MODIFIED ACRYLIC HYDROGELS AS CONTROLLED RELEASE SYSTEMS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

MAY 2006

Approval of the Graduate School of Natural and Applied Sciences

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ABSTRACT

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May 2006, 83 pages

In this study, pH-sensitive poly(acrylamide-co-acrylic acid) hydrogels were synthesized as controlled release systems in the presence of N,N-methylene bisacrylamide as crosslinker and ammonium persulfate as initiator. A set of hydrogels were used in the form they were prepared. One set of hydrogels were prepared as porous networks by incorporating sodium chloride into the reaction medium and then leaching of it after the completion of polymerization reaction. Two sets of hydrogels were modified by argon-plasma at different discharge powers. Hydrogels were characterized by ^{13}C-NMR, XPS, SEM, ATR-FTIR, ESR as well as equilibrium degree of swelling (EDS) and contact angle measurements. Prepared hydrogels were loaded with a model antibiotic, ciprofloxacin-HCl (CPFX), and in-vitro release of CPFX from hydrogel matrices were examined in buffer solutions of varying pH values. There are two factors determining the release rates of CPFX; one is the pH-dependent solubility of CPFX and the other is EDS of the hydrogel samples. For porous samples drug loading and release rates were higher when compared to the control samples and CPFX solubility dominated over release kinetics. Plasma treatment resulted in prolonged release rates in acidic medium.

Keywords: Hydrogel, plasma surface modification, poly(acrylamide-co- acrylic acid), controlled release.

ÖZ

KONTROLLU SALIM SİSTEMLERİ OLARAK MODİFİYE EDİLMİŞ AKRİLİK HİDROJELLER

Pınardağ, Fatma Esra

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Mayıs 2006, 83 sayfa

Bu çalışmada, kontrollu salım sistemi olarak N,N-metilen bisakrilamid çapraz bağlayıcı ve amonyum persülfat başlatıcı eşliğinde pH-duyarlı poli(akrilamid-ko-akrilik asit) hidrojelleri sentezlenmiştir. Bir set hidrojel hazırlandığı şekilde kullanılmıştır. Bir set hidrojel reaksiyon ortamına sodyum klorür eklenmesi ve polimerleşme reaksiyonu tamamlandıktan sonra uzaklaştırılması ile gözenekli olarak hazırlanmıştır. İki set hidrojel farklı deşarj güçlerinde argon-plazma ile modifiye edilerek hazırlanmıştır. Hidrojeller ^{13}C-NMR, XPS, SEM, ATR-FTIR, ESR, dengede şişme derecesi (EDS) ve değme açısı ölçümleri ile incelenmiştir. Hazırlanan hidrojeller model antibiyotik, siprofloksasin-HCl (CPFX), ile yüklenmiş ve CPFX'nin hidrojel matrislerden in-vitro salımı değişen pH değerlerine sahip tampon çözeltilerde incelenmiştir. CPFX salım hızını etkileyen iki ana faktör vardır; biri CPFX'nin pH'ya bağlı çözünürlüğü, diğeri de hidrojellerin EDS değerleridir. Gözenekli hidrojellerde kontrol örneği ile kıyaslandığında ilaç yükleme kapasitesi ve CPFX salım hızı yüksek ve CPFX çözünürlüğü salım kinetiğinde başlıca etken olarak gözlenmiştir. Plazma uygulaması asidik ortamda salımı yavaşlatıcı etki yapmıştır.

Anahtar Kelimeler: Hidrojel, plazma yüzey modifikasyonu, poli(akrilamid–ko-akrilik asit), kontrollu salım.

To My Dear Family,

ACKNOWLEDGEMENTS

I would like to express my appreciation to Prof. Dr. Nesrin Hasırcı for her guidance and encouragement.

I also wish to give my special thanks to Tuğba Endoğan, Cantürk Özcan, Aysel Kızıltay, Eda Ayşe Aksoy and Sinan Güven for their valuable help, friendship and moral support.

Especially, I am grateful to my family and my fiancée Serkan for their support, understanding, and love.

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LIST OF SYMBOLS AND ABBREVIATIONS

AA	Acrylic acid
AAm	Acrylamide
APS	Ammonium persulfate
Ar	Argon
BIS	N,N-methylene bisacrylamide
CASING	Crosslinking by activated species of inert gases
CDD	Controlled drug delivery
CP	Cross-polarization
CPFX	Ciprofloxacin-HCl
CR	Controlled release
DDS	Drug delivery system
EDS	Equilibrium degree of swelling
ESR	Electron spin resonance
GI	Gastrointestinal tract
HEMA	2-Hydroxyethyl methacrylate
MAS	Magic-angle sample spinning
NMR	Nuclear magnetic resonance
SD	Standard deviation
SEM	Scanning electron microscopy
XPS	X-ray photoelectron spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Controlled Drug Delivery Systems

Biomaterial is defined as "the material used to replace part of a living system which is not functioning properly or to help the biological system in intimate contact with living tissue". Biomaterials are used for the production of various biomedical systems including pacemakers, sutures, bone plates, intraocular lenses, controlled drug delivery systems and so on [1]. Controlled drug delivery systems (DDSs) are designed to deliver drugs at predetermined rates for predefined periods of time. DDSs may be classified into two general concepts; one is targeting, the other is controlled release. Systems delivering active agent to the desired tissues and organs are called as "targeted DDSs" and systems controlling the release rate of active agent are called as "controlled DDSs" [2].

Controlled release systems were first developed in the 1950s and were originally used to administer nonmedical agents, such as antifouling substances and pesticides. They were first used in medical research in 1960s and in the 1970s systems for slow release of large molecules were developed. The earliest drug delivery systems, first introduced in the 1970s, were based on polymers formed from lactic acid. Today, polymeric materials still provide the most important parameters for drug delivery research, primarily because of their ease of processing and the ability of researchers to readily control their chemical and physical properties via molecular synthesis [3]. By the 1980s, several polymer-drug systems became available in clinical use [4].

Controlled release formulations are mentioned under different terminologies which differs slightly from each other, namely, (1) delayed release, (2) prolonged release, (3) sustained release, and (4) repeat action dosage forms.

In delayed release products; release of active substance is delayed for a finite "lag time", after which release is unhindered [e.g. enteric coated or "gastro resistant" oral tablets or capsules which remain intact in the stomach and only disintegrate in the higher pH of the small intestine].

In prolonged release products; the rate of release of active substance from the formulation after administration has been reduced, in order to maintain therapeutic activity, to reduce toxic effects, or for some other therapeutic purposes [5].

Sustained release products are designed to release loading dose to produce an immediate response, followed by a constant dose (maintenance dose) required to maintain a therapeutically effective level for some desirable period. Generally sustained release systems emit drugs in less than a day, and environmental conditions influence release rates, which leads to patient to patient variations.

A repeat action dosage form is designed to release initially the equivalent of a usual single dose of drug. And then after a certain period another single dose of the drug is released [6].

1.1.1 Conventional Drug Therapy versus Controlled Release

Providing control over the drug delivery can be the most important factor at times when traditional oral or injectable drug formulations cannot be used. These include situations requiring the slow release of water-soluble drugs, the fast release of low-solubility drugs, drug delivery to specific sites, etc. The ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove, and easy to fabricate and sterilize.

Controlled release systems aim to achieve a delivery profile that would yield a high blood level of the drug over a long period of time. With traditional formulations, the drug level in the blood follows the profile shown in Figure 1a, in which the drug blood level rises after each administration of the drug and then decreases until the next administration. With traditional drug administration the

blood level of the drug exceeds toxic level immediately after drug administration, and falls down below effective level after some time. Controlled drug delivery systems are designed for long-term administration where the drug level in the blood follows the profile shown in Figure 1b, remaining constant, between the desired maximum and minimum, for an extended period of time [7].

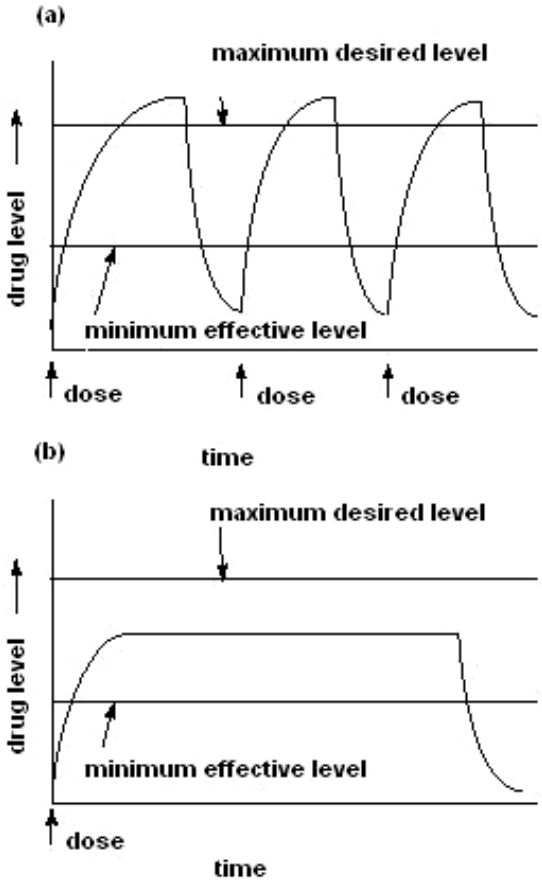


Figure 1.1 Drug levels in the blood plasma (a) traditional drug dosing, (b) controlled-delivery dosing

1.1.2 Controlled Release Mechanisms

Mechanisms for controlled release of drugs involve: (1) solvent activation, (2) diffusion, and (3) chemical reaction. Solvent activated systems may be either: osmotic or swelling controlled. A simple osmotic device (for water soluble agents) consists of a semipermeable membrane with an orifice, surrounding an osmotic drug core. When the device is introduced to an aqueous environment, water is absorbed at a controlled rate and a volume of saturated drug solution is released. The rate of drug release is constant as long as excess solid is present in the osmotic drug core. In swelling controlled systems release rate of the active agent is controlled by swelling rate of the polymer matrix. In diffusion controlled systems, release rate of the active agent is controlled by diffusion of active agent from an insoluble polymer. Diffusion controlled and swelling controlled systems may be either reservoir-type devices in which a drug formulation is present as a core surrounded by a polymer membrane, or monolithic devices where a dispersed or dissolved drug is uniformly distributed through a polymer matrix (Figures 2a, 2b). Chemically controlled systems may release drugs via polymer degradation or cleavage of drug from a polymer chain [8].

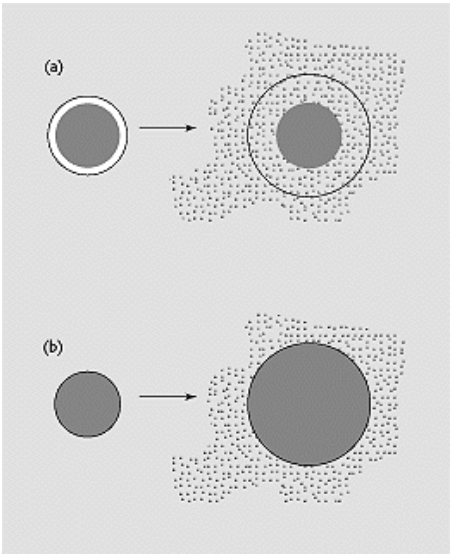


Figure 1.2 Drug release from (a) reservoir and (b) monolithic swelling-controlled release systems [7]

1.1.3 Controlled Drug Delivery Routes

Drugs are introduced into the body by several routes. They may be taken by mouth (orally); given by injection (parenteral route); or implanted beneath the skin (subcutaneously); placed under tongue (sublinguilly); instilled in the eye (ocular route) for local (topical) or bodywide (systemic) effects [9].

Drug delivery through the oral route has been the most popular method in pharmaceutical applications. In peroral administration drug is delivered to mouth, stomach, small intestine and colon. Drug carrier systems may be designed to release drugs in a controlled manner at the desired site. Gastrointestinal, GI, tract with its large surface area for systemic absorption is an attractive site of targetting drugs whereas colon-spesific delivery has significance for the delivery of peptide and proteins [10].

Drug delivery to the skin (transdermal route) has been conducted for topical use of dermatological drugs. It has generated much interest, beginning with the introduction of scopolamine (a sedative drug depressing nervous system) patch in 1983. Skin is also considered as a possible site for systemic delivery of drugs. However only eight drugs, which follow transdermal delivery, have been commercialized untill the year of 2000 [10, 11].

Ocular route is rather difficult subject due to blinking and low permeability of cornea. Conventional formulations fail because of being rapidly eliminated from the eye. Therefore controlled release systems are preferred since they offer prolonged ocular residence time [10].

Parenteral administration has advanced greatly in recent years for systemic and local drug delivery. Systems for parenteral route have evolved from simple polymers to colloidal systems which provide extended time for the circulation of drugs in the blood stream.

Nasal mucosa is considered to be the most permeable one of all the mucosal tissues, offering tremendous scope for peptide delivery particularly for vaccines. Several peptide formulations are on the market, but, high dose requirements and

low efficiency of deposition on alveolar surfaces limits the use of nasal route [11].

1.1.3.1 Colon Delivery

The development of drug delivery systems capable of selective release of drugs in the colon has received much attention in the last decades [12]. In addition to providing more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, colon specific delivery has an importance in oral delivery of macromolecular drugs. The colon may be the preferred absorption site for oral administration of protein and peptide drugs, because of the relatively low proteolytic enzyme activities in it. A colon-specific drug delivery system should minimize drug release in the stomach and small intestine, and release the drug abruptly in the colon. This requires a system that can respond to physiological changes in the colon. However, the physiological factors along the gastro-intestinal (GI) tract changes gradually. Enzymatic activity, motility, and fluid content decrease while pH increases. Since there is not a sudden change in the properties of the tract, colon-specific delivery becomes somewhat difficult. However, the presence of specific bacteria in the colon and an increasing pH gradient may be utilized as triggering components in colon-specific drug release. Several approaches have been proposed for targeted colon delivery, namely, prodrugs, coating of pH-sensitive polymers, use of colon-specific biodegradable polymers, timed released systems, osmotic systems, bioadhesive systems, pressure controlled drug delivery systems, and microflora-activated systems [13, 14].

pH sensitive polymers have been used in delivery of low-molecular-weight drugs as well as high-molecular-weight protein drugs. pH sensitive hydrogels have potential in formulating site-specific drug delivery systems to GI tract due to pH gradient throughout the tract [15]. For example, treatment of colon cancer has been aimed by approaches of oral drug administration. A pH-sensitive polymer Eudragit P-4135F (methacrylic acid, methyl acrylate and methyl methacrylate copolymer) was used to prepare microspheres, and relatively high drug load for 5-Fluorouracil was achieved [16].

1.1.4 Hydrogels

Hydrophilic polymers when crosslinked chemically or physically forming three dimensional networks; swell but do not dissolve in aqueous media. They are termed as hydrogels and are capable of absorbing water from 10-20% up to thousands times of their dry weight. Due to their unique characteristics, they have found applications in personal hygiene products, agriculture and other specialized areas like controlled drug delivery systems [17, 18].

First report on biomedical hydrogels was made in 1960 by Wichterle and Lim. They suggested that a hydrogel based on poly(2-hydroxy ethyl methacrylate) could be a synthetic biocompatible material [19]. Since then, hydrogels have found a wide range of biomedical applications including controlled drug delivery systems, replacement blood vessels, wound dressings, soft tissue substitutions, contact lenses and a variety of other related and potential uses. Hydrogels are generally found to be very well tolerated when implanted in vivo and can be easily tailored to suit the many functions of prosthetics in contact with blood or tissues. The success of hydrogels as biomaterials lies in their resemblance to living tissue because of their relatively high water content which minimizes the frictional irritation of surrounding tissue [20].

The relatively high water content of hydrogels makes them also permeable to small molecules like oxygen, nutrients, and metabolites. This high solute permeability makes them ideal materials of choice as devices for the controlled release of many drugs and other active agents. Much of the research on hydrogels has been focused on the application in controlled drug delivery. By proper design of hydrogels it is possible to control the kinetics of delivery of active ingredients [21]. Preparation of typical hydrogel-based drug delivery systems is given in Figure 1.3.

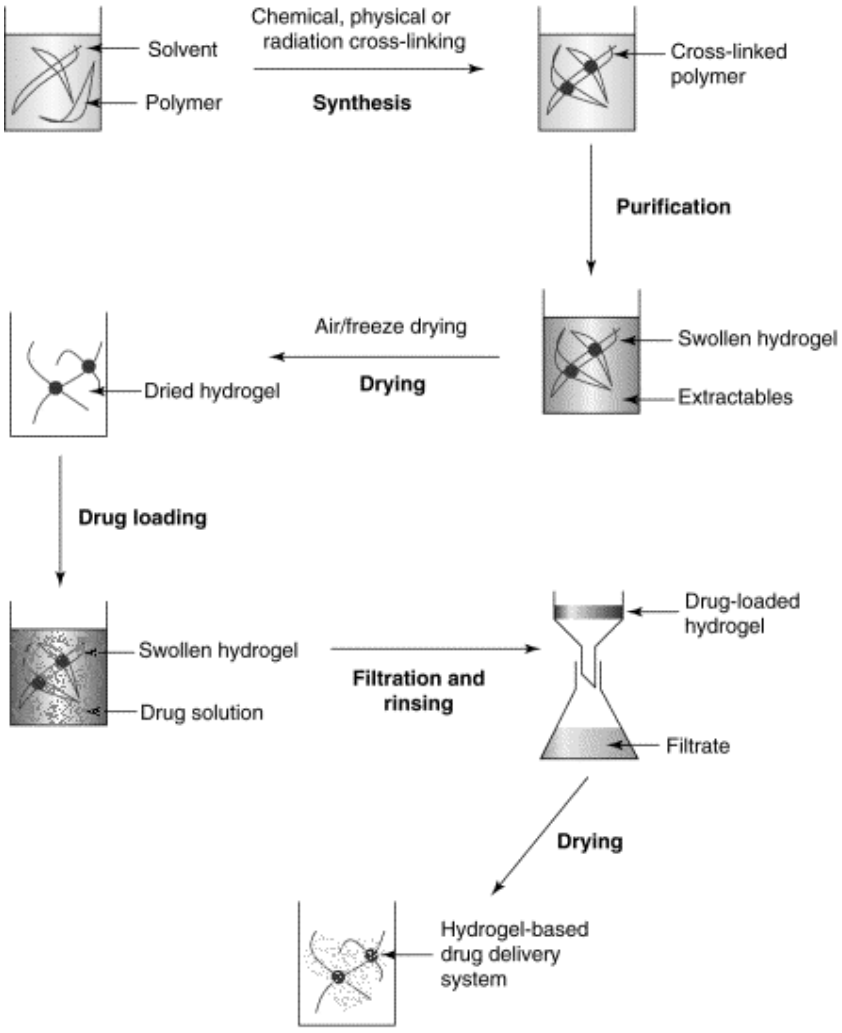


Figure 1.3 Preparation of hydrogel-based drug delivery systems [21]

1.1.4.1 Stimuli Responsive Hydrogels

The most characteristic property of hydrogels is their ability to swell in the presence of water and to shrink in the absence of it. The two most important factors controlling the extent of swelling are the hydrophilicity of polymer chains and the crosslink density. By incorporating some stimuli-responsive comonomers either into the backbone of the network structure or as pendant groups it is possible to prepare hydrogels with responsive properties. These hydrogels possess the ability to swell, shrink, bend or even degrade in response to a signal. These stimuli-responsive hydrogels are also called "intelligent hydrogels". They reversibly swell and shrink with small changes in the environmental conditions. The most common environmental factors that cause an abrupt volume changes in hydrogels are pH, temperature, electric field, ionic strength and type of salt (Figure 1.4). In addition to wide range of applications of these hydrogels in biomedical applications, they are also used in the separation and purification processes in industry.

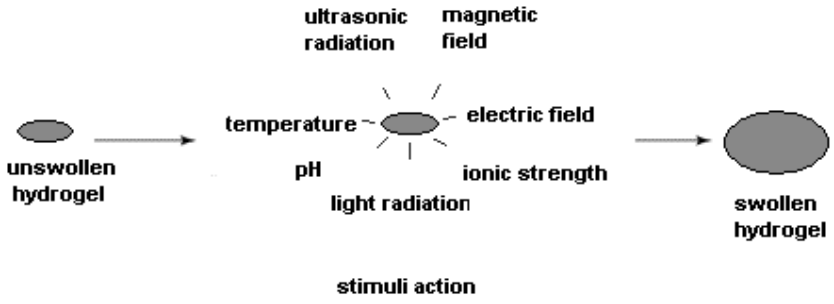


Figure 1.4 Stimuli that hydrogels respond

Hydrogels have been used extensively in the development of smart delivery systems. In these systems, polymeric matrix can protect drug from hostile environments (such as low pH and enzymes), while controlling drug release by changing the gel structure in response to environmental stimuli. The stimuli that hydrogel respond to may be physical or chemical. Drug release is triggered by various mechanisms as described in Table 1.1 [22].

Table 1.1 Stimuli responsive mechanisms of drug release from hydrogels [7]

Stimulus	**Hydrogel**	**Mechanism**
pH	Acidic or basic hydrogel	pH change causes swelling and release of drug
Ionic strength	Ionic hydrogel	Change in concentration of ions inside the gel causes swelling and release of drug
Chemical	Hydrogel containing electron-accepting groups	Formation of charge-transfer complex cause swelling and release of drug
Enzyme–substrate	Hydrogel containing immobilized enzymes	Product of enzymatic conversion causes swelling and release of drug
Magnetic	Magnetic particles dispersed in the hydrogel matrix	Applied magnetic field causes pores in gel and swelling followed by drug release
Thermal	Thermo-responsive hydrogel e.g. poly (N-isopropylacrylamide)	Change in polymer–polymer and polymer–water interactions cause swelling and drug release
Electrical	Polyelectrolyte hydrogel	Change in charge distribution causes swelling and drug release

The application of hydrogels in controlled drug delivery is sometimes limited by their low mechanical strength and slow response to stimuli. To increase the response rate, micro- and nanoporous hydrogels have been developed to offer responsive rates several orders of magnitude higher than non-porous gels. Size reduction is another approach to fast response. Hydrogels with a nanometer size could undergo volume change within a fraction of second upon exposure to a stimulus, because the time for polymer to diffuse is inversely proportional to square of dimension. The porosity favors faster water diffusion through the hydrogel network and higher swelling rate [23].

Crosslinking density is another factor affecting water absorption. Higher concentration of crosslinking agent will cause lower water absorbency. Higher crosslinker concentration produces more crosslinked points in polymeric chains and increases the extent of crosslinking of the polymer network, which results in less swelling when it is brought into contact with the solvent. High swelling capacity is also obtained with high ratio of ionizable groups. The presence of the ionic groups in polymer chains results in increasing of swelling because the ions are more strongly solvated rather than non-ionic groups in the aqueous medium. Generally, the extent to which the hydrogel swells at equilibrium increases with an increase in the concentration of functional ionizable groups on the network, and with a decrease in the extent of crosslinking occurred during the synthesis [24].

1.1.4.2 pH-Responsive Hydrogels

pH-responsive hydrogels are one of the most important class of stimuli-responsive hydrogels. All the pH-responsive polymers contain pendant acidic or basic groups which either accept or release protons in response to changes in environmental pH. The pH dependent swelling or shrinking response of polyelectrolyte gels is driven by the osmotic pressure resulting from a net difference in concentration of mobile ions between the interior of the polymer network and surrounding solution. pH-sensitive hydrogels can be weakly-acidic (anionic) or weakly-basic (cationic) depending on the nature of the ionizable moieties on their polymer backbones. The polymer swelling and shrinking characteristics depend on several factors, including their hydrophobic–hydrophilic nature, elasticity, charge density, and p*K*a values [25].

1.1.4.3 Acrylamide and Acrylic Acid Polymers

The industrial use of acrylic polymers has gained popularity due to the pioneering works by Pechmann and Rohm in the late 1930s. Acrylic polymers can be synthesized using different combinations of monomers to achieve distinctive properties. In 1950s the acrylic polymers such as Eudragit® (copolymers derived from esters of acrylic and methacrylic acid) and Carbopol® (polymers of acrylic acid) became popular and they are still currently being used worldwide in the pharmaceutical industry to control drug release, repel moisture, and develop special drug delivery systems [26].

Hydrogels prepared from polyacrylamide (PAAm) have many useful chemical and physical properties and consequently have been investigated for applications as smart polymers. These applications include biomimetic actuators, immobilisation of biocatalysts, drug delivery systems, and bioseparators [27]. Another application for PAAm hydrogels has been developed in the field of environmental analysis with the DGT (diffusive gradients in thin films) technique [28].

In the last years, research in the hydrogel delivery systems has focused primarily on systems containing poly(acrylic acid) (PAA) backbones. PAA hydrogels are known for their super-absorbency and ability to form extended polymer networks through hydrogen bonding. In addition, they are excellent bioadhesives, which means that they can adhere to mucosal linings within the gastrointestinal tract for extended periods, releasing their encapsulated medications slowly over time [3].

There are numerous studies covering acrylamide and acrylic acid homo- and copolymers in controlled release applications. Some selected recent studies are given in Table 1.2.

Table 1.2 Acrylamide and acrylic acid homo- and copolymers used in drug delivery

Polymer	**Active agent**	**Reference**
Poly(acrylamide-co-acrylic acid) hydrogels	Theophylline	[29]
Poly(acrylamide/maleic acid) hydrogels	Terbinafine hydrochloride	[30]
Polyacrylamide-grafted-guar gum	Diltiazem hydrochloride	[31]
Poly(2-hydroxyethyl methacrylate-co-acrylamide) hydrogels	5-Fluorouracil	[32]
Poly(vinylpyrrolidone-co-acrylic acid) hydrogel nanoparticles	FITC-dextran	[33]
Poly(pluronic-g-acrylic acid) tablets	Theophylline, hydrochlorothiazide, nitrofurantoin	[34]
Poly(ethylene oxide-g-acrylic acid) hydrogels	Insulin	[35]
Poly(acrylic acid-co-gelatin) hydrogel	Gentamicin sulphate	[36]
Poly(acrylic acid-co-methyl methacrylate) microparticles	Cisplatin	[37]
Poly(acrylic acid-co-dimethyl aminoethyl methacrylate)	Ketoprofen	[38]
Poly(acrylic acid)/poly(butyl acrylate) interpenetrating network	N-acetyl-5-methoxytryptamine	[39]

1.2 Surface Modification

Polymers have long been used in industrial fields for years. In the last decades their use in the synthesis of biomaterials and the applications in medical area gained great attention. Polymers can be obtained properly with the excellent required bulk properties. But sometimes the surface may not satisfy the desired surface properties for a specific application. In these cases surface modifications are needed to alter the properties of the surfaces in order to increase their performances in biological medium to be used in medical applications [40].

Surface modification is useful in:

1. producing functional groups at the surface
2. increasing surface energy
3. changing hydrophilicity/hydrophobicity
4. improving chemical inertness
5. introducing surface-crosslinking
6. increasing surface lubricity
7. removing contaminants
8. changing surface morphology (crystallinity, roughness)
9. increasing surface electrical conductivity [41]

Surface modification falls into two categories (1) chemically or physically altering atoms, compounds or molecules on the surface (etching and chemical alteration) and (2) coating the surface with a material having a different composition (coating, grafting and thin film formation). The results of surface modification are addition of materials, removal of materials, or alteration of materials [42].

Surface modification can be done by physically, chemically, or physico-chemically working processes as mentioned in Table 1.3. A Langmuir-Blodget film, which includes transfer of a monolayer on a solid substrate, is an example of physical surface modification. If the procedure is made several times, several layers of molecules over the substrate can be obtained in a very ordered self-assembly organization. Chemical etchants can be used to convert smooth hydrophobic polymer surfaces to rough hydrophilic surfaces by dissolution of amorphous regions and surface oxidation [41]. Physico-chemical modifications can be achieved by treatment with different energy sources which result in the

formation of radicals on the surfaces of polymers. Ionizing radiations, electric discharges are used in this kind of modifications [43]. Various chemical reactions are known to occur during ion-bombardment of polymers, namely, reduction, oxidation, crosslinking, ion implantation, loss of heteroatoms, etc [41].

Table 1.3 Surface modifications of polymeric material

Physically	Physical adsorption; Langmuir–Blodgett films
Chemically	Alkaline or acid etching; oxidation e.g. through ozone; other chemical transformations
Physico-chemically	Photo activation (UV); corona treatment; treatment with electron or ion irradiation; laser treatment; plasma treatment

1.2.1 Plasma Treatment

Irving Langmuir and his collaborators were the first to study phenomena in plasma in 1920's while working on the development of vacuum tubes for large current, and the term "plasma" was first used by Langmuir in 1929 [44]. Plasma contains activated species of electrons, charged particles, neutral atoms and molecules which are energetically reactive. These species cause some chemical and physical reactions at a solid surface which is placed in the reaction chamber. Therefore, alterations of the surface chemistry and morphology of the solid material is provided [45]. However, plasma technology has its own advantages and disadvantages, namely;

Advantages:

1. Surface is modified without altering bulk properties
2. All polymers can be modified by plasma application
3. Desired chemical reaction can be activated by the choice of suitable plasma gas
4. There are no reaction residuals, clean technology
5. Modification takes place uniformly

Disadvantages:

1. Necessity for vacuum environment increases the cost of application
2. Process parameters are highly system dependent
3. Large-scale production is not easy
4. Interactions between plasma and surface are complex [41]

Plasma application parameters like choice of carrier gas, flow rate, base pressure, discharge power and application time determines the final effects of plasma treatment.

1.2.2 Plasma Surface Modification of Biomaterials

Plasma surface modification is an effective and clean technology for many materials and of growing interest in biomedical engineering.

Compatibility of an artificial implant with the body is the most desired property. The implant and the host tissues should not be inducing undesired reactions such as inflammation. This necessitates biomaterials with very good surface properties in desired direction. Since it is not likely to have biomaterials fulfilling this requirement while already having adequate mechanical strength, etc.; surface modification is an important tool in improving surface properties of biomaterials such as biocompatibility and tribological properties.

Plasma-based technologies have some advantages for biomaterials engineering;

- Plasma homogeneity
- Plasma engineering is usually reliable, non-line-of-sight and applicable to different sample geometries as well as to different materials
- Plasma process can be monitored accurately
- Plasma treatment can result in various alterations on the surface. Chemical, tribological, mechanical properties of the surface changes
- Plasma processing can provide sterilization
- Plasma technologies are compatible with masking applications

Plasma-deposited films on biomaterials offer the advantages of easy preparation, unique film chemistry, coated on unique substrates with good adhesion, conformal and pin-hole free films, and excellent permeation barriers with low

level of leachables, sterile upon preparation. Beyond these, plasma treatment is utilized in many different areas of biomaterials science as summarized in Table 1.4 [42].

Table 1.4 Common research areas and applications of plasma treatment in biomaterials engineering

Research area	**Applications**
Blood compatible surfaces	Vascular grafts, catheters, stents, heart-valves, membranes (e.g. for hemodialysis), filters (e.g. for blood cell separation), biomolecules immobilized on surfaces
Non-fouling surfaces	Intraoculars (IOLs), contact lenses, wound healing, catheters, biosensors
Tissue engineering and cell culture	Cell growth, antibody production, essays, vascular grafts
Sterilization of surgical tools and devices	Cutting tools of surgeon, tweezers
Biosensors	Biomolecules immobilized on surfaces
Barriers coatings	Drug-release, gas-exchange membranes, device protection, corrosion protection, reduction of leaches

1.2.3 CASING

CASING stands for "Crosslinking by Activated Species of INert Gases". Inert gas plasmas create free radicals at surface but do not add new functional groups. Free radicals created by these plasmas can only react with other surface radicals or with other chains (excluding post-plasma reactions). This results in recombination, unsaturation, branching and crosslinking [46].

CASING was one of the earliest recognized plasma treatment effects [47]. When using an inert gas, such as argon, the main effect of plasma is to produce a crosslinked layer. Plasma induced crosslinking reaction can be considered as a thin film layer formation on the surface of the sample, since effect of the plasma treatment is limited to surface [48].

The exposure of the polymer to the inert gas plasma (such as argon) is sufficient to abstract hydrogen and to form free radicals at or near the surface which then interact to form the crosslinks and unsaturated groups with the chain scission. The plasma also removes the low-molecular-weight materials or converts them to a high-molecular-weight by crosslinking reactions. As a result, the weakly bound layers formed by the low-molecular-weight materials are removed [49].

Surface-crosslinking was utilized in increasing O_2-plasma-induced hydrophilicity. Hyun et al. used Ar^+ ion beam irradiation prior to O_2 plasma treatment in order to reduce polymer chain mobility from the surface to the bulk [50].

The effect of plasma-induced surface crosslinking of poly-(vinyl chloride) (PVC)-based flexible films was investigated to limit its migration (plasticizers, etc.) from packaging into fatty foodstuffs, and migration was prevented by surface crosslinking of films [51].

1.2.4 Plasma Surface Modification in Controlled Delivery Applications

Plasma-induced surface-crosslinking can be considered as a thin film formation process on the surface of polymeric materials. This phenomenon can be utilized in formulating sustained-release systems in order to extend the release durations of the drugs [48].

Formation of barrier coatings by plasma treatment is used in the design of controlled release systems. An earlier study held at the University of California involves CASING by means of which the surface of a hydrogel is modified to create a barrier membrane in situ. The initial attempts to crosslink surface of poly(2-hydroxyethyl methacrylate) (PHEMA) itself did not produce the anticipated retardation of pilocarpine-HCl release rate, but the related technology of generating a polyolefin film in situ did yield favorable results. Films on the surface of water swollen hydrogels of HEMA homo- and copolymers did modify the release kinetics from first order to zero order [52].

Plasma techniques which were used in the preparation of double-compressed tablets for reservoir type drug delivery systems of sustained- and delayed-release are shown in Figure 1.5. For Theophylline delivery which was conducted from reservoir type O_2- and Ar-plasma-treated samples, it was reported that polymers were more susceptible to surface-crosslinking than degradation when Ar is used as plasma gas. It was also reported that plasma-induced surface-crosslinking can be used to supress drug release from systems made of water-soluble polymers like poly(methacrylic acid) and polyacrylamide [53].

Matrix type drug carrier systems were also prepared with the aid of plasma treatment (Figure 1.5). For their preparation, mechanical vibration of plasma-irradiated polyethylene powder was carried out in the presence of theophylline powder in order to immobilize theophylline onto polyethylene matrix formed by inter-particle linkage. Theophylline release was supressed when compared to non-plasma-irradiated polyethylene [53].

Another investigated effect of plasma treatment on controlled release systems is the alteration of hydrophilicity of drug carrier. It is reported that the surface free energy and work of adhesion of O_2 plasma modified tocopherol loaded poly(ε-

caprolactone) microcapsules were increased as compared with unmodified ones, and release rate was increased with increasing the total application time of plasma treatment, due to the increase of hydrophilic groups containing oxygen on the microcapsules during the plasma treatment [54].

In another study, it was aimed to make poly(dl-lactic-co-glycolic acid) (PLGA) capsule surface enough hydrophobic, so that they would not have initial burst release and provide controlled slow release speed. The CF_4/He plasma and C_3F_6/He plasma treatments were applied in order to form a fluorinated layer on the PLGA capsule surface. It was reported that 5 cycles application of the C_3F_6/He plasma showed the prevention of the initial burst release and the slow release speed, but 7 cycles of treatment resulted in faster release due to the fact that excessive long time plasma treatment caused degradation and destroyed the PLGA capsules. Insulin which was loaded into PLGA capsules was not released completely from the treated PLGA capsules. The reason was explained as the treated PLGA capsules were condensed in the water because of their hydrophobicity [55].

RF-plasma-deposition of n-butyl methacrylate (BMA) onto drug-loaded polyurethane (PU) was conducted as to create a rate-limiting barrier to provide a constant, sustained release for ciprofloxacin. Deposition power and deposition time determined release kinetics. Increased crosslinking of BMA layer reduced release rates. Burst effect was eliminated, release kinetics approached zero-order with plasma-coating of PU [56].

Plasma technology is an important tool in the production of controlled release systems. With the aid of plasma treatment, it is possible to fasten release rate (increasing hydrophilicity of drug carrier), to prevent burst effect, to slow down release (making drug carrier hydrophobic, surface crosslinking, or thin film formation on the surface) and to modify kinetics of release.

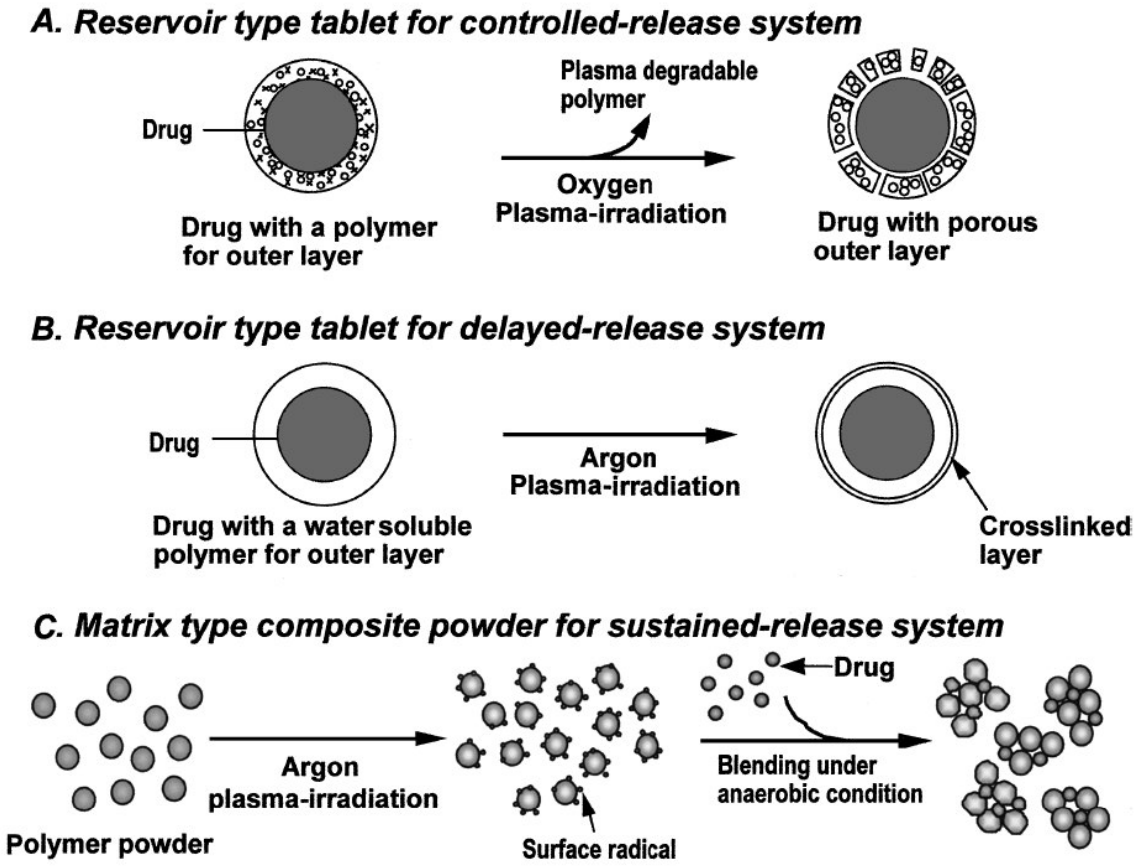


Figure 1.5 Drug delivery systems prepared by plasma applications [53]

1.3 Aim of the study

In this study the aim was to prepare pH-sensitive acrylic-based hydrogels as drug delivery systems and to be able to control the release rates by modifying the structures by various methods. For this purpose hydrogels were prepared using acrylamide and acrylic acid. The prepared matrices were modified by two different techniques. To one set of hydrogels, Ar-plasma was applied for surface modification and crosslinking for suppressing drug release and preventing burst effect. Another set of hydrogels were prepared by salt-leaching technique to form extra-porous systems to enhance release rates. A model drug ciprofloxacin-HCl (CPFX) was used in the release experiments. Effects of modifications on the release kinetics of CPFX, in different buffer solutions with varying pH values, were examined.

CHAPTER 2

EXPERIMENTAL

2.1 Materials

Acrylic acid (stabilized, 99.5%) and ammonium persulfate (98%) were purchased from Acros, USA. Acrylamide (99.9%) was purchased from J.T. Baker, Netherlands. N,N-methylene bisacrylamide (99%) was purchased from Aldrich, USA. Sodium Chloride was supplied from Merck, Germany. Ciprofloxacin-HCl (MW=385.8) was supplied from Deva, Turkey. All chemicals were used without further purification.

2.2 Instrumentation

2.2.1 Plasma Instrument

Plasma system (Advanced Plasma Systems Inc., USA) was used for surface modification of polymers. A picture of the instrument is given in Figure 2.1. Instrument consists of vacuum chamber, manifold unit, vacuum pump, power distribution box, RF power supply and matching network.

Vacuum chamber is the aluminum container in which the actual plasma process takes place. It bears electrodes, purge inlet board, vacuum break and chamber door. Electrodes are paired aluminum assemblies located inside the chamber. During the plasma cycle, these assemblies are excited by RF generator, creating the actual plasma used to treat the products. Manifold contains all the inlet and exhaust equipment to vent gases to and from the chamber. Vacuum pump unit is a two-stage mechanical pump that removes gases from the chamber, insuring a continuous flow. Seren R300 13.56 MHz power generator is used as RF power supply. Matching network is used to match the impedance of the RF source to the impedance of the chamber, eliminating reflected power.

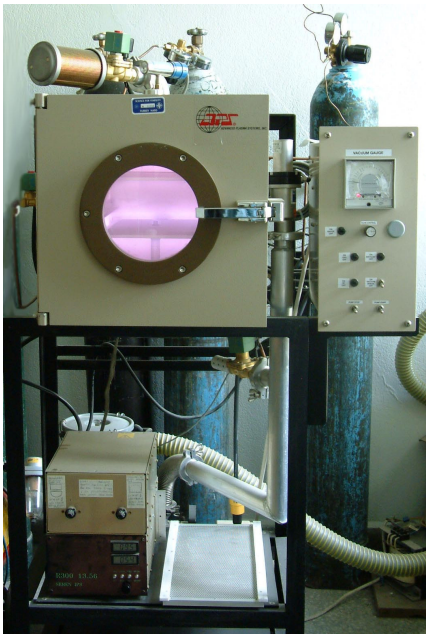


Figure 2.1 Plasma instrument

2.2.2 Solid State ^{13}C Nuclear Magnetic Resonance (^{13}C-NMR)

Solid state ^{13}C-NMR experiments were carried out using a Bruker Advance spectrometer at 300 MHz (Germany). High resolution ^{13}C-NMR was performed using magic-angle sample spinning (MAS) and high power spin decoupling. To enhance the signal to noise ratio, the cross-polarization (CP) technique was applied. The chemical shifts of ^{13}C spectra are reported in ppm relative to tetramethyl silane. Ultrashield superconducting magnet with 4 mm MAS probe was operated at a carbon frequency of 75.38 MHz and proton frequency of 299.77 MHz.

2.2.3 Attenuated Total Reflactance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Bruker VERTEX 70 spectrometer (Germany) equipped with PIKE Single Reflection MIRacle ATR component was used. ATR crystal was diamond.

2.2.4 X-Ray Photoelectron Spectroscopy (XPS)

XPS spectra of the plasma modified samples and the control were acquired on a SPECS SAGE spectrometer (Germany) with a monochromatic Mg-K alpha radiation source (1253.6 eV) at a power of 200 W. Base pressure was $1.3x10^{-9}$ mbar and a take-off angle of 90° with respect to the samples surface was used. The wide scan spectra for identification of elements were obtained over the range 0–1000 eV, using pass energy of 48 eV. The same pass energy was also used in obtaining the high-resolution spectra.

2.2.5 Scanning Electron Microscopy (SEM)

Physical appearances of the prepared samples were examined by Scanning Electron Microscope (JEOL JSM-6400, NORAN Instruments, Tokyo, Japan).

2.2.6 Electron Spin Resonance (ESR) Spectroscopy

Electron Spin Resonance spectra of the plasma-modified hydrogel samples were recorded at room temperature using a Bruker ESP-300 X-band spectrometer (Germany). The concentration of the paramagnetic species was evaluated from the double integrations of the spectra obtained with the following settings: magnetic field sweep=20 mT, microwave power=1–3 mW and modulation frequency=100 kHz. The ESR measurement was carried out in order to detect the free radicals if present after glow-discharge applications.

2.2.7 UV-VIS Spectrophotometer

CPFX concentrations were detected spectrophotometrically by using Agilent 8453 UV-Vis spectrophotometer (USA).

2.3 Experimental Procedure

2.3.1 Synthesis of Hydrogels

For polymer synthesis, acrylic acid (AA) and acrylamide (AAm) monomers were reacted in 50:50 (w/w) ratio and polymerization was carried out in the presence of N,N-methylenebisacrylamide as crosslinker (2.5% with respect to total monomer weight) and ammonium persulfate as initiator (5% with respect to total monomer weight). 6 grams of monomer (3 g AA+ 3 g AAm), crosslinker (150 mg) and initiator (300 mg) were dissolved in 10 mL distilled water and poured into glass test tubes. N_2 was purged through the monomer solution for 10 minutes in order to remove dissolved O_2 which inhibits polymerization reaction. Polymerization was held in a water bath of 37°C for three hours.

Resultant hydrogel rods were removed from tubes and cut into discs. Then they were washed in distilled water for three days (water refreshed at least 15 times) in order to remove any possible residual monomers. Hydrogel discs were dried at ambient temperature until constant weight. The thicknesses of the dry discs were about 2 mm, and the diameters for all were about 11 mm. Disc-shaped hydrogel samples weighed 194.19±21.23 mg. The monomer conversion after the polymerization was determined from the weight differences of the total initial monomer and the purified polymer. Polymer was purified by the extraction of the hydrogels in an excess of water and then drying the insoluble polymer to constant mass.

2.3.2 Modification of Hydrogels

Hydrogels were modified either by salt-leaching technique or by plasma discharge application.

2.3.2.1 Salt Leaching

In order to obtain porous hydrogels, 1 g NaCl salt was added into the polymerization medium. NaCl powder was crashed and sieved through 125-250 μ

mesh prior to addition. After the polymerization reaction completed, polymer rods were cut and washed with distilled water for three days (water refreshed at least 15 times) for the removal of residual monomers and salt crystals. Hydrogel discs were dried at ambient temperature until constant weight. The thicknesses of the dry discs were about 2 mm, and the diameters for all were about 11 mm. Disc-shaped hydrogel samples weighed 170.50±25.66 mg. This set of hydrogels are mentioned under the sample code "prs" throughout the thesis.

2.3.2.2 Plasma Treatment

Washed and dried disc-shaped hydrogel samples were subjected to argon plasma treatment at two different discharge powers of 20 and 100 W. Plasma treatment parameters are given in Table 2.1. After the samples were placed into the plasma chamber, the system was evacuated to a pressure of 20 mTorr. Then Ar gas was inlet into the system and Ar flow rate was controlled in order to set the plasma pressure at the 20 mTorr and discharge was applied for 15 minutes. After the treatment the reactor was kept under Ar atmosphere for 30 minutes, to protect the surface against contamination which could have taken place in the reactor immediately after the exposure to air.

Table 2.1 Plasma treatment parameters

Sample code	**Gas**	**Input Power (W)**	**Time (min)**
Control	-	0	0
Pls 20	Ar	20	15
Pls 100	Ar	100	15

2.3.3 Swelling Studies

Swelling experiments were performed by placing the prepared polymer discs in buffer solutions of varying pHs of 2-10 in a water bath at 37°C and measuring

weight gain as a function of time. Preparation of buffer solutions is given in Appendix A. The discs were withdrawn from the buffer solutions and weighed after removal of excess surface water by gentle blotting with a tissue.

Percent swelling is expressed as the percent weight ratio of water held in hydrogel to dry hydrogel at any instant during swelling.

$$\% \text{ swelling} = \frac{(\text{weight of swelled hydrogel} - \text{weight of dry hydrogel})}{\text{weight of dry hydrogel}} \times 100$$

Equilibrium degree of swelling valus are the maximum swelling values of the samples.

2.3.4 Contact Angle Measurements

Control sample and plasma-modified hydrogel samples were used in contact angle measurements for the investigation of hydrophobicity-hydrophilicity change at the surface by plasma treatment. Contact angles of the samples were calculated from the photographs of the deionized distilled water droplets on the polymer surfaces which were taken immediately after plasma treatment at room temperature. At least ten measurements were achieved for each sample. A Windows Excel computer program was used in the calculations of contact angles.

2.3.5 SEM Characterization

Hydrogel samples were swelled in acidic (pH 2) and basic (pH 10) buffer solutions for 24 hours and then freeze-dried in their swollen forms. After being sputter-coated with gold, SEM micrographs were obtained.

2.3.6 Drug Loading and Release Experiments

Dried disc-shaped hydrogels were allowed to swell in ciprofloxacin-HCl (CPFX) solutions (5mg/mL distilled water) for 72 hours for drug loading, then the polymers were separated and let to dry to constant weight at ambient conditions.

Amount of drug loaded was determined spectrophotometrically from the drug solutions that hydrogels swelled in and were found to be about 45 mg for the porous ones (prepared by salt-leaching) and about 23 mg for the control and plasma applied ones. For release experiments, dried hydrogel discs containing CPFX were placed in 15 mL buffer solutions of varying pH (2, 4, 6, 8, 10) at 37°C. Release experiments of CPFX were carried out under sink conditions where the buffer solutions were refreshed for every 24 hours and the amount of CPFX was detected spectrophotometrically. The corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release (calculated from the total amount of CPFX contained in each matrix) versus time.

Amphoteric quinolones, such as CPFX (Figure 2.2), have pH-dependent UV absortion spectra which depend on the protonation state of the carboxyl group. Figure 2.6 shows the UV absorption spectra of CPFX solution in the wavelength interval from 200 nm to 400 nm at different pH values. Three main maxima are observed in all spectra. With increasing pH, the first maximum shifts to a lower value while the second and third maxima shift to higher values. These changes can be attributed to the extent of ionization of the carboxylic group as a consequence of the removal of a proton.

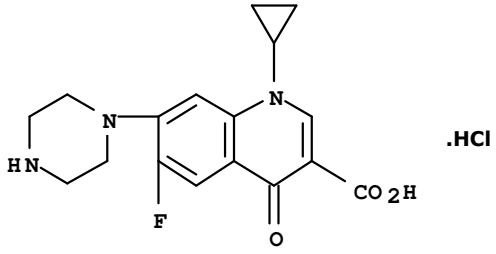


Figure 2.2 CPFX molecule

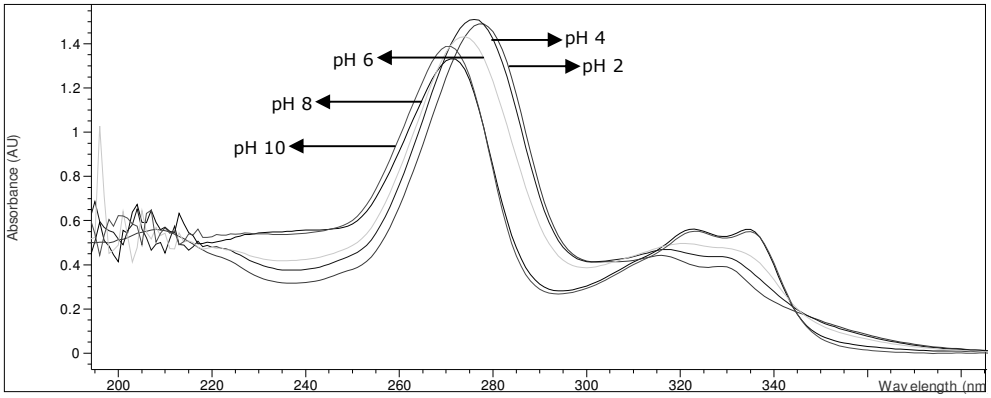


Figure 2.3 UV-Vis spectra of CPFX solution at varying pH values

To avoid systematic errors, the concentration of each sample was not determined spectrophotometrically by the standard procedure at a single wavelength, but instead the absorbance of the solution was systematically taken at the position of the first maximum in the spectrum as given in Table 2.2. Calibration curves for CPFX at varying pHs are given in Appendix B.

Table 2.2 Maximum wavelengths versus pH

pH of buffer solution	2	4	6	8	10
First maximum wavelength (nm)	277	276	274	271	271

For the investigation of release kinetics; zero-order (1), first-order (2) and Higuchi (3) equations, which are given below, were used;

$$Q_t = Q_0 + k_0 t \qquad (1)$$

where Q_t is the amount of drug released at time t, Q_0 the amount of drug in the solution at t=0, (usually, Q_0=0) and k_0 the zero-order release constant

$$Q_t = Q_\infty (1 - e^{-k_1 t}) \qquad (2)$$

Q_∞ being the total amount of drug in the matrix and k_1 the first-order kinetic constant

$$Q_t = k_H t^{1/2} \quad (3)$$

k_H representing the Higuchi rate constant. Furthermore, in order to better characterize the drug-release behavior for the studied polymeric systems, the Korsmeyer–Peppas (4) semi-empirical model was applied

$$Q_t/Q_\infty = kt^n \quad (4)$$

where Q_t/Q_∞ is the fraction of drug released at time t; k is a constant comprising the structural and geometric characteristics of the tablet; and n is the release exponent where it is a parameter which depends on the release mechanism. In the calculations, only the points within the interval of $0.1<Q_t/Q_\infty<0.7$ were used.

Codes of all the samples prepared and used in release experiments are given in Table 2.3.

Table 2.3 Sample codes of the prepared samples

Sample code	**Control**	**Pls 20**	**Pls 100**	**Prs**
Modification	No modification	Subjected to Ar plasma at 20 W for 15 minutes	Subjected to Ar plasma at 100 W for 15 minutes	Salt-leached

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Polymerization Reaction

After the copolymerization of acrylamide and acrylic acid, percent polymerization was found to be ~91% for the porous (prepared by salt-leaching) and ~92% for the other samples. Hydrogels are formed by copolymerization of acrylamide and acrylic acid. The reaction is a vinyl addition polymerization initiated by ammonium persulfate. The persulfate free radicals convert acrylic monomers to free radicals which react with unactivated monomers to begin the polymerization chain reaction (Figure 3.1). The elongating chains are randomly crosslinked by N,N-methylene bisacrylamide resulting in closed loops and a complex "web" polymer with a characteristic porosity which depends on the polymerization conditions and monomer concentrations. Termination reactions are given in Figure 3.2 and structure of the copolymer is given in Figure 3.3.

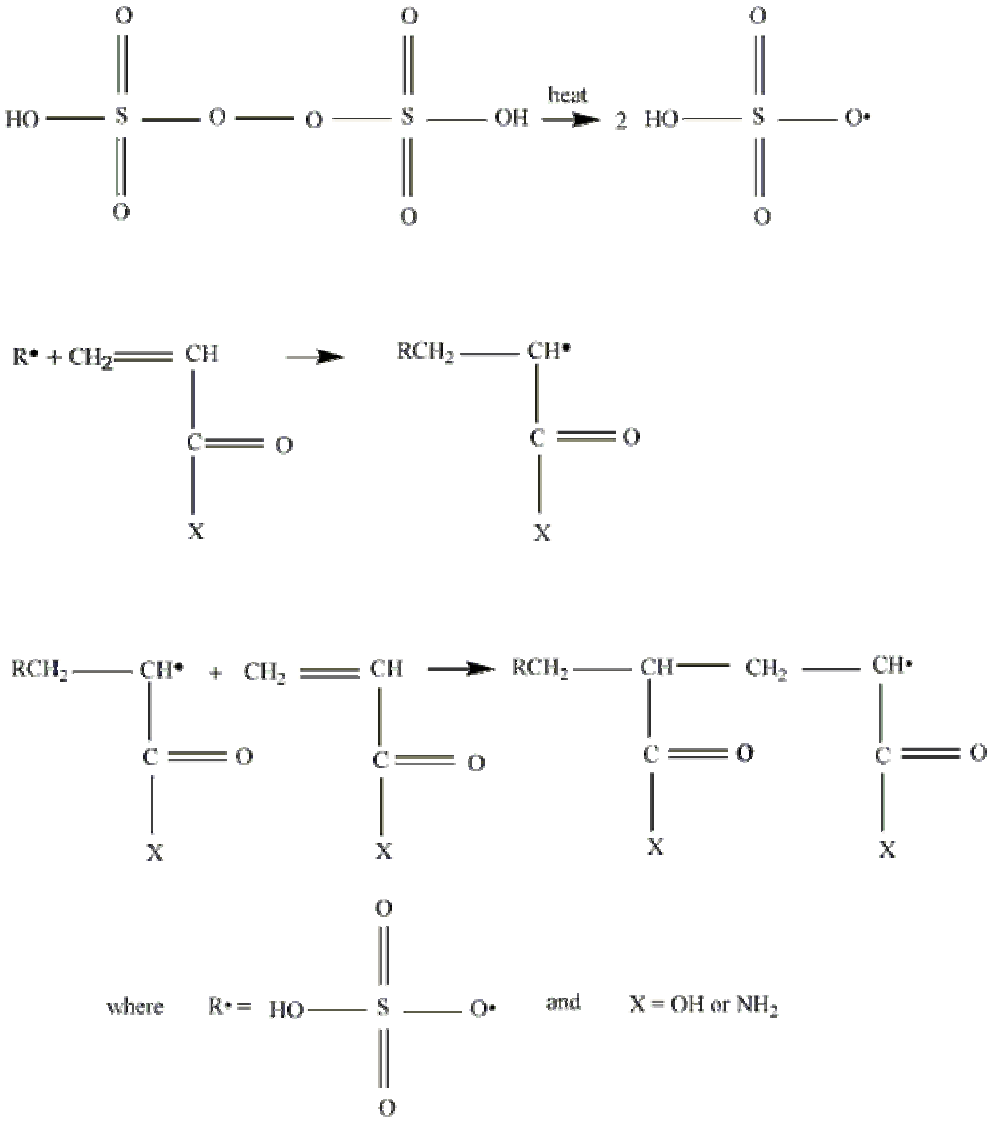


Figure 3.1 Initiation and propagation steps of the polymerization reaction

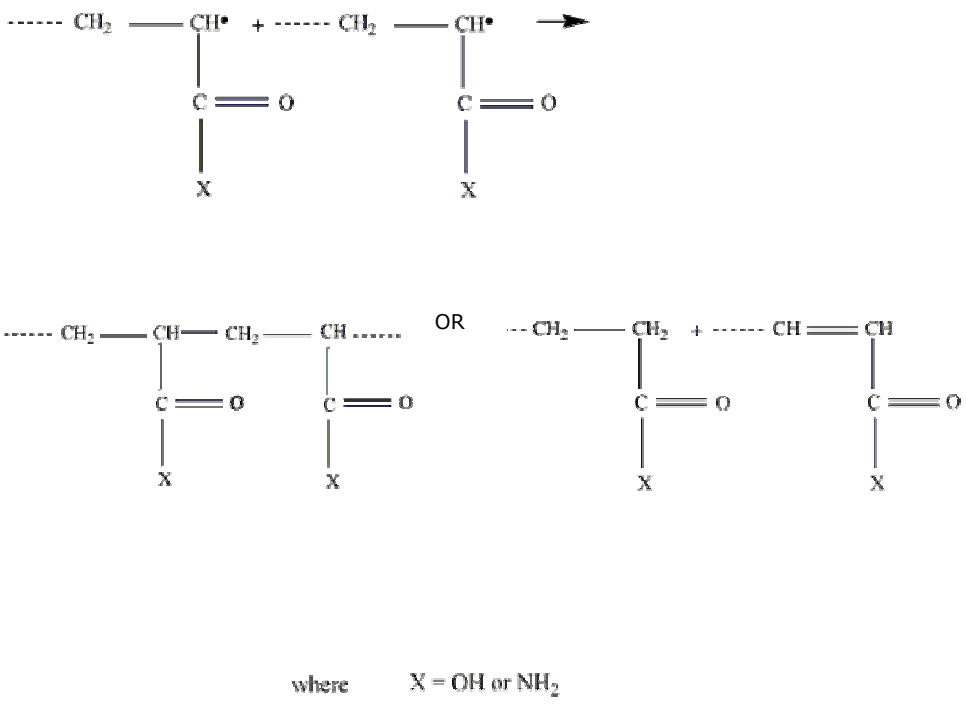


Figure 3.2 Termination step of the polymerization reaction

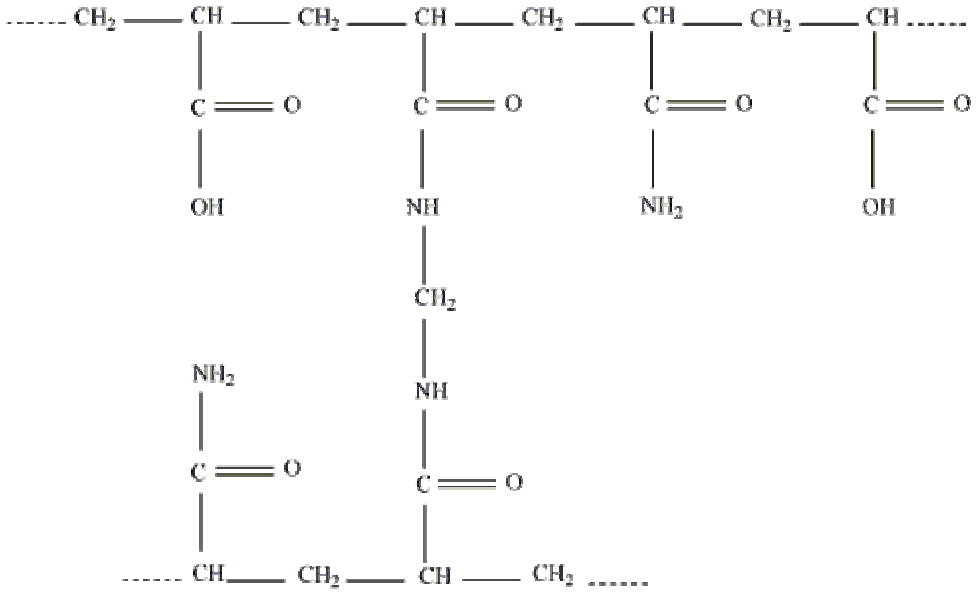


Figure 3.3 Structure of the copolymer

3.2 Swelling Studies

Equilibrium degree of swelling (EDS) values and swelling profiles of the samples are given in Figures 3.4-3.12 and Table 3.1. Acrylic acid has carboxylic acid moiety which deprotonates at basic medium and result in swelling, so higher EDS values are obtained for the copolymeric hydrogel samples at higher pH values (Figure 3.4). As a general trend, all the samples showed higher EDS values at higher pH values compared to lower ones, as expected. Swelling profiles could not have been investigated for pH 8 and 10 due to disintegration of hydrogels during the experiments.

EDS values for control sample is given in Figure 3.5. Control samples swell up to 197.0% and 849% of their dry weights at pH 2 and 10, respectively. As in all the samples; EDS increases with increasing pH.

For pls 20 sample EDS values are given in Figure 3.6. Pls 20 samples swell up to 198.6% and 850.0% of their dry weights at pH 2 and 10, respectively. In Figure 3.7 EDS values of pls 100 samples are given; which shows an increase from 194.9% to 862.1% for pH 2 and 10, respectively.

EDS values for prs sample is given in Figure 3.8. It reveals that prs sample has the highest EDS values among all the samples. EDS values for prs samples increase from 393.6 to 999.4 as the pH increases from 2 to 10. For pH values of 2 and 4, EDS values for the prs samples were almost the double of the other samples at the same pH values. On the other hand, control and plasma modified samples demonstrated very similar values of EDS at every pH media (Figures 3.5-3.7).

Swelling profiles of the control and plasma-treated samples are given in Figures 3.9-3.11. For all samples it was observed that, the sample gets double value in 4-5 hours and reach to maximum swelling in about 24 hours. Plasma-treated samples did not show apparent differences with the control sample in terms of swelling characteristics and EDS. The reason for that may be the fact that plasma modification is limited to a very fine surface layer that has no effect on swelling.

Swelling profile of the prs sample is given in Figure 3.12. Swelling profile shows that prs sample was fast-swelling when compared to control sample (Figure 3.9). Prs samples swelled up to 999.4% of their dry weight at pH 10.

Table 3.1 Equilibrium degree of swelling values of the samples

Sample	**EDS**				
	pH 2	**pH 4**	**pH 6**	**pH 8**	**pH 10**
Control	197.0	298.5	355.0	761.3	849.0
Pls 20	198.6	334.5	365.8	725.8	850.0
Pls 100	194.9	291.5	363.8	749.3	862.1
Prs	393.6	495.4	596.3	922.2	999.4

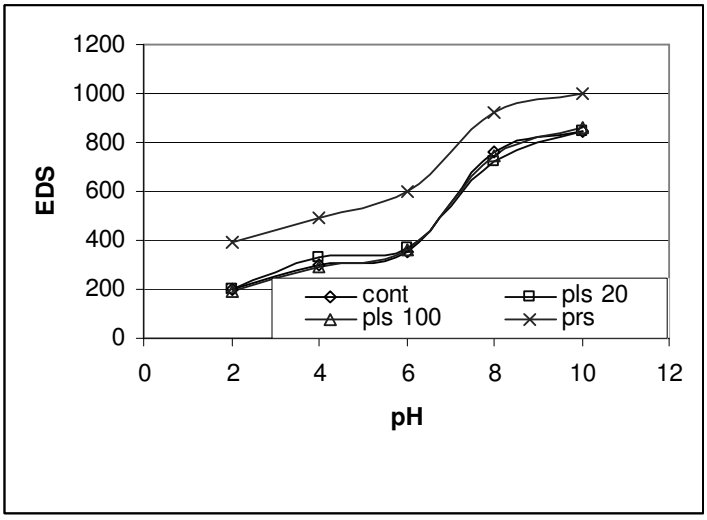


Figure 3.4 EDS values of the samples versus pH

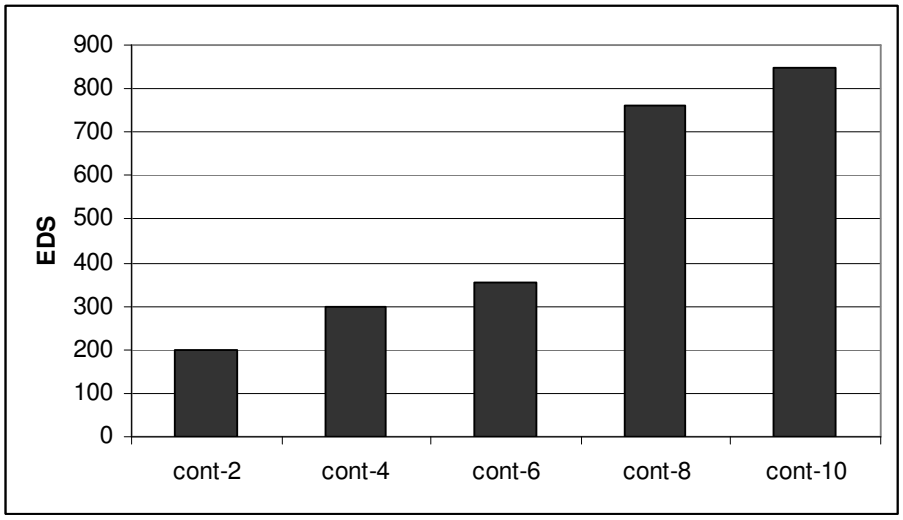


Figure 3.5 Equilibrium degrees of swelling values for control sample

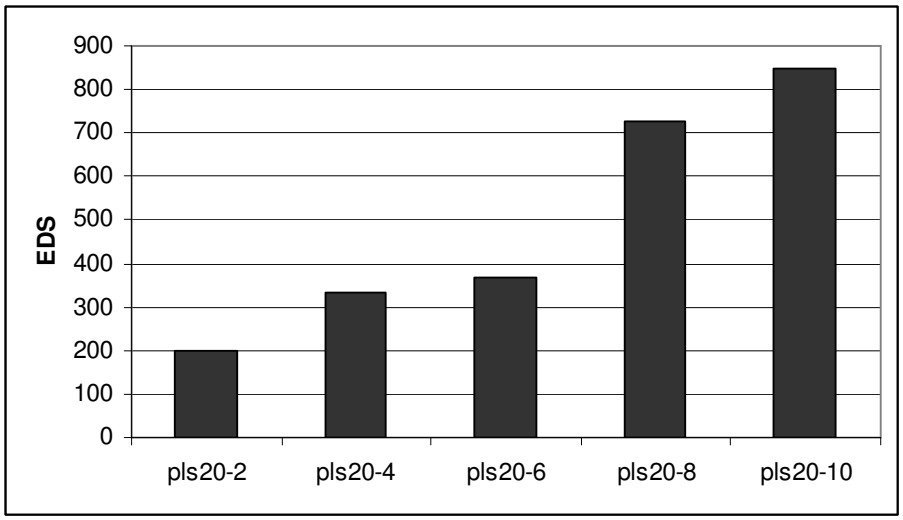


Figure 3.6 Equilibrium degrees of swelling values for pls 20 sample

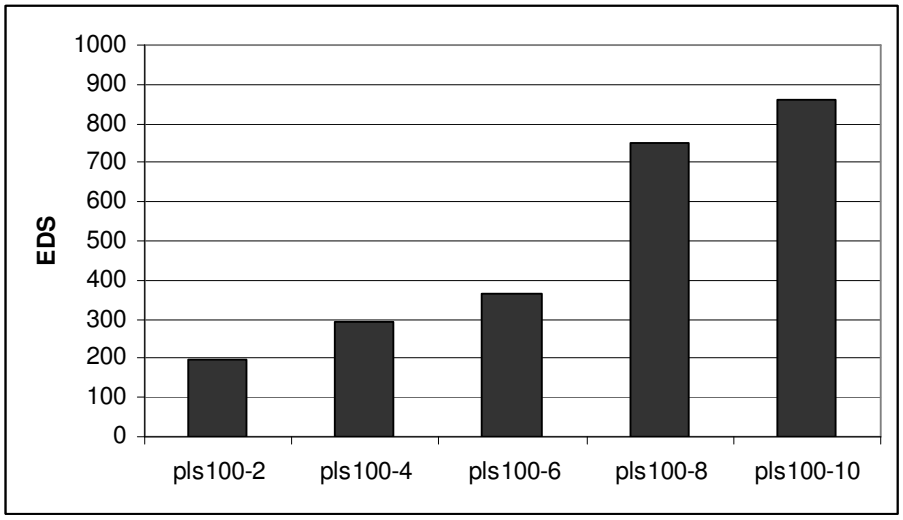


Figure 3.7 Equilibrium degrees of swelling values for pls 100 sample

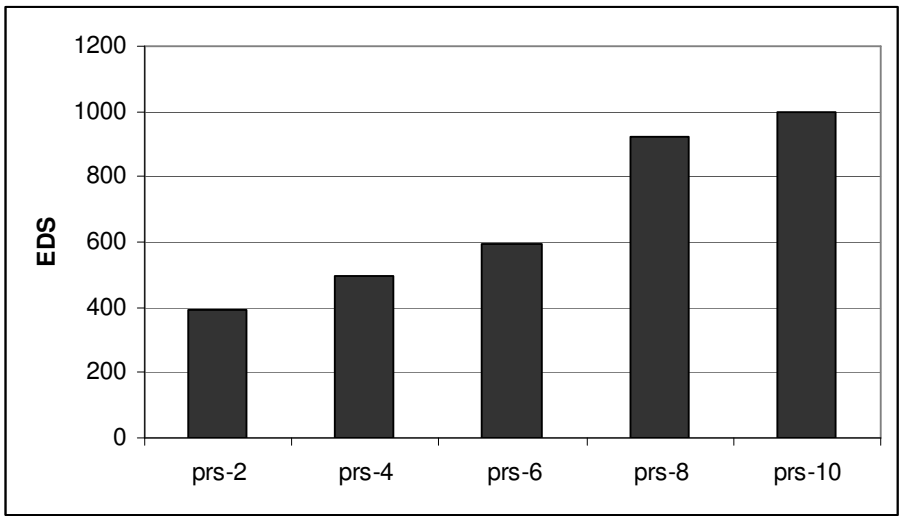


Figure 3.8 Equilibrium degrees of swelling values for prs sample

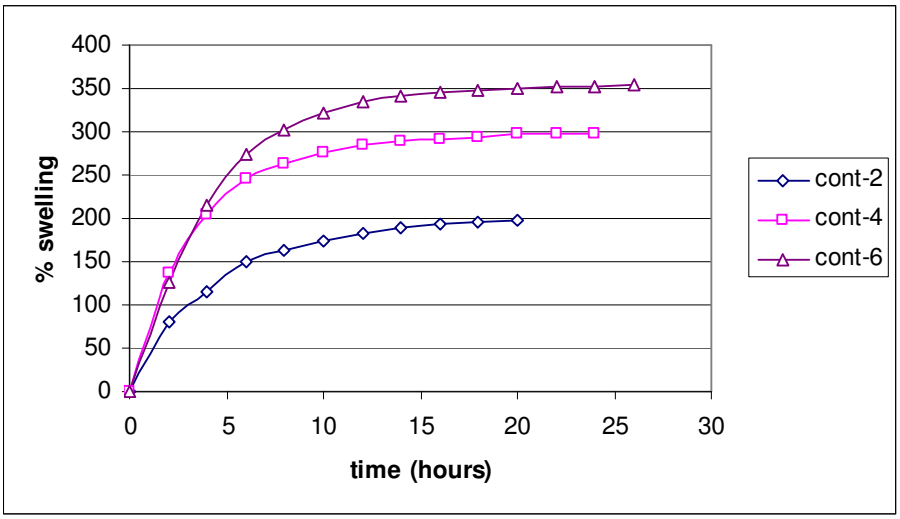


Figure 3.9 Percent swelling change by time for control sample

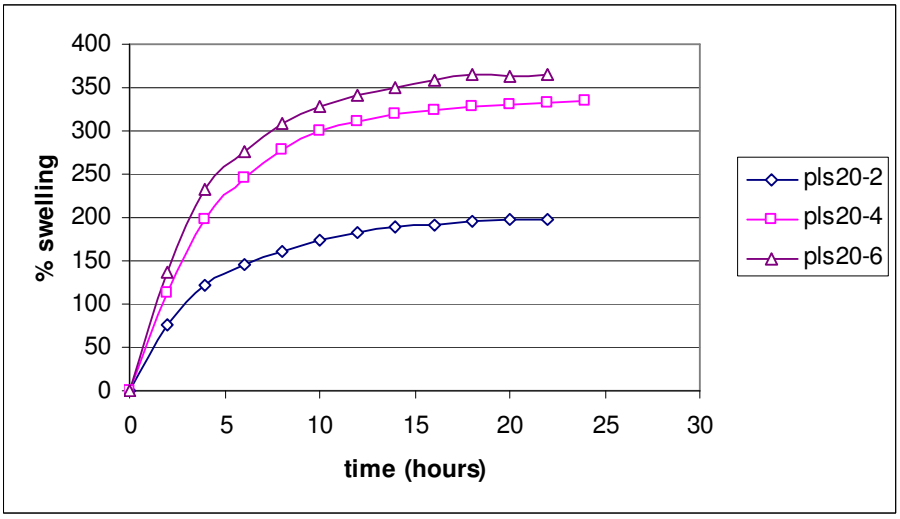


Figure 3.10 Percent swelling change by time for pls 20 sample

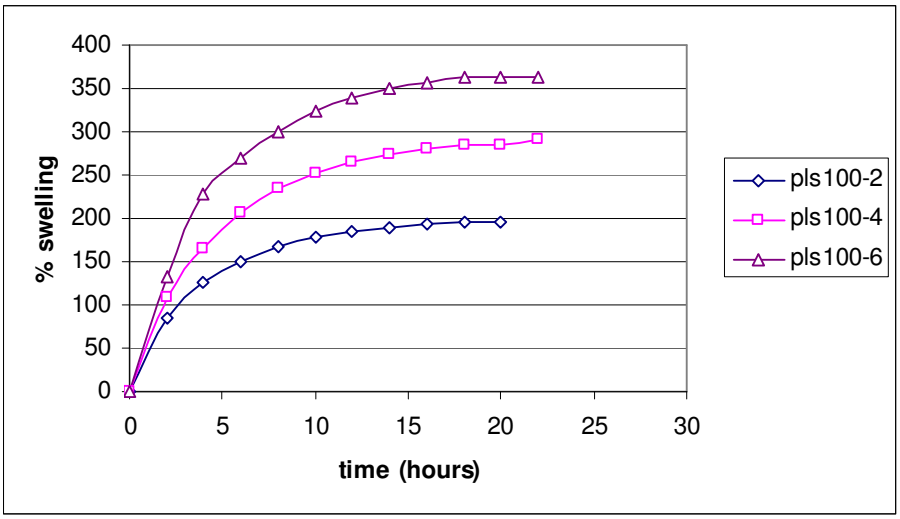


Figure 3.11 Percent swelling change by time for pls 100 sample

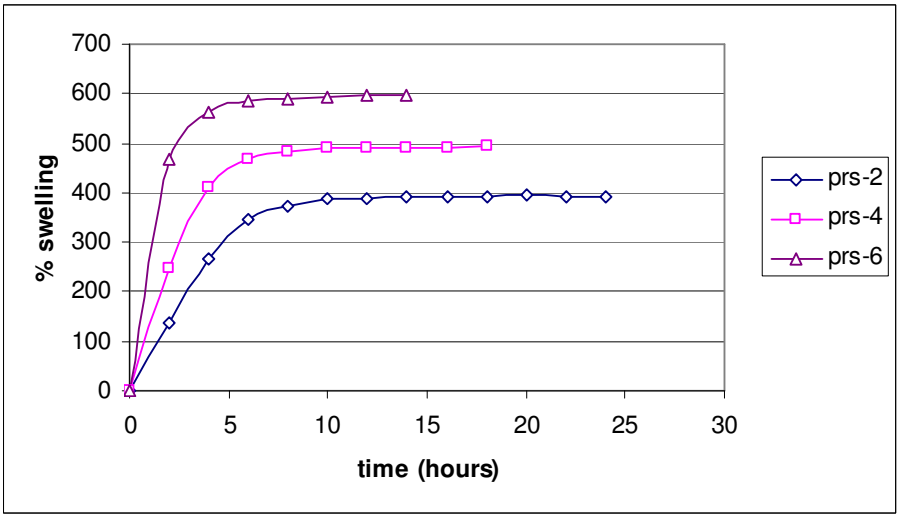


Figure 3.12 Percent swelling change by time for prs sample

3.3 Solid State ^{13}C-NMR

The ^{13}C-NMR CPMAS spectrum of the hydrogel is shown in Figure 3.13. The large peak with a shoulder at 37.05 ppm represents the main carbon backbone. The peak at 175.00 ppm depicts the carbon atoms of the amide and carboxylic groups. This last peak is actually the combination of two peaks overlapping with each other, because the range of the appearing carbon peak for both monomers (in poly(acrylic acid) and polyacrylamide) is approximately the same. The results are in good agreement with the literature [57].

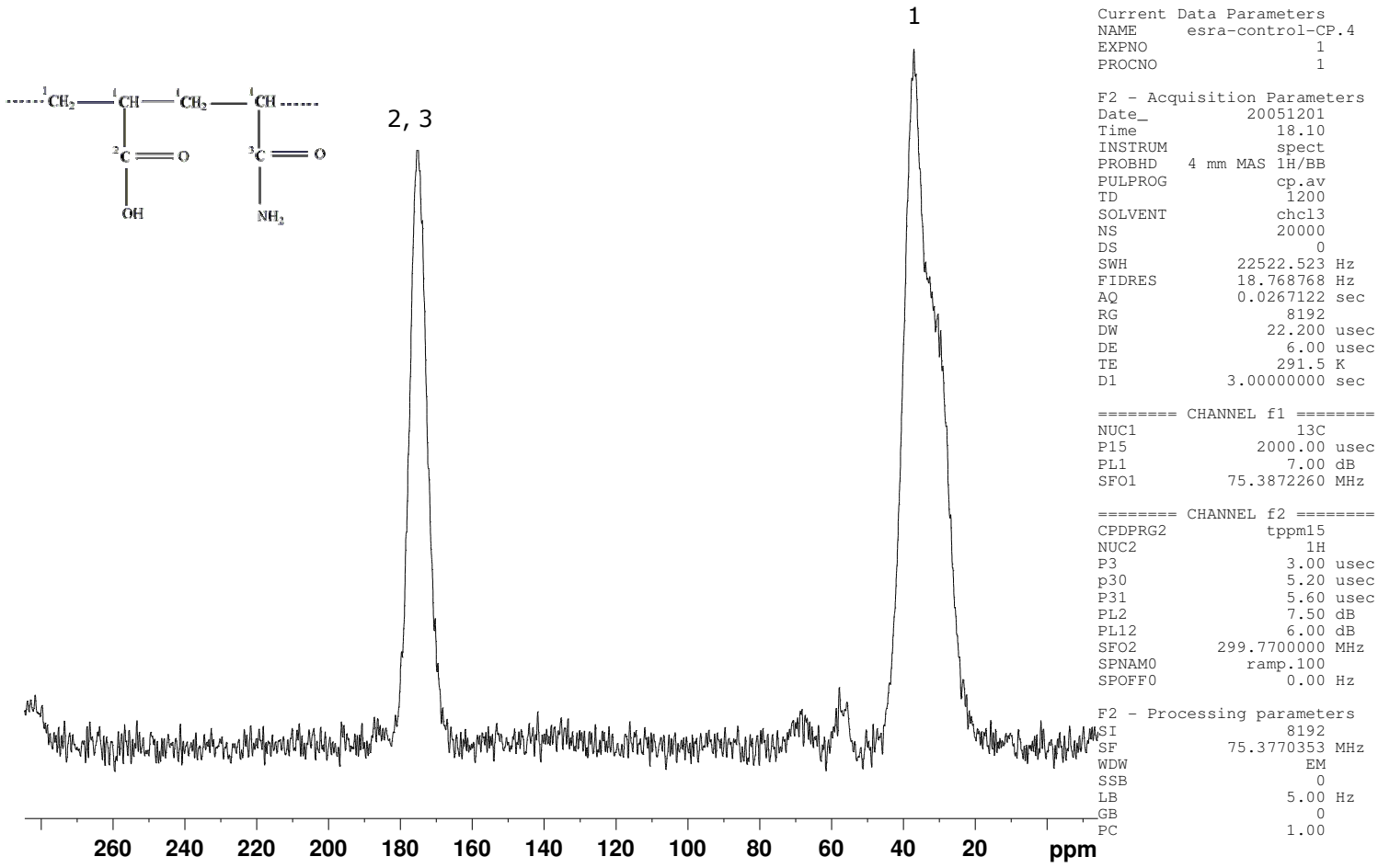


Figure 3.13 Solid state ^{13}C-NMR of the hydrogel

3.4 ATR-FTIR Characterization

The ATR-FTIR spectra of the samples are given in Figures 3.14-3.16. All the spectra show characteristic peaks for acrylamide-acrylic acid copolymer. The three sharp peaks observed between 1710–1600cm^{-1} can be identified as C-O bending of acrylic acid, C-O bending of amide and N-H bending of primary amide respectively. Peaks due to N-H stretching of amide groups and O-H stretching are observed at around 3200 and 3340 cm^{-1}, respectively. The peak at around 2920 cm^{-1} is due to C-H stretching of polymer backbone.

For plasma-treated samples an additional peak around 1084 cm^{-1} is observed which can be interpreted as an indication of anhydride structure (Figure 3.15 and 3.16). Polymer is thought to be crosslinked through the carbonyl moieties resulting in anhydride structures after plasma application (Figure 3.18). Possible plasma-induced radical formation and plasma-induced crosslinking reactions are given in Figures 3.17 and 3.18.

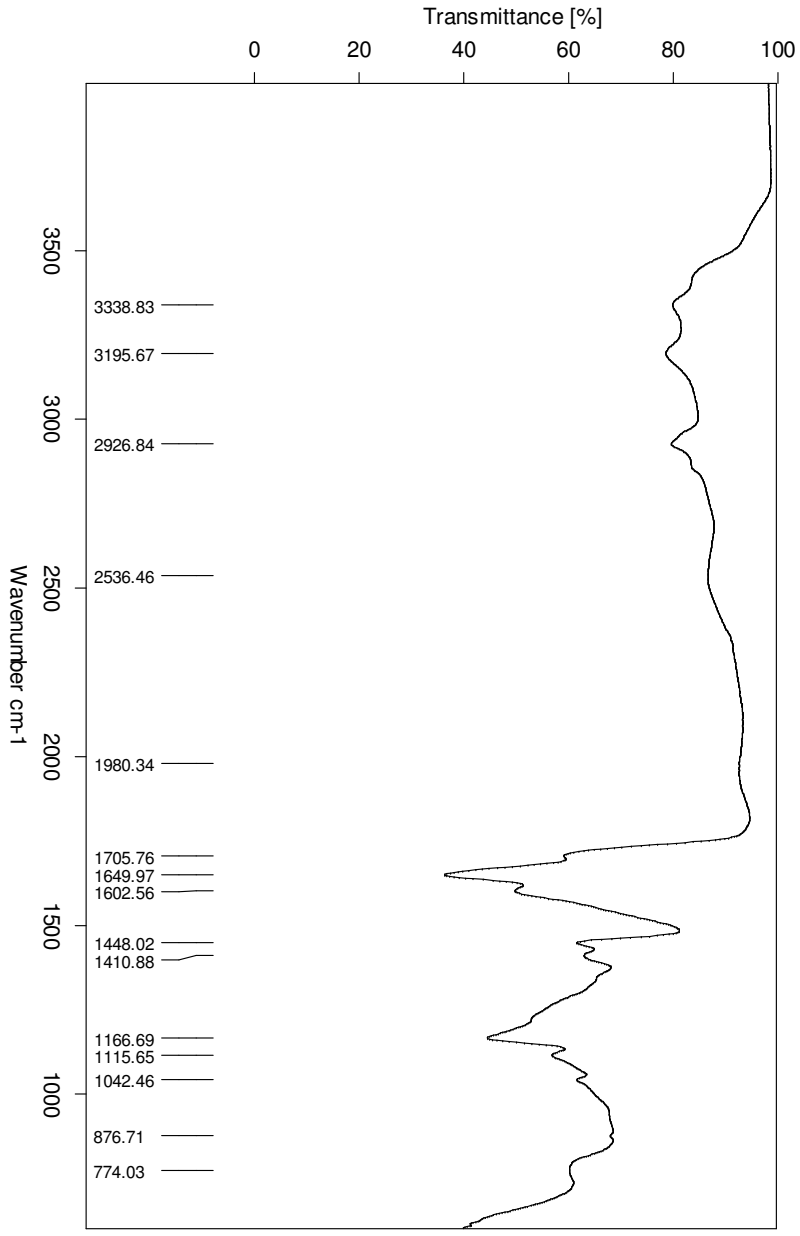


Figure 3.14 ATR-FTIR spectrum for control sample

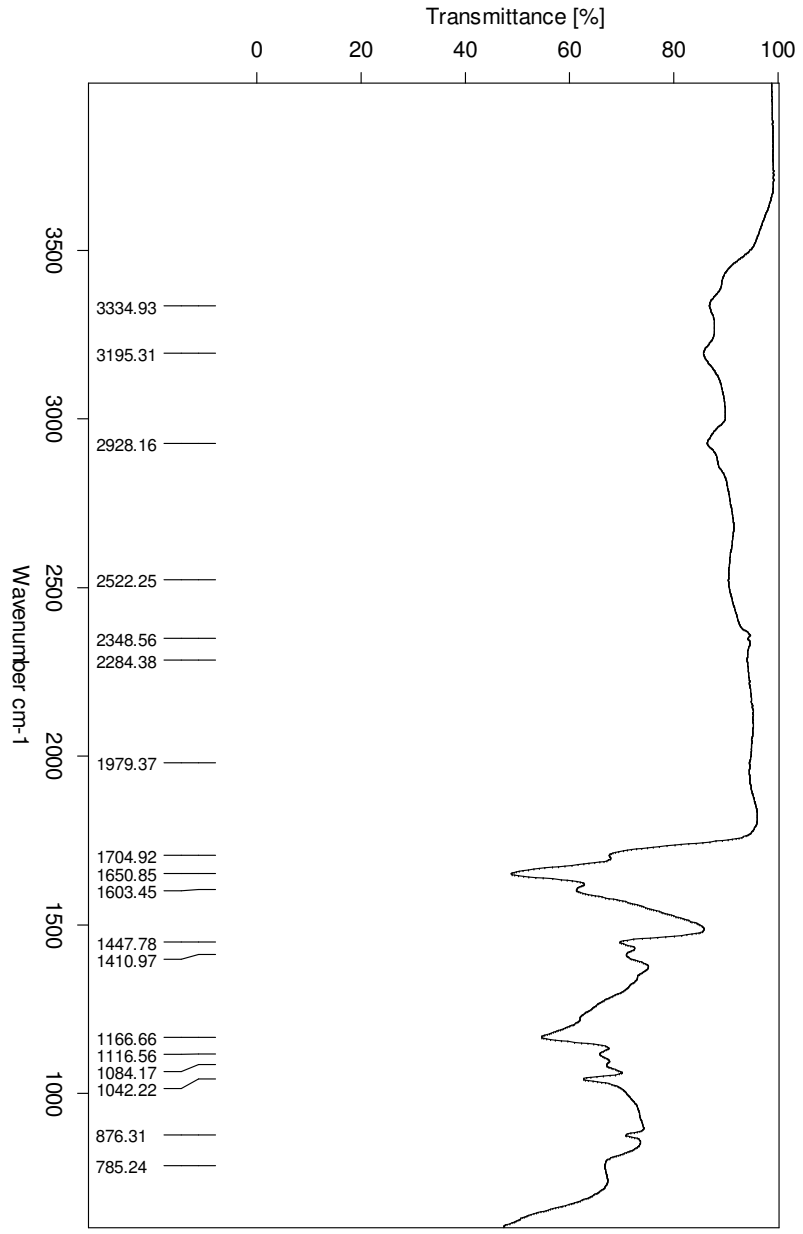


Figure 3.15 ATR-FTIR spectrum for pls 20

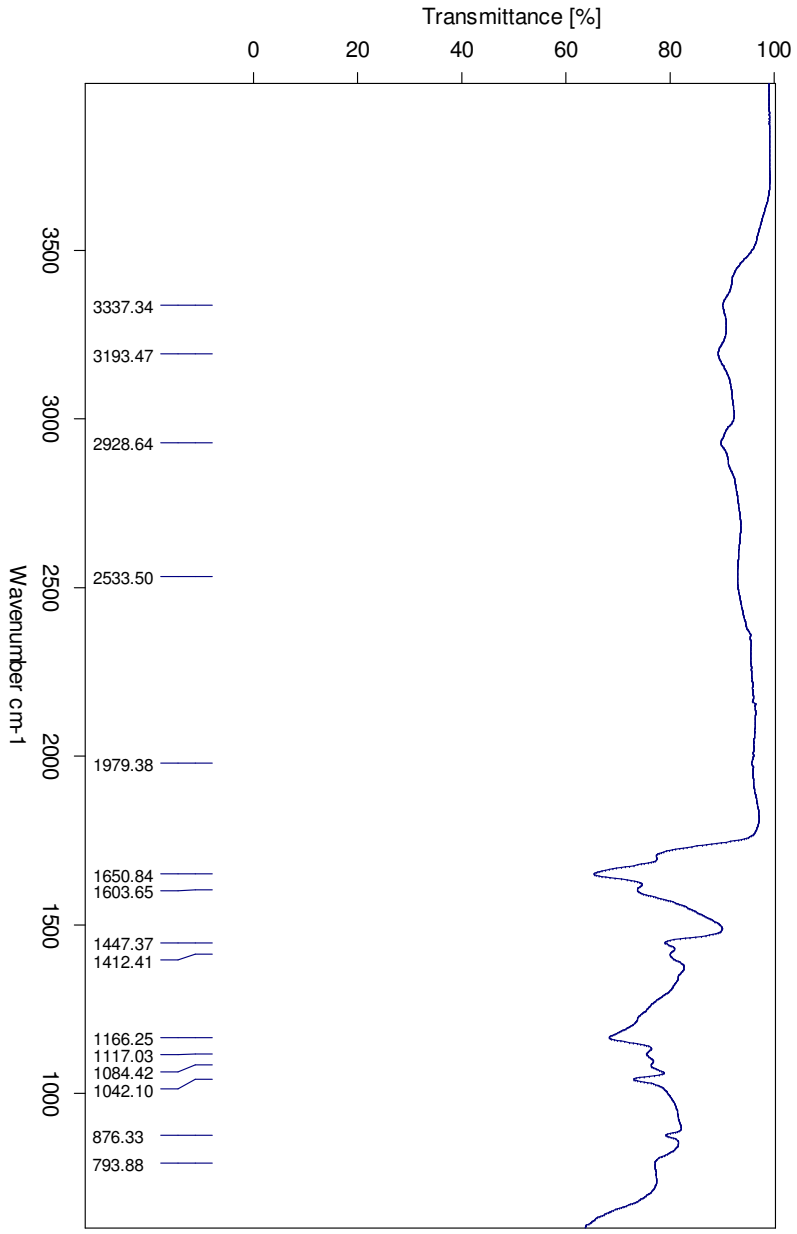


Figure 3.16 ATR-FTIR spectrum for pls 100

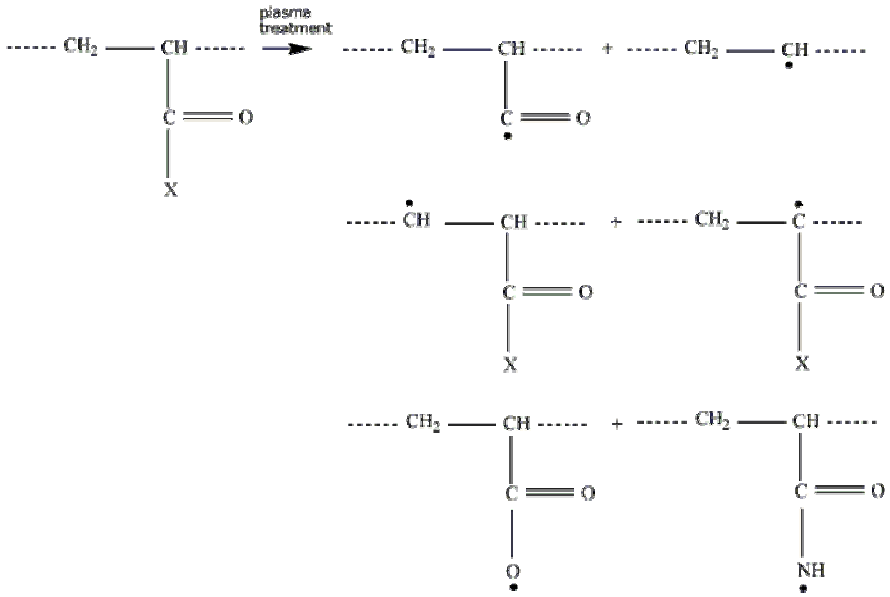


Figure 3.17 Possible plasma-induced radical formation reactions

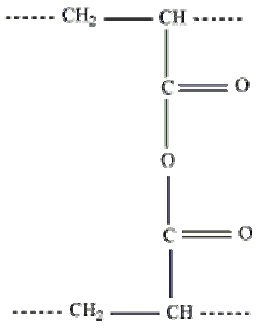


Figure 3.18 Possible plasma-induced crosslinking reaction

3.5 X-Ray Photoelectron Spectroscopy Characterization

X-ray Photoelectron Spectroscopy (XPS) measurements were carried out in order to investigate surface composition alteration of the samples by plasma treatment. Survey scans of the samples are given in Figures 3.19-3.21. All the samples contain peaks originating from C, N and O 1s levels. The spectrum for control sample contains a single peak with a shoulder centered at 284.5 eV due to electrons originating from C 1s level (Figure 3.19). However the spectra for plasma-treated samples contain two peaks belonging to their C 1s levels (Figures 3.20 and 3.21). These secondary peaks are located at higher binding energies; 287.0 and 287.2 eV for pls 20 and pls 100, respectively. The high binding energy structure is indicative of the formation of C-O and C-N bonds at polymer surfaces.

Percent elemental compositions obtained from XPS survey scan measurements are given in Table 3.2. These results reveal apparent oxidation and nitrogenation at the surfaces of plasma-treated samples. Oxygen content is found to be 21.6% for the control sample. Oxygen content of plasma treated samples increased up to 29.9 and 32.0% for pls 20 and pls 100 samples, respectively. Both the O/C and N/C ratios increase by plasma treatment.

Table 3.2 Percent elemental compositions obtained from survey scans

Element	Control (%)	Pls 20 (%)	Pls 100 (%)
C	72.7	59.2	58.5
N	5.7	10.9	9.5
O	21.6	29.9	32.0

For pls 100 sample, which was modified with application of 100 W discharge for 15 min under Ar atmosphere, oxidation ratio was slightly higher than for pls 20 sample, which was modified in similar way except the applied power was 20 W. This observation is explained by the fact that plasma-treated surface thickness increases with increasing treatment time and RF power [58].

Increase in oxygen and nitrogen content at the surface is attributed to post-plasma reactions. It was reported that, plasma-treated films exhibit oxygen and

nitrogen functionalities even when treated with inert gas plasmas [46]. Incorporation of oxygen functionalities during plasma modification is an inevitable phenomenon even when the plasma gas is inert. This fact does not exclude CASING. Free radicals, formed through the polymer surface during inert gas plasma treatment, may be stable for long durations after the plasma treatment is completed. Therefore it is possible that these radicals react with species present in the environment they are kept in. When inert gas plasma treated polymer samples are exposed to the atmosphere after the treatment, the plasma activated surface may readily react with the oxygen and nitrogen that are present in the environment atmosphere [49].

Depth profiling after ion bombardment was also conducted for plasma-treated samples. Depth profiling spectra of pls 20 and pls 100 samples are given in Figures 3.22-3.23. It is observed that secondary peaks in C 1s regions of plasma-treated samples disappear after ion bombardment. Percent elemental compositions obtained for the plasma-treated samples after ion-bombardment are given in Table 3.3. It is found that O/C and N/C ratios at plasma-modified samples drop below the ones belonging to control sample. It is known that the main effect of Ar plasma treatment is removal of functional groups and decreases in O/C and N/C ratios are expected. It can be said that there is a layer just below the surface that is modified by plasma but not involved in post-plasma reactions. It is argued that crosslinking may inhibit oxygen diffusion to deeper layers of the surface [48].

Table 3.3 Percent elemental compositions obtained after ion-bombardment

Element	**Pls 20 (%)**	**Pls 100 (%)**
C	87.4	84.4
N	8.0	7.3
O	4.6	8.3

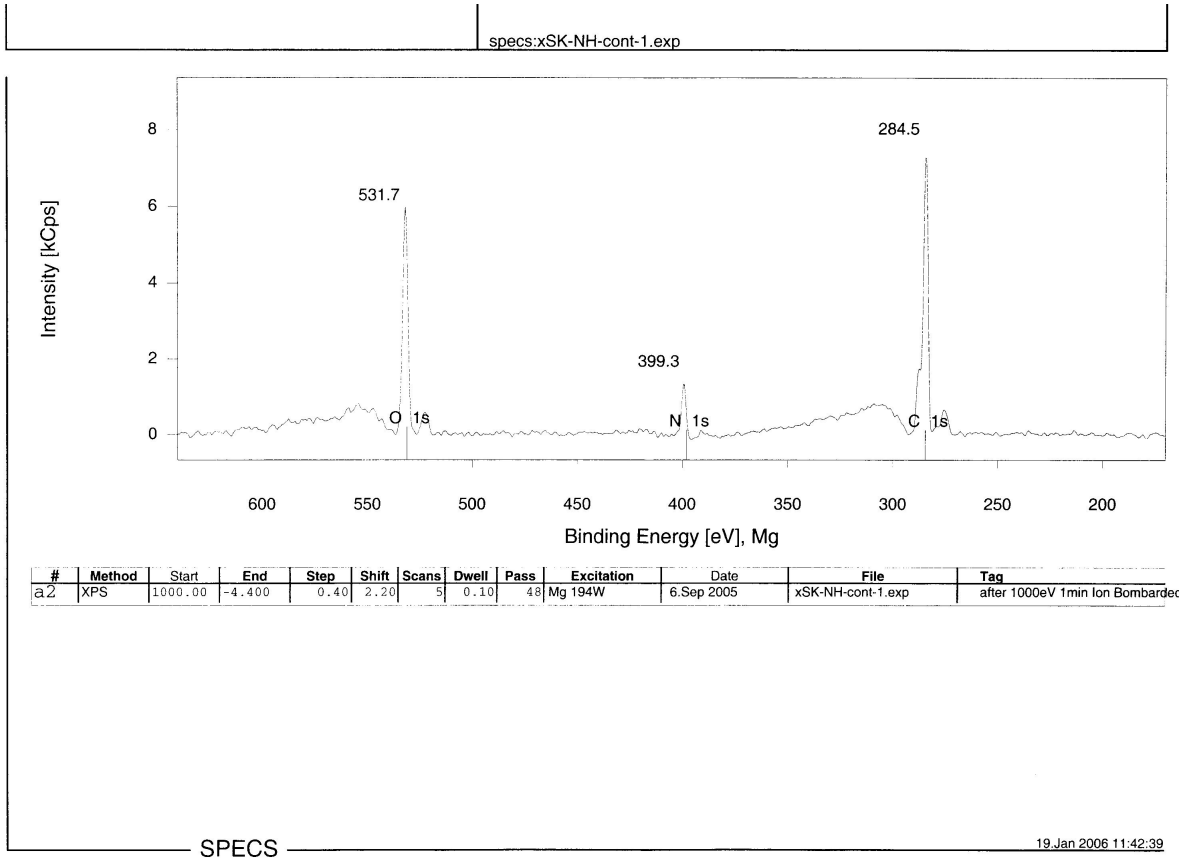
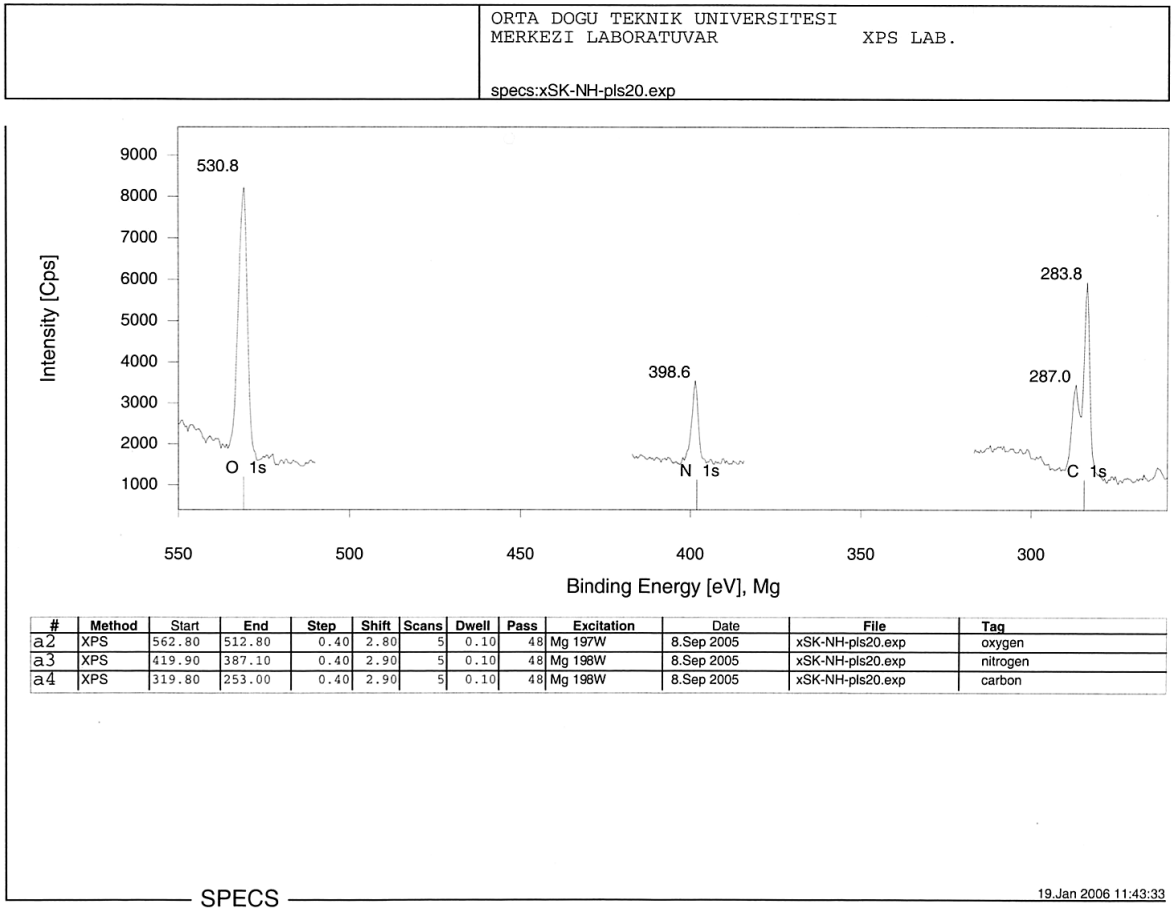
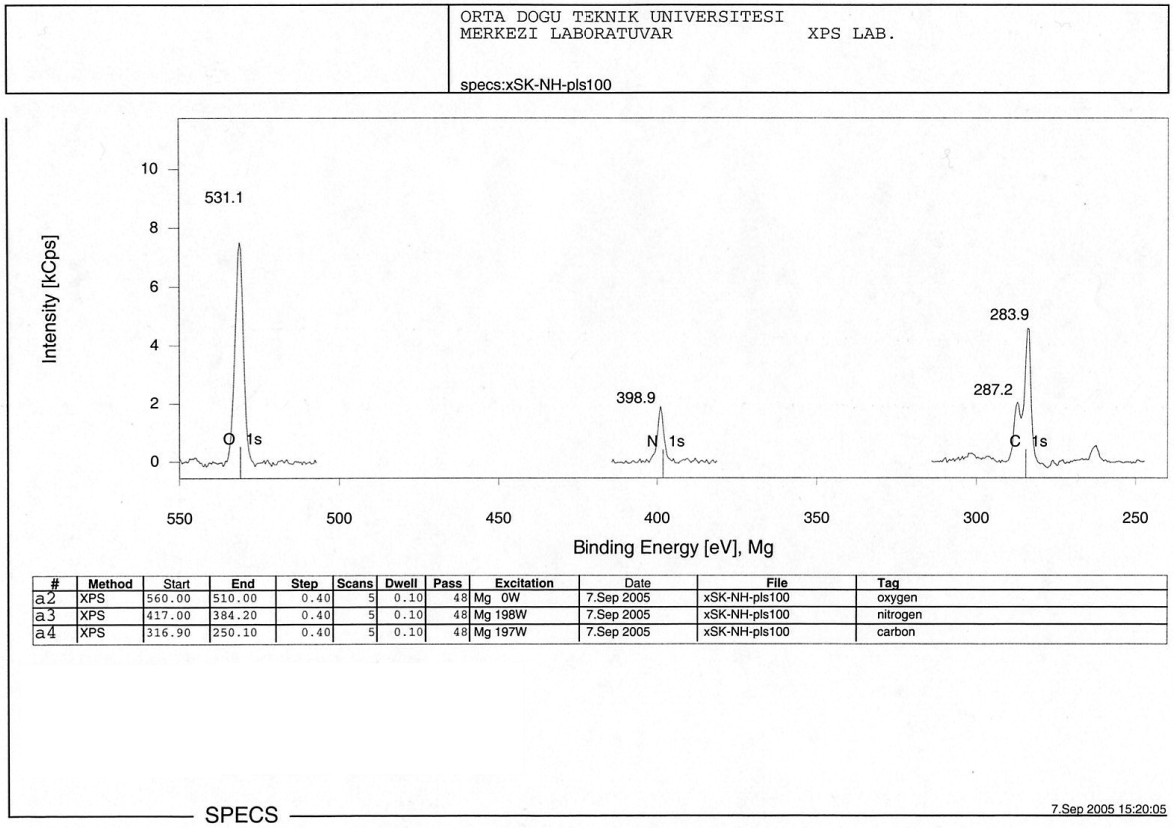


Figure 3.19 XPS survey scan spectrum for control sample



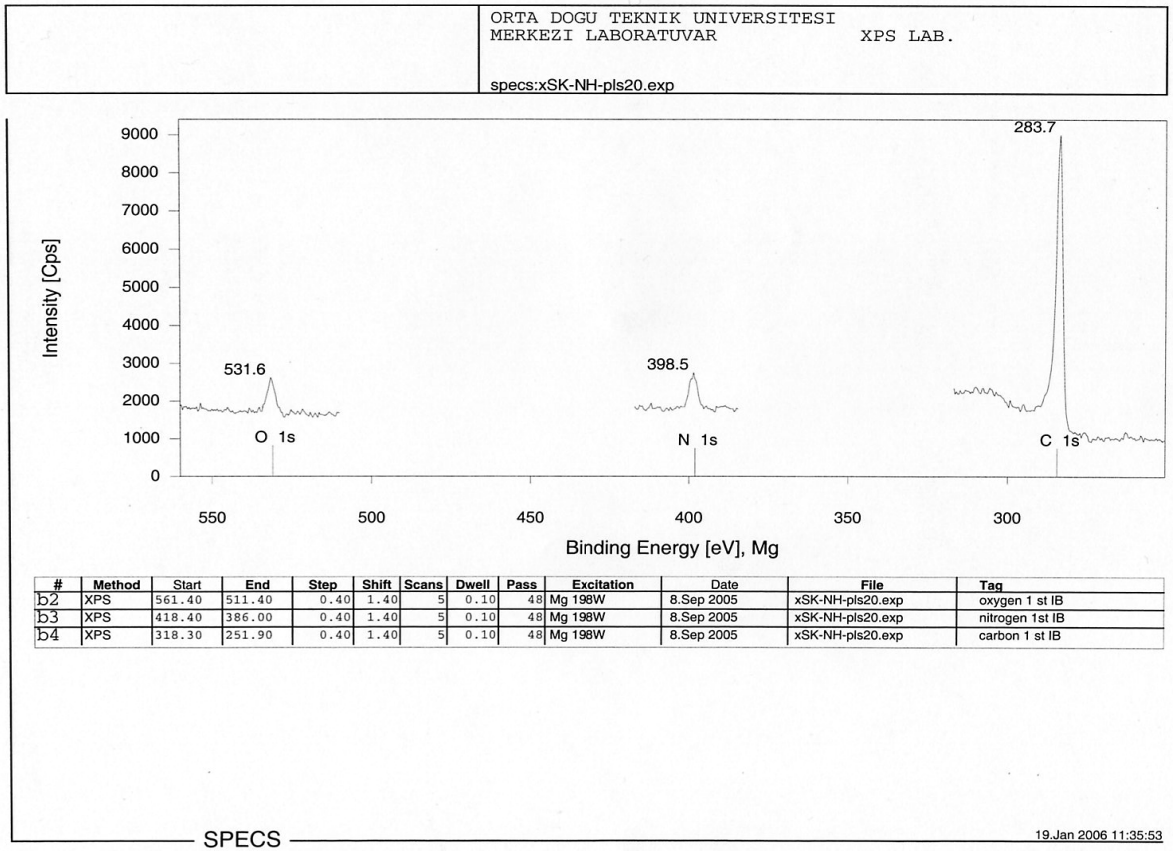
#	Method	Start	End	Step	Shift	Scans	Dwell	Pass	Excitation	Date	File	Tag
a2	XPS	562.80	512.80	0.40	2.80	5	0.10	48	Mg 197W	8.Sep 2005	xSK-NH-pls20.exp	oxygen
a3	XPS	419.90	387.10	0.40	2.90	5	0.10	48	Mg 198W	8.Sep 2005	xSK-NH-pls20.exp	nitrogen
a4	XPS	319.80	253.00	0.40	2.90	5	0.10	48	Mg 198W	8.Sep 2005	xSK-NH-pls20.exp	carbon

Figure 3.20 XPS survey scan spectrum for pls 20 sample



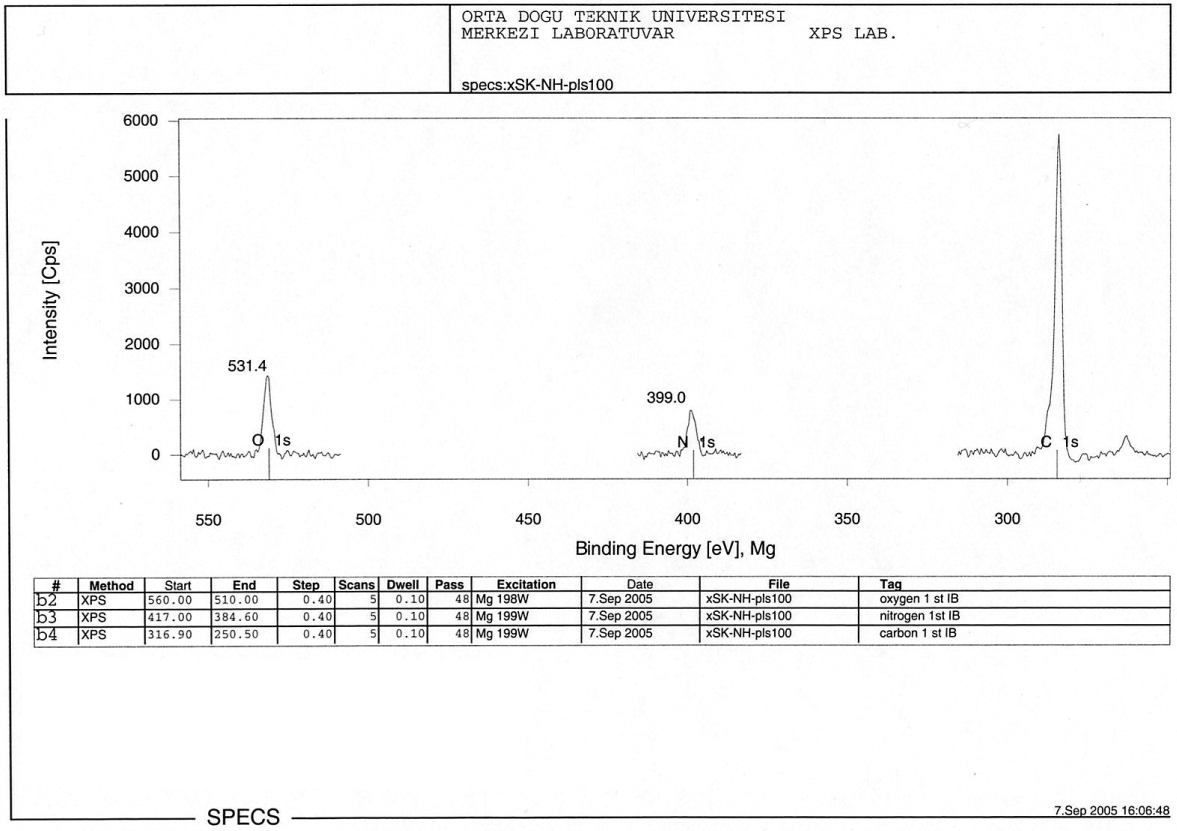
#	Method	Start	End	Step	Scans	Dwell	Pass	Excitation	Date	File	Tag
a2	XPS	560.00	510.00	0.40	5	0.10	48	Mg 0W	7.Sep 2005	xSK-NH-pls100	oxygen
a3	XPS	417.00	384.20	0.40	5	0.10	48	Mg 198W	7.Sep 2005	xSK-NH-pls100	nitrogen
a4	XPS	316.90	250.10	0.40	5	0.10	48	Mg 197W	7.Sep 2005	xSK-NH-pls100	carbon

Figure 3.21 XPS survey scan spectrum for pls 100 sample



#	Method	Start	End	Step	Shift	Scans	Dwell	Pass	Excitation	Date	File	Tag
b2	XPS	561.40	511.40	0.40	1.40	5	0.10	48	Mg 198W	8.Sep 2005	xSK-NH-pls20.exp	oxygen 1 st IB
b3	XPS	418.40	386.00	0.40	1.40	5	0.10	48	Mg 198W	8.Sep 2005	xSK-NH-pls20.exp	nitrogen 1st IB
b4	XPS	318.30	251.90	0.40	1.40	5	0.10	48	Mg 198W	8.Sep 2005	xSK-NH-pls20.exp	carbon 1 st IB

Figure 3.22 XPS spectrum for pls 20 sample after surface-etching ion-bombardment



#	Method	Start	End	Step	Scans	Dwell	Pass	Excitation	Date	File	Tag
b2	XPS	560.00	510.00	0.40	5	0.10	48	Mg 198W	7.Sep 2005	xSK-NH-pls100	oxygen 1 st IB
b3	XPS	417.00	384.60	0.40	5	0.10	48	Mg 199W	7.Sep 2005	xSK-NH-pls100	nitrogen 1st IB
b4	XPS	316.90	250.50	0.40	5	0.10	48	Mg 199W	7.Sep 2005	xSK-NH-pls100	carbon 1 st IB

Figure 3.23 XPS spectrum for pls 100 sample after surface-etching ion-bombardment

3.6 SEM Characterization

Scanning electron microscopy (SEM) was used to monitor pore sizes and their appearances in hydrogels which were swelled and freeze-dried at two different pH values. The SEM micrographs of the samples swelled in pH 2 buffer are given in Figure 3.24. Pore diameters for the control sample are observed to be ~5 μm (Figure 3.24 a). It is seen in Figures 3.24 b and c that plasma treatment resulted in degradation of polymer matrix. It is known that when polymers are exposed to plasma radiation for a long time, weight loss takes place and topmost layers of polymer matrix are etched [45]. Plasma-treated pls 20 sample which is exposed to lower energy plasma exhibits pore structure similar to control sample with additional pores throughout the polymer surface, with the pore diameters of ~10 μm. Morphology of high energy plasma-treated pls 100 sample does not resemble to either, its surface is homogenous throughout the matrix and harsh etching is observed. Pore diameters for pls 100 sample are observed to be ~25 μm. Plasma-treated, porous and control samples differ in morphology. SEM micrograph of prs sample swelled in pH 2 buffer is given in Figure 3.24 d. Porous hydrogel prepared by salt-leaching exhibits a pore structure which is attributed to voids left by salt particles, which are ~50 μm. Pore sizes for the samples are smallest for the control, and largest for the prs samples. For plasma-treated samples, pore sizes increase with the increase of applied discharge power.

SEM micrographs of samples swelled in pH 10 buffer are shown in Figure 3.25. While different pore sizes were observed in SEM micrographs of control and pls 20 samples swelled in pH 2 buffers; the ones swelled in pH 10 buffers seem to have similar pore sizes (diameters of ~50-60 μm). This can be explained by extensive swelling of hydrogels (up to ~1000% of their dry weight) at high pH values. Pls 100 sample demonstrated holes created during plasma induced degradation (Figure 3.25 c). Prs sample still demonstrated the largest pores (Figure 3.25 d).

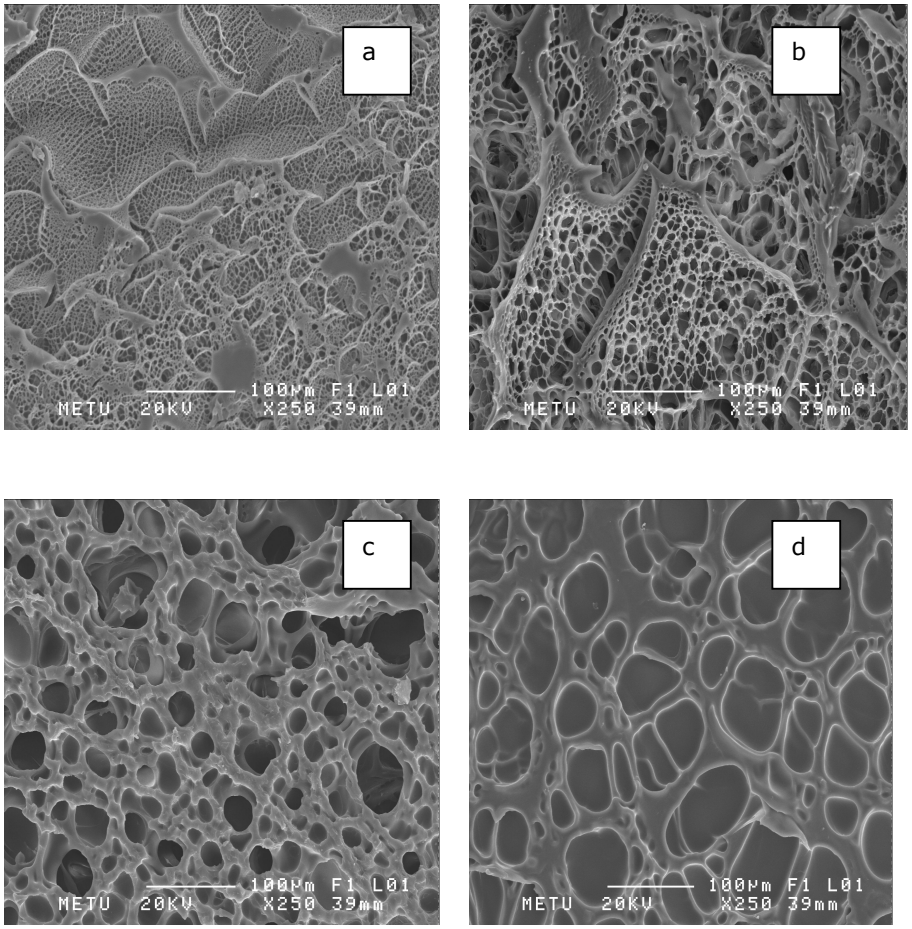


Figure 3.24 SEM images of a) cont; b) pls 20; c) pls 100; d) prs samples swelled in pH 2 buffer

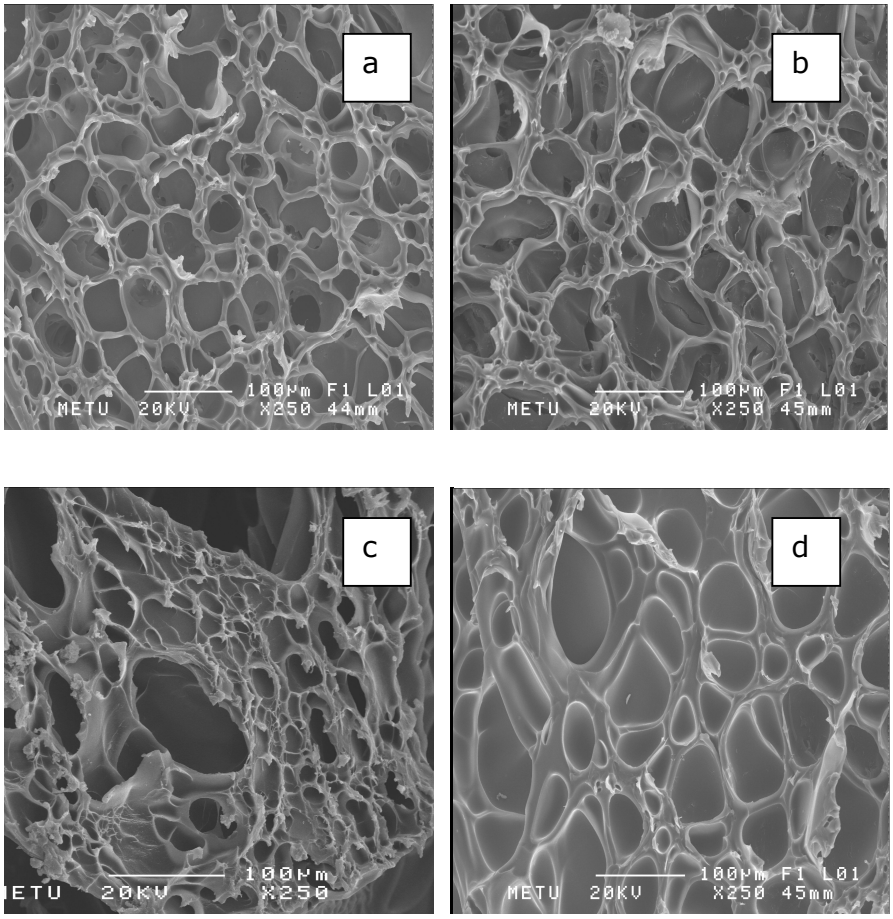


Figure 3.25 SEM images of a) cont; b) pls 20; c) pls 100; d) prs samples swelled in pH 10 buffer

3.7 ESR Characterization

In order to support XPS results suggesting post-plasma reactions; ESR measurements were carried out. It is seen in ESR spectra of the samples, which are given in Figures 3.26 and 3.27; the radicals are slowly disappearing in air. Two measurements were taken for the plasma-treated samples, one right after and one ten days after the plasma treatment. Living radicals are observed even on the 10^{th} day of plasma application.

Presence of free radicals explains post-plasma reactions. Although the hydrogel samples were flushed with argon for 30 min after the plasma discharge is turned off, free radicals were detected in the samples right after the plasma application. This may be due to limited mobility of radicals. All kinds of organic radicals eventually undergo a termination reaction, producing stable molecules. Unlike radicals in the liquid or gas phase, however, the recombination of solid state radicals is significantly suppressed due to the restriction of their mobility. It is reported that long-living radicals may be attributed to intermolecular crosslinking which makes oxygen diffusion into the surface layer difficult [48].

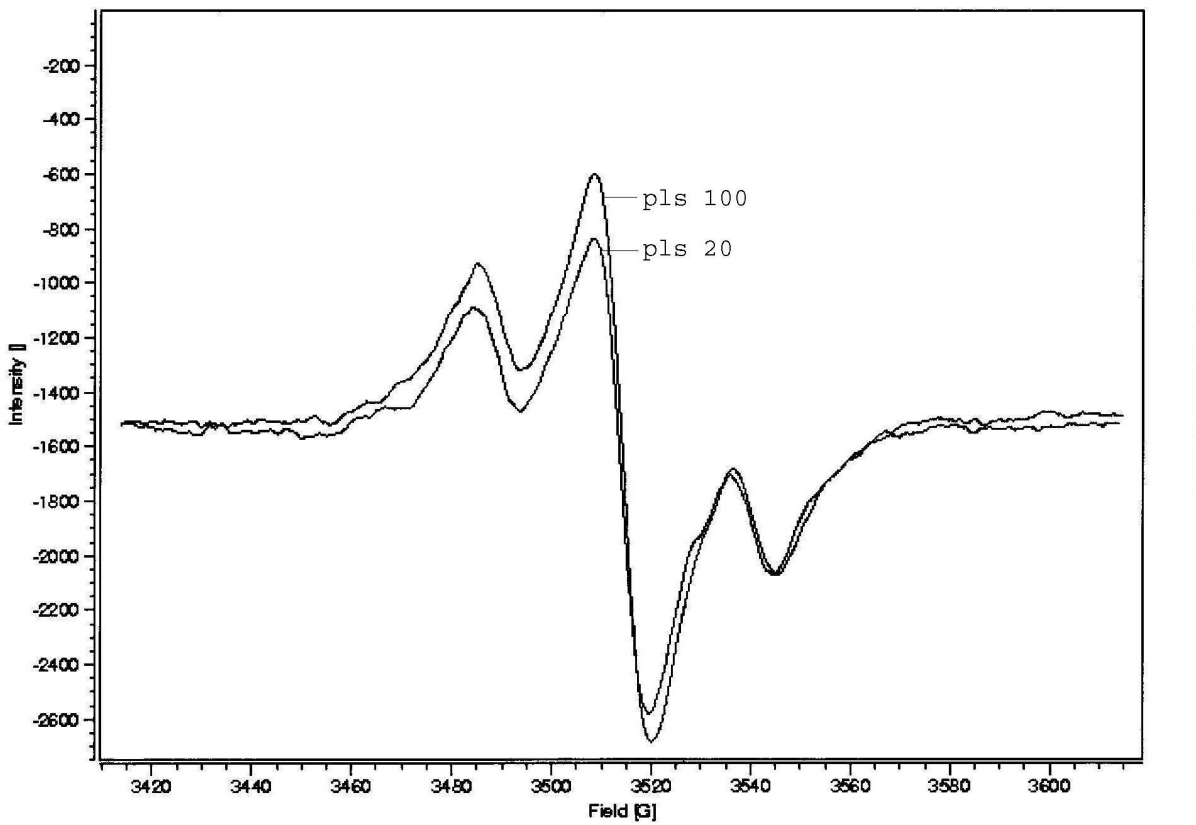


Figure 3.26 ESR spectrum of pls 20 and pls 100 samples right after plasma treatment

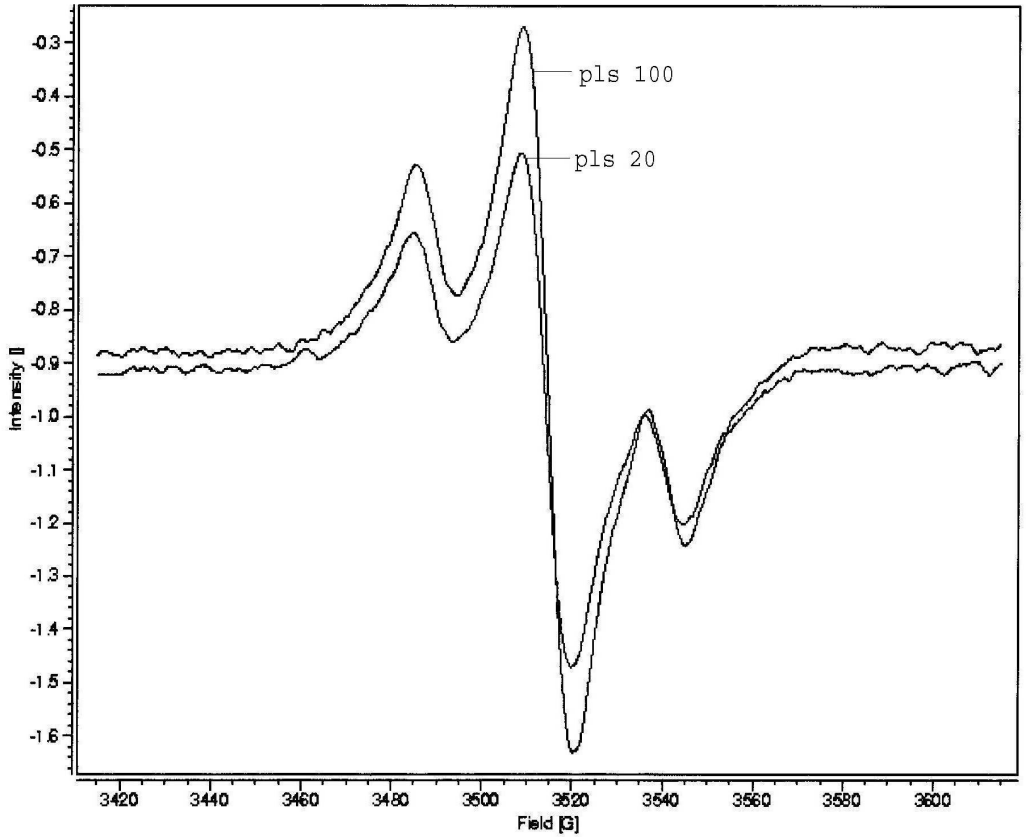


Figure 3.27 ESR spectrum of pls 20 and pls 100 samples on the 10th day of plasma

3.8 Contact Angle Measurements

Contact angles were measured by distilled water and the obtained values are given in Table 3.4. For control groups, the contact angle was about 49°, and for prs it was quite close but about four degrees higher. On the other hand plasma applied samples demonstrated a significant drop in contact angles down to 18°. This shows that plasma treatment leads to an increase in hydrophilicity of the samples.

Table 3.4 Contact angle values of the samples

Sample	Contact Angle
Control	48.72°±2.18
Pls 20	18.93°±2.48
Pls 100	18.89°±3.55
Prs	51.92°±2.88

3.9 Release Experiments

CPFX releases from different systems at varying pH values are given in Figures 3.28-3.31. There are two factors determining the release rates; one being the solubility of CPFX and the other is equilibrium degree of swelling of the hydrogel. CPFX has pH-dependent solubility and at low (<5) and high (>10) pH values its solubility is maximum. On the other hand, at high pH values hydrogels swell up to ~1000% of their dry weight and release their content easily. As the releases from control samples are examined (Figure 3.28), it is seen that releases are slower at pH 4 and 6. At pH 4 and 6; hydrogel samples release halves of their CPFX content in seven days. However, faster release profile is observed at pH 2, which is attributed to high solubility of drug at lower pH values. At pH 2, 50% of the CPFX loaded is released in four days. At pH 8 and pH 10 a delay in the release was observed, most probably because of the time lag between swelling of the matrix and dissolution of CPFX. Halves of the CPFX loaded was released in six days.

When release profiles from plasma-treated pls 20 sample are examined (Figure 3.29); almost the same patterns with the control sample are observed, except for the release at pH 2. CPFX releases from these hydrogels at pH 2 are suppressed due to plasma-modification. For pls 20 sample 25% of the drug loaded was released in three days at pH 2, whereas this takes less than two days for control sample. In seven days 50% and in fourteen days 75% of the loaded drug was released at pH 2. However, at high pH values suppressing effects of the modification are disappeared most probably due to very high swelling degree of the hydrogels.

Release profiles for pls 100 samples are given in Figure 3.30. As it was for pls 20 sample; no apparent differences in release rates are observed for control and pls 100 samples, except at pH 2. The same suppressing effect is observed for pls 100 sample at pH 2. Although the release rate was decreased when compared to control sample; suppressing effect of the plasma treatment was more prominent for pls 20 samples. 25% of the CPFX loaded was released in two days, whereas it was three and less than two days for pls 20 and control samples, respectively. 50% of the drug content was released in less than six days. In eleven days, release of 75% of the drug content was completed. For pls 100 samples, release profiles at basic media could not be obtained due to disintegration of the hydrogels.

Release data of prs samples are given in Figure 3.31. As release profiles are investigated it is surprisingly seen that release rates decreases as pH of the environment increases. Since the prs samples are fast-swelling with high equilibrium swelling degrees, CPFX solubility dominates over the release kinetics. It is also observed here that, at pH 8 and pH 10, only the halves of the drug loaded are released on the contrary. This might be explained by a probable drug-matrix interaction at this pH. Presence of salt crystals during the polymerization reaction might be affecting the matrix structure which in turn interacts with the drug molecule. Release rates of CPFX from prs samples seem to be decreased when compared to the other samples. However, as the amount of CPFX loaded into prs sample is concerned and if the release data were given in mg/mL, it could be seen that CPFX release rate was actually higher for the prs samples when compared to control sample.

Figures 3.32-3.36 show CPFX release profiles at different pH values. Drug release at pH 2 is slower from matrices modified by plasma as compared with that from control sample. At other pH values no significant differences in release rates are observed between matrices either modified by plasma or not. The equilibrium establishes about in 20 days at these acidic pH values. At basic media, at pH 8 and pH 10, as a general trend, increased CPFX release rates are observed which is attributed to high equilibrium swelling degree of hydrogels at these pH values where the equilibrium establishes about in 10 days. Release rates of CPFX at pH 2 and pH 4 are also unexpectedly high. This phenomenon is attributed to high solubility of the drug at acidic pH.

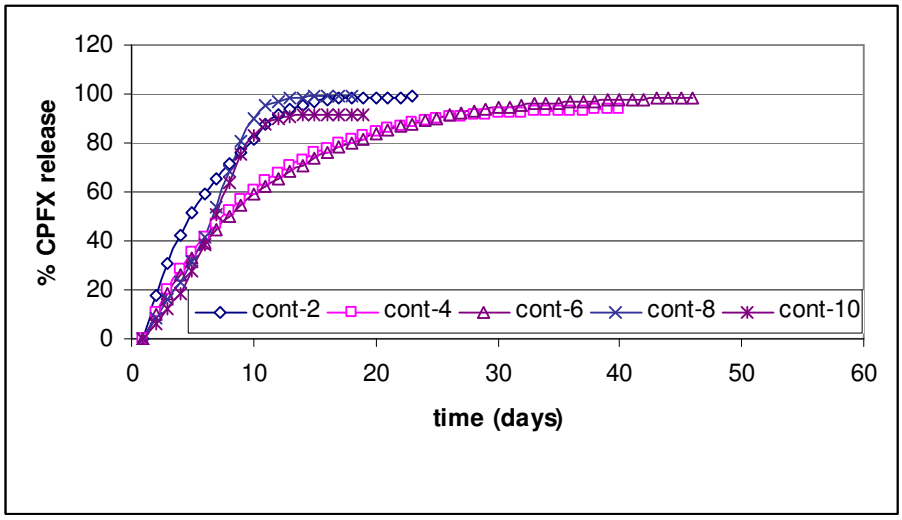


Figure 3.28 CPFX release from control samples

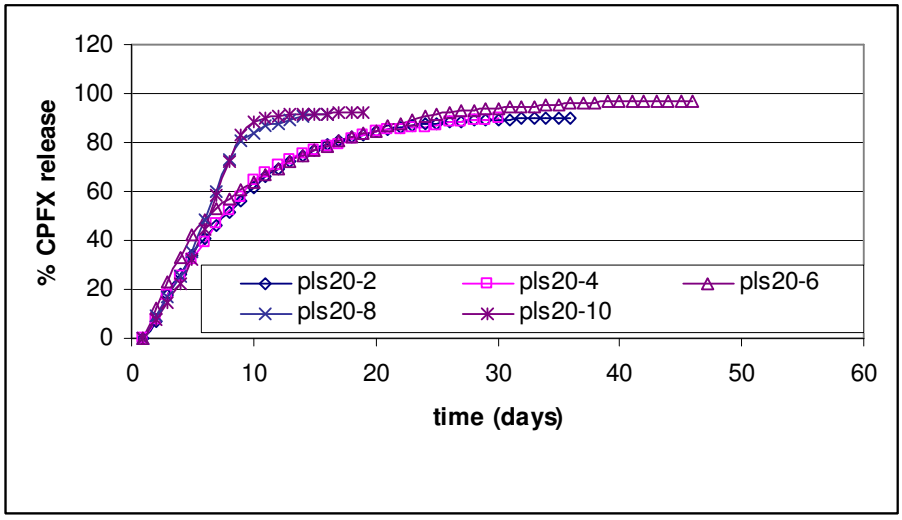


Figure 3.29 CPFX release from pls 20 samples

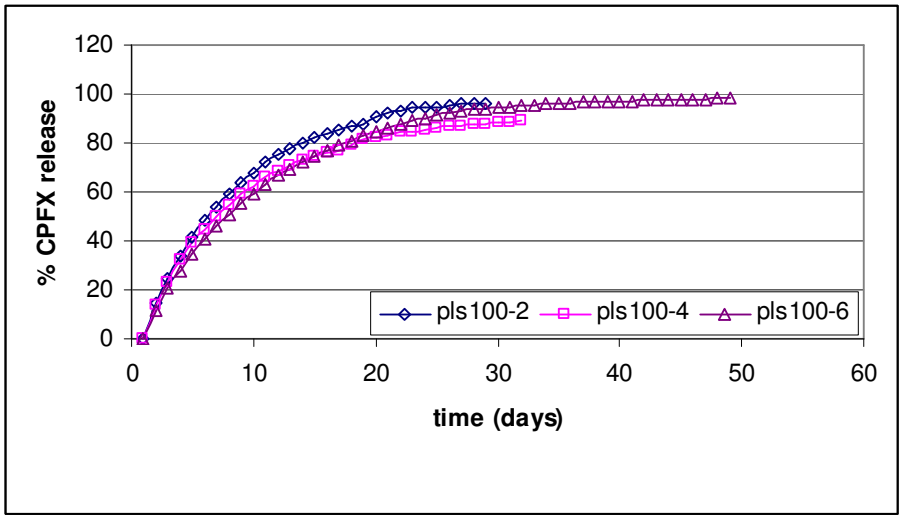


Figure 3.30 CPFX release from pls 100 samples

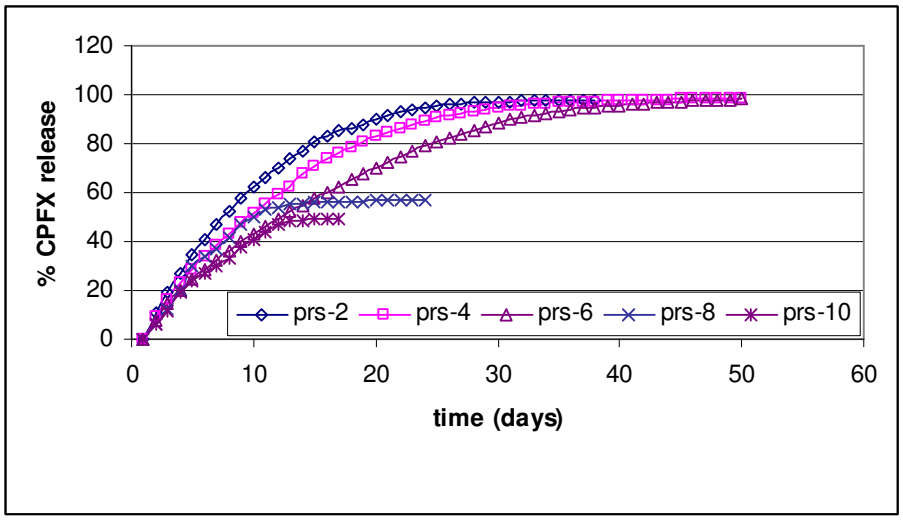


Figure 3.31 CPFX release from prs samples

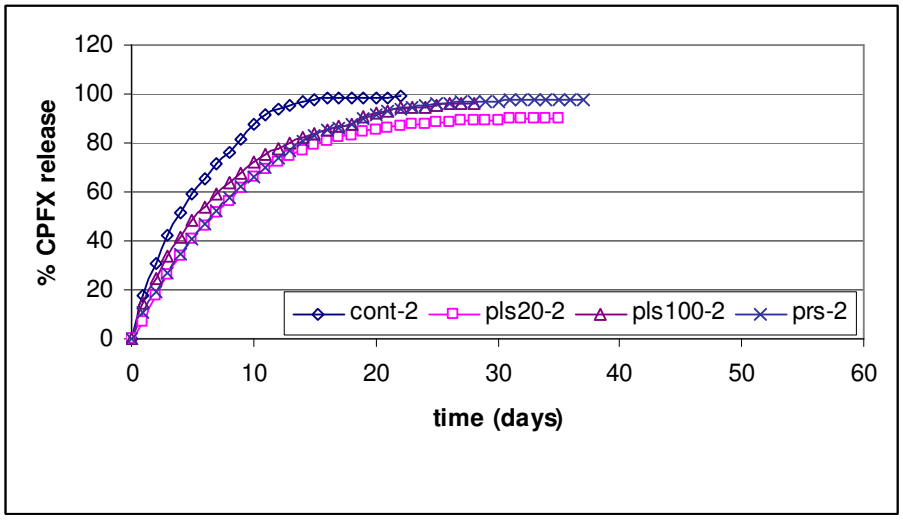


Figure 3.32 CPFX release at pH 2

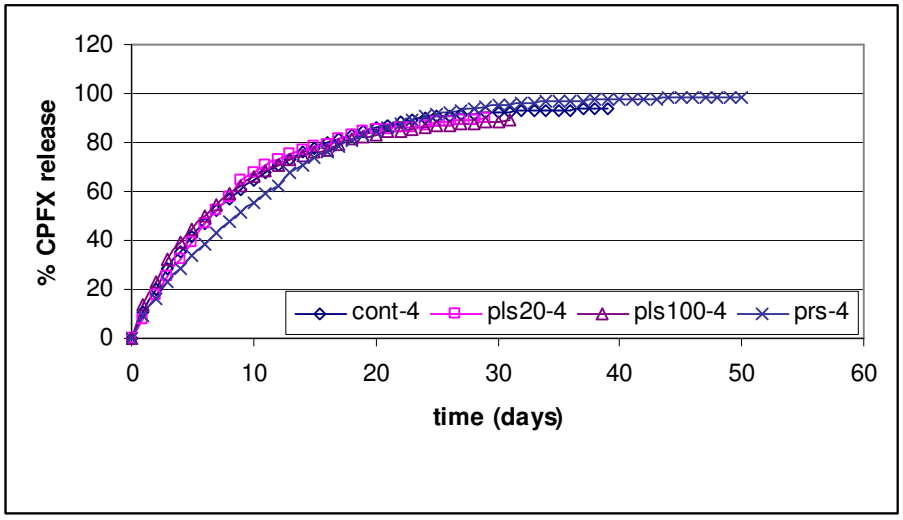


Figure 3.33 CPFX release at pH 4

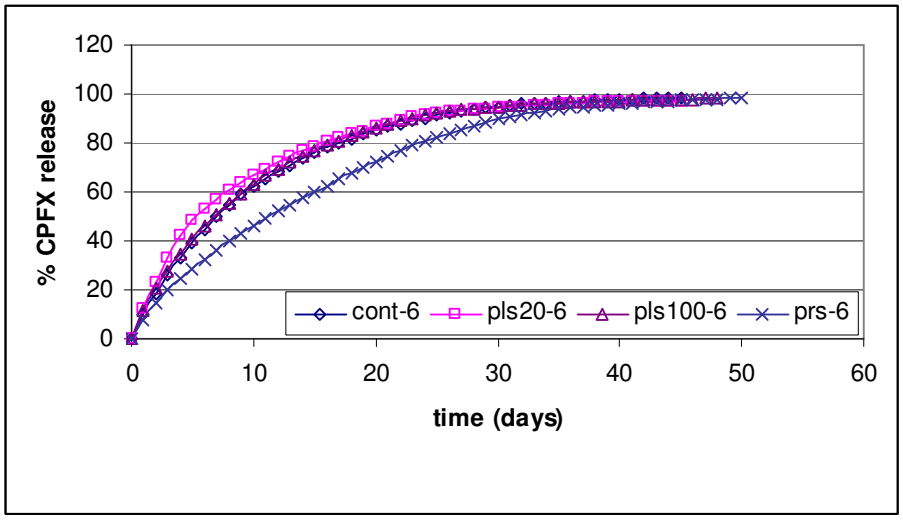


Figure 3.34 CPFX release at pH 6

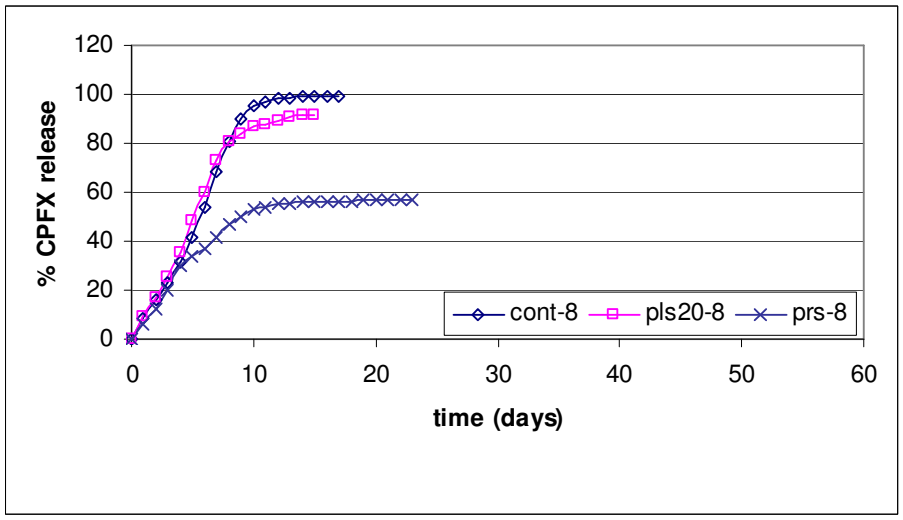


Figure 3.35 CPFX release at pH 8

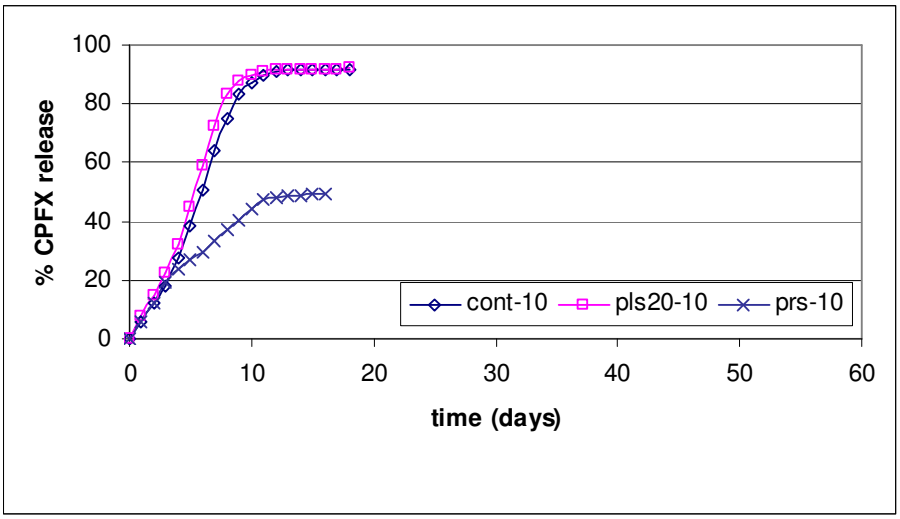


Figure 3.36 CPFX release at pH 10

The release rate kinetics data for all the systems are given in Table 3.5. Drug release data of all the control, pls 20 and pls 100 samples at pH 2, 4 and 6 were fitted in Higuchi equation. At low pH values (2, 4, 6) swelling of hydrogel samples are low and the releases are diffusion-controlled. Cont-10 and pls 20-8 have good correlation with Korsmeyer and also with first-order kinetics. Cont-8 and pls 20-10 also follow first-order kinetics.

Prs-2, prs-6 and prs-10 samples showed high linearity with Higuchi equation whereas prs-4 follows first-order and prs-8 follows Korsmeyer kinetics. In terms of Korsmeyer equation, all the systems demonstrated non-Fickian release ($n>0.5$).

Table 3.5 Release kinetics

Sample	Zero order		First order		Higuchi		Korsmeyer		
	K_0	R^2	K_1	R^2	K_H	R^2	K_{KP}	R^2	n
Cont-2	2.1414	0.9824	0.2502	0.9051	7.5167	**0.9992**	0.1825	0.9964	0.7413
Cont-4	1.2835	0.9798	0.1724	0.862	5.5397	**0.9993**	0.0971	0.9554	1.3759
Cont-6	1.2353	0.9737	0.1499	0.8395	5.7761	**0.9986**	0.1086	0.9932	0.7782
Cont-8	2.1766	0.9814	0.2895	**0.9952**	8.7361	0.9503	0.0675	0.9875	1.1592
Cont-10	2.5722	0.9845	0.3331	0.9887	10.333	0.955	0.0490	**0.9912**	1.3407
Pls20-2	1.4255	0.9879	0.166	0.919	6.3958	**0.9996**	0.1167	0.9936	0.8197
Pls20-4	1.3514	0.9963	0.1937	0.9503	5.78	**0.9972**	0.0986	0.99	1.4712
Pls20-6	1.3602	0.9425	0.1587	0.8091	5.9571	**0.9898**	0.1443	0.9801	0.7217
Pls20-8	2.193	0.9866	0.3952	0.9818	6.9932	0.9504	0.1051	**0.9902**	0.9874
Pls20-10	2.5362	0.9845	0.1497	**0.995**	9.6922	0.9587	0.0643	0.9909	1.2542
Pls100-2	1.5921	0.9802	0.1927	0.881	6.2669	**0.9993**	0.1560	0.9972	0.711
Pls100-4	1.5681	0.9795	0.2148	0.8914	5.8531	**0.9994**	0.1714	0.996	0.7164
Pls100-6	1.2393	0.9784	0.1515	0.8616	5.572	**0.9994**	0.1249	0.9965	0.7254
Prs-2	2.6901	0.9857	0.1829	0.8729	11.569	**0.9981**	0.1108	0.9971	0.8077
Prs-4	1.9694	0.9927	0.1165	0.9238	10.123	0.9979	0.1008	**0.999**	0.746
Prs-6	1.3895	0.9857	0.0801	0.8944	8.3601	**0.9996**	0.0926	0.9983	0.6941
Prs-8	2.8794	0.9764	0.3533	0.9021	10.061	0.984	0.1093	**0.9906**	1.0455
Prs-10	2.0172	0.9691	0.2617	0.8553	7.552	**0.9948**	0.1304	0.985	0.886

CHAPTER 4

CONCLUSION

In this study four different systems were prepared from acrylamide and acrylic acid as controlled release systems and their release profiles in varying pH medium were examined. Two modification methods were used for either sustaining or fastening release rate of the model drug ciprofloxacin. Prepared hydrogel samples were characterized in terms of swelling and release characteristics as well as chemical and physical structures.

As a general trend, EDS and swelling rate of the hydrogel samples increased with increasing pH. In acidic medium, the swelling values were about 195% for the control and plasma-treated ones, while these values increased upto ~860% at pH 10. For prs samples the same trend was observed with higher swelling values. Swelling values were 393.6 and 999.4% for pH 2 and pH 10, respectively.

Plasma-modified samples were characterized by ATR-FTIR, SEM, contact angle, XPS and ESR. ATR-FTIR spectra of plasma-modified samples demonstrated anhydride peaks (1084 cm^{-1}), which were interpreted as indication of crosslinking. However, scanning electron micrographs of the same samples revealed degradation at the polymer surfaces. It is concluded that crosslinking and degradation on the surface are competitive reactions, taking place at the same time. Plasma modification of discharge power of 100 W leaded to harsh etching.

XPS results revealed increased oxygen and nitrogen population on the surface, which were attributed to post-plasma reactions. ESR signals were observed for plasma-modified samples even after 10^{th} day of the application. Presence of living radicals supported the occurence of post-plasma reactions.

Plasma treatment also resulted in increase of hydrophilicity. Whereas the contact angle values were ~50° for control and prs samples; decreased to ~19° with the application of plasma. However the increase in hydrophilicity did not have an effect on swelling and release profiles.

For control samples drug release rate was higher at pH 2, which is attributed to high solubility of the drug at acidic medium. At pH 2, 25% of the loaded drug was released in less than two days and 50% of it was released in four days. At basic medium a delay in the release was observed, most probably because of the time lag between swelling of the matrix and dissolution of CPFX. Halves of the CPFX loaded was released in six days. At pH 4 and 6; hydrogel samples released halves of their CPFX content in seven days.

Although the plasma-surface-modification was aimed to supress drug release by surface-crosslinking of the drug-loaded hydrogel matrices; no appearent differences were observed for the release profiles of plasma-treated and non-treated samples, except the release at pH 2. For pls 20 sample 25% of the loaded drug loaded was released in three days at pH 2. In seven days 50% of the loaded drug was released at pH 2. However, at high pH values suppressing effects of the modification are disappeared most probably due to very high swelling degree of the hydrogels. As it was for pls 20 sample; no apparent differences in release rates were observed between control and pls 100 samples, except at pH 2. The same suppressing effect was observed for pls 100 sample at pH 2. Although the release rate was decreased when compared to control sample; suppressing effect of the plasma treatment was less prominent for pls 100 sample. 25% of the loaded CPFX was released in two days, whereas it was three and less than two days for pls 20 and control samples, respectively. 50% of the drug content was released in less than six days. Release profiles for pls 100 samples could not be investigated at basic media due to disintegration of the hydrogels during the measurements.

Prs samples were prepared in order to achieve fast release. Since the prs samples are fast-swelling with high equilibrium swelling degrees, CPFX solubility dominates over the release kinetics. As the release profiles were investigated it is seen that release rates decreases as pH of the environment increases. It is also observed here that, at pH 8 and pH 10, only the halves of the drug loaded were

released on the contrary. This might be explained by a probable drug-matrix interaction at this pH. Presence of salt crystals during the polymerization reaction might be affecting the matrix structure which in turn interacts with the drug molecule. Release rates of CPFX from prs samples seem to be decreased when compared to the other samples. However, as the amount of CPFX loaded into prs sample is concerned, if the release data were given in mg/mL, it could be seen that CPFX release rate is actually higher for the prs samples when compared to control sample. For example at pH 2; control sample releases ~4, pls 20 sample releases ~1.7, pls 100 sample releases ~3.4 and prs sample releases ~4.5 mg CPFX in one day. On the 7^{th} day, released CPFX amounts were found to be ~16, ~12, ~13.5 and ~23 mg for the control, pls 20, pls 100 and prs samples respectively. On the 10^{th} day of the release experiments, ~19.5, ~15, ~16.5 and ~29 mg CPFX was released from control, pls 20, pls 100 and prs samples.

As a result, it is demonstrated that pH-sensitive poly(acrylamide-co-acrylic acid) hydrogels can be modified by plasma glow discharge application or salt-leaching techniques to modify and to control the release rates of bioactive agents. For the model drug CPFX, depending on the modification conditions and depending on the pH of the medium, release rates changing between days to weeks are obtained.

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APPENDIX A

For the preparation of buffer solutions, chemicals given in the table below were mixed and completed to 1 L with distilled water. All chemicals were purchased from Riedel-De Haen (Germany).

Table A.1 Chemicals used in the preparation of buffer solutions

Chemical	**Buffer solution**				
	pH 2	**pH 4**	**pH 6**	**pH 8**	**pH 10**
H_3PO_4	0.0043 mol (0.21 mL)				
NaH_2PO_4	0.0057 mol (0.787 g)	0.01 mol (1.38 g)	0.0094 mol (1.297 g)	0.0014 mol (0.193 g)	
Na_2HPO_4			0.0006 mol (0.107 g)	0.0086 mol (1.531 g)	0.01 mol (1.78 g)

APPENDIX B

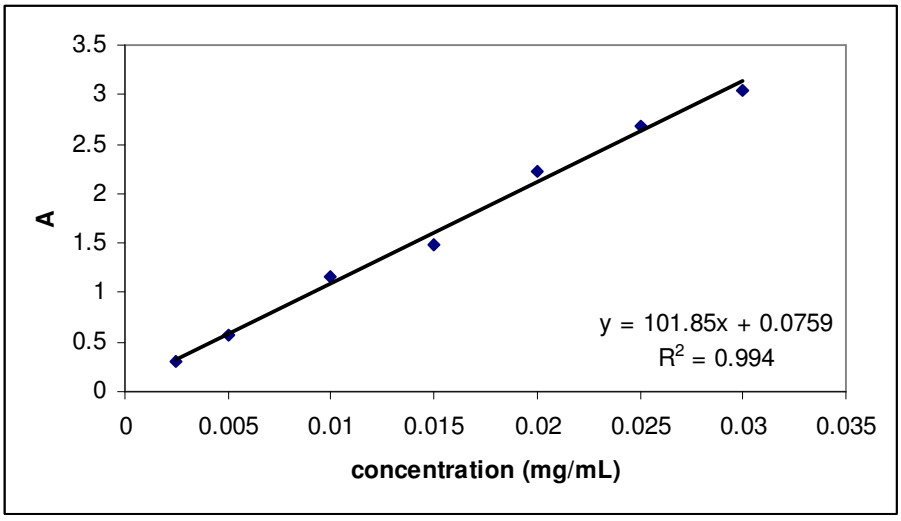

Figure B.1 Calibration curve for CPFX dissolved in pH 2 buffer solution

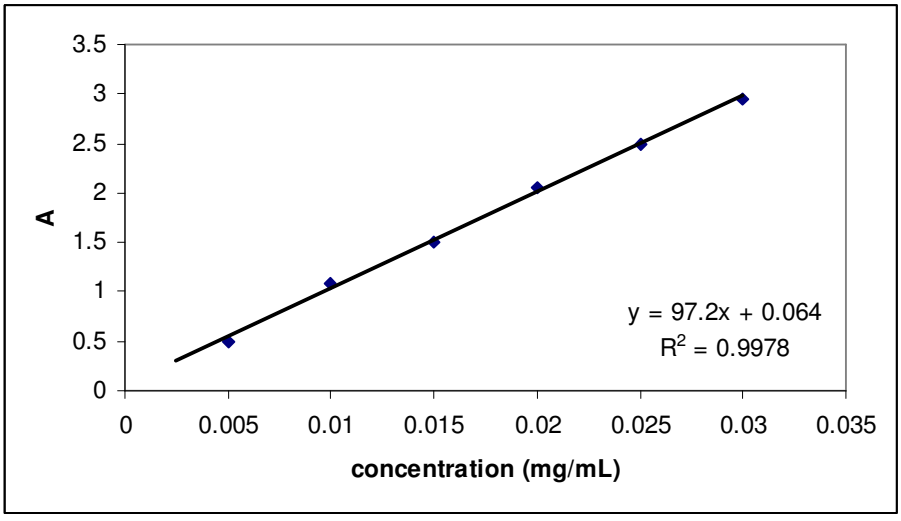

Figure B.2 Calibration curve for CPFX dissolved in pH 4 buffer solution

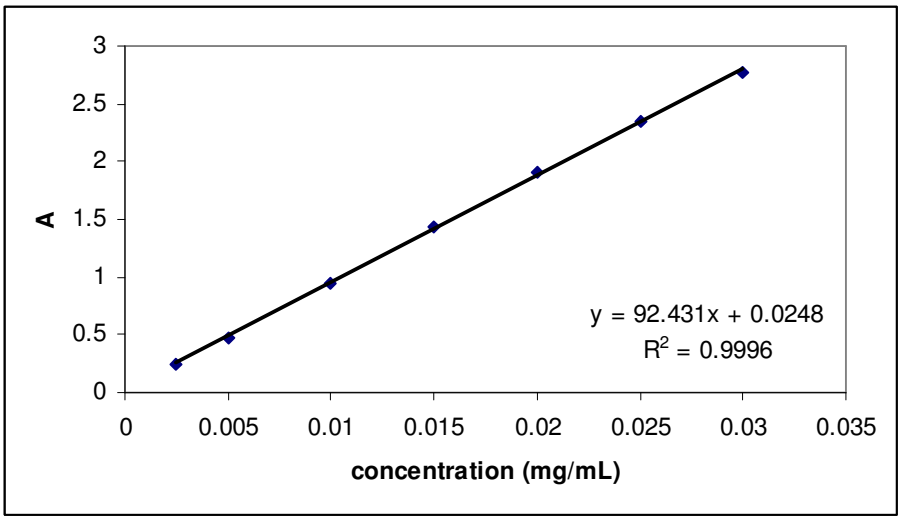

Figure B.3 Calibration curve for CPFX dissolved in pH 6 buffer solution

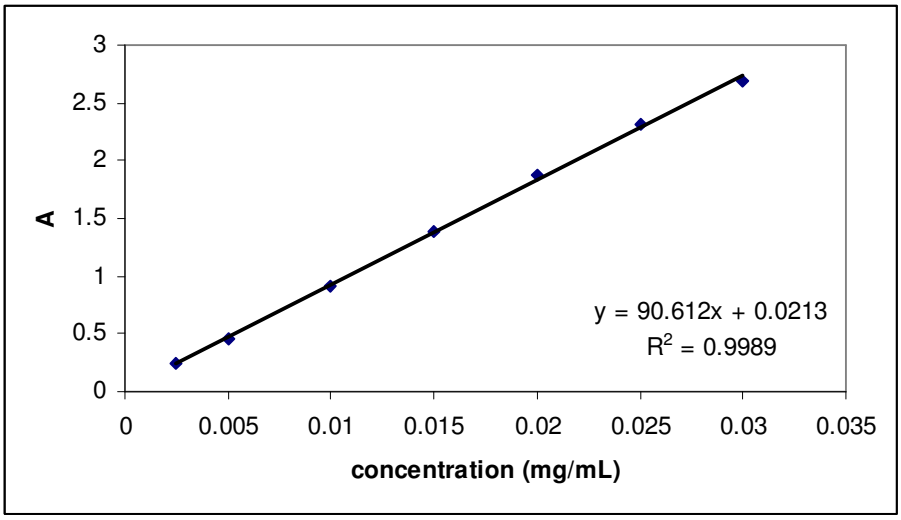

Figure B.4 Calibration curve for CPFX dissolved in pH 8 buffer solution

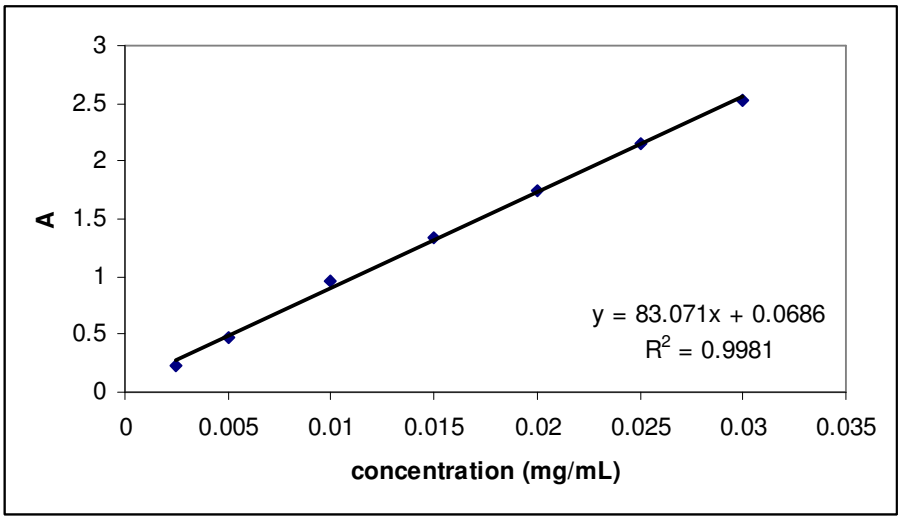

Figure B.5 Calibration curve for CPFX dissolved in pH 10 buffer solution