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SCIENCES

SYNTHESIS AND CHARACTERIZATION
OF NANOCOMPOSITE HYDROGELS FOR
CONTROLLED RELEASE

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SUMMARY

TITLE OF THE THESIS: SYNTHESIS AND CHARACTERIZATION OF NANOCOMPOSITE HYDROGELS FOR CONTROLLED RELEASE

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pH and temperature sensitive nanocomposite hydrogels were synthesized with sodium alginate (NaAlg), poly(N-isopropylacrylamide) PNIPA and nanoclay. The structure and morphology, thermal behavior, chemical characterization, swelling and deswelling behavior, and thymol release characteristics of the hydrogels were studied.

The NaAlg/PNIPA/Clay hydrogels revealed a highly porous structure in which the pore sizes decreased and the amount of pores increased with increasing the nanoclay content in the hydrogels as suggested by the SEM analysis. DSC studies showed that the T_g of the dried hydrogels decreased with increasing the NaAlg content, but the nanoclay content did not affect the T_g . FT-NIR spectrum showed the characteristic absorption peaks for nanoclay, NaAlg and PNIPA. XRD results suggested that the nanoclay sheets are both intercalated and exfoliated in the hydrogel network.

The swelling ratio of the nanocomposite hydrogels increased with increasing the pH value from 1.2 to 8. The nanocomposite hydrogels reached the maximum swelling ratio at pH=8. The swelling ratio of the hydrogels decreased as the nanoclay increased while the deswelling ratio increased with increasing the nanoclay. These results suggested that it is possible to design a hydrogel with a desired swelling/deswelling behavior by controlling the nanoclay content. Thymol release was controlled by the changing the nanoclay content. Results showed that pH and temperature sensitive nanocomposite hydrogels could be used in the pharmaceutical industry as the drug delivery system and in food industry as the antimicrobial releasing system.

ÖZET

TEZİN BAŞLIĞI: KONTROLLÜ SALINIM YAPABİLEN NANOKOMPOZİT HİDROJELLERİN SENTEZİ VE KARAKTERİZASYONU

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Bu çalışmada, sodyum aljinat (NaAlg), poly(N-isopropylacrylamide) PNIPA ve çapraz bağlayıcı olarak nanokil kullanılarak pH ve sıcaklık duyarlı nanokompozit hidrojeller sentezlenmiştir. Hidrojellerin yapı ve morfolojisi, termal davranışları, kimyasal karakterizasyonu, şişme ve büzülme davranışları ve thymol salınım karakteristikleri incelenmiştir.

SEM analizi sonuçları NaAlg/PNIPA/Clay nanokompozit hidrojellerin yapılarında yüksek oranda gözenek içerdiğini göstermiştir. Hidrojel içeriğindeki nanokil miktarının artmasıyla gözenek sayısının arttığı ve gözenek çapının küçüldüğü gözlenmiştir. DSC analizi, hidrojellerde T_g değerinin NaAlg içeriğinin artmasıyla azaldığını ancak nanokil içeriği ile değişmediğini göstermiştir. FT-NIR spektrumunda NaAlg, PNIPA ve nanokilin karakteristik absorpsiyon pikleri gözlenmiştir. XRD sonuçları, nanokil tabakalarının hidrojel yapısı içerisinde hem araya katılmış hem de dağılmış yapıda bulunduğunu göstermiştir.

Ortam pH değerinin 1,2'den 8'e artırılmasıyla hidrojellerin şişme oranı artmış ve maksimum şişme oranına pH=8'de ulaşılmıştır. Hidrojellerin şişme oranı nanokil miktarının artmasıyla azalmış, büzülme oranı ise nanokil miktarının artmasıyla artmıştır. Bu sonuçlar nanokil miktarının kontrol edilmesiyle istenen şişme/büzülme oranına sahip hidrojel tasarlanmasının mümkün olduğu göstermektedir. Salınım çalışmaları sonucunda thymol salınımının da nanokil içeriği ile kontrol edilebileceği gösterilmiştir. Sonuçlar, pH/sıcaklık duyarlı nanokompozit hidrojellerin, ilaç endüstrisinde ilaç taşıyıcı sistemlerde, gıda endüstrisinde ise antimikrobiyal salınım sistemlerinde kullanılmak üzere tasarlanabileceğini göstermiştir.

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TABLE OF CONTENTS

SUMMARY	iv
ÖZET	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	ix
LIST OF TABLES	xii
1 INTRODUCTION	1
1.1 Objective and Specific Objectives	1
1.2 Hypothesis	1
2 GENERAL BACKGROUND	2
2.1 Polymeric gels	2
2.2 Hydrogels	4
2.2.1 State of Water in Hydrogel	5
2.2.2 Classification of Hydrogels	6
2.2.3 Physical and Chemical Gels	8
2.2.4 Responsive Hydrogels	10
2.2.4.1 pH Responsive Hydrogels	11
2.2.4.2 Temperature Responsive Hydrogels	14
2.2.4.3 Magnetically Responsive Hydrogels	16
2.2.4.4 Electrically Responsive Hydrogels	17
2.2.4.5 Other Responsive Hydrogels	17
2.3 Controlled Release Systems	18
2.3.1 Release Mechanisms	19
2.3.1.1 Diffusion Controlled Release Mechanisms	19
2.3.1.2 Swelling Controlled Release Mechanisms	20

2.3.1.3 Chemically Controlled Release Mechanisms	23
2.4 Nanocomposite Hydrogels	24
3 MATERIALS AND METHODS	27
3.1 Materials	27
3.2 Methods	27
3.2.1 Preparation of Nanocomposite Hydrogels	27
3.2.2 Scanning Electron Microscope (SEM) Analysis	29
3.2.3 Thermal Analysis (Measurement of LCST and T_g)	29
3.2.4 Fourier Transform Near Infrared (FT-NIR) Analysis	30
3.2.5 X-ray Diffraction (XRD) Analysis	30
3.2.6 Determination of Swelling and Deswelling	30
3.2.7 Thymol Loading and Measurement of Thymol Release	31
4 RESULTS AND DISCUSSION	33
4.1 Structure and Morphology of the Hydrogels	33
4.2 Thermal Analysis	35
4.2.1 LCST of the Hydrogels	35
4.2.2 Glass Transition of the Hydrogels	37
4.3 FT-NIR Analysis	39
4.4 XRD Analysis	41
4.5 Swelling and Deswelling Behavior	44
4.5.1 Effect of pH on Swelling	44
4.5.2 Effect of Nanoclay on Swelling	50
4.5.3 Effect of Temperature on Deswelling	53
4.6 Thymol Release	55
5 CONCLUSION	58
6 FUTURE WORK AND RECOMMENDATIONS	59
REFERENCES	60
CURRICULUM VITAE	68

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
2.1 Polymer strands forming a gel and a hydrogel. Solid circles represent covalent crosslinks and hollow circles represent virtual crosslinks formed by entanglements [Gupta et al., 2002].	3
2.2 Different approaches to make physical hydrogels crosslinked by (a) ion-polymer complexation, (b) polymer-polymer complexation, (c) hydrophobic association, (d) chain aggregation and (e) hydrogen bonding.	9
2.3 Methods to prepare chemically crosslinked gels.	9
2.4 Representation of hydrogels stimuli responsive swelling [Gupta et al., 2002].	10
2.5 The swelling phenomenon of a hydrogel in a buffer solution [Bajpai et al., 2010].	11
2.6 The pH responsive swelling of (a): anionic and (b): cationic hydrogels [Gupta et al., 2002].	12
2.7 Representative alginate structure (a): chain conformation and (b): block distribution.	13
2.8 Schematic representation of the calcium-induced gelation of alginate in accordance with the “egg-box” structure [George and Abraham, 2006].	13
2.9 Structures of some temperature responsive polymers [Bajpai et al., 2010].	14
2.10 Phase transitions with respect to LCST and UCST for temperature sensitive polymeric hydrogels.	15
2.11 The chemical structure of PNIPA.	16
2.12 Schematic representation of magnetically induced drug release.	17
2.13 Schematic representation of drug release from a hydrogel-based reservoir delivery system.	20
2.14 Schematic representation of drug release from a hydrogel-based matrix delivery system.	20
2.15 Swelling of a hydrogel. (a) Hydrogel in unswollen or dry state; the arrow indicates pressure on the systems to swell, and (b) hydrogel in a swollen state; the mesh size is indicated by the dotted arrow [Bajpai et al., 2010].	21

2.16	Schematic representation of a polymer/clay nanocomposite gel network structure consisting of unmixed, intercalated and exfoliated inorganic clay.	25
3.1	Scheme of the formation process of NaAlg/PNIPA/Clay hydrogels.	28
3.2	UV calibration curve of thymol at 277 nm.	32
4.1	Dried (A_1-H_1) and swollen (A_2-H_2) hydrogels. Subscript 1 shows dried and subscript 2 shows swollen hydrogels based on the codes given in Table 3.1.	34
4.2	SEM micrographs of the nanocomposite hydrogels: (a) NaAlg10/PNIPA/Clay10, (b) NaAlg10/PNIPA/Clay15, (c) NaAlg10/PNIPA/Clay20 and (d) NaAlg10/PNIPA/Clay30. Scale bar is 50 μm . Magnification: x500.	35
4.3	DSC thermograms of PNIPA and swollen NaAlg10/PNIPA/Clay n nanocomposite hydrogels.	36
4.4	DSC thermograms of PNIPA and swollen NaAlg20/PNIPA/Clay n nanocomposite hydrogels.	37
4.5	DSC thermograms of dried NaAlg10/PNIPA/Clay n nanocomposite hydrogels.	38
4.6	DSC thermograms of dried NaAlg20/PNIPA/Clay n nanocomposite hydrogels.	38
4.7	FT-NIR spectra of NaAlg, Clay and NaAlg m /PNIPA/Clay n nanocomposite hydrogels.	40
4.8	FT-NIR spectra of nanocomposite hydrogels.	41
4.9	XRD profile for the nanoclay.	42
4.10	XRD profiles for nanoclay and NaAlg10/PINPA/Clay n nanocomposite hydrogels.	43
4.11	XRD profiles for nanoclay and NaAlg20/PINPA/Clay n nanocomposite hydrogels.	43
4.12	pH dependent swelling behavior of the NaAlg m /PNIPA/Clay n nanocomposite hydrogels at 5°C.	45
4.13	Schematic illustration of the NaAlg/PNIPA/Clay hydrogel at pH = 1.2 and pH = 8.	45

4.14	Swelling characteristics of the NaAlg10/PNIPA/Clay10 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	46
4.15	Swelling characteristics of the NaAlg20/PNIPA/Clay10 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	46
4.16	Swelling characteristics of the NaAlg10/PNIPA/Clay15 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	47
4.17	Swelling characteristics of the NaAlg20/PNIPA/Clay15 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	47
4.18	Swelling characteristics of the NaAlg10/PNIPA/Clay20 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	48
4.19	Swelling characteristics of the NaAlg20/PNIPA/Clay20 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	48
4.20	Swelling characteristics of the NaAlg10/PNIPA/Clay30 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	49
4.21	Swelling characteristics of the NaAlg20/PNIPA/Clay30 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.	49
4.22	Swelling ratios of NaAlg10/PNIPA/Clay n nanocomposite hydrogels at 5°C in buffer solutions with a pH of 4, 6 and 8.	51
4.23	Swelling ratios of NaAlg20/PNIPA/Clay n nanocomposite hydrogels at 5°C in buffer solutions with a pH of 4, 6 and 8.	52
4.24	The appearances of the nanocomposite hydrogel below and above the LCST.	53
4.25	Deswelling characteristics of the NaAlg10/PNIPA/Clay n hydrogels at 37°C in buffer solution with pH=8.	54
4.26	Deswelling characteristics of the NaAlg20/PNIPA/Clay n hydrogels at 37°C in buffer solution with pH=8.	55
4.27	Thymol release profiles of NaAlg10/PNIPA/Clay n nanocomposite hydrogels at 5°C and pH=8.	56
4.28	Thymol release profiles of NaAlg20/PNIPA/Clay n nanocomposite hydrogels at pH = 8 and 5°C.	57

LIST OF TABLES

<u>Table</u>	<u>Page</u>
2.1 Classification of hydrogels [Simões et al., 2012].	7
3.1 Feed compositions of nanocomposite hydrogels.	29

1 INTRODUCTION

1.1 Objective and Specific Objectives

The main objective of this study is to prepare NaAlg/PNIPA/Clay nanocomposite hydrogels and investigate the structure and morphology of these hydrogels. This work also aims to investigate the effect of content of nanoclay as a crosslinking agent on swelling and deswelling behaviors of the hydrogels and to evaluate the release characteristics of thymol from the hydrogels if they are used in food packaging applications.

The specific objectives of this study are to:

- prepare nanocomposite hydrogel systems sensitive to pH and temperature,
- investigate the swelling behaviors of the hydrogels at different pH mediums,
- evaluate the use of these hydrogels as drug delivery systems in thymol incorporated model hydrogel systems,
- get insight into the potential mechanism of prepared nanocomposite hydrogels.

1.2 Hypothesis

The hypothesis of this work is that when the pH responsive property of alginate and the temperature responsive property of PNIPA are combined with nanoclay as a crosslinking agent, the resulting combination could provide a new and effective drug delivery system with dual stimuli response.

2 GENERAL BACKGROUND

2.1 Polymeric gels

Polymeric gels are a class of macromolecular networks that contain a large fraction of solvent such as water within their structure. Polymer gels are usually formed by the free radical polymerization of monomers in the presence of a difunctional crosslinking agent and a solvent. They can be made either in bulk or in nano-or micro particles. The bulk gels are easy to handle, but usually have very slow swelling rate and amorphous structures arising from randomly crosslinked polymer chains, while the gel nanoparticles react quickly to an external stimulus, but are too small for some practical applications. Such structures include gels with embedded self-assembled solid polymer spheres and the complex formation of polyelectrolyte gels with oppositely charge surfactants [Hu, 2004].

A common misinterpretation in polymer science is the use of the terms ‘gel’ and ‘hydrogel’ synonymously. As polymeric networks, both gels and hydrogels might be similar chemically, but they are physically distinct. Technically, gels are semi-solid systems comprising small amounts of solid, dispersed in relatively large amounts of liquid, yet possessing more solid-like than liquid-like character. Sometimes, hydrogels are also described as aqueous gels because of the prefix ‘hydro’. Although the term ‘hydrogel’ implies a material already swollen in water, in a true sense hydrogels are crosslinked networks of hydrophilic polymers. They possess the ability to absorb large amounts of water and swell, while maintaining their three-dimensional structure. This definition differentiates hydrogels from gels, which are polymeric networks already swollen to equilibrium, and the further addition of fluids results only in dilution of the polymeric network. In Figure 2.1 polymer strands forming a gel and hydrogel are shown. The polymer strands show different behavior in aqueous environment. Although some of the gels are rigid enough to maintain their structure under a small stress, after exceeding the yield-value, gel fluidity is observed with loss of polymer structure. A hydrogel exhibits swelling in aqueous media for the same reasons that an analogous linear polymer

dissolves in water to form an ordinary polymer solution. Thus, the feature central to the functioning of a hydrogel is its inherent crosslinking. Conventional gels can also develop small levels of crosslinks as a result of a gain in energy under the influence of shear forces, but this is reversible because of the involvement of weak physical forces [Gupta et al., 2002].

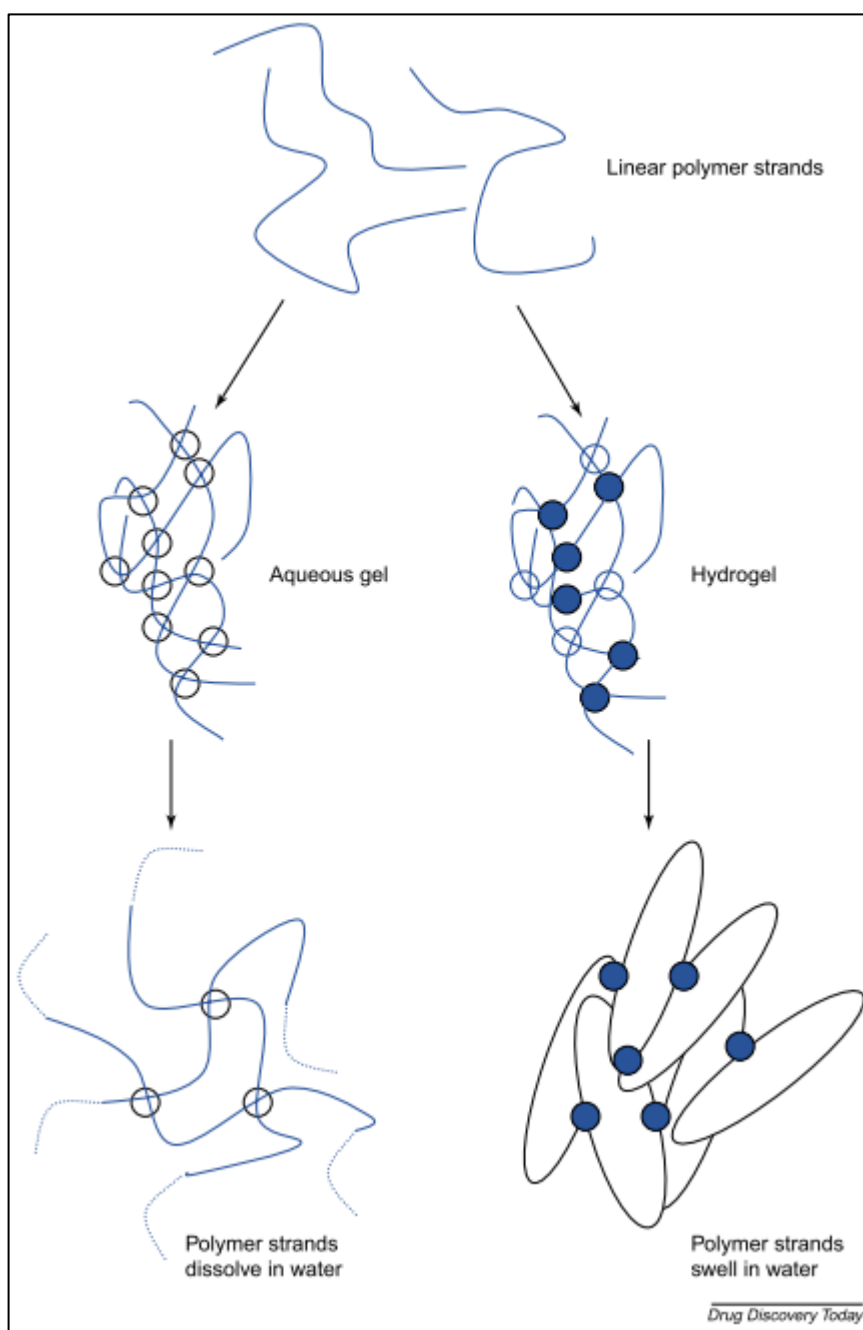


Figure 2.1 Polymer strands forming a gel and a hydrogel. Solid circles represent covalent crosslinks and hollow circles represent virtual crosslinks formed by entanglements [Gupta et al., 2002].

2.2 Hydrogels

In a simple binary system of a polymer and a liquid, a sol is formed when the polymer-liquid interaction is more favored than both polymer-polymer and liquid-liquid interactions. If the polymer is hydrophilic and the liquid is water, the product of the polymer-liquid interaction is called a hydrosol. The extent of this reaction is generally dependent on the polymer structure, functional groups, type, pH, temperature and amount of ions in the polymer structure as well as in the solution. The dissolution of a hydrophilic polymer in water can be prevented by adding crosslinks via either a physical or chemical process. A crosslinked hydrosol is called a hydrogel and can only swell in surrounding liquid to a certain swelling ratio, depending on the number of crosslinks, i.e., the crosslinking density [Omidian and Park, 2012].

Polymer hydrogels are three-dimensional hydrophilic networks that can absorb, swell and retain aqueous fluids up to hundreds of times of their own weight. Polymer hydrogel network structures consist of acidic, basic or neutral monomers. There is a wide variety of hydrogels made from natural or synthetic polymers.

The swelling properties of hydrogels are related to the elasticity of the network, the presence of hydrophilic functional groups such as -OH , -COOH , -CONH_2 and $\text{-SO}_3\text{H}$ groups in the polymer chains, the extent of crosslinking and porosity of the polymer [Dorkoosh et al., 2000].

Hydrogels may also have many different physical forms, including solid molded forms (e.g., soft contact lenses), pressed powder matrices (e.g., pills or capsules for oral ingestion), microparticles (e.g., as bioadhesive carriers or wound treatments), coatings (e.g., on implants or catheters, on pills or capsules, or coatings on the inside capillary wall in capillary electrophoresis), membranes or sheets (e.g., as a reservoir in a transdermal drug delivery patch, or for 2D electrophoresis gels), encapsulated solids (e.g., in osmotic pumps), and liquids (e.g., that form gels on heating or cooling) [Hoffman, 2002].

Hydrogels are capable of being devised as stimuli responsive. By the change including physical or chemical properties, they can respond to the environmental stimuli, such as temperature [Zhang et al., 2003], pH [George and Abraham, 2007], solvent composition [Kokufuta et al., 1998], ionic strength [Xu et al., 2008], electric field [Kim et al., 1999], etc. Because of their superior properties compared to traditional absorbents such as sponge, cotton, etc., hydrogels are widely used in many fields, such as sensors [Xu et al., 2008], artificial muscle [Liu et al., 2006], wound dressing [Kokabi et al., 2007], contact lenses [Ravi et al., 2005], drug delivery systems [Liu et al., 2008], enzyme immobilization [Liu et al., 1993], chemical valves [Sershen et al., 2005], metal particle preparation [Sahiner, 2006], dye adsorption and agriculture [Li et al., 2008] and filters [Haraguchi and Matsuda, 2005].

2.2.1 State of Water in Hydrogel

The character of the water in a hydrogel can determine the overall permeation of nutrients into and cellular products out of the gel. When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to '*primary bound water*'. As the polar groups are hydrated, the network swells, and exposes hydrophobic groups, which also interact with water molecules, leading to hydrophobically-bound water, or '*secondary bound water*'. Primary and secondary bound water are often combined and simply called the '*total bound water*'. After the polar and hydrophobic sites have interacted with bound water molecules, the network will imbibe additional water, due to the osmotic driving force of the network chains towards infinite dilution. This additional swelling is opposed by the covalent or physical crosslinks, leading to an elastic network retraction an equilibrium swelling level. The additional swelling water that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is called '*free water*' or '*bulk water*', and is assumed to fill the space between the network chains, and/or the center of larger pores, macropores or voids. As the network swells, if the network chains or crosslinks are degradable, the gel will begin to disintegrate and dissolve, at a rate depending on its composition. It should be

noted that a gel used as a tissue engineering matrix may never be dried, but the total water in the gel is still comprised of '*bound*' and '*free*' water [Hoffman, 2002].

2.2.2 Classification of Hydrogels

Hydrogels can be classified using various criteria depending on their preparation method and physicochemical properties. Natural polymers, such as proteins, polysaccharides and deoxyribonucleic acids (DNA's) are crosslinked by either physical or chemical bonds, and synthetic hydrogels can be easily prepared by crosslinking polymerization of synthetic monomers. In addition, natural polymers can be combined with synthetic polymers to obtain different properties in the same hydrogel [Simões et al., 2012]. Based on the nature of the pendant groups, hydrogels can be classified as neutral, anionic, cationic or ampholytic. According to their network structures, they can be classified as nonporous, microporous, macroporous and superporous. Additionally, they can be homopolymer, copolymer, multipolymer or interpenetrating networks. Finally, they can be classified based on the physical structure of the networks as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures and hydrocolloidal aggregates. Several classification methods of hydrogels are given in Table 2.1.

Table 2.1 Classification of hydrogels [Simões et al., 2012].

Classification	Contents
Origin	- Natural - Synthetic
Ionic charge (based on the nature of the pendent group)	- Neutral - Anionic - Cationic - Ampholytic
Water content or degree of swelling	- Low swelling - Medium swelling - High swelling - Superabsorbent
Network structure (Porosity)	- Nonporous - Microporous - Macroporous - Superporous
Network morphology	- Amorphous - Semicrystalline - Hydrogen bonded structures - Super molecular structures - Hydrocolloidal agregates
Crosslinking method	- Chemical (or covalent) - Physical (or noncovalent)
Component (based on the method of preparation)	- Homopolymer - Copolymer - Multipolymer - Interpenetrating
Function (based on the organization of the monomers)	- Biodegradable or non-biodegradable - Stimuli responsive - Superabsorbent
Mechanism or controlling the drug release	- Diffusion controlled release systems - Swelling controlled release systems - Chemically controlled release systems - Environment responsive systems.

2.2.3 Physical and Chemical Gels

Hydrogels can be divided into two main classes as chemical and physical crosslinked hydrogels [Haraguchi et al., 2003]. When hydrogels are covalently crosslinked network they are called “permanent” or “chemical” gels. They attain an equilibrium swelling state which depends on the polymer–water interaction parameter and the crosslink density. Accordingly, they are called “reversible” or “physical” gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic interactions. All of these interactions are reversible, and can be disrupted by changes in physical conditions or application of stress [Rosiak and Yoshii, 1999]. Physical hydrogels are reversible due to the conformational changes, but chemical hydrogels are permanent and irreversible as a result of configurational changes [Omidian and Park, 2012].

Physical crosslinked hydrogels can be formed by hydrophobic interactions, ion-polymer complexation, polymer-polymer complexation, chain aggregation or hydrogen bonding. Chemical crosslinked hydrogels can be formed by simultaneous polymerization and crosslinking or post-polymerization chemical crosslinking [Omidian and Park, 2012]. Some different approaches to make physical and chemical crosslinked gels are shown in Figure 2.2 and Figure 2.3, respectively.

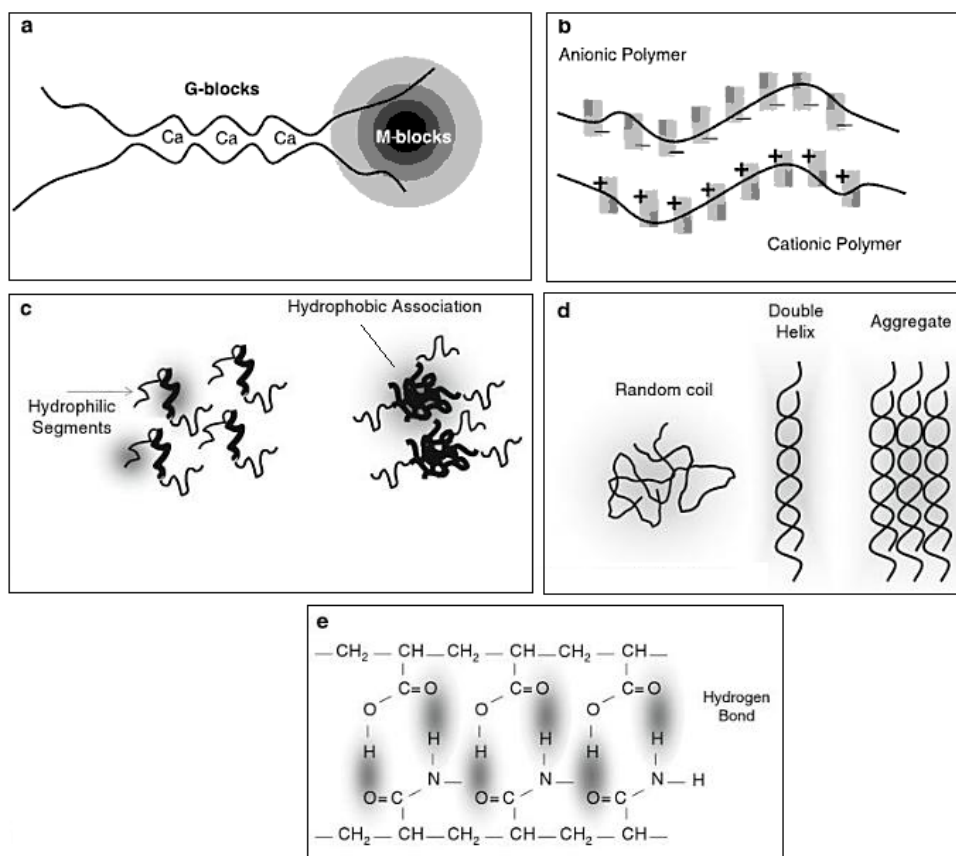


Figure 2.2 Different approaches to make physical hydrogels crosslinked by (a) ion-polymer complexation, (b) polymer-polymer complexation, (c) hydrophobic association, (d) chain aggregation and (e) hydrogen bonding.

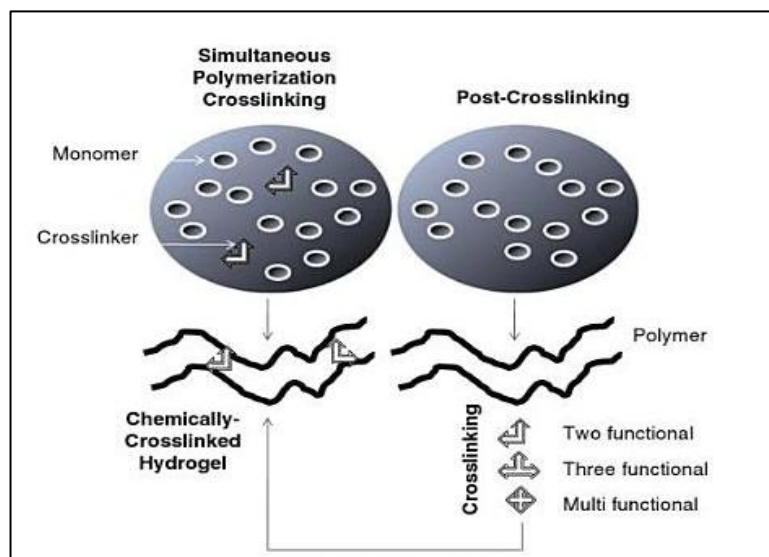


Figure 2.3 Methods to prepare chemically crosslinked gels.

2.2.4 Responsive Hydrogels

Depending its structure, a hydrogel can respond to environmental changes by changing its size or shape. These polymers are physiologically-responsive hydrogels, where polymer complexes can be broken or the network can be swollen as a result of the changing external environment. As a result, these systems tend to show drastic changes in their swelling ratio. Most important factors that trigger a hydrogel to give a response are pH, temperature and swelling medium [Omidian and Park, 2012]. Various factors that affect a hydrogel to respond can be seen in Figure 2.4.

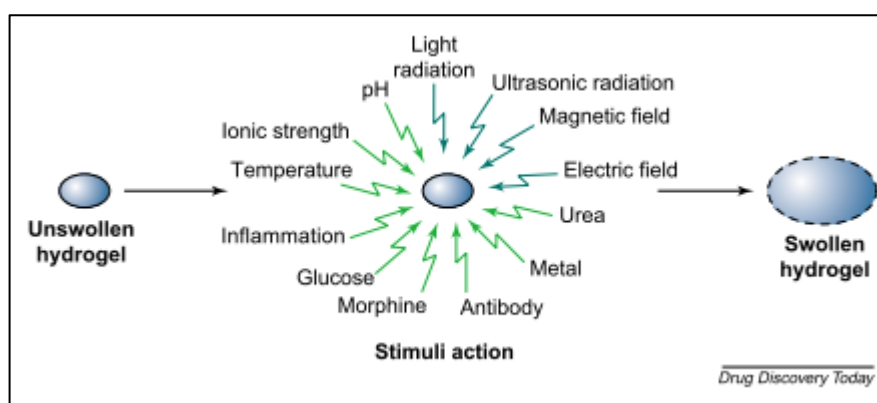


Figure 2.4 Representation of hydrogels stimuli responsive swelling [Gupta et al., 2002].

The responsive hydrogels are highly sensitive to changes in the environment and have been used in several applications, such as biosensors, superabsorbent polymers, site-specific drug delivery systems, emerging nanoscale technologies and tissue engineering [Simões et al., 2012].

Responsive hydrogels are unique concerning many different mechanisms for drug release and a lot of many different types of release systems based on these materials are formulated. For instance, in most cases drug release occurs when the gel is highly swollen, and swelling is typically controlled by the rate of swelling, the drug diffusion or a coupling of swelling and diffusion [Simões et al., 2012].

2.2.4.1 pH Responsive Hydrogels

pH sensitive hydrogels are composed of polymer chain networks crosslinked to each other and surrounded by a salt solution. A change in the pH of the solution surrounding the gel will initiate a physical process of either gel swelling or deswelling. The physical process, in general, is not instantaneous, and modeling the gel swelling/deswelling rate helps us to have a thorough understanding of the gel dynamics. This is particularly important when hydrogels are used in controlled drug-delivery devices, where the drug is released during the swelling process. Hydrogels that are pH sensitive contain acidic or basic groups bound to the polymer chains. The acidic groups on the chains deprotonate at high pH, whereas the basic groups protonate at low pH values. Figure 2.5 shows the general structure of a pH-sensitive gel. Not all ionisable groups (acidic or basic) become fully dissociated (protonated/deprotonated) as shown in Figure 2.5 when placed in an aqueous solution [Bajpai et al., 2010].

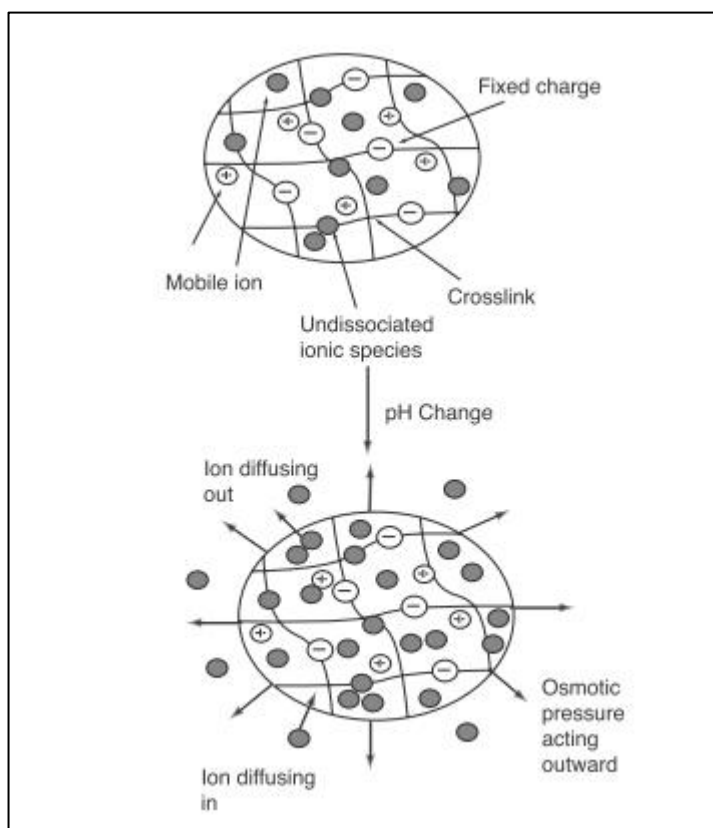


Figure 2.5 The swelling phenomenon of a hydrogel in a buffer solution [Bajpai et al., 2010].

Pendant groups of anionic hydrogels are unionized 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 values. Differential swelling of ionic hydrogels in acidic and alkaline buffers is presented in Figure 2.6 [Gupta et al., 2002].

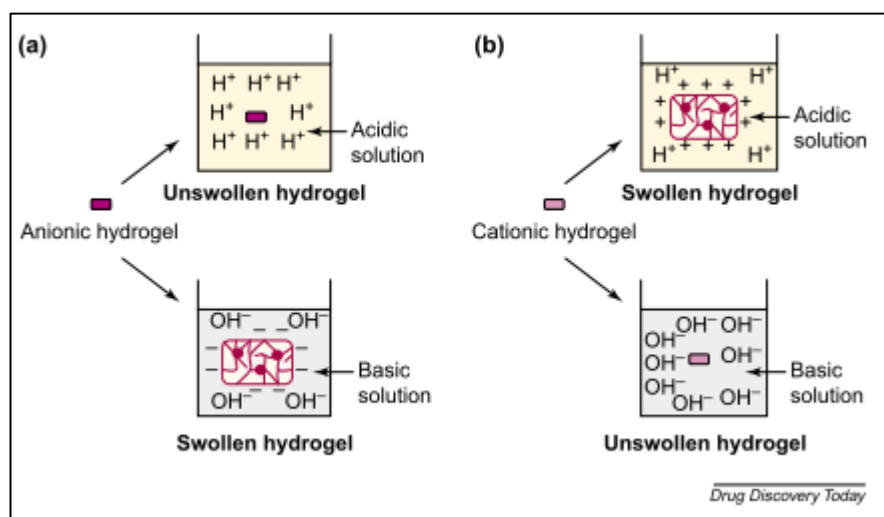


Figure 2.6 The pH responsive swelling of (a): anionic and (b): cationic hydrogels [Gupta et al., 2002].

Alginate is a water soluble linear polysaccharide extracted from brown sea weed and is composed of 1-4 linked β -D-mannuronic acid (M) and its C-5 epimer α -L-guluronic acid (G) [George and Abraham, 2007]. It is comprised of sequences of M (M-blocks) and G (G-blocks) residues interspersed with MG sequences (MG-blocks) [Pawar and Edgar, 2012]. In Figure 2.7, a polymer chain of two guluronic acid (G) monomers and two mannuronic acid (M) monomers with (1-4) linkages and M and G blocks of the alginates with their representative sequence are shown.

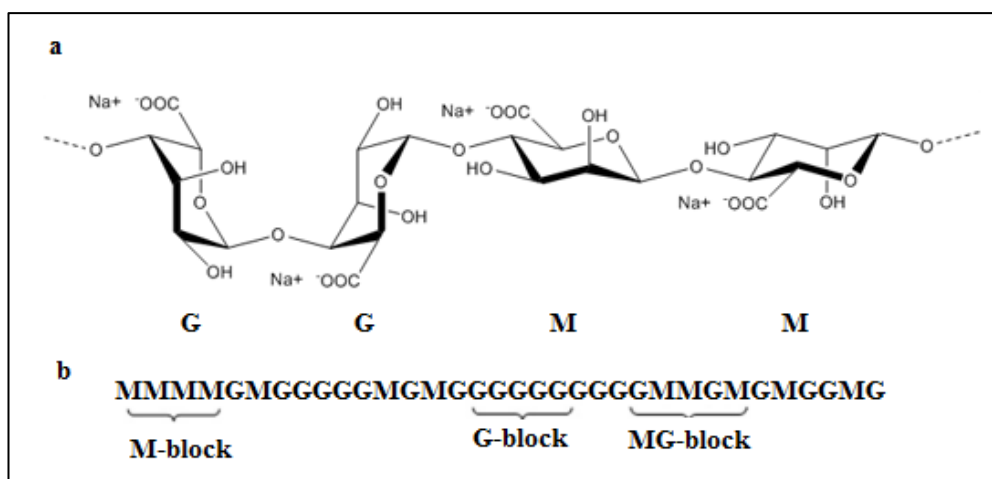


Figure 2.7 Representative alginate structure (a): chain conformation and (b): block distribution.

Alginate can be ionically crosslinked and form gels by the addition of divalent cations in aqueous solutions. Ca^{2+} is the most commonly used cation to induce alginate gel formation [Pawar and Edgar, 2012]. Monovalent cations and Mg^{2+} ions do not induce gelation. The gelation and crosslinking of the polymers are mainly achieved by the exchange of sodium ions from the guluronic acids with the divalent cations, and the stacking of these guluronic groups to form the characteristic egg-box structure shown in Figure 2.8 [George and Abraham, 2006]. Besides hydrogels induced by divalent cations, alginate can form acid gels at pH below the pK_a value of the uronic acid residues [Yang et al., 2011].

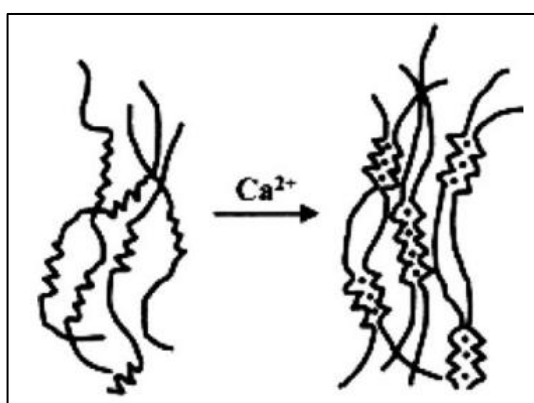


Figure 2.8 Schematic representation of the calcium-induced gelation of alginate in accordance with the “egg-box” structure [George and Abraham, 2006].

Alginates are included in a group of compounds that are generally regarded as safe (GRAS) by the FDA. Alginate is also used extensively in food industry as a thickener, emulsifier and stabilizer.

2.2.4.2 Temperature Responsive Hydrogels

Thermoresponsive hydrogels are crosslinked, three-dimensional polymer networks that are able to swell or deswell as a result of change in the temperature of the surrounding fluid [Peppas et al., 2000]. These hydrogels are the most commonly studied class of responsive polymer systems in drug delivery research.

The common characteristic of temperature sensitive polymers is the presence of hydrophobic groups, such as methyl, ethyl and propyl groups. The structures of some of these polymers are shown in Figure 2.9 [Bajpai et al., 2010].

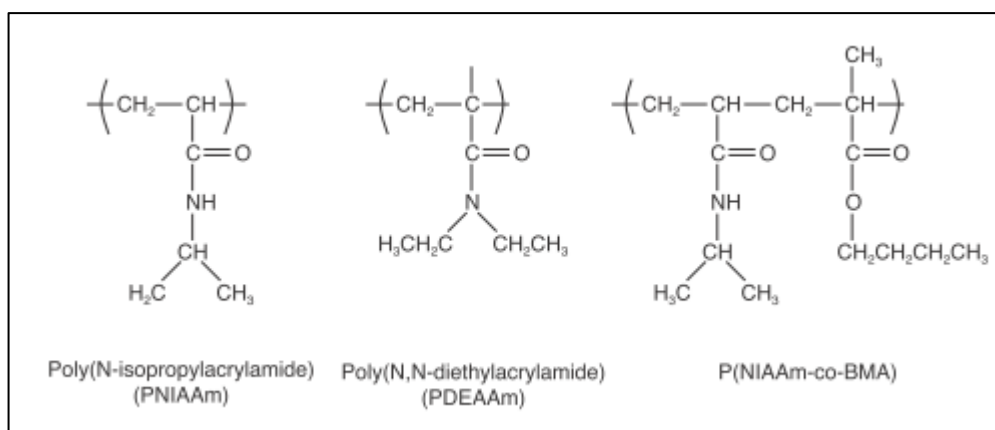


Figure 2.9 Structures of some temperature responsive polymers [Bajpai et al., 2010].

They present a fine hydrophobic–hydrophilic balance in their structure; and small temperature changes around a critical solution temperature make the chains collapse or extend, responding to adjustments of the hydrophobic and hydrophilic interactions between the polymer chains and the aqueous medium [Bajpai et al., 2008]. A critical solution temperature may be defined as a temperature at which the polymer solution undergoes separation from one phase to two phases. Thus, temperature sensitive polymers undergo an abrupt change in volume as the

temperature of the medium is varied above or below the critical solution temperature [Xu et al., 2006].

Polymers that become soluble upon heating have a so-called upper critical solution temperature. Those polymers that become insoluble in solutions upon heating possess a lower critical solution temperature. Figure 2.10 illustrates the phase transition with respect to LCST and UCST for temperature-sensitive polymeric hydrogels [Bajpai et al., 2010].

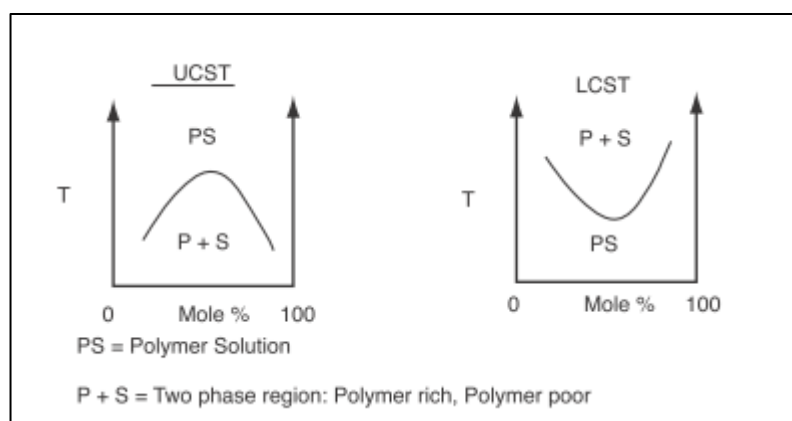


Figure 2.10 Phase transitions with respect to LCST and UCST for temperature sensitive polymeric hydrogels.

The LCST corresponds to the region in the phase diagram at which the enthalpy contribution of water hydrogen bonded to the polymer chain becomes less than the entropic gain of the system as a whole and is largely dependent on the hydrogen bonding capabilities of the constituent monomer units [Kumar et al., 2007].

Poly(N-isopropylacrylamide) (PNIPA) hydrogel, prepared from the monomer N-isopropylacrylamide (NIPA) and a suitable crosslinker, is a well-known thermoresponsive hydrogel. PNIPA molecules in aqueous solution exhibit a rapid and reversible hydration-dehydration change in response to small temperature changes around its LCST [Kaneko et al., 1998]. PNIPA has a hydrophilic/hydrophobic balance in the side chains (Figure 2.11), and thus hydrogen bonds can form between water molecules and hydrophilic groups of the hydrogel. These hydrogen bonds act cooperatively to form a stable shell around the hydrophobic groups, which means that the PNIPA hydrogel has good solubility at

low temperatures. As the temperature increases, these interactions become weak and the entrapped water molecules are slowly released. When the hydrogel reaches its LCST (around 33°C) or above, these interactions are decomposed and the hydrophobic interactions among the hydrophobic groups are strengthened dramatically and abruptly, which leads to the collapse of the polymer chains [Zhang et al., 2002].

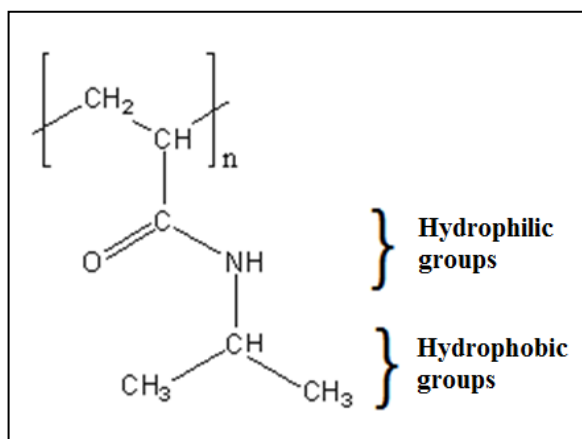


Figure 2.11 The chemical structure of PNIPA.

2.2.4.3 Magnetically Responsive Hydrogels

The magnetic targeted drug-delivery system is one of the most attractive strategies of delivering magnetic nanoparticulates to the area of interest. The goal of magnetic drug targeting is the concentration of drug at a target site, far away from the reticular endothelial system, with the drive of a magnetic field. Typically, the intended drug and a suitable magnetically active component are combined into a pharmacologically stable formulation. The development of magnetically responsive nanoparticulate has brought several additional advantages. Magnetic-controlled drug delivery by particulate carriers is a very efficient method of delivering drug to a localized disease site. Magnetic nanoparticulate can be used to remove cells and molecules by applying magnetic fields and/or to concentrate drugs at well-defined sites. Very high concentrations of chemotherapeutic or radiological agents can be achieved near the target site, such as a tumor, without any toxic effects to normal surrounding tissue or to the whole. The combination of these two advantages makes successful the magnetic nanoparticulate applications also in molecular and cell

biology, advancing both basic science and clinical practice. Magnetic particles have been shown to aid the release of encapsulated molecules from inside of a polymer matrix when they are magnetically induced by an oscillating or alternating current (AC) magnetic field (Figure 2.12) [Bajpai et al., 2010].

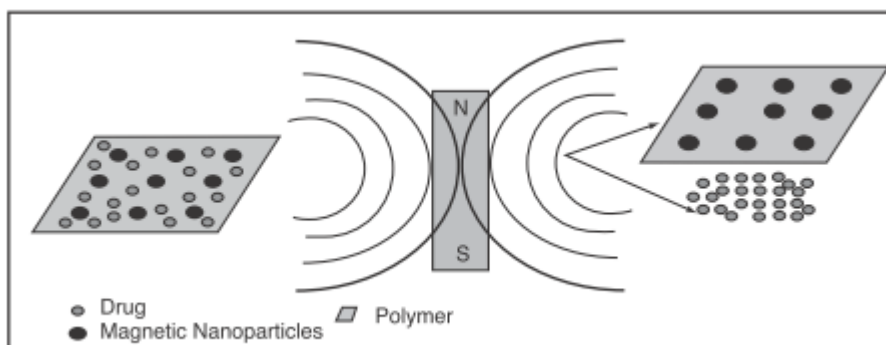


Figure 2.12 Schematic representation of magnetically induced drug release.

2.2.4.4 Electrically Responsive Hydrogels

Electrically responsive delivery systems are prepared from polyelectrolytes (polymers that contain a relatively high concentration of ionisable groups along the backbone chain) and thus are they pH responsive as well. Under the influence of an electric field, electroresponsive hydrogels generally deswell or bend, depending on the shape and orientation of the gel. The gel bends when it is parallel to the electrodes, whereas deswelling occurs when the hydrogel lies perpendicular to the electrodes. Several technologies are currently under investigation for the development of new drug-delivery devices that can achieve chronotherapy, specifically iontophoresis, infusion pumps and sonophoresis [Bajpai et al., 2010].

2.2.4.5 Other Responsive Hydrogels

The next generation of biomaterials and drug delivery systems will require biomolecule sensitive hydrogels that recognize specific biomolecules and respond to them. For example, glucose-sensitive hydrogels that undergo swelling in response to glucose can provide the tools for constructing self-regulated insulin delivery systems,

in which a necessary amount of insulin can be administered in response to the blood glucose concentration [Miyata et al., 2002].

Biomolecule sensitive hydrogels that undergo swelling changes in response to specific biomolecules (glucose, enzyme or antigen sensitive etc.) can be modified to design smart hydrogels that could degrade in response to an increase in concentration of specific biomolecule [Bajpai et al., 2010]. Even though most biomolecule sensitive hydrogels still require further research before practical application, they are likely to become quite important materials in the near future.

2.3 Controlled Release Systems

Stimuli responsive hydrogels have played a major role in the development of controlled release systems. They have been developed as controlled release carriers using water-soluble and biodegradable natural polymers such as chitosan, guar gum, sodium alginate, cellulose, etc. These natural polymers are often preferred over synthetic polymers due to their non-toxic, ease of availability and biodegradable characteristics.

Traditionally, controlled release polymeric systems have been classified into 'matrix' and 'reservoir' types. Matrix systems are most commonly employed because of their ease in development, cost-effectiveness and better performance. However, these systems tend to follow Higuchi's model, wherein drug release is proportional to the square root of time ($t^{1/2}$). This leads to non-uniform release rates, continuously decreasing in the beginning and more rapidly thereafter. The key benefit of hydrogels for controlled drug delivery lies in the near constant release rates [Gupta et al., 2002]. In reservoir type delivery systems, drug diffusion through the reservoir is rate limiting and controls the overall drug release rate. The drug transport mechanism through the reservoir is usually a solution-diffusion mechanism [Bajpai et al., 2008].

2.3.1 Release Mechanisms

Hydrogels have a unique characteristics combination that makes them useful in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amounts of water (>90%, w/v). Therefore, the molecular release mechanisms from hydrogels are very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of a drug from a hydrogel device as a function of time. These models are based on the rate limiting step for controlled release and are therefore categorized as follows [Bajpai et al., 2008].

2.3.1.1 Diffusion Controlled Release Mechanisms

Diffusion controlled is the most widely applicable mechanism for describing the drug release from hydrogels. Fick's law of diffusion with either constant or variable diffusion coefficients is commonly used in modeling diffusion-controlled release. Drug diffusivities are generally determined empirically or estimated a priori using free volume, hydrodynamic or obstruction-based theories [Amsden, 1998]. Diffusion controlled hydrogel delivery systems can be either reservoir or matrix systems [Vasile and Zaikov, 2009]. Release mechanisms can be described by Fick's law, according to equations [1] and [2].

$$J_A = -D \left(\frac{dC_A}{dx} \right) \quad [1]$$

$$\frac{\partial C_A}{\partial t} = D \left(\frac{\partial^2 C_A}{\partial x^2} \right) \quad [2]$$

Assuming a binary system, t , C_A , J_A , and D represents time, solute concentration, mass flux and solute diffusion coefficient, respectively.

For a reservoir system (Figure 2.13), where the drug is contained in a core which is surrounded by a rate controlling polymeric hydrogel membrane, Fick's first

law of diffusion (Eq. [1]) can be used to describe drug release through the membrane.

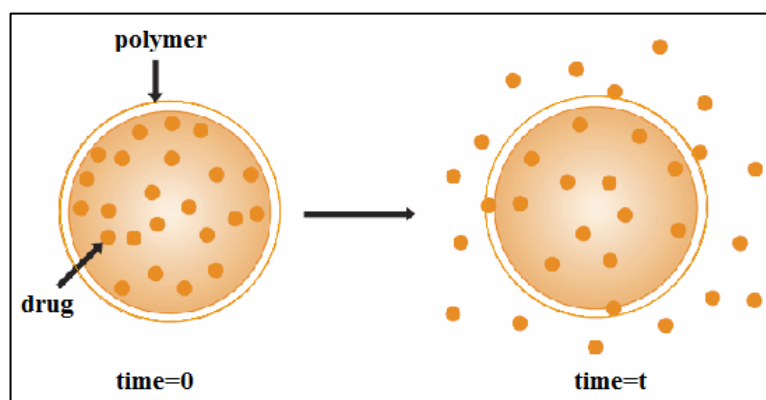


Figure 2.13 Schematic representation of drug release from a hydrogel-based reservoir delivery system.

For a matrix system (Figure 2.14), where the drug is uniformly dispersed throughout the matrix, unsteady-state drug diffusion in a one dimensional slab shaped matrix can be described using Fick's second law of diffusion (Eq. [2]) [Vasile and Zaikov, 2009].

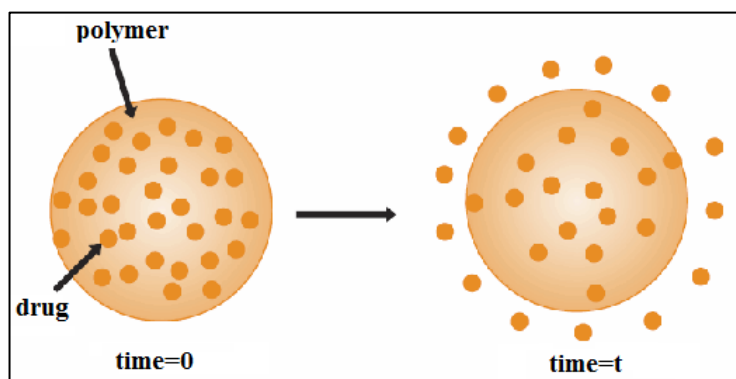


Figure 2.14 Schematic representation of drug release from a hydrogel-based matrix delivery system.

2.3.1.2 Swelling Controlled Release Mechanisms

Swelling controlled release occurs when diffusion of drug is faster than hydrogel swelling. The modeling of this mechanism usually involves moving

boundary conditions where molecules are released at the interface of rubbery and glassy phases of swollen hydrogels [Vasile and Zaikov, 2009].

When the hydrogel is in a dehydrated or unswollen state its polymer chains are in close proximity, with little room for diffusion of the molecule. As the material swells the polymer chains separate to an extent determined by the properties of the solvent in which the hydrogel has been placed. Figure 2.15 represents the change in mesh size after swelling of the hydrogel [Bajpai et al., 2010].

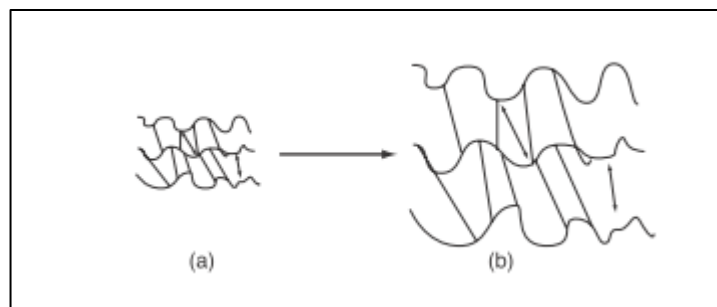


Figure 2.15 Swelling of a hydrogel. (a) Hydrogel in unswollen or dry state; the arrow indicates pressure on the systems to swell, and (b) hydrogel in a swollen state; the mesh size is indicated by the dotted arrow [Bajpai et al., 2010].

In swelling controlled devices, the drug can be released following two different mechanisms: diffusion and relaxation of polymer chains, which occur at the glassy rubbery interface. Ritger and Peppas (1987a) proposed a simple equation to determine the relative importance of diffusion and macromolecular relaxation on the overall drug delivery process:

$$F_R(t) = \frac{M_t}{M_\infty} = kt^n \quad [3]$$

$F_R(t)$ is the cumulative fraction of solute released in a time t , n is designated as a diffusional exponent from which the release mechanism can be evaluated and k is a structural/geometrical constant, M_t is defined as the mass of drug released at time t , and M_∞ is the mass of drug released as time approaches infinity [Ritger and Peppas, 1987b].

According to Ritger and Peppas (1987a) the value of release exponent n , is associated to the mechanism transport of drug through the hydrogel. Mechanisms have been established for diffusion phenomenon and categorized based on the exponent n , as follows;

If $n \cong 0,5$, the mechanism is called Fickian (Case I) and the release is diffusionally controlled.

If $0,5 < n < 1$, the mechanism is called non-Fickian (anomalous), and the release is dependent on both the diffusion and relaxation of the polymer.

If $n = 1$, the mechanism is called Case II transport, and the release is dependent only on the relaxation/swelling of the polymer.

Fickian diffusion (Case I) is often observed in polymer systems when the temperature is well above the glass transition temperature of the polymer (T_g). It represents a perfect Fickian process, during which the rate of solute penetration is slower than the chain relaxation rate [Gunasekaran et al., 2007].

The Case II diffusion occurs when diffusion is greater than the relaxation rate of the polymer chains and the solvent uptake is directly proportional with time.

Non-Fickian or anomalous diffusion occurs when the diffusion and relaxation rates are comparable. Solute release depends on two simultaneously rate processes, (1): water migration into the system and (2): solute diffusion through continuously swelling hydrogels is highly complicated [Ritger and Peppas, 1987b, a].

Case II and anomalous diffusion are usually observed for polymers whose glass transition temperatures are higher than the experiment temperature. The main difference between these two diffusion modes concerns the solvent diffusion rate [Masaro and Zhu, 1999].

2.3.1.3 Chemically Controlled Release Mechanisms

Chemically controlled release is used to describe drug release determined by reactions occurring within a delivery matrix [Simões et al., 2012]. These systems include all types of biodegradable or bioerodable systems. Drug release is controlled by the kinetics of the bioerosion/biodegradation reaction or by a combination of diffusion through the polymer and biodegradation [Peppas and Lustig, 1985]. The most common reactions that occur within hydrogel delivery systems are cleavage of polymer chains via hydrolytic degradation, enzymatic degradation or reversible/irreversible reactions occurring between the polymer network and the drug released. Under certain conditions, the surface or bulk erosion of hydrogels will control the rate of drug release. Chemically controlled release can be further categorized according to the type of chemical reaction occurring during drug release. Generally, the delivery of encapsulated or tethered drugs can occur through the degradation of pendant chains or during surface erosion or bulk degradation of the polymer backbone [Simões et al., 2012]. In pendant chain systems, the drug molecule is chemically bonded to a polymer backbone and the drug is released by hydrolytic or enzymatic degradation [Bajpai et al., 2008].

In erodible drug delivery systems the release of the drug is controlled by the dissolution or degradation of the polymer. Contrary to pendant chain systems, the drug diffuses from erodible systems. Depending on whether diffusion or polymer degradation controls the release rate, the drug is released following different mechanisms. If the erosion of the polymer is much slower than the diffusion of the drug through the polymer, then drug release can be treated as a diffusion controlled process. If the diffusion of the drug from the polymer matrix is very slow, then polymer degradation or erosion is the rate-controlling step. Two different types of erodible polymers can be found: hydrophilic and hydrophobic. Hydrophilic erodible polymers are completely permeated by water and they undergo a bulk erosion process. Erosion takes place throughout the polymer matrix. Hydrophobic erodible polymers can experience bulk or surface erosion. In bulk erosion, degradation occurs throughout the bulk of the polymer and generally the analysis of the drug release kinetics is complex since it comprises erosion and diffusion [Simões et al., 2012]. In

surface erosion, drug release is mediated by the rate of surface erosion. For hydrophobic polymer networks, surface erosion occurs when the rate of water transport into the polymer is much slower than the rate of bond hydrolysis. Due to the inherently high water content of hydrogels, surface erosion only occurs in enzymatic degrading systems where the transport of enzyme into the gel is slower than the rate of enzymatic degradation [Vasile and Zaikov, 2009].

2.4 Nanocomposite Hydrogels

In the last decades, polymeric hydrogels such as poly(N-isopropyl acrylamide) (PNIPA) and poly(N,N-dimethylacrylamide) (PDMAA) have attracted extensive attention as water-absorbing, soft and stimuli-sensitive materials. They were all prepared by chemical crosslinking reactions using an organic crosslinker such as methylene bisacrylamide (BIS) [Haraguchi et al., 2003]. The conventional chemically crosslinked hydrogels have several limitations in morphology and properties such as structural inhomogeneity, mechanical weakness, limited swelling at equilibrium and slow response to stimuli. [Haraguchi et al., 2003]. Furthermore, Shibayama et al. (1998) showed that structural inhomogeneities are strongly influenced by the degree of crosslinking. High crosslinker concentration also restricts the movement of polymer chains. As a result, they do not behave like flexible linear polymer chains in terms of their own characteristics such as sensitivity to external stimuli [Haraguchi and Takehisa, 2002]. Due to these limitations, many applications of polymeric hydrogels have been restricted.

In order to increase several properties like mechanical, optical and swelling/deswelling or to decrease other properties like electrical conductivity and permeability for gases, polymer materials have been filled with several inorganic synthetic or natural compounds [Haraguchi, 2007; Mansoori et al., 2010]. Clay powders are hydrated layered aluminosilicates which contain reactive –OH groups on the surface. Interaction of clay powders with the reactive sites of monomers and polymers results superabsorbent composite [Pourjavadi et al., 2007]. Inclusion of conventional clays into hydrogels produces aggregated and agglomerated points. This is attributed to the heterogeneous dispersion of clay particles. By using

nanoscale clays, intercalated and exfoliated clay-polymer composites can be produced. The resulting materials are known as nanocomposites. A nanocomposite gel composed of polymer/clay networks is shown schematically in Figure 2.16 [Koo, 2006].

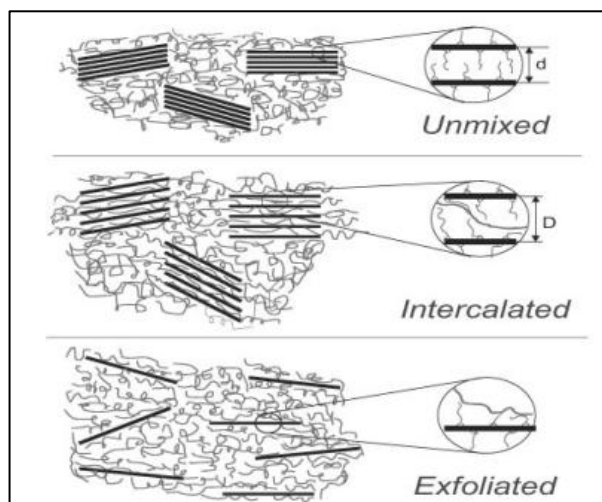


Figure 2.16 Schematic representation of a polymer/clay nanocomposite gel network structure consisting of unmixed, intercalated and exfoliated inorganic clay.

Messersmith and Znidarsich (1997) first prepared a stimuli sensitive polymer-clay hydrogel composite by copolymerization of N-isopropyl acrylamide with methylene bisacrylamide in an aqueous suspension of Na-montmorillonite. Hydrogels containing 3.5% by weight of montmorillonite showed a lower critical solution temperature (LCST) similar to unmodified PNIPA hydrogel, and showed a reversible volume shrinkage of 60-70% when heated from the ambient temperature to temperatures above the LCST. Unfortunately, only hydrogel nanocomposites containing montmorillonite up to 3.5 weight% exhibited a measurable LCST. Higher clay loading (10 wt%) eliminated the thermal transition and underwent considerably less shrinkage when heated.

Liang et al. (2000) demonstrated a new approach to prepare an organically modified clay-polymer nanocomposites with an enhanced thermal transition and an increased transition rate; thus, exhibiting higher swelling degree compared with conventional PNIPA hydrogels. The clay mineral used in this study was montmorillonite modified with N-tetradecyl trimethyl ammonium chloride.

Compared with previously reported polymer-clay nanocomposites, these advantages have been observed for the first time when a nanophase ceramic material is added to the polymer gel.

Xia et al. (2003) prepared PNIPA/Na-montmorillonite nanocomposite hydrogel and reported that 2.0-3.2 wt% Na-montmorillonite containing hydrogel had larger swelling ratio and shear modulus compared with the pure PNIPA gel. Xu et al. (2007) prepared hydrogels with ionic monomers and montmorillonite. The transparency, swelling degree and mechanical properties of these hydrogels did not improve simultaneously at high montmorillonite loadings, because the crosslinker N,N'-methylenebisacrylamide (BIS) caused structural inhomogeneity in the hydrogel network [Haraguchi et al., 2003; Nie et al., 2006; Xu et al., 2007; Zhang et al., 2009]. To overcome this problem Haraguchi et. al. (2002) prepared a special type of an inorganic (clay)/organic (PNIPA) thermo-sensitive nanocomposite hydrogel. In nanocomposite hydrogel, exfoliated inorganic clay (Laponite) particles act as a multifunctional crosslinker in place of any chemical crosslinker.

Zhu et al. (2006), Ma et al. (2007), and Mujumdar and Siegel (2008) also prepared various nanocomposite hydrogels by using Laponite as a multifunctional crosslinker. The resulting hydrogels exhibited not only excellent optical and ultrahigh mechanical properties, but also large swelling ratios and rapid shrinking capability. The high cost of laponite limited the use of laponite containing hydrogels in industrial applications [Zhang et al., 2009].

Clays, including montmorillonite [Kabiri and Zohuriaan-Mehr, 2004], [Messersmith and Znidarsich, 1997], kaolin [Wu et al., 2003], mica [Lee and Chen, 2005], attapulgite [Li and Wang, 2005] and sericite [Wu et al., 2000] have been used in many polymeric networks. Montmorillonite is extensively used as an inorganic layered clay because of its low cost and unique characteristics such as its good water adsorption, extensive swelling in water and cation exchange capacity [Mahdavinia et al., 2010].

3 MATERIALS AND METHODS

3.1 Materials

N-isopropylacrylamide (NIPA) was purchased from ABCR (Karlsruhe, Germany). Sodium alginate was kindly provided from GMT food (Istanbul, Turkey). Potassium peroxodisulfate (KPS), thymol (5-Methyl-2-(1-methylethyl)phenol) and tris(hydroxymethyl)aminomethane (TRIS) were all purchased from Merck (Darmstadt, Germany). Sodium acetate trihydrate was supplied from JT Baker (Phillipsburg, NJ, USA). N,N,N',N'-tetramethylethylene diamine (TEMED), acetic acid, calcium chloride (CaCl_2) and Nanomer®PGV (montmorillonite nanoclay, bentonite) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Nanomer®PGV has a thickness of 1 nm and individual sheets have aspect ratios (L/W) varying from 150-200, cation exchange capacity = 145 meq/100g). All reagents were analytical grades and used as received. All solutions used in the experiments were prepared in deionized water.

3.2 Methods

3.2.1 Preparation of Nanocomposite Hydrogels

Hydrogels were synthesized by free radical solution polymerizations of NIPA using KPS as the initiator, nanoclay as the crosslinking agent and TEMED as the accelerator. In the preparation of the NaAlg/PNIPA/Clay hydrogels, the initial dispersion consisting of TEMED (4 μl), deionized water (0.7 ml) and nanoclay (10, 15, 20 and 30 wt % with respect to NIPA) were stirred at 23°C for 18 hours. Then NIPA monomer (0.1 g), NaAlg (10 and 20 wt % with respect to NIPA) and deionized water (0.3 ml) were added to the dispersion. The dispersion was stirred in an ice-water bath for 30 min until a homogeneous dispersion was obtained. The dispersion was degassed with nitrogen for 15 min to remove oxygen. Finally, 50 μl KPS (0.06 g in 1 ml H_2O) was added to the dispersion. The free radical polymerization was

carried out in a water bath at 23°C for 48 hours. Hydrogels were synthesized in polypropylene (PP) tubes with a diameter of 1 cm and length of 3 cm. In Figure 3.1, a scheme to describe the formation process of NaAlg/NIPA/Clay nanocomposite hydrogels is shown.

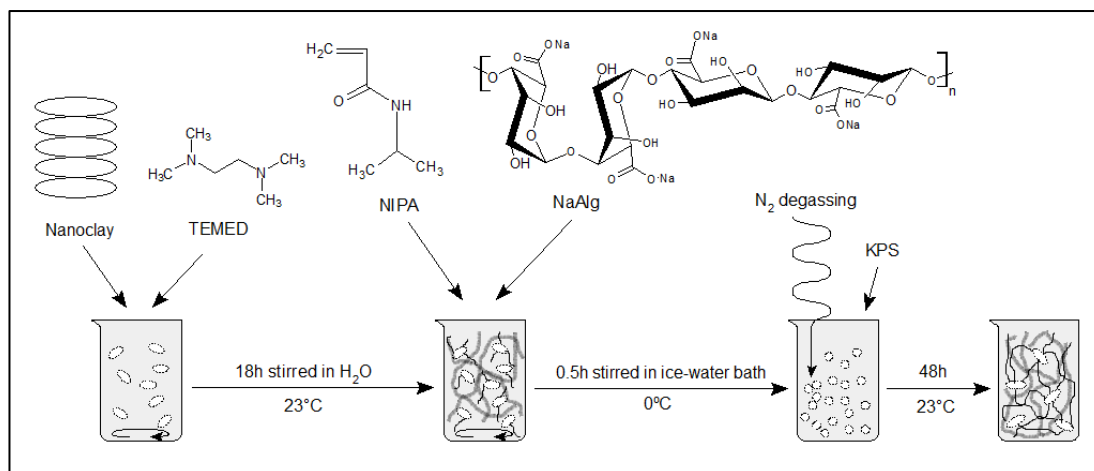


Figure 3.1 Scheme of the formation process of NaAlg/PNIPA/Clay hydrogels.

The hydrogels crosslinked by nanoclay are expressed as NaAlg_m/PNIPA/Clay_n hydrogel. The letters m and n correspond to the weight percent of NaAlg and nanoclay with respect to NIPA monomer, respectively. The feed compositions of prepared nanocomposite hydrogels are given in Table 3.1.

After polymerization was completed, the top plaque above the polymerized hydrogels was removed and the hydrogels were immersed in an aqueous CaCl₂ solution (0.03 g/ml) for 15 min to achieve the physical crosslinking of the outer surface of the hydrogels. The nanocomposite hydrogels were then immersed in an excess of deionized water for 4 days to remove linear polymer chains and residual unreacted constituents by changing the water daily. Finally, the equilibrium swollen hydrogels except for SEM and XRD analysis were dried in a desiccator at room temperature for a week. The dried hydrogels were kept in vacuum desiccators until they were used.

Table 3.1 Feed compositions of nanocomposite hydrogels.

Formulation Code	Polymer system	Compositions		
		NaAlg (g)	NIPA (g)	Nanoclay (g)
A	NaAlg10/PNIPA/Clay10	0.01	0.1	0.01
B	NaAlg20/PNIPA/Clay10	0.02	0.1	0.01
C	NaAlg10/PNIPA/Clay15	0.01	0.1	0.015
D	NaAlg20/PNIPA/Clay15	0.02	0.1	0.015
E	NaAlg10/PNIPA/Clay20	0.01	0.1	0.02
F	NaAlg20/PNIPA/Clay20	0.02	0.1	0.02
G	NaAlg10/PNIPA/Clay30	0.01	0.1	0.03
H	NaAlg20/PNIPA/Clay30	0.02	0.1	0.03

3.2.2 Scanning Electron Microscope (SEM) Analysis

The equilibrium swollen hydrogels in deionized water were then freeze-dried under vacuum using a freeze dryer (Virtis Ultra Super XL, New York, USA) at -35°C for overnight. The dried samples were fractured and coated with a thin layer of gold prior to analysis. The structure and morphology of the hydrogels were analyzed by a SEM (Philips XL30 FEG, Oregon, USA) an operating at a voltage of 2 kV.

3.2.3 Thermal Analysis (Measurement of LCST and T_g)

The LCST and T_g of the hydrogels were measured using a differential scanning calorimeter (DSC) (Perkin Elmer Jade DSC, Shelton, CT, USA). The LCST determination of the swollen hydrogels was performed from 20° to 45°C at a heating rate of 3°C/min under nitrogen with a flow rate of 25 ml/min to avoid sample degradation in aluminum pans. Deionized water was used as the reference in DSC analysis. The LCST was the onset point of the endothermal peak, which was determined by intersecting point of two tangent lines from the baseline and slope of the endothermic peak.

For the determination of T_g values of the dried hydrogels, approximately 10 mg of dried samples was scanned at a heating rate of 15°C/min under nitrogen with a flow rate of 25 ml/min to prevent oxidation. The first scan was done from 50° to

150°C to remove all residual moisture and erase any thermal history. The T_g values were determined from the second scan performed from 30° to 300°C.

3.2.4 Fourier Transform Near Infrared (FT-NIR) Analysis

The dried hydrogel samples were directly analyzed using a Fourier transform near infrared spectrometer (Bruker Optics, Ettlingen Germany) in the range of 12500-4000 cm^{-1} .

3.2.5 X-ray Diffraction (XRD) Analysis

XRD patterns of dried and milled hydrogel samples were determined with an X-ray diffractometer (Rigaku D-max 2200PC, Tokyo, Japan) equipped with an X-ray source of Cu K_α radiation with wavelength, $\lambda = 0.15418$. Data were collected from 2θ of 5° to 70° (θ being the angle of diffraction) with a scanning rate of 1.2°/min. The voltage and current of the X-ray tubes were 40 kV and 40 mA, respectively.

3.2.6 Determination of Swelling and Deswelling

Dried hydrogels were weighed and placed in 50 ml of buffer solutions with pH values of 4, 6 and 8. They were placed in an incubator at $5 \pm 0.5^\circ\text{C}$ for 72 h. The hydrogels were removed from the buffer solutions periodically, blotted with filter paper to remove surface water and weighed. The measurements were made in triplicate.

The swelling capacities of hydrogels are expressed in terms of swelling ratios (SR) as follows:

$$SR (\%) = \frac{W_t - W_d}{W_d} \times 100 \quad [4]$$

where W_t is the weight of the hydrogel at a given time during swelling and W_d is the weight of the dry hydrogel.

The deswelling behavior of the hydrogels was studied by recording the weight of water in the hydrogels. Water retention (WR) is given as:

$$WR (\%) = \frac{(W'_t - W_d)}{(W_s - W_d)} \times 100 \quad [5]$$

where W'_t is the weight of the hydrogel at a given time during the course of deswelling after the swollen hydrogels at 5°C were quickly transferred into TRIS buffer at a 37°C and pH=8, and W_s is the weight of the equilibrium swollen hydrogel at 5°C.

3.2.7 Thymol Loading and Measurement of Thymol Release

A thymol solution was prepared by dissolving thymol (85 mg) in 100 ml TRIS buffer (0.05M and pH=8). For loading the thymol, dried hydrogel samples were dried and then soaked in aqueous thymol solution and keeping them immersed at 5°C until they reached equilibrium. The thymol loaded hydrogels were removed and dried in a desiccator placed in an incubator at room temperature for a week.

For the release studies accurately weighed thymol loaded dried hydrogels were placed in conical flasks containing TRIS buffer (0.05M and pH=8). The samples were placed in a refrigerator at 5±0.5°C. At predetermined time intervals, samples of 5 ml were withdrawn from the release medium and replaced with the same amount of fresh TRIS buffer solution to keep the solution volume constant. The concentration of thymol in the buffer solution was assayed by an UV-VIS spectrophotometer (Perkin Elmer Lambda 35, Massachusetts, USA) at 277 nm based on the previously build calibration curve ($R^2=0.9991$) as shown in Figure 3.2 in which the absorbance was plotted against known concentrations of thymol.

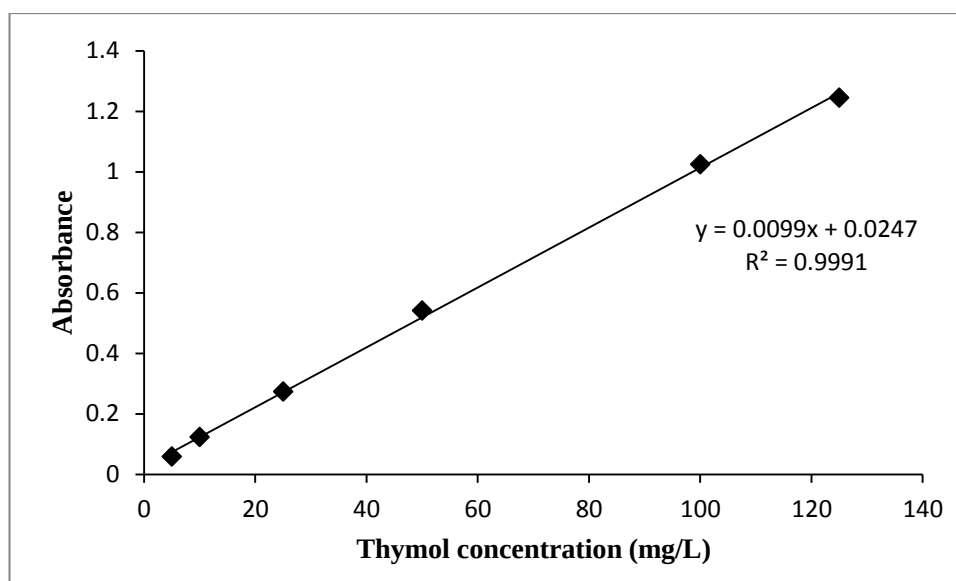


Figure 3.2 UV calibration curve of thymol at 277 nm.

4 RESULTS AND DISCUSSION

4.1 Structure and Morphology of the Hydrogels

The prepared NaAlg/PNIPA/Clay hydrogels did not dissolve when they were put into an excess of water for a week at room temperature, but they swelled and kept their original shapes. This behavior is consistent with the results obtained by Haraguchi and Takehisa (2002), Ma et al. (2007), Ma et al. (2008a, b). Pictures of dried and swollen hydrogels are shown in Figure 4.1. This result showed that NIPA and nanoclay formed network structures without any conventional crosslinker such as N,N-methylenebisacrylamide (BIS). Ma et al. (2008a) reported that the initiator KPS could strongly interact with nanoclay sheets through ionic interactions and their molecules were closely associated on the nanoclay surface in aqueous suspension. At the initial stage of the polymerization, radical formation was initiated and then the propagation reaction was proceeded, thus the PNIPA chains attached to the nanoclay sheets forming a network structure [Ma et al., 2007].

In order to prove that the nanoclay was crosslinked with the PNIPA and not the NaAlg, a mixture of the aqueous solution of nanoclay and NaAlg was prepared with the same procedure used in the preparation of NaAlg/PNIPA/Clay hydrogels. A turbid gel like material was formed and when it is further immersed in an excess of water, it dissolved and turned to a turbid solution. This showed that NaAlg was not crosslinked by the nanoclay when the PNIPA did not exist in the hydrogels.

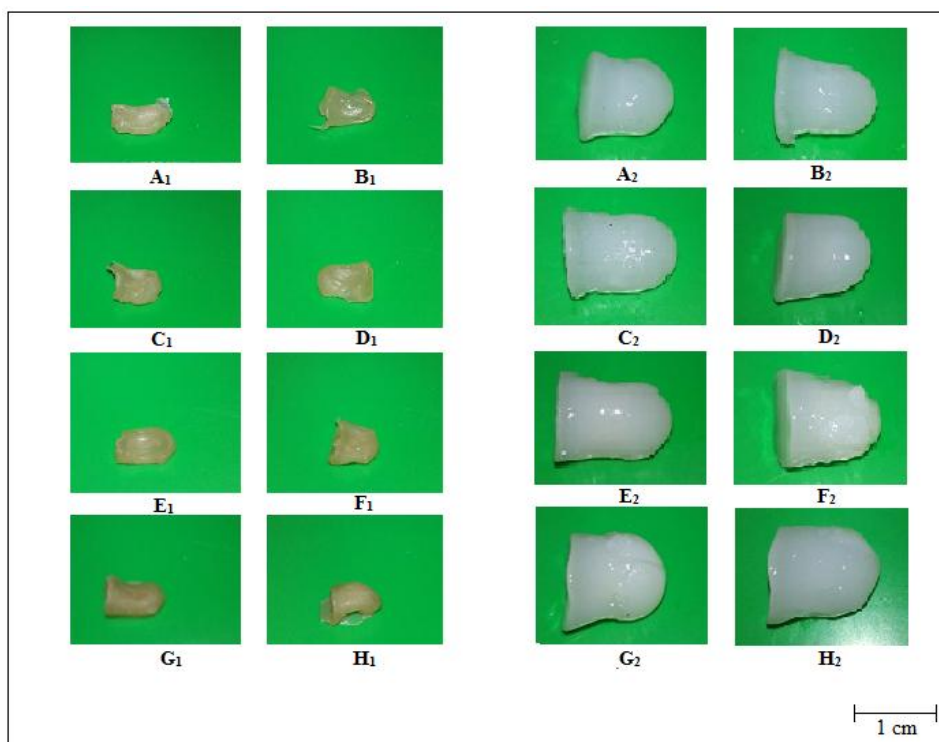


Figure 4.1 Dried (A_1 - H_1) and swollen (A_2 - H_2) hydrogels. Subscript 1 shows dried and subscript 2 shows swollen hydrogels based on the codes given in Table 3.1.

SEM pictures of the microstructure of the freeze-dried NaAlgm/PNIPA/Claynan hydrogels are shown in Figure 4.2. The SEM pictures of the nanocomposite hydrogels revealed a highly porous structure. As seen in SEM micrographs, the pore sizes decreased and the amount of pores increased with increasing the nanoclay content in hydrogels. As mentioned above, the nanoclay sheets acted as a crosslinker. The high nanoclay concentration produced more crosslinking points in the polymer network and increased the crosslinking density, resulting in hydrogels with lower pore size. Lee and Fu (2003) also reported that the pore sizes of PNIPA/Clay nanocomposite hydrogels decreased and the amount of pores increased with an increase in the nanoclay content in the hydrogels. Ma et. al. (2008a) also reported similar results for carboxymethylcellulose/PNIPA/Clay nanocomposite hydrogels. They observed that pore sizes in the hydrogels with a lower nanoclay content are larger than the hydrogels with a higher nanoclay content.

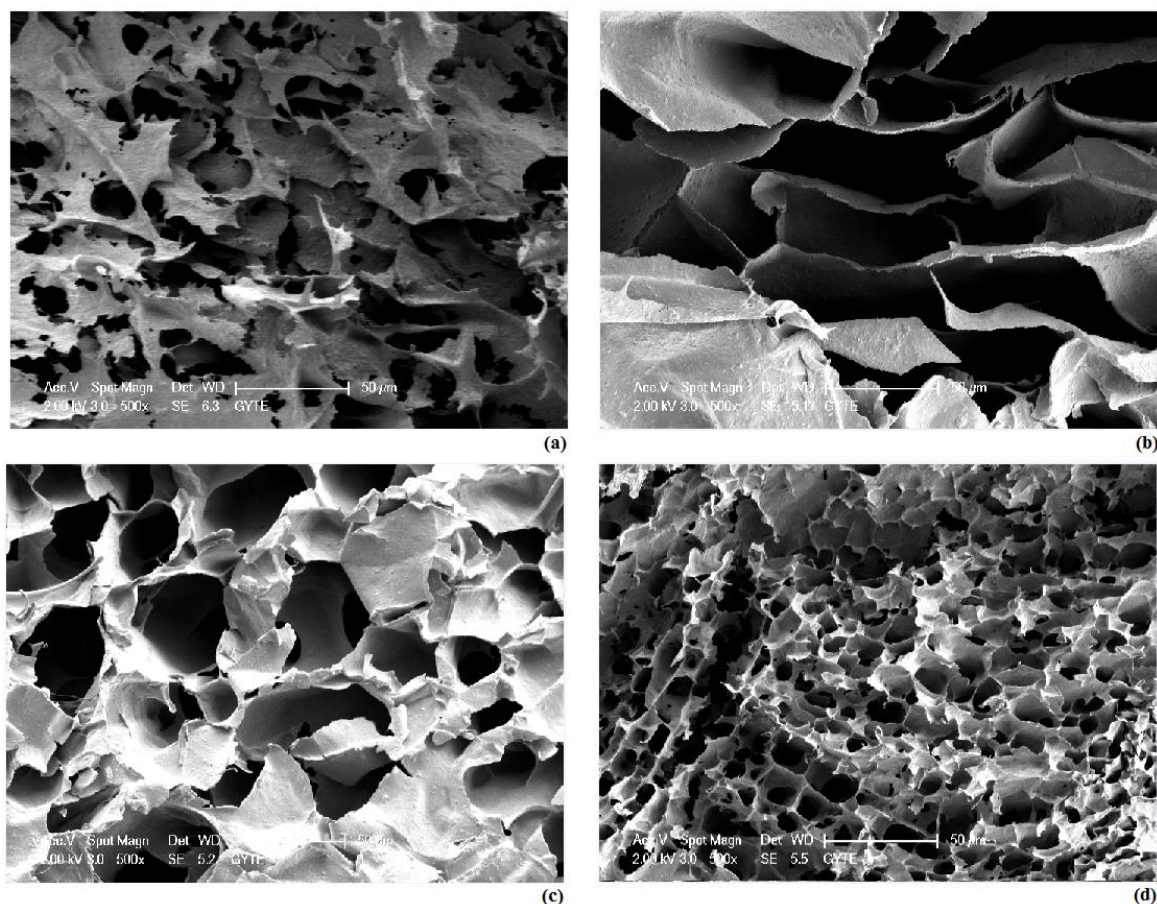


Figure 4.2 SEM micrographs of the nanocomposite hydrogels: (a) NaAlg10/PNIPA/Clay10, (b) NaAlg10/PNIPA/Clay15, (c) NaAlg10/PNIPA/Clay20 and (d) NaAlg10/PNIPA/Clay30. Scale bar is 50 μm . Magnification: x500.

4.2 Thermal Analysis

4.2.1 LCST of the Hydrogels

The DSC thermograms of conventional PNIPA and NaAlg/PNIPA/Clay hydrogels are shown in Figures 4.3 and 4.4. The temperature at the onset point of the DSC endotherm is referred as the LCST of the hydrogel [Otake et al., 1990]. At the LCST, water in the hydrogels is separated from the system, leading to a smaller heat capacity as suggested by Zhang et al. (2005).

As seen from the Figures 4.3 and 4.4 all the hydrogels exhibited a similar LCST between 32.02 and 33.10°C, and the LCST of the PNIPA is 31.54°C which is

very close to the LCST of the hydrogels. These results are consistent with the findings found by Zhang et al. (2005), Shi et al. (2006) and Ma et al. (2007). These hydrogels can be considered as semi-IPN systems because LCST of PNIPA and hydrogels are very close to each other indicating there is no chemical bond between NaAlg and PNIPA network, which may change the balance between the hydrophobic and hydrophilic interactions in PNIPA. It can also be concluded that the LCST of the hydrogels is not dependent on the amount of the nanoclay.

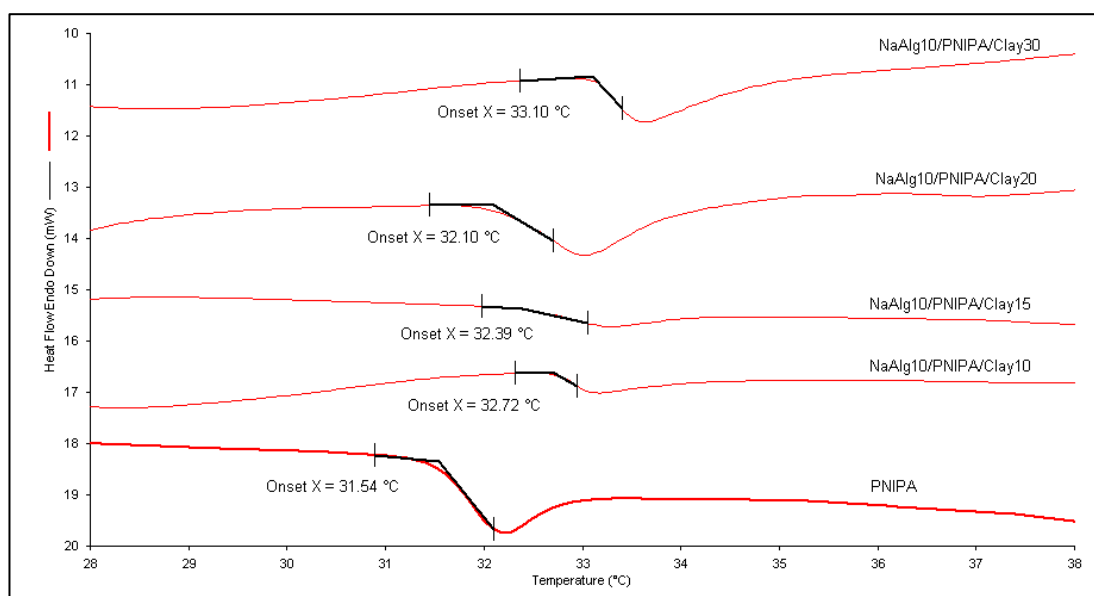


Figure 4.3 DSC thermograms of PNIPA and swollen NaAlg10/PNIPA/Clay n nanocomposite hydrogels.

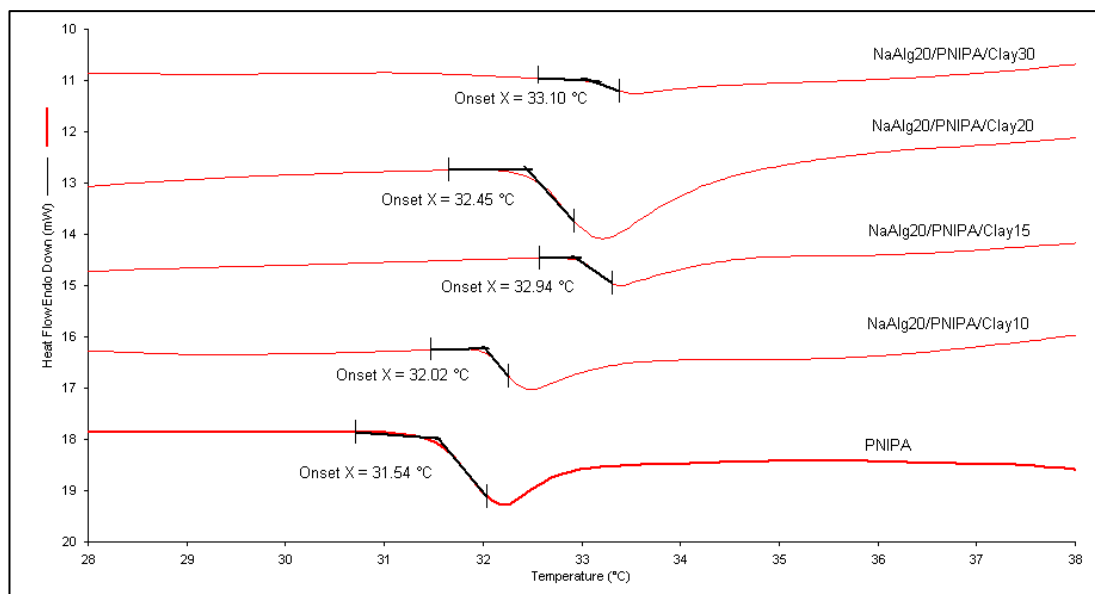


Figure 4.4 DSC thermograms of PNIPA and swollen NaAlg20/PNIPA/Clay nanocomposite hydrogels.

4.2.2 Glass Transition of the Hydrogels

As shown in Figures 4.5 and 4.6, the T_g values of the dried NaAlg/PNIPA/Clay hydrogels ranged from 138.20 to 142.87°C. The T_g values of the dried hydrogels did not change by increasing the amount of nanoclay in the hydrogels. The same observation was also made by Haraguchi and Takehisa (2002) who showed that nanoclay content did not affect T_g in PNIPA/Clay hydrogels. In contrast, when they used BIS, which is an organic crosslinker, the T_g increased with the amount of BIS in the hydrogels.

The relationship between the T_g and the NaAlg content of the hydrogels was also investigated. As shown in Figures 4.5 and 4.6, the T_g of the dried hydrogels decreased from about 142°C to 138°C with increasing the NaAlg content from 10 to 20%. This can be interpreted that additional NaAlg might act as a plasticizer, hence lowering the T_g .

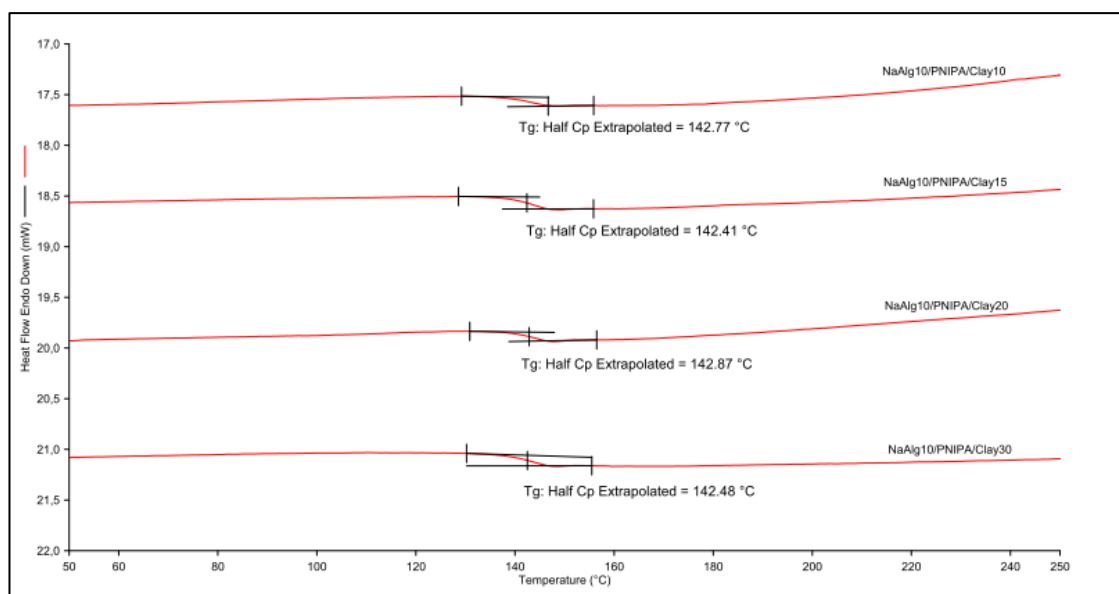


Figure 4.5 DSC thermograms of dried NaAlg10/PNIPA/Clay nanocomposite hydrogels.

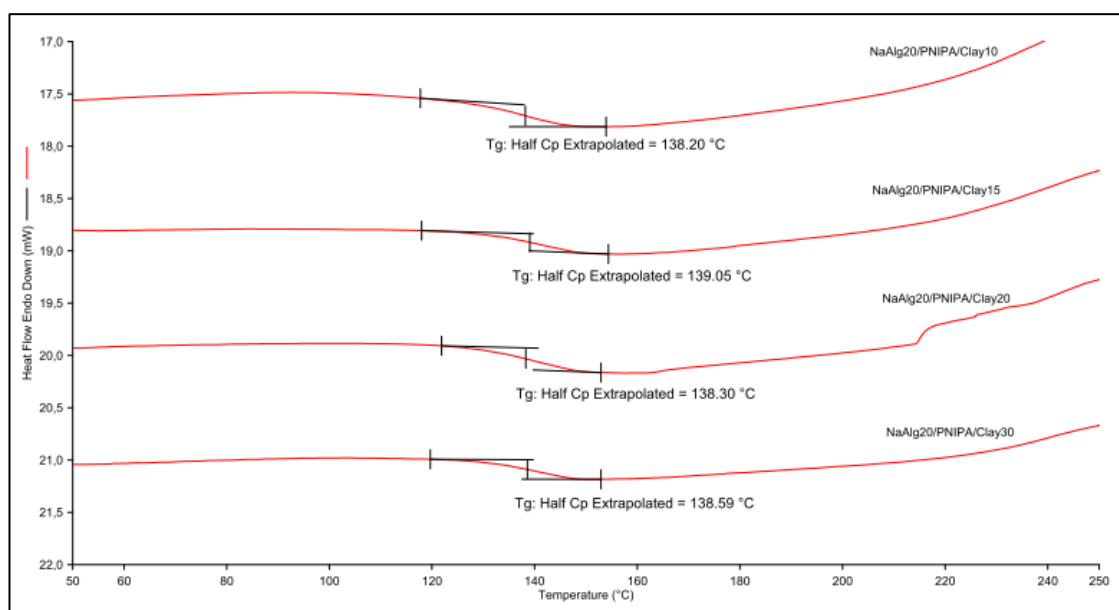


Figure 4.6 DSC thermograms of dried NaAlg20/PNIPA/Clay nanocomposite hydrogels.

4.3 FT-NIR Analysis

The molecular structure of the nanocomposite hydrogels was investigated using FT-NIR spectroscopy which measures over- and combination-tones predominantly of the fundamental vibrations that involve hydrogen [Salomonsen et al., 2008]. Figure 4.7 shows the combined FT-NIR spectra of NaAlg, Nanoclay and NaAlg/PNIPA/Clayn nanocomposite hydrogels. In the FT-NIR spectrum of NaAlg, a band between 5000 and 5200 cm^{-1} was attributed to O-H stretching vibrations. The peaks at around 4700 and 5100 cm^{-1} indicated the stretching vibrations of R-OH and R-COOH, respectively. A broad band between 6100 and 7300 cm^{-1} and the peak around 5700 cm^{-1} can be ascribed as the first overtone of -CH stretching vibration. Moreover, the band at 4290 cm^{-1} is due to combined stretching of -CH groups for NaAlg.

FT-NIR spectrum of nanoclay was examined in two main regions corresponding to water signals. The first domain between 4500 and 5500 cm^{-1} attributes to the combination of water stretching and water bending modes. The second domain around 7000-7300 cm^{-1} is corresponding to combinations and overtones of structural hydroxyl groups [Rinnert et al., 2005; Tomic et al., 2012]. The shoulder near 6848 cm^{-1} is due to the first overtone of water molecules involved in strong hydrogen bonds [Madejová et al., 2012].

The FT-NIR spectrum of NaAlg/PNIPA/Clayn hydrogel shows characteristic absorptions bands present in nanoclay and NaAlg, indicating the presence of these substances in the hydrogel network. In the spectrum of NaAlg/PNIPA/Clay hydrogel, a typical amide (CO-NH-R) peak appears at 6732 cm^{-1} which belongs to the PNIPA component in the hydrogel. In the spectra of both PNIPA and NaAlg/PNIPA/Clayn hydrogel, an intense complex band in the region of 5600-6100 cm^{-1} involves the contributions of overtones of asymmetric and symmetric stretching vibrations of CH_3 and CH_2 groups.

The peak at 5245 cm^{-1} in the nanoclay spectrum is broader compared with the peak at 5152 cm^{-1} in the spectrum of NaAlg/PNIPA/Clayn hydrogel. This indicated

that the intermolecular hydrogen bonding interactions increased among the molecules by the incorporation of nanoclay in the hydrogel structure. The peak at 7054 cm^{-1} in the spectrum of NaAlg/PNIPA/Clayn hydrogel, shifts to 7045 cm^{-1} which could be attributed to the existence of intermolecular hydrogen bonding interactions between PNIPA and nanoclay. The FT-NIR spectra of all nanocomposite hydrogels are shown in Figure 4.8 in which they exhibited very similar pattern.

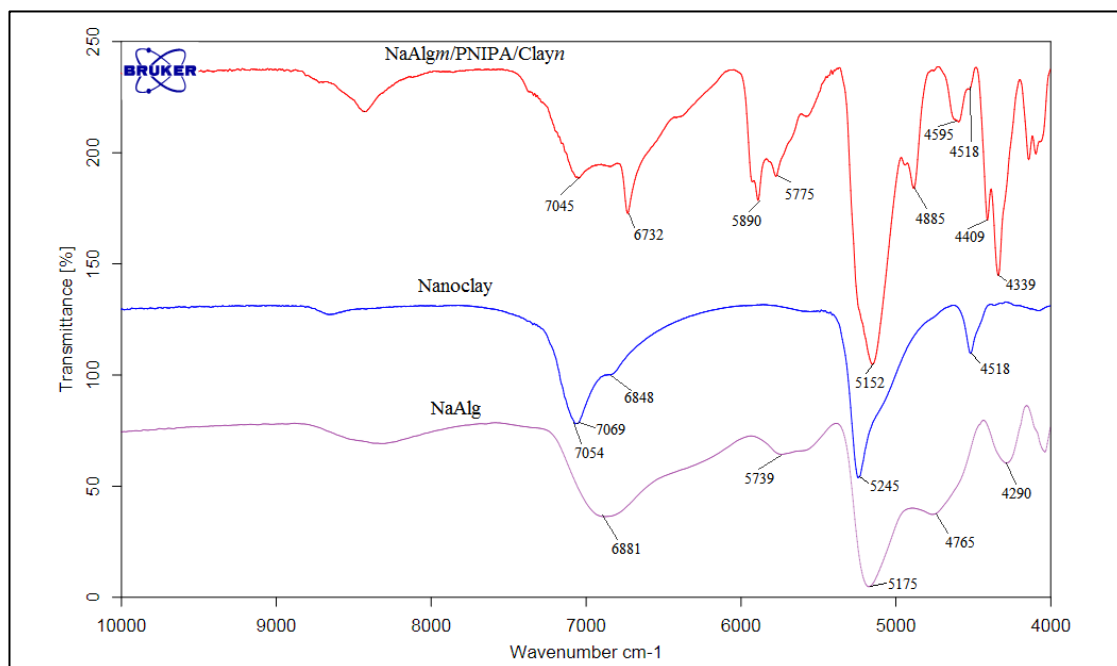


Figure 4.7 FT-NIR spectra of NaAlg, Clay and NaAlg/PNIPA/Clayn nanocomposite hydrogels.

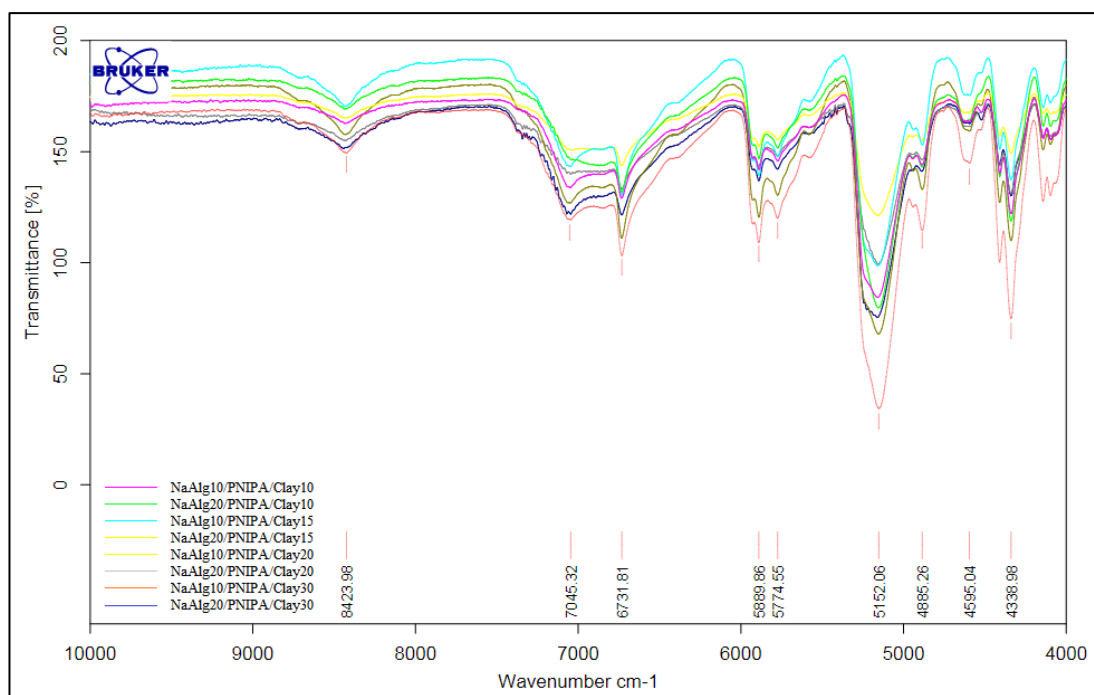


Figure 4.8 FT-NIR spectra of nanocomposite hydrogels.

4.4 XRD Analysis

The XRD patterns of both nanoclay and freeze-dried nanocomposite hydrogels are shown in Figures 4.9-4.11. The basal spacing values for all samples were calculated by Bragg's Law ($n\lambda = 2d\sin\theta$). The nanoclay pattern displayed two distinct diffraction peaks at 2θ angle of 5.98° and 19.78° , corresponding to a nanoclay interlayer spacing value of 1.48 nm and 0.45 nm, respectively (Figure 4.9).

As shown in Figures 4.10 and 4.11, the strength of the reflections of all NaAlg/PNIPA/Clay nanocomposite hydrogels tend to be weaker compared with the nanoclay itself. The reflections of the nanoclay did not shift after polymerization. According to Xu et al. (2007), this indicates that the nanoclay particles in the hydrogel composites consist of relatively large numbers of layers. They have suggested that there exist some disorder of the nanoclay sheets in the nanocomposite. These results are in agreement with the results obtained by Chen and Evans (2005). If the polymer chains were intercalated between the nanoclay sheets, the XRD peak would be shifted to a lower angle. If the nanoclay sheets were completely exfoliated in the hydrogel network, there would be no distinct diffraction peak. Based on the

XRD analysis, it could be suggested that the nanoclay sheets were in the form of intercalated and exfoliated, and dispersed randomly in the hydrogel network. Liang et al. (2000) prepared PNIPA/Clay hydrogels and found that the nanoclay minerals are both intercalated and mostly exfoliated in dried nanocomposite hydrogels based on the XRD and transmission electron microscopy (TEM) analysis. In order to get more detailed information regarding the structure of nanoclay sheets in the hydrogel network, other techniques such as TEM or small-angle X-ray scattering (SAXS) can be used.

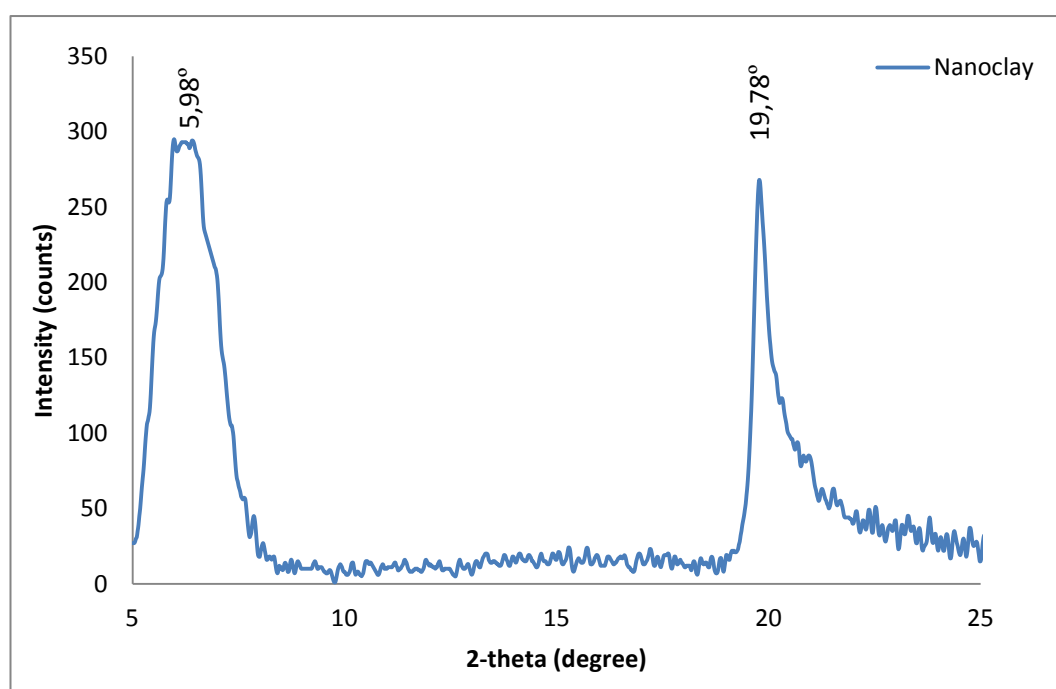


Figure 4.9 XRD profile for the nanoclay.

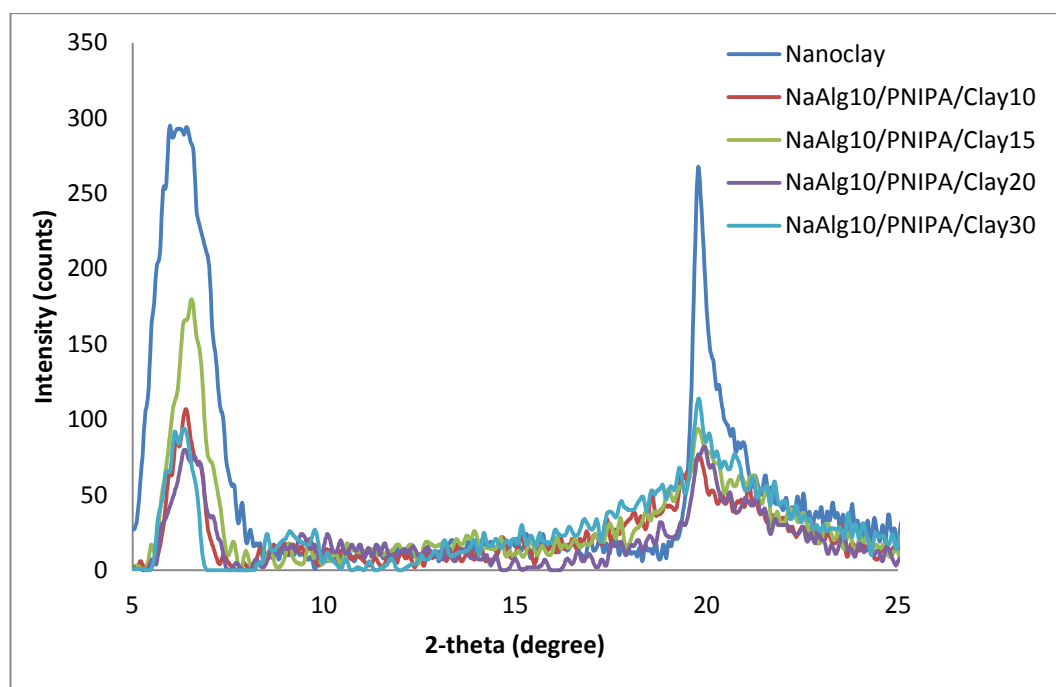


Figure 4.10 XRD profiles for nanoclay and NaAlg10/PNIPA/Clay n nanocomposite hydrogels.

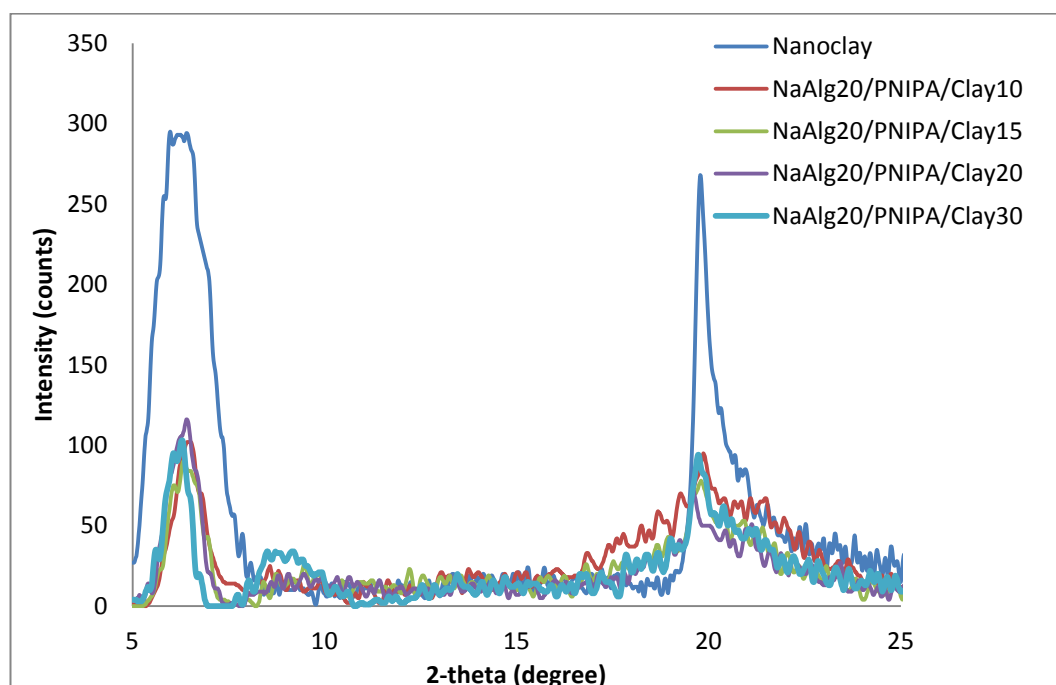


Figure 4.11 XRD profiles for nanoclay and NaAlg20/PNIPA/Clay n nanocomposite hydrogels.

4.5 Swelling and Deswelling Behavior

4.5.1 Effect of pH on Swelling

The effect of pH of the medium on the swelling ratios of the hydrogels was studied between a pH range of 1.2 and 10 (Figure 4.12)

The swelling ratio of the nanocomposite hydrogels increased with increasing the pH value from 1.2 to 8. At low pH values (below the pK_a ($\cong 3.2$) of the carboxylic groups), the $-\text{COO}^-$ groups in NaAlg are protonated to form $-\text{COOH}$ groups, and the hydrogen bonds between the $-\text{COOH}$ groups in alginate and $-\text{CONH}$ groups in PNIPA are formed [Zhang et al., 2005]. Hydrogen bonds lead to polymer-polymer interactions predominating over the polymer-water interactions which result in a decrease of swelling ratios [Zhang et al., 2005]. The lower the pH of the medium, the stronger the hydrogen bonds and thus the smaller the swelling ratios of the hydrogels. When the pH value is increased above the pK_a value of the carboxylic groups, the carboxylic groups become ionized and the electrostatic repulsion between the molecular chains is predominated which leads to the network to expand. This increases the diffusion of water molecules into the hydrogel [Ma et al., 2007; Ma et al., 2008b; Zhang et al., 2005]. Until the maximum swelling ratio is attained at a pH of 8. At pH=8, all the $-\text{COOH}$ groups were converted to $-\text{COO}^-$ groups, and this resulted in high anion-anion repulsion and high swelling capacity. Beyond pH of 8, a screening effect of counter ions, such as Na^+ , shielded the $-\text{COO}^-$ groups and prevented anion-anion repulsions. As a result, a remarkable decrease in equilibrium swelling was observed. Pourjavadi et al. (2007) was also found that alginate-g-poly(sodium acrylate)/kaolin nanocomposite hydrogels reached the maximum swelling ratio at pH = 8. Ma et al. (2007 and 2008a) reported similar results with Carboxymethylcellulose/PNIPA/Clay nanocomposite hydrogels. They achieved maximum swelling ratio at pH=5.9 due to higher pK_a ($\cong 4.6$) of carboxylic groups in carboxymethylcellulose. The swelling ratio of the hydrogels at pH=7.4 is higher than the swelling ratio at pH=1.2 because more expanding network structure is generated in the hydrogel under the non-acidic conditions.

A schematic representation of the swelling and deswelling behaviors of NaAlg/PNIPA/Clayn under the acidic and basic conditions is shown in Figure 4.13.

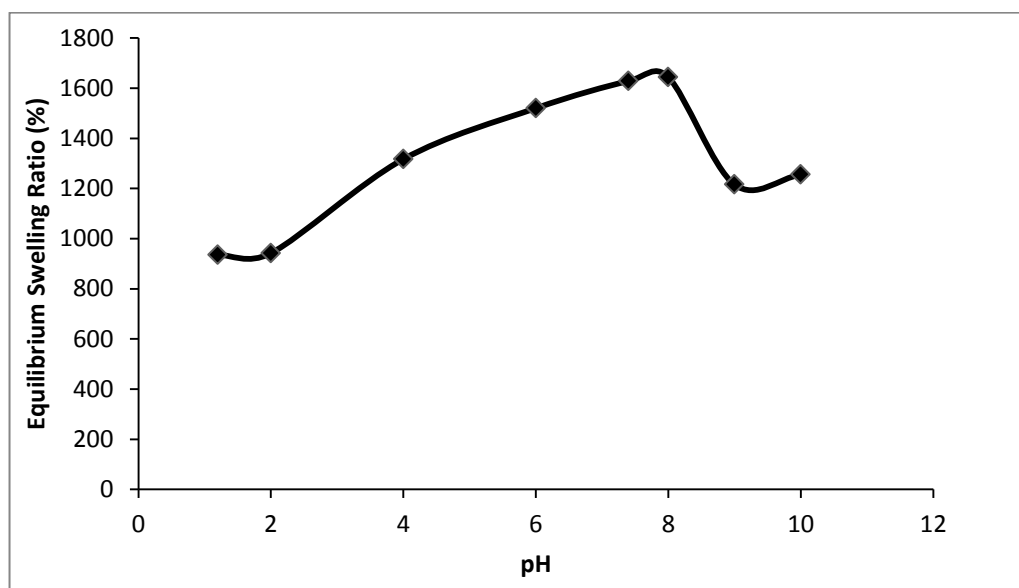


Figure 4.12 pH dependent swelling behavior of the NaAlg/PNIPA/Clayn nanocomposite hydrogels at 5°C.

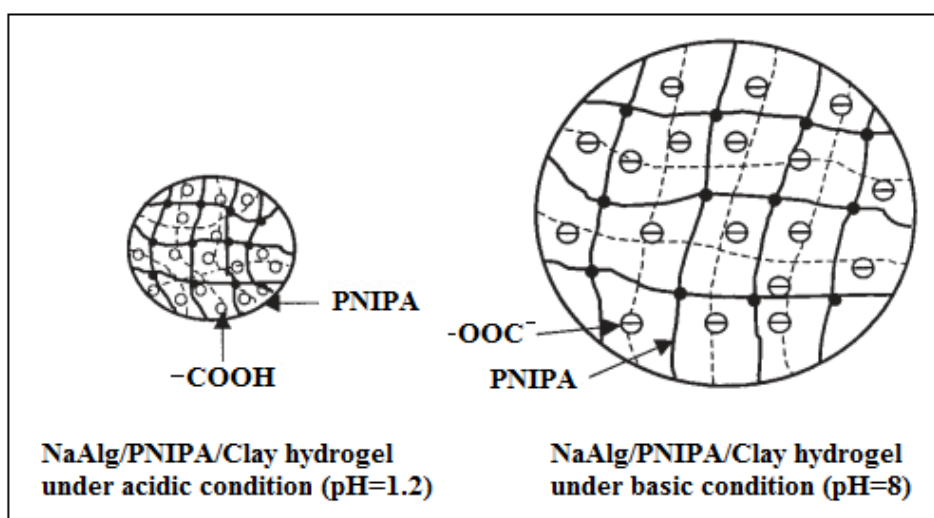


Figure 4.13 Schematic illustration of the NaAlg/PNIPA/Clay hydrogel at pH = 1.2 and pH = 8.

Swelling characteristics of the hydrogels with different nanoclay contents are shown in Figures 4.14–4.21. The swelling ratios mostly increased with increasing the pH during the period of 8h. After 8h, the hydrogels start to disintegrate due to the high water diffusion to the hydrogels. The disintegration in crosslinked chains is

higher for the hydrogels in buffer solution with a pH of 8, in which the maximum swelling is attained.

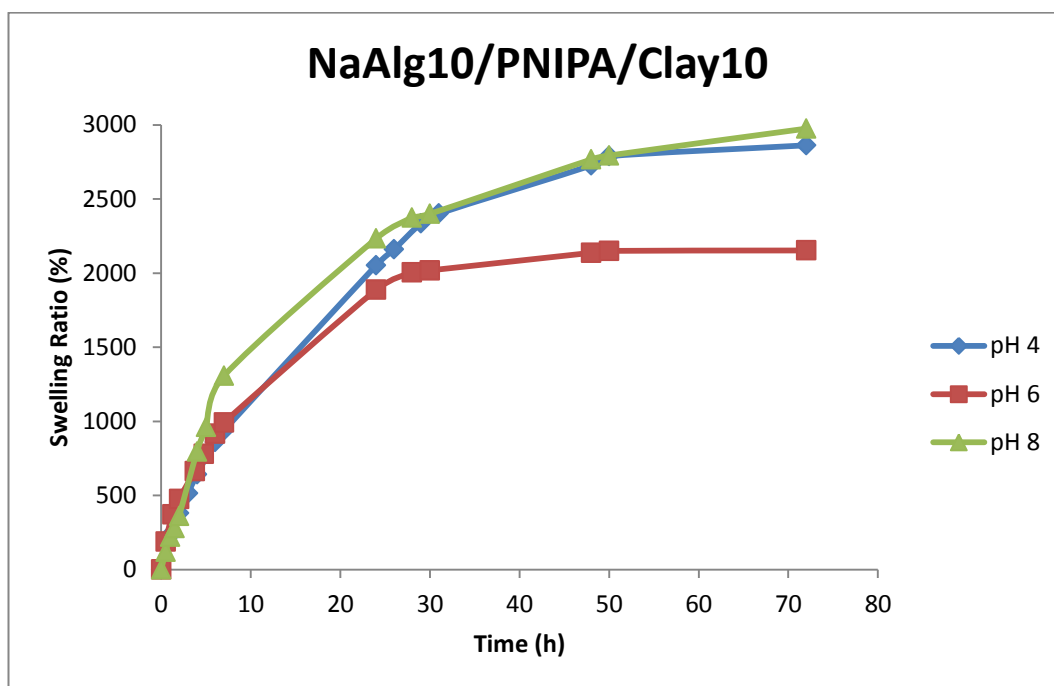


Figure 4.14 Swelling characteristics of the NaAlg10/PNIPA/Clay10 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

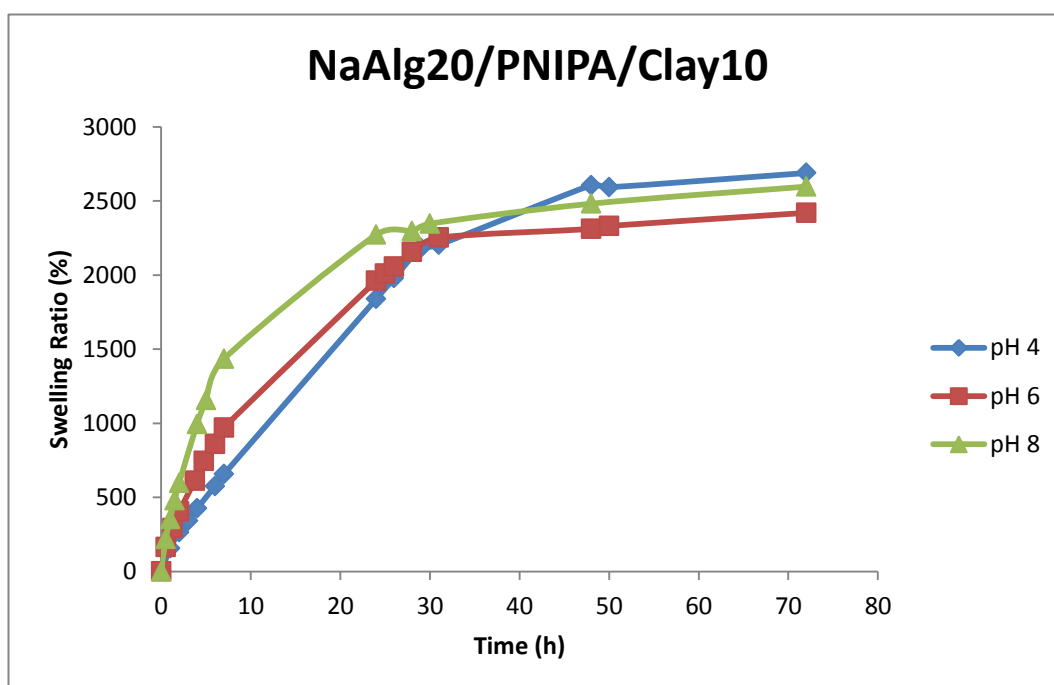


Figure 4.15 Swelling characteristics of the NaAlg20/PNIPA/Clay10 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

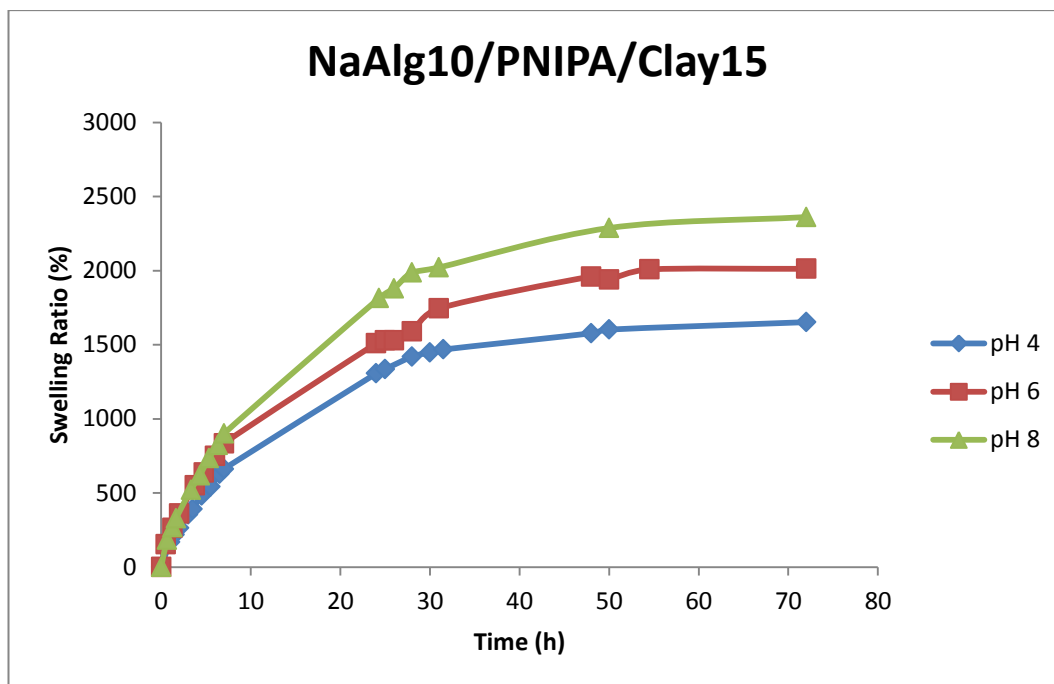


Figure 4.16 Swelling characteristics of the NaAlg10/PNIPA/Clay15 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

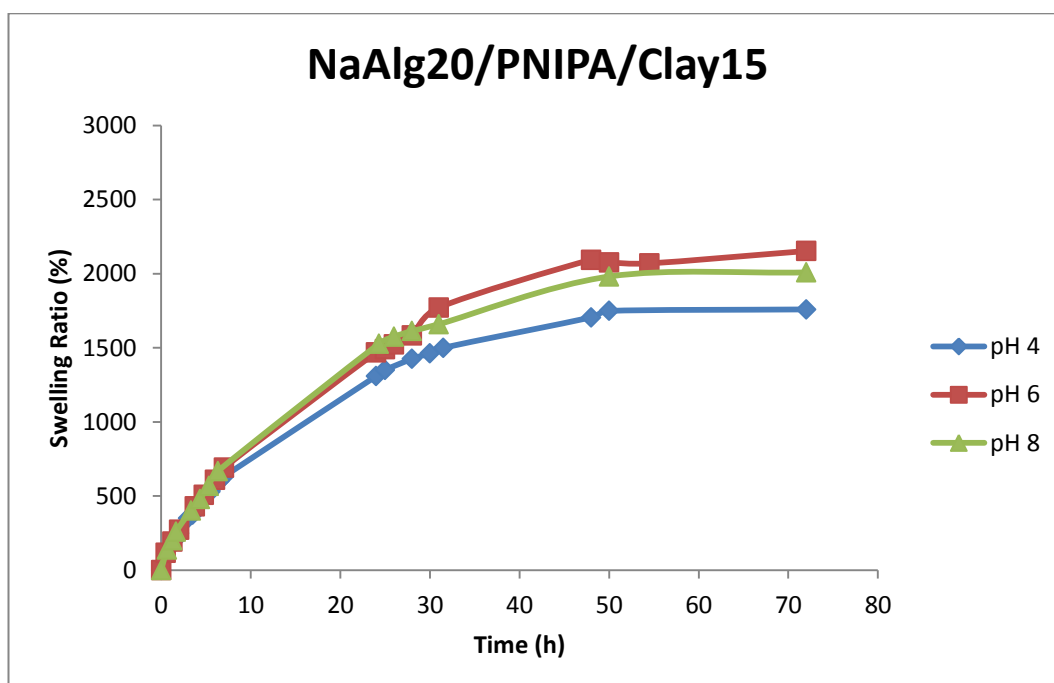


Figure 4.17 Swelling characteristics of the NaAlg20/PNIPA/Clay15 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

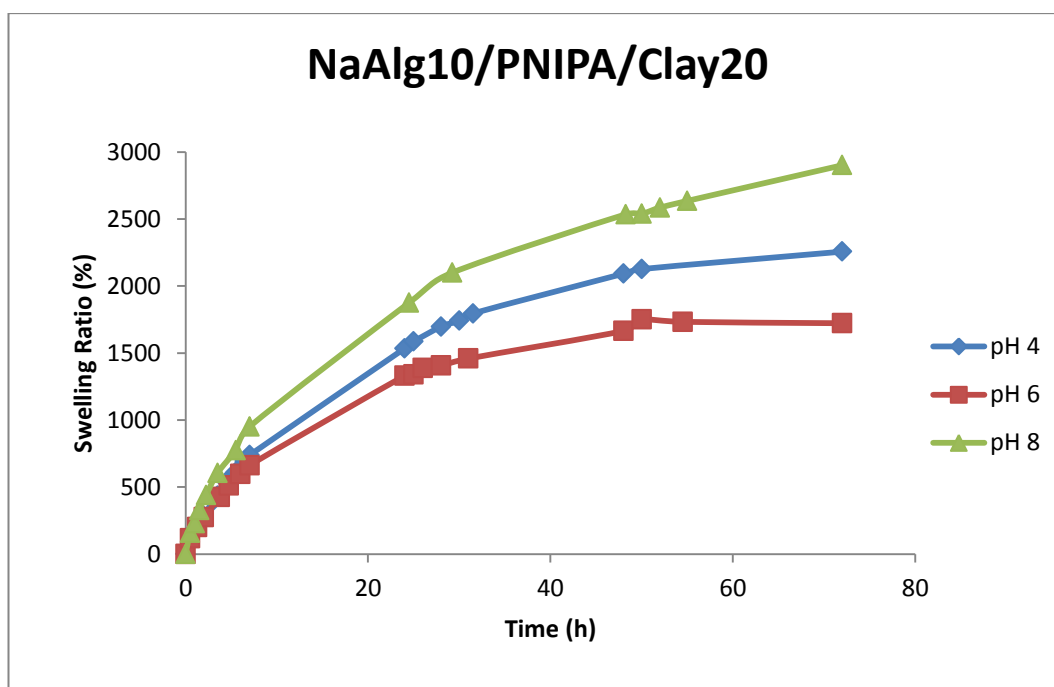


Figure 4.18 Swelling characteristics of the NaAlg10/PNIPA/Clay20 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

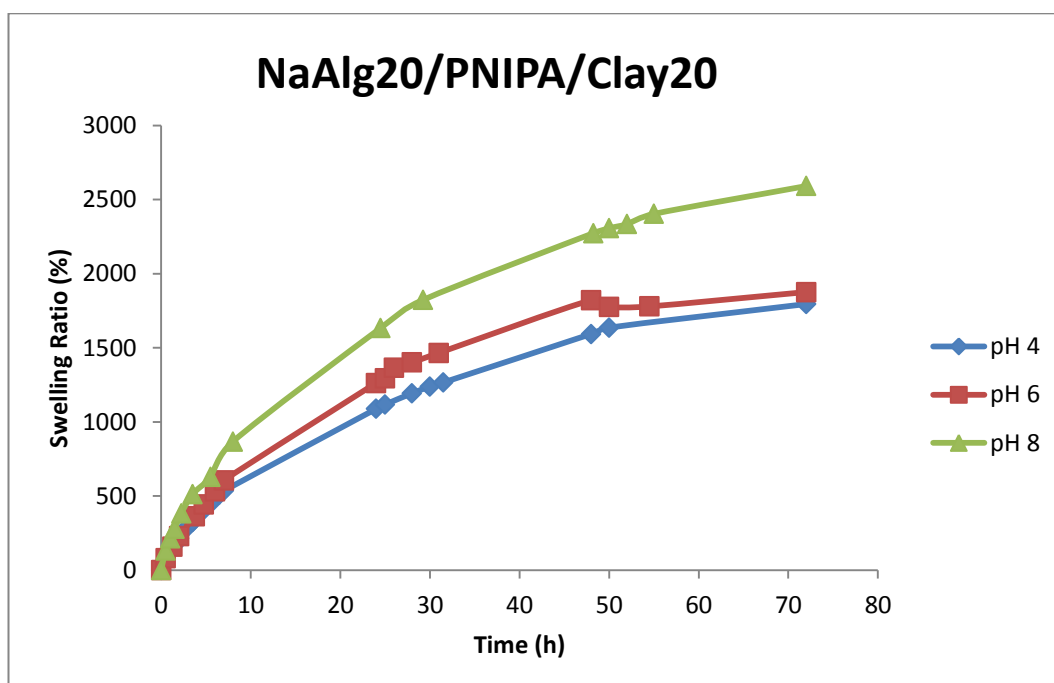


Figure 4.19 Swelling characteristics of the NaAlg20/PNIPA/Clay20 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

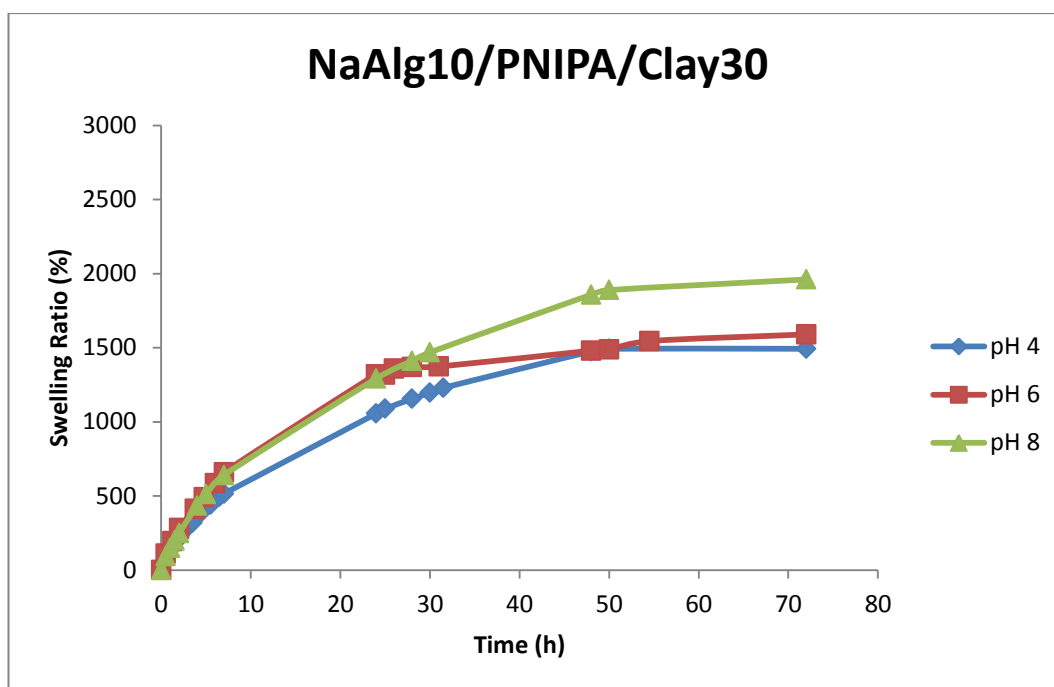


Figure 4.20 Swelling characteristics of the NaAlg10/PNIPA/Clay30 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

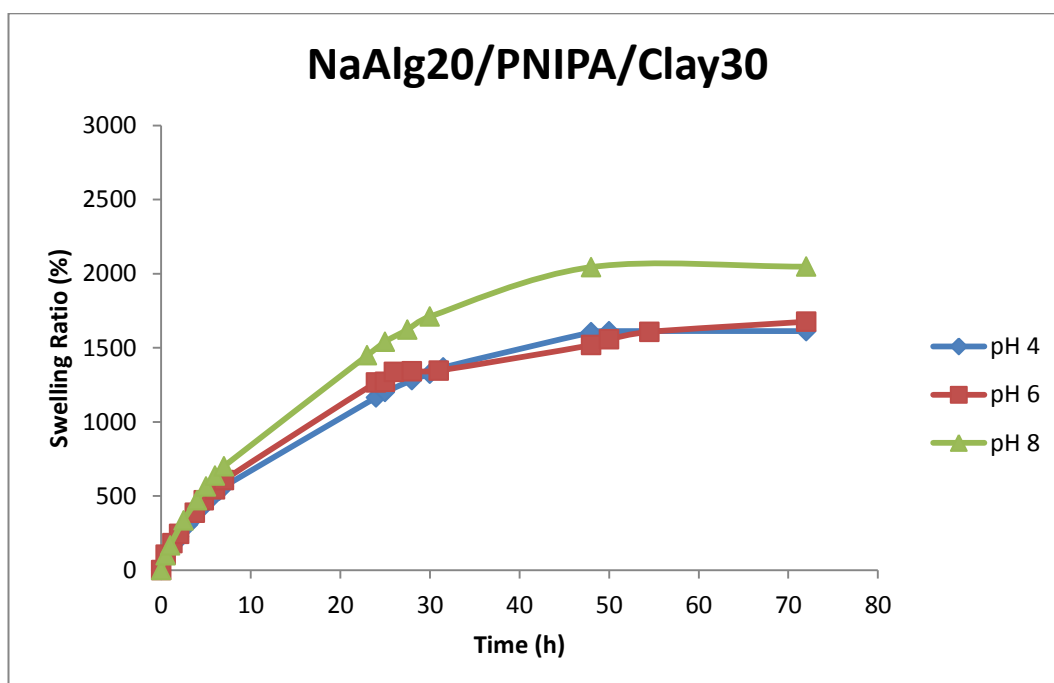


Figure 4.21 Swelling characteristics of the NaAlg20/PNIPA/Clay30 hydrogel at 5°C in buffer solutions, with pH = 4, 6 and 8.

4.5.2 Effect of Nanoclay on Swelling

The swelling ratios of the hydrogels gradually decreased with increasing the nanoclay content (Figures 4.22 and 4.23), which is due to the high crosslinking as confirmed by SEM analysis. NaAlg m /PNIPA/Clay30 hydrogels showed the lowest degree of swelling while NaAlg m /PNIPA/Clay10 hydrogels showed the highest degree of swelling. This is because the polymer chains in NaAlg m /PNIPA/Clay30 hydrogels were restricted by a large number of chemical crosslinks unlike NaAlg m /PNIPA/Clay10 hydrogels which have less crosslinking, resulting in more flexibility.

Li et al. (2009) found that the equilibrium swelling ratio of poly(acrylamide)/PNIPA/Clay hydrogels decreased as nanoclay content increased. Mahdavinia et al. (2010) also observed the same swelling behavior in Carrageenan/poly(acrylamide)/Montmorillonite Clay hydrogels. The authors claimed that high clay concentration increases the extent of crosslinking of the polymer network, resulting in less swelling.

Similar results were also reported by several researchers. Ma et al. (2008a) found that the equilibrium swelling ratio of Carboxymethylcellulose/PNIPA/Laponite Clay hydrogels in a medium at pH=1.2 and 7.4 decreased as the clay content increased from 20% to 60%. Although CMC was replaced by poly(acrylamide) in Carboxymethylcellulose/PNIPA/Laponite Clay hydrogels, the equilibrium swelling ratio also decreased with increasing the clay content [Li et al., 2009].

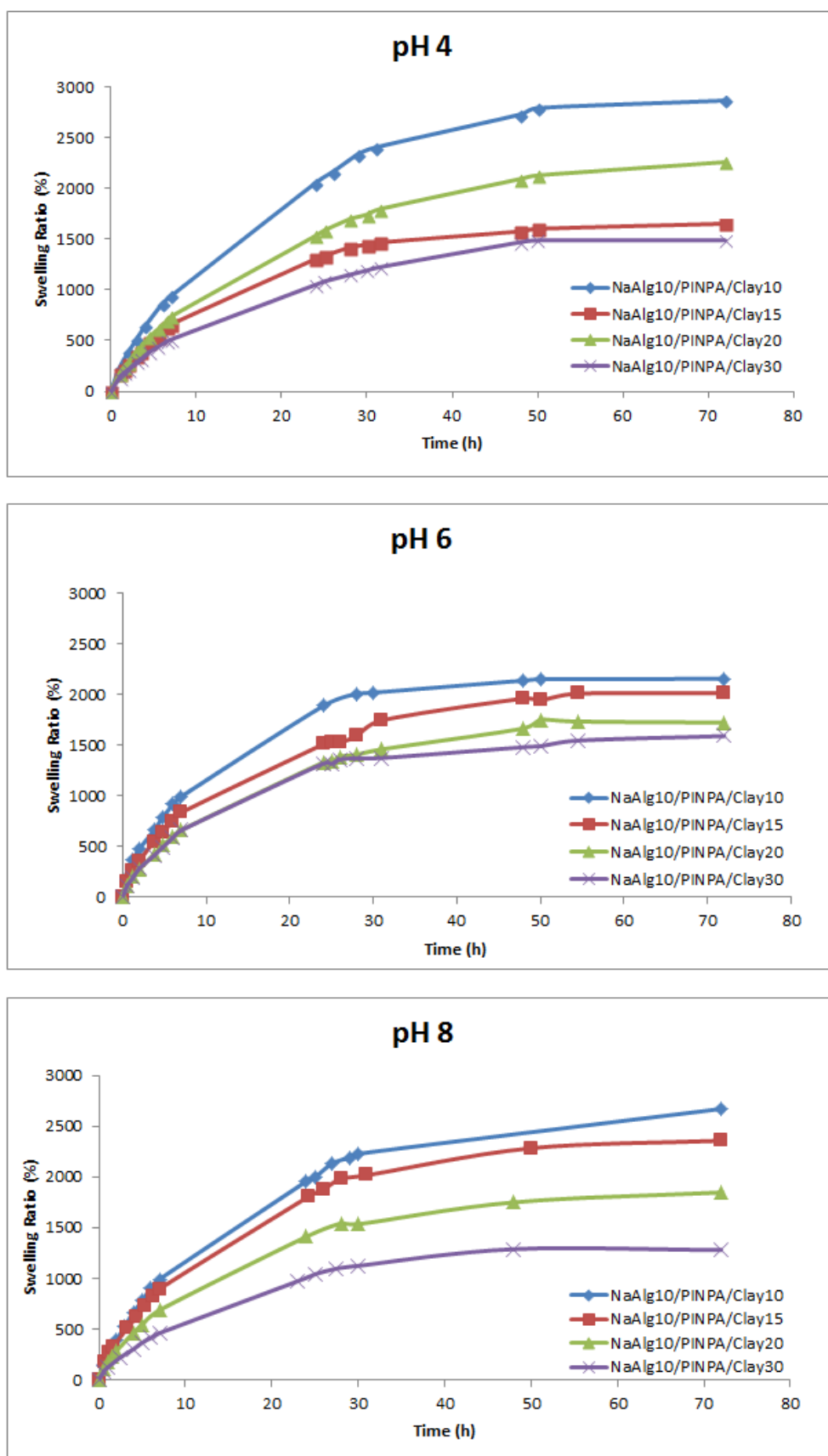


Figure 4.22 Swelling ratios of NaAlg10/PINPA/Clay n nanocomposite hydrogels at 5°C in buffer solutions with a pH of 4, 6 and 8.

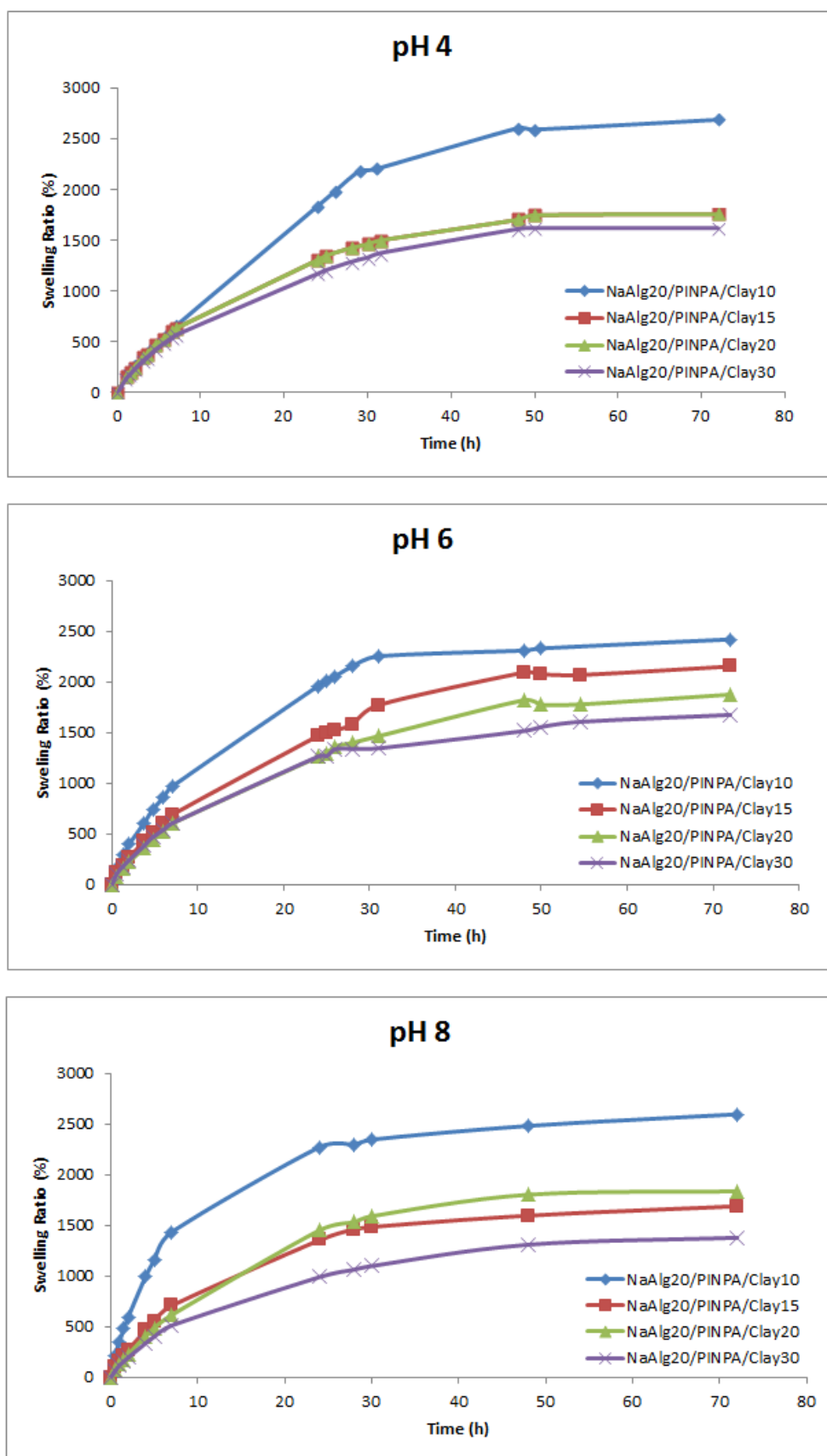


Figure 4.23 Swelling ratios of NaAlg20/PINPA/Clay n nanocomposite hydrogels at 5°C in buffer solutions with a pH of 4, 6 and 8.

4.5.3 Effect of Temperature on Deswelling

By increasing the temperature from 5°C to 37°C, the NaAlg/PNIPA/Clay nanocomposite hydrogels changed their appearances from translucent to opaque indicating that hydrogels were temperature sensitive (Figure 4.24). Virtanen et al. (2000) reported that if the temperature was increased from the temperatures below the LCST to the temperatures above the LCST, PNIPA chains collapsed and shrunk due to phase separation of PNIPA from the water. Phase separation causes a noticeable change in appearance of the PNIPA hydrogel.



Figure 4.24 The appearances of the nanocomposite hydrogel below and above the LCST.

NaAlg/PNIPA/Clay hydrogels were taken from the swelling medium at 5°C and put in a medium at 37°C, and their deswelling behaviors were investigated (Figures 4.25 and 4.26). The deswelling rates increased with increasing the nanoclay content. For example, when the nanoclay content was increased from 10% to 20%, approximately 50% more water was released within 25 h for NaAlg10/PNIPA/Clay nanocomposite hydrogels while NaAlg20/PNIPA/Clay nanocomposite hydrogels released about 30% more water.

These findings conform to the results reported by Lee and Fu (2003). They also observed that the deswelling ratio of PNIPA/Clay nanocomposite hydrogels increased as the clay content increased from 1% to 20%.

The improvement in deswelling with the increase in nanoclay content could be attributed to the formation of more porous structure within the hydrogels as confirmed by SEM studies. More nanoclay incorporation into the hydrogels led to formation of more porous structure. Large number of pores provides more water releasing channels, so the water molecules can be squeezed out of the hydrogel more easily at temperatures above the LCST [Lee and Fu, 2003; Ma et al., 2008a]. These results suggest that it is possible to design a hydrogel with a desired deswelling behavior by controlling the nanoclay content in the hydrogel.

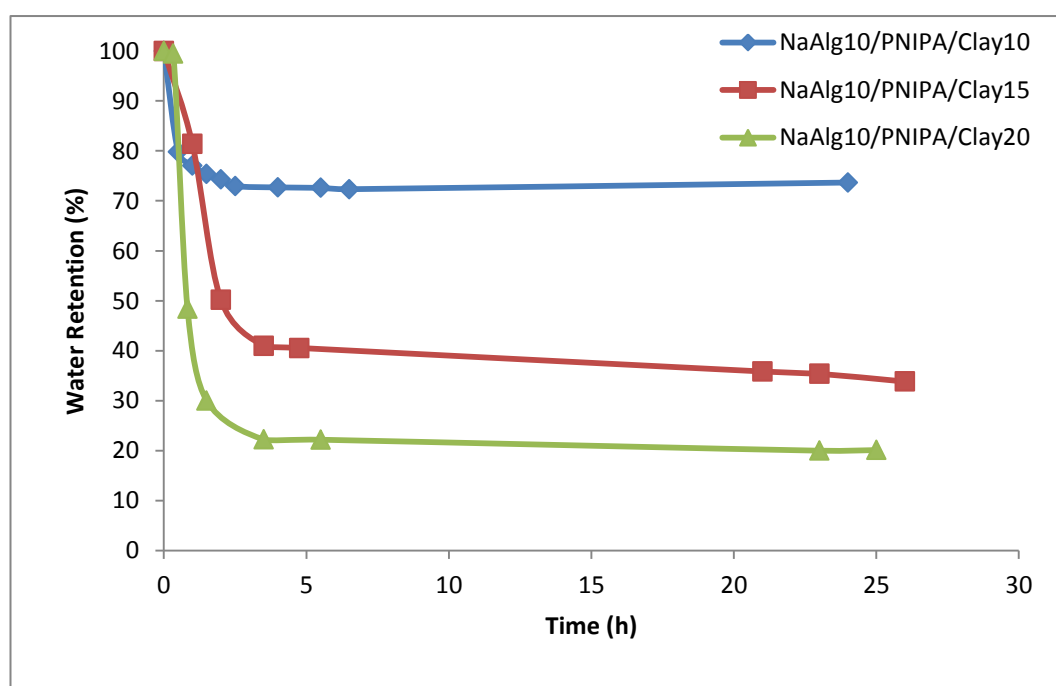


Figure 4.25 Deswelling characteristics of the NaAlg10/PNIPA/Clayn hydrogels at 37°C in buffer solution with pH=8.

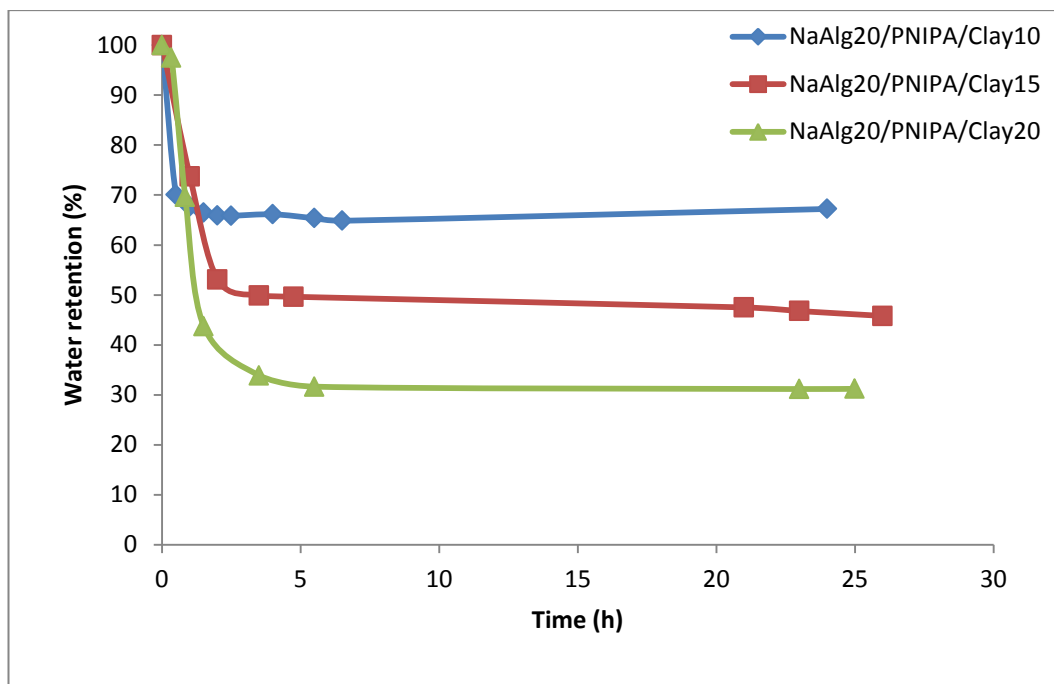


Figure 4.26 Deswelling characteristics of the NaAlg20/PNIPA/Clay n hydrogels at 37°C in buffer solution with pH=8.

4.6 Thymol Release

The release behaviors of thymol from nanocomposite hydrogels with different nanoclay contents were studied at pH=8 and 5°C (Figures 4.27 and 4.28). The release profiles of the hydrogels generally exhibited different release rates, and the released thymol amount decreased with increasing the nanoclay content. These results are in consistent with the results reported by Lee and Fu (2003) who found that the release of caffeine, neutral red and crystal violet decreased with increasing the nanoclay content in PNIPA/Clay hydrogels.

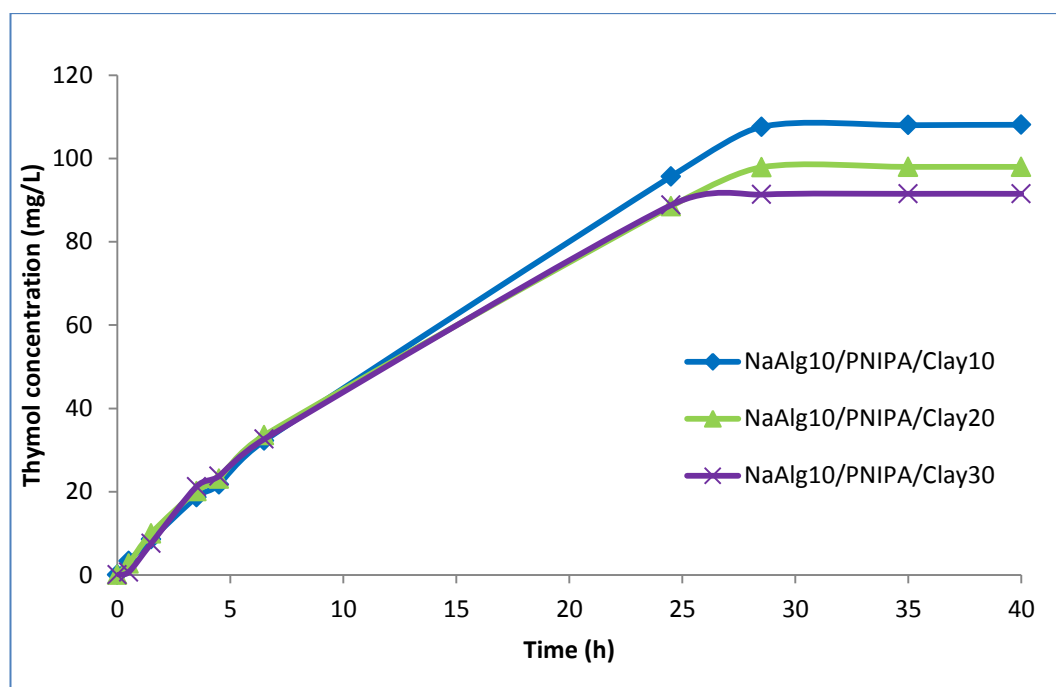


Figure 4.27 Thymol release profiles of NaAlg10/PNIPA/Clay n nanocomposite hydrogels at 5°C and pH=8.

Up to nanoclay content of 20%, the thymol release from the hydrogels containing 10 and 20% NaAlg was almost the same. When the nanoclay content was increased to 30%, the NaAlg20/PNIPA/Clay30 hydrogel released less thymol than the NaAlg10/PNIPA/Clay30 hydrogel. This suggested that the release rate of thymol can be controlled by changing the nanoclay content in the hydrogels.

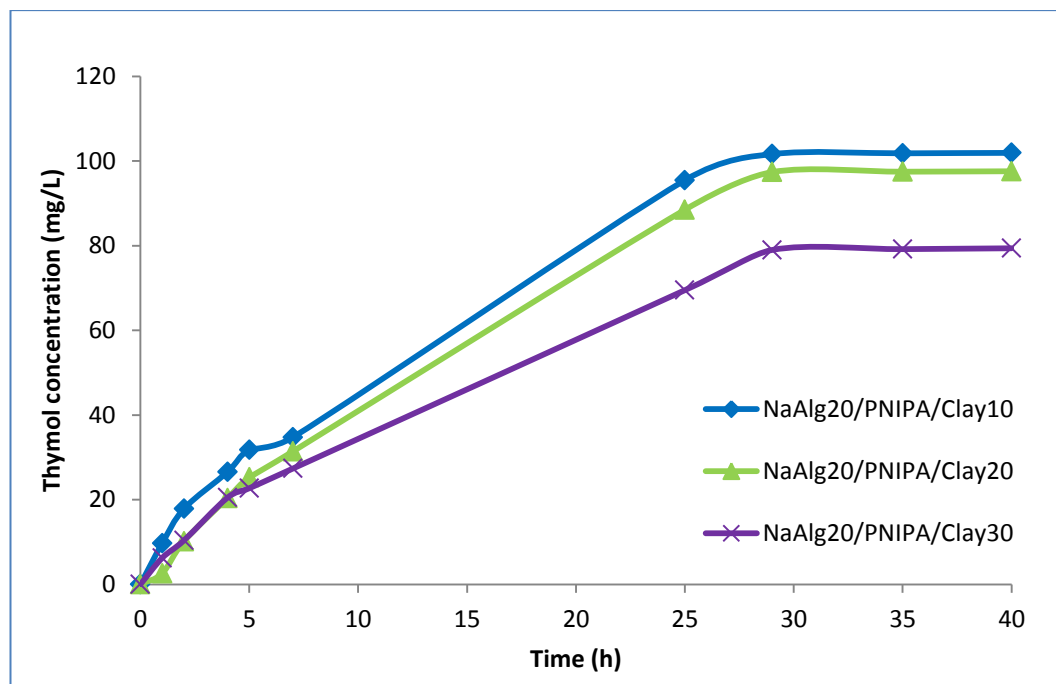


Figure 4.28 Thymol release profiles of NaAlg20/PNIPA/Clay n nanocomposite hydrogels at pH = 8 and 5°C.

Some hydrogel systems in the bead form [Deng et al., 2010; Shi et al., 2006] or containing large molecular weight drugs [Li et al., 2009] have been reported to show an initial burst release time. The burst release is a non-steady state and high release of materials that is mostly encountered at the beginning of release [Hasan et al., 2007; Huang and Brazel, 2003]. The burst release is one of the major problems encountered in the drug delivery systems. The lack of prolonged release would severely limit the applicability of a system for drug delivery. The sudden release of the drug can be pharmacologically dangerous and economically insufficient [Huang and Brazel, 2001]. NaAlg m /PNIPA/Clay n nanocomposite hydrogels did not exhibit a burst release time. It is thought that thymol penetrated into the hydrogel network because it has a low molecular weight and thus prevented the burst release. This result clearly showed that NaAlg m /PINPA/Clay n hydrogels could be used in pharmaceutical industry as the drug delivery system and in food industry as the antimicrobial releasing system.

5 CONCLUSION

The nanoclay sheets acted as a crosslinker in the hydrogels. SEM pictures of the nanocomposite hydrogels revealed a highly porous structure. The pore sizes decreased and the amount of pores increased with increasing the nanoclay content in hydrogels.

The NaAlg/PNIPA/Clayn nanocomposite hydrogels exhibited the same LCST around 33°C indicating that there is no chemical bond between NaAlg and PNIPA network. The hydrogels can be considered as semi-IPN systems. The T_g of the dried hydrogels decreased with increasing the NaAlg content. Additional NaAlg acted as a plasticizer.

FT-NIR spectrum of NaAlg/PNIPA/Clayn hydrogels showed the characteristic absorptions of nanoclay, NaAlg and PNIPA, indicating the presence of these substances in the hydrogel network. The XRD profiles of nanocomposite hydrogels revealed that nanoclay layers are both intercalated and exfoliated.

The results of the influence of pH value on swelling behaviors showed that the maximum swelling attained at the pH of 8. The swelling ratios mostly increased with increasing the pH during the period of 8 h. The deswelling rates increased with increasing the nanoclay content at 37°C due to the increase in water releasing channels. The swelling ratios of the hydrogels gradually decreased with increasing the nanoclay content, which is due to the high crosslinking.

The thymol release studies indicated that the release rate of thymol can be controlled by changing the nanoclay content in the hydrogels. These results suggest that the NaAlg/PNIPA/Clayn nanocomposite hydrogels have a great potential to be used as effective pH/temperature responsive drug delivery systems in pharmaceutical industry and as antimicrobial releasing systems in active packaging applications in the food industry.

6 FUTURE WORK AND RECOMMENDATIONS

Over the last 30 years, hydrogels have become popular carriers for drug delivery applications. But, the high water content and large pore sizes of the most hydrogels often result in relatively rapid drug release, over a few hours to rarely a few days [Simões et al., 2012]. Recently, there has been an increased interest in using nanomaterials to develop new systems in the pharmaceutical and food industries.

Although it has been shown that NaAlg μ /PNIPA/Clay μ n hydrogels are pH and temperature responsive, and they can be used as drug/antimicrobial delivery systems, some future work is required to obtain more detailed information on these systems. The amount of NaAlg and nanoclay in the hydrogels could be increased to obtain hydrogels with more superior properties. Proper design schemes and optimization techniques can be used to optimize hydrogel characteristics with respect to final use of the hydrogel. The more detailed release profiles could provide practical information to researchers, and drug and packaging manufacturers to design controlled release systems with improved properties. Therefore, drug loading at different concentrations could be prepared to investigate release profiles more thoroughly. Release kinetic studies at different temperatures and pH values should also be done to determine the drug release rates with analytical methods which provide more precise information on release rates and contribute to the release certainly and development of more sophisticated delivery systems. In addition, the effectiveness of the thymol loaded hydrogels in controlling bacterial spoilage could be investigated in agar plates or in real foods.

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