DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE BASED HYDROGELS

by
Merve DİLAVER

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İZMİR

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE BASED HYDROGELS"** completed by **MERVE DİLAVER** under supervision of **PROF. DR. KADİR YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE BASED HYDROGELS

ABSTRACT

Carboxymethylcelusose (CMC) based hydrogels were synthesized by crosslinking methot in presence of poly(vinylalcohol) (PVA) and fumaric acid (FA) used as a crosslinker. NaCMC and PVA cross-linked with different concentrations of FA. Hydrogels were characterized with different methods (FTIR, TGA, XRD and SEM) of analysis. The water absorbencies of these hydrogels in different pH solutions were investigated. As the FA concentration was increased, equilibrium swelling capacity reduced due to increasing crosslinking between CMC and PVA. Also pH sensitivity and reversibilty of hydrogels were investigated. The results indicated that the hydogels in the base medium had higher swelling behavior than the acidic medium. More over the hydrogels had pH reversibilty behavior which swelled at pH=8 and shrinked at pH=2.6. The FTIR results indicated the cross-linking between carboxyl group of FA with hydroxyl group of PVA and NaCMC through ester formation. The thermal behavior of the hydrogels was examined by TGA/DTG. The crosslinking improved the thermal stability of the hydrogels. Furthermore the SEM analysis showed that a highly porous structure was observed for 10FA and 15FA hydrogels while for 20FA and 25FA samples firstly high fibrous structure, then dispersion was observed.

Keywords: carboxymethylcellulose, fumaric acid, poly(vinylalcohol), crosslinking, swelling behavior, hydrogel

KARBOKSİMETİLSELÜLOZ TABANLI HİDROJELLERİN HAZIRLANMASI VE KARAKTERİZASYONU

ÖZ

Karboksimetilselüloz (CMC) tabanlı hidrojeller çapraz bağlanma metodu ile polivinilalkol (PVA) varlığında sentezlenmiştir ve çapraz bağlayıcı olarak fumarik asit (FA) kullanılmıştır. Sodyumkarboksimetilselüloz (NaCMC) ve polivinilalkol farklı konsantrasyonlarda FA kullanılarak çapraz bağlanmştır. Hidrojeller farklı karakterizasyon yöntemleri (FTIR, TGA, XRD ve SEM) kullanılarak incelenmiştir. Hidrojellerin su absorblayabilme özelliği farklı pH çözeltilerinde incelenmiştir. FA konsantrasyonu arttıkça; CMC ve PVA arasındaki çapraz bağlamanın artmasından dolayı, denge şişme kapasitesi azalmıştır. Ayrıca hidrojellerin pH duyarlılıkları incelenmiştir. Sonuçlar hidrojellerin bazik ortamda asidik ortama göre daha çok şişme davranışında bulunduğunu göstermiştir. Buna ek olarak hidrojeller pH tersinir özelliğine sahiptir. pH=8 de şiştiği ve pH=2.6 da büzüldüğü gözlenmiştir. FTIR spektrumları, FA'in karboksil grupları ile PVA'ün ve NaCMC'nin hidroksil grupları arasında çapraz bağlanmanın ester formunda oluştuğunu göstermiştir. Hidrojellerin termal davranışları, TGA/DTG analizi ile incelenmiştir. Hidrojellerdeki termal kararlılıkları çapraz bağlanma ile arttmıştır. SEM analizi, 10FA ve 15FA hidrojellerinde yüksek gözenekli bir yapının oluştuğunu, 20FA ve 25FA örneklerinde lifli yapının önce arttığı sonra ise çözüldüğünü göstermiştir.

Anahtar sözcükler: karboksimetilselüloz, fumarik asit, poli(vinilalkol), çapraz bağlanma, şişme davranışı, hidrojel

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CHAPTER ONE
INTRODUCTION

1.1 Hydrogels

Hydrogels are defined as three-dimensional polymer networks that are able to absorb large quantities of water, but remain insoluble due to chemical or physical crosslinks between individual polymeric chains (Peppas, Bures, Leobandung & Ichikawa, 2000). In the polymeric network hydrophilic groups or domains are present which are hydrated in an aqueous environment thereby creating the hydrogel structure. As the term 'network' implies, crosslinks have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase. Hydrogels can also be described in a rheological way. Aqueous solutions of hydrophilic polymers at low or moderate concentrations, where no substantial entanglement of chains occurs, normally show Newtonian behavior. On the other hand, once crosslinks between the different polymer chains are introduced, the so obtained networks show viscoelastic and sometimes pure elastic behavior (Park K., Shalaby & Park H., 1993; Peppas, 1986). It also provides required mechanical strength and physical integrity to the hydrogels (Rowley, Madlambayan, Faulkner & Mooney, 1999). Thus, hydrogels can imbibe water nearly 10-20 times its molecular weight and hence become swollen (Kim, Bae & Okano, 1992). The swelling capacity of hydrogels has been studied by different authors. It is well known that the presence of fixed charges, typical of polyelectrolyte gels, determines a significant swelling of the polymer in water. This behavior is due to a Donnan equilibrium established between gel and the external solution, whose ionic strength strongly affects the swelling degree (Esposito, Nobile, Mensitieri & Nicolias, 1996). Because of their water-absorbing capacity, hydrogels are not only subject of investigation of researchers interested in fundamental aspects of swollen polymeric networks, but have also found widespread application in different technological areas, e.g. as materials for contact lenses and protein separation, matrixes for cell-encapsulation and devices for the controlled release of drugs and proteins (Park et al., 1993; Peppas,1986).

Since the favorable properties of hydrogels stem from their hydrophilicity, the characterization of their water-sorption capabilities is the first step towards understanding the nanoscopic structure of hydrogel networks. Generally, three parameters are critical in describing the nanostructure of crosslinked hydrogel networks (Peppas, Huang, Torres-Lugo, Ward & Zhang, 2000):

1- Polymer volume fraction in the swollen state
2- Number average molecular weight between crosslinks
3- Network mesh size (ξ)

For non-porous hydrogels, the amount of liquid being retained in the hydrogel, the distance between polymer chains, and the flexibility of those chains together determine the mobility of encapsulated molecules and their rates of diffusion within a swollen hydrogel matrix.

Theoretically, no solute diffusion is possible within the hydrogel matrix when mesh size approaches the size of the solute as shown in Figure 1.1 (Peppas, Keys, Torres-Lugo & Lownan, 1999). Mesh size is affected by several factors including;

- Degree of crosslinking of the gel
- Chemical structure of the composing monomers
- External stimuli such as temperature, pH and ionic strength.

Mesh size is important in determining the physical properties of the hydrogels including mechanical strength, degradability, and diffusivity of the releasing molecule (Mason et al., 2001; Amsden, 1998). Typical mesh sizes reported for biomedical hydrogels range from 5 to 100 nm in their swollen state (Mason et al., 2001; Cruise, Scharp & Hubbell, 1998). These size scales are much larger than most small-molecule drugs and therefore diffusion of these drugs are not significantly retarded in swollen hydrogel matrices. However, the release of macromolecules such as peptides, proteins, and oligonucleotides can be sustained from swollen hydrogels due to their significant hydrodynamic radii. When designed appropriately, the structure and mesh size of swollen hydrogels can be tailored to obtain desired rates of macromolecule diffusion (Lustig & Peppas, 1998). Alternatively, the rate and degree of gel swelling or degradation can also be tailored to control the release of molecules much smaller than the gel mesh size.

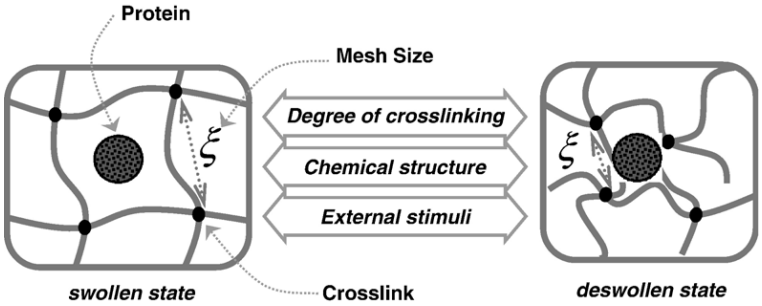


Figure 1.1 Schematic of mesh size in hydrogels at swollen or shrunken states (Peppas et al., 1999).

1.1.1 Classification of Hydrogels

Concerning definitions of hydrogel types, according to the source, hydrogels can be divided into those formed from:

- Natural polymers
- Synthetic polymers

On the basis of the cross-linking method, the hydrogels can be divided into:

- Chemical gels
- Physical gels (Silva, Richard, Bessodes, Scherman & Merten, 2009).

1.1.1.1 Hydrogel Types According to the Source

Hydrogels can be prepared from natural or synthetic polymers (Davis & Anseth, 2002). Those based on natural polymers present, generally, weak mechanical properties. These disadvantages are corrected on one hand by their biocompatibility and biodegradability and on the other hand by the fact they allow the sequence of cellular activity without any repulsion inflammatory response from the 'host' organism. Currently, polysaccharides are interesting due to the fact that they come from living organisms; they are biocompatible, nontoxic, and present major physicochemical properties necessary for controlled release applications (Coviella, Matricardi, Marianecci & Alhaique, 2007). The most studied polysaccharides are alginate (Tonnesen & Karlsen, 2002; Liang et al., 2004), dextran (Hennink, Franssen, Van Dijk-Wolthuis & Talsman, 1997; Stubble, Maris, Van Den Mooter,

De Smendt & Demeester, 2001), gellan (Agnihotri & Aminabhavi, 2005), xanthan (Dumitriu & Chornet, 1998), hyaluronic acid (Barbucci, Consumi, Lamponi & Leone, 2003; Ha et al., 2006), and chitosan derivatives (Kumari & Kundu, 2007; Guo & Gao, 2007). Synthetic hydrogels, on the other hand, do not possess these inherent bioactive properties. Fortunately, synthetic polymers usually have well-defined structures that can be modified to yield tailorable degradability and functionality. Table 1.1 lists natural polymers as well as synthetic monomers that are commonly used in hydrogel fabrication.

Table 1.1 Natural polymers and synthetic monomers used in hydrogel fabrication (Peppas et al., 2000; Davis et al., 2002)

Natural Polymer	**Synthetic Monomer**
Chitosan	Hydroxyethyl methacrylate (HEMA)
Alginate	N-(2-hydroxypropyl) methacrylate (HPMA)
Fibrin	N-vinyl-2-pyrrolidone (NVP)
Collagen	N-isopropyl acrylamide (NIPAAm)
Gelatin	Vinyl acetate (VAc)
Hyaluronic acid	Acrylic acid (AA)
Dextran	Methacrylic acid (MAA)
	Polyethylene glycol acrylate/methacrylate (PEGA/PEGMA)
	Polyethylene glycol diacrylate/dimethacrylate (PEGDA/PEGDMA)

1.1.1.2 Hydrogel Types According to the Cross-linking Method

Various physical and/or chemical cross linking mechanisms have been used for *insitu* network formation.

Physical gels involved in the formation of in-situ hydrogels are as follows:

- Hydrogen bonding
- Hydrophobic – hydrophobic interactions
- Electrostatic interactions

For example, sodium alginate hydrogels are formed physically by cross-linking due to addition of calcium ions but are unstable and disintegrate rapidly and unpredictably (Zhou, Chu, Wu, 2004).

Chemical gels are formed by covalent bonds. Chemical cross linking methods performed under physiological conditions produce relatively stable hydrogel networks with predictable degradation behavior. For example, photo polymerization of multi- vinyl macromers. It is a fast process and can be conducted at room temperature without organic solvents (Gombotz & Wee, 1998). Photo polymerization of degradable hydrogels may be applied to protein and gene delivery (West & Hubbell, 1995; Burdick, Mason, Hinman, Thorne & Anseth, 2002).

1.1.2 Preparation of Hydrogels

1.1.2.1 Use of Crosslinkers

Copolymerization of monomers using multifunctional co-monomer, which acts as cross linking agent, chemical initiator initiates the polymerization reaction which can be carried out in bulk, solution or suspension.

Cross linking of linear polymers by irradiation or by chemical compounds. Monomers used here contain an ionizable group that can be ionized or can undergo a substitution reaction after the polymerization is completed.

Thus, the hydrogels synthesized may contain weakly acidic groups like carboxylic acids or weakly basic groups like substituted amines or a strong acidic and basic group like sulfonic acid and quaternary ammonium compounds.

Cross linkers incorporated are glutaraldehyde, calcium chloride and oxidized konjac glucomannan (DAK). They impart sufficient mechanical strength to the polymers and thus prevent burst release of the medicaments (Ta, Dass & Dunstan, 2008).

1.1.2.2 Use of gelling agent

Gelling agents like glycophosphate-1,2-propanediol, glycerol, trehalose, mannitol etc have been used in the preparation of hydrogels. However, presence of negative charged moieties and turbidity are the problems associated with the method (Schuetz, Gumy & Jordan, 2008).

1.1.2.3 Use of irradiation and freeze thawing

Irradiation method is suitable as well as convenient but the processing is costly along with the poor mechanical strength of the product. Freeze thawing method imparts sufficient mechanical strength and stability to the hydrogels except that they are opaque in appearance with little swelling capacity. However, hydrogels prepared from microwave irradiation are more porous than conventional methods (Yang, Liu, Chen, Feng & Zhu, 2008).

1.1.2.4 Synthesis of hydrogel in industry

Formulation of monomer along with initiators and additives lead to polymerization which forms the gel. The gel is dried, sieved and mixed with other additives and post treatment is done if needed. The final formulation is packed and dispatched (Singh, Sharma, V. K. Gard & G. Gard, 2010).

1.1.3 Smart Hydrogels

Several terms have been coined for hydrogels, such as “intelligent gels” or “smart hydrogels” (Dagani, 1997). The smartness of any material is the key to its ability to receive, transmit or process a stimulus, and respond by producing a useful effect (Harvey, 1995). Once acted on, stimuli can result in changes in phases, shapes, optics, mechanics, electric fields, surface energies, recognition, reaction rates and permeation rates. Hydrogels are ‘smart’ or ‘intelligent’ in the sense that they can perceive the prevailing stimuli and respond by exhibiting changes in their physical or

chemical behavior, resulting in the release of entrapped drug in a controlled manner (Kost, 1999).

The past few years have witnessed enormous advances in polymer-based controlled-release drug delivery systems. Several products displaying constant or decreasing release rates have progressed from the laboratory to the clinic in this short period of time. Most of these systems are therapeutically advantageous over conventional systems, but are insensitive to changing metabolic states in the body. To synchronize the drug-release profile with physiological conditions, mechanisms responding to physiological variations must be provided. An ideal drug delivery system should respond to physiological requirements, sense the changes and accordingly alter the drug-release profile. The symptoms of most of the disease states follow a rhythmic pattern and require drug delivery as per the rhythms. Above all, if the drug possesses some side effects, drug release when not required poses an extra burden on the body's metabolic system. Thus, drug delivery patterns need to be optimized for pulsed or self-regulated mechanisms.

Hydrogels can exhibit dramatic changes in their swelling behavior, network structure, permeability or mechanical strength in response to different stimuli, both internal and external to the body (Lowman, Peppas, 1999). Various stimuli that have been explored for modulating drug delivery are represented in Figure 1.2 (Kost, 1999).

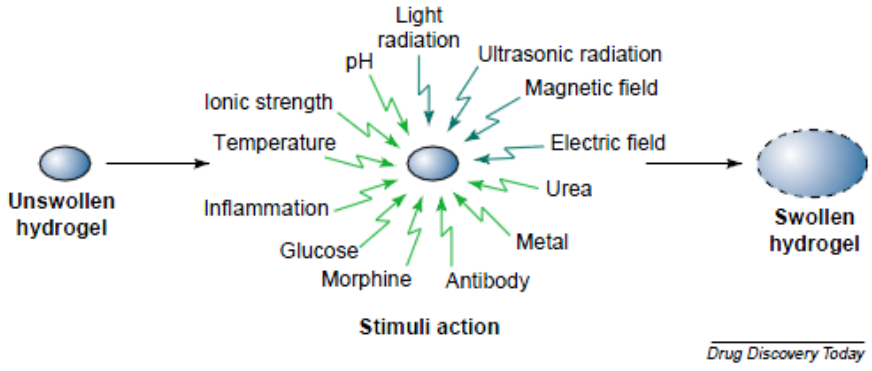


Figure 1.2 Stimuli responsive swelling of hydrogels (Gupta, Vermani, & Garg, 2002).

External stimuli have been produced with the help of different stimuli generating devices, whereas internal stimuli are produced within the body to control the structural changes in the polymer network and to exhibit the desired drug release.

Most of the time, drug release is observed during the swelling of the hydrogel. However, a few instances have been reported for drug release during syneresis of the hydrogel, as a result of a squeezing mechanism (Gutowska et al., 1997). Another interesting characteristic about many responsive hydrogels is that the mechanism causing changes in network structure can be entirely reversible in nature. This imparts elastic deformability with "shape-memory" behavior so that hydrogels return back to their original shape at the end of triggering stimuli.

Much research has been directed towards single-stimulus-responsive hydrogels for drug delivery. This might not be advantageous in pathological conditions with more than one physiological stimulus present, where drug release is required in the presence of both stimuli rather than a single one. An interpenetrating network (IPN) of gelatin and dextran has been proposed as a dual-stimuli-responsive biodegradable hydrogel (Kurisawa & Yui, 1998), wherein lipid microspheres (LM) have been incorporated as drug micro-reservoirs. The hydrogel prepared below the sol–gel transition temperature was found to release LM in the presence of both α-chymotrypsin and dextranase, whereas it hindered the release in the presence of either enzyme alone.

Table 1.2 summarizes various applications of stimuli-responsive drug delivery systems. Because of feasibility in therapeutic applications, product scale-up and cost considerations, internal stimuli-responding systems have gained wider attention compared with those governed by external stimuli. Among this category, the application and development of temperature-responsive and pH-responsive systems have been extensively studied for stimuli-responsive drug delivery. The temperature has to be altered externally in most cases except maybe hyperthermia therapy within narrow limits. But the pH changes within the body and it can therefore be used to direct the response to a certain tissue or cellular compartment (Table 1.3).

Table 1.2 Stimuli-responsive hydrogels in drug delivery Gupta, P., Vermani, K., & Garg, S. (2002).

Stimuli	Polymer	Drug
Magnetic field	Ethylene-co-vinyl acetate (EVAc)	Insulin
Ultrasonic radiation	EVAc, Ethylene-co-vinyl alcohol	Zinc bovine insulin, insulin
Electric field	Poly(2-hydroxyethyl methacrylate) (PHEMA)	Propranolol hydrochloride
Glucose	EVAc	Insulin
Urea	Methyl vinyl ether-co-maleic anhydride	Hydrocortisone
Morphine	Methyl vinyl ether-co-maleic anhydride	Naltrexone
Antibody	Poly(ethylene-co-vinyl acetate)	Naltrexone, ethinyl estradiol
pH	Chitosan–poly (ethylene oxide) (PEO)	Amoxicillin, metronidazole
	Poly(acrylic acid):PEO	Salicylamide, nicotinamide, clonidine hydrochloride, prednisolone
	Gelatin–PEO	Riboflavin
	PHEMA	Salicylic acid
	Poly(acrylamide-co-maleic acid)	Terbinafine hydrochloride
	N-vinyl pyrrolidone, polyethylene glycol diacrylate, chitosan	Theophylline, 5-fluorouracil
Temperature	Poly(*N*-isopropyl acrylamide)	Heparin
pH and temperature	Poly(*N*-isopropyl acrylamide-co-butyl methacrylate-co-acrylic acid)	Calcitonin

1.1.3.1 pH-Responsive Hydrogels

The pH is an important signal, which can be addressed through pH-responsive materials. The physiological pH changes have been mentioned earlier (Table 1.3). Ionisable polymers with a pKa value between 3 and 10 are candidates for pH-responsive systems (Siegel, 1993). Weak acids and bases like carboxylic acids, phosphoric acid and amines, respectively, exhibit a change in the ionization state upon variation of the pH. This leads to a conformational change for the soluble polymers and a change in the swelling behavior of the hydrogels when these ionisable groups are linked to the polymer structure.

Table 1.3 pH in various tissues and cellular compartments (Florece & Attwood, 1998; Watson & Jones, 2005; Grabe & Oster, 2001).

Tissue/cellular compartment	pH
Blood	7.35–7.45
Stomach	1.0–3.0
Duodenum	4.8–8.2
Colon	7.0–7.5
Early endosome	6.0–6.5
Late endosome	5.0–6.0
Lysosome	4.5–5.0
Golgi	6.4
Tumour,extracellular	7.2–6.5

Variations in pH are known to occur at several body sites, such as the gastrointestinal tract (Guyton & Hall, 1998), vagina (Deshpande, 1992) and blood vessels, and these can provide a suitable base for pH-responsive drug release. In addition, local pH changes in response to specific substrates can be generated and used for modulating drug release. The pH-responsive drug delivery systems have been targeted for peroral controlled drug delivery (Bilia et al., 1996; Patel & Amiji, 1996), taste-masking of bitter drugs (Ito et al., 1994) and intravascular drug release during elevated blood pH in certain cardiovascular defects (Brazel & Peppas, 1996).

pH-responsive hydrogels are composed of polymeric backbones with ionic pendant groups. Most commonly studied ionic polymers for pH-responsive behavior include poly(acrylamide) (PAAm), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(diethylaminoethyl methacrylate) (PDEAEMA) and poly(dimethylaminoethyl methacrylate) (PDMAEMA) (Lowman & Peppas, 1999). In aqueous media of appropriate pH and ionic strength, the pendant groups ionize and develop fixed charges on the polymer network, generating electrostatic repulsive forces responsible for pH-dependent swelling or deswelling of the hydrogel (Kost, 1999), thereby controlling the drug release. Small changes in pH can result in significant change in the mesh size of the polymeric networks. Pendant groups of anionic hydrogels are un-ionized below and ionized above the pK_a of the polymeric

network, leading to swelling of the hydrogel at a pH above the polymer pK_a because of a large osmotic swelling force by the presence of ions. The reverse is the case for cationic hydrogels, which swell at lower pH. Differential swelling of ionic hydrogels in acidic and alkaline buffers is presented in Fig. 1.3.

The effect of drug product size on its swelling kinetics has been favorably exploited for rapid response to changing environments. The development of chemically modified polyacrylamide-g-guar-gum-based anionic spherical hydrogels of micron size has been tried as pH- and ionic strength-sensitive drug delivery systems for diltiazem hydrochloride and nifedipine (Soppimath, Aminabhavi, Kulkami, & Rudzinski, 2001). The micron-sized spherical hydrogels were found to respond rapidly to a changing environment.

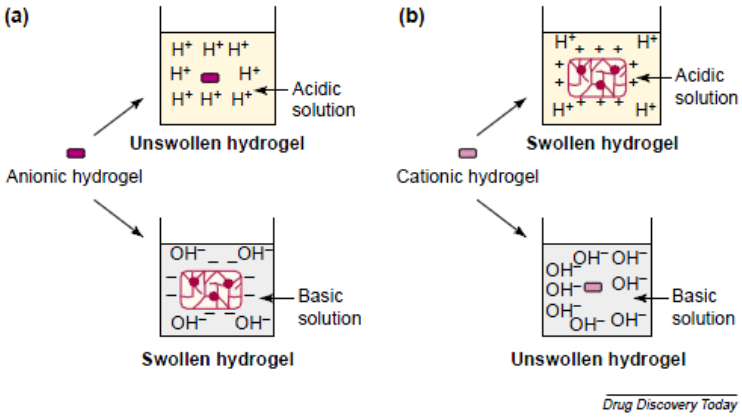


Figure 1.3 The pH-responsive swelling of **(a)** anionic and **(b)** cationic hydrogels. (Gupta, Vermani, & Garg, 2002).

1.1.3.2 Temperature-Responsive Hydrogels

Another important stimulus for causing hydrogel responsiveness is temperature. Temperature-responsive polymers and hydrogels exhibit a volume phase transition at a certain temperature, which causes a sudden change in the solvation state. Polymers, which become insoluble upon heating, have a so-called lower critical solution temperature (LCST). Systems, which become soluble upon heating, have an upper critical solution temperature (UCST). LCST and UCST systems are not restricted to an aqueous solvent environment, but only the aqueous systems are of interest for

biomedical applications. The change in the hydration state, which causes the volume phase transition, reflects competing hydrogen bonding properties, where intra- and intermolecular hydrogen bonding of the polymer molecules are favored compared to a solubilisation by water. Thermodynamics can explain this with a balance between entropic effects due to the dissolution process itself and due to the ordered state of water molecules in the vicinity of the polymer. Enthalpic effects are due to the balance between intra- and intermolecular forces and due to solvation, e.g. hydrogen bonding and hydrophobic interaction. The transition is then accompanied by coil-to-globule transition. There are also systems, which exhibit both LCST and UCST behavior, but that is usually not occurring within the setting of the intended biomedical applications. The corresponding hydrogels have similar transitions, the so-called lower gel transition temperature (LGTT) or upper gel transition temperature (UGTT) (Schmaljohann, 2006).

The most commonly used synthetic polymer for fabricating temperature sensitive hydrogels is poly(N-isopropylacrylamide) (poly(NIPAAM)), which possesses a lower critical solution temperature (LCST) at around 32 °C. The value of the LCST can be increased or decreased by copolymerizing hydrophilic or hydrophobic polymers with poly(NIPAAM). When the bulk temperature is higher than the LCST of the polymer, the polymer chains lose their bound-water. Hydrophobic interactions between the polymer chains lead to a rapid collapse (deswelling) of the gel (Schild, 1992). Readers are directed to other more thorough reviews discussing the mechanisms and applications of thermo-sensitive hydrogels (Ruel-Gariepy & Leroux, 2004; Jeong, Kim & Bae, 2002). Temperature-responsiveness is particularly useful for in-situ formation of drugdelivery devices since it allows handling of the formulation in the sol-phase at room temperature and solidification of the carrier upon injection (Ruel-Gariepy et al. 2004).

1.1.4 Application of Hydrogels

Wound Healing: Modified polysaccharide found in cartilage is used in formation of hydrogels to treat cartilage defects. For example, the hydrogel of gelatin and

polyvinyl alcohol (PVA) together with blood coagulants are formulated.

Soft Contact Lenses (silicon hydrogels and polyacrylamides): The first commercially available silicon hydrogels adopted two different approaches. First approach by Bausch and Lomb was a logical extension of its development of silicon monomers with enhanced compatibility in hydrogel forming monomers. The second by Ciba vision was the development of siloxy monomers containing hydrophilic polyethylene oxide segments and oxygen permeable polysiloxane.

Industrial Applicability: Hydrogels are used as absorbents for industrial effluents like methylene blue dye. Another example is adsorption of dioxins by hydrogel beads.

Tissue Engineering: Micronized hydrogels are used to deliver macromolecules (phagosomes) into cytoplasm of antigen-presenting cells. This property is also utilized in cartilage repairing. Natural hydrogel materials used for tissue engineering include agarose, methylcellulose and other naturally derived products.

Drug Delivery in GI Tract: Hydrogel deliver drugs to specific sites in the GIT. Drugs loaded with colon specific hydrogels show tissue specificity and change in the pH or enzymatic actions cause liberation of drugs. They are designed to be highly swollen or degraded in the presence of micro flora.

Rectal Delivery: Hydrogels showing bioadhesive properties are used for rectal drug delivery. Miyazaki et al. explored the xyloglucan gel with a thermal gelling property as matrices for drug delivery.

Ocular Delivery: Chitoni et al. reported silicon rubber hydrogel composite ophthalmic inserts. Cohen et al. developed ***in-situ*** forming gelling system of alginate with high gluconic acid contents for the ophthalmic delivery of pilocarpine.

Transdermal Delivery: Swollen hydrogels can be used as controlled release devices in the field of wound dressing. Hydrogel based formulations are being explored for transdermal iontophoresis to obtain enhanced permeation of products viz. hormones and nicotine (Singh et al., 2010).

Subcutaneous Delivery: Hydrogel formulations for subcutaneous delivery of anticancer drugs are being prepared viz. crosslinked PHEMA was applied to cytarabine (Ara-c). Implantable hydrogels are now leading towards the development of biodegradable systems which don't require surgical removal once the drug has been administered (Lee & Mooney, 2001; Van der Linden, Herber, Olthuis, Bergveld, 2003).

Novel Hydrogel For Controlled Drug Delivery: HYPAN is the novel hydrogel having properties useful controlled drug delivery. Physical network of crystalline clusters distinguishes HYPAN hydrogels from others (Azad, Sermsintham, Chandrkrachang & Stevens, 2004).

Hydrogel For Gene Delivery: Modification of hydrogel composition leads to effective targeting and delivery of nucleic acids to specific cells for gene therapy. Hydrogel versatility has potential application in the treatment of many genetic and/or acquired diseases and conditions (Van der Linden et al., 2003).

Cosmetology: Hydrogels when implanted into breast accentuate them for aesthetic reasons. These implants have silicon elastomer shell and are filled with hydroxyl propyl cellulose polysaccharide gel.

Tropical Drug Delivery: Instead of conventional creams, hydrogel formulations are employed to deliver active components like Desonide, a synthetic corticosteroid used as an anti – inflammatory for better patient compliance.

Protein Drug Delivery: Interleukins conventionally administered as injection are now given as hydrogels which show better compliance and form *in-situ* polymeric network and release proteins slowly (Singh et al., 2010).

1.1.5 Hydrogels and Bionanotechnology

The design and synthesis of "smart" hydrophilic polymers and hydrogels has significant potential in future biomedical and nanotechnology applications. The future success of these materials relies on the development of novel materials that can address specific biological and medical challenges. This development will occur through synthesis of new polymers or by modifying natural polymers. For tissue engineering, the desired tissue should be used as the model to engineer the desired chemical, mechanical, and biological properties into the hydrogel. Hydrogels being used for cartilage or tissue engineering should be capable of providing mechanical properties and loading as well as the molecular signals that are present in the native or regenerating organ. In addition, other properties of gels, such as pore sizes and degradation properties, must also be optimized. Novel tissue-engineering approaches should incorporate temporal and spatial signals that are present during the normal healing process.

With respect to drug delivery, the continued development of "smart" biocompatible materials that can respond to their environments will provide new and improved methods of delivering molecules for therapeutic applications. Finally, advancing the knowledge and the use of hydrogels and smart polymers for nanotechnology is an important area with significant potential that remains to be fully investigated. The incorporation of functional hydrogels into microdevices and the use of microdevices to engineer hydrogels will continue to provide new methods for fabricating improved hydrogelbased systems.

The above examples represent some of the approaches that can be used to synthesize and use hydrophilic polymers and hydrogels for biological and medical problems. With the development of new materials and novel methods of engineering chemical, mechanical, and biological functionality into hydrophilic molecules, we

anticipate that in the future hydrophilic polymers will play an even greater role in biomedical applications and nanotechnology (Peppas, Hilt, Khademhosseini & Langer, 2006).

1.2 Properties of Carboxymethyl Cellulose (CMC)

Cellulose is the most abundant renewable resource on earth. It will be expected that it will become the main chemical resource in the future (Schurz, 1999; Eichhorn, Young, & Davies, 2005). Moreover, numerous new functional materials from cellulose are being developed over a broad range of applications, because of the increasing demand for environmentally friendly and biocompatible products (Klemm, Heublein, Fink, & Bohn, 2005). Cellulose having abundant hydroxyl groups can be used to prepare hydrogels easily with fascinating structures and properties. Cellulose-based hydrogels have many favorable properties such as hydrophilicity, biodegradability, biocompatibility, transparency, low cost, and non-toxicity. Therefore, cellulose based hydrogels have wide applications in tissue engineering (Vinatier et al., 2009), controllable delivery system (Chang et al., 2010), blood purification (Ye, Watanabe, Iwasaki, & Ishihara, 2003), sensor (Sannino, Pappada, Giotta, & Maffezzoli, 2007), agriculture (Ibrahim et al., 2007), as well as water purification (Zhou et al., 2005) and chromatographic supports (Xiong, Zhang, & Wang, 2005). Figure 1.4 shows prospects for the various applications of cellulose-based hydrogels. Moreover, cellulose is environmental friendly and low-cost hydrogels, which will form a viable substitute for petroleum-based materials in near future.

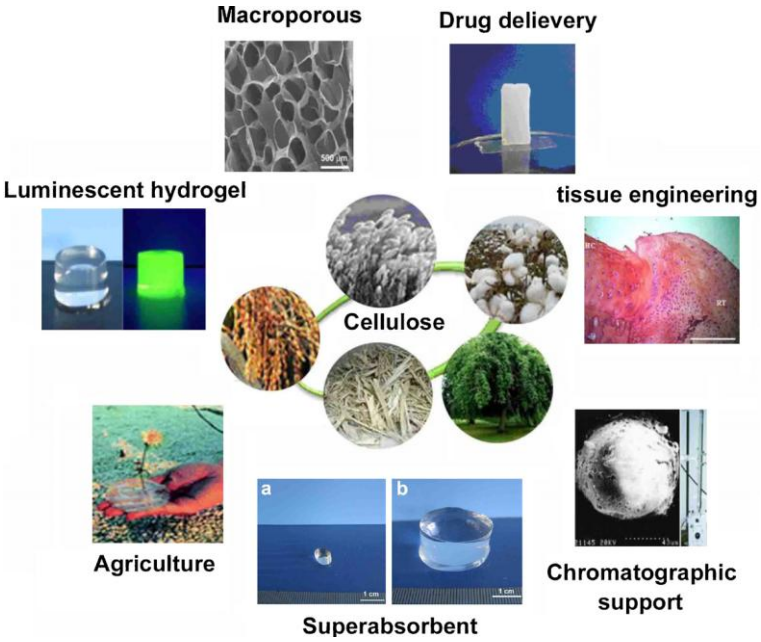


Fig 1.4 Prospects for applications and developments of cellulose based hydrogels (Chang et al., 2009a; Chang et al., 2009b; Chang et al., 2010; Liang et al., 2007; Marcì et al., 2006; Vinatier et al., 2009; Xiong et al., 2005).

Cellulose hydrogels can be prepared from a cellulose solution through physical cross-linking. Because cellulose has many hydroxyl groups which can form hydrogen bonding linked network easily. However, cellulose is very difficult to be dissolved in common solvents due to its highly extended hydrogen bonded structure (Edgar et al., 2001), so the major problem for preparing cellulose hydrogel is a lack of appropriate solvents. Due to the dissolving problem, water soluble cellulose derivatives can be used. Water soluble cellulose derivatives are mostly biocompatible which can be used as thickener, binding agents, emulsifiers, film formers, suspension aids, surfactants, lubricants and stabilizers, especially as additives in food, pharmaceutical, and cosmetic industries (Weng, Zhang, Ruan, Shi & Xu, 2004). Carboxymethyl cellulose (CMC) is one of the water soluble cellulose derivatives. CMC is a biocompatible, biodegradable, non-toxicity and low cost ionic polysaccharide that contains carboxyl and hydroxyl groups, more over exhibits pH sensitivity (Charpentier et al., 1997; Mitsumata et al., 2003).

Carboxymethylation of polysaccharides is a widely studied conversion since it is simple and leads to products with a variety of promising properties. In general, the polysaccharide is activated with aqueous alkali hydroxide, mostly sodium hydroxide, and converted with monochloroacetic acid or its sodium salt according to the Williamson ether synthesis yielding the corboxymethyl (CM) polysaccharide derivative (Heinze & Koschella, 2005). CMC is also a cellulose ether behaved as typical polyelectrolytes.

While the mostly applied sodium salt form of CMC is water soluble, a conversion by treating the polymer with a mineral acid leads to the water insoluble free carboxylic acid of the polymer. The pK_a value was determined to be 3.2 (Hoogendam et al., 1998). However, it should be pointed out that this pK_a represents an average value because it depends on the distance of the carboxylic group from the polymer backbone, i.e., it can be controlled by the pattern of functionalization. A CMC with a preferred carboxymethylation at O-2 possesses a pK_a of 3.0 while preferred O-6 modified samples give a pK_a of 3.3 (Kötz, Philipp, Nehls, Heinze & Klemm, 1990). This increase in pK_a value with decreasing distance of ionic group from the polymer backbone is even more pronounced for oxidized cellulose(6-corboxyl cellulose, pKa 2.8) as expected.

1.2.1 Application of CMC

1) Controlled drug release was investigated with CMC. The polymer was used as carrier for, e.g., erythomycia as model drug (Sungur & Emregül, 1996). The CMC was cross-linked with ferric salt to get biodegradable beads. Controlled release was improved by coating with gelatin/CMC and by cross-linking.

2) The macroscopic properties of polymer solutions are determined by microscopic (molecular) parameters. For example, the viscosity of solution of CMC and CMS in influenced by the molar mass of the polymers, their degree of branching, and their radius of gyration, Rg, as well as their flexibilty. Thickening properties of

the solution s are related to the rigidity of the polymer backbone, which can be characterized by the persistence length (Hoogendam et al., 1998).

3) CMC was studied as component in blends by mixing the aqueous solution of the polymer and further polysaccharides, e.g., konjac glucomannan (Xiao et al., 2001). The properties of such products like mechanical stability depend on the formation of hydrogen bonds, which can be controlled by the blending ratio.

4) The CMC (H-form) is of interest for metal ions adsorption, e.g., in wastewater treatment (Heinze, Helbig & Klemm, 1993). Experiments with Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, and Al^{3+} indicate a high adsorption capacity of up to 0.32 mmol Ni^{2+}/g and a very high rate of absorption. About 90% of binding is accomplished within 1 h. Applying beads of CMC optimal for separation by filtration, the spherical shape is not destroyed and hence it can be regenerated and reused.

5) The interaction of carboxylic groups with multivalent metal cations can be used to form so-called ionotropic gels, which are predominately stabilized by the electrostatic interactions. In addition interactions between the OH groups of the polymers and the metal ions contribute to the stability and the water insolubility of these polymeric aggregates. It should be pointed out that ionotropic gels can obtained from natural, biotechnological produced and chemically modified polysaccharides containing carboxyl functions as anionic groups and multivalent metal cations is covered by many review papers (e.g., Heinze, Klemm, Loth & Phillip, 1990 and references cited therein). An interesting area of application of these gels is cell immobilization and controlled release of bioactive compounds. It is even possible to dry the gel forming xerogels, which are still beads with a dense surface layer as exemplarily.

1.2.2 Hydrogels from Cellulose Derivatives

Selective cellulose derivatives, including methyl cellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), and carboxymethyl

cellulose (CMC) have been used to fabricate cellulose-based hydrogels through physical cross-linking and chemical cross-linking. In the case of physical cross-linked gels, there is no covalent bonding formation or breakage and the cross-linked network is formed through ionic bonding, hydrogen bonding, or an associative polymer-polymer interaction (Weng et al., 2004). In general, chemical cross-linked hydrogels are prepared through cross-linking two or more kinds of polymer chains with a functionalized cross-linker (Deng, He, Wu, & Yang, 2008) or under UV light (Guo & Chu, 2005).

1.2.2.1 Physical cross-linking

In the family of thermo-reversible hydrogels, hydrophobically modified cellulose is one of the largest members. When hydroxyl groups are substituted partly by methyl groups or hydroxylpropyl groups, some hydrogen bonds are prevented and the resultant derivatives become water soluble. MC aqueous solutions posses the unusual property of forming reversible physical gels, due to hydrophobic interactions when heated above a particular temperature (Li, Thangamathesvaran, Yue, Hu, & Lam, 2001). HPMC has a higher gelation temperature than MC, which forms firmer gels with equivalent substitution and molecular weight. There is evidence that the gelation of cellulose derivatives results from the exclusion of water from heavily methoxylated regions of polymer (Sammon, Bajwa, Timmins, & Melia, 2006).

1.2.2.2 Chemical cross-linking

1.2.2.2.1Cross-linking reagents The stable structure and effective swelling of cellulose-based hydrogels often require a chemically cross-linked network. Some di-functional molecules are employed as the cross-linker for cellulose or its derivatives to covalently bind different polymer molecules in a three dimensional hydrophilic network.

1.2.2.2.2 Radical cross-linking Irradiation is a useful method for obtaining chemical hydrogels, including irradiation of solid polymer, monomer (in bulk or in

solution) or polymer aqueous solution (Rosiak & Ulanski, 1999). The advantage of radical cross-linking is that cross-linkers, which limited the applications of hydrogels in the food, drug, and pharmaceutical industries due to their toxicity, are not required in the fabrication process, leading to high purity of the hydrogel product. The concentrated aqueous solutions of cellulose derivatives, such as CMC, HPC, and MC, can be cross-linked under ionizing radiation to prepare cellulose based hydrogels (Fei, Wach, Mitomo, Yoshii, & Kume, 2000).

1.3 Properties of Poly(vinyl alcohol) (PVA)

Another major synthetic polymer is PVA (Hassan & Peppas, 2000). PVA is water-soluble and Biocompatible (Chiellini, Corti, D'Antone & Solaro, 2003). PVA hydrogels are stable, and elastic gels that can be formed by the repeated freezing and thawing process or chemically crosslinked (Nuttelman, Mortisen, Henry & Anseth, 2001). They can be formed by both physical and chemical crosslinking methods (Peppas & Merrill, 1977). The physically crosslinked versions of PVA hydrogels are biodegradable, and thus can be used for various biomedical applications (Peppas et al., 1977; Shaheen & Yamaura, 2002). PVA must be crosslinked in order to be useful for a wide variety of applications, specifically in the areas of medicine and pharmaceutical sciences. Crosslinking may be achieved by chemical, irradiative, or physical mechanisms. PVA can be crosslinked through the use of difunctional crosslinking agents. Some of the common crosslinking agents that have been used for PVA hydrogel preparation include glutaraldehyde, acetaldehyde, formaldehyde, and other monoaldehydes. When these crosslinking agents are used in the presence of sulfuric acid, acetic acid, or methanol, acetal bridges form between the pendent hydroxyl groups of the PVA chains. As with any crosslinking agent, however, residual amounts are present in the ensuing PVA gel. It becomes extremely undesirable to perform the time-consuming extraction procedures in order to remove this residue. If the residue is not removed, the gel is unacceptable for biomedical or pharmaceutical applications because, if it were placed directly in the body, the release of this toxic residue would have obvious undesirable effects. Other methods of chemical crosslinking include the use of electron-beam or gamma irradiation.

These methods have advantages over the use of chemical crosslinking agents as they do not leave behind toxic, elutable agents. In addition, photocrosslinkable PVA hydrogels have been synthesized that facilitate cell adhesion in tissue-engineering applications (Schmedlen & Masters, 2002; Nuttelman, Henry & Anseth, 2002).

1.4 Properties of CMC/PVA Blend

Polyvinyl alcohol (PVA) is a good candidate for the preparation of hydrogels which can be cross-linked by using several methods, including chemical agents, electron beam, γ-irradiation, or physically thermal cycling. For biomedical applications, physical cross-linking has the advantage of avoiding residual amounts of toxic chemical cross-linker, and higher mechanical strength than PVA gels cross-linked by either chemical or irradiation techniques (Ivanov, Popa, Ivanov, & Popa, 2007). Also the effect of cross-linking methods on structure and properties of cellulose/PVA hydrogels were studied. Chemical hydrogels, prepared by cross-linking cellulose and PVA with ECH, have a high swelling ratio, but low mechanical strength as a result of the weak hydrogen bonding between cellulose and PVA (Chang, Lue, & Zhang, 2008). However, physical hydrogels prepared via solution blending of cellulose and PVA and repeating freezing/thawing cycles exhibit a dense structure between cellulose and PVA, leading to high mechanical strength. In addition, bacterial cellulose (BC) fibers of an average diameter of 50nmhave been used in combination with PVA, which can be transformed into a hydrogel through freezing/thawing cycles to form biocompatible nanocomposites. The nanocomposites possess a broad range of mechanical properties, and can be made with mechanical proper ties similar to that of cardiovascular tissue, such as aorta and heart valve leaflets (Millon&Wan, 2006). CMC/PVA copolymer hydrogels have been prepared by using electron beam irradiation technique, which can be used as dye removal materials. The results indicate that the hydrogel composition is an effective parameter in determining the dyes sorption character (Taleb, Abd El-Mohdy, & Abd El-Rehim, 2009).

1.5 Properties of Fumaric Acid

Fumaric acid or trans-butenedioic acid is the chemical compound with the formula $HO_2CCH=CHCO_2H$. This white crystalline compound is one of two isomeric unsaturated dicarboxylic acids, the other being maleic acid. In fumaric acid the carboxylic acid groups are trans (E) and in maleic acid they are cis (Z).

Fumaric acid was first prepared from succinic acid. A traditional synthesis involves oxidation of furfural (from the processing of maize) using chlorate in the presence of a vanadium-based catalyst. Currently, industrial synthesis of fumaric acid is mostly based on catalyticisomerisation of maleic acid in aqueous solutions at low pH. Maleic acid is accessible in large volumes as a hydrolysis product of maleic anhydride, produced by catalytic oxidation of benzene or butane.

The chemical properties of fumaric acid can be anticipated from its component functional groups. This weak acid forms a diester, it undergoes additions across the double bond, and it is an excellent dienophile.

Fumaric acid is used in many area like biology, medicine, food and hydrogels.

- Biology: Fumaric acid is found in fumitory, bolete mushrooms, lichen, and Iceland moss. Fumarate is an intermediate in the citric acid cycle used by cells to produce energy in the form of adenosine triphosphate (ATP) from food. It is formed by the oxidation of succinate by the enzyme succinate dehydrogenase. Fumarate is then converted by the enzyme fumarase to malate. Human skin naturally produces fumaric acid when exposed to sunlight. Fumarate is also a product of the urea cycle.
- Medicine: Fumaric acid esters are used to treat psoriasis, as it has been suggested that the condition is caused by an impairment of fumaric acid production in the skin. A starting dose is 60-105 mg. daily, which may be gradually increased to as much as 1290 mg. per day. Side effects include kidney or gastrointestinal disorders, as well asa skin flushing; these are mainly caused by excess intake. Decreased white blood cell (WBC) counts have been reported with prolonged use. A fumaric acid ester is currently under investigation for treatment of multiple sclerosis.

- Food: Fumaric acid is a food acidulent used since 1946. It is non-toxic. It is generally used in beverages and baking powders for which requirements are placed on purity. It is generally used as substitute for tartaric acid and occasionally in place of citric acid. It is also used as a coagulant in stovetop pudding mixes.
- Hydrogels: Fumaric acid-based macromers including poly(propylene fumarate-co-ethylene glycol) (P(PF-co-EG)) and oligo(poly(ethylene glycol) fumarate) (OPF) are used as biocompatible biodegradable hydrogels. Degradation released fumaric acid is a well known product that is naturally formed in the Krebs cycle and is found in mammalian cell metabolism. The presence of vinyl groups along the polymer backbone makes it an ideal substrate for crosslinking. Hydrogels based on P(PF-co-EG) or OPF macromers are formed in situ in the presence of an initiator by photo- or redox-initiated crosslinking either between macromers or using crosslinking agents, such as N-vinyl-2-pyrrolidinone (NVP) and PEG diacrylate. These hydrogels generally form mechanically strong networks that are biodegradable both in vitro and in vivo. Mechanical and swelling properties as well as degradation rates of the hydrogels are adjustable by changing the hydrophilicity and crosslinking density; these are controlled by changes in the molecular weight and molar ratio of the PEG, and the amount of macromers and crosslinkers, respectively. The biocompatibility of these hydrogels is well-documented with respect to various cell types, such as chondrocytes, endothelial cells and marrow stromal cells. In cartilage and bone tissue engineering, these materials are used as carriers for marrow stromal cells or chondrocytes and have been shown to promote cellular differentiation and matrix production in vitro (Ottenbrite, Park, & Okano, 2010).

1.6 Purpose of the Study

Cellulose and CMC are biocompatible and biodegradable, so they are ofen used in biomedical field. Recently, cellulose-based hydrogels prepared by using different synthesis methods.

Chang, Duan, Cai & Zhang (2009) have synthesized superabsorbent hydrogels successfully from CMC and cellulose in NaOH/urea aqueous solution by cross-linking with epichlorohydrin (EPC). The experimental results proved that the cellulose/CMC hydrogels exhibited superabsorbent capacity and high equilibrium swelling ratio, which could be improved by changing the amount of CMC. The hydrogels were sensitive to inorganic salts aqueous solution, physical saline water and synthetic urine, showing smart swelling and shrinking behaviors.

Buhus, Popa & Desbrieres (2009) were prepared hydrogels based on GEL and CMC by crosslinking with glutaraldehyde. The hydrogels have a macroporous morphology and their swelling degree was ~6000% depending on the hydrogel composition (GEL contenr) and the parameters of the cross-linking reaction. The ability of the hydrogels to include and release water-soluble drugs depend on the swelling degree in water and, hence, on the reaction parameters.

Xiao, Li & Gao (2008) prepared Fe-CMC/PVA double-network microparticles via ionic crosslinking and freezing-thawing technology. Such a process is physical and mild, and is suitable for entrapping bioactive substances.

Demitri et al. (2008) synthesized new environmentally friendly hydrogels from CMCNa and hydroxyethylcellulose (HEC) in distilled water by crosslinking with citric acid (CA). An optimal degree of swelling (900) for practical applications was achieved using low-CA concentrations. The hydrogel obtained through the method described in this article has the great advantage to reduce primary and production costs and avoid any toxic intermediate during its synthetic process.

The aim of this study is to synthesise and characterization carboxymethyl cellulose (CMC) based hydrogels by using biocompatible, biodegradable components for drug delivery. During the study, swelling behaviors of the hydrogel as a function of pH and amount of cross-linker will be investigated. The structural analysis of hydrogel will be studied using XRD, SEM and FTIR spectroscopy. The thermal behavior of the hydrogel will be determined by using TGA.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Materials

NaCMC was purchased from Sigma (low viscosity) and used without further purification. PVA was bought from Fluka (degree of polymerization: ~1600 and degree of hydrolysation 97.5-99.5 mol%). Fumaric acid was obtained from Sigma Aldrich.

2.2 Preparation of Hydrogels

Hydrogel synthesis reactions were performed in distilled water. The hydrogels were synthesized as follows: 2% polymer of the total weight of the water prepared by mixing NaCMC and PVS with weight ratio equal to 3/1. PVA and NaCMC were solved in 50 mL. of distilled water. Then solution of PVA was added solution of NaCMC the clear solution was expected to occur for 24 hours. Finally, fumaric acid (FA) was added to the mixture as the cross-linker contents of 10wt%, 15wt%, 20wt%, 25wt% and stirred at room temperature for 24 hours. These last solutions were poured into petri dishes. Then they were dried at drying oven at 60ºC for 6.30 h. and 21.30 h. at vacuum oven.

2.3 Characterization of Hydrogels

2.3.1 Swelling Studies

The hydrogel cuttings were put in the 10 mL. of different pH solution (pH= 2.6, 4, 6, 8), different pH buffer solution (HCl/KCl, PBS) and distilled water at 37ºC. The swelling values of hydrogels were determined at intervals, after removal of the surface water using tissue paper, until equilibrium swelling value was reached. The % swelling values were determined by following equation (Eq. 2.1).

$$\%Swelling = \%S = 100\left[\frac{m_t - m_0}{m_0}\right] \quad \text{(Eq. 2.1)}$$

Where m_0 is the initial mass and m_t the final mass of the gel at time t. Data points are means of three measurements.

2.3.2 Fourier Transform Infrared (FTIR) Spectra of the Samples

FTIR spectroscopy was used to obtain the information about the interactions between FA and NaCMC, PVA polymers chains. FTIR spectra of the hydrogels samples were taken as KBr pellets. For this purposes, 1 mg's of hydrogels were mixed with 100 mg KBr. After dried at 60 ºC, FTIR analyses were conducted.

Fourier transform infrared (FTIR) spectra (transmission) were measured on a Perkin-Elmer FTIR spectrophotometer. Spectrum BX-II n the range 4000-400 cm^{-1} at resolution of 4 cm^{-1}.

2.3.3 Thermal Analysis

Thermal behaviors of hydrogels were investigated by thermogravimetric analysis (TGA). TGA was performed under nitrogen flow and atmosphere air from 30 ºC to 525 ºC at a rate of 15ºC/min with Perkin Elmer Diamond TG/DTA instrument.

2.3.4 SEM Analysis

The surface morphologies of hydrogels were studied using a scanning electron microscope at an accelerating voltage of 20kV. The samples were coated with a thin gold layer two times before scanning to increase conductivity. SEM photographs were taken at different magnification (in the range of 50×, 100×, 250×, 500×, 1000× and 3000×) by using Jeol JSM 60 model SEM apparatus. These analysis were carried out partly at the Laboratories of Materials Research Center of İzmir Institute of Technology and Metallurgy and Materials Engineering Department of Dokuz Eylul University, İzmir.

2.3.5 XRD Analysis

The X-ray diffraction (XRD) patterns of the hydrogel films were recorded with oriented mounts, in a Philips X'Pert Pro X-Ray diffractometer using Cu K_α radiation at 45 kV and 40 mA in the 2θ range of 5-60º. These analysis were carried out at the Laboratories of Materials Research Center of İzmir Institute of Technology.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared (FTIR) Spectra of Samples

The structure of hydrogel films were analyzed by using FTIR spectroscopy. Figure 3.1 and 3.2 show FTIR spectra of NaCMC and NaCMC/PVA blend, hydrogels films, respectively.

In the evaluation of the FTIR data of the samples, one should know about the FTIR spectra of NaCMC in which showed bands at 3432 cm^{-1}, due to the stretching frequency of the –OH group. The band around 2920 cm^{-1} is due to C-H stretching vibration. The peresence of a strong absorption band at 1608 and 1423 cm^{-1} are due to the asymmetric and symmetric stretching of COO- group, respectively. The band around 1325 cm^{-1} are assigned to –OH bending vibration. The band at 1060 cm^{-1} is due to CH-O-CH_2 stretching (Qiu & Yu, 2007; Ma, Xu, Fan & Liang, 2007).

On the other hand, the IR spectrum of PVA shows characteristic broad band at 3456 cm^{-1} corresponding to the O-H stretching of the hydroxyl group of the PVA. The sharp band at 1650 cm^{-1} corresponds to the C-O stretching of the acetate group of PVA. On the other hand, the backbone aliphatic C-H stretching vibrations for PVA give sharp bands at 2924 and 2851 cm^{-1}. The strong absorption band at 1099 cm^{-1} has been assigned to the C-O in stretching mode for PVA and the bands observed at 1440 cm^{-1} have been attributed to combination frequencies of (CH-OH).

When we evaluate the FTIR spectra of the samples, as can be seen from Figure 3.1 that NaCMC shows bands at 3333 cm^{-1}, due to the stretching frequency of the –OH group. The band around 2918 cm^{-1} is due to C-H stretching vibration. The peresence of a strong absorption band at 1602 and 1423 cm^{-1} are due to the asymmetric and symmetric stretching of COO- group, respectively. The band around 1328 cm^{-1} are assigned to –OH bending vibration. The band at 1060 cm^{-1} is due to CH-O-CH_2 stretching. The FTIR spectra of 10FA, 15FA, 20FA and 25FA shows that the band

around 1730-1740 cm^{-1} appeared after synthesis of hydrogel by using fumaric acid. This band attributed to C=O stretching of ester carbonyl. While the FA content of hydrogels increased, C=O stretching of carboxylic acid for FA disappeared. These results may confirm the cross-linking between carboxyl group of FA with hydroxyl group of PVA and NaCMC through ester formation. Also cross-linking may reach the degree of saturation over 20FA hydrogels.

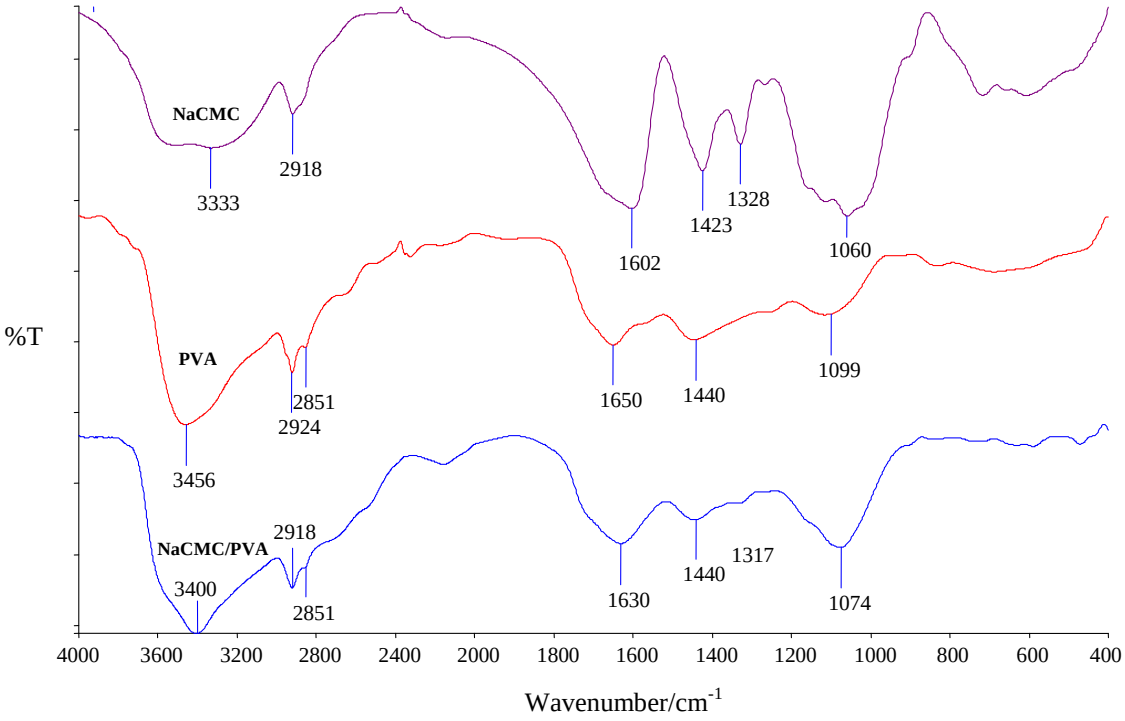


Figure 3.1. FTIR spectrum of the NaCMC, PVA and NaCMC/PVA.

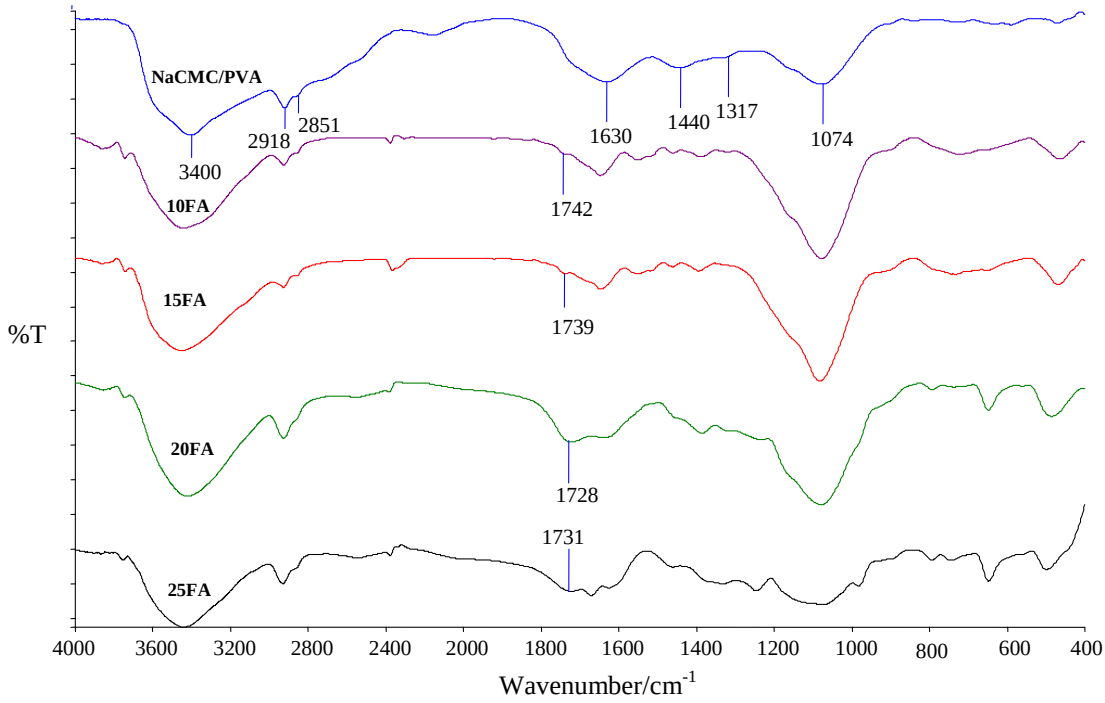


Figure 3.2 FTIR spectrum of the NaCMC7PVA blend, 10FA, 15FA, 20FA and 25FA.

3.2 Thermal Analysis

The TGA/DTA curves of NaCMC can be seen from Figure 3.3 as follows: there are two steps of degradation of NaCMC. The first step shows weight loss about 10% within the temperature range of 46–190 °C and the major weight loss about 40% occurs in the second step in 240-300 ºC, furthermore maximum decomposition temperature appears 290 ºC (Yang et al., 2009).

Pure PVA showed two-step decomposition: the first step began around 200 °C, and the second step began around 382 °C. The first step of weight loss could be attributed to the loss of loosely bound water, accompanied by the formation of volatile disintegrated products. The residue was predominantly macromolecules of a polyene structure. Further heating yielded carbon and hydrocarbons. The endset temperature was around 460 °C (Sreedhar, Sairam, Chattopadhyay, Syamala Rathnam & Mohan Rao, 2005).

The thermal stability of NaCMC/PVA blend and hydrogels has been investigated by TGA and DTA under nitrogen flow (Figures 3.4, 3.5). There are three steps of degradation of NaCMC/PVA blend. The first range (40-140 ºC) is associated with the loss of moisture about 10 wt%. The second range (200-340ºC) corresponds to the degradation NaCMC and PVA about 51 wt%. The third range (417-477 ºC) shows weight loss of polyene of PVA about 8 wt%. The DTA analysis of hydrogels two shoulder is appear between 200-361 ºC at figure 3.5. The first and second shoulders relate to degradation of NaCMC and PVA, respectively. The maximum degradation temperature of PVA is 200 ºC but this temperature is shifted to about 250 ºC while FA concentration is increased. Also the maximum degradation temperature of NaCMC is 290 ºC but this temperature is shifted to about 310 ºC while FA concentration is increased.

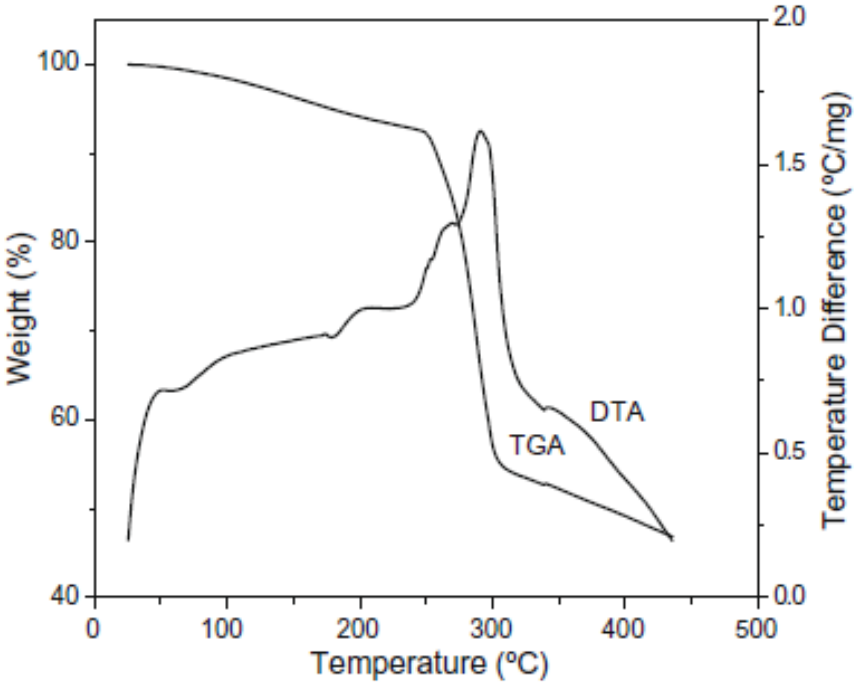


Figure 3.3 Thermogravimetric curves of NaCMC

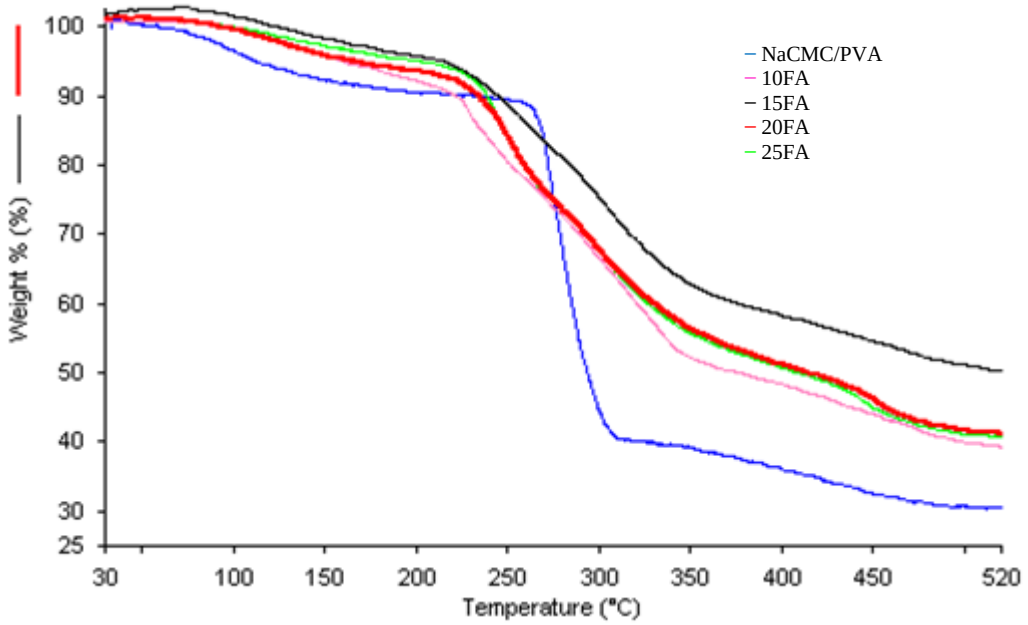


Figure 3.4 Thermogravimetric curves of NaCMC/PVA, 10FA, 15FA, 20FA and 25FA in nitrogen flow.

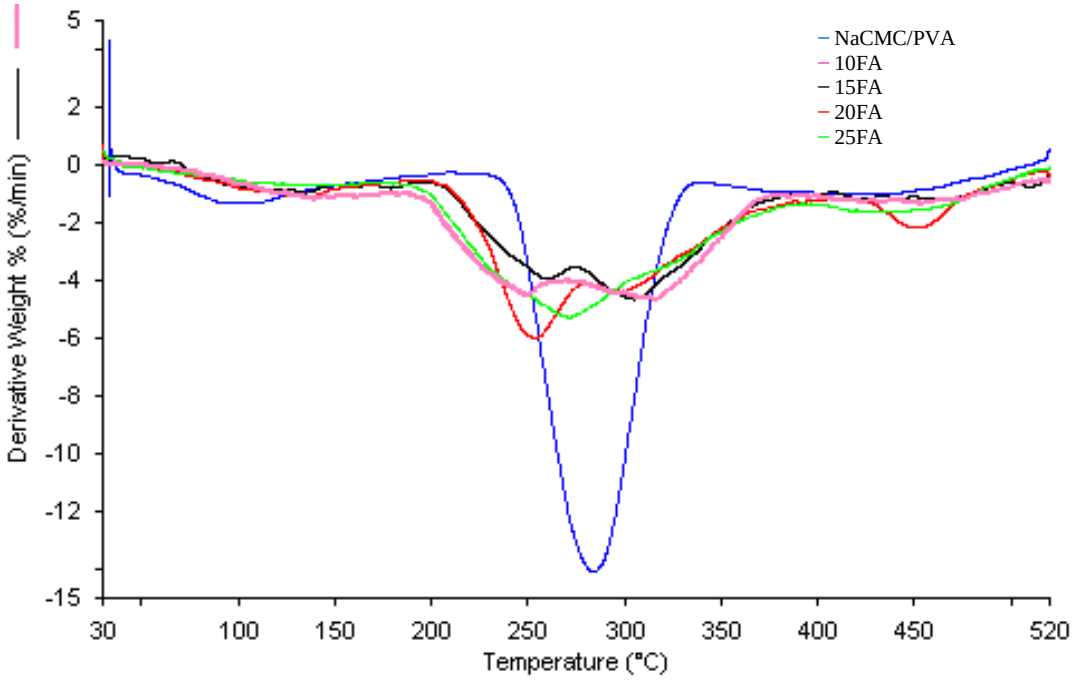


Figure 3.5 DTG curves of NaCMC/PVA, 10FA, 15FA, 20FA and 25FA in nitrogen flow.

Table 3.1 Results of thermogravimetric analysis of hydrogels in nitrogen flow.

Sample	First Stage		Second Stage				Third Stage	
			Shoulder 1		Shoulder 2			
	T(ºC)	Mass Loss %	T (ºC)	Mass Loss %	T (ºC)	Mass Loss %	T(ºC)	Mass Loss %
10FA	117	6.1	243	17.6	317	26.3	523	8.9
15FA	113	6.3	253	13.8	307	23.6	468	8.9
20FA	127	7.7	250	20.5	302	21.8	451	9.1
25FA	108	5.4	249	22.1	306	22.6	523	10.5

3.3 XRD Analysis

Hydrogels and NaCMC are characterized by of X-ray diffraction (XRD) pattern shows a board peak at 20º associated with the low crystallinity of NaCMC stucture. NaCMC and PVA have main caracteristic peak at 2θ=20.5 and 20 respectively, so the peak intensity of NaCMC/PVA blend is higher than the peak of pure NaCMC at 20º. It is clear that NaCMC is not completely amorphous, but have a relatively higher degree of crystallinity. The diffractogram of the NaCMC/PVA blend and hydrogels are similar to pure NaCMC and PVA, which indicate that this blend is miscible. Perhaps, the miscibility of this blend can result due to hydrogen bonding interaction. While the fumaric acid (FA) is added the cross-linking occurs and the peak intensity of hydrogels have been decreased at 20º. This could be explained that the crystallinity is decreased while cross-linking occurs between NaCMC and PVA. The FA concentration is increased its own peak appears at 29.5º. There is no shift of the 2θ values of the hydrogels this indicates that internal spaces of the NaCMC structure has not been changed.

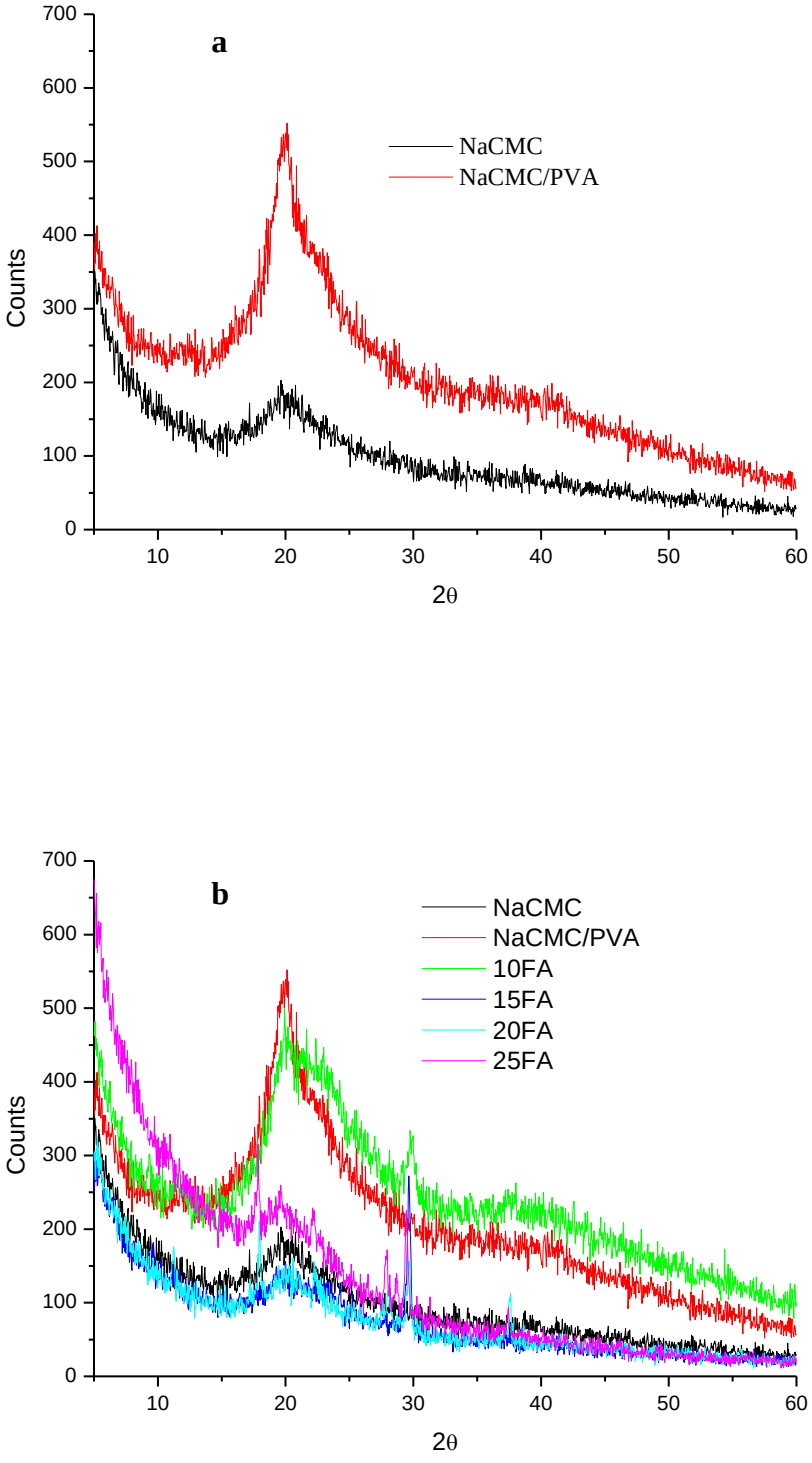


Figure 3.6 XRD pattern of the samples (a) NaCMC and NaCMC/PVA, (b) NaCMC, NaCMC/PVA and hydrogels.

3.4 Scanning Electron Microscopy Analysis (SEM)

The SEM micrographs of hydrogels, NaCMC/PVA blend and pure NaCMC were given in Figure 3.7.

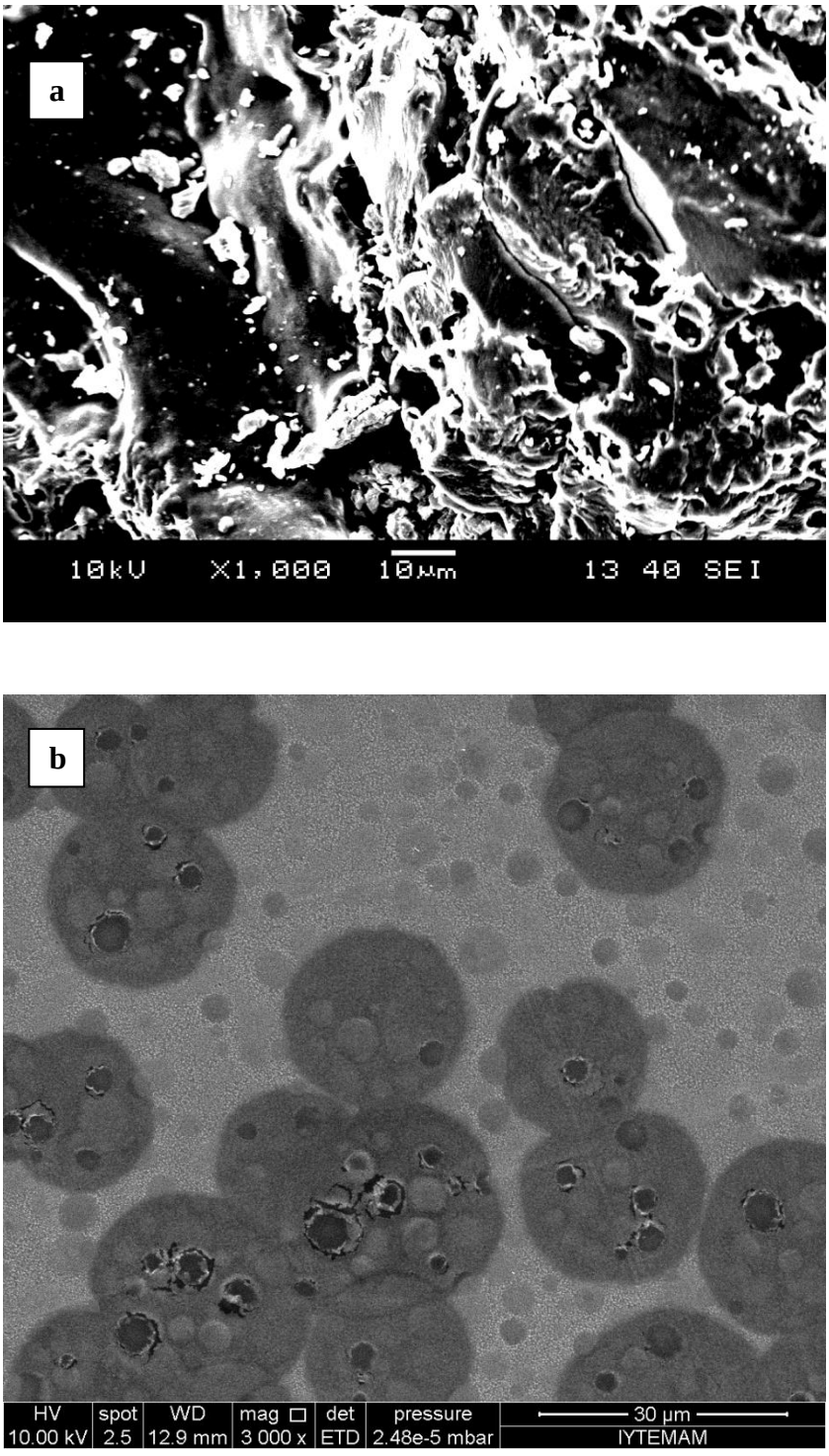


Figure 3.7 SEM micrograph of the morphologies of the samples (a) pure NaCMC, (b) NaCMC/PVA

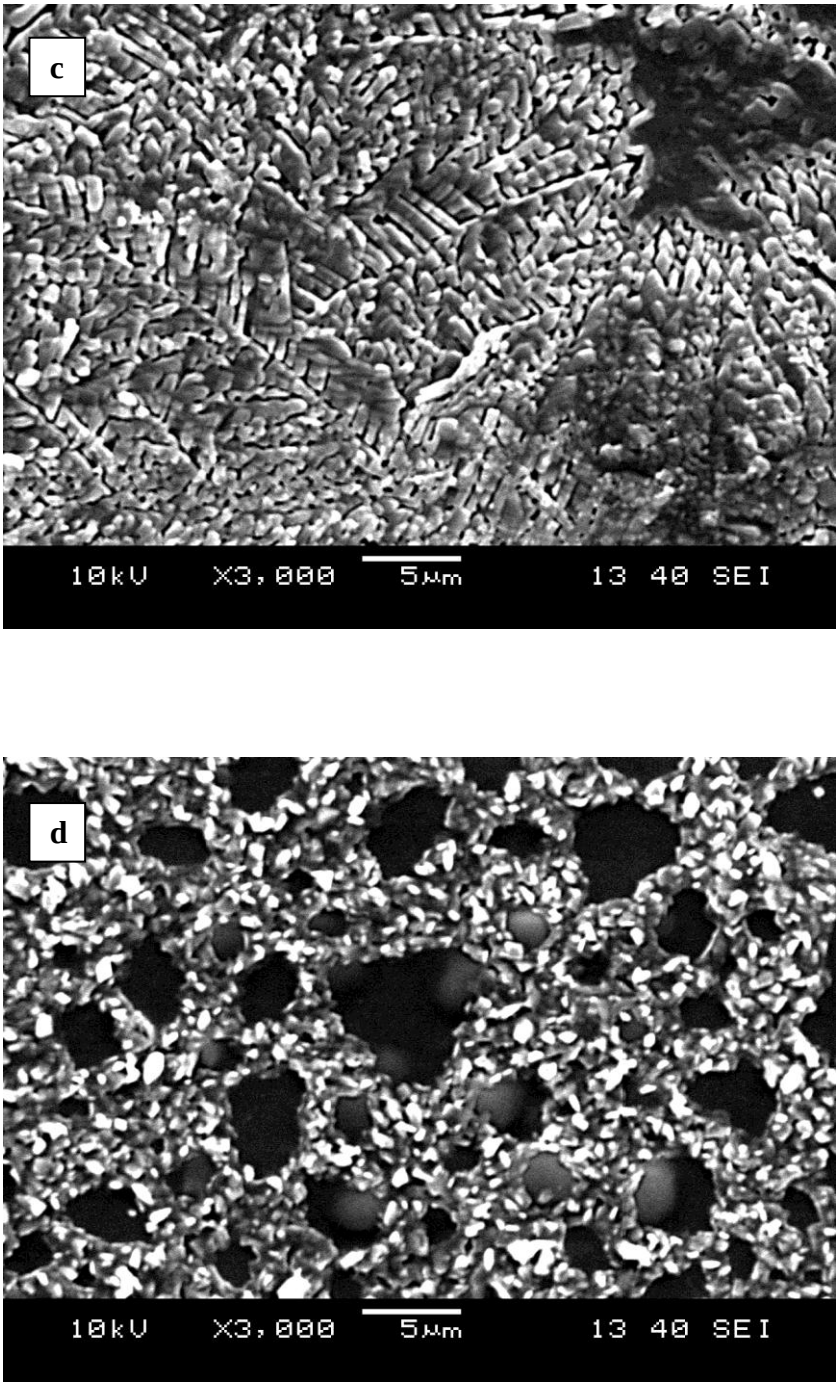
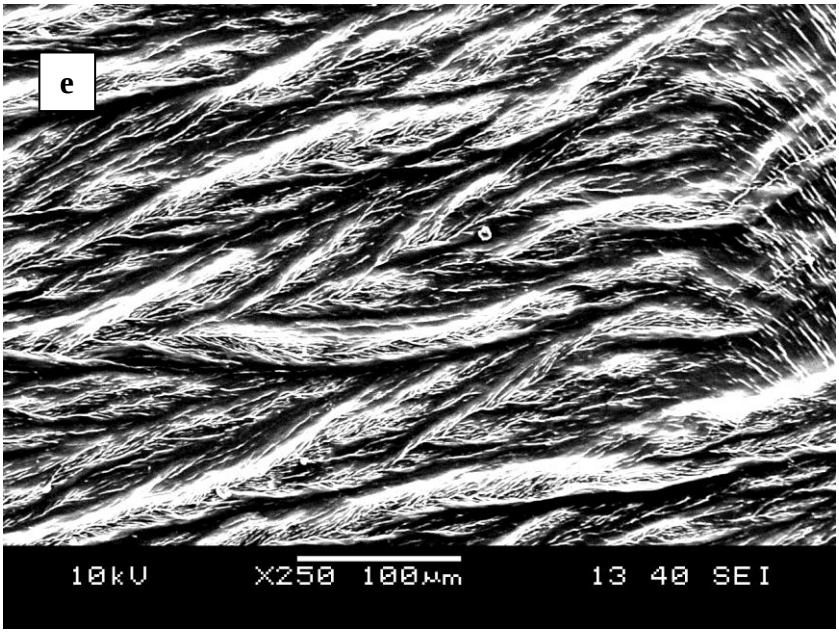


Figure 3.7 SEM micrograph of the morphologies of the samples (c) 10FA, (d) 15FA



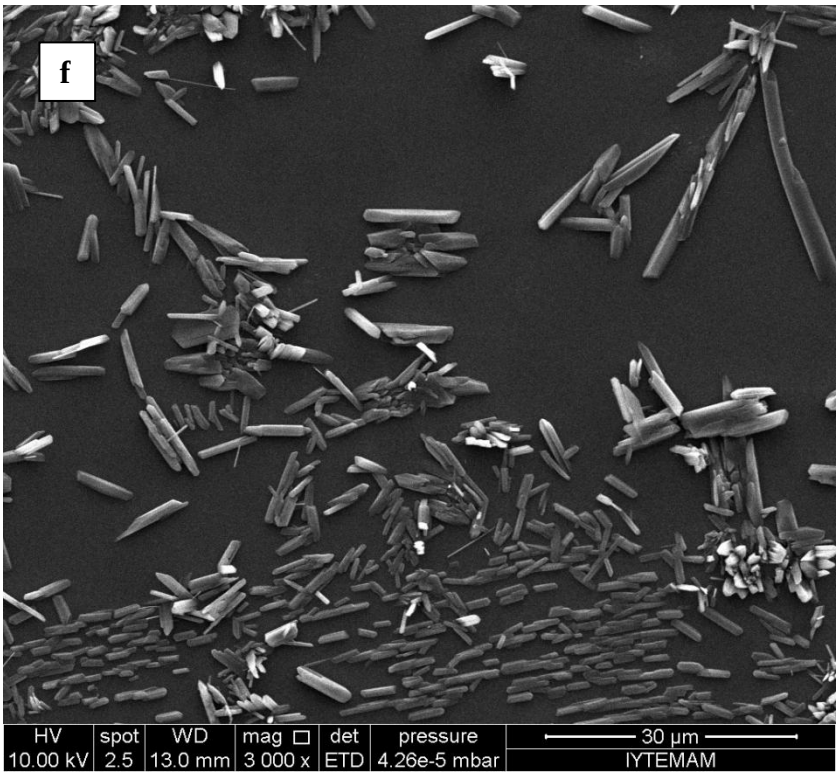


Figure 3.7 SEM micrograph of the morphologies of the samples (e) 20FA, (f) 25FA.

SEM micrographs showed that cross-linking of NaCMC occured when compared to pure NaCMC. While the cross-linker (FA) content of hydrogels increased, cross-linking increased too, also hydrogels have more stiff structure. A highly porous structure was observed owing to formation of gaps at 10FA and 15FA. After hydrogels were placed into the different pH solutions, water molecules diffused into these gaps and hydrogels belongs to rather high swelling capacity. However, 10FA hydrogel decomposed because of cross-linking structure not enough which could be seen at Figure 3.7.c. More over at Figures 3.7.e. and 3.7.f.; the structures of 20FA and 25FA hydrogels deformed due to increasing of FA content.

3.5 Swelling Capacity

Four main forces have influence on swelling capacity of ionized superabsorbents. They are as follows; polymer solvent interaction which depends on solubility tendency of a polymer in solvent; elastic interaction which is proportional to average molecular weight between two consecutive junctions; osmotic pressure due to mobile ion concentration difference between gel and solution phase, and the electrostatic repulsion between ionic groups located on macromolecular chains. The latter is usually negligible comparing to three other forces. (Buchholz, 1990).

15FA, 20FA, 25FA hydrogels are anionic as CMC is negatively charged polyelectrolyte.

Swelling capacity in phosphate buffer (PBS), HCl/KCl buffer and distilled water at different cross-linking hydrogels (15FA, 20FA, 25FA) at 37 ºC is illustrated in Figure. 3.8.

At PBS buffer, HCl/KCl buffer and distilled water; equilibrium swelling capacity is decreased from 296 to 100 g/g, from 91 to 46 g/g, from 182 to 41 g/g respectively because cross-linker (FA) content is increased from 15% (w/w polymer%) to 25%. As FA content is increased, cross-linking of CMC and PVA is increased too. The stucture of hydrogels are being more stiff so swelling capacity is decreased.

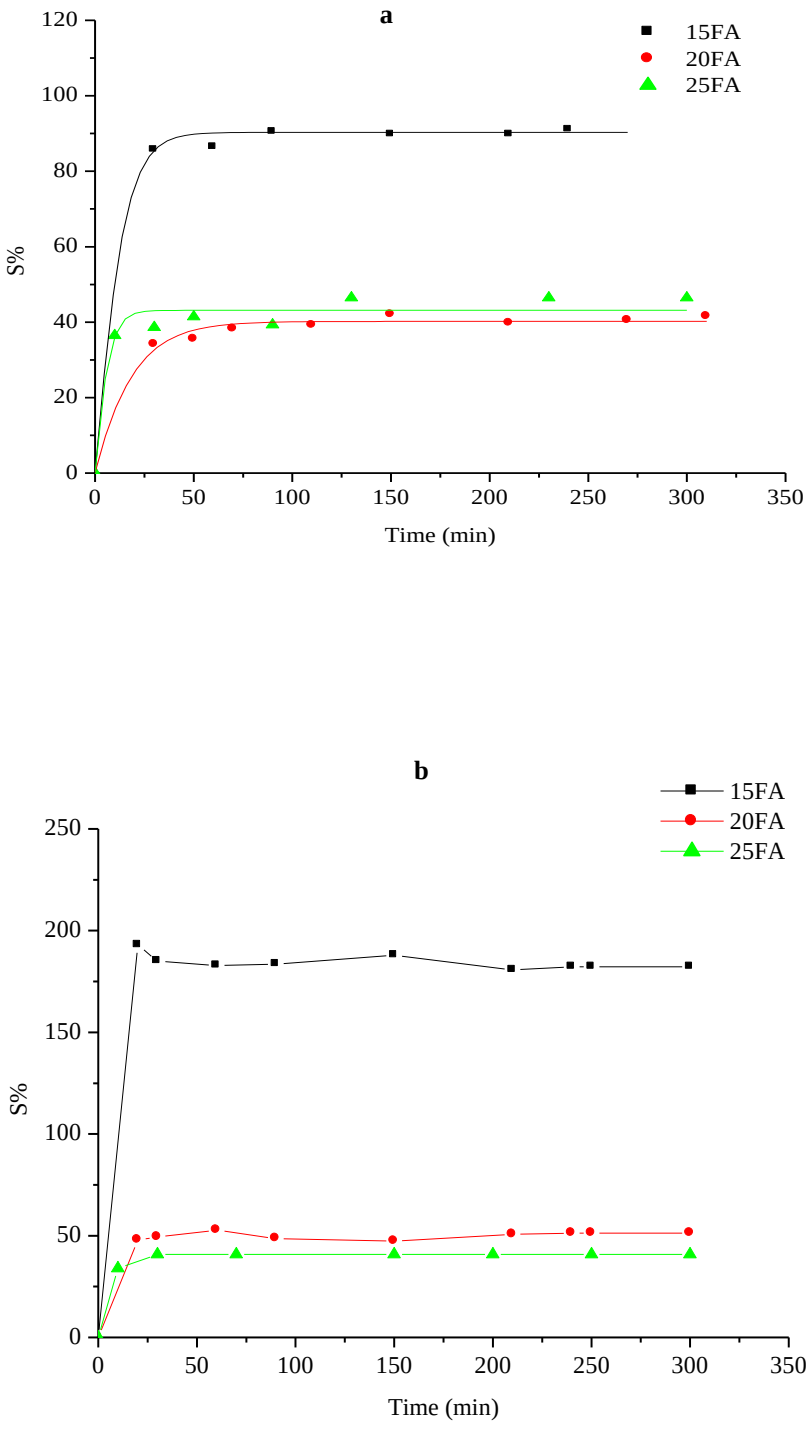


Fig 3.8 Percentage mass swelling as a function of time for the series of different amount of crosslinking hydrogels at 37 ºC at (a) HCl/KCl, (b) PBS

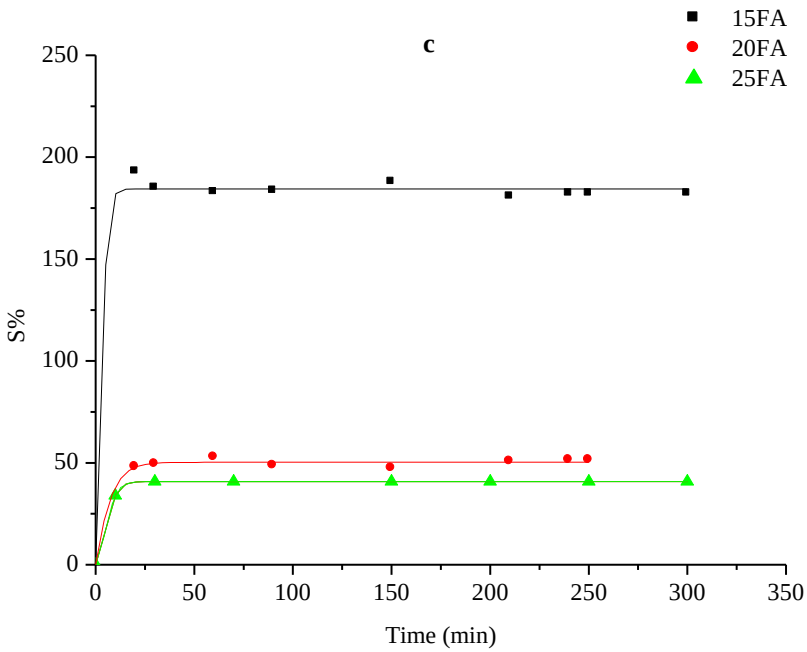


Fig 3.8 Percentage mass swelling as a function of time for the series of different amount of crosslinking hydrogels at 37 ºC at (c) Distilled Water.

3.6 pH Sensitivity of Hydrogels

Figure 3.9 represents pH dependence of the equilibrium mass swelling percentage for hydrogels at 37ºC in phosphate buffer solution from pH= 2.6 to 8. Consistent with poly-electrolyte behavior, the swelling of hydrogels was found to increase with the higher pH values.

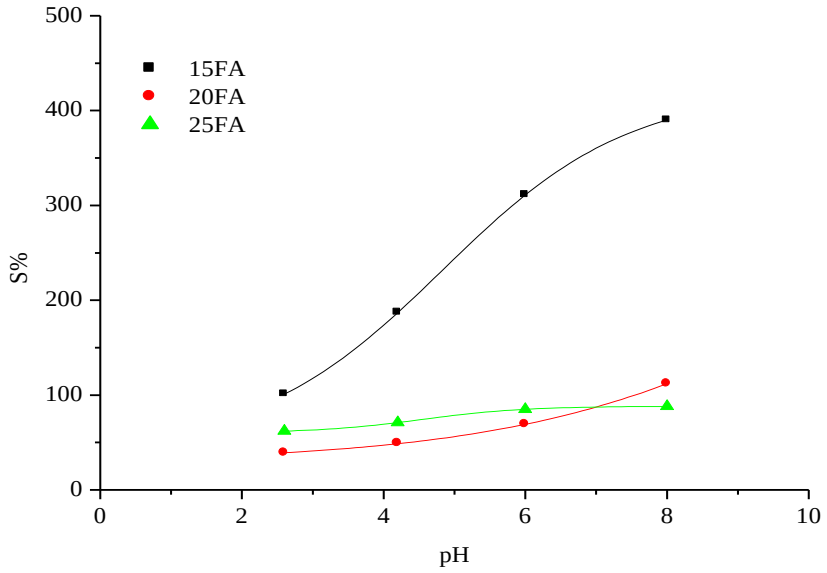


Fig 3.9 pH dependence of equilibrium mass swelling percentage.

The results indicate that under acidic conditions, anionic carboxylate groups are protonated, and the copolymeric network collapsed. Ionic moieties are incorporated into hydrogels, the swelling depends not only on the chemical composition of the gel but also on the pH of the surrounding medium. Generally, anionic hydrogels deprotonate and swell more when external pH is higher than pKa of the ionizable groups tethered on polymer chains. At high pH values, the concentration of anionic groups in the polymer network increases. This occurrence makes the percentage mass swelling of the hydrogels increase with an increase in the ionizable constituent.

3.7 pH Reversibility of Hydrogels

Figure 3.10 shows pH reversibility of hydrogels at 37ºC in acidic and basic medium (pH=2.6 to 8). The 20FA hydrogel swell to 115 g/g at pH=8 and shrink to 62 g/g at pH=2.6. Also the 25FA hydrogel swell to 100 g/g at pH=8 and shrink to 57 g/g at pH=2.6. 20FA hydrogel swell more percentage mass swelling due to having more swelling capacity than 25FA. Reversible swelling of 15FA hydrogel has not been carry out because of its desultory swelling and floppy structure while put into pH=2.6 and 8 solutions over and over again.

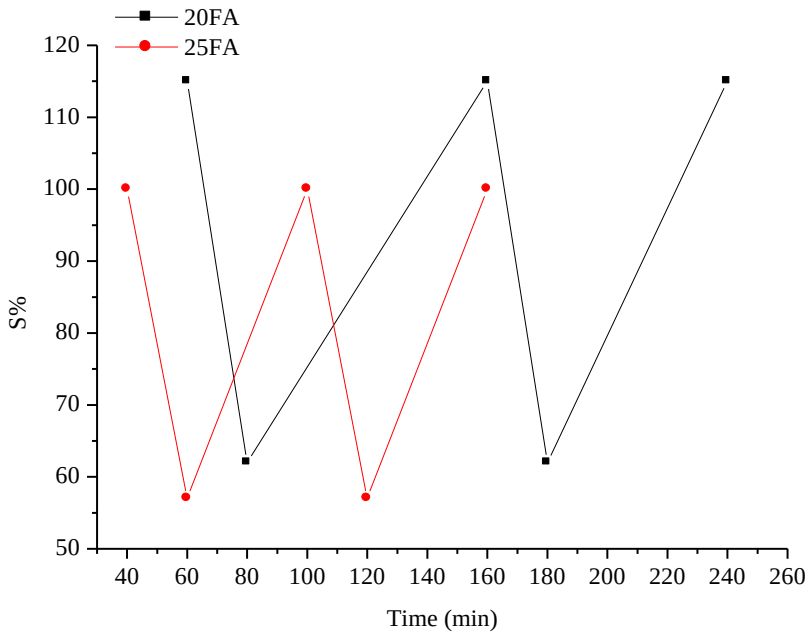


Figure 3.10 Reversible swelling of hydrogels to relate to pH.

3.8 Swelling Kinetics

Swelling kinetic of the hydrogels had been calculated from the equations.

$$t/S_t = A + Bt \quad \text{(Eq. 3.1)}$$

$$B = 1/S_{max} \quad \text{(Eq. 3.2)}$$

$$A = 1/(ds/dt)_0 = 1/S_{max}^2\, k_s \quad \text{(Eq. 3.3)}$$

$$A = 1/r_0 \quad \text{(Eq. 3.4)}$$

Where, S_t is the amount of solution absorbing at time t, B is the inverse of the maximum amount of swelling solution and also slope of the line which can be obtained from the plot of t/S_t result. r_0 is the initial swelling rate, A is the inverse of the initial swelling rate (r_0), and k_s is the constant of the kinetic of swelling. The relation represents second-order kinetics.

The kinetic parameters calculated from slope and interceps of the lines in Figure 3.11 is presented in Table 3.2.

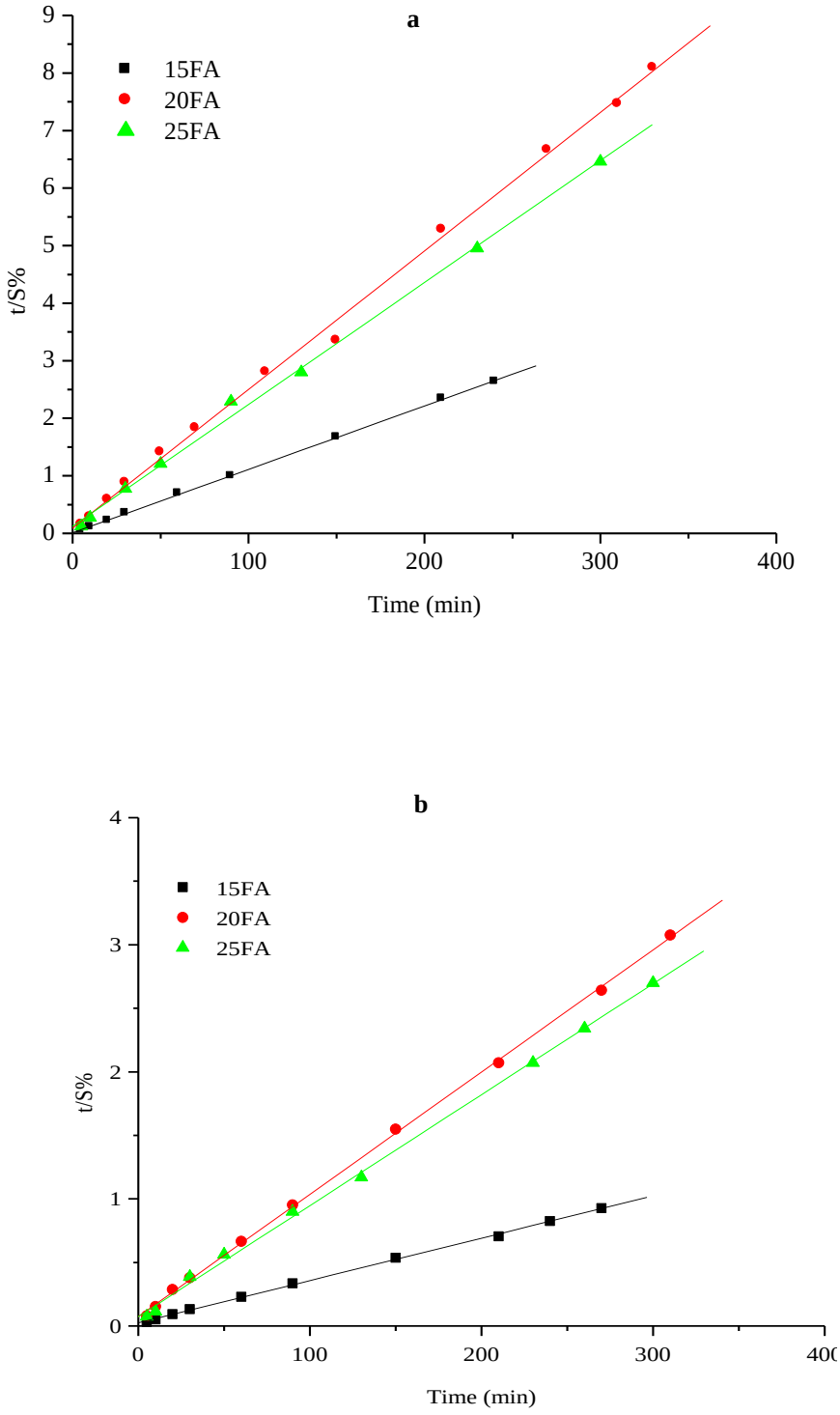


Fig 3.11 Swelling kinetic curves for the series of different amount of crosslinking hydrogels at 37 ºC at (a) HCl/KCl, (b) PBS.

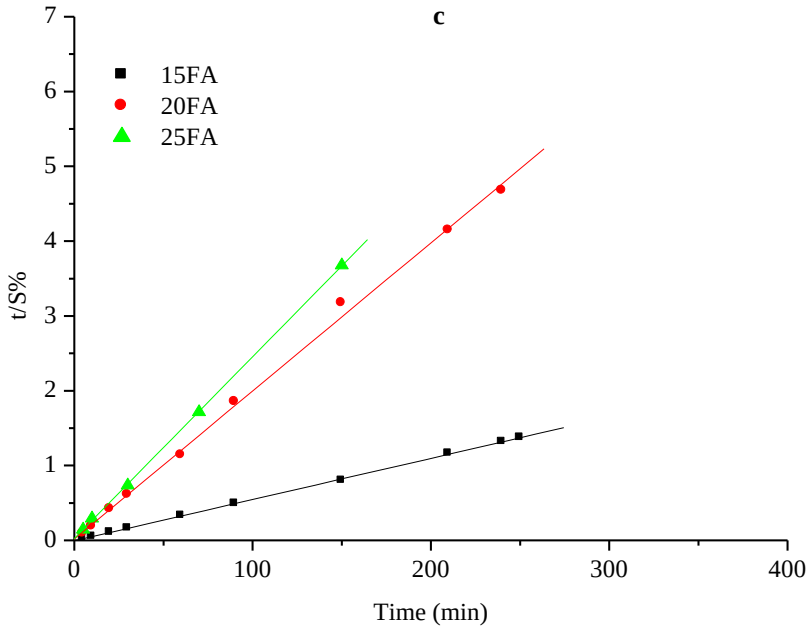


Fig 3.11 Swelling kinetic curves for the series of different amount of crosslinking hydrogels at 37 ºC at (c) Distilled Water.

Figure 3.11a-c show the linear regression of swelling kinetics curves of the samples at HCl/KCl buffer, PBS buffer and distilled water, respectively. Maximum swelling value, S_{max} were calculated from the slope, the value of initial swelling rate, r_0 and swelling rate constant, k_s values were calculated from the intersection of the lines.

It showed that increase in the fumaric acid (FA) content of hydrogels resulted decrease in the swelling of hydrogels. This result is due to the cross-linking between fumaric acid, sodium carboxymethyl cellulose and poly(vinyl alcohol).

Table 3.2 Kinetic parameters for hydrogels at PBS, HCl/KCl buffers and distilled water at 37ºC.

PBS

	S_{eq}%	S_{max} $g_{solution}(g_{hydr.})^{-1}$	$k_s \times 10^5$ $g_{Hydrogel}(g_{Solution})^{-1}s^{-1}$	r_0 $g_{Solution}(g_{Hydrogel})^{-1}s^{-1}$
15FA	296	300	46	41
20FA	101	104	125	14
25FA	111	114	102	13

HCl/KCl

	S_{eq}%	S_{max} $g_{solution}(g_{hydr.})^{-1}$	$k_s \times 10^5$ $g_{Hydrogel}(g_{Solution})^{-1}s^{-1}$	r_0 $g_{Solution}(g_{Hydrogel})^{-1}s^{-1}$
15FA	91	91	1400	112
20FA	41	41	630	11
25FA	46	47	362	8

Distilled water

	S_{eq}%	S_{max} $g_{solution}(g_{hydr.})^{-1}$	$k_s \times 10^5$ $g_{Hydrogel}(g_{Solution})^{-1}s^{-1}$	r_0 $g_{Solution}(g_{Hydrogel})^{-1}s^{-1}$
15FA	181	181	Not detected	Not detected
20FA	51	50	2327	59
25FA	41	41	2273	39

CHAPTER FOUR
CONCLUSIONS

The aim of the study was to synthesize the carboxymethyl cellulose based hydrogels and their characterization.

FTIR spectra of the samples were given in Figure 3.1 and 3.2. As can be seen from the Figure 3.2 that ester bands which are very important for the interaction of CMC/PVA and FA. This band appeared around 1740 cm^{-1} which attributed to C=O stretching of ester carbonyl. These bands indicated the cross-linking between carboxyl group of FA with hydroxyl group of PVA and NaCMC through ester formation.

On the other hand, XRD diffraction profiles of the samples were shown in Figure 3.6. In the patterns, increasing the ratio of crosslinker FA indicated a decrease in the peak intensity of main reflections at $2\theta=20°$. This result was in agreed with the above FTIR data.

As far as TGA/DTG data concerned as in Table 3.1 and also in Figure 3.5, it was observed that the second stage losses in temperature range of 200-340ºC, corresponding to the degradation NaCMC and PVA about 51 wt%. The maximum degradation temperature of NaCMC and PVA are 290 and 200ºC, respectively but these temperatures are shifted to about 310 and 250 ºC, respectively while FA concentration is increased. It was also observed that crosslinking improved the thermal properties of hydrogels. As a conclusion, thermal stabilities of the samples were improved by the addition of crosslinker FA.

SEM images of the samples were given in Figure 3.7. SEM micrographs showed that a highly porous structure was observed for 10FA and 15FA. After hydrogels were placed into the different pH solutions, water molecules diffused into these gaps and hydrogels belongs to rather high swelling capacity. On the other hand, increase

in the amount of crosslinker FA caused for the formation of fibrous morphology which might be decline for further additions of FA.

In the swelling experiments which were monitored in Figure 3.8 showed that, at PBS buffer, HCl/KCl buffer and distilled water; equilibrium swelling capacity is decreased from 296 to 100 g/g, from 91 to 46 g/g, from 182 to 41 g/g respectively because cross-linker (FA) content is increased from 15% (w/w polymer%) to 25%. While cross-linker concentration was increased, the stucture of hydrogels was being more stiff so swelling capacity was decreased accordingly.

In order to investigate the behavior of the samples in solutions with different pH values, one should look at Figure 3.9. The samples had pH sensitivity which observed that the swelling of hydrogels was found to increase with the higher pH values. Also in Figure 3.10 shows pH reversibility of hydrogels at 37ºC in acidic and basic medium (pH=2,6 to 8). The hydrogels were swollen at pH=8 and shranken at pH=2.6. It has been well-known that anionic hydrogels deprotonate and swell more when external pH is higher than pKa of the ionizable groups tethered on polymer chains. At high pH values, the concentration of anionic groups in the polymer network increases This occurrence makes the percentage mass swelling of the hydrogels increase with an increase in the ionizable constituent. In contrast under acidic conditions, anionic carboxylate groups are protonated, and the copolymeric network collapsed.

As a conclusion, these hydrogels may be used as drug transport systems as pH sensitive materials for intestinal area. Further experiment should be done related with the biocompatibility and biodegradability purposes.

REFERENCES

Agnihotri, S. A., & Aminabhavi, T. M. (2005). Development of novel interpenetrating network gellan gum-poly(vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. *Drug Development and Industrial Pharmacy*, *31*, 491–503.

Amsden, B. (1998). Solute diffusion within hydrogels. *Macromolecules, 31,* 8382-8395.

Anderson, J. M. (1994). In vivo biocompatibility of implantable delivery systems and biomaterials. *European Journal of Pharmaceutical and Biopharmaceutics*, *40*, 1-8.

Azad, A. K., Sermsintham, N., Chandrkrachang, S., & Stevens, W.F. (2004). Chitosan membrane as a wound healing dressing: characterization and clinical applications. *Journal of Biomedical Materials Research Part B Applied Biomaterials*, *69* (B), 216-222.

Barbucci, R., Consumi, M., Lamponi, S. & Leone, G. (2003). Polysaccharides based hydrogels for biological applications. *Macromolecular Symposium, 204*, 37–58.

Bilia, A., Carelli, V., Di Colo, G., & Nannipieri, E. (1996). In vitro evaluation of a pH-sensitive hydrogel for control of GI drug delivery from silicone-based matrices. *International Journal of Pharmaceutics, 130*, 83–92.

Brazel, C. S. & Peppas, N. A. (1996). Pulsatile local delivery of thrombolytic and antithrombotic agents using poly(Nisopropylacrylamide-co-methacrylic acid) hydrogels. *Controlled Release*, *39*, 57–64.

Buchholz F.L. (1990). Ed. Peppas, L. B., Harland, R. S. *Absorbent polymer technology*, pp. 288. Elsevier Science & Technology, Oxford/GB. Section 11. Kinetics of swelling of absorbent polymers.

Buhus, G., Popa, M., & Desbrieres, J. (2009). Hydrogels based on carboxymethylcellulose and gelatin for inclusion and release of chloramphenicol. *Bioactive and Compatible Polymers, 24*, 525-545.

Burdick, J. A., Mason, M. N., Hinman, A. D., Thorne, K., & Anseth, K. S. (2002). Delivery of osteoinductive growth factors from degradable PEG hydrogels influences osteoblast differentiation and mineralization. *Controlled Release, 83*, 53–63.

C. W. Hoogendam, A. de Keizer, M. A. Cohen Stuart, B. H. Bijsterbosch, J. A. M. Smit and J. A. P. P. van Dijk, P. M. van der Horst & J. G. Batelaan. (1998). Persistence length of carboxymethyl cellulose as evaluated from size exclusion chromatography and potentiometric titrations. *Macromolecules, 31*, 6297–6309.

Canal, T., & Peppas, N.A. (1989). Correlation between mesh size and equilibrium degree of swelling of polymeric networks. *Biomedical Materials Research, 23*, 1183-1193.

Chang, C., Duan, B., & Zhang, L. (2009). Fabrication and characterization of novel macroporous cellulose-alginate hydrogels. *Polymer, 50 (23)*, 5467-5473.

Chang, C., Duan, B., Cai, J., & Zhang, L. (2010). Superabsorbent hydrogels based on cellulose for smart swelling and controllable delivery. *European Polymer Journal, 46*, 92-100.

Chang, C., Lue, A., & Zhang, L. (2008). Effect of crosslinking methods on structure and properties of cellulose/PVA hydrogels. *Macromolecular Chemistry and Physics, 209 (12)*, 1266-1273.

Chang, C., Peng, J., Zhang, L.,&Pang, D.W.(2009). Strongly fluorescent hydrogels with quantum dots embedded in cellulose matrices. *Journal of Materials Chemistry, 19*, 7771-7776.

Charpentier, D., Mocanu, G., Carpov, A., Chapelle, S., Merle, L., & Muller, G. (1997). New hydrophobically modified carboxymethylcellulose derivatives. *Carbohydrate* Polymers, *33*, 177-186.

Chiellini, E., Corti, A., D'Antone, S., & Solaro, R. (2003). Biodegradation of poly(vinylalcohol) based materials. *Progress in Polymer Science, 28 (6),* 963-1014.

Coviella, T., Matricardi, P., Marianecci C., & Alhaique, F. (2007). Polysaccharide hydrogels for modified release formulations. *Journal of Controlled Release, 119,* 5-24.

Cruise, G. M., Scharp, D. S., & Hubbell, J. A. (1998). Characterization of pemeability and network structure of interfacially photo polymerized poly(polyethylene glycol) diacrylate hydrogels. *Biomaterials, 19,* 1287-1294.

Dagani, R. (1997). Intelligent gels. *Chemical & Engineering News, 75,* 26–36.

Davis, K. A., & Anseth K. S. (2002). Controlled release from crosslinked deradable networks. *Critical Reviews in Therapeutic Drug Carrier Systems, 19,* 385-423.

Demitri, C., Del Sole, R., Scalera, F., Alessandro, S., Vasapollo, G., Maffezzoli, A., Ambrosio, L., & Nicolais, L. (2008). Novel superabsorbent cellulose-based hydrogels crosslinked with citric acid. *Applied Polymer Science, 110,* 2453-2460.

Deng, J., He, Q., Wu, Z., & Yang, W. (2008). Using glycidyl methacrylate as crosslinking agent to prepare thermosensitive hydrogels by a novel one-step method. *Journal of Polymer Science Part A: Polymer Chemistry, 46,* 2193.

Deshpande, A. A., Rhodes, C. T., & Danish, M. (1992). Intravaginal drug delivery. *Drug Development and Industrial Pharmacy, 18,* 1225-1279.

Dumitriu, S., & Chornet, E. (1998). Inclusion and release of proteins from polysaccharide-based polyino complexes. *Advances in Drug Delivery Reivew, 31,* 223-246.

Edgar, K. J., Buchanan, C. M., Debenham, J. S., Rundquist, P. A., Seiler, B. D., Shelton, M. C., et al. (2001). Advances in cellulose ester performance and application. *Progress in Polymer Science, 26*, 1605-1688.

Eichhorn, S. J., Young, R. J., & Davies, G. R. (2005). Modeling crystal and molecular deformation in regenerated cellulose fibers. *Biomacromolecules, 6*, 507-513.

Esposita, F. E., Nobile, M. A., Mensitieri, C., & Nicolais, L. (1996). Water sorption in cellulose-based hydrogels. *Journal of Applied Polymer Science*, *60*, 2403-2407.

Fei, B., Wach, R. W., Mitomo, H., Yoshii, F., & Kume, T. (2000). Hydrogel of biodegradable cellulose derivatives. I. Radiation-induced crosslinking of CMC. *Journal of Applied Polymer Science*, *78*, 278-283.

Florence, A. T., & Attwood D. (1998). Physicochemical principles of pharmacy, pp. 380, London: Macmillon Press.

Fumaric acid, (05/2011), http://en.wikipedia.org/wiki/Fumaric_acid.

Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. *Advances in Drug Delivery Reivew*, *31*, 267-285.

Grabe, M., & Oster, G. (2001). Regulation of organelle acidity. *Journal of General Physiology, 117*, 329–344.

Guo, B. L. & Gao, Q.Y. (2007). Preparation and properties of a pH/temperature-responsive carboxymethyl chitosan/poly(N-isopropylacrylamide) semi-IPN hydrogel for oral delivery of drugs. *Carbohydrate Research, 342*, 2416–2422.

Guo, K, & Chu, C. C. (2005). Synthesis and characterization of novel biodegradable unsaturated poly(ester amide)/poly(ethylene glycol) diacrylate hydrogels. *Journal of Polymer Science Part A: Polymer Chemistry, 43 (17)*, 3932-3944.

Gupta, P., Vermani, K., & Garg, S. (2002). Hydrogels: from contolled release ro pH-responsive drug delivery. *Drug Discovery Today, 7,* 569-579.

Gutowska, A., Bark, J. S., Kwon, I. C., Bae, Y. H., Cha,Y., & Kim, S. W. (1997). Squeezing hydrogels for controlled oral drug delivery. *Journal of Controlled Release, 48*, 141-148.

Guyton, A. C., & Hall, J. E. (1998). Guyton, A. C., Hall, J. E. (Eds.). Secretory functions of the alimentory tract. In *Textbook of Medical Physiology*. pp. 815-832, W. B. Saunders Co.

Ha, D. I., Lee, S. B., Chong, M.S., Lee, Y. M., Kim, S. Y. & Park, Y. H. (2006). Preparation of thermo-responsive and injectable hydrogels based on hyaluronic acid and poly(N-isopropylacrylamide) and their drug release behaviours. *Macromolecular Research, 14*, 87–93.

Harvey J.A. (1995). Kroschwitz, J. I., Howe-Grant, M. (Eds.). *Smart materials in encyclopaedia of chemical technology*. pp. 502-514, John Wiley Sons.

Hassan, C. M., & Peppas, N. A. (2000). Structure and morphology of freeze/thawed PVA hydrogels. *Macromolecules*, *33*, 2472-2479.

Heinze, T., & Koschella, A. (2005). Carboxylmethyl ethers of cellulose and starch- a review. *Macromolecular Symposia*, *223*, 13-39.

Heinze, T., Helbig, K., & Klemm, D. (1993). Investigation of metal ion adsorption of carboxymethyl cellulose gel beads. *Acta Polymerica, 44*, 108-109.

Hennink, W. E., Franssen, O., Van Dijk-Wolthuis, W. N. E., & Talsman, H. (1997). Dextran hydrogels fort he controlled release of propeties. *Journal of Controlled Release*, *48*, 107-114.

Hoogendam, C. W., de Keizer, A., Cohen Stuart, M. A., Bijsterbosch, B. H., Smit, J. A. M., van Dijk, J. A. P. P., van der Horst, P. M., & Batelaan, J. G. (1998). Persistence length of carboxymethyl cellulose as evaluated from size exclusion chromatography and potentiometric titrations. *Macromolecules*, *31*, 6297-6309.

Sayeda, M. I., El Salmawi, K. M., & Zahran, A. H. (2007). Synthesis of crosslinked superabsorbent carboxymethyl cellulose/acrylamide hydrogels through electron-beam irradiation. *Journal of Applied Polymer Science, 104*, 2003-2008.

Ito, A., Dobashi, Y., Obata, K., & Sugihara, M. (1994). Investigation of compressed coating tablet swelling with water as a new dosage form for elderly patients. *Japanese. Journal Hospital Pharmacy, 20*, 41–49.

Ivanov, C, Popa, M., Ivanov, M., & Popa, A. A. (2007). Synthesis of poly(vinyl alcohol)- methyl cellulose hydrogel as possible scaffolds in tissue engineering. *Journal of Optoelectronics and Advanced Materials, 9,* 3440-3444.

Jeong, B., Kim, S. W., & Bae, Y. H. (2002). Thermosensitive sol–gel reversible hydrogels. *Advanced Drug Delivery Reviews*, *54*, 37–51.

Kim, S.W., Bae Y.H., & Okano, T. (1992). Hydrogels: Swelling, drug loading and release. *Pharmaceutical Research, 9 (3),* 283-290.

Klemm, D., Heublein, B., Fink, H., & Bohn, A. (2005). Cellulose: Fascinating biopolymer and sustainable raw material. *Angewandate Chemie International Edition, 44 (22),* 3358-3393.

Kost, J. (1999). Mathiowitz, E. (ed.). Intelling drug delivery systems. In *Encyclopaedia of Controlled Drug Delivery.* pp. 445-459, John Wiley Sons.

Kötz, J., Philipp, B., Nehls, I., Heinze, T., & Klemm, D. (1990). Zum polyelektrolytverhalten einer C-6-substituierten carboxycellulose im vergleich zu carboxymethylcellulose. *Acta Polymerica, 41 (6),* 333-338.

Kumari, K. & Kundu, P. P. (2007). Semiinterpenetrating polymer networks of chitosan and L-alanine for monitoring the release of chlorpheniramine maleate. *Journal of Applied Polymer Science, 103*, 3751–3757.

Kurisawa, M., & Yui, N. (1998). Dual-stimuli-responsive drug release from interpenetrating polymer network-structured hydrogels of gelatin and dextran. *Controlled Release*, *54*, 191–200.

Lee, K. Y., & Mooney, D. J. (2001). Hydrogels for tissue engineering. *Chemical Reviews*, *101 (7)*, 1869-1880.

Li, L., Thangamathesvaran, P., Yue, C., Hu, X., & Lam, Y. (2001). Gel network structure of methylcellulose in water. Langmuir, *17 (26)*, 8062-8068.

Liang, H. F., Hang, M. H., Ho, R. M., Chung, C. K., Lin, Y. H., & Chen, C.H. (2004). Novel method using a temperature-sensitive polymer (methycellulose) to thermally gel aqueous Alginate as a pH-senstve hydrogel. *Biomacromolecules*, *5*, 1917-1925.

Liang, S., Zhang, L., Li, Y., & Xu, J. (2007). Fabrication and properties of cellulose hydrated membrance with unique structure. *Macromolecular Chemistry and Physics, 208*, 594-602.

Lowman, A. M., & Peppas N. A. (1999). Hydrogels. In *Encyclopaedia of Controlled Drug Delivery*. pp. 397-418, John Wiley Sons.

Lustig, S.R., Peppas, N. A. (1998). Solute diffusion in swollen membranes. 9. Scaling laws for solute diffuse in gels. *Journal of Applied Science, 36*, 735-747.

Ma, J., Xu, Y., Fan, B., & Liang, B. (2007). Preparation and characterization of sodium carboxymethylcellulose/poly(N-isopropylacrylamide)/clay semi-IPN nanocomposite hydrogels. *European Poylmer Journal*, *43*, 2221-2228.

Marcì, G., Mele, G., Palmardo, L., Pulito, P., & Sannino, A. (2006). Environmentally sustainable production of cellulose-based superabsorbent hydrogels. *Green Chemistry, 8*, 439-444.

Mason, M. N., Metters, A.T., Bowman, C. N., & Anseth, K.S. (2001). Predicting controlled-release behavior of degradable PLA-b-PEG-B-b-PLA hydrogels. *Macromolecules, 34*, 4630-4635.

Millon, L. E., & Wan, W. K. (2006). The polyvinyl alcohol-bacterial cellulose system as a new nanocomposite for biomedical applications. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 79 (B)*, 245-253.

Mitsumata, T., Suemitsu, Y., Fujii, K., Fujii, T., Taniguchi, T., & Koyama, K. (2003). pH-response of chitosan, K-carrageenan, carboxymethylcellulose sodiu salt complex hydrogels. *Polymer, 44*, 7103-7111.

Nuttelman, C. R., Henry, S. M., & Anseth, K. S. (2002). Synthesis and characterization of photocrosslinkable, degradable poly(vinyl alcohol)-based tissue engineering scaffolds. *Biomaterials, 23*, 3617-3626.

Nuttelman, C. R., Mortisen, D. J., Henry, S. M., & Anseth, K. S. (2001). Attachment of fibronectin to poly(vinyl alcohol) hydrogels promotes NIH3T3 cell adhesion, proliferation, and migration. *Journal of Biomedical Materials Research, 57*, 217-223.

Ottenbrite, R. M., Park, K., & Okano, T. (2010). Biomedical applications of hydrogel handbook. 05/2011, http://www.springerlink.com/content/978-1-4419-5918-8#section=761629&page=3&locus=10.

Park K., Shalaby W. S. W. & Park, H. (1993). *Biodegradable Hydrogels for Drug Delivery*. Lancaster, PA: Technomic Publishing Co.

Park, H., & Park, K. (1996). Biocompatibilty issues of implantable drug delivery systems. *Pharmaceutical Research, 13*, 1770-1776.

Patel, V.R. & Amiji, M.M. (1996). Preparation and characterization of freeze-dried chitosan-poly(ethylene oxide) hydrogels for site-specific antibiotic delivery in the stomach. *Pharmaceutical Research, 13*, 588–593.

Paul J. Flory. (1953). *Principles of Polymer Chemistry (George Fisher Baker Non-Resident Lec)*, Ithaca, NY: Cornell Univ Press.

Peppas N. A. (Ed.). (1986). *Hydrogels in medicine and pharmacy*. Boca Raton, Florida: CRC Press Inc., , 1986 (Vol. l), 1987 (Vols 2 and 3). Vol. 1. Fundamentals, pp. vii + 180; Vol. 2. Polymers, pp. vii + 171; Vol. 3. Properties and Applications, pp. vii + 195.

Peppas, N. A. (1987). *Hydrogels in medicine and pharmacy: polymers Hydrogels in Medicine & Pharmacy*, pp. 184, CRC.

Peppas, N. A., & Merrill, E. W. (1977). Development of Semicrystalline PVA Networks for Biomedical Applications. *Journal of Biomedical Materials Research*, *11*, 423-434.

Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutical and Biopharmaceutics*, *50*, 27-46.

Peppas, N. A., Hilt, J. Z., Khademhosseini, A., & Langer, R. (2006). Hydrogels in biology and medicine: from molecular principles to bionanotechnology. *Advanced Materials*, *18*, 1345-1360.

Peppas, N. A., Huang, Y., Torres-Lugo, M., Ward, H., & Zhang, J. (2000). Physicochemical, foundations and structral design of hydrogels in medicine and biology. *Annual Review of Biomedical Engineering*, *2*, 9-29.

Peppas, N. A., Keys, K. B., Torres-Lugo, M., & Lowman, A. M. (1999). Poly(ethylene glycol)-containing hydrogels in drug delivery. *Controlled Release*, *62*, 81-87.

Qiu, H., & Yu, J. (2008). Polyacrylate/(carboxymethylcellulose modified montmorillonite) superabsorbent nanocomposite. *Journal of Applied Polymer Science*, *107*, 118-123.

Rosiak, J. M., & Ulanski, P. (1999). Synthesis of hydrogels by irradiation of polymers in aqueous solution. *Radiation Physical and Chemistry*, *55*, 139-151.

Rowley, J., Madlambayan, G., Faulkner, J., & Mooney, DJ. (1999). Alginate hydrogels as synthetic extracellular matrix materials. *Biometerials*, *20*, 45-53.

Ruel-Gariepy, E., & Leroux J. C. (2004). In situ-forming hydrogels-review of temperature-sensitive systems. *European Journal of Pharmaceutical and Biopharmaceutics*, *58*, 409-426.

Sammon, C., Bajwa, G., Timmins, P., & Melia, C. D. (2006). The application of attenuated total reflectance fourier transform infrared spectroscopy to monitor the concentration and state of water in solution of a thermally responsive cellulose ether during gelation. *Polymer*, *47 (2)*, 577-584.

Sannino, A., Pappada, S., Giotta, L., & Maffezzoli, A. (2007). Spin coating cellulose derivatives on quartz crystal microbalance plates to obtain hydrogel-based fast sensors and actuators. *Journal of Applied Polymer Science*, *106*, 3040–3050.

Schild, H. G. (1992). Poly (n-isopropylacrylamide)-experiment theory and application. *Progress in Polymer Science*, *17*, 163–249.

Schmaljohann, D. (2006). Thermo- and pH-responsive polymers in drug delivery. *Advanced Drug Delivery Reviews*, *58*, 1655-1670.

Schmedlen, R. H., Masters, K. S., & West, J. L. (2002). Photopolymerizable Polyvinyl Alcohol Hydrogels That Can Be Modified with Cell Adhesion Peptides for use in tissue engineering. *Biomaterials*, *23*, 4325-4332.

Schuetz, Y. B., Gumy, R., & Jordan, O. (2008). A novel thermoresponsive hydrogel of chitosan. *European Journal of Pharmaceutical and Biopharmaceutics*, *68*, 19-25.

Schurz, J. (1999). "Trend in polymer science" a bright future for cellulose. *Progress in Polymer Science*, *24*, 481-483.

Shaheen, S.M., & Yamaura, K. (2002). Preparation of theophylline hydrogels of atactic poly (vinyl alcohol)/NaCl/H_2O system for drug delivery system. *Journal of Controledl Release, 81,* 367-377.

Siegel, R. A. (1993). Dušek, K. (Ed.). Responsive Gels: Volume Transitions I, Hydrophobic weak polyelectrolyte gels: Studies of swelling equilibria and kinetics. *Advances in Polymer Science, 109,* 233-267.

Silva, A. K. A., Richard, C., Bessodes, M., Scherman, D., & Merten, O. W. (2009). Growth factor delivery approaches in hydrogels. *Biomacromolecules, 10,* 9-18.

Singh, A., Sharma, P. K., Garg, V. K., & Garg, G. (2010). Hydrogels: A review. *International Journal of Pharmaceutical Sciences Review and Research, 4,* 97-105.

Soppimath, K.S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Chemically modified polyacrylamide-gguar gum-based crosslinked anionic microgels as pH-sensitive drug delivery systems: preparation and characterization. *Journal of Controlled Release, 75,* 331–345.

Sreedhar, B., Sairam, M., Chattopadhyay, D. K., Syamala Rathnam P. A. & Mohan Rao, D. V. (2005). Thertmal, machanic, and surface characterization of starch-poly(vinyl alcohol) blends and borax-crosslinked films. *Journal of Applied Polymer Science, 96,* 1313-1322.

Stubble, B., Maris, B., Van Den. Mooter, G., De Smendt, S. C., & Demeester, J. (2001). The ınvitra evalution of Azo containing polysaccharide gels for colon delivery. Journal of *Controlled Release, 75,* 103-114.

Sungur, S., & Emregül, E. (1996). Application of carboxymethylcellulose in controlled drug release. *Macromolecular Science, 33 (A),* 319-325.

Szepes, A., Makai, Z., Blumer, C., Mader, K., Kasa, P., & Revesz, P. S. (2008). Characterization and drug delivery behavior of starch based hydrogels prepared via isostatic ultrahigh pressure. *Carbohydrate Polymers, 72,* 571-575.

T. Heinze, D. Klemm, F. Loth & B. Philipp. (1990). Herstellung, struktur und anwendung von ionotropen gelen aus carboxygruppenhaltigen polysacchariden fortschrittsbericht. *Acta Polimerica, 41*, 259–269.

Ta, H. T., Dass, C. R., & Dunstan, D. E. (2008). Injectable chitosan hydrogels for localized cancer therapy. *Controlled Release, 126*, 205-216.

Taleb, M., Abd El-Mohdy, H., & Abd El-Rehim, A. (2009). Radiation preparation of PVA/CMC copolymers and their application in removal of dyes. *Journal of Hazardous Materials, 168*, 68-75.

Taşdelen, B., Kayaman-Apohan, N., Güven, O., & Baysal, B. M. (2004). pH-thermoreversible hydrogels. 1. Sythesis and characterization of poly(N-isopropylacrylamide/maleic acid) copolymeric hydrogels. *Radiation Physics and Chemistry, 69*, 303-310.

Tonnesen, H. H., & Karlsen, J. (2002). Alginate in drug delivery systems. *Development and Industrial Pharmacy, 28*, 621-630.

Van der Linden, H. J., Herber, S., Olthuis, W., & Bergveld, P. (2003). Patterned dual pH responsive core shell hydrogels with controllable swelling kinetics and volume. *Analyst, 128*, 325-331.

Vinatier, C., Gauthier, O., Fatimi, A., Merceron, C., Masson, M.,Moreau, A., et al. (2009). An injectable cellulose-based hydrogel for the transfer of autologous nasal chondrocytes in articular cartilage defects. *Biotechnology and Bioengineering, 102*, 1259-1267.

Wang, M., Xu, L., Hu, H., Zhai, M., Peng, J., Nho, Y., Li, J., & Wei, G. (2007). Radiation synthesis of PVP/ CMC hydrogels as wound dressing. *Nuclear Instruments and Methods, 265(B),* 385-389.

Watson, P., Jones, A. T., & Stephens, D. J. (2005). Intracellular trafficking pathways and drug delivery: fluorescence imaging of living and fixed cells. *Advanced Drug Delivery Reviews, 57*, 43–61.

Weng, L., Zhang, L., Ruan, D., Shi, L., & Xu, J. (2004). Thermal gelation of cellulose in a NaOH/thiourea aqueous solution. *Langmuir*, *20*, 2086-2093.

West, J.L., & Hubbel,l J.A. (1995). Photopolymerized hydrogel materials for drug-delivery applications. *Reactive Polymers*, *25*, 139–147.

Xiao, C., Li, H., & Gao, Y. (2008). Preparation of fast pH-responsive ferric carboxymethylcellulose/poly(vinyl alcohol) double-network microparticles. *Society of Chemical Industry*, *58*, 112-115.

Xiao, C., Lu, Y., Liu, H., & Zhang, L. (2001). Preparation and characterization of konjac glucomannan and sodium carboxymethylcellulose blend films. *Applied Polymer Science*, *80*, 26-31.

Xiong, X., Zhang, L.,&Wang, Y. (2005). Polymer fractionation using chromatographic column packed with novel regenerated cellulose beads modified with silane. *Journal of Chromatography A*, *1063*, 71–77.

Yang, F., Li, G., He, YG., Ren, FX., & Wang, GX. (2009). Synthesis, characterization and applied properties of carboxymethyl cellulose and polyacrylamide graft copolymer. *Carbohydrate Polymers*, *78*, 95-99.

Yang, X., Liu, Q., Chen, X., Feng, Y., & Zhu, Z. (2008). Investigation of PVA/ws-chitosan hydrogels prepared by combined γ-irradiation and freeze-thawing *Carbohydrate Polymers*, *73 (3)*, 401-408.

Ye, S. H., Watanabe, J., Iwasaki, Y., & Ishihara, K. (2003). Antifouling blood purification membrane composed of cellulose acetate and phospholipid polymer. *Biomaterials*, *24*, 4143–4152.

Zhou, D., Zhang, L., & Guo, S. (2005). Mechanism of lead biosorption on cellulose/ chitin beads. *Water Research*, *39*, 3755-3762.

Zhou, Y., Chu, J. S., & Wu, X. Y. (2004). Theoretical analysis of drug release into a finite medium from sphere ensembles with various size and concentration distributions. *European Journal of Pharmaceutical* Sciences, *22* , 251–259.