Synthesis of Alginate-Graft-Poly(benzyl methacrylate) Copolymer by Chemical and UV Initiation

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ABSTRACT

The subject of this thesis is to synthesize alginate-graft-poly(benzyl methacrylate) copolymer by two different approaches including chemical and UV initiation. The pros and cons of the grafting methods were examined by evaluating benzyl methacrylate: alginate ratio, type and amount of initiator, reaction duration and type of solvent on grafting yield. Grafting percentage was calculated gravimetrically. The highest grafting yield was obtained as 32.1% by UV initiation under 0.1g beads, 0.5 mL BMA with 2.5g 2.2-Dimethoxy-2-phenylacetophenone (DMPA) and 2.5mL hexane. The dissolution properties tested in buffer media of pH 1.2, 7 and 11. Ciprofloxacin loading in water and release studies were performed in water and acidic media (pH 1.2). The ciprofloxacin release kinetics was investigated with four different models. The release kinetics in water is dependent on the diffusion rate as such fits into the Higuchi's Model while that for the acidic media fitted best for the Korsmeryer-Peppas Model. In-vitro antibacterial activity of the products was examined. Grafted beads exhibited antibacterial activity however non-grafted beads did not show any inhibition.

Keywords: Alginate beads, Benzylmethacrylate, UV initiation, Antibacterial activity.

ÖZ

Bu çalışmanın konusu aljinate-aşı-poli(benzil metakrilat) kopolimer sentezini kimyasal ve UV polimerizasyon yöntemleri ile sentezini gerçekleştirmekti. Seçilen aşılama yöntemlerinin artılarını ve eksilerini değerlendirmek için benzilmetakrilat: aljinat oranı, başlatıcı türü ve miktarı reaksiyon süresi ve çözücü türünün aşılama verimine etkisi incelendi. Yüzde aşılama verimi gravimetrik olarak hesaplandı. En yüksek aşılama verimi UV polimerizasyon yöntemi ile 0.1 g aljinat boncuklarının 0.5 mL BMA in 2.5 mL heksanda çözülmesi ile 2.5 g 2,2- Dimetoksi- 2 - fenilasetofenon (DMPA) varlığında 32.1 % verim elde edildi. Ürünlerin pH 1.2 , 7 ve 11 tampon çözeltilerdeki şişme özellikleri tespit edildi. İlaç yükleme ve salımı için siprofloksasin seçilmiş ve ilaç yükleme suda, salımı için su ve asidik ortam (pH 1.2) 'de gerçekleştirildi. Siprofloksasin salım kinetikleri dört farklı modellerle araştırılmıştır. In vitro olarak LB besiyer üzerinde E.coli bakterilerine karşın ürünlerin antibakteriyel aktiviteleri inhibisyon çapı ölçümleri ile incelendi . BMA aşılı boncuklar inhibisyon oluştururken aşılama olmayan aljinat boncukların antibakteriyal aktivite göstermediği tayin edildi

Anahtar Kelimeler: Aljinat boncuklar, benzilmetakrilat, UV başlatma , Antibakteriyel aktivite .

I dedicate this thesis work to the ever faithful, ever loving and ever righteous God.

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INTRODUCTION

Its expedient to alter the characteristics of a polymer to perfectly fit into requirements outlined for purposed applications in this polymeric age. Various methods known to alter the characteristics of polymers include; blending, grafting and curing. The blending entails the physical incorporation of two or more polymers to obtain the needed characteristics whereas grafting is the covalent bonding of monomers to the chain of the polymer, then in curing, a covering (produced by the polymerization of an oligomer combination) clings to the substrate and gives it an unwrinkled completion by stuffing into the gorges on the surface (Bhattacharya & Misra, 2004). Currently, advancement in natural and synthetic polymer composite materials has earned great scrutiny. Of all the qualifications of polymers, the age-old and effective grafting method stands out as one of the hopeful methods. Fundamentally, copolymerization by grafting is an appealing means of conveying various functional groups to a polymer. Products of graft copolymerization have been used as, thermoplastic elastomers, impact resistant materials, compatibilizers or emulsifiers for the development of stable blends or alloys and a number of expanded potential applications.

Distinct copolymers of grafting are commonly manufactured either by, grafting-through, grafting-from or grafting-to methods. Grafting-through method is a method which is an easy means to incorporate copolymers of grafting with suitable side chains on polymer or oligomer. This allows for the inclusion of polymer or oligomers

(possessing a functional group which enables for additional polymerization) manufactured by other controlled processes of polymerization inside backbones like polystyrene or poly(methyl acrylate) also prepared by a controlled or living radical polymerization. This connection of regulated processes of polymerization grants for mastery of polydipersity, functionality, composition of copolymer, length of backbone, length and spacing of branch by considering the mole-ratio and reactivity ratio of both the monomer and polymer.

The basic necessity for a favorable grafting-from response is an intended polymer with assigned radically interchangeable atoms alongside the polymer backbone. The grafting-to approach has proven to be more productive in the production of graft copolymers. In this way, useful monomers react with the backbone of the polymer to produce the copolymer grafted with structures which can be loose or dense grafted copolymers or well defined star-like molecules.

1.1 Techniques of Grafting

The techniques of graft copolymerization include mainly chemical and radiation techniques, others are photochemical, plasma-induced and enzymatic grafting techniques; (Bhattacharya & Misra, 2004)

1.1.1 Chemical Grafting

In grafting introduced by chemical routes, the importance of initiators is not neglected as it decides the course of the whole grafting process.

1.1.1.1 Redox Initiation

This is a common indirect chemical route where redox reagents are used to bring about the free radicals that are transmitted to the polymer in order to enable grafting. Redox reactions are easily carried out and there are no restrictions at points of

reaction. They are feasible in aqueous media and also at room temperature. The extent of the grafting copolymerization can be regulated by adjusting the reaction variables like temperature, reaction time and composition of mixture. Redox reactions occur by persulfates, Mn^{2+}/H_2O_2, reducing agents, direct oxidation and metal chelates.

Fenton's Reagent (Fe^{2+}/ H_2O_2) produces a hydroxyl radical ($HO^{\cdot}$) which has the potential to take away a hydrogen atom from the polymer molecule thereby introducing a free radical on the polymer for further reaction with neighboring monomers.

$Fe^{2+} + H_2O_2$ -----> $Fe^{3+} + OH^- + OH^{\bullet}$

Fe^{2+}/ persulfate serves as a source of $SO_4^{-\bullet}$ which either reacts with water to produce a hydroxyl radical that afterwards introduces a free radical on the polymer or directly reacts with polymer molecule to bring about the needed radicals (Bhattacarya, Rawlins, & Ray, 2009).

$S_2O_8^{2-} + Fe^{2+}$ -----> $SO_4^{-\bullet} + Fe^{3+} + SO_4^{2-}$

$SO_4^{-\bullet} + H_2O$ -----> $HSO_4^- + OH^{\bullet}$

$SO_4^{-\bullet} + R_{polymer}$-OH ------> $HSO_4^- + R_{polymer}$ –$O^{\bullet}$

Reducing agents such as Ag^+, sodium bisulphate or thiosulphate combine with persulphates to also give SO_4^- which further reacts to create a free radical on the polymer

Persulfate/Reducing agent: $S_2O_8^{2+} + Ag^+$ -----> $SO_4^{-\bullet} + Ag^{2+} + SO_4^{2-}$

Persulfate/bisulphate: $S_2O_8^{2-} + HSO_3^-$ -----> $SO_4^{-\bullet} + HSO_3^{\bullet} + SO_4^{2-}$

Persulfate/thiosulphate: $S_2O_8^{2-} + S_2O_3^{2-}$ -----> $SO_4^{-\bullet} + HSO_3^{\bullet} + SO_4^{2-}$

Hydroperoxides react with Fe^{2+} by thermal decomposition to produce the free radicals required for further reaction. The free radicals can also result on the polymer

substrate by direct oxidation reactions by the aid of some transition metal ions. Low oxidation potential is better chosen for greater efficiency hence, the potentials of the metal ion is a major determining parameter using this pathway.

$Ce^{4+} + R_{polymer}\text{-}OH$ -----> Complex -----> $R_{polymer}\text{-}O^{\bullet} + Ce^{3+} + H^{+}$

Reactions by metal chelates are not commonly used although it has some advantages. In order to avoid undesired reactions like increased occurrence of homopolymerization, metal chelates are used.

Pretreatment of polymeric backbone by ozonation, diazotization or xanthation may also result in free-radical sites for grafting although, secondary free radical sites may inappropriately occur for grafting to take place (Bhattacarya, et al., 2009).

1.1.1.2 Living Radical Polymerization

In the view of Szwarc, polymers that maintain their capability to propagate and increase their size while the degree of termination as well as the chain transfer remain insignificant, are known as living polymers (Szwarc, 1998).

The polymerization continues as long as the monomers are present. Additional monomers will lead to extended polymerization. The rate of initiation is far lesser than the rate of propagation and an active equilibrium occurs between a dormant species and a propagating radical. The living polymers possess controlled molecular weights and low polydipersities. Living polymers can be obtained from atom transfer, nitroxide transfer, and degenerative transfer reactions.

The basic molecule for atom transfer radical polymerization (ATRP) is a molecule that contains a halogen at the alpha position of a cyano, carbonyl, phenyl, and alkoxy-carbonly, groups. The ATPR process involves the activation of dormant

species by transition metal complex to produce radicals by one electron transfer process and the transition metal itself oxidized to higher oxidation state. This changeable process immediately sets up an equilibrium that is mainly shifted to the low radical concentration area. The number of initiators determines the number of polymer chain. The ATRP process can take place both in solution and suspensions.

Reactions are satisfactory with styrene, methyl methacrylate, aqueous styrene sulfonate, and butadiene. It is practicable with initiators produced commercially and in situ. Production of dendrimers, telechelics and functionalized polymers are possible and no gel effect is encountered.

Grafting reaction takes place by chain transfer agents like alkyl iodides, thiol compounds and unsaturated polymethacrylates in reversible addition-fragmentation chain transfer also known as degenerative transfer. The polymer forms active and dormant species by the invasion of the propagating radical. Chemical grafting can also proceed through ionic modes by cationic and anionic mechanisms.

1.1.2 Radiation Grafting

This is a simple, precise and easy to control process with low energy consumption. Catalysts or initiators are not required for initiation of the process. It involves the absorption of energy by a polymer from a radiation source like high-energy gamma radiation and ion beam.

1.1.2.1 Free-radical Initiation

Free radicals are introduced on the macromolecules by exposure to homolytic fission. The space of time that the free radical can stay before it is grafted is determined by the type of polymer backbone. The free radical initiation progresses in three mechanisms;

- pre-irradiation,
- peroxidation, and
- simultaneous irradiation.

The polymer is first radiated in vacuum in the pre-irradiation mechanism, to form comparatively stable free radicals that react with monomers. Homopolymerization can be avoided since the monomers do not undergo the irradiation process although instead of the resulting grafted product, a block copolymer may emerge possibly due to the inability of polymer substrate to retain the radicals for a long duration of time.

The core of the polymer is irradiated with high energy with the existence of oxygen in the peroxidation method to bring about diperoxides or hydroperoxides depending on the type of backbone of polymer and state of exposure to radiation. The substantial peroxy products react with monomer at very high temperatures, whereas peroxides are decomposed to radicals for further grafting. This mechanism enables the substrates have the ability to stay long enough before grafting takes place.

As the name implies, simultaneous irradiation mechanism involves the simultaneous exposure of the polymer and the monomer to radiation to produce free radicals. Depending on the radiation yield value on either the monomer or polymer, homopolymerization or grafting may commonly occur respectively.

Just like in chemical grafting, radiation grafting also originates through ionic modes via cationic or anionic. The irradiated polymer produces the polymer ion that reacts with the monomer to give the grafted copolymer. Ionic grafting through radiation has a high rate of reaction.

1.1.3 Photochemical Grafting

This is accomplished via two methods. It could be either directly (without sensitizers) i.e. polymer goes to an excited state by absorption of light to its chromophore and dissociates into free radicals for further grafting or indirectly (with sensitizers) i.e. by the addition of photosensitizers, e.g. metal ions, dyes, benzoinethylether and aromatic ketones, that diffuse into the polymer backbone, abstract hydrogen atoms and produce radical sites which are essential for grafting. In the direct method, homopolymerization could occur if the generated radical is also reactive toward the monomer. The magnitude of chain termination through disproportionation or through combination determines the chemical attribute of the resulting product. Chain combination actually leads to crosslinks.

Lower activation energy is needed for photochemical grafting than for chemical reaction. It has rapid rates of reaction. It proceeds at low temperatures and results in high monomer conversion as such the monomer residue will be low. Grafting is mainly on the surface of the polymer due to poor penetration of light as such the bulk properties are not affected. Obtaining an appropriate optimum condition as well as the best sensitizer is cumbersome. There also exists the possibility of scission of polymer backbone via excessive dose application (Bhattacarya, et al., 2009).

1.1.4 Plasma Radiation Induced Grafting

Plasmas are charged ion beams or neutral gas-like clouds. Radicals (generated from the energy provided from the gas discharge) on the polymer chains undergo grafting similar to that of free radical radiation grafting. Plasma induced grafting has lower energy than the common high energy sources thus has milder effects because of the low level of high activated species, electrons, and ions. The radicals only form on the surface of the polymer as such the bulk properties of the polymer are not greatly

affected. Plasmas mostly used for grafting are oxygen and argon plasma. This method is limited by its expensive nature.

1.1.5 Enzymatic Grafting

In this technique, grafting is initiated by enzymes like horseradish peroxidase, Tyrosinase, etc. which produce free radical by either providing molecular oxygen to the molecules or by taking away electrons from the molecules. These oxidative enzymes are mostly used for natural polymers than for synthetic polymers and can be coupled with other techniques. Apart from being expensive, they are useful in narrow temperature ranges.

1.2 Alginates

Alginates are hydrocolloids and water soluble biopolymers obtained from brown seaweed (algae). They naturally occur in seaweed usually in the form of sodium, calcium and magnesium salts. Alginates are commercially manufactured from algal sources by three stages namely; pre-extraction, neutralization and precipitation (Siddhesh & Kevin, 2012). Alginates are carbohydrate polymers i.e. polysaccharides with building blocks made up of two uronate sugars, the salts of guluronic and mannuronic acid. The uronic acids are transformed into their salt forms, guluronate and mannuronate by neutralization. The length, relative amount and dispersion of the blocks of alginates dictate their physical and chemical features.

Generally alginates are known to be a class of polyanionic linear copolymers of α-l-guluronic acid (G) and β-d-mannuronic acid (M) linked by the 1- and 4- positions respectively by the ether-oxygen bridges (Dettmar, Strugala, & Richardson, 2011). They are reputed to be a family because the portion and order of the two monomers differs over a broad range. Both monomers are stereoisomers differing in their

configuration at the carbon-5 hence are C5 epimers and can give rise to a conversion of the monomer chair shape, which gives rise to the four likely glycoside linkages at the molecular level (Draget, Skják-braek, & Smidsrod, 1997). The M and G blocks differ in their shapes where M block is nearly a straight polymer while G block is like a fastened (buckled) chain as a result of equatorial and axial groups at the 1 and 4 positions respectively.

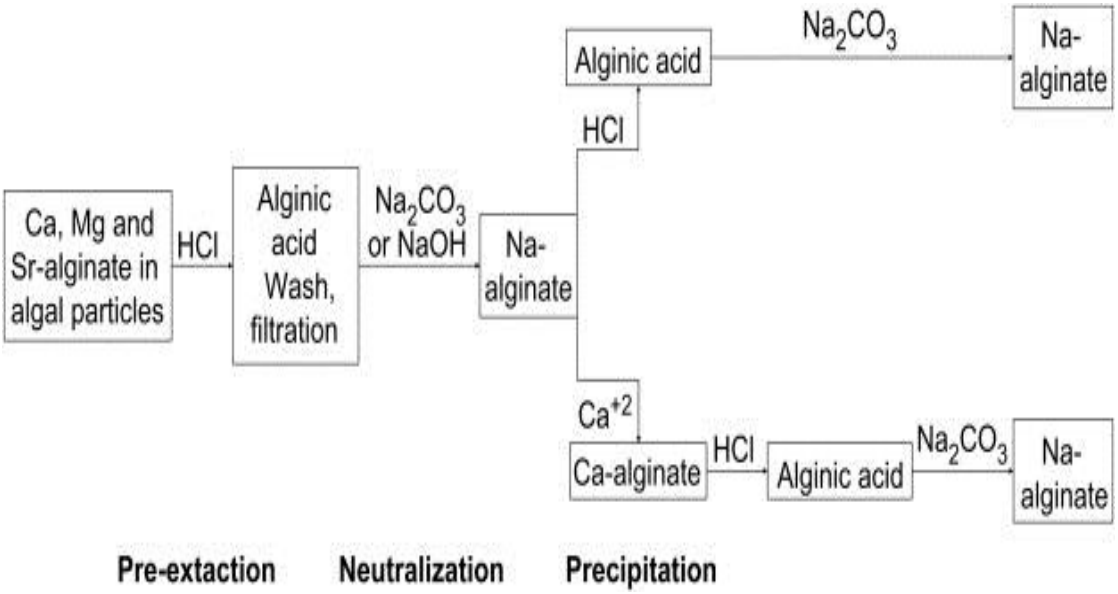

Figure 1. Diagrammatic representation of the abstraction process of alginates from algae (Siddhesh & Kevin, 2012)

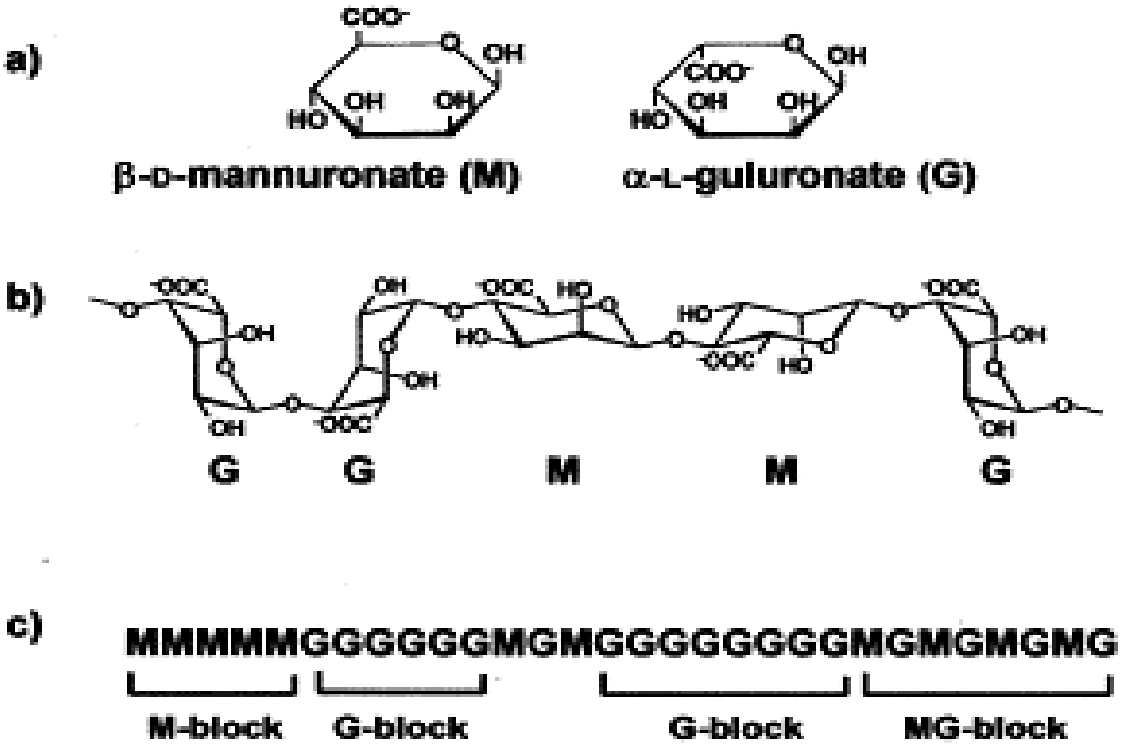

Figure 2. Fundamental features of alginates: (a) monomers of alginates (b) chain shape and (c) dispersion of blocks

1.2.1 Properties of Alginates

1.2.1.1 Solubility

Alginate solubility is actively determined by the condition of the backbone of carboxylic groups. Generally there are three guidelines for alginates solubility in water. Firstly, it is the mediums pH which affects the solubility of the alginates as a result of the electrostatic charges found on the uronic acid substrate. Another is the ionic strength of the environment from salting out effects of non-gelling cations (Alistair, Glyn, & Peter, 2006). A change in the ionic strength of alginates affects the behavior of the aliginates like its viscosity, conformation of polymer, extension of chain and consequently the solubility. Lastly is the existence of gelling ions in the medium which restricts the solubility of alginates and instead forms gels. The alginic acid itself does not dissolve in any solvent but sodium alginates are hydrocolloids i.e.

form a sticky solution or gel in the presence of water. The sodium alginates are not completely soluble in organic medium.

1.2.1.2 Stability

The pure, dried and powdered form of sodium alginate can have a shelf life that stretches to several months as long as it remains in a cool, dark and dry place. Under these conditions, the sodium alginate could even be preserved for several years and there will still be no significant molecular weight reduction. Alginic acid is very different from sodium alginate as it is barely stable even at regular temperatures as a result of catalyzed intermolecular degradation of acid (Alistair, et al., 2006). When degradation is favoured viscosity of the solution reduces in a short while. The glycosidic linkages are liable to oxidation due to degradation by acids and alkali. Other techniques used in sterilization that can cause degradation of alginates are gamma irradiation, treatment with heat and ethylene oxide and also autoclaving (Siddhesh & Kevin, 2012).

1.2.2 Capabilities of Alginates

Alginates have found applications in food and beverage industries, pharmaceutical industries, textile industries and other industries, due to its properties.

1.2.2.1 Viscosity

The molecules of alginates get hydrated when dissolved in water and the solution becomes viscose. The dissolved molecules are not completely flexible due to the hindered rotation around the glycosidic linkages of the G-block regions which gives rise to a stiffening of the chain that is highly viscous. The viscosity of alginates in a solution is determined by the length of alginate molecules or average molecular weight and concentration of alginate. Thus at same concentration and increased chain length, viscosity increases. Also as the shear rate or speed of stir increases the

viscosity increases. This phenomenon is known as psuedoplasticity or non-Newtonian flow. Temperature decreases viscosity so that it can be mixed at moderate temperatures.

1.2.2.2 Formation of Gel

Alginates possess the ability to form gels when there are adequate amount of guluronate monomers in the block to enable it to react with divalent cations. Thus the reaction with calcium and the resultant formation of gel is determined by the average level of GG portions. The divalent cation appropriately enters the G block feature just like the way an egg suitably fits into an egg box. This gives rise to the gelation by forming convergence areas where there is a binding. The alginate gel may be seen as partly solid and partly solution. Water molecules and other molecules are captured inside the matrix of the alginate by capillary action although they are still free to migrate by means of diffusion. This characteristic feature of alginate gels have been utilized in various ways like cell immobilization and encapsulation, wound treatment, treatment of anti-reflux diseases etc.

1.2.2.3 Film-Forming Capability

Alginates have the ability to form films like other biopolymers. In combination with plasticizers, the films produced are flexible, strong, have an outstanding transparency and impermeable to oxygen. The films could be either soluble or insoluble. The sodium alginate films which are soluble are produced by casting and drying. On the other hand, the gelled or insoluble films are made by using a layer of alginate solution and thereafter cross-linked with calcium salts and further dried. This property provides a terrace for drug delivery systems (Biolpolymers, 2003).

1.2.3 Application of Alginates

Alginates have various applications in many industries as a result of their versatile properties.

Table 1**.** Applications of alginates

Applications	**Uses**
Food and beverage industry	
Drinks, Ice-cream and Jelly	As stabilizers and thickeners
Production of Ethanol	For Encapsulation material of yeast cells
Pharmaceutical industry	
Transplantation and cell culture	A material for encapsulation
Material for dental impression	Used as a mould
Drugs	An adhesive agent and sustained-release
Dressing of wounds	As a haemostatic and an absorbent
Other industries	
Textiles	Thickeners
Paper	As an adhesive agent and a filler
Paint	As a stabilizer and suspending agent
Toothpaste	As a stabilizers and thickeners

1.2.4 Chemical Modification of Alginates

The solubility, reactivity and characterization of alginates are guidelines for the modification of alginates. Solvent chosen for the modification reaction depicts the type of reagents that could be used and affect the pattern of substitution. Dispersed on the backbone of alginates are free carboxyl and hydroxyl groups which make them perfect for chemical modification. Selective modification can be done where two functional groups possess different reactivity. Modification of the hydroxyl groups include oxidation, reductive-amination of oxidized alginate, sulfation, copolymerization and covalent linking with α-cyclodextrin while that for carboxyl

acid groups include, esterification, ugi reaction (the use of combinational chemistry to form a compound with two amide groups from a ketone or aldehyde, an amine, an isocyanide and carboxylic acid) and amidatiom (Ji-Sheng, Ying-Jian, & He, 2011). Alginates reactivity towards acids, bases and reducing agents are also taking into consideration. Most importantly is the comprehensive knowledge of patterns of substitution because of the complicated nature of the backbone of alginates.

1.2.4.1 Modification by Acetylation

Calcium alginates put in an aqueous media formed calcium alginate beads after which the acetylation process was done by solvent exchange using pyridine. The acetylation reaction took place as the beads were suspended at 38^{o}C in a mixture of pyridine-acetic anhydride. This gave rise to the selective acetylation of only M residues. Calcium ions were exchanged with sodium ions after its being carefully washed. The resulting product is dialyzed and freeze dried. Water is of paramount importance in the acetylation process as it determines the degree of substitution. This method of acetylation contributed significantly in describing the polymer structure. Another method of acetylation of alginates is by dissolution of TBA-Alg in polar aprotic solvents like DMSO, DMF, DMAc and DM, containing tetrabutylammonium floride (TBAF). Under homogenous condition a partial dissolution in DMSO and acetylation of both M and G residues resulted while under heterogeneous conditions a complete dissolution in DMSO/TBAF and a selective acetylation of M residues resulted.

1.2.4.2 Modification by Sulfation

Sulfation of alginates can be done either enzymatically or chemically. Sulfated alginates of both methods improve blood compatibility and anticoagulant activity. The sulfation can be done by reacting sodium alginates with chlorosulfonic acid in

formamide. Another method uses carbodiimide coupling chemistry such that the hydroxyl groups are the only ones sulfated in the reagent system. An uncommon reagent used also in sulfating alginates is obtained from the aqueous solution mixture okof sodium nitrate and sodium bisulfite. Other sulfating reagents like sulfuryl chloride sulfamic acid, sulfuric acid, sulfur trioxide and chlorosufonic acid are rarely used since they cause alginates to degrade hydrolytically. Sulfated alginates possess better binding abilities than unmodified alginates thus they bind to heparin and boost its blood clotting inhibitory properties. However excess of the sulfation has adverse effects.

1.2.4.3 Modification by Phosphorylation

Phosphorylation can be done by using a phosphoric/urea acid reagent. The resultant alginates upon series of analysis showed regioselectivity (i.e. substitution of phosphate on M residues with a greater magnitude of substitution on the hydroxyl group at position 3 than on the hydroxyl group on position 2) due to greater reactivity and higher accessibility on the equatorial sites than on the axial sites. The phosphorylated alginates were unable to form gels due to the conformational changes and molecular weight degradation since phosphoric acid is a strong acid. However a mixture of the phosphorylated alginate and the unmodified alginates gave stable calcium-cross linked gels

1.2.4.4 Modification by Attaching Cell Signaling Molecules

Alginates are biocompatible, non-immunogenous, hydrophilic and their gels are able to encapsulate most biological entities but natural cells do not adhere to alginates and thus, the need for alginates to be modified with extracellular signaling molecules.

Covalent bonding of galactose on alginate backbone using aqueous carbodiimide chemistry occurs by the reaction of the carboxylic groups on the alginate with Gal-1-

NH_2 using FDS/NHS coupling reagent. This enhances interaction with hepatocyte cell that performs various metabolic activities in the liver. At the exterior of the liver, hepatocyte cell loses its activity and can be applicable for brief moment thus the need for immunoprotection and an mechanical support. The galactose modified alginates results in beads with greater volumes due to conformational disordering within the gels as a result of net gain in hydration. For a better mechanically strong product, various methods of galactosylation of alginate can be undertaken.

1.2.4.5 Modification by Hydrophobic Moiety

Alginates being predominantly hydrophilic in nature due to the hydroxyl and carboxylate groups can be modified to an amphiphilic or a hydrophobic polysaccharide. This can be accomplished by covalent linkage of aromatic groups or long alkyl chains to the backbone of the alginates. An example is the use of sodium metaperiodate to activate sodium alginates by oxidizing the hydroxyl groups to aldehyde groups. This increases its reactivity and since the carboxylate groups don't react, the modified alginates can still form ionic gels. Alginates can be hydrophobically modified by intramolecular and intermolecular interactions.

1.2.4.6 Modification by Covalently Crosslinking of Alginates

Although the alginate gels which are of ionic nature are of great importance, their stability in polar solvents with high ionic strength as well as an improved mechanical and chemical property is obtained by the covalent crosslinking with alginates. This can be obtained by a reaction involving epichlorohydrin and calcium alginate beads in aqueous sodium hydroxide solution and then the removal of calcium by treatment with sodium citrate solution. The covalent crosslinking occurs with only the alginate hydroxyl groups since the carboxylic acid groups have been used up in ion crosslinking. Thus the modified alginates still maintain their ion binding properties.

Gluteraldehyde was used to covalently crosslink sodium alginates which were used for isomer separation, encapsulation and for the controlled release of biomaterials. When the crosslinking was allowed to reach equilibrium, a pH-responsive and thermodynamically regulated network of alginate gels were produced.

Covalently crosslinked alginates were also produced by formation of amide bonds and used in treating traumatic disorders of the intervertebral disc. Water soluble carbodiimide chemistry was also used to produce covalently crosslinked alginate hydrogels, where the hydroxyl groups react with carboxylic acid groups. The crosslinking can also be done by partial oxidation of neighboring hydroxyl groups using sodium periodate.

1.2.4.7 Modification by Graft Copolymerization

This modification technique is an effective method to adjust physical and chemical properties of alginates by increased steric effects and hydrophobicity thus aiding prevention of disintegration and a continuous discharge of biomolecules from the matrix of the alginate. Grafting of certain synthetic polymers like PMMA, PAN, and PMA unto alginates by a ceric-induced system resulted in homopolymerization. Although grafting of PAAm unto alginates, did not form homopolymers. The use of CAN or Fenton's reagent for grafting synthetic polymer unto alginates is unspecific as the C-H bonds are susceptible to cleavage as a result of the oxygen functionality attached.

PNIPAAm, a temperature responsive polymer which shows lower critical solution temperature (LCST) behavior in aqueous solution at $32^{o}C$ is grafted unto alginates which are pH responsive because of the carboxylic acid groups, to form

pH/temperature responsive hydrogels with vast applications. The terminal amine reacts with the carboxyl acid group by EDS/NHS coupling.

Poly((2-dimetylamino)ethyl methacrylate) PDMAEMA which also possess terminal amine groups, show LCST behavior and is water soluble as well is grafted unto oxidized sodium alginate (OAlg) in order to be useful for biomedical applications (Siddhesh & Kevin, 2012).

Acrylamide and 2-acrylamide-2-methylpropanesulfonic acid were grafted simultaneously unto alginates in an aqueous medium using ammonium persulfate as the initiator (Mohammad, Esmat, Fatemeh, Laleh, & Hadis, 2014). Graft copolymer of sodium alginate and poly(itaconic acid) were done by free radical polymerization using cerium(iv)ammonium nitrate/nitric acid in a redox system. The resultant copolymer had increased thermal stability, was soluble in NaOH solution but insoluble in other solvent (Nuran & Fatma, 2012).

Polyacrylonitrile was grafted on chitosan and loaded with silver nanoparticles then coated fully on alginate beads. Silver nanoparticles possess special biological, physical and chemical features especially a broad surface area. It is a potential antibacterial agent used as disinfectants. However, its nanosized particles are a stumbling block to its usability and so needs to be integrated into a variety of substrates. The coated beads have lower risk as a water treatment agent than chlorine which has adverse effects like cancer (Hebieish, Ramadan, Montaser, Krupa, & Farag, 2015).

N-vinylcaprolactam was grafted unto sodium alginate by chemical free radical initiation method. The graft copolymer was used to produce spherical and smooth surfaced micro gels. These micro gels were loaded and useful as well for drug delivery purpose like colon cancer(Rao, Rao, Sudhakar, Rao, & Subha, 2013).

1.3 Poly (benzyl methacrylate)

Poly(benzyl methacrylate) also known as benzyl methacrylate resins are acrylic polymers which are amongst the most used polymers due to their properties which can be adjusted for various applications. They possess good environmental stability and are used in coatings, adhesives, and fibers. PBMA have a repeat unit of molecular weight 176.22g/mol with a glass transition temperature of 54^{o}C. It is hydrophobic in nature.

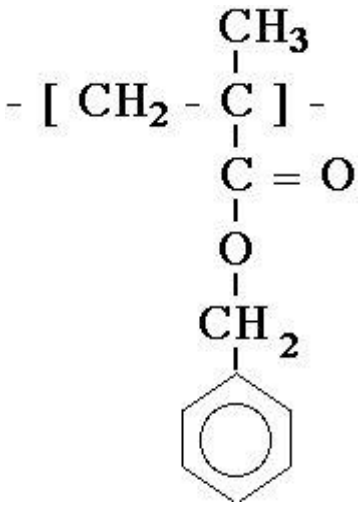

Figure 3. Repeat unit of Poly (benzyl methacrylate)

PBMA is gotten from the homopolymerization of benzyl methacrylates (BMA) also known as methacrylic acid benzyl ester. BMA is light sensitive, flammable and stable acrylic monomers that differ with oxidizing agents, reducing agents and free radical initiators. BMA has low toxicity but toxic to aquatic habitat. This is the reason why its use is restricted to close systems. Furthermore BMAs are

hydrophobic, and possess high impact resistance, low viscosity, hardness properties and high refractive index which make them useful as composites, structural adhesives, acrylic flooring, UV curing systems, chemical fixings and anchor bolts.

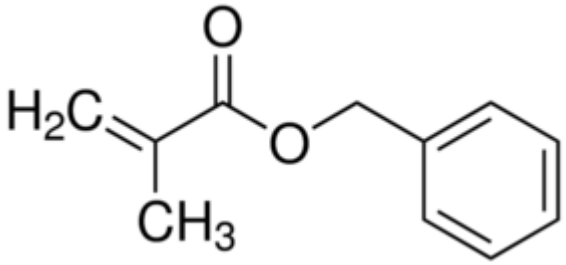

Figure 4**.** Structure of benzyl methacrylate

PBMA can be used in making color gels that are used in videography for color correction and color light. It is also used in Nano imprint lithography (Bharathwaj, Natarajah, & Dhamodharan, 2010).

1.4 Comparison of Chemical and Radiation Methods of Graft copolymerization

Radiation method is an inadequate method but it is useful since there is absence of successive steps. Acrylic acid was grafted unto rayon fibers by pretreatment with ceric ion induced system and high energy radiation (pre-irradiation) methods and a correlation of the retaining ability, elongation, ability to withstand pressure and sorbency properties of both modified rayon fibers were carried out. Homopolymerization which is consistent with acrylic acid was considerably reduced by the pre irradiation methods. The pre irradiated graft generally showed better properties than the ceric ion pretreatment graft as a result of degradation from oxidation and acidic effects (McDowall, Gupta, & Stannett, 1987).

Rayon fibers were well modified through graft copolymerization with acrylonitrile by chemical (ceric ion induced) and radiation (mutual) methods. The grafting percentage yield was higher for that of the chemically modified under the best reaction conditions (Kaur, Sharma, & Kumari, 2013).

1.5 Ciprofloxacin

The fifth largest and the most commonly used antibacterial is ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid). It's a second-generation amphoteric quinolone antibiotic belonging to the fluoroquinolone group which possesses a piperazine group at position 7 of the 4-quinolone nucleus and has an extended array of activity against the Gram-negative and the Gram-positive bacteria. It is an anti-infective agent for nucleic acid synthesis inhibitors in respiratory, gastrointestinal and urinary tracts infections (Cazedey & Salgado, 2012). It is a faint to light yellow crystalline powder which is soluble in dilute hydrochloric acid and also in water to a concentration of about 30,000mg/L (20^{o}C), but virtually insoluble in ethanol. Ciprofloxacin's mechanism of action differs from other antibiotics and has a greater affinity for bacteria DNA gyrase. As such its capability to particularly exhibit the activity of bacteria in a case that is difficult to diagnose. Thus it will be effective in diagnosing pneumonia, tuberculosis, abdominal abscess, obscure fever, osteomyelitis, appendicitis and wound infection.

Ciprofloxacin has a characteristic absorbance of 275nm and linear absorbance-concentration interval of 0.085- 2.29 absorbance units (Hornyák et al., 2014).

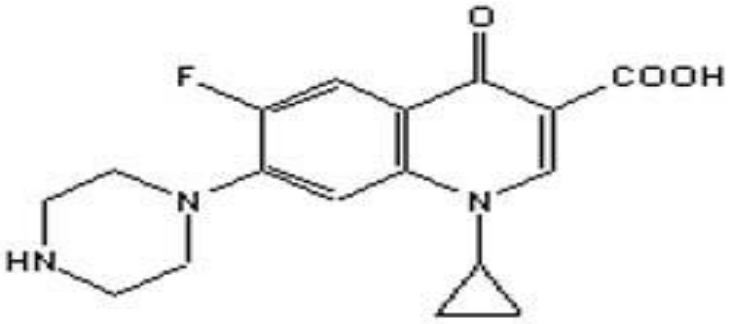

Figure 5**.** The chemical structure of ciprofloxacin

1.6 Release Kinetic Models

This is employed in the dissolution of drug from solid dosage forms and also in the interpretation of the mechanisms of drug release from a matrix. It is grouped into three classes

- Model independent methods; which include pairwise procedure (resign index, similarity factor, difference factor) and ratio tests
- Statistical methods; this includes repeated measures design, exploratory data analysis method, multivariate approach (MANOVA: multivariate analysis of variance)
- Model dependent methods; this includes zero order, first order, Higuchi, Korsmeyer-Peppas model, Hixson Crowell, Baker-Lonsdale model, Weibull model, etc.

For this study, the main emphasis will be on the model dependent methods.

1.6.1 Model dependent Models

They are also known as the mathematical models since they are based on various mathematical functions that explain the profile of the dissolution.

1.6.1.1 Zero-order Model

This model evaluates the dissolution of drug from dosage forms that can not separate and it releases the drug slowly. It can be denoted by the equation;

$Q_o - Q_t = K_o t$

This equation can be reorganized to give;

$Q_t = Q_o + K_o t$

Where, Q_t gives the amount of drug dissolved in the solution at time t, Q_o is the amount of drug initially in the solution (usually it is equal to zero) while K_o is the zero order release constant with units of concentration per unit time. To obtain the release kinetics a graph was plotted with cumulative amount of drug released versus time. The correlation describes the dissolution of drug from various types of modified release pharmaceutical dosage forms. (Lokhandwala, 2013).

1.6.1.2 First Order Model

This model describes the absorption and/or desorption of some drugs, this mechanism is usually difficult to theorize. It can be denoted by the equation;

$\frac{dc}{dt} = -Kc$

Taking logarithm of both sides will result in

$\log C_t = \log C_o - \frac{Kt}{2.303}$

Where C_o is the initial concentration of drug in solution, C_t is the amount of drug released in time t, K is the first order rate constant, and t is the time. A graph is plotted of the log cumulative percentage of drug remaining versus time to obtain a straight line. This describes the dissolution of drugs in pharmaceutical dosage forms like those comprising of water-soluble drugs in permeable matrices.

1.6.1.3 Higuchi Model

This model was developed to study the release of low and water soluble drugs integrated into solid and semisolid matrices. The dissolution from a planer system with a homogeneous matrix can be studied using the equation;

$Q = [D (2C-C_s) C_s t]^{1/2}$

Where Q is the amount of drug released in time t per unit area, C_s is the solubility of drug in the matrix media, C is the initial concentration and D is the diffusivity of drug molecules in the matrix substance. In cases where the concentration of the drug in the matrix is less than its solubility and the pores located in the matrix are used for the release, the equation becomes

$Q = D\varepsilon/\tau (2C-\varepsilon C_s) C_s t$

All other parameters remain the same while ε is the porosity factor of the matrix and τ is the tortuosity factor of the capillary system. Generally, Higuchi model can be abridged as,

$Q = K_H t^{1/2}$

K_H is the Higuchi dissolution constant of the drug. A graph is then plotted as cumulative percentage release of drug versus square root of time. The model describes the dissolution of drug from various kinds of improved release pharmaceutical dosage forms, like in some transdermal systems and matrix tablets with water soluble drugs (Dash, Murthy, Nath, & Chowdhury, 2010).

1.6.1.4 Korsmeyer-Peppas Model

Korsmeyer et al. obtained a simple relationship that described the release of drug from a polymeric system equation. In order to ascertain the drug release mechanism, the data containing the first 60% drug release is to be fitted in Korsmeyer-Peppas model.

$\frac{M_t}{M_\infty} = kt^n$

The logarithm form of this equation is;

$\text{Log } \frac{M_t}{M_\infty} = \text{Log } k + n \text{ Log } t$

Where $\frac{M_t}{M_\infty}$ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. Here, the value of n characterizes the mechanism of drug release. From the data obtained a graph is plotted as log cumulative percentage drug release versus log time.

Chapter 2

EXPERIMENTAL

2.1 Materials

The commercially available chemicals given in the table below were used as received without further purification.

Table 2. Materials used

Material	Manufacturer
Sodium alginate	AppliChem
Hexane	Merck-Germany
Tetrahydrofuran	AnalarR- England
2,2-Dimethoxy-2-phenylacetophenone	Aldrich-UK
α,α'-Azoisobutyronitrile	Fluka-Switzerland
Benzyl methacrylate	Aldrich-USA
Dimethyl sulfoxide (DMSO)	Sigma Aldrich
Hydrochloric acid	BDH
Potassium chloride	Sigma Aldrich
Sodium bicarbonate	BDH
Sodium hydroxide	Aldrich
Sodium hypo phosphite	Aldrich
Calcium chloride	Sigma Aldrich

2.2 Preparation of Solution

2.2.1 Sodium Alginate Solution

2.0 % w/v sodium alginate solution was prepared by dissolving 4.0 g of sodium alginate powder in a 200mL volumetric flask, and distilled water was added to mark.

2.2.2 Calcium Chloride Solution

Calcium chloride solution of 3.0 % w/v was prepared by dissolving 6.0 g of $CaCl_2$ in a 200mL volumetric flask, and distilled water was added to mark.

2.2.3 Buffer Solutions

The buffer solutions prepared were used for both the drug release studies and swelling experiments. Some were also used for the release studies. A pH meter was employed to ratify the correct pH values before use.

To prepare the buffer solution of pH1.2, 50mL of 0.2M KCl and 85mL of 0.2M HCl were mixed in a 200mL volumetric flask with distilled water added to the mark.

To prepare pH 7.0, 29.63mL of NaOH and 50mL of KH_2PO_4 were mixed together in a 200mL volumetric flask and diluted with distilled water to mark

To prepare pH 11, 0.42g of sodium bicarbonate were dissolved in 45.4mL of NaOH in a 500mL volumetric flask with distilled water added to mark.

2.3 Preparation of Calcium Alginate Beads

Sodium alginate beads were prepared at room temperature by dropwise addition of 2.0% (w/v) aqueous sodium alginate solution into a 3% (w/v) aqueous calcium chloride solution stirred at 400rpm. The stirring continued for one hour after which the beads were obtained by filtration and then washed thoroughly with distilled

water, and dried in vacuum at 40^{o}C overnight (Mandal, Kumar, Krishnamoorthy, & Basu, 2010).

2.4 Graft Copolymerization of Benzyl Methacrylate onto Sodium Alginate Beads

2.4.1 UV Source

A Luzchem Photoreactor, of the Luzchem Research Inc., Canada (LZC4) equipped with UV lamps of 350nm wavelength and 7670 uW/cm2 power (in the UV region) was used for the irradiation of samples.

2.4.2 Preparation of Alginate-graft-poly(Benzyl Methacrylate) by UV Initiation

The weighed mass of alginate beads were conditioned by leaving in hexane overnight. The conditioned beads were put in a glass and pre-irradiated for one hour from both sides. The beads were placed at 15cm from the lamps. DMPA was dissolved in hexane and subsequently benzyl methacrylate (BzMA) was added to the mixture. This was then poured into the glass containing the beads and irradiated for 15 minutes from both sides. The product was washed two times with 50 ml THF for removal of homopolymer.

Some of the beads were not pre-irradiated but put together with the mixture and irradiated for 15 minutes on both sides. No photoinitiator as well as solvent was used for some radiation reactions.

2.4.3 Preparation of Alginate-graft-poly(BzMA) by Chemical Initiation

The beads (0.5g) were immersed in a mixture of 25ml hexane and 2.5mg DMPA in a three-necked round bottom flask under reflux. Nitrogen gas was passed through for 30 minutes after which the 0.5ml benzyl methacrylate was added and the grafting

was allowed for a period of time at 60^{o}C temperature. The grafted beads were washed two times with 50 ml THF for removal of homopolymer.

2.5 Percentage Graft Yield Determination

The level of graft copolymerization can be obtained from the percentage graft copolymer yield which is obtainable with utility of the equation

% graft yield = $\frac{M_{2-}M_1}{M_1} \times 100$

Where M_2 is the mass of the grafted beads after removal of homopolymer and M_1 is the initial mass of the non-grafted beads.

2.6 Scanning Electron Microscope (SEM) Analysis

SEM pictures were taken in CIU (Cyprus International University, Nicosia) using JEOL JSM-6510 scanning electron microscope. It is used to create high-resolution images of the beads, as well as it's modified and drug loaded forms.

2.7 Dissolution and Swelling Properties of Samples

The dissolution and swelling properties of the samples were undertaken by gravimetric analysis. 10mg bead samples were placed in a beaker containing 10mL of solution at room temperature. The bead sample was taken out at various time intervals, immediately washed free of solution with distilled water, weighed on an analytical balance, and then put back into the swelling medium. The percentage swelling was calculated from the equation.

Percentage of swelling = $\frac{W_2 - W_1}{W_1} \times 100$

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

2.8 Drug Release Studies

2.8.1 Preparation of Ciprofloxacin Loaded Beads

50 mg beads of average diameter 500 µm were placed in a beaker containing 50 mL aqueous ciprofloxacin solution of concentration 0.01mg/mL and kept at room temperature for 24 hours. The concentration of drug loading was determined spectrophotometrically at 275 nm.

2.8.2 Ciprofloxacin Release

50 mg ciprofloxacin loaded bead sample was placed in a beaker containing 50 mL of water and stirred at 60 rpm at 37 °C. The amount of drug release was determined by measuring the absorbance of the solution at 275nm at various time intervals. The spectrum of ciprofloxacin in water of concentration 0.01mg/mL with cell length of about 16mm is as follows.

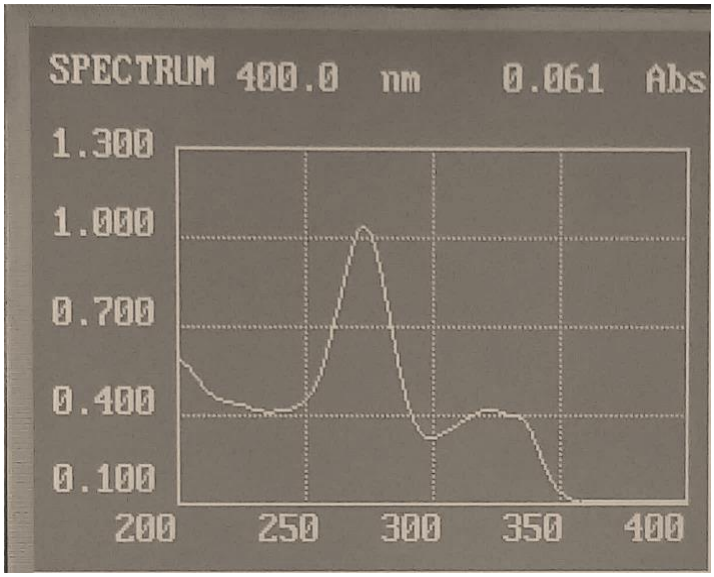

Figure 6. Spectrum of Ciprofloxacin in Water

The calibration curves differ in molar absorptivity possibly as a result of interactions between the ciprofloxacin with water and with pH 1.2. The following calibration curves were used in the release studies.

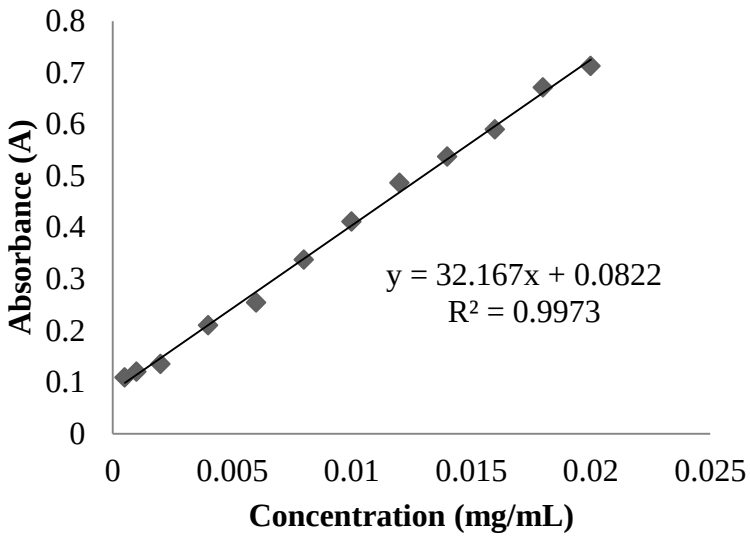

Figure 7**.** Calibration curve for Ciprofloxacin in Water

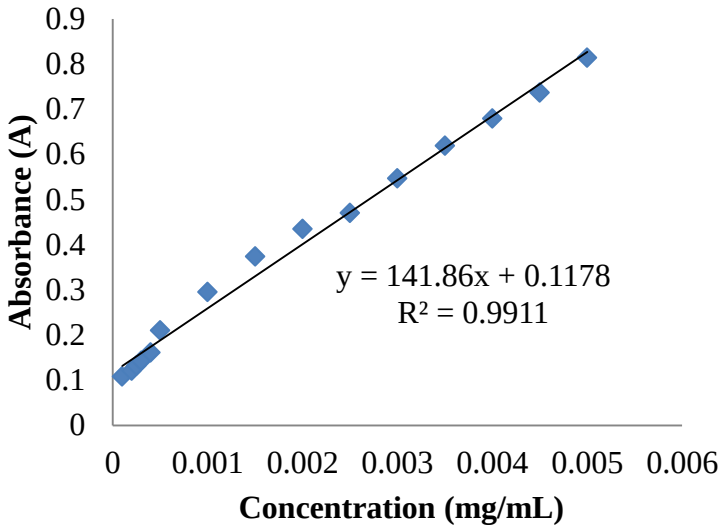

Figure 8. Calibration Curve for Ciprofloxacin in pH 1.2

2.8.3 Ciprofloxacin Percentage Release

The equation for calculating percentage release obtained from the calibration curve is as follows:

$$\% \text{ Release} = \frac{C_{release}}{C_{loaded}} \times 100$$

Where $C_{release}$ is the concentration of drug released and C_{loaded} is the concentration of drug loaded.

2.8.4 Ciprofloxacin Percentage Loading

The equation for calculating percentage loading obtained from the calibration curve is as follows:

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

$C_{initial}$ is the initial concentration of drug solution before the loading of the beads.

2.9 Antibacterial activity test

E.coli was incubated first at 37 ºC for 24 h on a nutrient agar plate. The added microorganisms were then coated on the surface of Luria-Bertani (LB) agar, petri dishes. The prepared beads were then added to the surface of the agarose plates containing cultures of bacteria in Luria-Bertani medium. The mean diameters of the zone of inhibition measurements were calculated.

CHAPTER 3

RESULTS AND DISCUSSION

Alginate-graft-poly(benzyl methacrylate) copolymer was synthesized by two different approaches including chemical and UV initiation. Grafting percentage was calculated gravimetrically. Characterization was carried out by SEM analysis. The dissolution properties were tested in aqueous media. In-vitro ciprofloxacin release from samples was investigated in water. Antibacterial activity of the products was examined against E.coli.

3.1 Synthesis of Alginate-graft-poly(benzyl methacrylate) by Chemical Initiation

Sodium alginate beads were grafted with benzyl methacrylate (as described in section 2.4.3) in the presence of free radical initiator AIBN in two different solvents, hexane and DMSO. The results are summarized in Table 3.

The initiations at one hour showed higher percentage grafting and grossly decreased as the hours increased in both hexane and DMSO. This could be due to decomposition of initiator which decreases the concentration of initiator and consequently a decrease in generation of radicals needed for grafting. There could also be insufficient monomer. The beads that were conditioned gave higher grafting than those not conditioned indicating the conditioning of the beads improved the grafting as a result of better diffusion due to possibly a little swelling of the beads. The grafting yields were also higher in hexane solution than in DMSO.

Table 3. Grafting percentage of Alginate-graft-poly(BzMA) by Chemical Initiation. (* denotes without conditioning.)

Sample ID	**Beads(g)**	**BMA(mL)**	**AIBN(mg)**	**Hexane(mL**	**DMSO(mL)**	**Reaction time (hr)**	**% G**
Alg- *graft* (CI)-poly BzMA 1 (21.4)	0.5	0.5	2.5	25	---	1	21.4
Alg-*graft* (CI)-poly BzMA 2 (6.1)	0.5	0.5	2.5	25	---	2	6.1
Alg-*graft* (CI)-poly BzMA 3 (4.0)	0.5	0.5	2.5	25	---	3	4.0
Alg-*graft* (CI)-poly BzMA1 * (7.5)	0.5	0.5	2.5	---	25	1	7.5
Alg-*graft* (CI)-poly BzMA2 *(2.8)	0.5	0.5	2.5	---	25	2	2.8

3.2 Synthesis of Alginate-graft-poly(BzMA) by UV Initiation

Sodium alginate beads were exposed to UV light under the following different conditions

A- Beads + benzyl methacrylate + conditioning in hexane overnight

B- Beads + benzyl methacrylate +chemical initiator (AIBN or DMPA) +solvent (hexane or DMSO) with or without conditioning of beads in solvent overnight.

C- Beads + benzyl methacrylate +chemical initiator (AIBN or DMPA) +solvent (hexane) by) by conditioning of beads in solvent overnight and pre-irradiation of beads.

The results of synthesis of alginate-graft-poly(BzMA) by UV Initiation with their percentage grafting are shown in Table 4.

The beads that were conditioned gave higher grafting than those not conditioned indicating the conditioning of the beads improved the grafting as a result of better diffusion possibly due to a little swelling of the beads. Under the same reaction conditions and a smaller amount of beads, the grafting percentage yield increased indicating more grafting sites.

The percentage grafting in hexane were higher than in DMSO which shows that hexane is a better solvent than DMSO when reacting with alginates. Alginates tend to shrink in polar solvent but are practically unaffected in non-polar solvents and DMSO being a polar solvent will instead shrink the beads and thus a decrease in graft yield.

The graft percentages were higher for those initiated AIBN in comparison with those initiated with DMPA. Pre-irradiated products gave lower percentage yields than those not pre-irradiated.

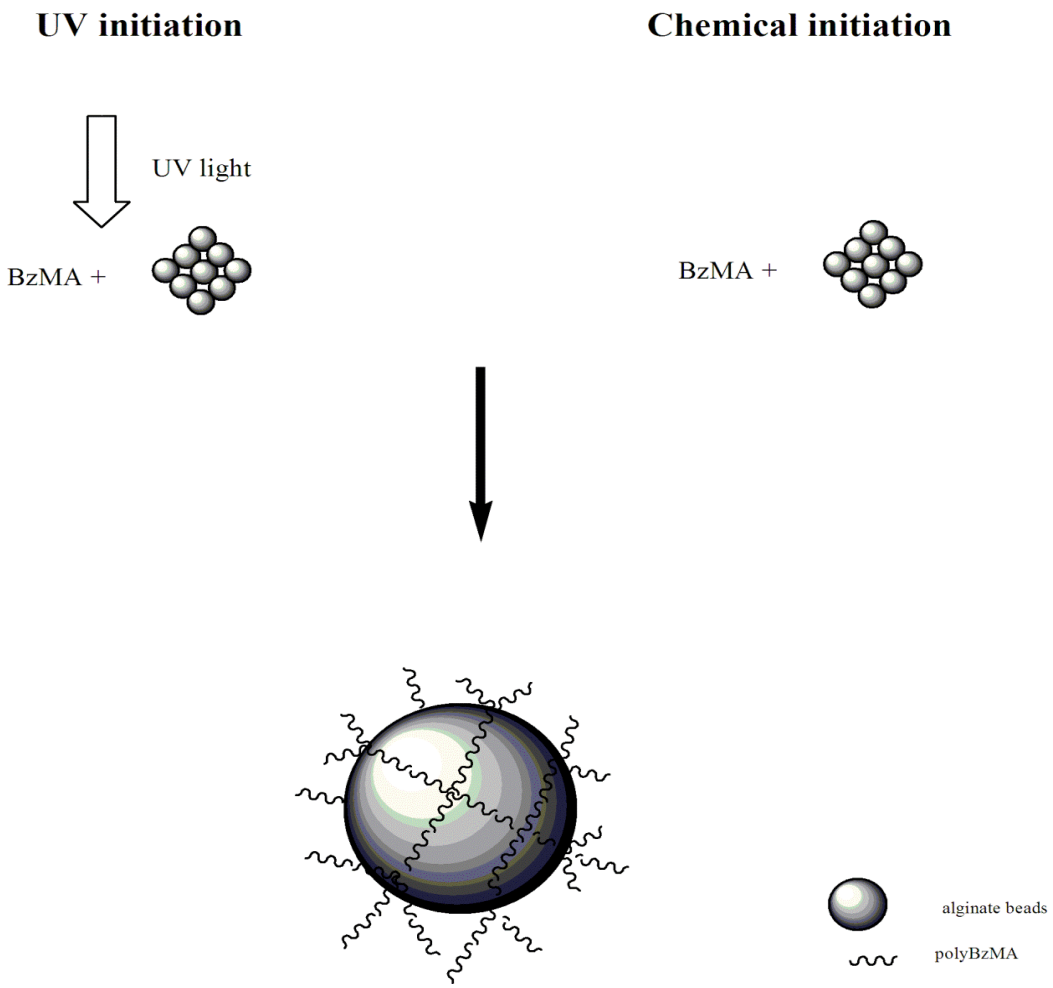

Figure 9. Hydrophobic modification on the alginate bead surface via benzyl methacrylate

Table 4. Grafting percentage of Alginate-graft-poly(BzMA) by UV Initiation.(*without conditioning, # pre-irradiated)

Sample ID	**Beads(g)**	**BMA(ml)**	**AIBN(mg)**	**DMPA(mg)**	**Hexane(ml)**	**DMSO(ml)**	**% G**
Alg-*graft (UV)*-poly(BzMA) (9.6)	0.5	0.5		2.5	2.5	-	9.6
Alg-*graft (UV)*-poly(BzMA)(2.0)#	0.5	0.5		2.5	2.5	-	2.0
Alg-*graft (UV)*-poly(BzMA)(14.6)	0.5	0.5		25	2.5	-	14.6
Alg-*graft (UV)*-poly(BzMA)(3.7)*	0.5	0.5		25	2.5	-	3.7
Alg-*graft (UV)*-poly(BzMA)(6.5)*	0.5	0.5		25		2.5	6.5
Alg-*graft (UV)*-poly(BzMA)(8.8)*	0.1	0.5		2.5		2.5	8.8
Alg-*graft (UV)*-poly(BzMA)(32.1)	0.1	0.5		2.5	2.5	-	32.1
Alg-*graft (UV)*-poly(BzMA)(10.8)#	0.5	0.5	2.5		2.5	-	10.8
Alg-*graft (UV)*-poly(BzMA)(10.7)	0.5	0.5	2.5		2.5	-	10.7
Alg-*graft (UV)*-poly(BzMA)(10.0)	0.5	0.5	2.5		2.5	-	10.0
Alg-*graft (UV)*-poly(BzMA)(2.5)	0.5	0.5	25		2.5	-	2.5
Alg-*graft (UV)*-poly(BzMA)(21.3)	0.5	0.5				-	21.3
Alg-*graft (UV)*-poly(BzMA)(13.2)	0.5	0.5				-	13.2

3.3 SEM Analysis

SEM images of grafted/non-grafted beads as well as ciprofloxacin loaded and released beads are shown in Figure 10 and the SEM images of half cut beads of ciprofloxacin loaded and release grafted and non-grafted beads are shown in figure 11.

White particles as shown in the figure below were seen adhering to the surface of the beads due to the grafting of BMA. The Alg-*graft (UV)*-poly(BzMA)(32.1) beads possess a heterogeneous surface while is also indicative of its higher release percentages while the alginate beads possess a more homogenous surface.

The ciprofloxacin drug appears as spherical particles on the surface of the alginate beads but tends to penetrate the Alg-*graft (UV)*-poly(BzMA)(32.1) due to its heterogeneous nature which create pores for passage.

The ciprofloxacin molecules were found on both the alginate and the Alg-*graft (UV)*-poly(BzMA)(32.1) beads even after release.

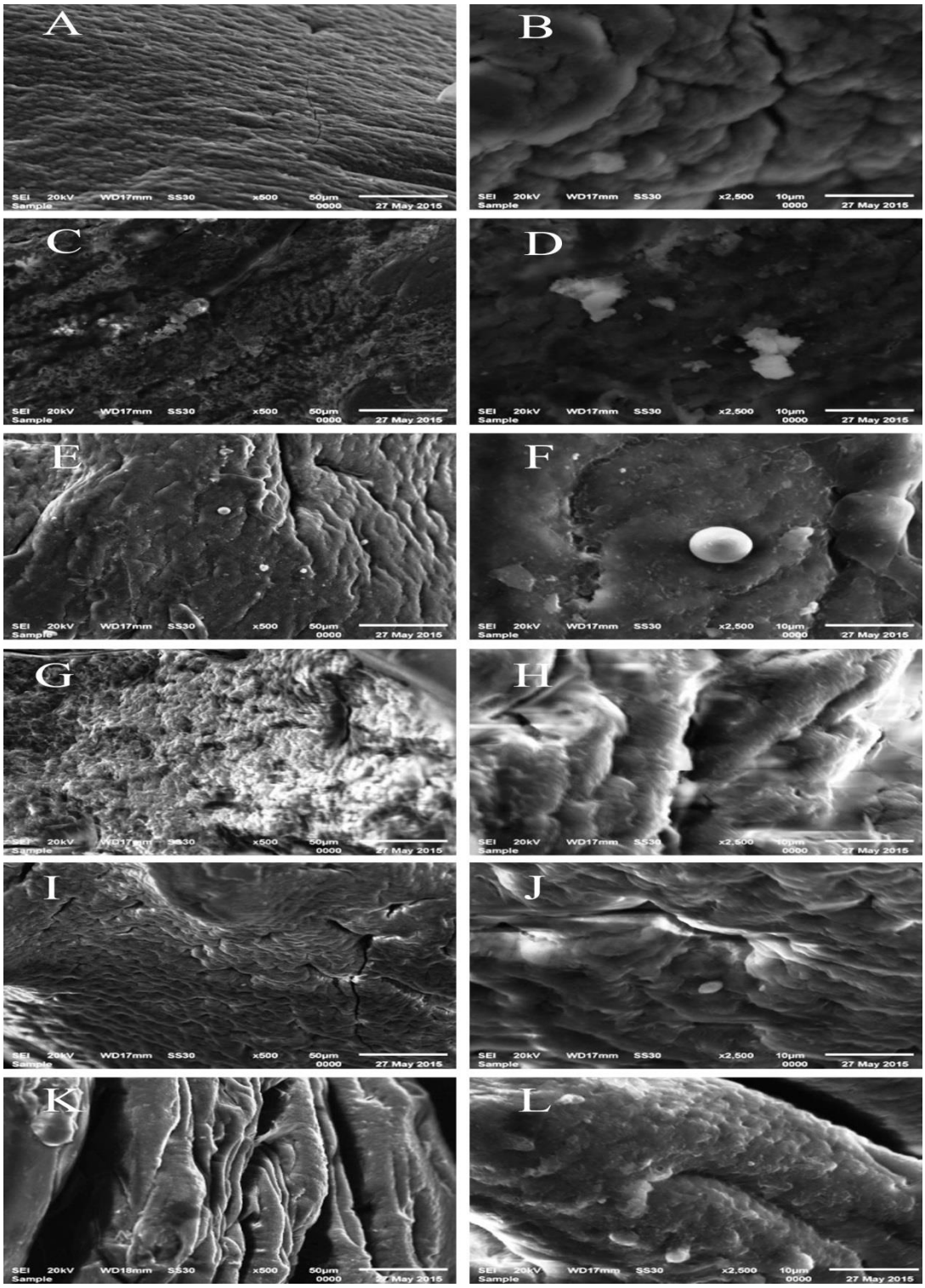

Figure 10. SEM Images of A and B- Alginate beads, C and D-Alg-graft (UV)-poly(BzMA)(32.1) , E and F- Ciprofloxacin loaded Alginate beads, G and H-ciprofloxacin loaded Alg-graft (UV)-poly(BzMA)(32.1), I and J- non grafted beads after ciprofloxacin release for 24 hours , K and L- grafted beads after ciprofloxacin release for 24 hours

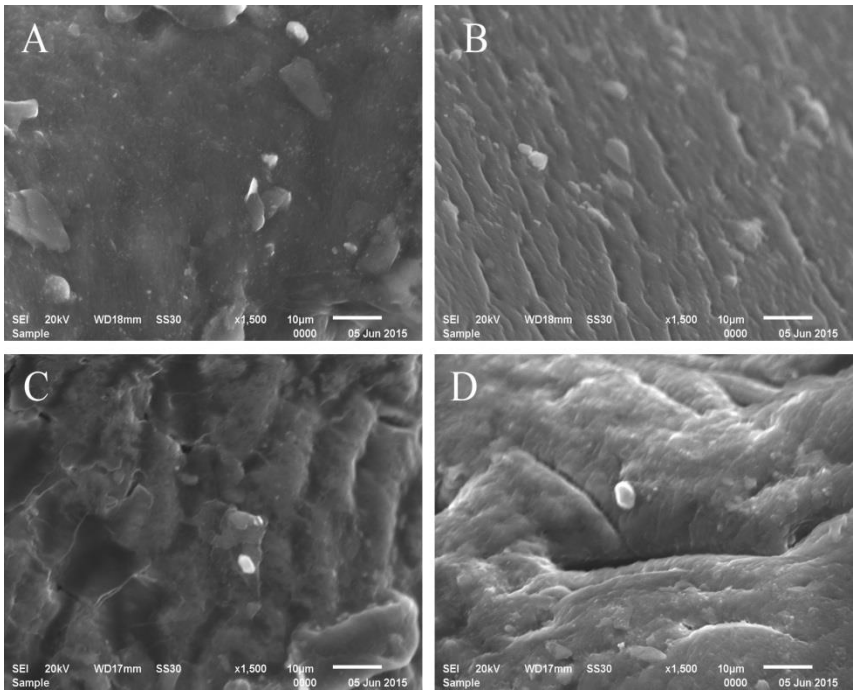

Figure 11. SEM images of half cut beads of (A) alginate ciprofloxacin loaded bead, (B) Alg-graft (UV)-poly(BzMA)(32.1) ciprofloxacin loaded bead, (C) alginate ciprofloxacin loaded bead after drug release (D) Alg-graft (UV)-poly(BzMA)(32.1) ciprofloxacin loaded bead after drug release

3.4 Dissolution and Swelling Properties of Products

Swelling and dissolution properties of alginate beads and grafted products were examined in solutions at pH values of 1.2, 7.0, and 11.0. The results are shown on Table 5.

Alginates are sensitive to pH due to the presence of carboxylic groups on its backbone. The percentage swelling is higher and better at pH 1.2, possibly due to repulsive forces and it decreases as the pH increases for both the grafted and non-grafted product. Although at each pH the grafted product gave a better percentage swelling than the non-grafted product, for every time interval. This shows that the beads will be better effective only in the stomach hence has a specific property which makes it a potential drug carrier in the intestinal tract.

Table 5. Swelling percentage in buffer solutions

Time (hr)	**% Swelling in pH 1.2**		**% Swelling in pH 7**		**% Swelling in pH 11**	
	Alginate Beads	Alg-graft (UV)-poly(BzMA)(32.1)	Alginate beads	Alg-graft (UV)-poly(BzMA)(32.1)	Alginate Beads	Alg-graft (UV)-poly(BzMA)(32.1)
1	72	76	10	12	8	11
2	57	70	25	25	23	25
3	77	71	45	39	39	36
4	69	70	55	47	52	47
5	67	69	62	49	61	55
6	71	72	Dissolved	Dissolved	Dissolved	58

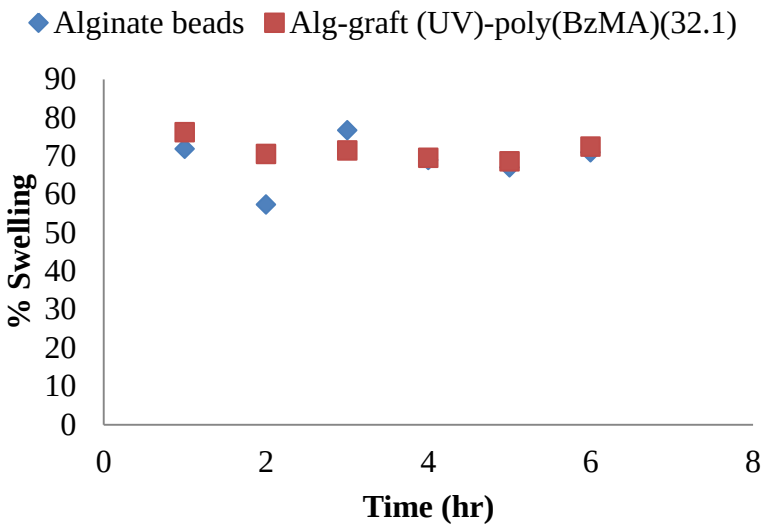

Figure 12. Percentage swelling in pH 1.2

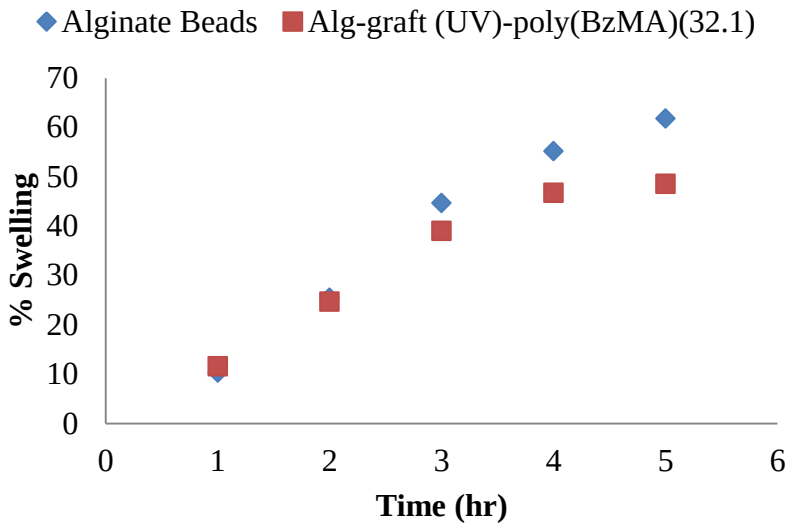

Figure 13**.** Percentage swelling in pH 7

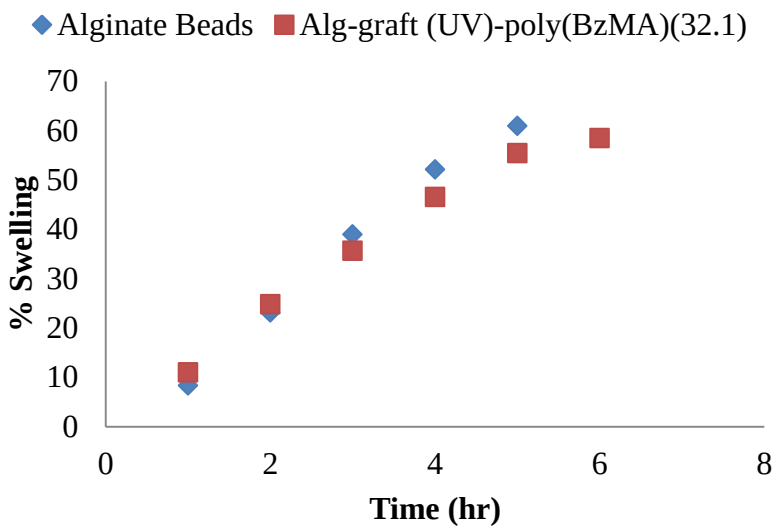

Figure 14**.** Percentage swelling in pH 11

3.5 In-vitro Percentage Ciprofloxacin Loading and Release Study

Grafted and non-grafted alginate beads were loaded with ciprofloxacin drug solution. The loading and release behavior of the grafted and non-grafted alginate beads were examined as described in sections 2.9.1 and 2.9.2. The results are shown in Tables 6 and 7 as well as Figures 16 and 17.

The radiation initiated product had a higher percentage ciprofloxacin loading than the non-grafted and chemically initiated graft product although the percentage loading amongst the three samples did not differ much. This shows that the grafting does not really affect the loading properties as well as its encapsulation as both have hydrophobic character as such it's a situation of "like dissolve like".

Table 6. Ciprofloxacin loading percentage

Sample ID	**% Loading**
Alginate Beads	23
Alg-graft (UV)-poly(BzMA)(32.1)	24
Alg- graft (CI)-poly BzMA 1 (21.4)	19

Alginate beads released the ciprofloxacin more rapidly in water than the grafted beads because it shows higher swelling percentage as a result of its higher hydrophilicity. In the pH 1.2 the grafted products gave higher ciprofloxacin release also as a result of higher percentage swelling in pH 1.2.

Table 7. Percentage Release of Ciprofloxacin in Water and pH 1.2

Time (h)	**% Release in water**			**% Release in pH 1.2**		
	Alginate Beads	**Alg-graft (UV)-poly(BzMA)(32.1)**	**Alg- graft (CI)-poly BzMA 1 (21.4)**	**Alginate Beads**	**Alg-graft (UV)-poly(BzMA)(32.1)**	**Alg- graft (CI)-poly BzMA 1 (21.4)**
1	14	10	5	13	19	18
2	17	11	18	16	26	28
3	18	16	19	17	28	31
4	36	18	22	17	36	26
5	22	19	8	19	36	56
6	25	23	22	17	39	34
24	36	20	17	19	43	42
48	36	26	19	24	35	52
72	38	26	23	26	52	60

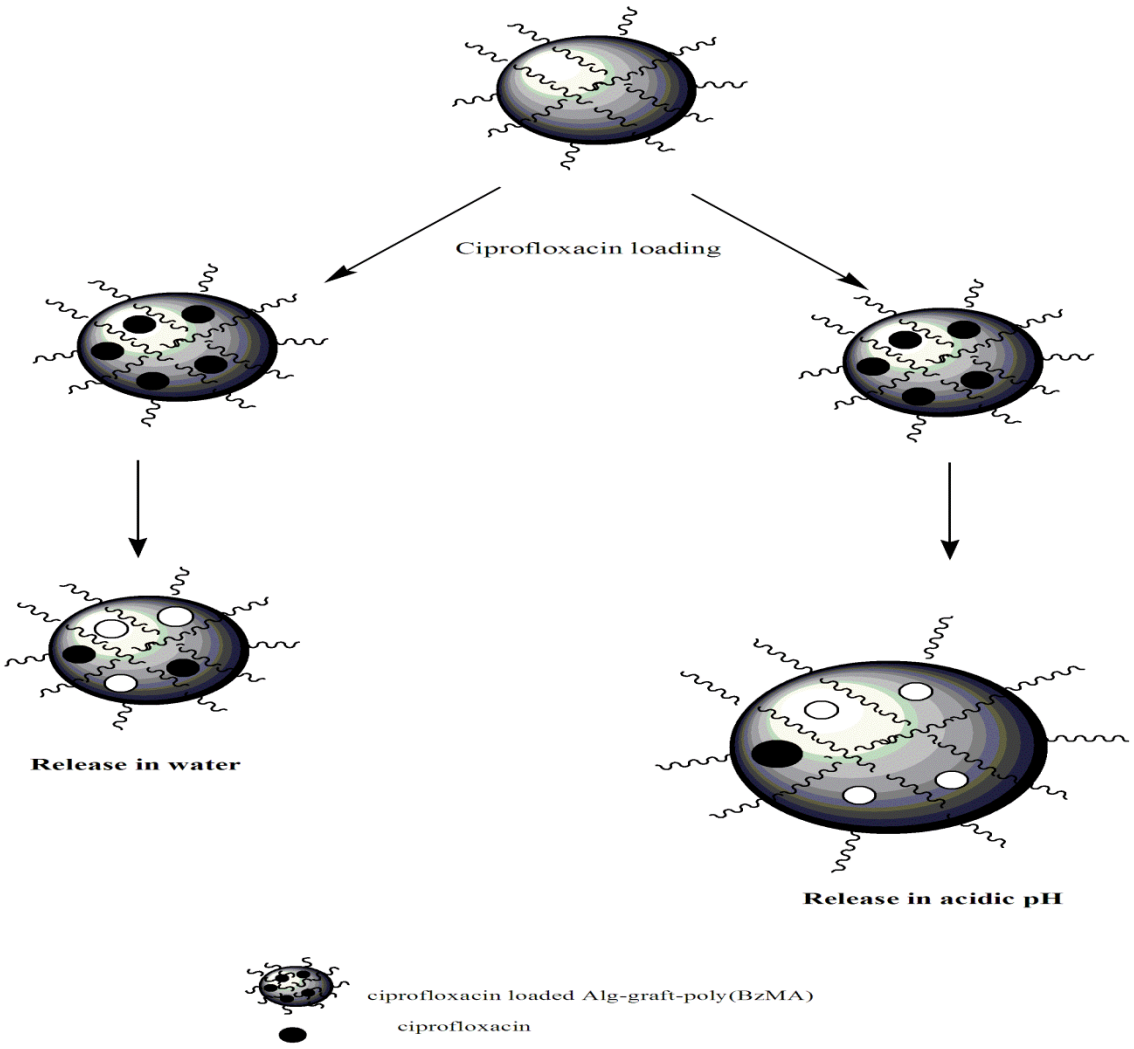

Figure 15**.** Ciprofloxacin Release from Alg-graft-poly(BzMA) in water and acidic media

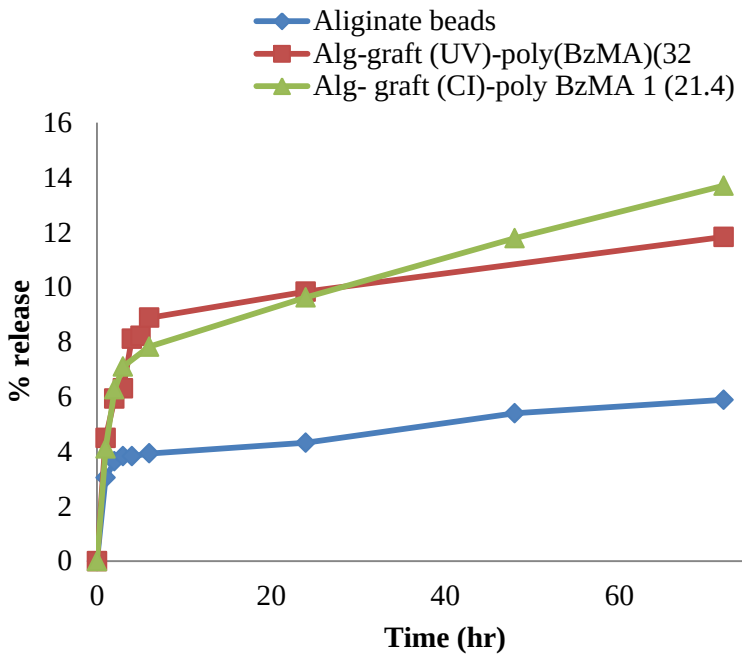

Figure 16. Percentage Release of Ciprofloxacin in pH 1.2

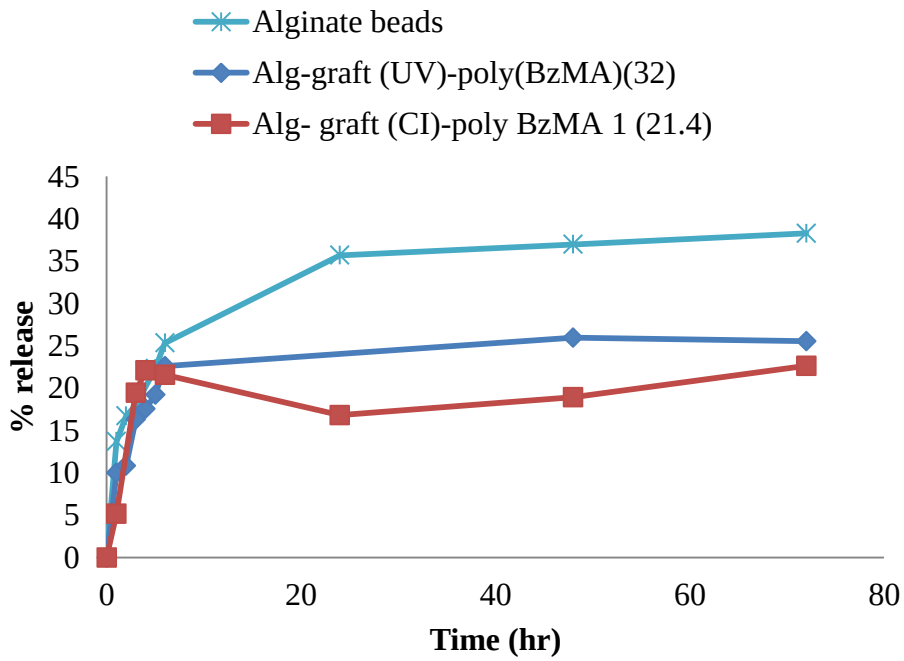

Figure 17. Percentage Release of Ciprofloxacin in Water

3.6 Release Kinetics

The summary of the release kinetics using the different mathematical methods is shown in table 8. The best fit were chosen based on the closeness of the R^2 values to unity (1)

The release kinetics in water is dependent on the diffusion rate as such fits into the Higuchi's Model.

Table 8. R^2 values from the various mathematical methods

	Alginate Beads		**Alg-graft (UV)-poly(BzMA)(32.1)**		**Alg- graft (CI)-poly(BzMA) 1 (21.4)**	
	Water	pH 1.2	Water	pH 1.2	Water	pH 1.2
Zero-order Model	0.8122	0.5281	0.9057	0.8381	0.8362	0.6931
First Order Model	0.9843	0.6432	0.9351	0.9187	0.6851	0.9110
Higuchi Model	0.9714	0.8198	0.9819	0.9953	0.9107	0.9797
Korsmeyer-Peppas Model	0.9684	0.8426	0.9245	0.9684	0.3829	0.9110

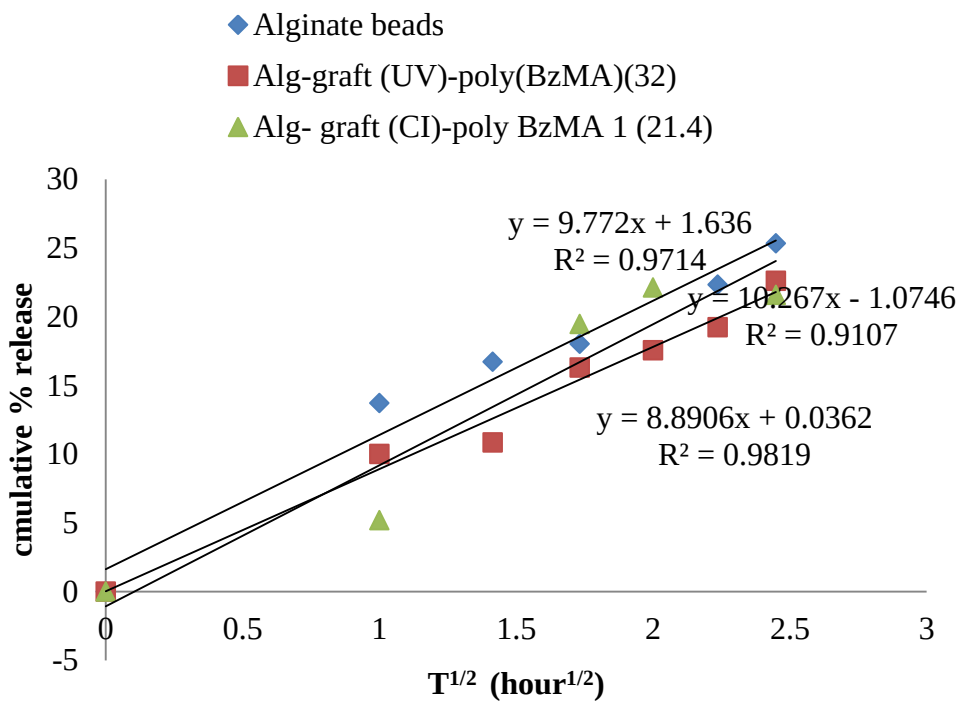

Figure 18. Higuchi's model release of samples

The release kinetics in pH 1.2 solution best fitted In for Korsmeryer-Peppas Model

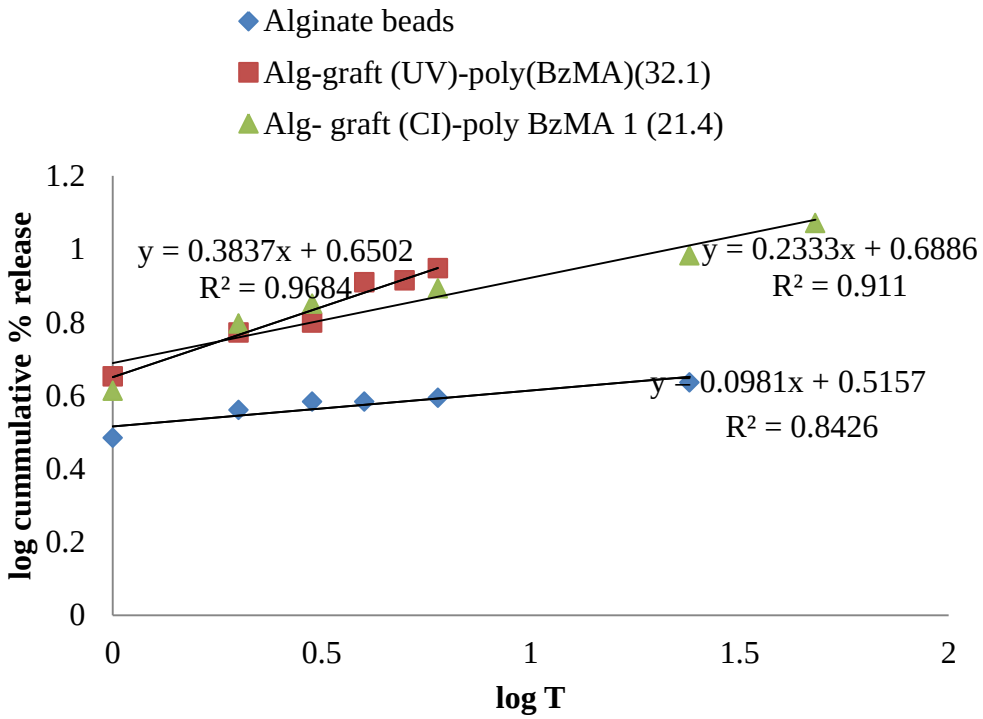

Figure 19. Korsmeryer-Peppas Model release for samples

3.7 Antibacterial Tests

Antibacterial activity tests of the products were studied as described in section 2.10. The results are summarized in Table 8. Figure 20 shows the inhibitory zones of the samples.

Grafting increased inhibition while alginate beads do not show any inhibitory effect. The ciprofloxacin loaded alginate beads showed better inhibitory effect than the ciprofloxacin loaded grafted beads due to higher percentage swelling of alginate beads at pH 7 (pH at which bacteria grows) which allowed for rapid ciprofloxacin release.

The grafted beads gave higher inhibitory effect than the ciprofloxacin loaded grafted beads possibly due to the increased hydrophilicity and consequently decrease in hydrophobicity which enhances inhibition since the ciprofloxacin is hydrophilic in nature.

Table 9**.** Inhibitory Effect of Samples

Sample ID	**Antibacterial Effect(cm)**
Alginate Bead	No Inhibitory Effect
Alginate Ciprofloxacin loaded Bead	2.95
Alg-*graft (UV)*-poly(BzMA)(32.1)	3.05
Alg-*graft (UV)*-poly(BzMA)(32.1) Ciprofloxacin loaded	1.60

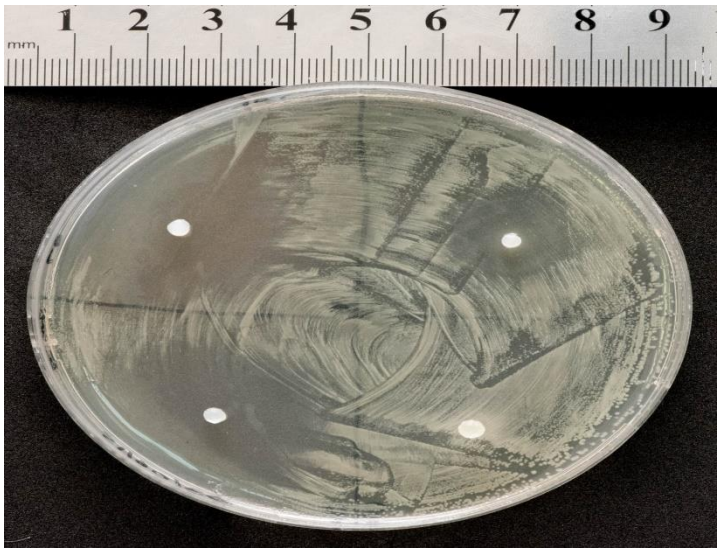

Figure 20**.** Inhibitory zone measurement of the samples

Chapter 4

CONCLUSION

In this thesis an alternative alginate-based antibacterial agent was synthesized by introducing amphiphilic character onto the alginate to enhance penetrating bacteria cell alginate-graft-poly(benzyl methacrylate) copolymer. This was synthesized by two different approaches including chemical initiation and UV initiation. Hydrophobic chains on the alginate bead surface were created by grafting of BMA.

The synthesis with UV initiation gave higher percentage graft yields than that with chemical initiation as a result of a greater influence of solvent on the chemical initiated approach.

Alginates are sensitive to pH due to the presence of carboxylic groups on its backbone. The percentage swelling is higher and better at pH 1.2, possibly due to repulsive forces and it decreases as the pH increases for both the grafted and non-grafted product. This shows that the beads will be better effective only in the stomach hence has a specific property which makes it a potential drug carrier in the intestinal tract

The release kinetics in water is dependent on the diffusion rate as such fits into the Higuchi's Model while that for the acidic media fitted best for the Korsmeryer-Peppas Model.

The Alg-*graft (UV)*-poly(BzMA)(32.1) product showed a greater inhibition zone than the non-grafted product as well as the drug loaded samples due to the hydrophilic nature of drug which reduces effect.

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