Hydrogels of Chitosan-*graft*-Poly(diethylamino ethyl methacrylate)

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ABSTRACT

Poly(diethylamino) ethyl methacrylate, poly(DEAEM), was grafted onto chitosan, CH, under homogenous and heterogeneous conditions by using potassium persulphate, KPS, as the initiator to obtain pH responsive copolymers. The product, soluble in aqueous solution, was crosslinked with glutaraldehyde, GA. Gelation times of GA crosslinked CH and GA crosslinked CH-*graft*-poly(DEAEM) gels were experimentally found, and their swelling properties in aqueous solution were followed. Obtained samples were characterized by Scanning Electron Microscopy (SEM) analysis and FT-IR analysis.

CH-TPP beads were prepared by coagulating CH in acetic acid solution in aqueous tripolyphosphate, TPP, solution. Then the beads were grafted by poly(DEAEM) by redox initiation.

The antibiotic ciprofloxacin was loaded into the gel and drug release from CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads was investigated.

Keywords: chitosan gel, copolymerization, drug release, smart polymer

ÖZ

Poly(dietilamino) etil metakrilat, poli(DEAEM), kitosan (CH) üzerine homojen ve heterojen ortamda potasyum persülfat (KPS) redoks başlatıcı kullanılarak aşılanmıştır. Bu yöntemle pH'a duyarlı kopolimerler elde etmek amaçlanmıştır. Asitli sulu çözeltide çözünen kopolimer glutaraldehit (GA) çapraz bağlayıcı ile reaksiyona sokularak hidrojeller elde edilmiştir. GA-CH ve GA-CH-*aşı*-poli(DEAEM) örneklerinin GA ile çapraz bağlanmak suretiyle jelleşme süreleri ölçülerek birbirleriyle karşılaştırılmıştır. Elde edilen jellerin sulu asitli, nötral ve bazik sulu ortamda şişme davranışları incelenmiştir. Örnekler taramalı electron mikroskobu (SEM) ve FTIR töntemleri ile karakterize edilmiştir.

CH-TPP jel boncuklar kitosanın sodium tripolifosfat çözeltisi içinde koagülasyonu ile elde edilerek poli(DEAEM) aşılanarak modifiye edilmiştir.

Elde edilen örnekler yukarda analtıldığı gibi karakterize edilmiş ve siprofloksasin yüklenerek sistemin ilaç salım davranışı incelenmiştir.

Anahtar Kelimeler: kitosan jel, kopolimerizasyon, ilaç salımı, akıllı polimer

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”Your best shot at happiness, self-worth and personal satisfaction - the things that constitute real success - is not in earning as much as you can but in performing as well as you can something that you consider worthwhile.”

William Raspberry

I have this priceless feeling of happiness, self-worth and personal satisfaction since i have finished my thesis, the first serious scientific work in my life.

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Chapter 1

INTRODUCTION

1.1 Chitosan

Chitosan [poly-(β-1→4)-2-amino-2-deoxy-D-glucopyranose] (Figure 1) is deacetylated form of the natural polymer called chitin. Chitosan is a polycationic polymer, obtained usually through deacetylation of chitin applying 40–50% sodium hydroxide at 110–115°C for a few hours. In order to obtain a soluble derivative in aqueous medium with pH lower than 7, the degree of deacetylation of chitin should reach around 50%, forming a copolymer (Dutta & Singh, 2008) known as chitosan. It is only possible to achieve solubilization due to protonation of the amino group, which is located on the C-2 position of the D-glucosamine repeating part. Hence, in acidic media the polysaccharide turns into a polyelectrolyte.

MW, DD, purity, viscosity, molecular weight and solubility influence the properties of chitosan and are possible to alter according to the preparation process.

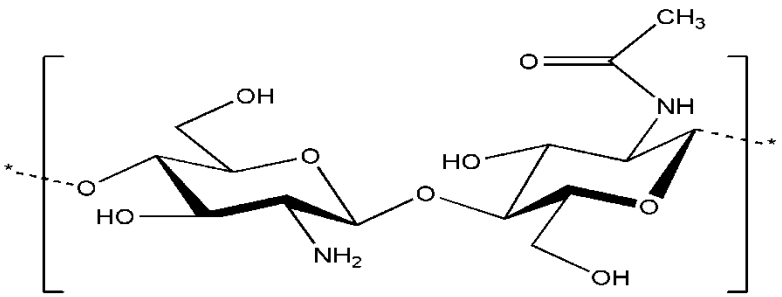

Figure 1. Chemical structure of chitosan

1.2 DEAEM and poly(DEAEM)

Diethyl amino ethyl methacrylate (DEAEM) is a monofunctional acrylate monomer containing a polar tertiary amine functional group which is soluble in aqueous medium (Figure 2). Poly[2-(diethylamino) ethyl methacrylate], poly(DEAEM), (Figure 3), is able to be influenced by temperature as well as pH. This polymer is mainly used for drug delivery mainly due to its property of phase transition in aqueous solution in response to pH and temperature changes. The polymer bears a lower critical solution temperature (LCST) in aqueous media varying from 38 to 50°C, and it has a pKa of 7.6. These are close to the physiological values.

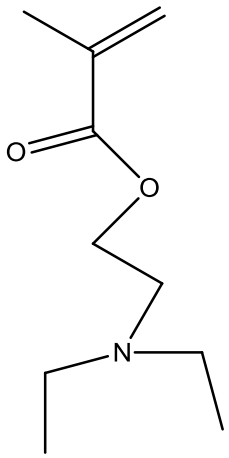

Figure 2. Chemical structure of DEAEM

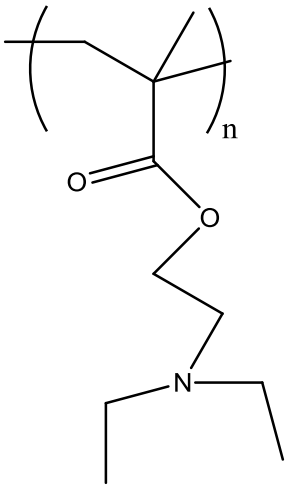

Figure 3. Chemical structure of poly(DEAEM)

This polymer is pH-responsive and it has a very low level of toxicity (Agarwal et al., 2007). That is why we can use it in various biomedical fields. Also poly(DEAEM) collapses at neutral conditions ensuring the encapsulation of species. Moreover, it is capable of expanding in order to release encapsulated drugs at acidic conditions (Agarwal et al., 2007).

1.3 pH Responsive Chitosan and its Biomedical Application

Chitosan is easily soluble in aqueous medium up to pH 6.2. It has pH-responsive properties because of the "protonation–deprotonation equilibrium" of $–NH_2$ group (Guo, Yuan, & Gao, 2008). According to the various changes in the environmental pH, the degree of ionization in a polymer changes. Such fast alteration of the net charge among the pendant groups results in the change of its hydrodynamic volume. pH-responsive nature makes possible the idea of controlled drug release carriers based on chitosan (Drozdov, 2015). Recently, there have been a lot of studies regarding pH-sensitive applications in microform or nanoform. They examine for drug delivery systems against tumor cells which tend to be of a lower pH compared to the healthy cells at 37 °C (Chen et al., 2015). So we can say the drug carriers now may be manipulated and altered according to pH differences.

Due to biocompatible and biodegradable nature and relatively low toxicity of chitosan (Araujo, Davidenko, Danner, Cameron, & Best, 2014) scientists can widely apply chitosan and its derivatives in biomedicine, especially in surgical applications, biocompatible sponges, wound healing bandages, in addition to drug release applications (Jahren, Butler, Adams, & Cameron, 2010). Wide range of choices of chitosan's applications are mainly due to its outstanding characteristics when in contact with human body; for example bioactivity or antibacterial properties (Espadin et al., 2014). It also is known for its wide application for wound treatment as well as various ulcers and burns. It is recommended to use chitosan for tissue regeneration and restoration, due to its cell affinity and biodegradability.

1.4 Chemical and Physical Crosslinked Chitosan

Chitosan is a polyfunctional polymer containing amino and hydroxyl groups, which allow chemical and/or physical crosslinking of the polymer.

1.4.1 Covalently crosslinked chitosan hydrogels

In order to prepare covalently crosslinked chitosan hydrogels there are several chemical crosslinkers available. Among the crosslinkers that researchers typically apply together with chitosan are dialdehydes such as glutaraldehyde (Ostrowska-Czubenko, Pierog, &Gierszewska-Druzynska, 2013). The aldehyde units are able to interact with chitosan's -NH_2 groups forming covalent imine bonds. The reaction is possible in aqueous media, under mild conditions. We can say that one of the most important disadvantage of glutaraldehyde is its toxicity.

Covalent crosslinking forms permanent network leading to the free diffusion of water molecules and improving the physical characteristics of the hydrogel. Generally, there are two ways for application of covalently crosslinked chitosan gels: drug delivery purpose and as a permanent networks (Grolik et al., 2015).

One parameter which determines swelling and drug release from covalently crosslinked chitosan gels is the pore structure (Yin et al., 2000). Furthermore, the density of crosslinking determines the diffusion of small molecules in and out of crosslinked chitosan matrix. Moreover, chemical functionalities available on the polymer are of critical importance. For example, pH-responsive swelling mechanism presupposes protonation of chitosan containing -NH_2 groups in case the level of pH goes down. Such protonation causes chain repulsion, interaction of positive and negative ions accompanied by the aqueous molecules in the hydrogel.

1.4.2 Ionically crosslinked chitosan hydrogels

A method to overcome toxicity and in order to skip the step of purification is to form gels by reversible ionic crosslinking. Chitosan is a polycation polymer, which is capable to chelate (Guibal, Sweeney, Zikan, Vincent, & Tobin, 2001). Therefore, interactions with negatively charged compounds result in creation of a network through ionic bridges of reacted polymeric groups. Ionic crosslinking is usually characterized by entities (ions or ionic molecules) with defined molecular weight which can interact with chitosan.

The ionic crosslinking of chitosan can be obtained by integration of negatively charged multivalent ions within positively charged chitosan chains. One example to such negatively charged ions is tripolyphosphate (TPP). It is possible to create physical hydrogels by using many different multivalent ions interacting with the polyelectrolyte. As a result it is possible to obtain hydrogels by ionic interactions or by secondary interactions.

Either anions or anionic molecule groups can create ionically crosslinked chitosan networks. The crosslinker chosen determines the structure of the created compound.

The ionic interactions of negative charge of the applied crosslinker on the one hand and the positively charged chitosan groups on the other are the fundamental interactions within the compound. The process of the ionic crosslinking is not complicated and requires mild conditions. The opposite of covalent crosslinking, there are no ancillary molecules needed to conduct a reaction.

The most widely spread ionic crosslinkers are metallic ions, lead to the creation of coordinate covalent bonds within the nucleophilic $–NH_2$ units of chitosan. It is well-documented that the network formed by ionic crosslinking is more stable than the hydrogel created by anionic molecules when electrostatic interactions takes place inside the hydrogel. Beside the positively charged $-NH_2$ units of chitosan, alternative units along the chitosan molecule are able to interact with the ionic crosslinker (e.g.–OH groups of chitosan). The most widely used as anionic molecules are phosphate-containing groups, for example ß-glycerophosphate or tripolyphosphate (TPP).

To prepare an ionic crosslinked network one needs to mix a negatively charged ionic crosslinker with the chitosan solution. As a result of irregular crosslinking interactions a homogeneous hydrogel forms. The essential characteristics for ionically crosslinked hydrogel are physical stability, swelling and drug release. The process of ionic crosslinking primarily depends on the size of the crosslinker, the global charge, densities, concentration of both crosslinker and chitosan, MM and DD of chitosan and duration of the reaction.

1.4.3 Grafted chitosan hydrogels

Chitosan contains two kinds of the main reactive units that allow grafting. These are vacant $–NH_2$ unit on deacetylated part of chitosan and the –OH unit on the C-3 and C-6 carbons on acetylated or deacetylated units (Baser, Demirel, & Caykara, 2011).

In order to improve chitosans' properties grafting is applied. As a result improved solubility in aqueous solution as well as in organic solvents, chelating characteristics, antibacterial activity may be obtained. After grafting the level of many crucial characteristics of the hydrogel such as mucoadhesivity, biocompatibility and biodegradability is still high.

We can name different methods of grafting. They are free radical, radiation induced enzymatic and cationic or anionic grafting methods.
It should be noted that the process of grafting will not necessarily always induce the creating of a network. Grafting can be conducted by copolymerization onto vacant reactive groups of chitosan a functional group of the graft. Extra additional molecules might be needed with the purpose of catalyzing the grafting reaction.

Grafting of the molecules that contain a reacting unit that can react with the covalent links of chitosan is the most applicable. Palmitic acid, lactic acid or glycolic acid, as well as aldehydes could be listed as an examples. In order to achieve further reactions, as in the case of covalent crosslinking some extra processes might be needed after grafting. The characteristics of the hydrogel usually are determined by the nature of interactions occurring between polymeric chains during the process of grafting. So, we should not neglect the modulation of the density. For example swelling and drug release can be considered as these processes. The density of a solution is influenced by the kind and quantity of grafts as well as by the concentration of the grafted chitosan. The ratio of each component, the solvent properties, time and temperature of the reaction, the original concentration of the catalyst, the structure of the graft molecule, the molecular weight and degree of deacetylation of chitosan are the factors that affect the process of grafting. A few

disadvantages of the process can be listed. The process of grafting is quite complicated, dependent on various aspects. So, is crucial to monitor the grafting reaction. In order to fix such drawbacks a further research and investigation is needed.

1.5 Ciprofloxacin

Ciprofloxacin is an antibiotic which is useful in many fields like the skin infections, respiratory diseases, urinary tract disease, the gastrointestinal operative treatment, gonococcal urethritis, and sepsis. (Crump, Wise, & Dent, 1983). The chemical formula of the component is $C_{17}H_{18}FN_3O_3$. Its chemical structure is represented in Figure 4.

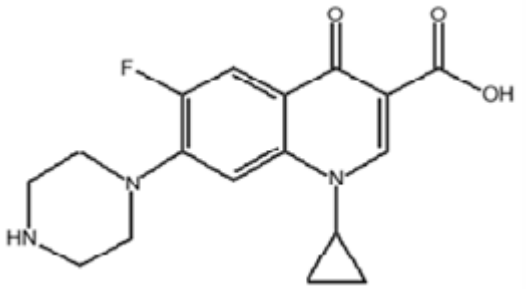

Figure 4. Chemical structure of ciprofloxacin

Ciprofloxacin (CFX), is the antibiotic which belongs to the third generation of fluoroquinolone antimicrobials with superior properties such as wide antibiotic spectrum, strong bactericidal activeness and low-level restriction concentration. All these properties lead to wide range of medical applications. CFX is used in the form of capsule, tablet, and through injection. It is a highly potent material with broad antibacterial activity against Gram-negative pathogens. The applicability of CFX for clinical purposes is well-documented.

Scientists created a new compound which contain chitosan and cysteine as the carriers of thiol groups with 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) as catalyst. The compound releases ciprofloxacin when applied, showing antibiotic characteristics to the microbes *E.Coli* which can be used to heal and protect wounds for a prolonged time (Chang et al., 2015).

Chapter 2

EXPERIMENTAL

2.1 Materials

The materials given in the Table 1 were used without any further purification step.

Table 1. Chemicals and manufacturers

Material	**Company**
Chitosan (medium molecular weight, molar mass 4.0x105 g/mol, degree of deacetylation of 85%)	Aldrich, Germany
2-(diethyl amino)ethylmethacrylate (DEAEM)	Aldrich, Germany
Glutaraldehyde (GA)	Aldrich, Germany
Potassium persulfate (KPS)	Aldrich, Germany
Acetic acid	Riedel-de Häen, Germany
Ethanol	Riedel-de Häen, Germany
Acetone	Riedel-de Häen, Germany
Pentasodiumtripolyphosphate (TPP)	Aldrich, Germany

2.2 Preparation of CH-*graft*-poly(DEAEM) under Homogeneous Conditions

Grafting of DEAEM onto chitosan was carried out according to a method described before (Yahya Covan Ibrahim Ali, 2014). Aqueous CH solution of volume 25 mL and concentration 1% (w/v) was mixed with acetic acid solution of concentration 1% (v/v). The reaction solution was placed in a two-neck reaction flask and brought to a temperature of 70^{o}C. The initiator KPS and monomer (2-diethylamino)ethyl methacrylate, DEAEM, were then added into CH solution. The process was conducted under homogeneous conditions. Nitrogen atmosphere and temperature of 70^{o}C was applied for 4 hours using vigorous magnetic stirring at 1200 rpm. Afterwards, acetone was added into the final solution for precipitation of the polymer and left overnight at 50^{o}C to get dried. The product was purified by dialysis against distilled water using a dialyzing membrane of MWCO 6000-8000 Da to remove any unreacted monomer, initiator or any oligomers. Then the soxhlet extraction in dichloroethane was conducted. Data for preparation of CH-*graft*-poly(DEAEM) under homogeneous conditions are shown in Table 2.

Table 2.Studied conditions for all CH-*graft*-poly(DEAEM).

Sample ID	**DEAEM (mL)**	**T (°C)**	**Time (hr)**	**KPS (g)**
CH-*graft*-poly(DEAEM)(294)	0.25	70	4	0.1250
CH-*graft*-poly(DEAEM)(361)	0.50	70	4	0.1250
CH-*graft*-poly(DEAEM)(356)	0.75	70	4	0.1250
CH-*graft*-poly(DEAEM)(221)	1.00	70	4	0.1250

2.3 Preparation of GA crosslinked CH-*graft*-poly(DEAEM) gels

The gelation time for CH and CH-*graft*-poly(DEAEM) samples dissolved in acetic acid (pH=3.0) and pH=1.0 (HCl/KCl buffer) was measured by placing 4.0 mL solution in glass test tube by vigorous magnetic stirring. The process of gelation is considered as complete when the magnet stops turning and the product in the tube does not flow when the tube is flipped. The preparation conditions for the GA crosslinked CH-*graft*-poly(DEAEM) gels are shown in Table 3.

Table 3. The preparation conditions of GA crosslinked CH-*graft*-poly(DEAEM) gels.

Sample ID	**Volume (mL) of CH**	**GA Volume (μL)**
GA(1)-CH-*graft*-poly(DEAEM)(294)	3.96	40
GA(2)-CH-*graft*-poly(DEAEM)(294)	3.92	80
GA(3)-CH-*graft*-poly(DEAEM)(294)	3.88	120
GA (4)-CH-*graft*-poly(DEAEM)(294)	3.84	160

2.4 Preparation of CH-TPP Beads and CH-TPP-*graft*-poly(DEAEM)

Preparing of CH-TPP beads was carried out according to a method described before (Covan Yahya, 2014). A CH solution of concentration 2% (w/v) was prepared in 1% (v/v) acetic acid. The solution was added dropwise into 5% (w/v) TPP aqueous solution. Formation of CH-TPP beads occurred immediately due to coagulative process at room temperature under magnetic stirring of 20 rpm. The obtained beads were purified with water and left overnight to dry in the oven at 50°C.

2.5 Ciprofloxacin Loading

CH-TPP Beads and CH-TPP-*graft*-poly(DEAEM) beads sample with mass of 50 mg and average diameter 500 μm was put in a 50 mL aqueous ciprofloxacin solution under magnetic stirring 60 rpm at 37 °C. The concentration of ciprofloxacin loading was computed by spectrophotometry at 275 nm.

$$\%\ Loading = \frac{C_{loaded}}{C_{initial}} * 100$$

2.6 Ciprofloxacin Release

50 mg ciprofloxacin loaded beads were put in 50 mL of distilled water under magnetic stirring at 60 rpm at 37 °C. The value of ciprofloxacin release was measured by measuring the absorbance of the solution at 275 nm. The UV spectrum of CFX in water and the calibration curve obtained at 275 nm are shown in Figure 5 and 6 respectively.

$$\%\ Release = \frac{C_{released}}{C_{loaded}} * 100$$

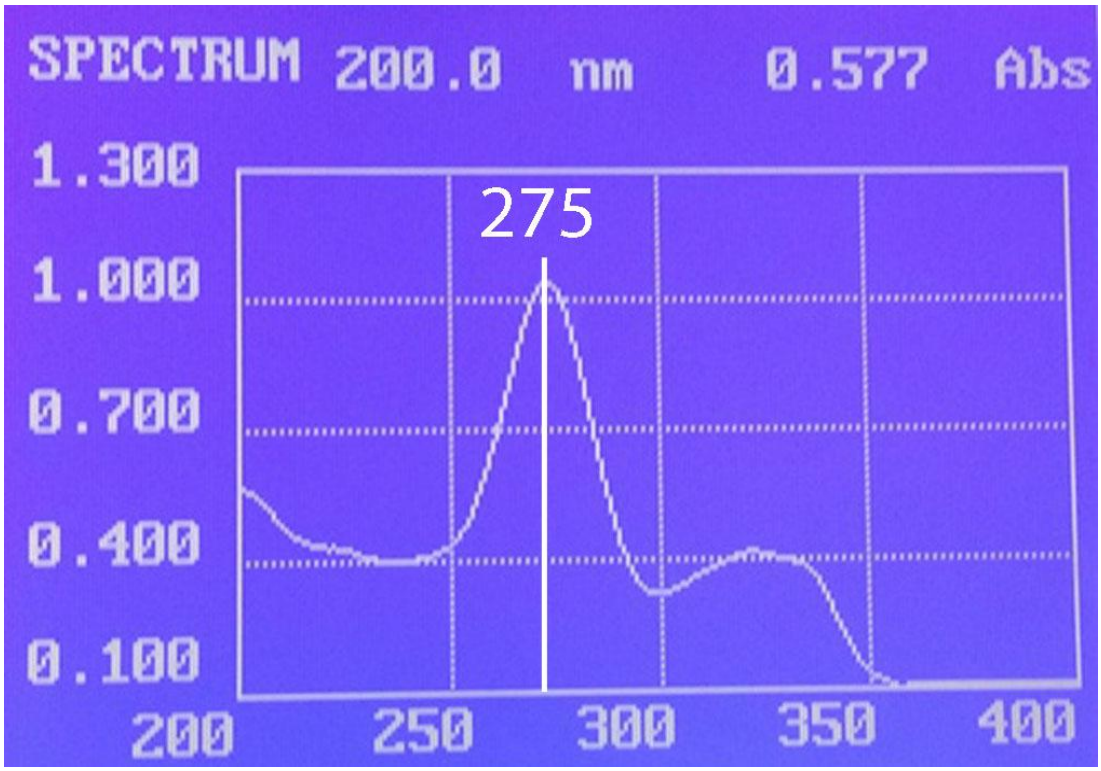

Figure 5.The spectrum of CFX in water.

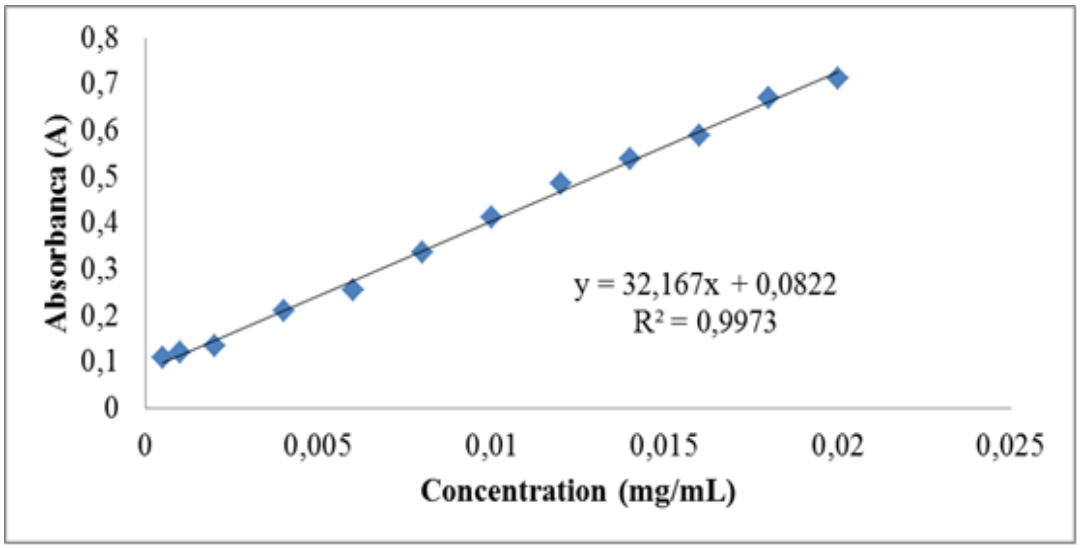

Figure 6. The calibration curve of CFX in water

2.7 Gravimetric Analysis

The grafting yield, yield of homopolymerization, and crosslinking degree were calculated by the following equations.

$$Homopolymer\ (\%) = \frac{m\ (product\ after\ grafting) - m\ (product\ after\ purification)}{m\ (product\ after\ purification)} x\ 100$$

$$Grafting\ (\%) = \frac{m\ (grafted\ chitosan\ after\ purification) - m\ (chitosan)}{m\ (chitosan)} x\ 100$$

$$Crosslinking\ (\%) = \frac{m\ (product\ after\ gelation) - m\ (chitosan)}{m\ (chitosan)} x\ 100$$

2.8 Swelling Capacity Determination

The swelling and dissolution nature of the products were determined with respect to time and solution pH. The swelling characteristics of the samples' were investigated by immersing the samples in solution for various periods of time at 37 °C.

$$Swelling\ (\%) = \frac{W_2 - W_1}{W_1} \times 100$$

Where W_2 is the mass of sample at the various time intervals while, W_1 is the initial mass of beads before the swelling.

Beads (approximately 0.05 g) were brought into contact with the buffer solution (pH=7) for 1 hour. Then, the swollen gels were blotted with paper and weighed. Then the beads were immersed in the buffer solution (pH=1.0) for 1 hour. The % swelling of the beads was determined. Three consecutive treatments were performed. An identical approach was conducted at pH=1.0 and pH=11.0.

2.9 SEM analysis

Samples were evaluated using SEM analysis (JEOL/JSM-6610LV scanning electron microscope) at Cyprus International University.

Chapter 3

RESULTS AND DISCUSSION

Poly(DEAEM) was grafted onto CH under homogenous and heterogeneous conditions by using potassium persulphate, KPS, as the initiator to obtain pH responsive copolymers. An analogous method had been used to modify the surface of CH-TPP gel beads.

3.1 Preparation of CH-*graft*-poly(DEAEM)

The grafting conditions has been studied at several different volumes of DEAEM (0.25mL, 0.50 mL, 0.75 mL) under homogenous conditions as shown in Table 2. The grafting yield increases with increasing monomer concentration up to 0.50 mL monomer. The maximum grafting percentage of poly(DEAEM) onto CH has been calculated as 361% under homogeneous conditions using 0.50 mL of monomer with 1.00 g CH dissolved in 1.0 mL of 1.0% (w/v) acetic acid solution by using 0.125 g KPS, at 70◦C and 4 hour duration. At 1.00 mL monomer the grafting yield decreases probably due to the formation of the oligomers and some homopolymers. Percent grafting (%G) and percent homopolymerization (% H) values do not compete with each other, since oligomers formed were lost into the dialysis solution during cleaning process. As redox initiation usually produces polymers with a lower molecular weight, %H values obtained are low. The optical pictures of the products obtained before and after dialysis are shown in Figure 7 and 8 respectively.

Table 4.Values %H and % G of CH-*graft*-poly(DEAEM).

Sample ID	**DEAEM (mL)**	**%H**	**%G**
CH-*graft*-poly(DEAEM)(294)	0.25	3.37	294
CH-*graft*-poly(DEAEM)(361)	0.50	4.95	361
CH-*graft*-poly(DEAEM)(356)	0.75	3.26	356
CH-*graft*-poly(DEAEM)(221)	1.00	1.69	221

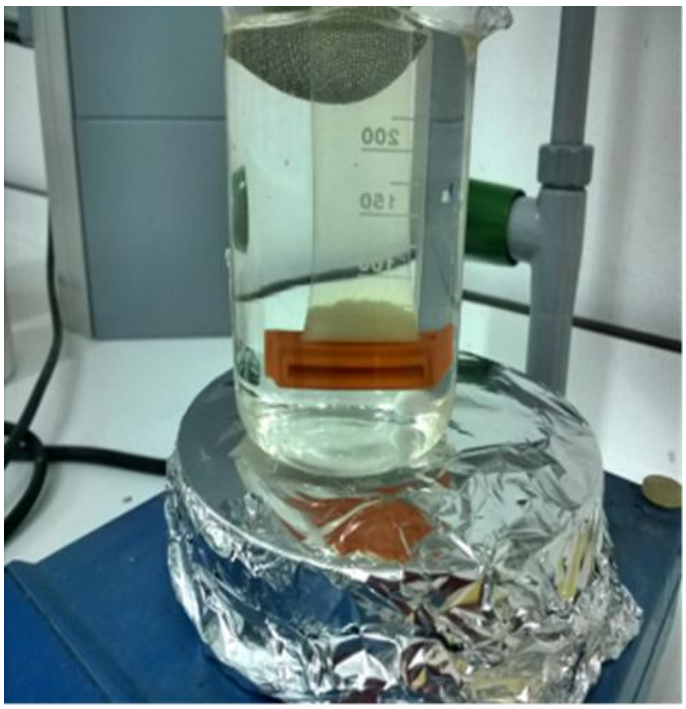

Figure 7. Image of dialysis of CH-*graft*-poly(DEAEM) for 12 hours.

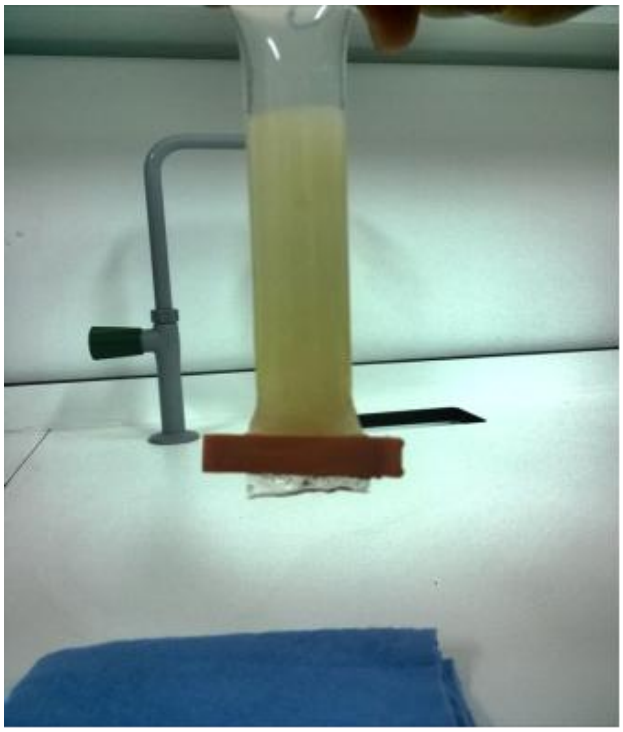

Figure 8. Image of dialysed CH-*graft*-poly(DEAEM).

3.2 Preparation of GA crosslinked poly(DEAEM) CH gels

The grafted product which is soluble in acidic medium is the sample with 294% grafting yield, CH-*graft*-poly(DEAEM)(294). Therefore, GA crosslinking was carried out on this product to prepare chemically crosslinked poly(DEAEM) CH gels. The gelation conditions are summarized in Table 5. Figure 9 shows the optical pictures of the gels obtained.

Table 5. Gelation time determination of GA crosslinked CH and GA crosslinked CH-*graft*-poly(DEAEM) gels.

GA%	**Gelation Time (minutes)**		
	CH dissolved in acetic acid	**CH* dissolved in pH=1.0**	**CH*-*graft*-poly(DEAEM)(294) dissolved in pH=1.0**
1	35	Complete gel formation not detectable.	305
2	26	190	155
3	17	43	20
4	9	22	4

*dissolved in pH=1.0

The gelation times for grafted products are longer when compared to the gelation times of CH. Since DEAEM bears tertiary amine groups, crosslinking reaction with GA is not expected from those functional groups. Crosslinking occurs only due to the imine formation between the free $-NH_2$groups of CH and the aldehyde functionalities of GA. As some amine groups of CH may have served as grafting sites, the fraction of free amine groups available for imine formation is less in the grafted products. Another factor is that due to the branched nature of grafted chains the solution viscosity decrease increasing gelation time.

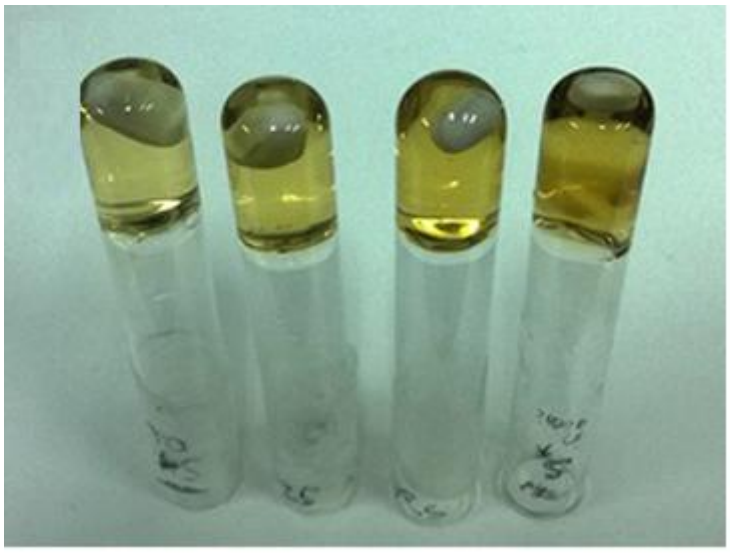

Figure 9. Left to right: CH gel dissolved in acetic acid (pH=3) crosslinked with 1%GA, 2%GA, 3%GA, 4%GA

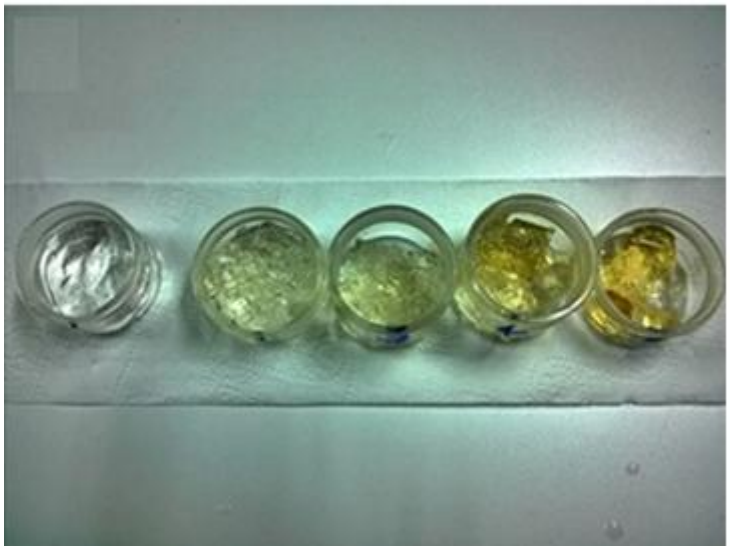

Figure 10. Left to right: CH gel dissolved in HCl solution (pH=1) with 4%GA, CH gel dissolved in acetic acid(pH=3) crosslinked with 1%GA, 2%GA, 3%GA, 4%GA

Table 6. Comparison of gelation time and % Crosslinking of GA crosslinked CH and GA crosslinked CH-*graft*-poly(DEAEM) gels.

4% GA Gelation Conditions		
	Gelation Time, minutes	**%Crosslinking (by mass)**
CH	9	54.2
CH*	22	14.3
CH-*graft*-Poly(DEAEM)	4	15.4

It can be followed from Table 5 that complete gel formation is not detectable for CH in pH=1.00 HCl solution using 1.0 % GA solution. Also, the gelation times for CH are higher in HCl solution than in acetic acid solution. Furthermore, it was not possible to obtain gels of CH-*graft*-poly(DEAEM) in acetic acid solution due to the low viscosity of the solution prepared.

Since at pH=1.0, the amines are protonated at a higher fraction that at pH=3.0 (1.0% acetic acid solution), imine formation reaction is less probable. For CH solution in 1.0 % acetic acid % crosslinking has been calculated as 54.2% as shown in Table 6 and the gelation time was measured as 9 minutes using 4% GA. On the other hand, in pH=1.0 solution, % crosslinking was obtained as 14.3% with a gelation time of 22 minutes. The crosslinking degree of CH*-*graft*-poly(DEAEM) was obtained as 15.4%, a value very close to that of CH*. This result can easily be explained by the fact that grafting reaction occurs under acidic conditions. As amine groups are either protonated or occupied by inter/intra molecular hydrogen bonding. The monomer molecules cannot approach the CH chains from the amine groups. Moreover, due to the bulky nature of monomers, C-6 is more available for the reaction with the monomer molecules. Hence, amine groups are still available for imine formation

with GA, after the grafting occurs.

3.3 Preparation of CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads

To prepare CH-TPP beads the following procedure was conducted. A CH solution of concentration 2% (w/v) was prepared in 1% (v/v) acetic acid. The solution was added dropwise into 5% (w/v) TPP aqueous solution. Formation of CH-TPP beads occurred immediately due to the process of coagulating at room temperature under magnetic stirring of 20 rpm. The obtained beads were washed with water, left overnight to dry in the oven at 50 °C.

Grafting of poly(DEAEM) onto CH-TPP beads, CH-TPP-*graft*-poly(DEAEM) was obtained using following way. The beads of 0.25 g were immersed in 25 mL 1.0% acetic acid solution. The monomer, 0.25 mL, combined with 1.0 mL ethanol was placed into the reaction substance consisted of 0.25 g CH-TPP beads and 0.125 g KPS in 25 mL acetic acid. The process of grafting was conducted under nitrogen atmosphere for 4 h at 70°C. The grafting yield was found to be 41%.

3.4 Scanning Electron Microscopy (SEM) Analysis

SEM micrographs of the a) CH gel by 1% GA b) CH* gel by 1% GA c) CH* gel by 4% GA d) CH*-*graft*-poly(DEAEM)(294) by 1% GA , (e) CH-TPP beads and (f) CH-TPP-*graft*-poly(DEAEM) are given in Fig. 11. (a), (b), (c), (d), (e) and (f).

In Fig. 11. (a), it is illustrated that the surface morphology of gels obtained by using %1.0 GA are smooth in comparison to the surface of the gel obtained by using 4% GA. The SEM image of gels of chitosan (Fig. 11. (a) and 11. (b)) shows smooth surface by 1% GA, increasing GA% the roughness of the gels increase ((Fig. 11. (c))

the surface morphology of CH*-*graft*-poly(DEAEM)(294) ((Fig. 11 (d)) reveals that grafting sites on the less smooth surface. As shown in Fig. 11(e) the surface morphology of CH-TPP is rougher than that of CH-TPP-*graft*-poly(DEAEM) (Fig. 11 (f)). Since surface modification of CH-TPP gel beads by grafting, leading to smoother surface. The SEM micrograph of grafted CH-TPP showed fractured and rough surface morphology than that of gels that CH-TPP providing fast diffusion of ions and ciprofloxacin. However surface modified CH-TPP has smooth surface leads to more controllable release of ions and ciprofloxacin compared to CH-TPP, as will be explained in more detail below.

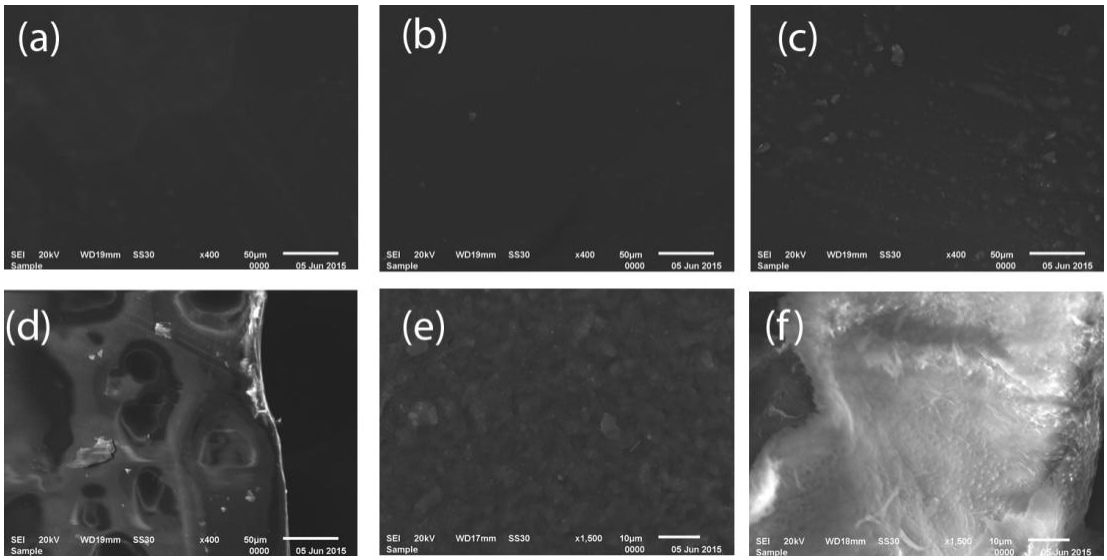

Figure 11. SEM micrographs of the a) GA(1)-CH, b) GA (1)-CH* c) GA (4)-CH* d) GA (4)-CH*-*graft*-poly(DEAEM)(294) (e) CH-TPP beads and (f)CH-TPP-*graft*-poly(DEAEM).

3.5 Swelling Properties of the Gels

Swelling properties of pure chitosan gels crosslinked with GA, CH-*graft*-poly(DEAEM) gels exhibits improved pH responsive swelling when compared to pure CH gels. Under acidic conditions CH-GA gel exhibits an equilibrium swelling of 280% while CH-*graft*- poly(DEAEM) gel has an equilibrium swelling of 350%.

Similarly, at pH=7.0 and pH=11.0 the GA-crosslinked pure chitosan gel shows an equilibrium swelling value of 117%, and 171% respectively while the grafted product swells by 448% and 560% at pH=7.0 pH=11.0 respectively. The equilibrium % swelling value in basic conditions competes with the value in acidic conditions. This behavior can be explained by basic and acidic hydrolysis of imine bonds giving rise to less crosslinked network.

The swelling behavior of CH-TPP beads was followed in pH=1.0, 7.0 and 11.0. CH-TPP gel swells with an equilibrium swelling capacity %8825 and CH-TPP grafted beads have a swelling capacity of %5742. The reason why CH-TPP beads have higher swelling capacity than CH-TPP-*graft*-poly(DEAEM) beads can be attributed to screening effect of ethyl groups in between protonated tertiary amine groups on poly(DEAEM). In pH=7.00 and pH=11.0 both CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads have swelling capacity % 470.

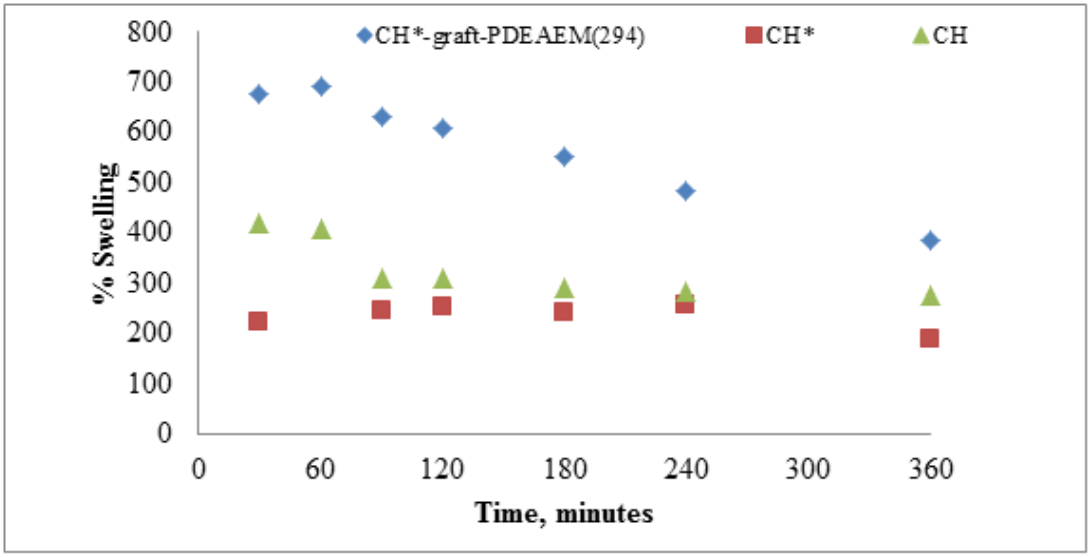

Figure 12.Swelling behavior of GA (4)-CH*-*graft*-PDEAEM(294), GA(4)-CH, b) GA (4)-CH* in pH=1.0.

Table 7. Swelling behavior of GA (4)-CH*-*graft*-PDEAEM(294), GA(4)-CH, b) GA (4)-CH* in pH=1.0.

Time, min	**Swelling %**		
	CH*-*graft*-PDEAEM(294)	**CH***	**CH**
30	676	223	419
60	692	230	408
90	629	245	309
120	608	251	308
180	551	240	291
240	483	256	282
360	390	250	280

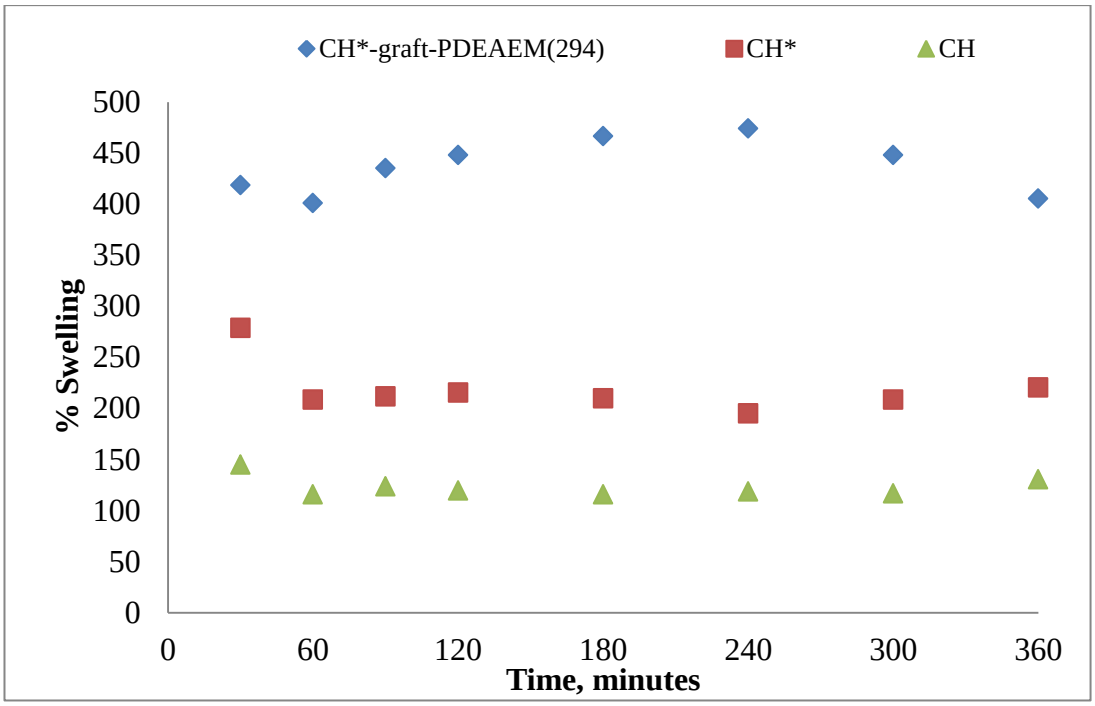

Figure 13. Swelling behavior of GA (4)-CH*-*graft*-PDEAEM(294), GA(4)-CH, b) GA (4)-CH* in pH=7.0.

Table 8. Swelling behavior of GA (4)-CH*-*graft*-PDEAEM(294), GA(4)-CH, b) GA (4)-CH* in pH=7.0.

Time, min	**Swelling %**		
	CH*-*graft*-PDEAEM (294)	**CH***	**CH**
30	419	279	145
60	401	209	116
90	435	212	124
120	448	216	120
180	467	210	116
240	474	195	119
360	448	209	117

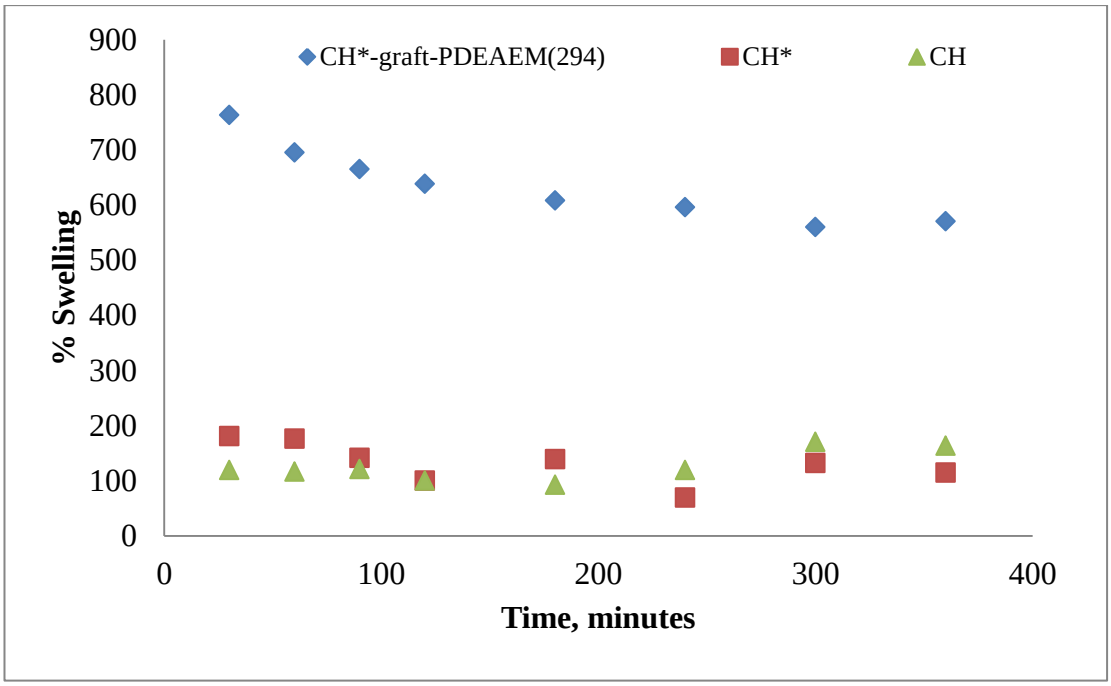

Figure 14. Swelling behavior of GA (4)-CH*-*graft*-poly(DEAEM)(294), GA(4)-CH, b) GA (4)-CH* in 11.0.

Table 9. Swelling behavior of GA (4)-CH*-*graft*-poly(DEAEM)(294), GA(4)-CH, GA (4)-CH* in pH=11.0.

Time, min	**Swelling %**		
	CH*-*graft*-poly(DEAEM)(294)	**CH***	**CH**
30	763	181	119
60	695	176	117
90	665	141	121
120	639	100	100
180	608	139	93
240	596	70	119
360	560	132	171

Table 10. Swelling of CH-TPP beads and CH-*graft*-TPP beads in pH=1 medium.

Time, min	**Swelling %**	
	CH-TPP	**CH-*graft*-TPP**
30	93	568
60	929	620
90	2772	638
120	3556	1008
180	5562	1631
240	7493	2605
300	8236	3565
360	8825	5742

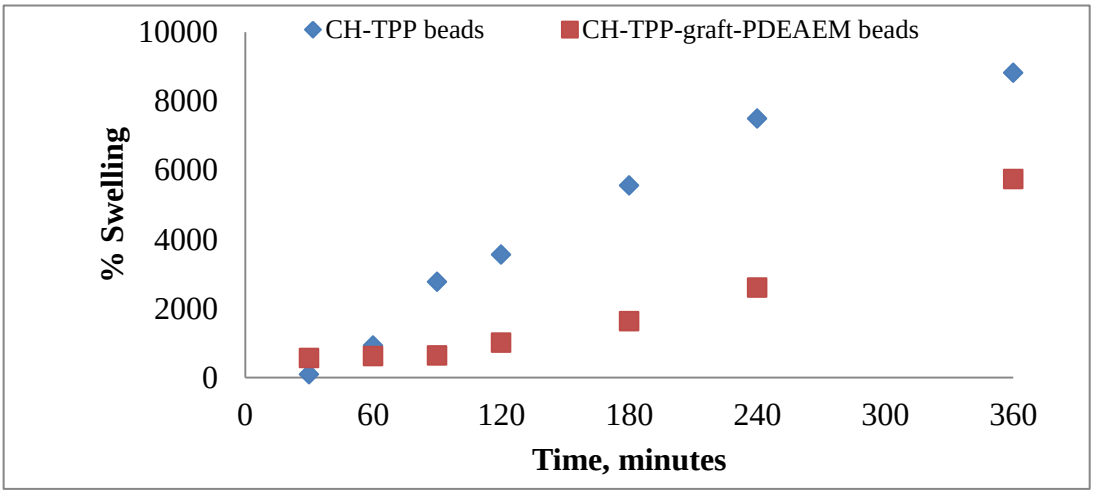

Figure 15. Swelling behavior of CH-TPP and CH-TPP-*graft*-poly(DEAEM) in pH=1.0.

Table 11. Swelling of CH-TPP beads and CH-*graft*-TPP beads in pH=7 medium.

Time, minutes	Swelling %	
	CH-TPP	CH-*graft*-TPP
30	106	102
60	169	169
90	182	178
120	217	186
180	269	266
240	353	344
300	386	360
360	536	400

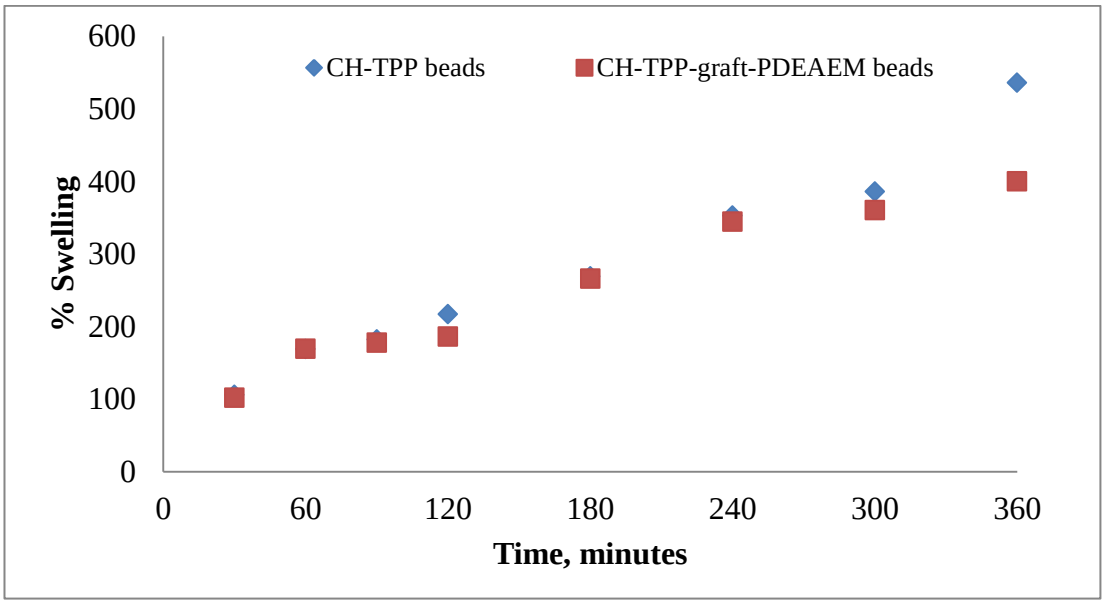

Figure 16. Swelling of CH-TPP and CH-TPP-*graft*-poly(DEAEM) in pH=7.0.

Table 12. Swelling of CH-TPP beads and CH-*graft*-TPP beads in pH=11 medium.

Time	Swelling %	
	CH-TPP	CH-*graft*-TPP
30	125	95
60	183	125
90	222	157
120	357	225
180	372	277
240	388	350
300	444	435
360	508	441

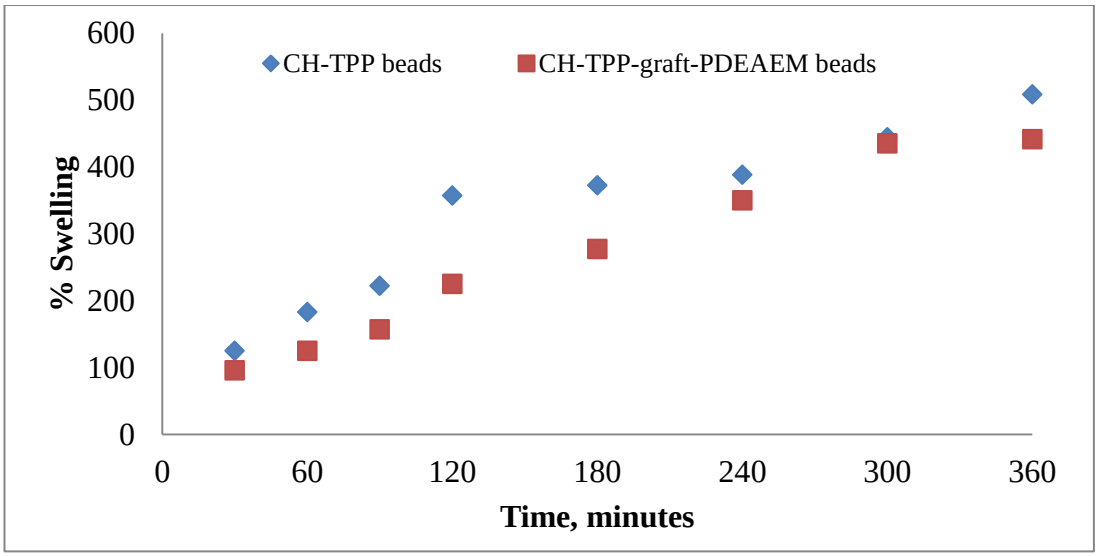

Figure 17. Swelling of CH-TPP and CH-TPP-*graft*-poly(DEAEM) in pH=11.0.

Swelling behavior of CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads was studied upon repeated steps of immersing in pH=7.00, pH=11.00 and pH=1 solutions. This type of swelling behavior between pH=7.0 and pH=1.0 as shown in Fig 18, 19, 20.

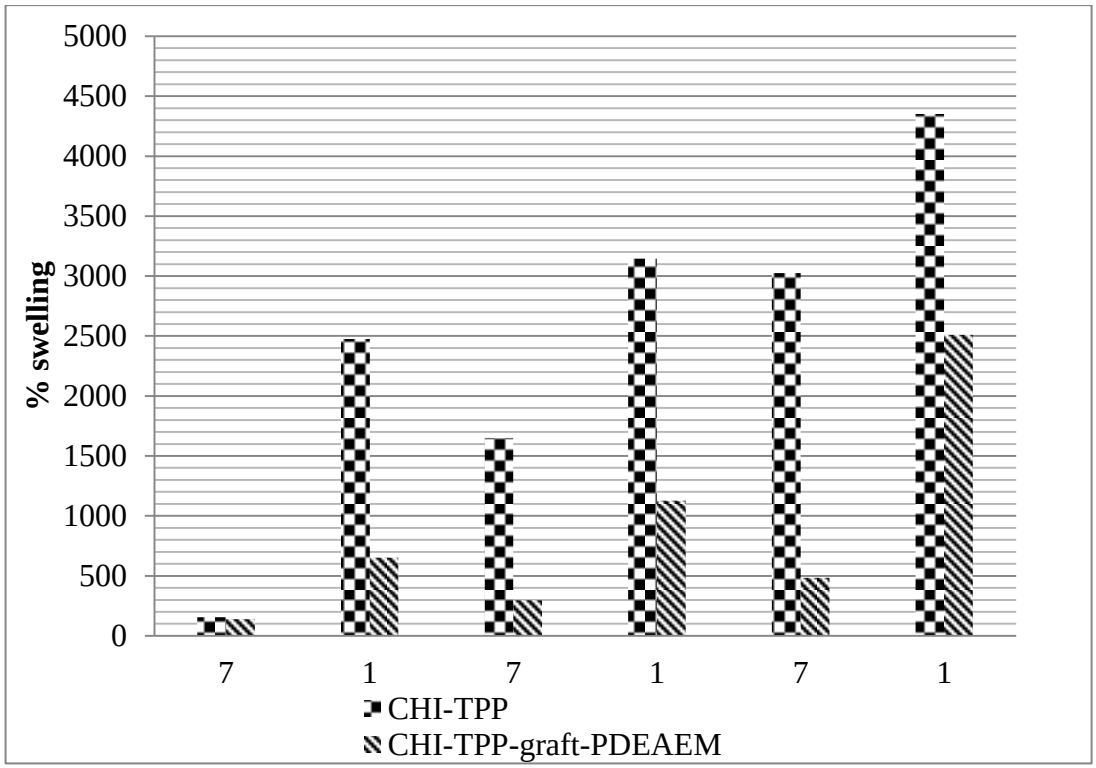

Figure 18. The comparison of swelling behavior of CH-TPP and CH-TPP-*graft*-poly(DEAEM) beads upon repeated steps of immersing in pH=7.0 and pH=1.0.

When the beads are initially swollen in pH=7.0, the swelling capacity increases to 2473% for CH-TPP beads and 650% CH-TPP-*graft*-poly(DEAEM) beads following immersion in pH=1.0 as shown in Fig. 18 and 19. The same trend with increasing % swelling values are observed at repeated steps. The similar swelling behavior is observed for CH-TPP-*graft*-poly(DEAEM) beads for consecutive immersion in pH=11.0 and pH=1.00 as shown in Fig .20.

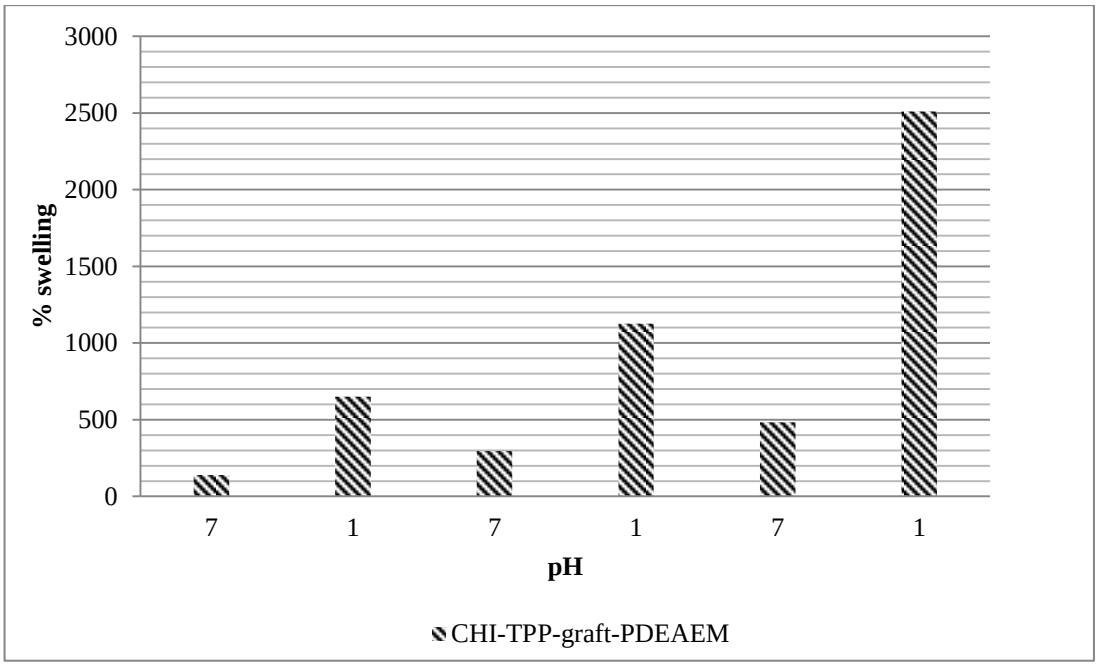

Figure 19. Swelling behavior of CH-TPP-*graft*-poly(DEAEM) upon repeated steps of immersing in pH=7.0 and pH=1.0.

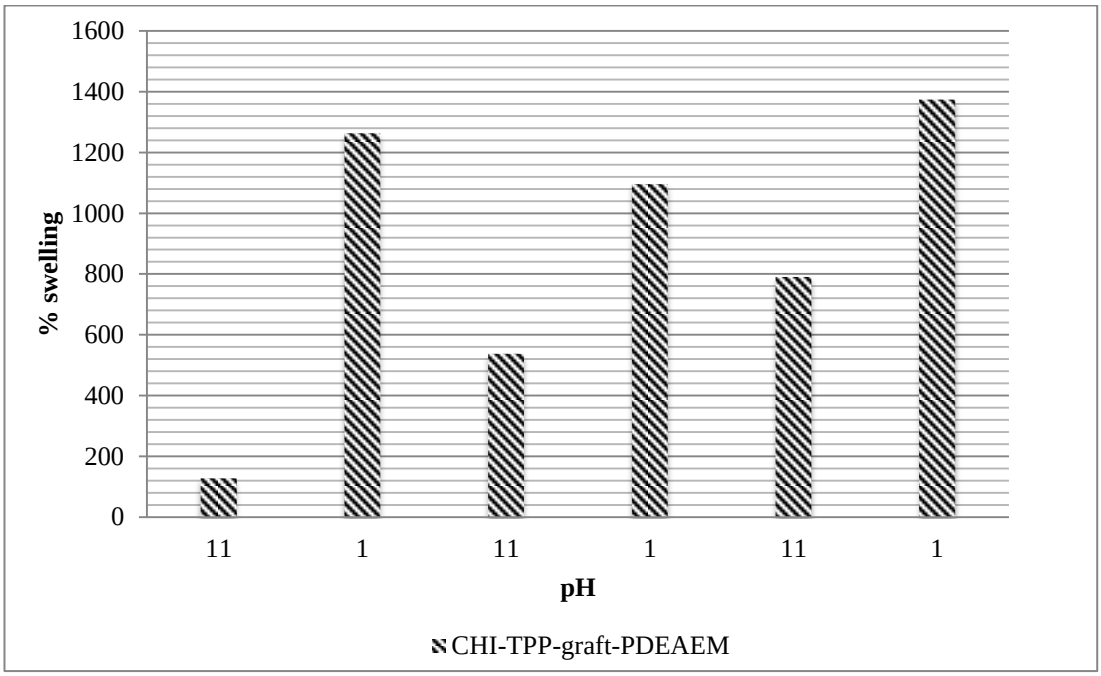

Figure 20. Swelling behavior of CH-TPP-*graft*-poly(DEAEM) upon repeated steps of immersing in pH=11.0 and pH=1.0

3.6 *In-vitro* Ciprofloxacin Loading and Release

Table 13.Ciprofloxacin loading percentage.

Sample ID	% Loading
CH-TPP Beads	48
CH-TPP-*graft*-poly(DEAEM)	48

Ciprofloxacin release from the beads was followed in water. The release profiles shown in Figure 21, reveal that both beads release about 8% of the drug loaded within first three hours in water. CH-TPP-*graft*-poly(DEAEM) beads release 12% of the drug after 12 hours whereas the non-grafted CH-TPP bead releases the same amount about 7% after 12 hours. Hence, poly(DAEM) grafting onto the beads does not create a considerable difference in either drug loading capacity or drug release behavior in water. The fact that the two type of beads have the same loading capacity and release behavior, indicate that the drug interacts with the matrix on the surface via physical interactions rather than chemical interactions and/or drug diffusion into the matrix. The schematic representation of CFX loading onto the chitosan based beads and CFX release from the beads are shown in Figure 22.

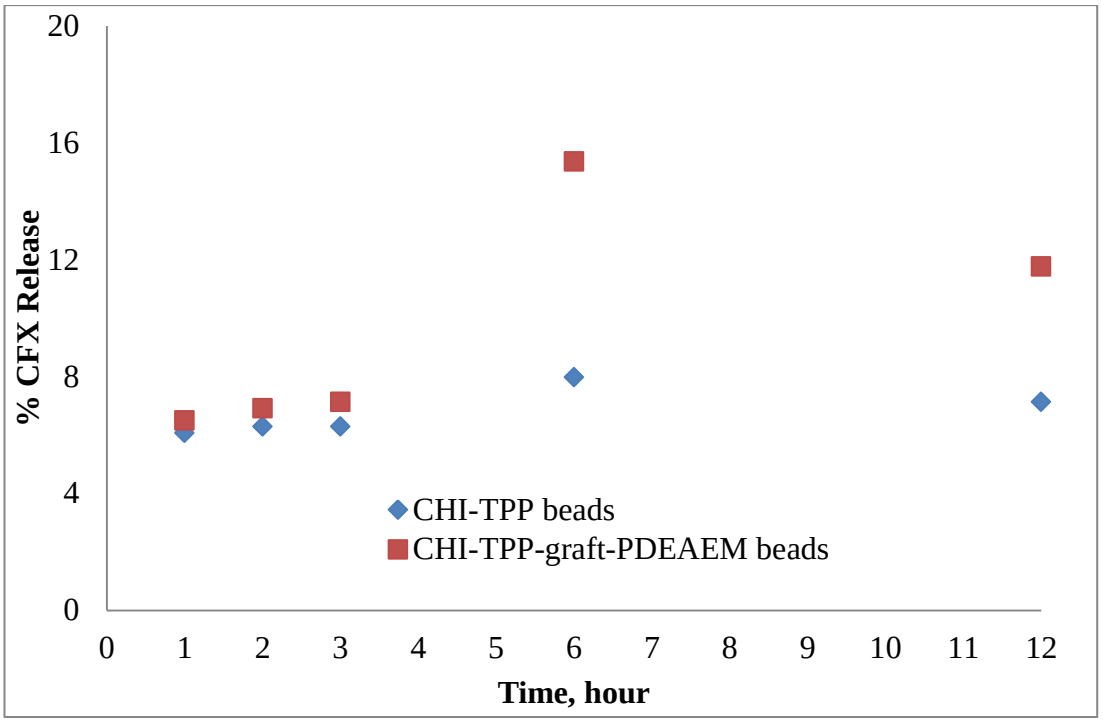

Figure 21. *In-vitro* ciprofloxacin release in water from (a) CH-TPP and (b) CH-TPP-*graft*-poly(DEAEM).

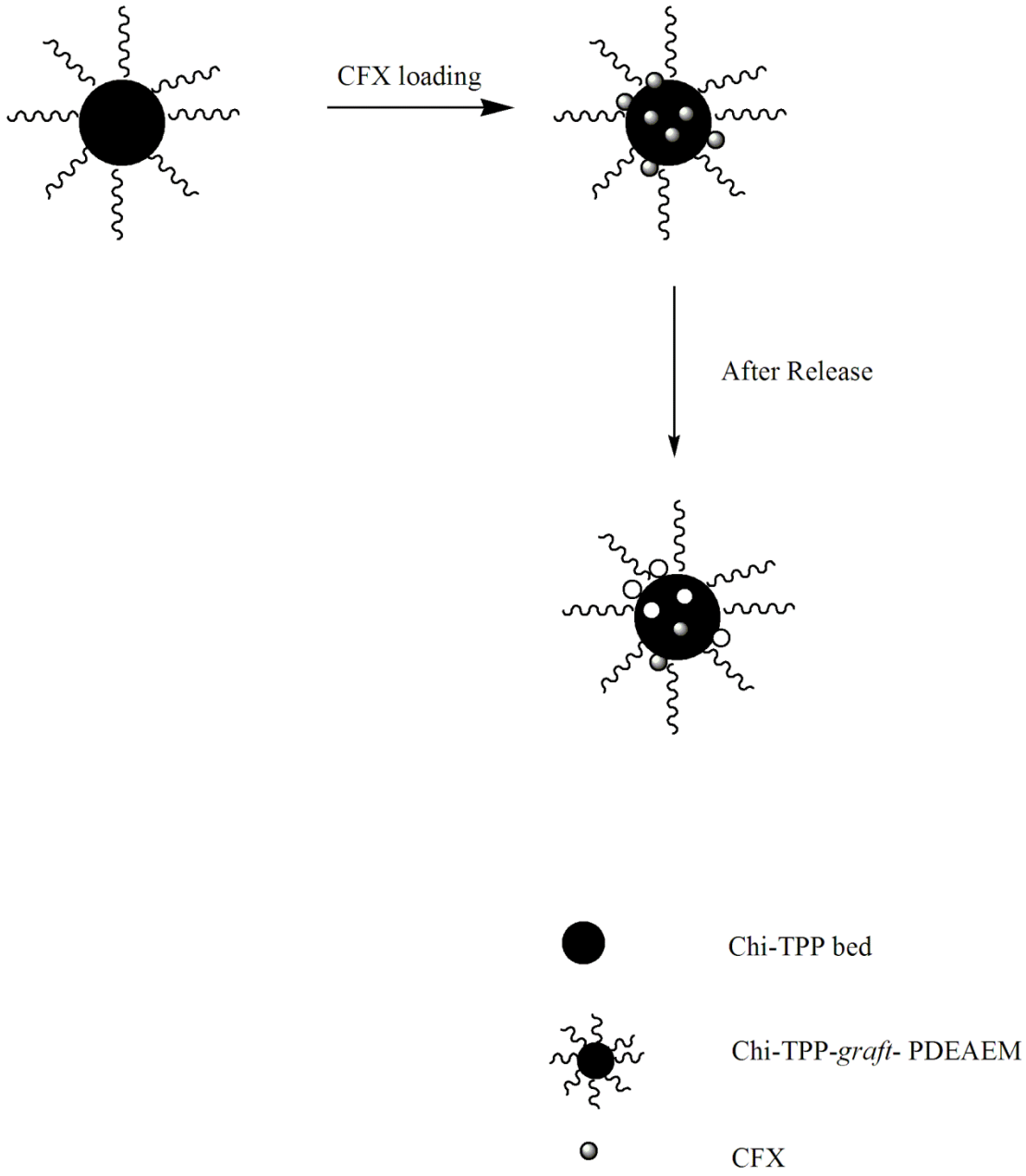

Figure 22. *In-vitro* ciprofloxacin release in water from CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads.

3.7 Antibacterial Test against E.coli

Antibacterial activities of the beads and the drug-loaded beads have been tested against *E.Coli* as illustrated on Table 15.

Table 14.Inhibition zone measurement of samples.

Sample ID	**Inhibition zone, cm**
CH-TPP bead	1.4 cm
CH-TPP-*graft*-poly(DEAEM) bead	No inhibition
CFX loaded CH-TPP bead	1.8 cm
CFX loaded CH-TPP-*graft*-poly(DEAEM) bead	2.6 cm
CFX loaded CH-TPP bead (after release)	1.4 cm
CFX loaded CH-TPP-*graft*-poly(DEAEM) bead (after release)	1.8 cm

CH-TPP bead shows antibacterial activity with an inhibition zone diameter of 1.4 cm. However, CH-TPP-graft-poly(DEAEM) bead does not exhibit antibacterial activity. For an unclarified reason poly(DEAEM) grafting onto CH-TPP beads prevents antibacterial activity. CFX loading improves antibacterial efficiency of CH-TPP bead with 40% increase due to the synergistic effect of the bead and the drug together. There is no data, however, to compare the inhibition zone diameters measured to that of pure CFX. CFX loaded CH-TPP-*graft*-poly(DEAEM) bead shows an inhibition zone of 2.6 cm indicating that a greater amount of drug is released from the grafted beads compared to the non-grafted ones on the agar, as observed with *in-vitro* drug release experiments shown in Figure 21, and as explained in section 3.8.

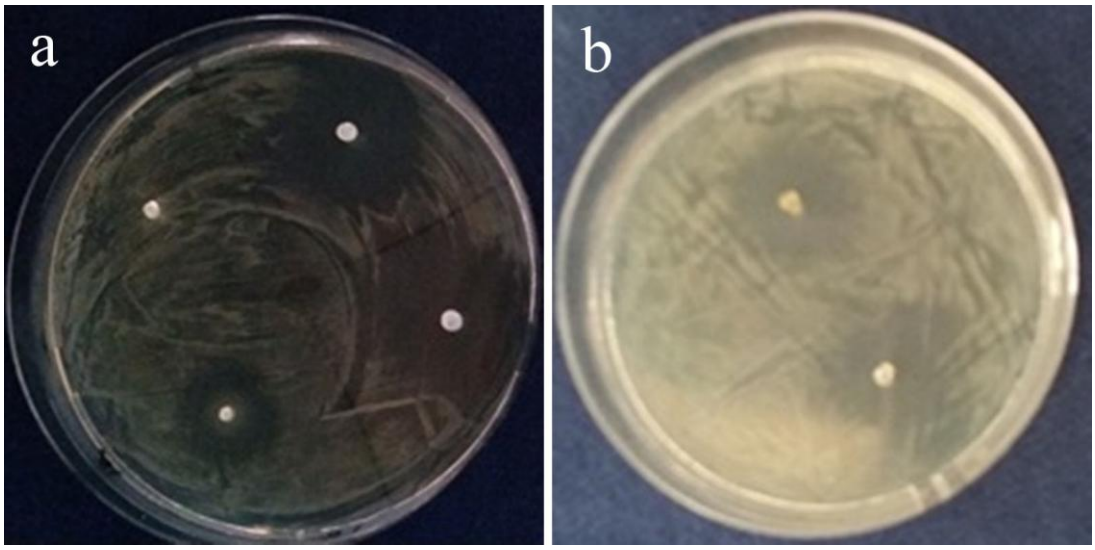

Figure 23. Inhibition zone measurement of (a) CH-TPP beads and CH-TPP-*graft*-poly(DEAEM) beads CFX loaded CH-TPP beads and CFX loaded CH-TPP-*graft*-poly(DEAEM) beads (b) CFX loaded CH-TPP beads and CFX loaded CH-TPP-*graft*-poly(DEAEM) beads after drug release for 12 hours

CONCLUSIONS

DEAEM can be graft copolymerized onto chitosan using KPS as the redox initiator under homogeneous and heterogeneous conditions.

Solubility of the products in aqueous acidic solution is controlled by the grafting yield, in the case of products obtained under homogeneous conditions. Chitosan-*graft*-poly(DEAEM) sample, which is soluble in aqueous acidic solution can be chemically crosslinked by glutaraldehyde at pH=1. Gels with improved pH sensitive swelling capacity compared to glutaraldehyde crosslinked pure chitosan gels are obtained.

Graft copolymerization of DEAEM onto chitosan-TPP gel beads can modify the surface morphology of the chitosan based gels. In pH=1 solution, chitosan-TPP-*graft*-poly(DEAEM) gel beads swell to perform as superabsorbent gels.

The antibiotic ciprofloxacin can be loaded into chitosan-TPP and chitosan-TPP-*graft*-poly(DEAEM) hydrogel beads. Controlled release of the drug from the beads has been achieved. The bead/drug system exhibits antibacterial activity against *E.Coli* as determined by inhibition zone measurements.

Obtaining the poly(DEAEM) grafted chitosan gels is the promising idea for drug delivery as well as novel bio-based adsorbents after further detailed in-vitro and in-vivo detailed investigation.

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