otherwise known as chemical crosslinking or physical otherwise known as reversible crosslinked hydrogels.

5. Appearance: Some hydrogels appear as films, microspheres, matrix etc. This is largely influenced by the polymerization techniques.

6. Electrical charge: Hydrogels can be further classified as ionic (anionic or cationic), non-ionic or amphoteric (Ahmed, 2015).

7. Hydrogels are also classified by gelation mechanism as shown in Figure 1.4 (Gulrez and Al-Assaf, 2011):

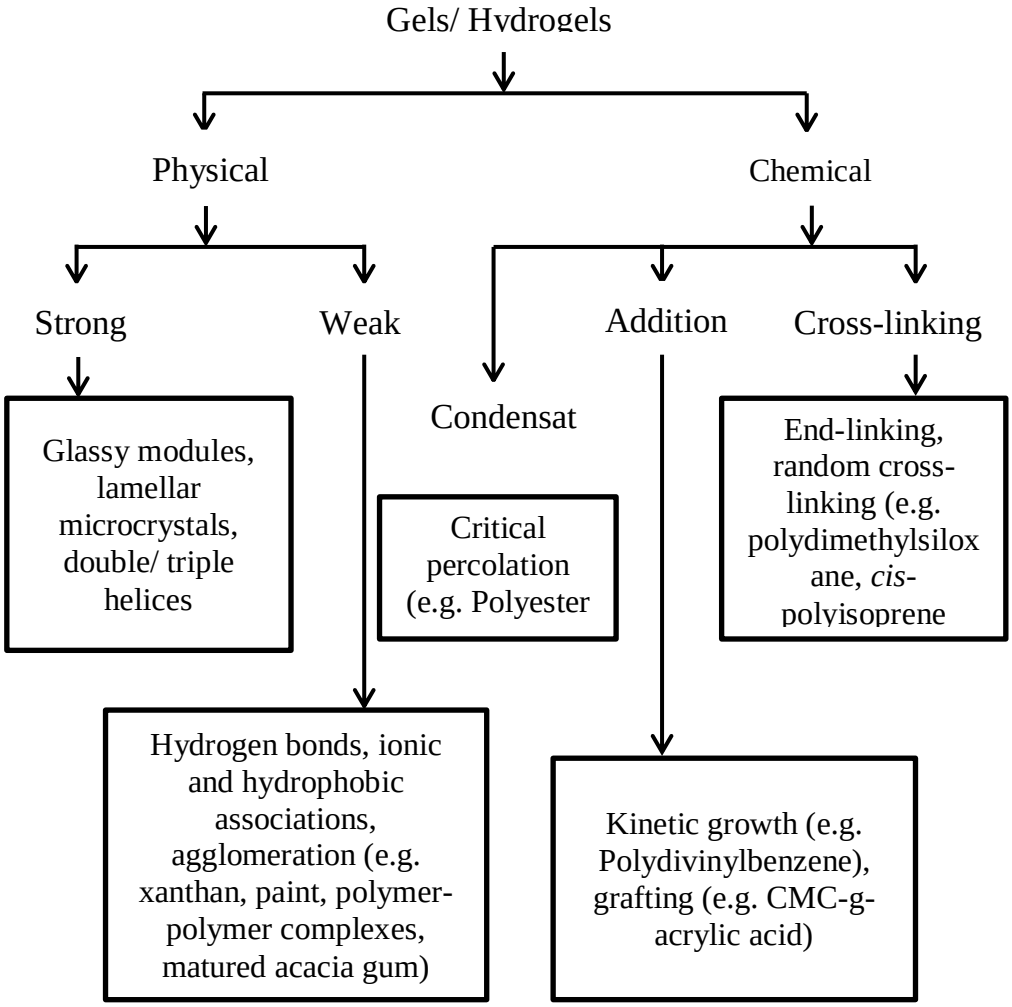

Figure 1.4: Classification of hydrogel by preparation mechanism

1.3.1 Swelling Principles of Hydrogels

Hydrogels are of great importance in research today due to their swelling and de-swelling behaviour. When hydrogels swell, they absorb and retain water hence substances of choice are incorporated into the hydrogels due to the swelling activity. Hydrogels also release whatever is entrapped in them when they de-swell. The swelling and de-swelling properties of hydrogels are however influenced by a number of conditions which are classified as chemical or physical stimuli. They generally include pH, electric field, magnetic field, temperature, solvent composition and ionic strength amongst others. Figure 1.5 shows the swelling and de-swelling mechanism of a hydrogel under the influence of an external stimulus.

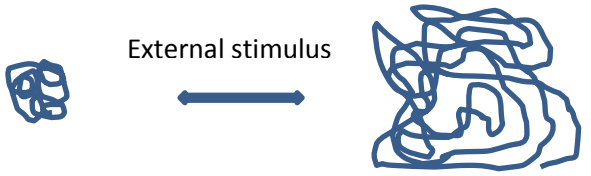

Figure 1.5 Swollen and un-swollen hydrogel

1.3.2 Application of Hydrogels

Hydrogels have various applications and a few of them are highlighted in Table1.2 (Gulrez and Al-Assaf, 2011):

Table1.2: Examples of Polymeric Hydrogels and their Applications

Purpose of use	**Type of hydrogels**
Wound care	Polyurethanes, Polyethylene glycol
Drug delivery/Pharmaceuticals	Carboxylmethyl cellulose, chitosan
Dental materials	Hydrocolloids
Tissue engineering implants	Poly(acrylic acid), Collagen
Injectable polymeric system	Polyesters, Polyphosphazenes
Technical products	Xanthan, Polyvinyl alcohol

1.3.3 Hydrogels of PDEAEM

The hydrogels of PDEAEM synthesized through crosslinking and copolymerization has made the polymer fit for gene/drug delivery systems and other biomedical applications due to modifications or tuning of its properties such as cytotoxicity which affect cells negatively. These hydrogels are also synthesized through the same polymerization techniques as the homopolymer as already described earlier. The hydrogels of PDEAEM synthesized in this research is poly[2-(diethylamino ethyl

methacrylate)/(ethylene glycol dimethacrylate)] PDEAEM/EGDMA as shown in Figure 1.6:

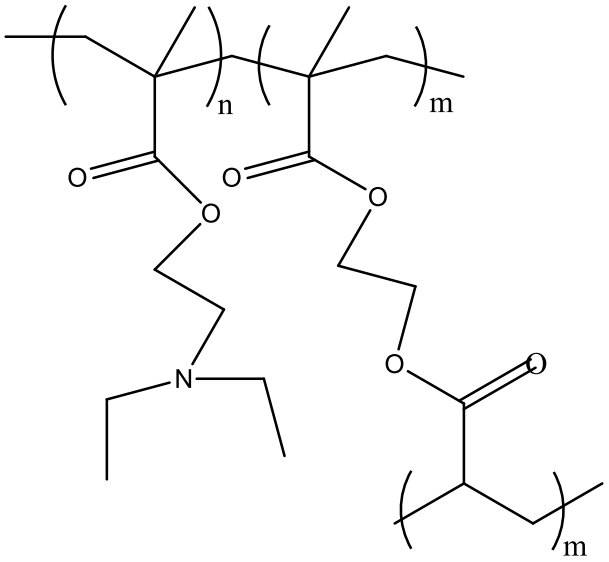

Figure 1.6: PDEAEM/EGDMA

The following are some of the hydrogels of PDEAEM and their properties as discovered in the literatures currently available:

1. Poly(dimethylamino ethyl methacrylate-b-diethylamino ethyl methacrylate) [P(DMAEM-b-DEAEM)]

This hydrogel, as reported by Bütün et. al., 2001, exhibits pH induced micellization. The hydrogels show hydrophilic-hydrophilic behavior in acidic solution (pH 2) and hydrophobic-hydrophilic behavior in slightly basic solution (pH 8) (Butun et al., 2001). The copolymer becomes micelles at pH 8 and at pH 2, it becomes unimers is illustrated in Figure 1.7:

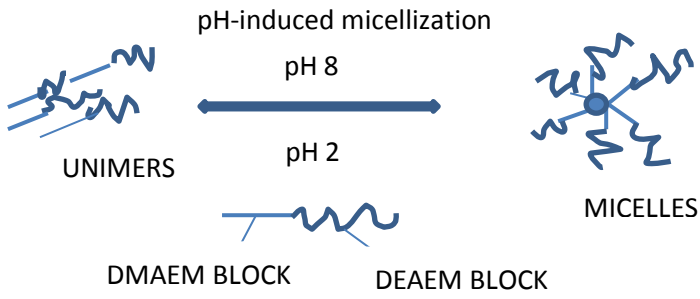

Figure 1.7: Diagrammatic illustration of pH induced micellization for P(DMAEM-b-DEAEM)

2. Poly[2-(diethylamino ethyl methacrylate)]-b-Poly(N-isopropylacrylamide) (PDEAEM-b-PNIPAM)

Poly[2-(diethylamino ethyl methacrylate)]-b-Poly(N-isopropylacrylamide) was synthesized by Liu et. al., in 2008. Their research reveals that PDEAEM-b-PNIPAM shows double sensitivity (including pH-sensitive and thermo-sensitive behavior).

3. The solubility of PDEAEM at pH 7.4 was made possible through copolymerization with ethylene glycol methyl ether methacrylate (EGMEM). P(EGMEM-b-DEAEM) 30:70 mass fraction was fully soluble at pH 7.4. (Anderson and Mallapragada, 2002).

4. Marek et. al., 2013, showed that nanoparticles of poly[(diethylamino ethyl methacrylate)-b-poly ethylene glycol] [P(DEAEM-g-PEG)] swelled from 100nm in their collapsed state to about 800nm in the swollen state as measured by Dynamic Light Scattering (Marek and Peppas, 2013).

5. Methyl methacrylate and diethylaminoethyl methacrylate (7:3) copolymer dispersion which is registered as Kollicoat® Smartseal 30 D produced by BASF is an aqueous dispersion of a film forming polymer. It is used for taste masking, that is, reducing undesirable taste and moisture barrier applications. The polymer safeguards active protection from unpleasant taste and humidity.

1.4 Controlled Drug Delivery

Drug delivery can be described as a route of administering a medication to accomplish a therapeutic result in humans or animals (Tiwari et al., 2002). Drug delivery becomes controlled when the concentration of a drug is being maintained with respect to time until it reaches the peak concentration and is sustained in its therapeutic window with respect to time. The progression of controlled delivery of drugs started in 1952. The first set of drug delivery was on how to develop oral and transdermal continuous release system thereby establishing mechanisms for controlled release. This happened between 1950 and 1980. The second set (1980-2010) was characterized by developing a release system of the "zero-order kinetics", self-controlled delivery system and delivery systems using nanomaterials amongst others. Smart materials and polymers which were produced by several means of synthesis accessible at macro, micro and nano states played major roles in this era. The kinetics of drug release of these materials were also studied. The study of nanoparticles for drug delivery characterized the latter period of this era. The third generation, (2010 and 2040) is expected to have immediate and direct impact on realities. This set would be expected to marry the *in vitro* release systems with the *in vivo* release (Park, 2014).

1.5 Ciprofloxacin-HCl

According to *WebMD and Drugs.com*, Ciprofloxacin is a drug belonging to the fluoroquinolones. It is an antibiotics and basically used for the treatment of bacterial contaminations. Fluoroquinolones are known antibiotics with a wide range of antibacterial applications to infections such as gastro-intestinal infections, respiratory tract contaminations, skin contaminations and sexually transmitted infections (Talan et al., 2004). Its mode of action is to prevent the growth or development of bacteria.

Ciprofloxacin HCl basically combats all kinds of bacteria present in almost any part of the body. Figure 1.8 gives the molecular structure of ciprofloxacin-HCl. Ciprofloxacin has lots of side effects which the patients need to watch out for. Some of them include skin color change, bloody stools or black stools, itching, general discomfort etc.

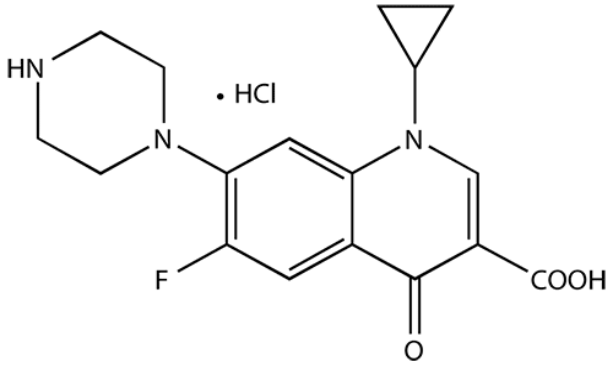

Figure 1.8: Molecular structure of ciprofloxacin-HCl

1.6 Drug Release Kinetics

The dissolution, release or diffusion of drugs have been studied and described using kinetic models. These kinetic models are zero order, first order, Hixson-Crowell and Higuchi model. Drug release is affected by a number of parameters such as drug solubility, solvent, polymer permeability and so on (Chime et. al., 2013). Equations 1.1, 1.2, 1.3,and 1.4 represents the equations of the zero-order release equation, first-order release equation, Higuchi model, and the Korsmeyer-Peppas model respectively.

1. Zero-order release equation

$$Q_t = Q_o + K_o t \quad (1.1)$$

Where Q_t is the cumulative amount of drug releases at time t

Q_o is the initial amount of drug

K_o is zero-order release constant

t is time (h)

The zero-order kinetics is represented graphically by plotting the cumulative drug release vs time. The plot should be linear. This model is applicable to systems in which the rate of drug release is independent of its concentration (Lokhandwala et al., 2013).

2. First-order release equation

$$logC_o = logC + \frac{Kt}{2.303} \quad (1.2)$$

Where C is the cumulative amount of drug releases at time t

C_o is the initial amount of drug

K first-order release constant

t is time (h)

The first-order kinetics is represented graphically by plotting the log of %cumulative drug release vs time. The plot should be linear. This model is applicable to systems in which the rate of drug release is dependent of its concentration (Dash et al., 2010).

3. Higuchi model

$$Q = K_H t^{1/2} \quad (1.3)$$

Where Q is the cumulative amount of drug releases at time t

K_H Higuchi dissolution constant

t is time (h)

The Higuchi model is represented graphically by plotting the cumulative drug release vs $t^{1/2}$. The plot is expected to be linear (Chime et al., 2013).

4. Korsmeyer-Peppas

$$\frac{M_t}{M_\alpha} = Kt^n \quad (1.4)$$

Where M_t is the cumulative amount of drug releases at time t

M_α is the total amount of drug in dosage form

K is kinetic constant

t is time

The Korsmeyer-Peppas kinetics is represented graphically by plotting the log M_t/M_α vs log of time. The plot should be linear. The value of n is obtained from log M_t/M_α vs log t, and it determines what kind of behaviour is expressed by the delivery system (Dash et al., 2010).

1.7 Application of PDEAEM Hydrogels

One of the advantages of PDEAEM is its excellent DNA binding ability which has made it a desirable component of the gene/drug delivery systems. Its ease of polymerization, pH sensitivity and biodegradability are additional desirable properties of the polymer.

The polymer and it hydrogels can also be applied in dye adsorption/metal adsorption from solution or drug encapsulation as reported by Abdel-Halim (Abdel-Halim, 2013).

1.8 Aim

This thesis focuses on the synthesis and characterization of poly[2-(diethylamino ethyl methacrylate)] (PDEAEM) and its ethylene glycol dimethacrylate (EGDMA) cross-linked hydrogels by free radical polymerization. The thesis further investigates the swelling properties, the drug loading and delivery properties of the homopolymer and its hydrogels using ciprofloxacin HCl.

Chapter 2

EXPERIMENTAL

2.1 Materials

The materials used in this project are as listed in the Table 2.1. The materials were used as received. The distilled water used was from our laboratory.

Table 2.1: Chemicals and materials used and their respective manufacturers

S/No.	Materials (chemicals)	Manufacturer
1.	2-(diethylamino ethyl methacrylate)	Aldrich-Germany
2.	Ethylene glycol dimethacrylate (EGDMA)	Sigma-Aldrich-Germany
3.	Potassium Persulfate	Aldrich-Germany
4.	Hydrochloric Acid	Merck-Germany
5.	Sodium hydroxide	Sigma-Aldrich-Germany
6.	Potassium chloride	Sigma-Aldrich-Germany
7.	Monopotassium phosphate	Aldrich-Germany
8.	Acetic Acid	Merck-Germany

2.2 Synthesis of Homopolymer and Hydrogels

The homopolymer and the cross-linked hydrogels were synthesized as described below.

2.2.1 Homopolymerization

The homopolymerization was carried out by free radical polymerization using 20%(v/v) DEAEM solution in water with pH 5 adjusted using 1M HCl. The reaction was initiated by potassium persulphate (KPS) (monomer/initiator ratio is 100:1 mol/mol). Before the polymerization process, the monomer solution in water with pH 5 was first purged under nitrogen atmosphere for about 20 min. Afterwards the initiator was added and the mixture was further purged for another 10 min. The polymerization was carried out for 22 h at 60 ^{o}C. The polymer obtained was then extensively purified by dialysis against 1% acetic acid and distilled water respectively. The homopolymer were obtained by precipitation using 1M NaOH and dried at about 40 ^{o}C. The picture of the PDEAEM homopolymer obtained is shown in Figure 2.1.

Figure 2.1: Photograph of PDEAEM homopolymer

2.2.1.1 Hydrogel Preparation

Hydrogels of PDEAEM chemically crosslinked with ethylene glycol dimethacrylate (EGDMA) were prepared under conditions similar to homopolymerization of DEAEM. The amount of monomer and initiator used remained constant with varying amount of the EGDMA cross-linker. Table 2.2 shows the amount of chemicals/reagents used for the crosslinking experiments.

Table 2.2: Reagents for cross-linking

Monomer/Crosslinker ratio	DEAEM Monomer (mL)	EGDMA Crosslinker (mL)
1:2	2 (1×10^{-2} mol)	4 (2×10^{-2} mol)
1:4	2 (1×10^{-2} mol)	8 (4×10^{-2} mol)
1:7.5	2 (1×10^{-2} mol)	15 (7.5×10^{-2} mol)

2.3 Instrumental Analysis

2.3.1 FTIR Analysis

FTIR analysis of the PDEAEM homopolymer and EGDMA cross-linked hydrogels of PDEAEM were determined with Perkin Elmer Spectrum 2 FT-IR spectrometer.

2.3.2 Particle Size Analysis

The EGDMA cross-linked hydrogels of PDEAEM obtained were crushed into fine particles and the particles were separated into different sizes by the use of Retsch AS 200 Vibratory Sieve Shaker.

2.3.3 SEM Analysis

The SEM analysis of the PDEAEM homopolymer and the EGDMA cross-linked hydrogels of PDEAEM were taken at Cyprus International University, Nicosia using JEOL/JSM-6510LVF scanning electron microscope. The samples were analysed in their dried form and there was no pre-treatment done on the samples.

2.3.4 Swelling Properties

Samples of the homopolymer and each of the cross-linked hydrogels were weighed respectively and the swelling properties were examined at pH (1.2, 4 and 7). The percentage swelling for each hydrogel was calculated as in equation 2.1:

$$\% \text{ Swelling } = \frac{M_2}{M_1} X\ 100 \qquad (2.1)$$

Where:

M_1 = mass of hydrogels before soaking in buffer solution

M_2= mass gained by the hydrogels after soaking in buffer solution with respect to time

2.4 Drug Loading and Release

2.4.1 Phosphate Buffer Solution Preparation

Phosphate buffer solution of pH 1.2 and 4.0 were used. The composition of the buffer solutions are shown in Table 2.3.

Table 2.3: Buffer solution reagents

pH	Constituents	Volume
1.2	333.4 mL 0.2 M KCl + 166.6 mL 0.2 M HCl	0.5 L
4	333.4 mL of 1 M KH_2PO_4 + 166.6 mL of 0.1 M HCl	0.5 L

2.4.2 Ciprofloxacin HCl Calibration Curve

A stock solution of 5 mg/500 mL of ciprofloxacin HCl was prepared in pH 1.2, 4 and distilled water respectively. The drug stock solution was prepared by dissolving the drug in phosphate buffer pH 1.2, pH 4 and water at room temperature. Series of concentrations of ciprofloxacin HCl were prepared from the stock solution by serial dilution to obtain different concentrations. The Beer-Lambert calibration curve was prepared by measuring the absorbance of the prepared concentrations with UV-visible spectrometer at wavelength of 275 nm for pH 1.2; 240 nm for pH 4 and 275 nm for distilled water as obtained from the respective spectra.

2.4.3 Drug Loading

For the drug loading, 25 mL was taken from the stock solution and 25 mg of the hydrogels were weighed and added into the collected drug solution. This was done using buffers pH 1.2, pH 4 and distilled water separately. This was allowed to incubate for 48 h for the first set of experiments and 72 h for the second and third sets. The absorbance of the drug solution (A_i) after loading was measured using the UV-visible spectrometer and it was computed into the equation obtained from the calibration curve to calculate the concentration (C_i) of the loaded drug. The hydrogels used are the homopolymer, 1:2 PDEAEM/EGDMA and 1:7.5 PDEAEM/EGDMA respectively. The drug loading percentage was calculated as given in equation 2.2:

$$\% \text{ Loading } = \frac{C_i}{C_o} X\ 100 \qquad (2.2)$$

Where:

C_i = concentration of drug loaded into the hydrogels

C_o = initial concentration of drug available for loading

2.4.4 *In-vitro* Release

After the incubation time was over, the drug-loaded hydrogels were collected and washed with distilled water several times and air-dried. The dried and drug loaded hydrogels were placed in a beaker with 25 mL of fresh buffer pH 1.2, pH 4 and distilled water respectively. The beaker containing the buffer and the gels was then placed in a water bath maintained at 37^{o}C. About 3 mL was withdrawn from the beaker every 1 h and the absorbance measured at 275 nm for pH 1.2, 240 nm for pH 4 and 275 nm for distilled water respectively. The calibration curve was used to calculate the concentrations of the released drug at each interval. The percentage drug release was calculated as shown in equation 2.3:

$$\% \text{ Release } = \frac{C_r}{C_i} X\ 100 \qquad (2.3)$$

Where:

C_r = cumulative amount of drug release from the hydrogels

C_i = amount of drug loaded into the hydrogels

Chapter 3

RESULTS AND DISCUSSION

3.1 Homopolymerization

Poly[2-(diethylamino ethyl methacrylate)] homopolymer shown in Figure 1.2 was obtained by free radical polymerization in solution. The initiator potassium persulphate (KPS) dissociate to produce sulphate anion radical as shown in Figure 3.1. The radical produced initiates the polymerization of the monomer.

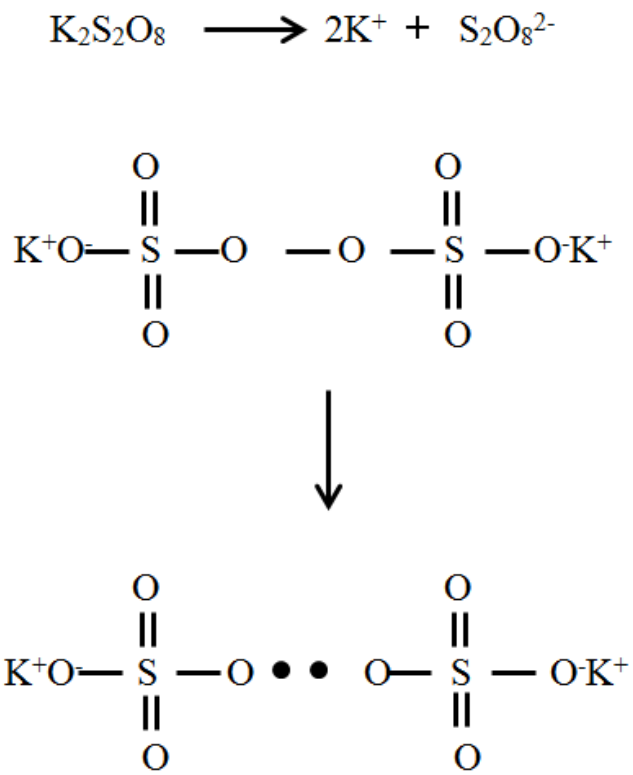

Figure 3.1: Dissociation of KPS

3.1.1 Hydrogel Preparation

EGDMA crosslinked hydrogels of PDEAEM are obtained by simultaneous free radical polymerization and chemical crosslinking in solution. The structure of the crosslinked polymer is given in Figure 1.6.

The products were white powders as shown in Figure 3.2

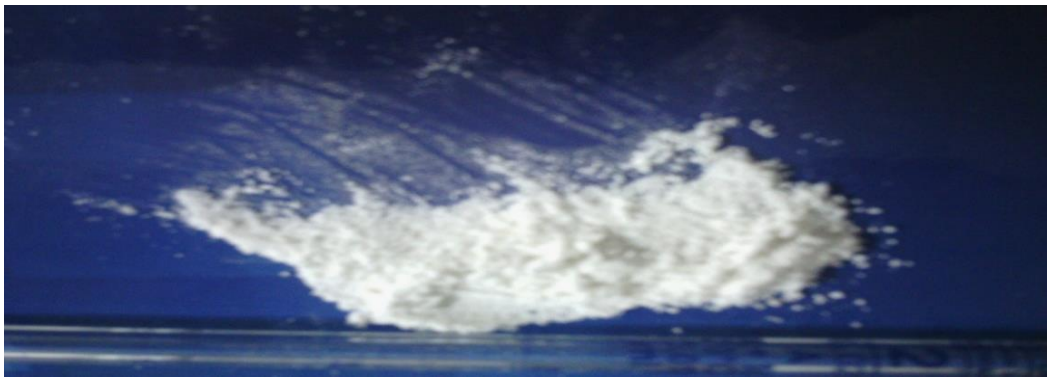

Figure 3.2 (a): Photograph of PDEAEM/EGDMA hydrogels (average diameter 250µm)

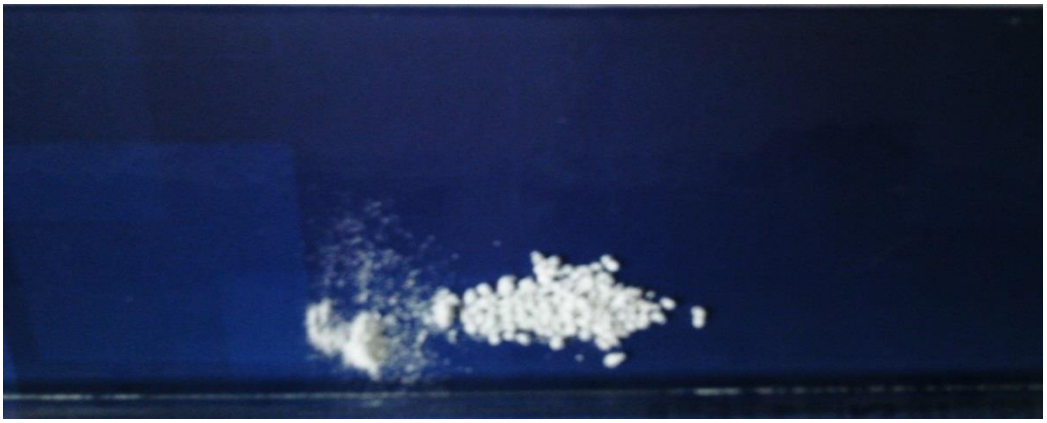

Figure 3.2 (b): Photograph of PDEAEM/EGDMA hydrogels (average diameter 500µm)

3.2 Instrumental Analysis

3.2.1 FTIR Analysis

The FTIR analysis for the Poly[2-(diethylamino ethyl methacrylate)] homopolymer and the EGDMA cross-linked hydrogels are shown in Figure 3.3

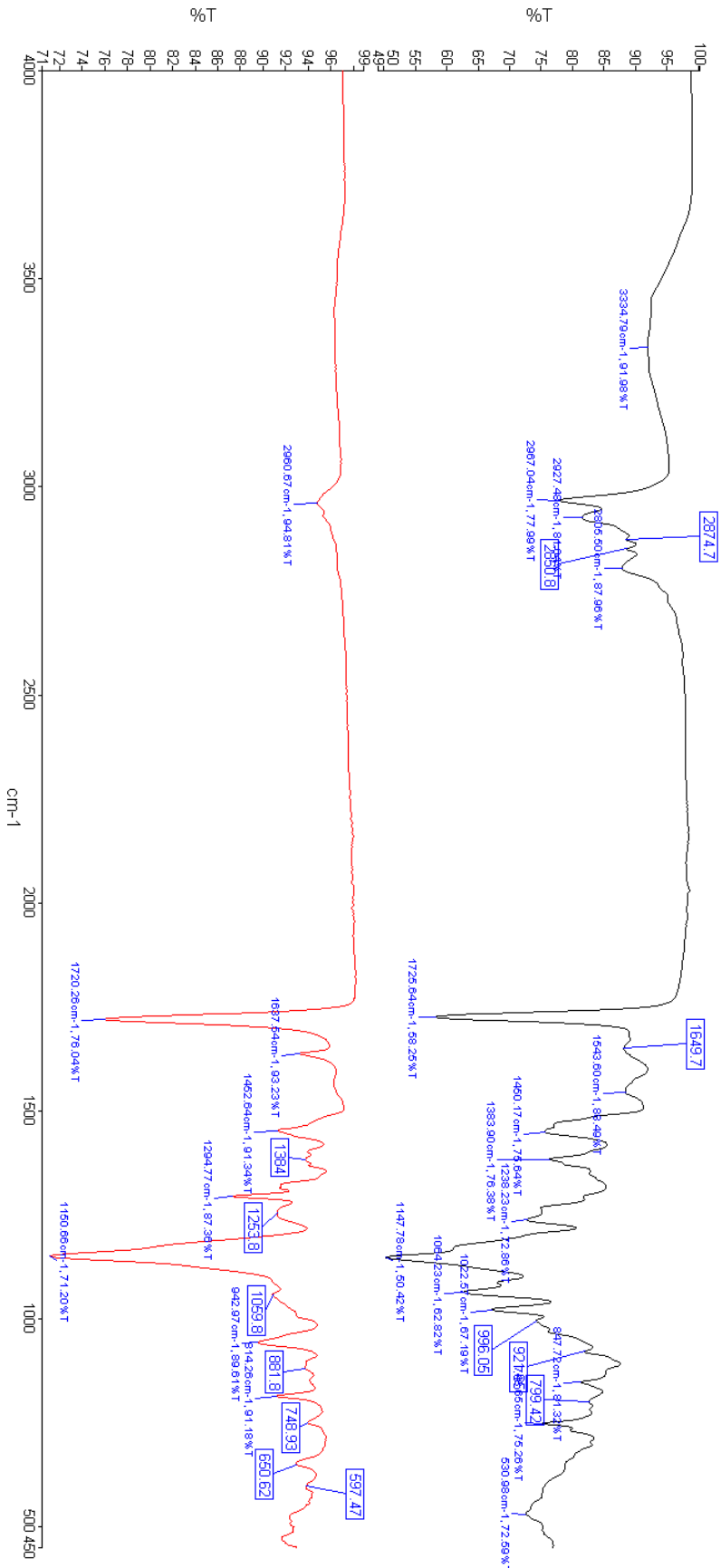

Figure 3.3: FTIR Spectra for (a) PDEAEM (b) PDEAEM/EGDMA hydrogel

The FTIR spectrum of the PDEAEM and that of the EGDMA crosslinked hydrogel is shown in Figure 3.3(a) and (b) respectively. The spectrum of the homopolymer exhibits characteristic bands as explained below:

In the region 2900 - 2800 cm^{-1} C-H stretching of the methylene groups are observed. Bending vibrations of the methylene groups and the methyl group are found in the region 1450 - 1380 cm^{-1} the C=O stretching of the carbonyl group is at 1725 cm^{-1} and the stretching vibrations C-O-C is identified in the region 1100 – 1000 cm^{-1}.

In the spectrum of the hydrogel shown in Figure 3.3(b), characteristics C-H stretching, C-H bending C-O stretching and carbonyl stretching band can be observed. As the functional groups added by the EGDMA to PDEAEM homopolymer structure are not different from the groups already present on the PDEAEM, it is not possible to observe very distinctive differences in the two spectra except for a shift in the carbonyl stretching from 1725 cm^{-1} to 1720cm^{-1} and a strong C-O-C stretching vibration at 1150 cm^{-1}.

3.2.2 SEM Analysis

Figure 3.4 (a), (b), (c), (d) and (e) represent SEM picture of poly[2-(diethylamino ethyl methacrylate)] homopolymer magnified by 250, SEM picture of poly[2-(diethylamino ethyl methacrylate)] homopolymer magnified by 2000, SEM picture of hydrogel magnified by 250, SEM picture of hydrogel magnified by 2000 and SEM picture of hydrogel magnified by 2500 respectively. The characteristics of the surface structure of the PDEAEM homopolymer and that of the crosslinked hydrogels were studied. As clearly seen from the SEM pictures in Figure 3.4 (a) and (b) , there is a drastic transformation from a partially smooth and seemingly impermeable surface of the PDEAEM homopolymer to a rough and clearly porous surface characteristics in the SEM pictures in Figure 3.4 (c), (d) and (e). The

irregular and highly porous surface in the latter SEM pictures are indicative of highly crosslinked hydrogels.

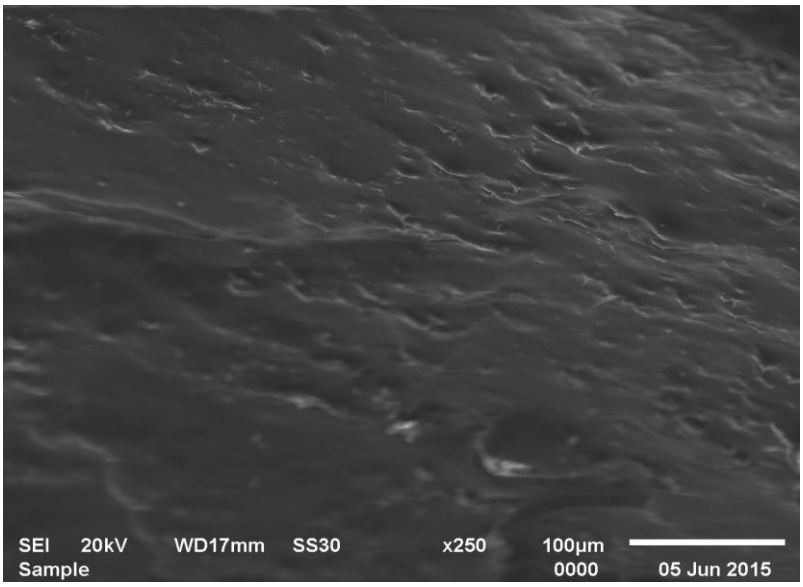

Figure 3.4 (a): SEM picture of PDEAEM x250

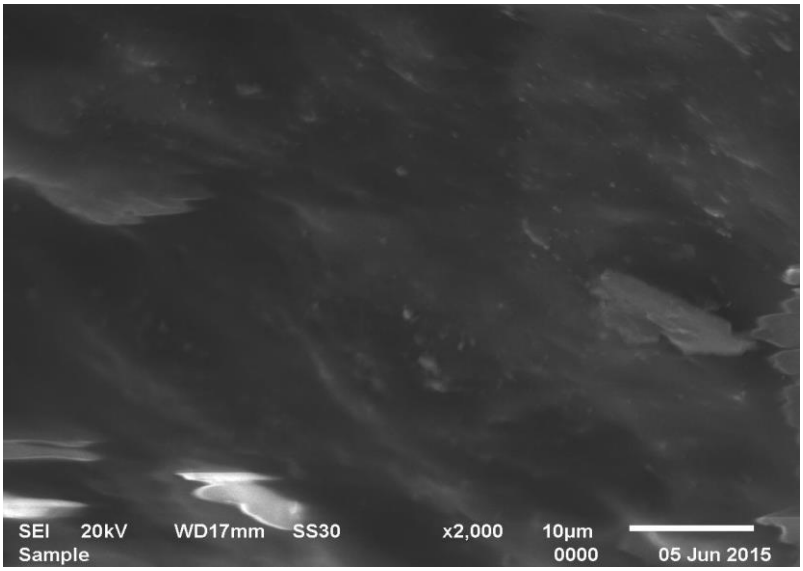

Figure 3.4 (b): SEM picture of PDEAEM x2000

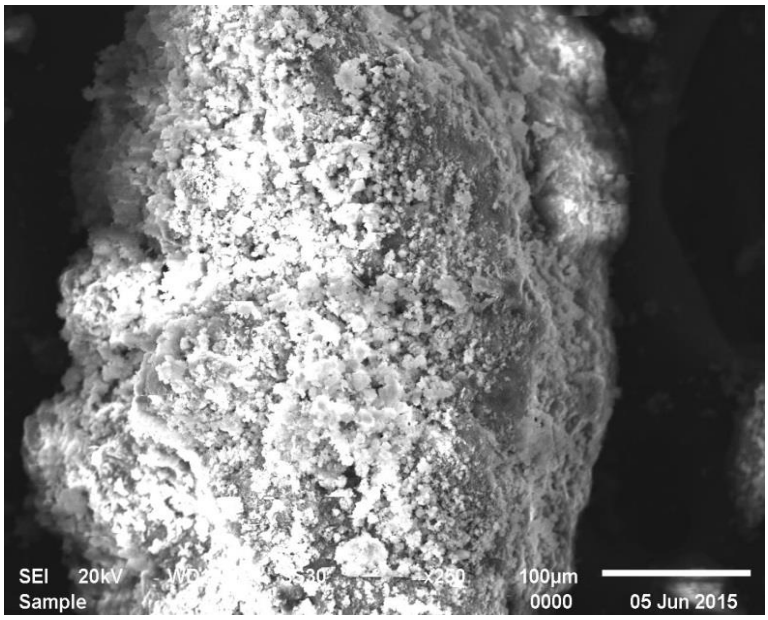

Figure 3.4 (c): SEM picture of PDEAEM/EGDMA hydrogel x250

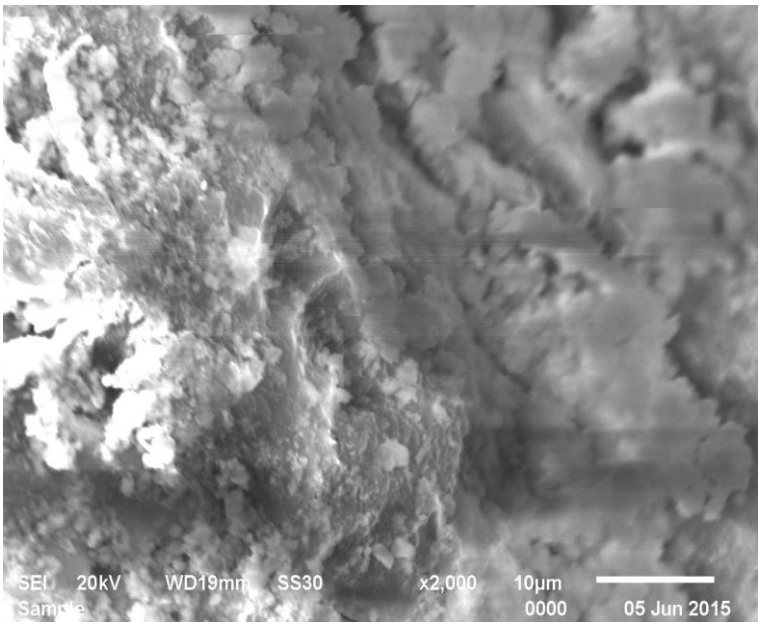

Figure 3.4 (d): SEM picture of PDEAEM/EGDMA hydrogel x2000

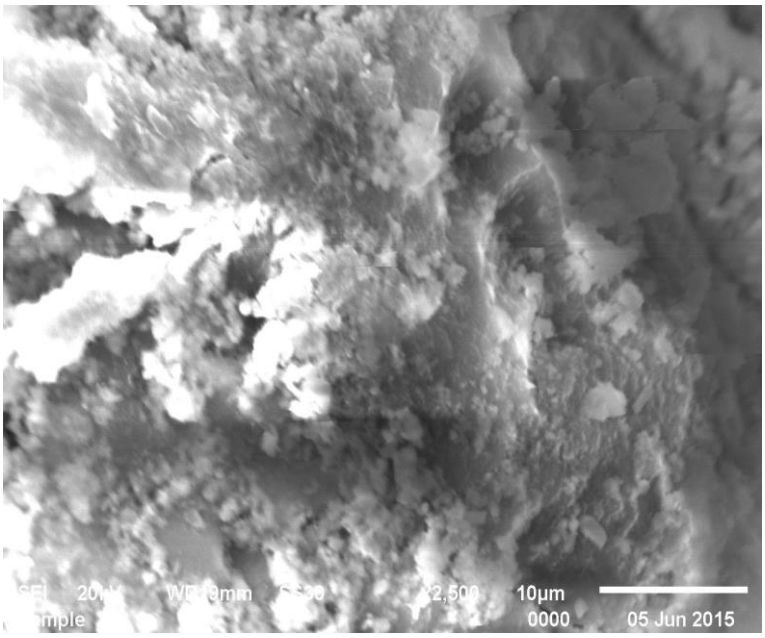

Figure 3.4 (e): SEM picture of PDEAEM/EGDMA hydrogel x2500

3.2.3 Swelling Properties

In order to investigate the swelling properties of the homopolymer and the hydrogels, the samples were introduced into buffer solutions with different pH values. The pH values of the buffering medium used are 1.2, 4 and distilled water at 25ºC. The swelling capacity of the homopolymer was only investigated in distilled water as it readily dissolves in pH 1.2 and pH 4 due to protonation. Table 3.1(a), (b) and (c) and Figure 3.5(a), (b) and (c) below give a clear picture of the swelling properties of the hydrogels.

Table 3.1(a): % Swelling at pH 1.2

Time h	% Swelling 1:2 hydrogels	% Swelling 1:7.5 hydrogels
1	8	7.
2	14	12
3	20	16
4	22	20
5	26	24
6	33	26
26	51	48

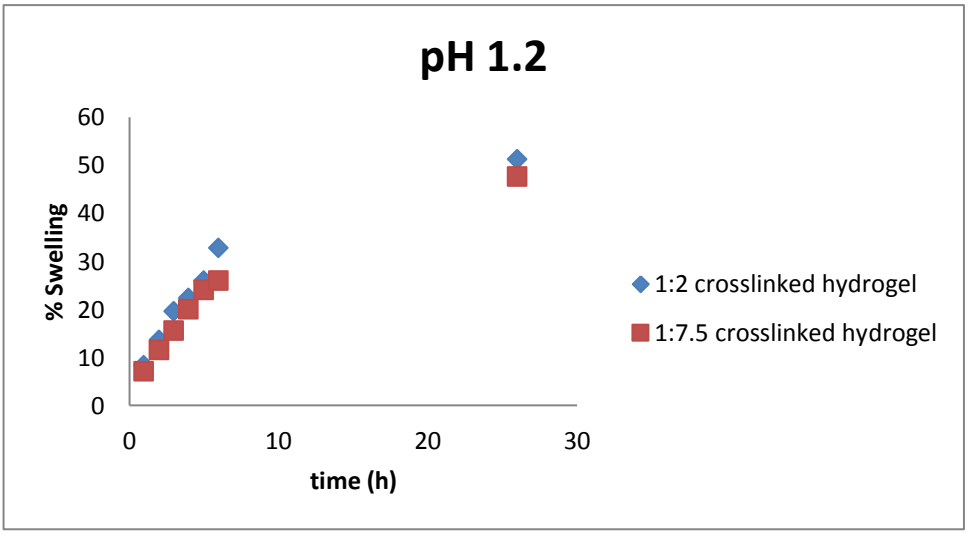

Figure 3.5(a): Swelling of hydrogels at pH 1.2

The homopolymer readily dissolves in acidic medium due to protonation. The hydrogels, which are cationic hydrogels, swells in acidic medium due to protonation. The swelling of the hydrogels is as a result of repulsion within the protonated amine groups. According to Figure 3.5(a), 1:2 hydrogels and 1:7.5 hydrogels reached equilibrium swelling capacity at 51% and 48% respectively within 30 h. Although the difference between the two hydrogels is not much but it can still be associated with the crosslinking density of the 1:7.5 hydrogels which is higher than in 1:2

hydrogels (Schwarte and Peppas, 1998). A similar trend is seen in Figure 3.5(b) below in pH 4.

Table 3.1(b): % Swelling at pH 4

Time h	%swelling 1:2 hydrogels	%swelling 1:7.5 hydrogels
1	8	8
2	12	12
3	19	16
4	22	20
5	25	24
6	30	28
26	50	45

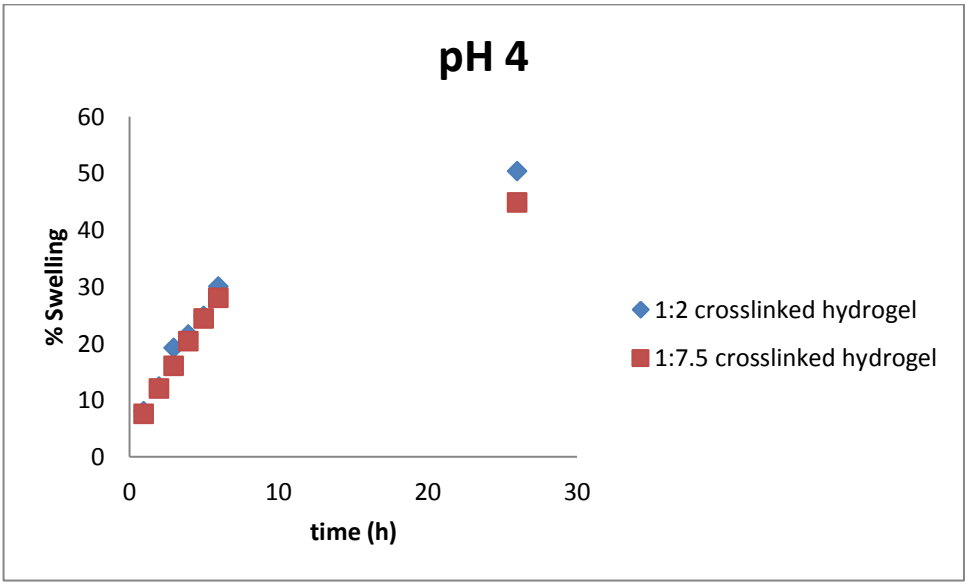

Figure 3.5(b): Swelling of hydrogels at pH 4

Table 3.1(c): % Swelling in distilled water

Time h	%swelling 1:2 hydrogels	%swelling 1:7.5 hydrogels	PDEAEM homopolymer
1	6	6	21
2	8	8	36
3	10	10	59
4	16	14	80
5	20	18	85
6	23	21	108
26	34	31	260

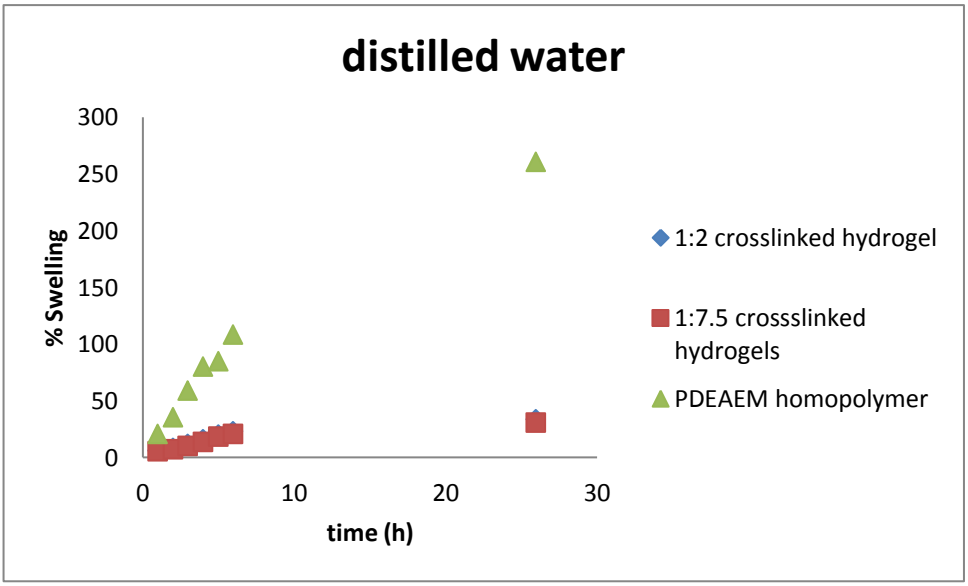

Figure 3.5(c): Swelling of hydrogels in distilled water

Figure 3.5(c) above shows a different principle where the homopolymer reached it maximum swelling after 26 h. The deprotonation results in repulsion within the polymer structure of the homopolymer thereby favouring good interaction with water. On the other hand, the hydrogels 1:2 and 1:7.5 reached equilibrium swelling capacity at 34% and 30% respectively within 30 h. The decrease in degree of swelling observed compared to the swelling properties of the homopolymer is due to

crosslinking in the hydrogels and poor or no ionic interaction because the hydrogels carry little or no electric charge in distilled water.

3.2.4 Particle Size Analysis for EGDMA Cross-linked Hydrogels

The masses of the hydrogels before and after the particle size experiment were recorded according to the each cross-linker ratio. The size distribution of the hydrogels for each cross-linker ratio was also recorded as shown below as shown in Table 3.2.

Table 3.2: Particle size distribution for cross-linked hydrogels

Sieve size (μm)	1:2 poly(DEAEM-co-EGDMA) (g)	1:4 poly(DEAEM-co-EGDMA) (g)	1:7.5 poly(DEAEM-co-EGDMA) (g)
125	0.0262	0.1116	0.0781
250	1.2197	0.2047	0.2432
500	1.2261	0.0630	0.1470
1000	0.4314	0.0043	0.0288

3.3 Drug Loading and Release

The antibacterial drug, ciprofloxacin-HCl was loaded into the hydrogels and the drug/hydrogel system was tested as a controlled drug release system.

3.3.1 Ciprofloxacin-HCl Calibration Curve in pH 1.2, pH 4 and Distilled Water

Ciprofloxacin-HCl calibration curve data in pH 1.2, pH 4 and distilled water are represented in Table 3.3(a), (b) and (c), and Figure 3.6(a), (b) and (c) respectively. Different concentrations of the ciprofloxacin-HCl were prepared by serial dilution of 5mg/500mL stock solution in phosphate buffers pH 1.2, 4 and distilled water

respectively. The corresponding absorbance for each concentration was measured and the calibration curves using the three buffers were obtained respectively.

Table 3.3 (a): Spectrometric data for the calibration curve of ciprofloxacin-HCl at pH 1.2, λ_{max} = 275nm

Concentration mg/mL	Absorbance (A)
0.0003	0.112
0.0005	0.135
0.0010	0.176
0.0013	0.199
0.0030	0.375
0.0050	0.569
0.0070	0.730
0.0090	0.896

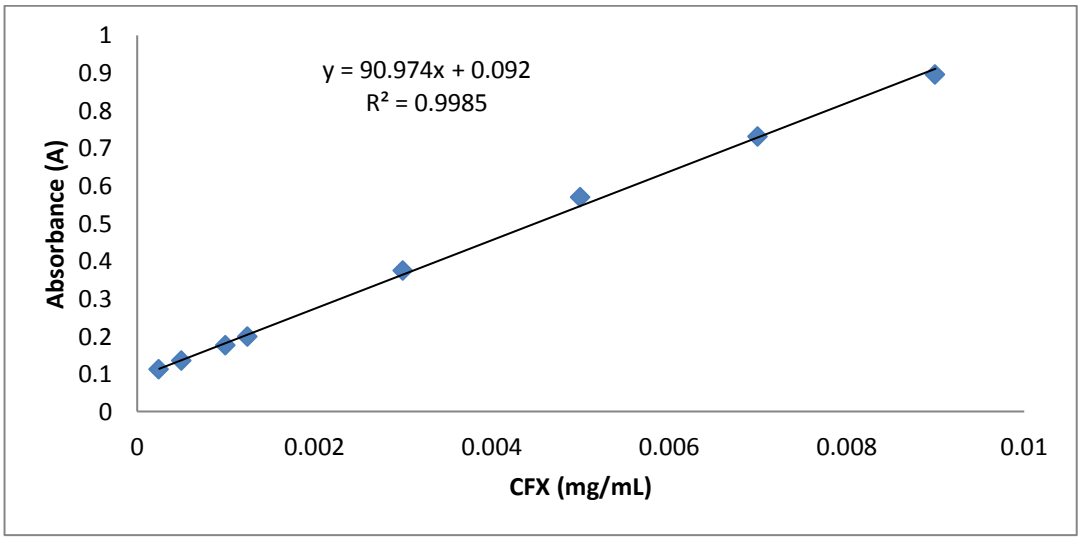

Figure 3.6 (a): Calibration curve of CFX in pH 1.2 buffer

Table 3.3 (b): Spectrometric data for the calibration curve of ciprofloxacin-HCl at pH 4, λ_{max} = 240nm

Concentration (mg/mL)	Absorbance (A)
0.0003	0.084
0.0005	0.093
0.0010	0.104
0.0013	0.109
0.0015	0.111
0.0023	0.122
0.0060	0.228
0.0080	0.263
0.0090	0.306

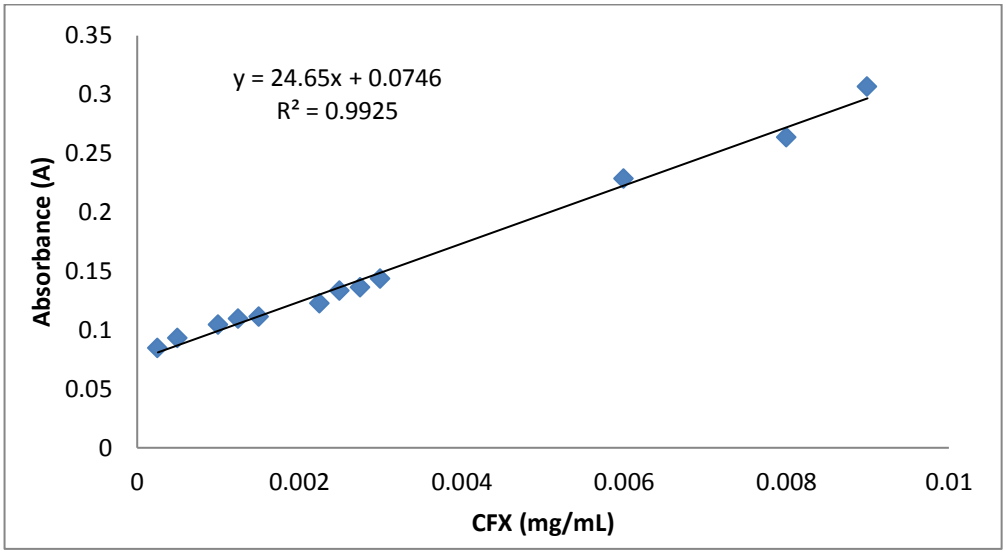

Figure 3.6 (b): Calibration curve of CFX in pH 4 buffer

Table 3.3 (c): Spectrometric data for the calibration curve of ciprofloxacin-HCl at pH 7, λ_{max} = 275nm

Concentration (mg/mL)	Absorbance (A)
0.0005	0.109
0.0010	0.120
0.0020	0.135
0.0040	0.210
0.0060	0.254
0.0080	0.337
0.0100	0.411
0.0120	0.486
0.0140	0.537
0.0160	0.590
0.0180	0.671
0.0200	0.713

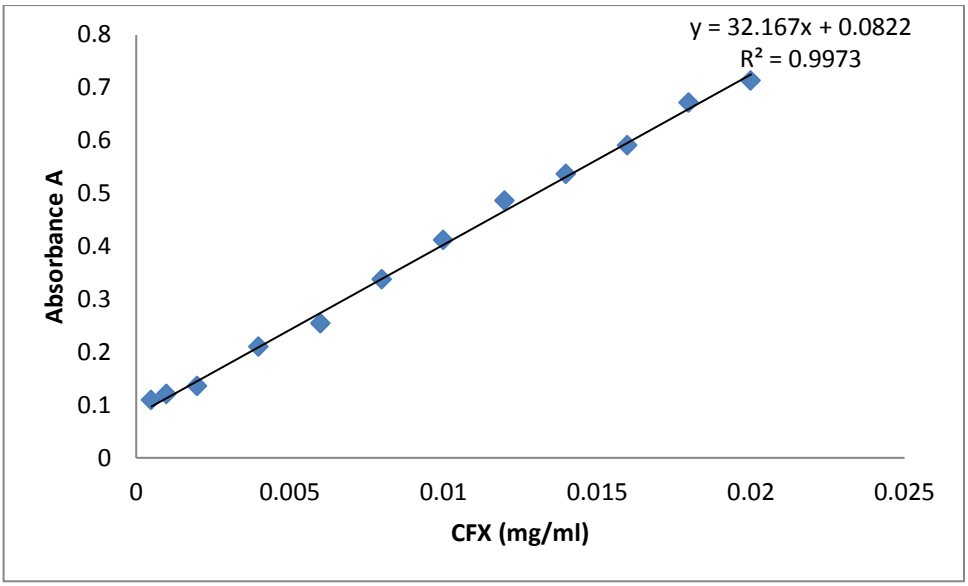

Figure 3.6 (c): Calibration curve of CFX in distilled water

The equations used for drug loading and release calculations as obtained by linear regression are as highlighted in equation 3.1 (a), (b) and(c) below. These equations were used to calculate drug loading, % drug loading, drug release and % drug release.

Buffer pH 1.2 (λ_{275}) $A = 90.974C + 0.092$ (3.1a)

Buffer pH 4 (λ_{240}) $A = 24.65C + 0.0746$ (3.1b)

Distilled water: (λ_{275}) $A = 32.167C + 0.0822$ (3.1c)

Where:

A is absorbance

C is concentration

3.3.2 Drug Loading

The values of concentration of drug loaded and released were calculated directly using the calibration curve equations.

Ciprofloxacin HCl was loaded into the homopolymer and hydrogels in 25 mL of the stock solution for 48 h and 72 h respectively. Table 3.3(a) gives the loading percentage values after 48 h of incubation in distilled water. The %loading values after incubation for 72 h are shown in Table 3.2 (b) and (b) in distilled water.

Table 3.4 (a): Ciprofloxacin-HCl Loading after 48 h in distilled water

Sample	% Loading
PDEAEM homopolymer	9.40
1:2 PDEAEM/EGDMA hydrogel	9.70
1:7.5 PDEAEM/EGDMA hydrogel	17.6

Table 3.4 (b): Ciprofloxacin-HCl Loading after 72 h in distilled water

Sample	% Loading
1:2 PDEAEM/EGDMA hydrogel	34.0
1:7.5 PDEAEM/EGDMA hydrogel	35.4

Table 3.4 (c): Ciprofloxacin-HCl Loading after 72 h in distilled water

Sample	% Loading
1:2 PDEAEM/EGDMA hydrogel	22.2
1:7.5 PDEAEM/EGDMA hydrogel	25.0

As clearly seen from Table 3.3.2 (a), (b) and (c), there was a higher loading efficiency in the hydrogels than the homopolymer and the loading efficiency of the hydrogels were increased after 72 hours than those loaded at 48 hours. An even distribution of the drug in the hydrogels is expected due to the carboxylic acid group on the drug and the amine group on the polymer/hydrogels. There is no established chemical reaction between the drug and the hydrogels.

Higher loading capacity of the EGDMA crosslinked hydrogels than the homopolymer can be attributed to the porous nature of the surface of the hydrogels as revealed by the SEM pictures. The pores available on the surface of the hydrogels allow penetration of the drug molecules into the hydrogels.

3.3.3 Drug Release Profile

The drug release profile in pH 1.2, 4 and distilled water are as shown in Table 3.5 (a), (b), (c) and Figure 3.7 (a), (b) and (c) respectively.

Table 3.5 (a): Cumulative % release of Ciprofloxacin HCl in distilled water

	Cumulative % release		
Time (h)	PDEAEM homopolymer	1:2 PDEAEM/EGDMA hydrogel	1:7.5 PDEAEM/EGDMA hydrogel
0	0	0	0
1	18.0	69.5	38.1
2	10.5	75.8	48.4
3	18.0	89.3	60.4
4	13.7	89.3	60.4
5	18.0	96.6	65.5
6	18.0	102.9	65.5
7	18.0	102.9	65.5

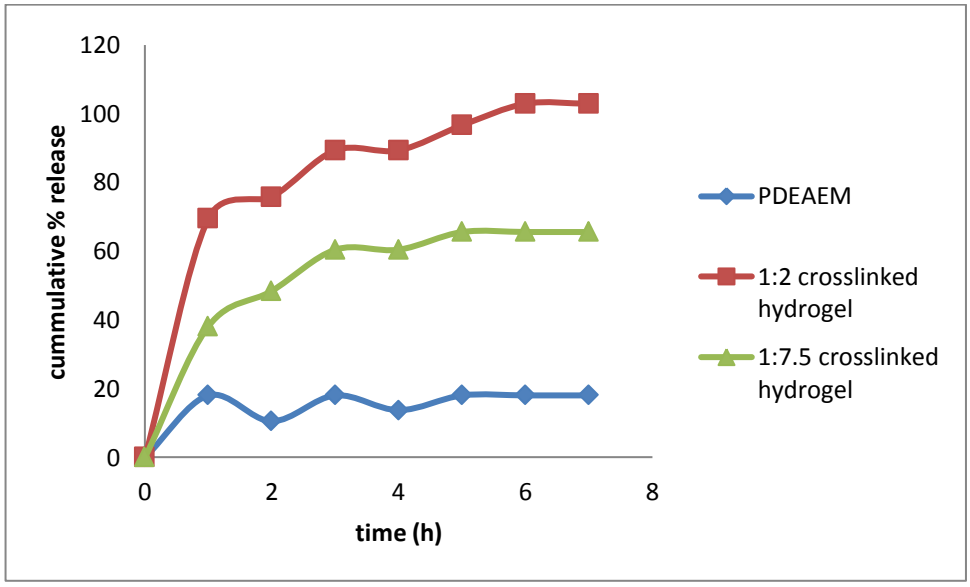

Figure 3.7 (a): Cumulative % release of Ciprofloxacin HCl in distilled water

It can be followed from Table 3.5 (a) and Figure 3.7 (a) that 18% of the drug is released from the polymer matrix within first hour. The cumulative amount of drug release did not change after 5 h of contact with the distilled water. The 1:2 hydrogel on the other hand releases 69% of the drug loaded within the first hour. All of the drug loaded is released in distilled water after 5 hours. The 1:7.5 hydrogel exhibits limited release compared to 1:2 hydrogel by releasing 38% of the drug after 1 hour and releasing only 65% of the ciprofloxacin-HCl loaded after 5 hours.

Though, the homopolymer exhibits higher swelling capacity (50%) compared to the hydrogels (~10%) within 5 hours in distilled water, the homopolymer released the least amount of drug (18%) compared to the hydrogels due to strong physical interaction between the homopolymer and the drug. The hydrogels allow the diffusion of the drug from porous surface giving rise to a greater percentage of drug release. The higher crosslinking density in the 1:7.5 hydrogel limited it drug release profile compared to the 1:2 hydrogel.

Table 3.5 (b): Cumulative % release of Ciprofloxacin HCl in buffer pH 4

	Cumulative % release	
Time (h)	1:2 PDEAEM/EGDMA hydrogel	1:7.5 PDEAEM/EGDMA hydrogel
0	0	0
1	70.7	54.9
2	75.8	56.1
3	85.3	57.7
4	90.2	61.3
5	97.0	67.7
6	101.9	70.9

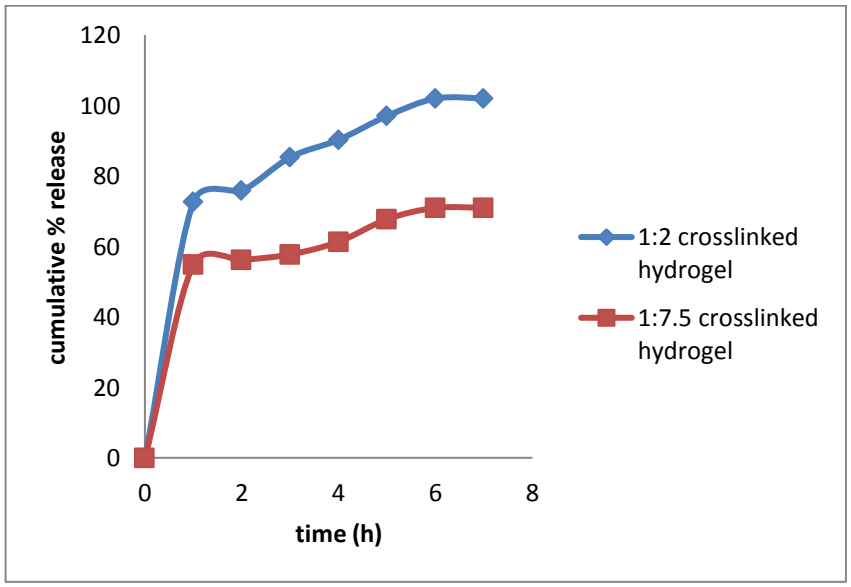

Figure 3.7 (b): Cumulative % release of Ciprofloxacin HCl in buffer pH 4

Drug release from the homopolymer was not followed in acidic medium as PDEAEM dissolves following swelling in pH 4 and pH 1.2 solutions. The drug

release profiles of the two hydrogels in pH 4 and pH 1.2 follows a similar trend to that observed in distilled water. The 1:2 hydrogel releases all the drug in pH 4 and pH 1.2 as shown in Table 3.5 (b), 3.5 (c) and Figure 3.7 (b) and Figure 3.7 (c) respectively. The 1:7.5 hydrogel releases 70% of the drug loaded after 6 hours in pH 4 and 60 % in pH 1.2 as illustrated in Table 3.5 (b), 3.5 (c) and Figure 3.7 (b) and Figure 3.7 (c). The drug release kinetics of the hydrogels have not been studied due to lack of enough data.

Table 3.5 (c): Cumulative % release of Ciprofloxacin HCl in buffer pH 1.2

	Cumulative % release	
Time (h)	1:2 PDEAEM/EGDMA hydrogel	1:7.5 PDEAEM/EGDMA hydrogel
0	0	0
1	89.5	42.8
2	103.5	60.6
3	109.5	61.4
4	109.2	61.7
5	111.0	61.6
6	111.0	61.6

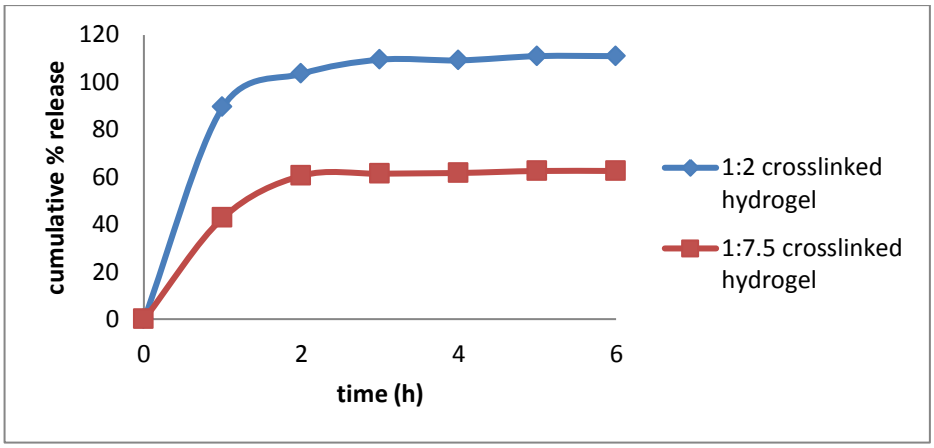

Figure 3.7 (c): Cumulative % release of Ciprofloxacin HCl in buffer pH 1.2

Chapter 4

CONCLUSION

Poly[2-(diethylamino ethyl methacrylate)] continues to be a polymer of interest due to its various applications especially in the field of biomedicine.

PDEAEM homopolymer was prepared by free radical polymerization in solution initiated by potassium per sulphate. Thus crosslinking ratios of EGDMA with a constant ratio of the DEAEM monomer were also prepared by free radical polymerization using the same initiator. The FTIR spectra and SEM micrograph reveal a successful polymerization of the homopolymer and the cross-linked hydrogels. Further characterization by swelling properties shows that the crosslinked hydrogels swelled better in acidic medium than in distilled water. This is due to ionic interaction in the acidic medium. There was also a slight difference observed in the swelling properties of the 1:2 hydrogels and 1:7.5 hydrogels due to difference in crosslinking density. The hydrogels with the lower crosslinking density swelled higher than the hydrogels with lesser crosslinking density. The homopolymer as expected swells very well in distilled water but dissolves in acidic medium due to protonation.

The drug release profiles of the hydrogels were better in acidic medium than in distilled water. This was expected because there is ionic interaction between the hydrogels and the acidic medium. Another reason is that ciprofloxacin-HCl has

higher solubility in acidic medium hence a better diffusion into the medium from the hydrogels.

Though, the preparation of EGDMA cross-linked hydrogels of PDEAEM and their drug delivery capacities were successful, there is a need to increase the cross-linker ratio for better cross-linking density to prevent the chemical interaction of hydrogels with the delivery system as seen with 1:2 hydrogels. There is also a need to investigate the loading of the ciprofloxacin-HCl in acidic medium and more data is needed to study the release kinetics.

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