Synthesis of Chitosan/Poly (Diethyl aminoethyl methacrylate) Interpenetrating Polymer Network Gels

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ABSTRACT

In this study, chitosan was cross-linked with glutaraldehyde to prepare semi-IPN (SIPN) and IPN hydrogel systems. Several interpenetrating polymer network (IPNs) type hydrogels from the mixture of chitosan, diethylamino ethylmethacrylate (DEAEM), potassium persulphate, N,N′- methylenebisacrylamide (MBA) of pH responsive swelling properties were synthesized by in-situ polymerization method.

The IPN and semi-IPN (SIPN) gels prepared were characterized by swelling characteristics at 37 °C, and also by scanning electron microscopy (SEM). The SEM image of the semi-IPN gel shows smoother morphology while that of the IPNs gives rougher surface with smaller pores. The hydrogels obtained exhibit pH sensitive swelling behavior. All samples swell highest in acidic medium. The equilibrium swelling values obtained at pH 1, 7 and 11 respectively are as follows: IPN (822%)> SIPN (783%) > CH (173%), IPN (453%)> SIPN (235%)> CH (119%) IPN (425%)> SIPN (242%) > CH(78%).

The drug ciprofloxacin was loaded into the IPN hydrogels in water. Drug release kinetics were followed in pH 1.2 buffer solution at 37 °C.

Keywords: Interpenetrating polymer network, glutaraldehyde, chitosan, ciprofloxacin, hydrogels

ÖZ

Bu çalışmada , kitosan glutaldehit ile çapraz bağlanıp yarı iç-içe girmiş polimerik ağ yapılar ile iç-içe girmiş polimerik ağ yapılarının sentezi ile hidrojeller hazırlandı. pH duyarlı çeşitli polimerik ağlar kitosanın dietilamino etilmetakrilat (DEAEM), potasyum persülfat , N , N'- metilenbisakrilamid (MBA) ile in-situ polimerizasyon yöntemi ile sentezlenmiştir. Hazırlanan yarı iç-içe girmiş polimerik ağ yapılar ile iç-içe girmiş polimerik ağ yapılarının 37 °C 'de şişme özellikleri incelenmiş ve taramalı elektron mikrokopu ile morfolojileri karakterize edilmiştir. Elektron mikroskopu analizlerinden iç-içe girmiş polimerik ağ yapılarının küçük gözeneklere sahip pürüzlü bir yüzeye sahip olduğu gözlemlenirken yarı iç-içe girmiş polimerik ağ yapılarının SEM görüntüsü daha az pürüzlü morfoloji göstermektedir . Hidrojellerin pH duyarlı şişme davranışı gösterdiği saptandı. Bütün numuneler, asidik ortam içinde en yüksek oranda şişme gösterirken. Denge şişme% değerleri pH 1 , 7 ve 11 de elde edilmiş ve aşağıda sırasıyla belirtilmiştir: IPN (822 %) > SIPN (783 %) > CH (173 %) , IPN (453 %) > SIPN (% 235) > CH (119 %) IPN (425 %) > SIPN (242 %) > CH (% 78).

Siprofloksasin antibiyotik ilaç suda hazırlanan hidrojellere yüklenerek ilaç salım kinetiği , 37 ° C'de pH 1.2 tampon çözeltisi içinde takip edilmiştir.

Keywords: iç-içe girmiş polimerik ağ yapılar, glutaraldehit, kitosan, siprofloksasin

DEDICATION

I dedicate this thesis to Mr. Alizor Benjamin Onuwa for making it a reality.

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INTRODUCTON

Polymer blends are mixtures of two linear or branched polymers, which are compatible with each other. Solution blending is one way of bringing two polymers together in a mutual solvent. Co-precipitation or coagulation of the polymers in a common non-solvent or film forming by solvent evaporation results in the formation of a blend of the two polymers. Bringing crosslinked polymers together is achieved by the formation of Interpenetrating Polymer Networks (IPN). It is possible to form an IPN by simultaneous or sequential crosslinking of the two polymers. In simultaneous IPN preparation the two monomers, initiator(s) and crosslinkers are mixed together; polymerization and crosslinking produces two crosslinked polymer systems entangled together. In the sequential addition method a crosslinked polymer network is prepared first, then the second monomer is polymerized and crosslinked in the presence of the first network.

The aim of this study is to prepare IPN's of chitosan/poly (diethylaminoethyl methacrylate) system. This should constitute a hybrid system of a natural and a synthetic polymer with pH responsive swelling properties. The IPN systems prepared are anticipated to form suitable matrices for drug encapsulation and controlled drug release as well as other biomedical applications such as precursor for scaffolds, for tissue regeneration. The gels may also serve as bio-adsorbents for toxic metal or dye removal from industrial wastewater.

The study covers preparation of chemically crosslinked chitosan network by glutaraldehyde (GA) crosslinking. The network then swollen in aqueous solution to absorb diethylaminoethyl methacrylate (DEAEM) monomer, the crosslinker, N, N′-methylenebisacrylamide (MBA) and the initiator potassium persulfate (KPS). The polymerization and crosslinking reactions of DEAEM carried out in the presence of crosslinked chitosan produces IPN gels. Semi-IPN gels are prepared under the same conditions with IPN gels, in the presence of uncrosslinked DEAEM. Swelling kinetics studies show pH responsive swelling behavior. The hydrogels produced proved to be suitable matrices for controlled release of the drug ciprofloxacin.

1.1 Blends, Semi -IPNs and IPNs

While mixtures of linear polymers are referred to as polymer blends, a mixture of a crosslinked and an uncrosslinked polymer is a semi-IPN and a mixture of two crosslinked polymers is an IPN. Polymers are mixed together to prepare new systems with modified properties for several industrial, environmental or biomedical applications to name a few.

1.1.1 Semi-IPNs

A system containing only one crosslinked component while the others are in linear or branched form is referred as semi-IPN (Murugesh & Badal, 2012). The component can be separated without any chemical bond destroyed. This a major difference between IPNs and SIPN.

1.1.2 Inter Penetrating Polymer Networks (IPNs)

Partly intertwined two polymer networks not covalently bonded to each other is referred to as an IPN. The polymer networks are brought together without forming any chemical bonds between the two crosslinked polymers, except for some degree of chemical grafting of one polymer onto the other one during polymerization of the

second monomer in the presence of the first polymer, or copolymerization of the two monomers during polymerization and crosslinking. The intertwined system cannot be separated except by breaking of the chemical bonds (Murugesh & Badal, 2012).

Table 1 further explains the classification of IPN based intermolecular forces.

Table 1. IPN classifications based on intermolecular forces

Covalent Semi-IPN	In this case single polymer network is formed following the polymerization of two systems which are isolated.
Non covalent Semi-IPN	A system comprising the crosslinking of a single polymer network.
Non covalent full-IPN	Independently crosslinking two separate polymers.

More so, IPNs are also classified based on the pattern of arrangement as shown in Table 2. The four types of IPNs according to pattern of arrangement are sequential IPNs, novel IPNs, semi-IPNs and simultaneous IPNs.

Table 2. IPN classifications based on arrangement pattern

Sequential-IPNs	In this case when the polymerization of the first polymer component system ends, the polymerization of the second polymer component follows afterwards.
Novel-IPN	The partly intertwining of at least two polymer system which are not joined together covalently and cannot be detached only by the destruction of chemical bonds.
Semi-IPNs	One of the constituent is in a linear form and the other is cross-linked.
Simultaneously-IPNs	Polymerization of the two system component is done at the same time.

1.1.3 IPN- Features and Characteristics

For a system to be called an ideal IPN; without dissolving in a solvent it should have the ability to swell in a solvent, when stress is applied on the IPN, separate phases must be kept together and should resist flow and creep.

1.1.4 IPNs -Advantages & Disadvantages

Interpenetrating polymer network systems are highly been regarded in modern age based on its advantages (Babu, Sairam, Hosamani, & Aminabhavi, 2007) & (Rokhade, Shelke, Patil, & Aminabhavi, 2007). The pros and cons of IPNs are summarized in Table 3.

Table 3. IPN -advantages & disadvantages

Advantages of IPNs	**Disadvantages of IPNs**
The final product mechanical properties are enhanced.	IPN can produce synergistic effect by sharing the properties of both the polymers consequently avoiding the limitations of natural as well as synthetic Polymers.
They increase the final product phase stability.	Interpenetrating polymer network (IPN) is not formed from normally mixing of two or more polymers and also does not produce from copolymers
Ability to overcome the thermodynamic incompatibility if the reacting components are carefully blended, based on the fact that the segmented networks are permanently intertwined.	

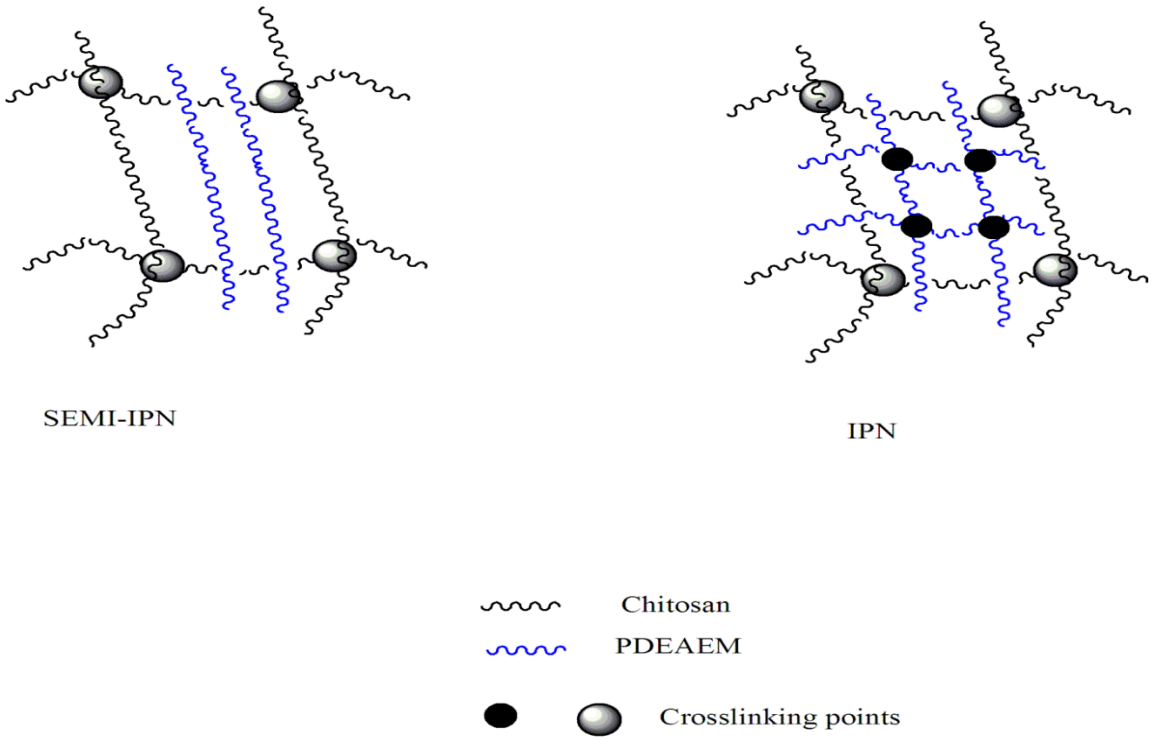


Figure 1. Semi- IPN and IPN formation

1.1.5 Semi-IPN and IPN -Synthetic Pathways

The preparation of interpenetrating and semi-IPNs can be done via; *in-situ* and sequential method. Figure 1 illustrates semi- IPNs and IPNs structures referring to chitosan and PDEAEM as sample polymers.

Polymerisation of IPNs and Semi-IPNs using the *in- situ* synthesis pathways is initiated only when the mixing of every reacting component is done. The formation of network sequentially or simultaneously occurs in this case when the initiation is either or not occurs at the same time for the two network that is been synthesised. Therefore, the production of various networks need be dissimilar in nature through the reaction pathway; else the morphology material gotten can be controlled and highly differently made through a change in its proportion of the polymer partners in the *in- situ* synthetic reaction pathway.

Polymerisation via the sequential synthesis method allows the synthesis of the initial polymer network and is then swollen with every necessary precursors leading to the second network formation and then carried out inside the first network. Generally, in this case then final material morphology is determined by the first network. Sequential synthesis method is denoted as semi-IPN based on the above explanation, because it allows crosslinked association of polymer with dissimilar properties into a further or fewer homogeneous materials (Chikh, Delhorbe, & Fichet, 2011).

1.2 Chitosan; Structure, Properties and Applications

Chitosan is linear polysaccharide linked by β (1-4) D-glucosamine units with N-acetyl glucosamine positioned at random positions on the polymer network depending on the polymer deacetylation degree (Figure 2). Chitosan is obtained from chitin a polymer that is fully acetylated, forming basically arthropod exoskeleton. It stands as the second most plentiful polysaccharide next to cellulose (Nair & Laurencin, 2007).

Chitosan has the ability to interact with the mucous membrane negative charge because of its solid positive charges, giving it the ability to have good mucous-adhesive properties. More so, chitosan can form complexes and ion adsorbing ions when in solution because of its cationic nature. Chitosan pKa is 6, contrasting to other polysaccharides which are at most time basic or natural, the only basic polysaccharides are chitin and chitosan (Yilmaz, 2004).

Chitosan is a multifunctional polymer because it possesses both the amine or acetamido group on the carbon 2 position with the primary and secondary hydroxyl

group present at the Carbon 3 and Carbon 6 positions on the polymer backbone. The amine content of the polymer is distributed randomly and is also a very important factor; since it gives the polymer its different physical and chemical properties making it possess intra and inter molecular hydrogen bonding (Zhang et al., 2010).

The usefulness of chitosan for the delivery of drug requires that it be cross-linked, thereby increasing its consistency and time for delivery of drugs (Campos, Satsangi, Rawls, & Mei, 2009). Chitosan is soluble in aqueous media; therefore it can be formed into different forms including nano - fibers, microsphere, nano-spheres and gels.

Chitosan is readily soluble in acetic acid and is processable into several forms like; gels, nano-fibers, microspheres and Nano spheres. Also its unique bio-adhesivity, compatibility, degradability, non-toxicity, poly-functionality and the sensitivity of pH sensitivity makes chitosan a hopeful medical and pharmaceutical candidate for the development of devices for delivery of drug and in tissue engineering as scaffolds. Chitosan having antimicrobial, anti-tumor and immune-enhancing properties, has found applications in the field of medicine as surgical sutures that are absorbable, physiological material, artificial skin and wound healing accelerator (Zhang, et al., 2010).

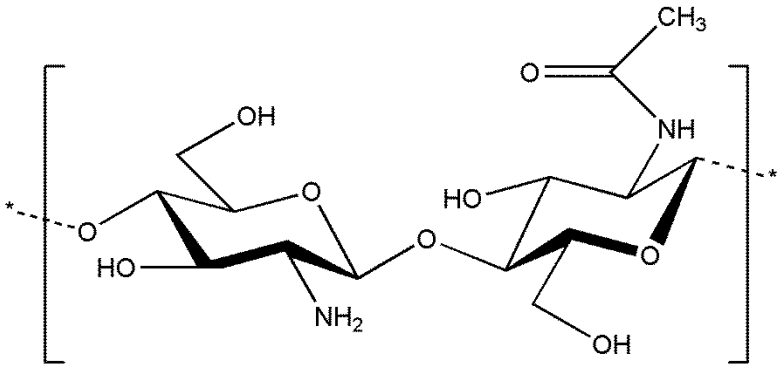


Figure 2. Structure of Chitosan

1.2.1 Chitosan- Chemical Modifications

Chitosan is water insoluble because it is crystalline in nature. However transforming chitosan into water soluble products will improve its potential as a biomaterial.

Owing to the poly functionality of chitosan, numerous kinds of alteration can be performed to get dissimilar constituents with dissimilar features. The benefits of chitosan is that it retains its properties after undergoing modification without the backbone affected (Mourya & Inamdar, 2008) .

Modifying chitosan through the hydroxyl linkage is always favored, since its cationic with biological property is lost in solution when modified through the amine group linkage; which is its utmost exiting property. Chitosan can undergo alchol-like reactions such as etherification and esterification reaction (Gorochovceva & Makuska, 2004; Liu, Wang, Shen, & Fang, 2005).

1.3 Hydrogels of Chitosan

Three dimensional polymer linkages having several hydrophilic functional groups are referred to as hydrogels (Kurita, 2001).

Hydrogels are prepared by crosslinking natural or synthetic polymers. They have the tendency of keeping fluid 1000 times of the dry mass when swollen in biological and aqueous media. Hydrogels of chitosan can be used as controlled drug release media, wound dressing material and for bio-active macromolecules transport. The most regularly used crosslinking agent for hydrogels of chitosan formation is glutaraldehyde.

Hydrogels are also called smart or intelligent materials owing to their response to changes in whatever media they find themselves. Dry hydrogels from bio-macromolecules display numerous properties such as biodegradability, incorporating cells, drugs and compounds that are bio-active. Based on their linkages they are divided into two: chemical and physical hydrogels.

Chemical gels are made through crosslinking reactions to yield covalent bonds. Numerous familiar cross-linkers used include formaldehyde, diglycidyl ether EDGE, dialdehydes; includes glutaraldehyde and glyoxal, epichorohydrin, ethylene glycol. This crosslinkers have limited application in pharmaceutical because of because of their toxicity. Non-poisonous cross-linkers frequently used are genipin and PEG (Wu, Liou, Yeh, Mi, & Lin, 2013). The bridging of chemical hydrogels through crosslinkers is done by covalently at designated precise site linking the biopolymer together.

The crosslinking of chitosan using glutaraldehyde has numerous benefits; this may be used for the adsorption of metal and immobilization of enzymes (Kurita, 2001). From reviews ordinary chitosan without crosslinker could not inhibit bacterial

growth, but when cross-linked with glutaraldehyde the bacterial *Burkholderia cepacia* was inhibited (Li et al., 2013).

The figures below (Figure 3 and 4); show the structural representation of chitosan chemically cross-linked with glutaraldehyde.

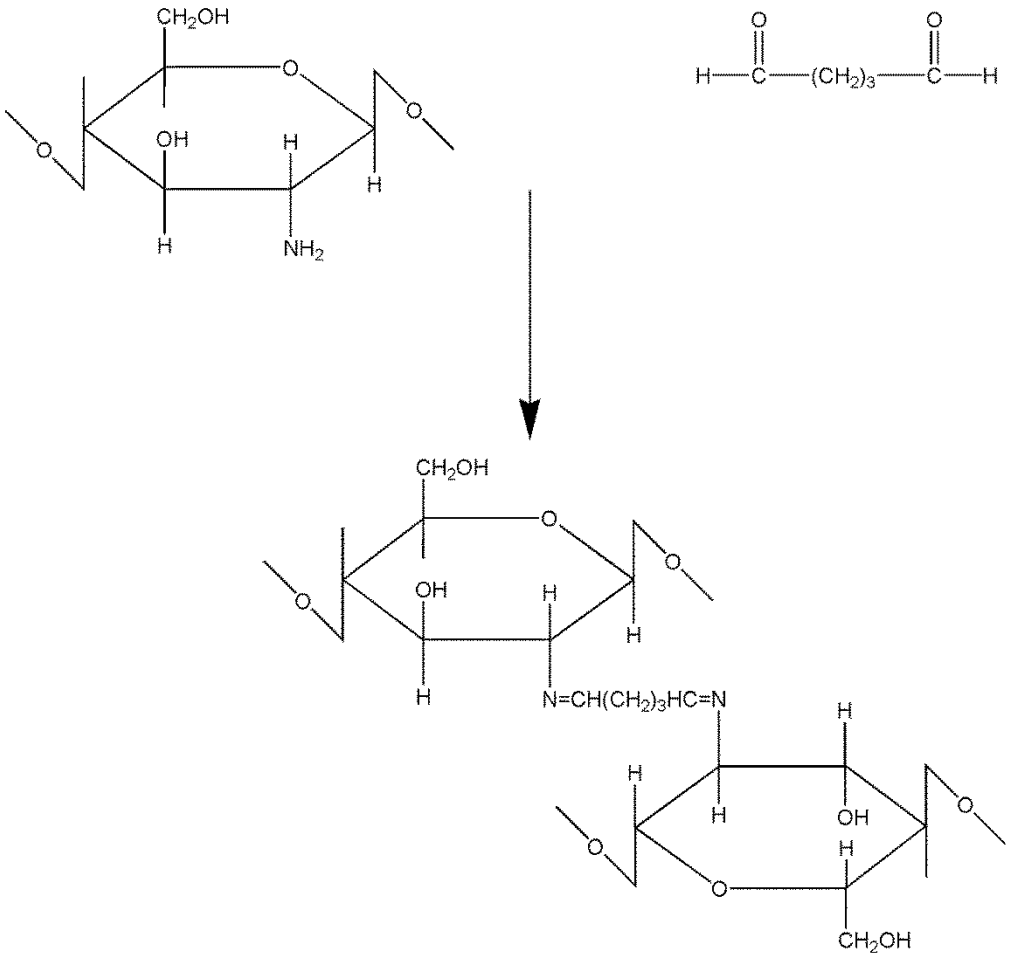


Figure 3. Chemical crosslinking of Chitosan with Glutaraldehyde

Physical gels of chitosan are not mechanically stable, they lose their integrity easily, and this factor limits their application. Knowing well that chitosan is polycationic, it therefore, has the ability of interacting with ions which are negatively charged, and via electrostatic attraction an ionic complex of the anion and polymer chain of

chitosan is formed. In the delivery of drugs physically cross-linked hydrogels are frequently used because no reagents which may be harmful to the human system during or after the loading and release of the drug is used. Anions commonly used include; sodium sulphate, sodium citrate and sodium tripolyphosphate(TPP) (Sashiwa & Aiba, 2004).

Physical gels can degrade completely in water, making it a highly sensitive material; it can also be reversed thermally.

Physical and chemical hydrogels are being prepared by combining both synthetic and natural polymers. Both hydrogel systems having independent domain organized heterogeneously. Physical hydrogels are heterogeneously structured in distinct domain of clusters, produced by entanglement of the molecule, molecular loop, free end chain joined together by interactions of ions, and weak hydrophobic interactions (Sashiwa & Aiba, 2004).

1.4 Poly Diethylamino Ethyl Methacrylate (DEAEM): Structure, Properties and Applications

Poly diethyl amino ethyl methacrylate (PDEAEM) whose chemical structure is shown in Figure 5 promising pH-sensitive material for tumour-targeted drug delivery it is also a cationic polyelectrolyte having a pKb of 6.9. By protonation of the amino groups it becomes soluble in acidic solution but at a neutral pH it is insoluble (Yang et al., 2013).

DEAEM properties and phase transition has been tuned, using poly (2-vinyl pyridine) – a pH sensitive counter blocks polymer to obtaining a micelles that are pH

reliant (Campos, et al., 2009), poly (2-(N-carbazolyl) ethyl methacrylate) – hydrophobic block polymer to produce holes transport properties (Krasia & Patrickios, 2002), and copolymers of amphiphilic di-blocks, Poly(oligo(ethylene glycol) methacrylate) POEGMA(Gohy, Antoun, & Jerome, 2001), and Poly(N-isopropylacrylamide) PNIPAM (Emileh, Vasheghani-Farahani, & Imani, 2007) a block polymer with temperature responsive property to adjust its lower critical solution temperature (LCST).

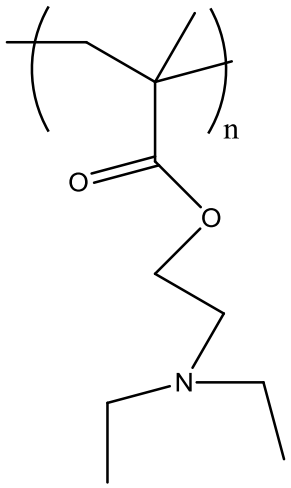


Figure 4. Chemical structure of PDEAEM

1.5 Ciprofloxacin Drug

Ciprofloxacin (1- cyclopropyl – 6 – fluoro – 1, 4 – dihydro – 4 –oxo – 7 – (1 – piperazinyl) – 3 -Quinoline carboxylic acid) is an effective synthetic fluoroquinolone wide spectrum antibiotic, having antibacterial effectiveness against gram-positive and gram-negative bacteria (Babu, et al., 2007). The chemical structure of ciprofloxacin is shown in Figure 6.

Ciprofloxacin is very effective in the treatment of a lot of infections, not limited to instigated multi-tough causing gastrointestinal infections, complex urinary tract

infections, bones, lower respiratory tract caused by malignant otitis, febrile neutropenia, intraabdominal infections and infections transmitted sexually such as cancroid and gonorrhea**.** Because of its high healing ability Ciprofloxacin is used for pneumonia treatment as lone agent (Rokhade, et al., 2007).

Moreover list of exceptional, although severe side effects has been linked to the administration of ciprofloxacin, these effects include; severe damage of the liver (hepatitis), drug fever, vision loss, severe damage of the arteries and acute pancreatitis (Nguyen et al., 2012).

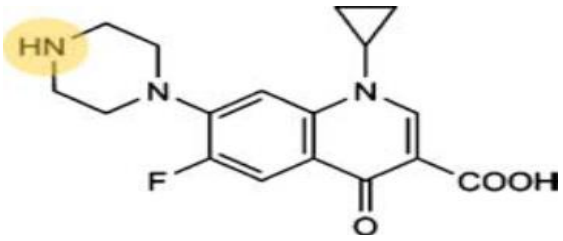


Figure 5. Structure of ciprofloxacin drug

1.6 Drug Release Kinetics

The availability of drugs for pharmacological actions occurs when a drug is exposed to metabolism, absorption, excretion, and distribution after exiting the product that contain the drug. This process is referred to as drug release (Gautam & Mahaveer, 2011).

The drug release can be described in several ways; Instant release of the drug products permitting dissolving without intention of delaying, extending and dissolution of the drug; improved dosage release methods comprise of postponed and

prolonged drug discharge products. In postponed release the drug is discharged at time (t) only after some period following administration; prolonged products release formulation are articulated to making the drug available over sustained period when administered; Lastly, pulsatile controlled product release includes discharge of fixed amount of the drug in different time recesses programed inside the product of the drug.

Drugs may be released through leaching in dissolution medium which is able to enter the polymer drug matrix system through pores, cracks and inter granular spaces. The diffusion rate of the fluid into the matrix may stay controlled via changes in pores present inside the matrix (Dash, Murthy, Nath, & Chowdhury, 2010). The release of drug from matrices may involve processes of diffusion, erosion, and leaching or dissolution (A, C, & I, 2013; Chime, Onunkwo, & Onyishi, 2013).

Numerous kinetics models such as; zero, first order, Higuchi and Korsmeyer-Peppas define drug dissolution from instant and improved dosage discharge methods have been proposed (Gautam & Mahaveer, 2011).

Drug release kinetics can be influenced by the drug type, size of the particle, polymorphic form, solubility, crystallinity and dosage in pharmaceutical form.

However the aim of controlled release systems is the ability to maintain the concentration of the drug at targeted domains and blood as possibly can in preferred value. Largely, in achieving speedily effective concentration of the therapeutic drug, the released controlled system in the beginning discharge portions of the contained dose (Hussain, Ashwini**,** & Shirish, 2013)**.**

Chapter 2

EXPERIMENTAL

2.1 Materials

Chemicals used are outlined in Table 4 below;

Table 4. Materials used and manufacturing company name

Materials	**Manufacturing company**
Chitosan	Aldrich
Acetic acid	Reidel-deHaen
Glutaraldehyde 25% (w/w)	Aldrich, Germany
Diethylamino ethylmethacrylate(DEAEM)	Aldrich, Germany
Ethanol, 96% (v/v)	Riedel-de Häen, Germany
potassium persulphate (KPS)	Aldrich, Germany
N,N'-methylenebisacrylamide (MBA)	Aldrich, Germany

2.2 Chitosan Gels Cross-Linked With Glutaraldehyde

Preparation conditions are shown in Table 5. In order to find the lower limits of both glutaraldehyde and chitosan concentration, a series of gelation studies were performed. Chitosan solution was added to glutaraldehyde in a test tube according to the volume in Table 5 given below. The gelation time was determined and recorded at 40 °C. Gelation is complete when the product inside the tube does not move at all

when turned upside down. Each sample was kept in water with magnetic stirring at a temperature of 40 °C and at a stirring speed of 750 rmp for additional 3 h after gelation is complete Bengisu, M., & Yilmaz, E. (2002). The test tube was removed and 10 mL of ethanol was added to the gel and allowed to stand without heat for 1 h, after which it was transferred into a beaker and 50 mL of ethanol was added and allowed to stand overnight. Additional 20 mL ethanol was added.

Table 5. The preparation conditions of chitosan gels

Sample ID	**Chitosan (mL)**	**Glutaraldehyde (µl)**	**% Glutaraldehyde**
GEL 1	19.50	200	1
GEL 2	19.50	400	2
GEL 3	19.50	600	3
GEL 4	19.50	800	4

The % crosslinking was calculated by the following equation.

$$Gelation\ (\%) = \frac{m\ product - m\ chitosan}{m\ chitosan}\ x\ 100 \qquad (1)$$

2.3 Buffer Solutions –Preparation

Swelling and drug release experiments were carried out using buffer solutions prepared as outlined below;

Buffer pH 1.2 was prepared by mixing 125 mL of 0.2 M potassium chloride and 212.5 mL of 0.2 M hydrochloric acid in a 500 mL volumetric flask and completed to mark with distilled water.

Buffer pH 7.0 was prepared by mixing 122 mL of 0.1 M hydrochloric acid and 378 mL 0.1 M sodium hydrogen phosphate in a 500 mL volumetric flask and completed to mark with distilled water.

Buffer pH 11.0 was prepared by dissolving 1.059 g of sodium bicarbonate in 113.5 mL of 0.1 M sodium hydroxide in 500 mL volumetric flask and completed to mark with distilled water.

2.3.1 Preparation of Semi-IPN

Potassium per sulphate (0.5g) was dissolved in 30 mL water and added to pre-weighed amount of filtered GA crosslinked chitosan gel. Then, 70 mL of ethanol and 0.5 mL of DEAEM were added onto the mixture above. The solution was stirred continuously at 40 °C for 3 h, filtered and, finally dried in the oven at 40 °C. The preparation conditions for two different semi-IPNs is presented in Table 6.

Table 6. Preparation condition for SIPNs

Sample ID	**Chitosan solution (mL)**	**Glutaraldehyde (µl)**	**DEAEM(mL)**
Semi-IPN 1	19.50	200	0.50
Semi-IPN 4	19.50	800	0.50

2.3.2 Preparation of IPN

The as-prepared chitosan gel was added into a beaker containing 0.5 mL DEAEM in a 70 mL ethanol solution and was mixed for 15 min and added to the swollen gel.

1.125 g MBA (N,N- methylene bis acrylamide) and 0.125g potassium persulphate was dissolved in 30 mL water and added to the filtered gel. Each chitosan gel was

stirred for 4 h at a temperature of 40 °C. The gel was filtered and dried at 40 °C in the oven. The preparation conditions of the IPN's are summarized in Table 7.

Table 7. Preparation conditions for IPNs

Sample ID	**Chitosan Solution (mL)**	**Glutaraldehyde (µL)**	**DEAEM (mL)**	**MBA (g)**
IPN(1)	19.50	200	0.50	0.125
IPN(2)	19.50	400	0.50	0.125
IPN(3)	19.50	600	0.50	0.125
IPN(4)	19.50	800	0.50	0.125

The percentage IPN formation was calculated using the following equation:

IPN formation (%) = $\frac{mproduct - m\ chitosan}{m\ chitosan} x\ 100$ (2)

2.4 Percentage Swelling Capacity Determination

The swelling capacity of the hydrogel was determined by dipping a known mass of the IPN and crosslinked gel in 20 mL of the prepared buffer solutions at different pH values at 37 °C. At predetermined time intervals, the samples were carefully removed from the buffer solution, surface water was wiped off with the use of filter paper and the samples were weighed to determine the mass.

$$\% \text{ Swelling Ratio} = \frac{(Mt - Mi)}{Mi} * 100 \quad (3)$$

M_t = mass of sample at time t

M_i= initial mass of sample

2.5 Ciprofloxacin Release Study

2.5.1 Preparation of Ciprofloxacin Solution

Ciprofloxacin solution was prepared by dissolving 5.0 mg of the powdered drug in 500 mL volumetric flask and completing to mark with distilled water.

2.5.2 Ciprofloxacin Loading

1.00 g of IPN was placed in 0.2 mg of aqueous ciprofloxacin solution in a beaker for 24 hours at 37°C. The amount of drug loading was determined spectrophotometrically at 275 nm by measuring the absorbance of the solution after loading.

The % loading was calculated using the following equation:

$$\text{Loading (\%)} = \frac{C\ loaded}{C\ initial}\ x\ 100 \qquad (4)$$

c loaded = concentration difference of ciprofloxacin solutions before and after loading

c initial = concentration of ciprofloxacin solution before loading

2.5.3. Ciprofloxacin Release

The drug loaded IPNs described in section 2.5.2 above were transferred into 20 mL of the release medium (water and pH 1.2) and stirred at 37 °C. The concentration of drug released with respect to time was determined by measuring absorbance of the solution at 275 nm.

The release percentage was calculated using the formula below;

$$\text{Release (\%)} = \frac{C\ release}{C\ loaded}\ x\ 100 \qquad (5)$$

c release = concentration of the ciprofloxacin solution after release

c loaded = concentration difference of ciprofloxacin solutions before and after loading

2.5.4 Drug Release Kinetics

Data obtained from the drug release experiments was used to evaluate kinetics of the release to determine if it was zero-order kinetics, first-order, or obeyed Higuchi release or Korsmeyer- Peppas equations. Equations used are depicted in the Table 8.

Table 8. Kinetic models and equation

Release Kinetic Model	**Equation**
Zero Order Release Kinetics	$Q_t = Q_o + K_o t$
First Order Release Kinetics	$\log Q_t = \log Q_o + K\,t/2.303$
Higuchi Equation	$Q = K_H t^{1/2}$
Korsmeyer- Peppas Equation	$K_M\, t^n = \frac{Mt}{M}$

Where;

Q_t, M_t= total amount of drug released at time t (hours).

Q_o = initial amount of drug

K_o = zero order release constant

K = first order release constant

M = total amount of drug in dosage form

K_M = kinetic constant

2.6 SEM Analysis

SEM analysis of the samples was carried out at Cyprus International University using JEOL/JSM-6510LVF scanning electron microscope.

Chapter 3

RESULTS AND DISCUSSION

3.1 Preparation of Glutaraldehyde Cross-Linked Chitosan Gels

Gelation times of the glutaraldehyde crossinked chitosan gels are given in Table 9 increasing amount of glutaraldehyde results in shorter gelation time as can be followed from Table 9. In Figure 7, it can be observed that the time to gelation decreases with increasing gutaraldehyde concentration. For the system of 1% chitosan in 1% (v/v) acetic acid at 40 °C, time to gelation decreases from 35 to 5 min when glutaraldehyde concentration is increased from 1% to 4% by volume.

Table 9. Effect of the amount of glutaraldehyde on gelation time

Sample ID	**Gelation Time (min)**	**Glutaraldehyde %**
GEL 1	31	1
GEL 2	15	2
GEL 3	7	3
GEL 4	5	4

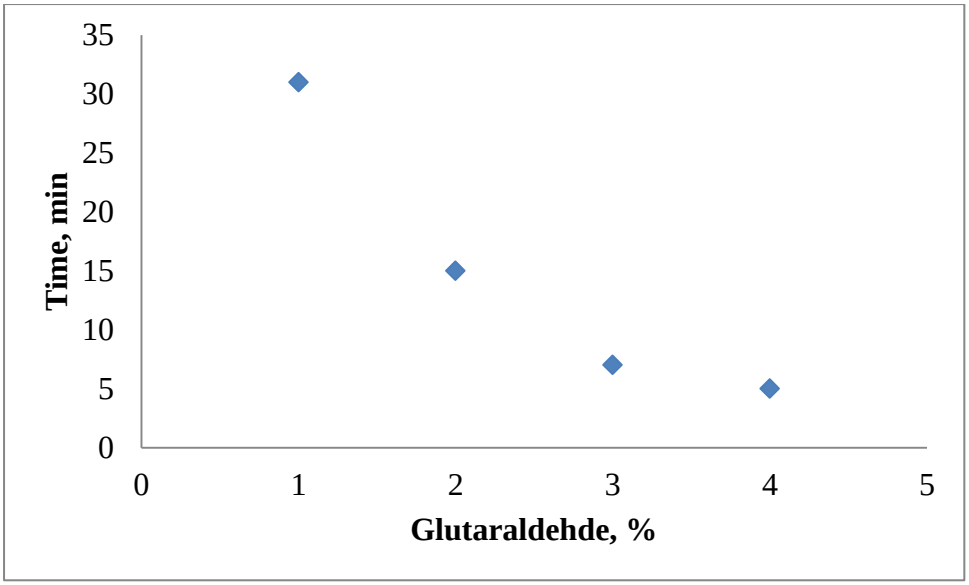


Figure 6. Effect of glutaraldehyde concentration on gelation time of chitosan solution

Figure 8 shows that as the concentration of the crosslinker (glutaraldehyde) is increased, the percentage crosslinking is also increased. G 1 hardly showed crosslinking as compared to G 4. The amount of glutaraldehyde in G 4 is higher thereby increasing the percentage crosslinking.

Table 10. Effect of the concentration of glutaraldehyde on % crosslinking

Sample ID	**Percentage crosslinking**	**Glutaraldehde %**
GEL 1	0.55	1
GEL 2	22	2
GEL 3	25	3
GEL 4	146	4

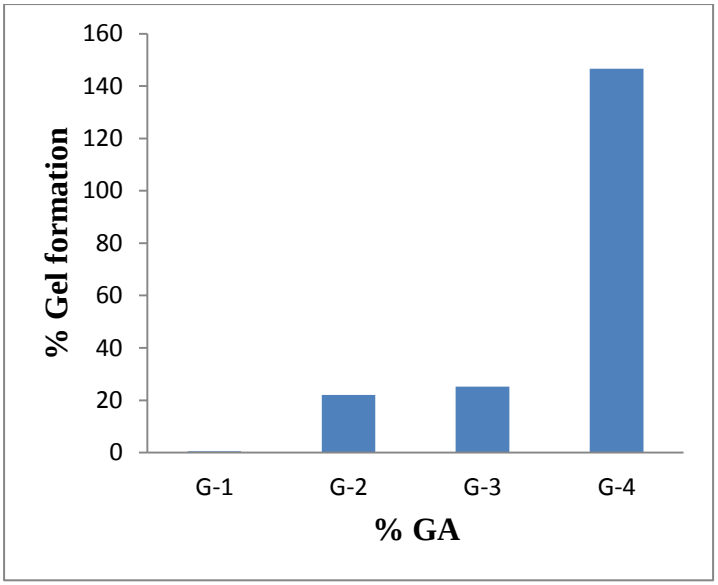


Figure 7. Percent glutaraldehyde crosslinking

3.2 Percentage IPN Yield

Figure 9 shows the effect of GA percentage of the chitosan gel on the IPN yield. As the glutaraldehyde concentration is increased, the degree of crosslinking of the chitosan gel increases as given in Table 10. Hence, the structure becomes more rigid and the amount of the monomer and its crosslinker that is able to penetrate into the polymer network decreases. Therefore, IPN formation decreases as the amount of glutaraldehyde is increased.

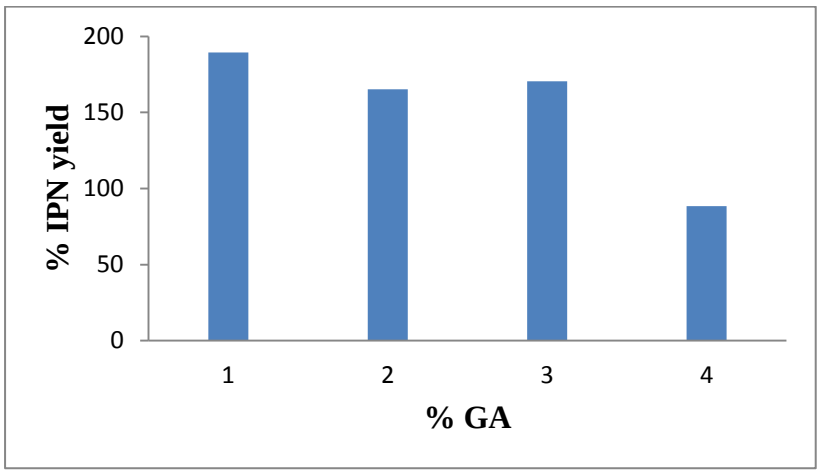


Figure 8. Effects of glutaraldehyde concentration on IPN yield

3.2.1 Percent Semi-IPN and IPN Yield

In Figure 10, % IPNs and SIPN yield values are shown in the presence and in the absence of MBA crosslinker respectively. The percentage interpenetrating network of the IPN is higher than the semi-IPN this is due to the effects of the second crosslinker (MBA) present in the IPN.

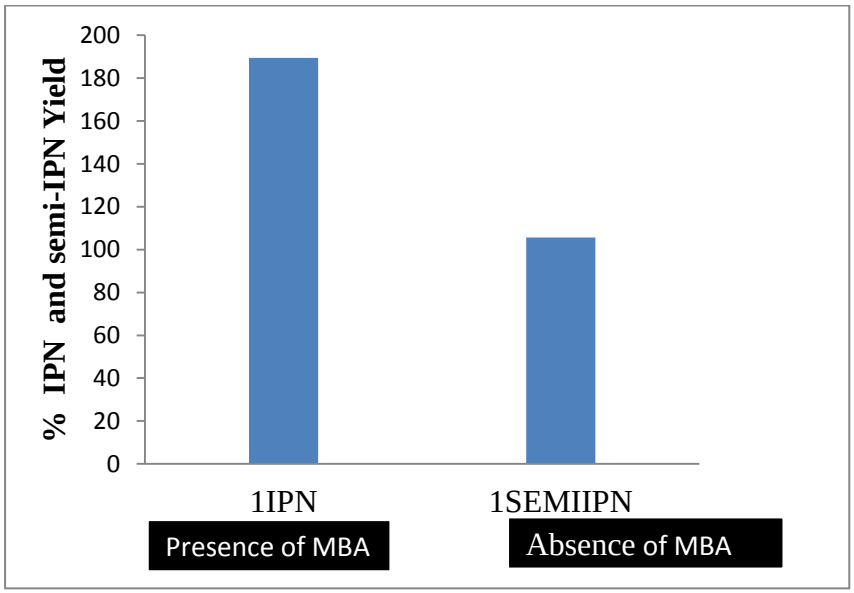


Figure 9. Effects of MBA crosslinker in % IPN formation

3.3 Swelling Kinetics

The time dependent swelling behavior of the hydrogels was followed in pH 1, 7 and 11 at 37 °C. The results are given in Table 11,12 and 13 respectively. The swelling kinetics are illustrated in Figures 11-20 below. When the swelling capacities of the glutaraldehyde crosslinked chitosan gels (GEL 1, GEL 3 and GEL 4) are compared to those of the IPNs (IPN 1, IPN 3, IPN 4) it can be observed that the IPN's exhibit higher swelling capacities than the gels at all pH values under study. Therefore it can be said that the swelling ratio decreases with increasing extent of crosslinking. Figure 11, illustrates the swelling behavior of semi-IPN at 37 ^{o}C and pH 1. The gels

swells up to around 800% within 120 minutes. Then an equilibrium swelling capacity of around 600% is obtained in 420 minutes. The gel is stable with the same swelling capacity after 1500 minutes. The swelling behaviors of IPN 1, IPN 3 and IPN 4 are shown in Figure 12 and those of chitosan gels in Figure 13. A similar trend to that of the semi-IPN is observed for the IPN gels and chitosan gels as well. It is noted with IPN gels or chitosan gels that the swelling capacity decreases with increasing crosslinking density. IPN-1 has an equilibrium swelling capacity of 600%, while IPN-3 and IPN-4 exhibit a value of around 300%. The chitosan gels, on the other hand, exhibit equilibrium swelling capacity values of the order of 50-100%. The fact that a decrease in swelling capacity is observed after an initial higher swelling could be attributed to the swelling followed by dissolution of some uncrosslinked polymer chains or some unreacted monomer, or crosslinker left in the samples. Swelling behaviors and swelling capacities of the semi-IPN, IPNs and gels of chitosan at pH 7 at 37 °C can be followed in Figure 14, 15 and 16 respectively. Similarly, the swelling behavior at pH 11 and at 37 °C is shown in Figures 17, 18 and 19.

All samples have their highest swelling ability in acidic medium (pH 1) as compared to pH 7 and 11, showing that the swelling ratio of all the hydrogels decreased as the pH is increased. At pH 1.2 which is acidic, there is protonation of the amine groups of chitosan or PDEAEM which leads to an electrostatic repulsion of the primary or tertiary amine groups present on chitosan and PDEAEM respectively. Diffusion of water molecules occures into the polymer network and swelling is observed. This protonation also leads to disruption of secondary interactions such as hydrogen bonding and this allows the diffusion of more water into the IPN network. As the pH

of the buffer solution increased, amine groups of chitosan become deprotonated, hydrogen bonding is restored and there is no electrostatic repulsion and hence an observable reduction in the swelling ratio of the hydrogels**.**

Table 11. Swelling behaviors of chitosan hydrogels at 37 °C and pH 1

Time, min	**% Swelling in pH 1**						
	Sample ID						
	Semi-IPN 1	**IPN 1**	**IPN 3**	**IPN 4**	**GEL 1**	**GEL 3**	**GEL 4**
30	683	712	530	598	134	173	77
60	596	357	368	415	104	134	62
120	783	757	308	351	183	134	81
180	623	822	283	146	150	76	14
240	587	215	395	323	111	91	64
300	577	507	188	544	119	73	62
360	550	782	277	340	116	45	47
420	565	518	264	282	109	60	54

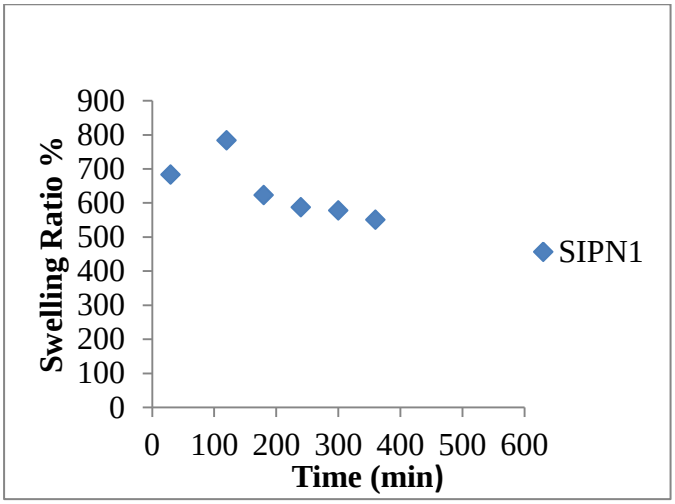


Figure 10. Swelling behavior of semi-IPN at 37 °C and pH 1

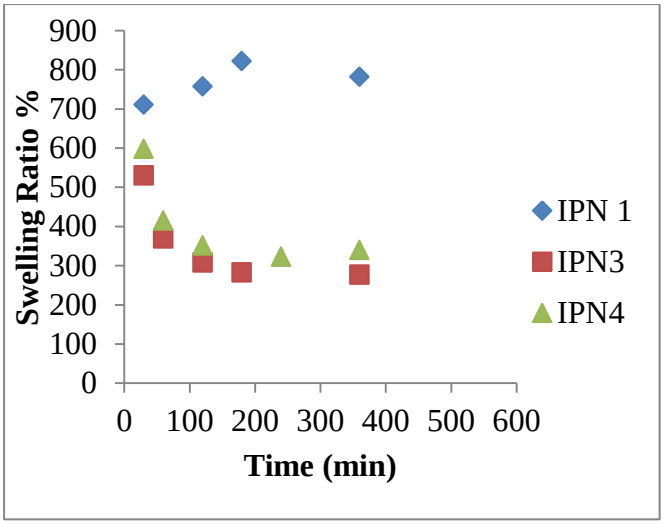


Figure 11. Swelling behaviors of IPNs at 37 °C and pH 1

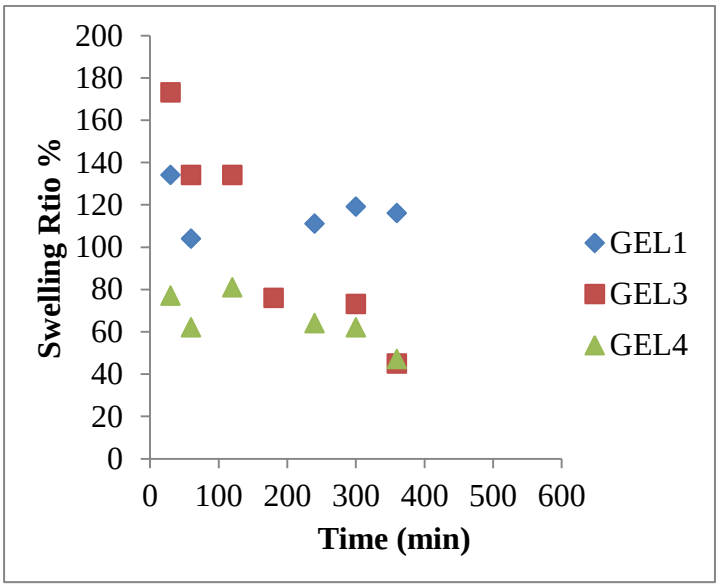


Figure 12. Swelling behaviors of chitosan based hydrogels at 37 °C and pH 1

Table 12. Swelling behaviors of chitosan hydrogels at 37 °C and pH 7

Time, min	% Swelling in pH 7						
	Sample ID						
	Semi-IPN 1	IPN 1	IPN 3	IPN 4	GEL 1	GEL 3	GEL 4
30	158	242	153	299	79	62	29
60	257	340	172	267	119	84	45
120	177	430	123	254	86	78	75
180	169	453	146	288	82	62	62
240	155	377	111	350	96	38	62
300	146	331	152	220	75	59	65
360	162	208	165	213	84	56	84
420	138	296	148	230	85	55	64

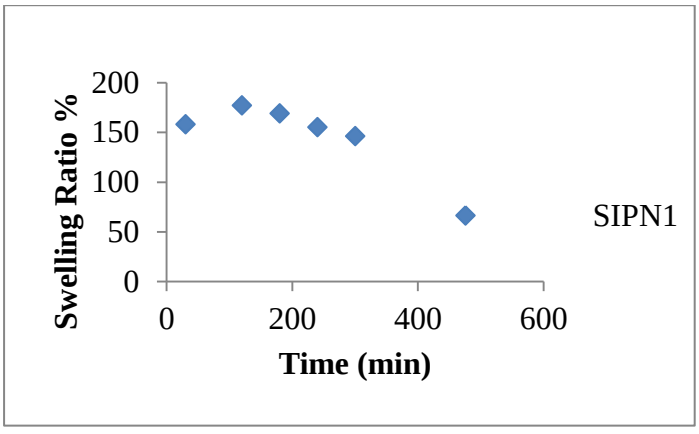


Figure 13. Swelling behavior of semi-IPN at 37 °C and pH 7

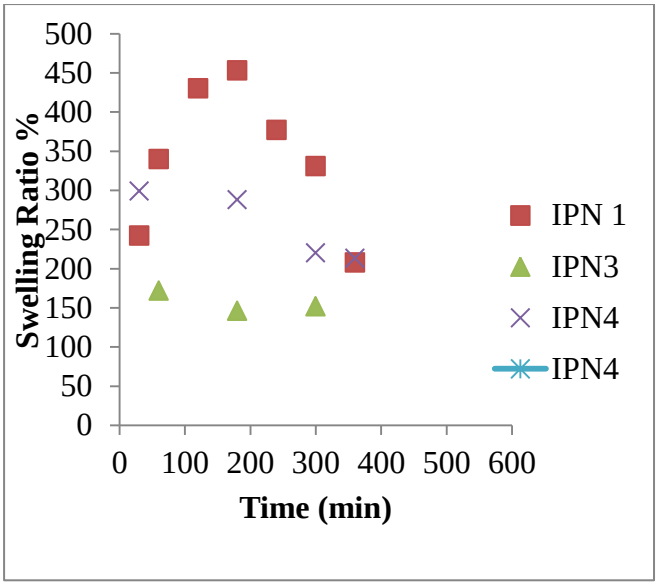


Figure 14. Swelling behaviors of IPNs at 37 °C and pH 7

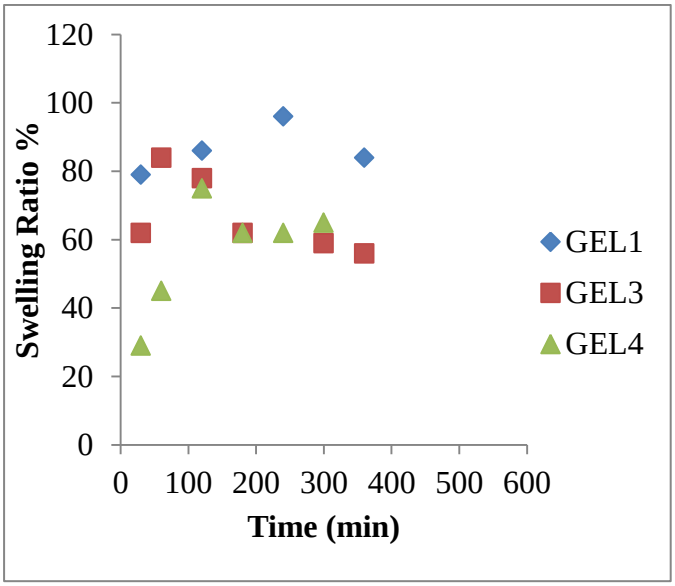


Figure 15. Swelling behaviors of chitosan based hydrogels at 37 °C and pH 7

Table 13. Swelling behaviors of chitosan hydrogels at 37 °C and pH 11

Time, min	% Swelling in pH 11						
	Sample ID						
	Semi-IPN 1	IPN 1	IPN 3	IPN 4	GEL 1	GEL 3	GEL 4
30	147	224	110	344	62	46	54
60	196	275	114	425	70	58	64
120	187	323	142	373	76	52	60
180	203	251	187	336	54	56	62
240	177	240	123	309	52	59	65
300	190	219	148	311	50	51	78
360	174	273	113	292	64	54	46
420	242	242	139	265	60	59	49

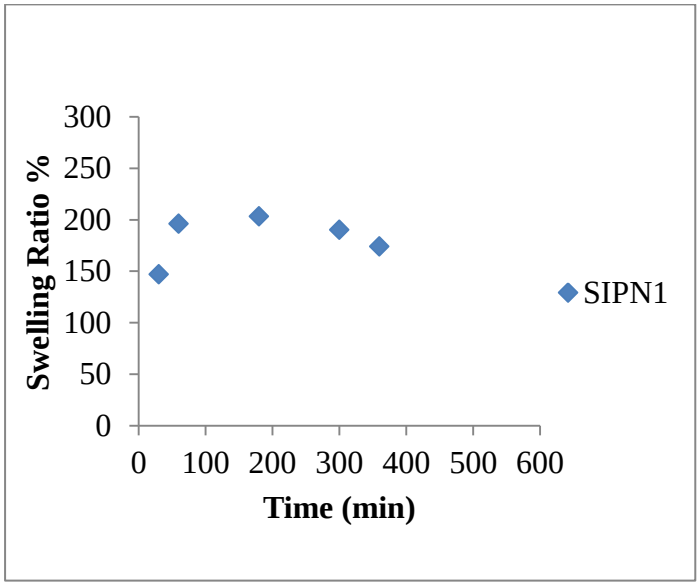


Figure 16. Swelling behaviors of semi-IPN at 37°C and pH 11

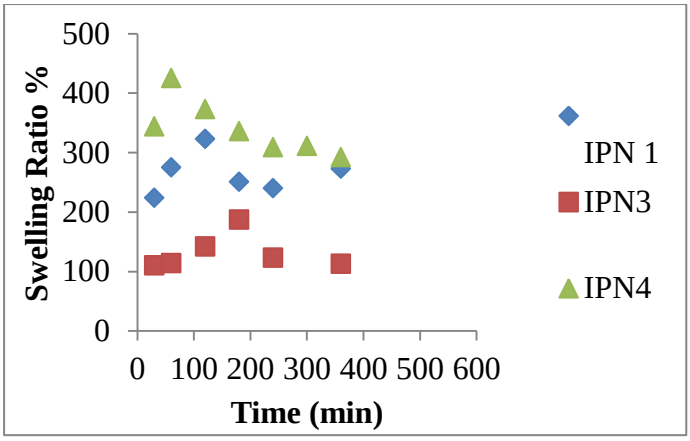


Figure 17. Swelling behaviors of IPNs at 37 °C and pH 11

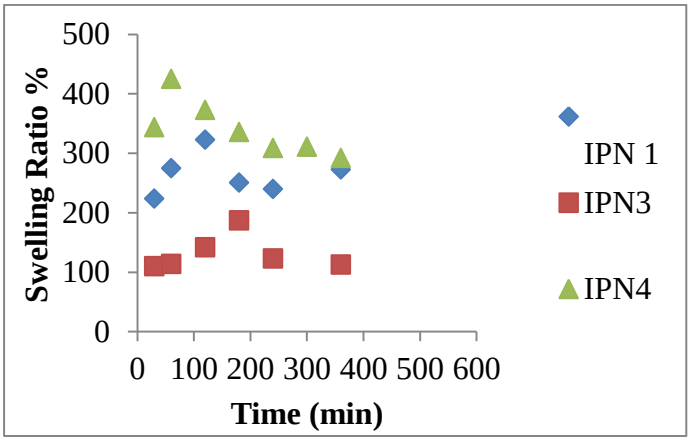


Figure 18. Swelling behaviors of chitosan based hydrogels at 37°C and pH 11

3.4 Ciprofloxacin Loading and Release Study

Drug was loaded on IPNs (1 and 4) through adsorption. The IPNs were immersed in the drug solution and kept for 24 hours. The drug loading efficiency for both IPNs was calculated to be 33%. This shows that the amount of crosslinker had no effect on the drug loading capacity of the hydrogels. Figures (20 - 23) shows the ciprofloxacin release of IPN 1 and IPN 4 in water and pH 1.2. As can be observed from the figures, there was an initial burst release in the first hour. This is due to the drug loaded on the surface of the hydrogel, followed by a controlled release of the drug at the time frame the study was carried out i.e. 6 hours. For the release carried out in water, IPN 1 showed a slightly higher release (61%) as compared to IPN 4 (55%).

This could be due to the effect of higher crosslinking density in IPN 4 glutaraldehyde present in the IPN 4 as compared to IPN 1 which resulted in less swelling of the former and hence lesser drug release. For pH 1.2, it was observed in both cases that both IPNs had similar release trend, with about 30% of the drug loaded drug released in the first 6 hours.

Table 14. Drug release in water for IPN 1

Time (hours)	**Absorbance**	**Cumulative amount of drug (g)**	**Cumulative % release**
1	0.197	0.0036	33
2	0.212	0.0040	38
3	0.257	0.0054	51
4	0.262	0.0056	52
5	0.272	0.0059	55
6	0.291	0.0065	61
24	0.260	0.0055	52
48	0.240	0.0049	46
72	0.225	0.0044	41

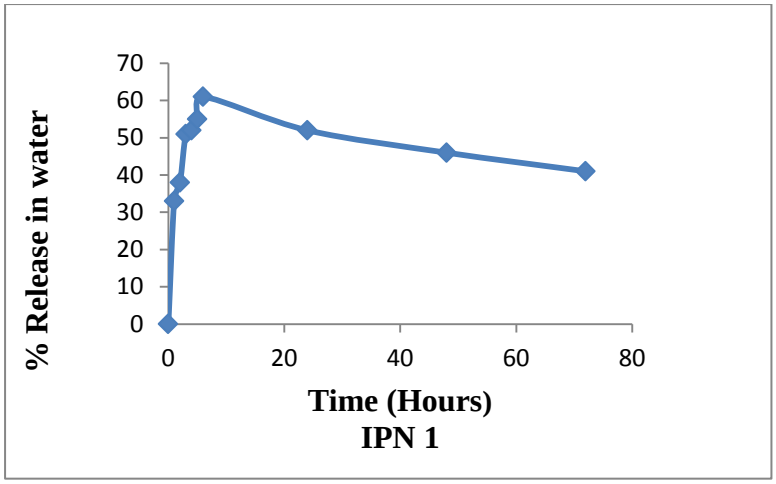


Figure 19. IPN 1 Hydrogel ciprofloxacin release in water

Table 15. Drug release in water for IPN 4

TIME (hours)	**Absorbance**	**Cumulative drug (g)**	**Cumulative % release**
1	0.177	0.0029	28
2	0.190	0.0034	32
3	0.242	0.0049	47
4	0.245	0.0051	48
5	0.265	0.0057	54
6	0.269	0.0058	55
24	0.220	0.0043	40
48	0.214	0.0041	39
72	0.205	0.0038	36

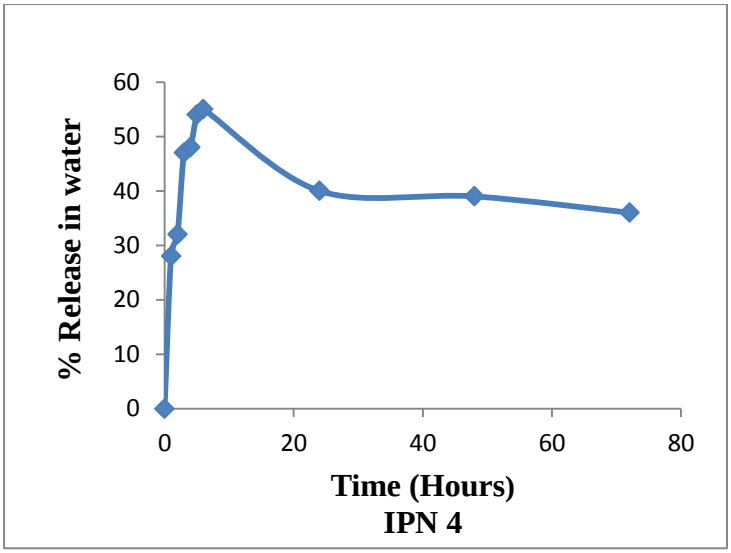


Figure 20. IPN 4 Hydrogel Ciprofloxacin Release in Water

Table 16. Drug release in pH 1.2 for IPN 1

TIME (hours)	Absorbance	Cumulative drug	Cumulative % release
1	0.483	0.0026	23
2	0.522	0.0028	25
3	0.559	0.0031	28
4	0.566	0.0032	28
5	0.621	0.0035	31
6	0.612	0.0035	31

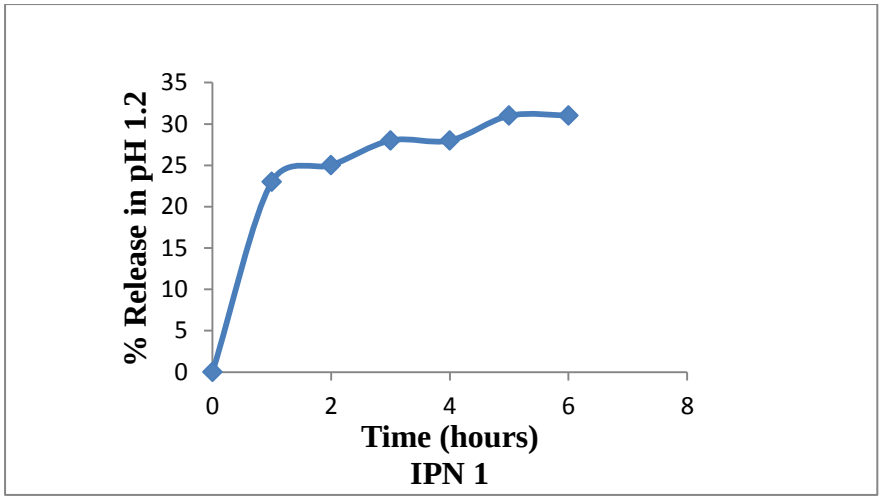


Figure 21. IPN 1 Hydrogel ciprofloxacin release in pH 1.2**Table 17. Drug release in pH 1.2 for IPN 4**

Time (Hours)	Absorbance	Cumulative drug	Cumulative % release
1	0.494	0.0027	26
2	0.523	0.0029	28
3	0.554	0.0031	30
4	0.572	0.0032	31
5	0.599	0.0034	33
6	0.599	0.0034	33
24	0.392	0.0019	19
48	0.428	0.0022	21
72	0.429	0.0022	21

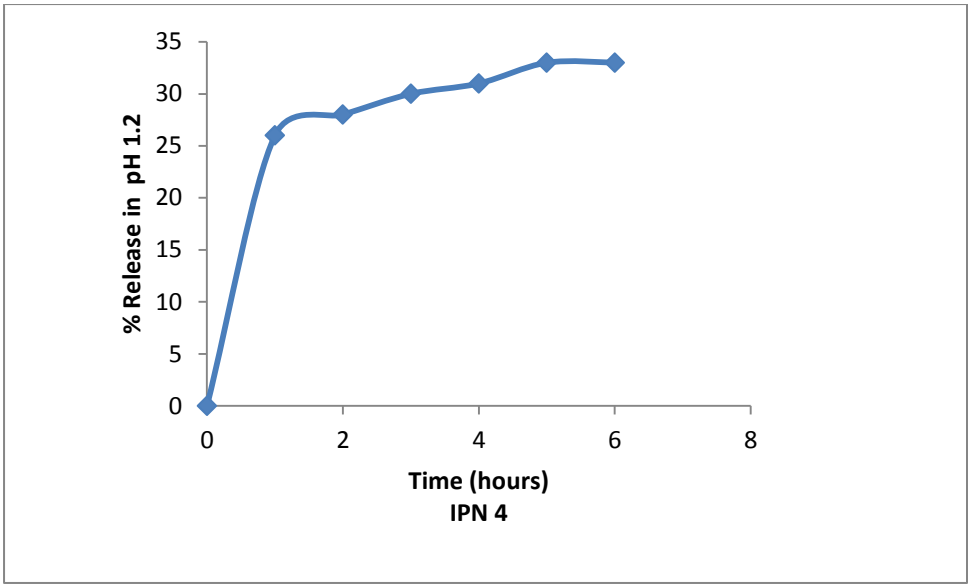


Figure 22. IPN 4 Hydrogel ciprofloxacin release in pH 1.2

3.5 Release Kinetics in Water and pH 1.2

The release kinetics of IPN samples at different concentration in water and in buffer pH 1.2 was plotted as can be seen in the graphs shown in Figure 24. It should be noted that it is not easy to distinguish between different kinetic models as they all produce close linear regression coefficient values R^2. More data and in-depth mathematical analysis are needed to clarify the mechanism.

Table 18. Drug release kinetics in water for IPN 1 and 4

Release order	**IPN 1** **R^2 value**	**IPN 4** **R^2 value**
Zero Order	0.9272	0.9011
First Order	0.9455	0.9114
Higuchi	0.8172	0.972
Korsmeyer-Peppas	0.9486	0.9306

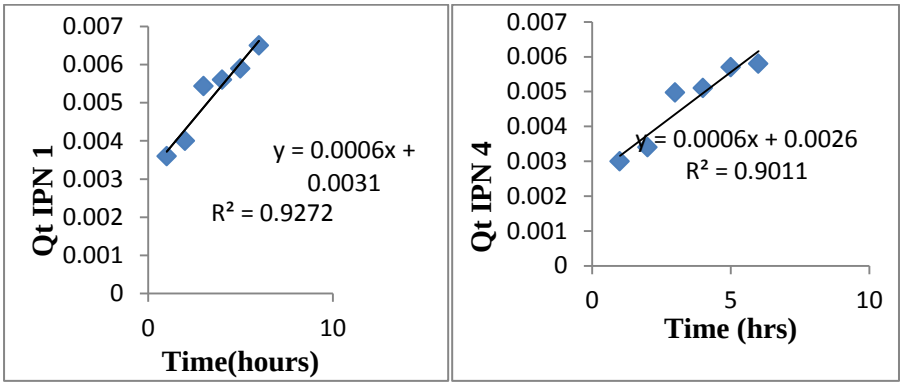


Figure 23. 24 Zero Order Drug release kinetics in water for IPN 1 and 4

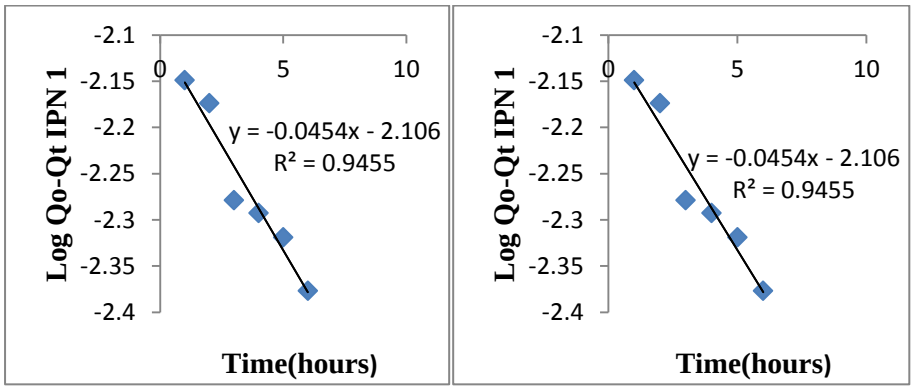


Figure 24. First Order drug release kinetics in water for IPN 1 and 4

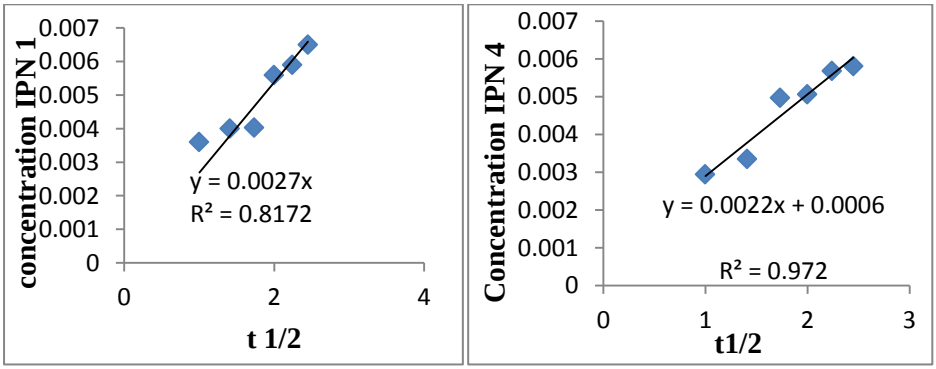


Figure 25. Higuchi Order drug release kinetics in water for IPN 1 and 4

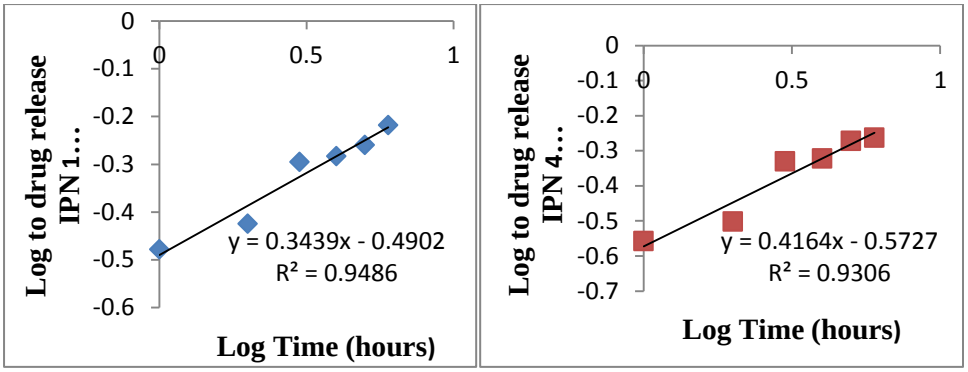


Figure 26. Korsmeyer-Peppas drug release kinetics in water for IPN 1 and 4

Table 19. Drug release kinetics in pH 1.2

Release order	IPN 1 R² value	IPN 4 R² value
Zero Order	0.954	0.9628
First Order	0.9613	0.9588
Higuchi	0.952	0.9829
Korsmeyer-Peppas	0.956	0.9816

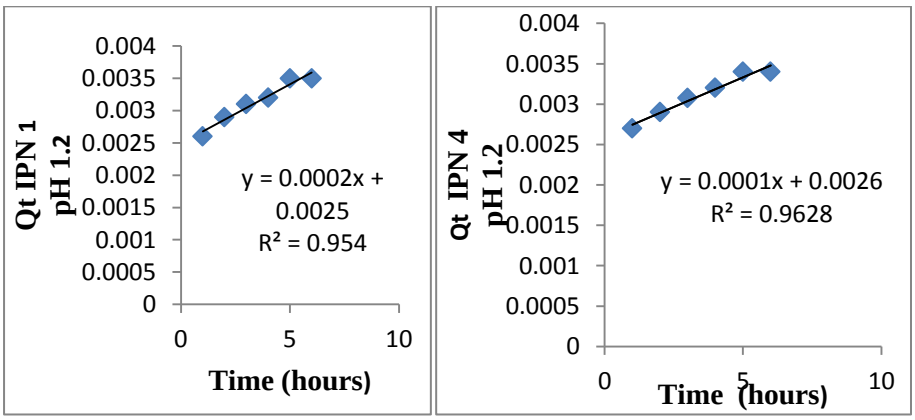


Figure 27. Zero order drug release kinetics in pH 1.2

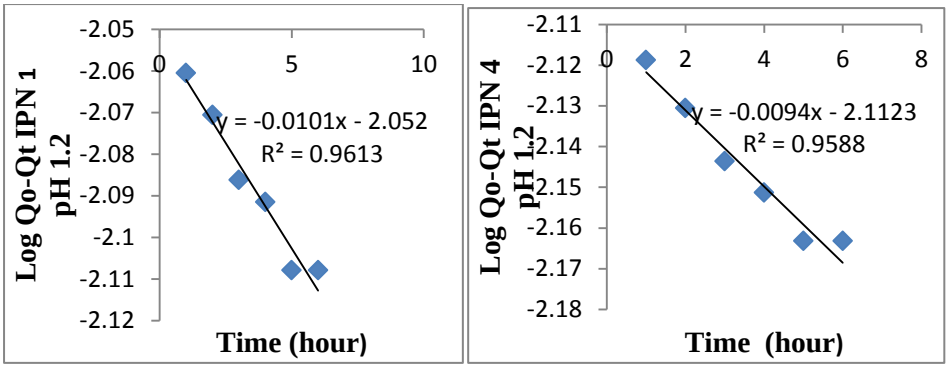


Figure 28. First order drug release kinetics in pH 1.2

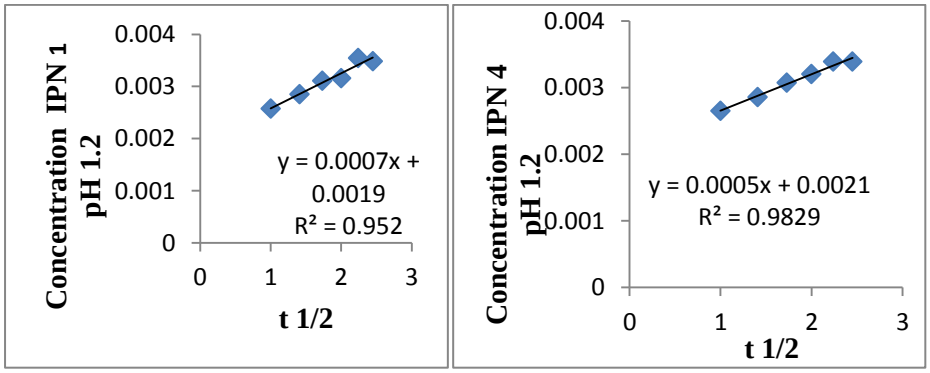


Figure 29. Higucci order drug release kinetics in pH 1.2

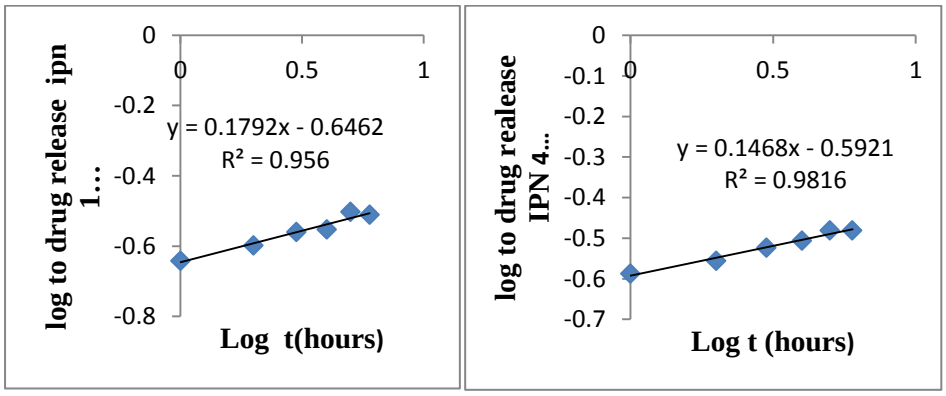
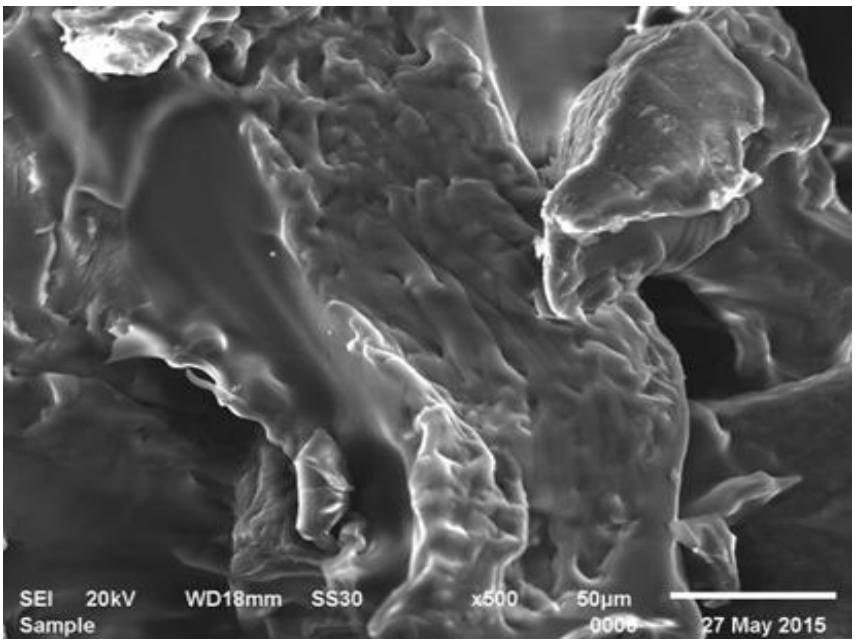


Figure 30. Korsmeyer-Peppas drug release kinetics in pH 1.2 for IPN 1 and 4

3.6 SEM Analysis

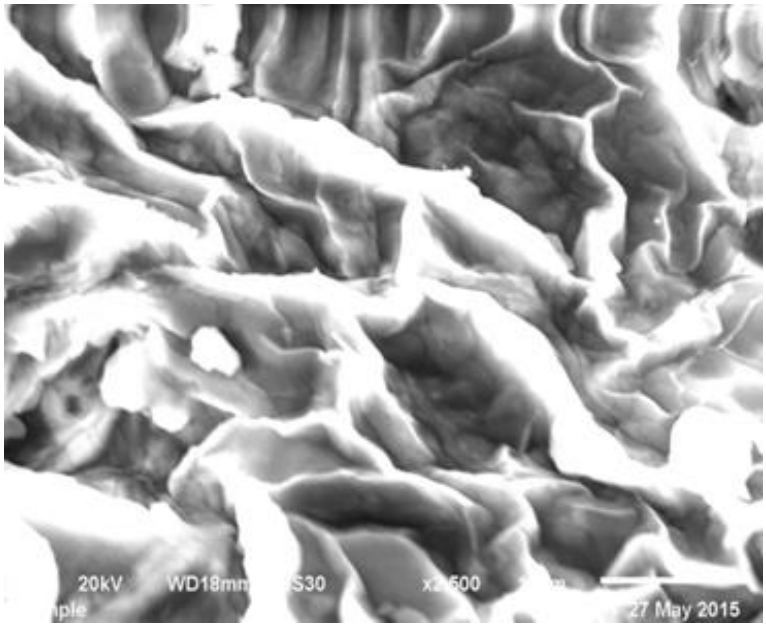
The SEM image of semi-IPN exhibit smoother morphology as compared with the IPN SEM images. Also, interconnected pores are evidently visible within the polymeric network of the semi-IPN. Figures 8 and 10 are the SEM images of IPN 1

and 4. As shown, IPN 4 exhibit a rough-like corrugated surface with smaller pores. The difference in the IPN morphology may be atributed to homogeneous network of chitosan and DEAM in the presence of MBA. It is obvious that the IPN1 and 4 maintain similar morphology, as compared with the semi-IPN, however IPN 4 shows more compact crosslinked network and denser morphology which may be ascribed to the presence of higher concentration of glutaradehyde . The swelling data is also constituent with the morphology.



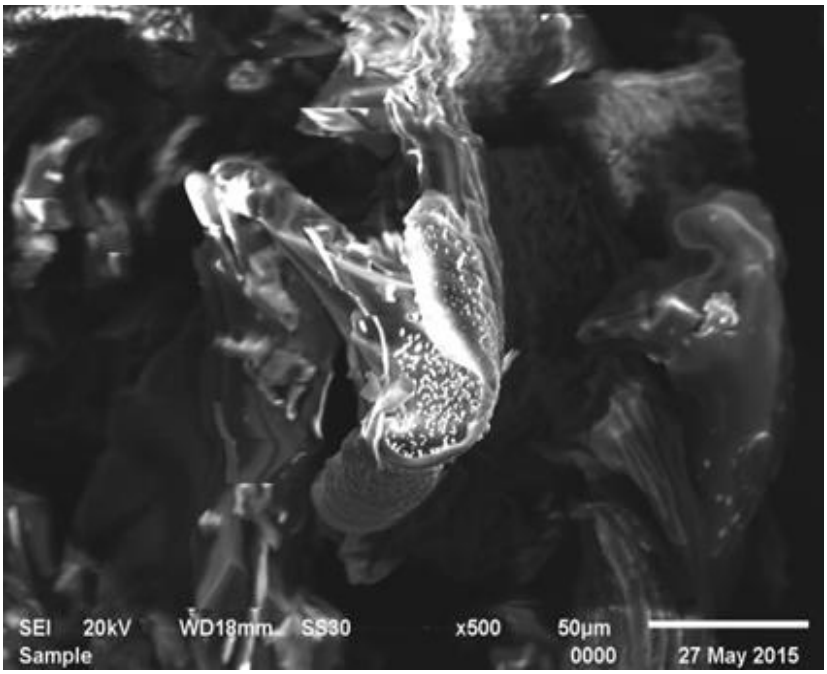
SEMI IPN 1

Figure 31. SEM image of glutaraldehyde crosslinked Chitosan with DEAEM



ES1 x500 IPN 1

Figure 32. SEM image of glutaraldehyde crosslinked chitosan/MBA IPN and glutaraldehyde crosslinked chitosan gel



IPN 4

Figure 33. SEM Image of glutaraldehyde crosslinked chitosan/MBA IPN and glutaraldehyde crosslinked chitosan gel

Chapter 4

CONCLUSION

In this study, glutaraldehyde crosslinked chitosan hydrogels, glutaraldehyde crosslinked chitosan/methylenebisacrylamide crosslinked PDEAEM interpenetrating polymer networks (IPNs) and glutaraldehyde crosslinked chitosan/PDEAEM semi-IPNs hydrogels were successfully synthesized and characterized. The hydrogels were subjected to swelling and drug delivery tests. The hydrogels bear pH sensitive swelling behavior. The highest swelling degree is obtained in acidic medium with the IPNs producing higher swelling capacities than the semi IPN or the chitosan gel alone. The antibacterial drug ciprofloxacin was successfully loaded into the IPNs produced with a drug loading efficiency of 33%. The drug is released from the gels in a controlled manner with 61% of ciprofloxacin released by IPN 1 in water and 55% release from IPN 4.

Further investigations are needed to elaborate drug release kinetics.

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