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**PREPARATION, CHARACTERIZATION AND *IN-VITRO*  
EVALUATION OF CHITOSAN-BASED SMART  
HYDROGELS FOR CONTROLLED DRUG RELEASE**

A thesis presented in partial fulfillment of  
the requirements for the degree of

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## *Abstract*

Controlled drug release enhances the safety, efficacy and reliability of drug therapy. Regulation of the drug release rate results in a reduction in the frequency of drug administration and should encourage patients to comply with dosing instructions.

Hydrogels are crosslinked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids. The number of polymers suitable for the controlled release of viable therapeutics is quite limited because of inherent toxicity or lack of certain properties such as biodegradability. In this thesis, chitosan was chosen as the base polymer for the development of new hydrogels that can be tailored for use in the site-specific delivery of drugs to the gastrointestinal tract. Chitosan is a non-toxic and biodegradable polymer obtained through the alkaline deacetylation of natural chitin. The interesting characteristics of chitosan make it an ideal candidate for use in controlled drug release formulations. However, chitosan exhibits some shortcomings such as hydrophobicity and a high pH-dependency for its physical properties. Hence, it is very difficult to control drug release with chitosan itself because of the various pH values of the internal organs of the human body. This may negatively affect the human body because of drug under- or over-release.

In a structured programme, some new chitosan-based hydrogels have been prepared for controlled drug release investigations by applying three main approaches to overcome the shortcomings of chitosan. The first approach was the incorporation of chitosan into interpenetrating polymer network hydrogels with either a hydrophilic polymer or with hydrophilic monomers treated to bring about in situ copolymerization in the presence of chitosan and a suitable crosslinking agent. The second approach was the chemical modification of chitosan by grafting of a suitable vinyl macromer such as poly(ethylene glycol)-diacrylate, then crosslinking this modified chitosan. The equilibrium swelling studies were carried out for the hydrogels prepared using these two approaches at 37 °C at pH 2.1 (simulated gastric fluid, *SGF*) and at pH 7.4 (simulated intestinal fluid, *SIF*). The swelling results showed a pH-responsive nature of these hydrogels. They attained higher swelling values in *SGF* than in *SIF*. 5-Fluorouracil (*5-FU*), an anti cancer drug,

was entrapped as a model drug in all the hydrogels prepared using these two approaches. The *in-vitro* drug release studies were carried out at 37 °C in *SGF* and *SIF*. From the preliminary investigations of the prepared hydrogels, they may be customized and used to expand the utilization of these systems in drug delivery applications.

In the third approach, chitosan was modified in such a fashion that the hydrogels produced were also pH-responsive but attained limited swelling in *SGF* and higher swelling in *SIF*. Hence, the resulting hydrogels could be tailored for utilization for intestine-targeted delivery of peptide and protein drugs with a potential protection of the drugs from the harsh acidity of the stomach. In this third approach the ionotropic gelation was used for the preparation of the hydrogels based on the modified chitosan with another natural polymer (sodium alginate) in the presence of a divalent ion. Bovine serum albumin (*BSA*) was entrapped as a model protein drug and the *in-vitro* drug release profiles were established at 37 °C in *SGF* and *SIF*. The results showed promising release profiles of *BSA*. However, this hydrogel study requires more effort to limit the swelling and consequently the loss of drug in the *SGF*, to act as an excellent candidate for intestine-specific delivery of peptide and protein drugs.

*Dedicated to Amal (my wife)  
and the spirit of my Dad*

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## List of Abbreviations

AA	Acrylic acid
AO	Amine oxide
APS	Ammonium persulfate
BIS	Methylene bisacrylamide
BMA	Butyl methacrylate
BSA	Bovine serum albumin
Ch	Chitosan
CMCh	Carboxymethyl chitosan
CAN	Ceric ammonium nitrate
Da	Dalton
2D-XRD	Two dimensional X-ray diffraction
DSC	Differential scanning calorimetry
EG	Ethylene glycol
EO	Ethylene oxide
E	Ethylene
FTIR	Fourier transform infrared
5-FU	5-Fluorouracil
-g-	Grafted copolymer
HPLC	High performance liquid chromatography
IPN	Interpenetrating polymer network
MAA	Methacrylic acid
MVE	Methyl vinyl ether
MMA	Methyl methacrylate
Mw	Average molecular weight
MPa	Mega Pascal
NIPA	N-isopropyl acrylamide
NVP	N-vinyl pyrrolidone
NAGly	N-acryloylglycine
<sup>1</sup> H-NMR	<sup>1</sup> H-Nuclear magnetic resonance
<sup>13</sup> C-NMR	<sup>13</sup> C-Nuclear magnetic resonance
PHEMA	Poly(2-hydroxyethyl methacrylate)

PEG	Poly(ethylene glycol)
PHB	Poly(hydroxy butyrate)
PBCPP	Poly(bis-carboxy-phenoxy-phosphazene)
PET	Poly(ethylene terephthalate)
PVA	Poly(vinyl alcohol)
PEO	Poly(ethylene oxide)
PAAm	Poly(acrylamide)
P(.....)	Poly(.....)
PAA	Poly(acrylic acid)
PMAA	Poly(methacrylic acid)
PNVP	Poly(N-vinyl pyrrolidone)
P(NIPA)	Poly(N-isopropyl acrylamide)
PDEAEMA	Poly(diethyl-aminoethyl methacrylate)
PAS	Positron annihilation lifetime spectroscopy
PEGDA	Poly(ethylene glycol diacrylate)
PEt	Polyether
Semi-IPN	Semi-interpenetrating polymer network
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SEM	Scanning electron microscope
SD	Standard deviation
TPP	Sodium tripolyphosphate
VAc	Vinyl acetate
VA	Vinyl alcohol
$\eta_r$	Relative viscosity
$\eta_{sp}$	Specific viscosity
$\eta_{red}$	Reduced viscosity
$[\eta]$	Intrinsic viscosity
$p$	Probability

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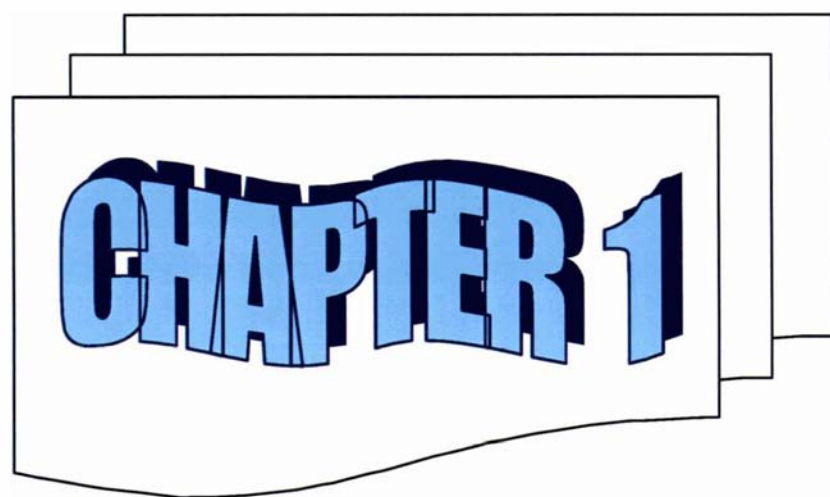
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**CHAPTER 1**

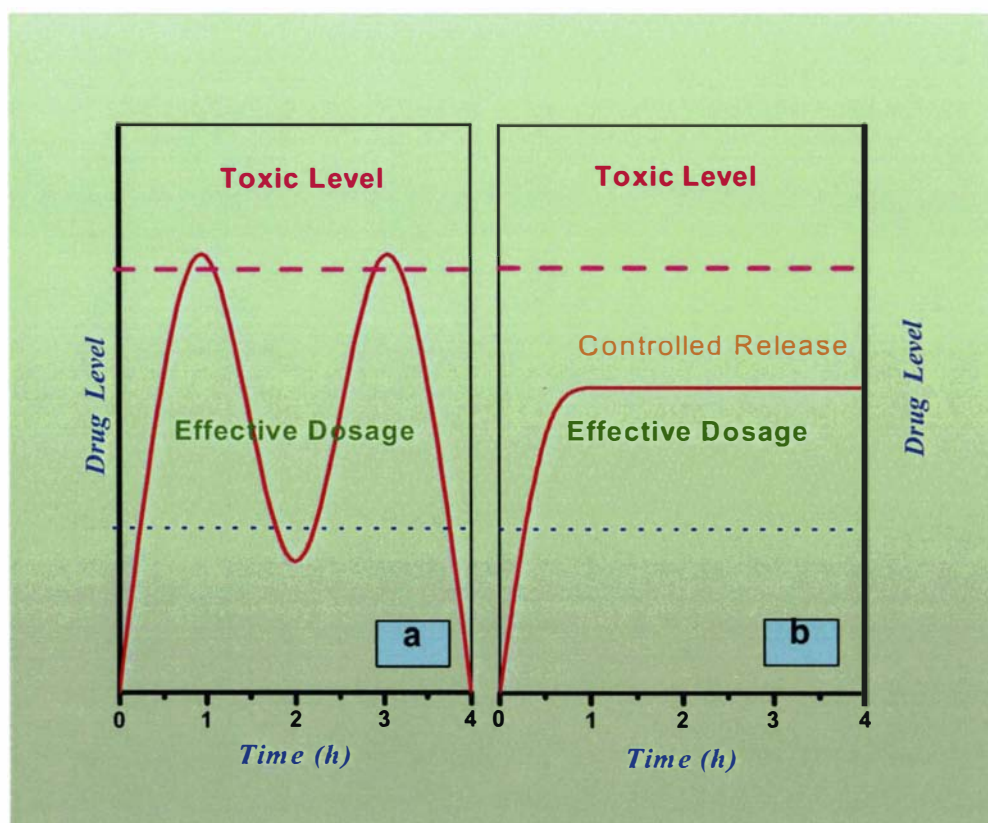
## CHAPTER 1

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### 1. INTRODUCTION

#### 1.1. Controlled drug release technology

In the last few decades, the ways of administering drugs have gained increasing attention. Development of administration methods that allow the patients to safely treat themselves is as important as any other health care development, particularly in poor countries where doctors, clean syringes, sterile needles, and sophisticated treatments are rare [1]. Conventional drug dosage forms, including pills, tablets, capsules, suppositories, injections, ointments, creams and aerosols, provide an instantaneous release of drug in a bolus form. For drugs which get cleared rapidly from the body, achieving and maintaining the drug concentration within the therapeutically effective range requires a multiple dosing treatment, often more than once a day. Such an inconvenient dosing treatment results in a deficiency in the patient compliance with dosing instructions. With conventional dosing formulations, the concentration of the released drug is initially high, peaks and then declines sharply below the minimum therapeutic level [1,2]. Consequently, for drugs whose actions correlate with their concentration in the serum, these fluctuations in the drug levels often cause unacceptable side effects at the peaks, followed by insufficient therapy in the troughs (**Figure 1.1a**). Moreover, conventional dosage forms, which are still predominant for the pharmaceutical products, are not able to control either the rate of drug delivery or the target area of drug administration. In contrast, regulating the rates of drug release with aid of controlled drug release formulations offers numerous advantages over conventional dosage forms (**Figure 1.1b**). Some of the main advantages [3] of controlled release forms are described below and in **Figure 1.2**:



**Figure 1.1.** Graphical representations showing the typical pharmacokinetic profiles in case of (a) conventional and (b) controlled drug release formulations [2].

..... Minimum therapeutic level

■ ■ Toxic level

(1) Controlled release of drugs results in a reduction in the frequency of the drug administration and should encourage patients to comply with the dosing instructions.

(2) The drug is released in a controlled fashion that is most appropriate for the application. The control could be in terms of the onset of release (delayed vs. immediate), the duration of release, and the release profile itself.

(3) The drug could be released in a targeted region. This can be carried out either by timed release of the drug or by tailoring the carrier matrix to release the drug in that desired site. This targeting of the drug release could maximize the drug efficacy.

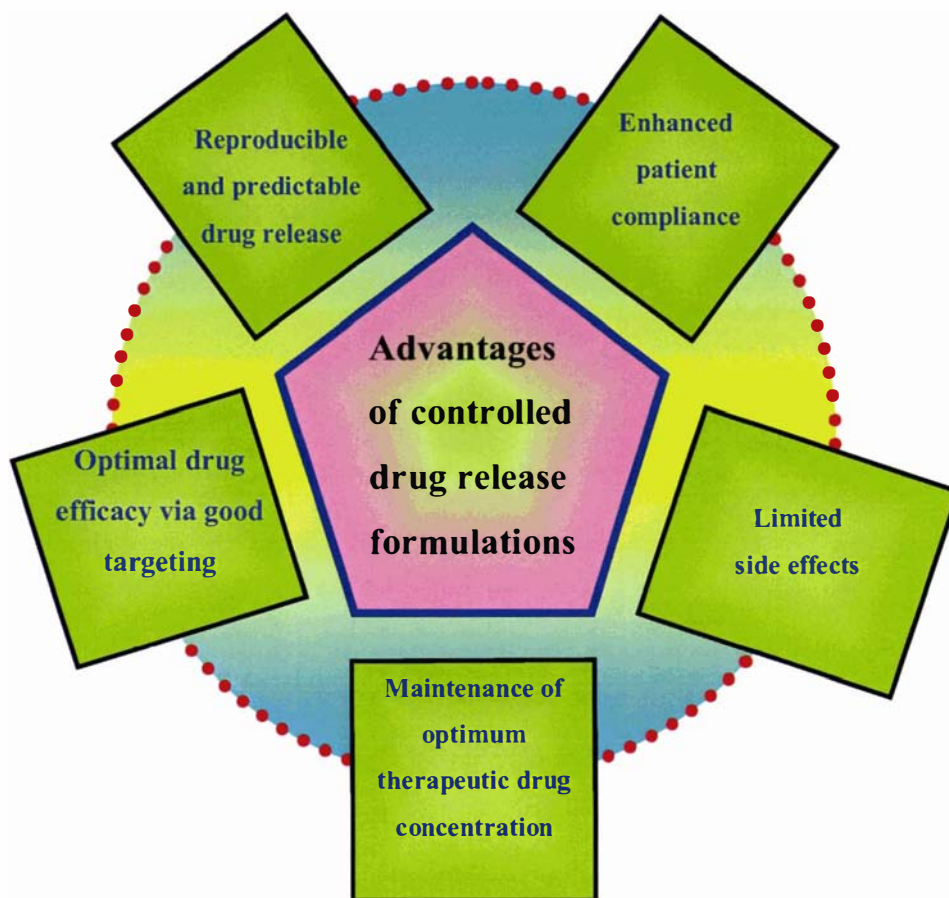
(4) Systemic exposure of the drug could be reduced upon its targeting to the desired environment. This results in decreasing the systemic side effects of the drugs (especially for toxic drugs).

(5) The controlled release formulations enable the drug to be protected from the physiological environment for longer durations. Consequently, the effective residence time of the drug should be extended.

However, the cost of controlled drug release formulations may be considerably higher than the conventional forms. Also, controlled drug release formulations exhibit some limitations. These limitations will be prominent in the following circumstances [4]:

- (1) Delayed onset
- (2) Increased potential for first-pass metabolism
- (3) Less accurate dose adjustment
- (4) Dependence on residence time in gastrointestinal (GI) tract

These shortcomings of controlled drug release devices may make some drugs unsuitable for such formulations [5]. However, controlled drug release technology may be beneficial in many cases.



**Figure 1.2.** Some of the main advantages of controlled drug release technology.

## **1.2. Hydrogels and their applications in controlled drug release**

Hydrogels are three-dimensional networks composed of hydrophilic polymers crosslinked through covalent bonds or held together via physical intermolecular attractions. Hydrogels can absorb large amounts of water or biological fluids, from 20% up to several thousand %, and swell readily without dissolving. The high hydrophilicity of the hydrogels is mainly due to the presence of a number of hydrophilic moieties such as amino, carboxyl, amide and hydroxyl groups distributed along the backbone of the polymeric strands. In the swollen state the hydrogels are soft and have a rubbery

structure, resembling to a great extent the living tissues. Some hydrogels, such as alginate-based gels, also offer excellent biocompatibility [6,7].

The appearance of synthetic hydrogels dates back more than forty years, when Wichterle et al (1955-1960) [8] prepared and investigated a hydrogel based on poly(2-hydroxyethyl methacrylate) (*PHEMA*) for contact lens applications. Since then, the research in the area of the hydrogels has expanded dramatically particularly in the last decade. Also the uses of the hydrogels have extended to cover various applications including drug delivery, wound dressing, ophthalmic materials and tissue engineering [9,10].

Hydrogels usually attain equilibrium swelling when a balance occurs between the osmotic driving forces, which encourage the entrance of water or biological fluids into the hydrophilic polymer, and the cohesive forces exerted by the polymer strands. These cohesive forces tend to resist the hydrogel expansion and the magnitude of these forces depends mainly on the crosslinking density [11,12]. In general, the more hydrophilic the polymer forming the hydrogel, the higher the total water absorbed by the hydrogel. Whereas, the higher the crosslinking extent of a particular hydrogel, the lower the extent of the gel hydration. The hydrogels in their dried forms are usually referred to as *xerogels*. When some drying techniques, such as freeze-drying or drying using solvent extraction are applied, the resulting hydrogels are extremely porous. These porous dried hydrogels are called *aerogels* [11]. Different techniques have been applied in the literature to characterize the hydrogels and to investigate their parameters. **Table 1.1** shows some examples of these techniques.

**Table 1.1.** Some examples of the techniques used for characterization of hydrogels.

<i>Parameter</i>	<i>Measurement techniques</i>	<i>Ref.</i>
Crosslinking extent	☐ <i>The change in polymer solubility with time</i>	[13,14]
	☐ <i>Ultimate compressive strength</i>	
Network pore size	☐ <i>Scanning electron microscopy</i>	[15]
	☐ <i>Quasi-elastic laser-light scattering</i>	
	☐ <i>Mercury porosimetry</i>	
	☐ <i>Rubber elasticity measurements</i>	
Degree of swelling	☐ <i>Equilibrium swelling measurements</i>	[16,17,18,19]
	☐ <i>Dimensional changes with time</i>	
	☐ <i>Equilibrium water content</i>	
Mechanical strength	☐ <i>Ultimate compressive strength</i>	[13,14,20]

### 1.2.1. Hydrogels as compared to gels

One of the common misconceptions in polymer science is the use of the concept “gel” instead of “hydrogel” and vice versa. The gels are semi-solid materials made of hydrophilic polymeric systems comprising small amounts of solids, dispersed in relatively large amounts of liquid. However, gels may appear more solid-like than liquid-like [21]. The hydrogels are also made of hydrophilic polymer strands but they are crosslinked and that enables them to swell while retaining their three-dimensional structure [16]. From that, the main feature of the hydrogels, that distinguishes them from conventional gels, is their inherent crosslinking. However, gels can also get a low level of virtual crosslinking under the influence of shear forces, but this kind of crosslinking is very weak and reversible. The distinct behaviors of gels and hydrogels in aqueous environment are shown in **Figure 1.3**.

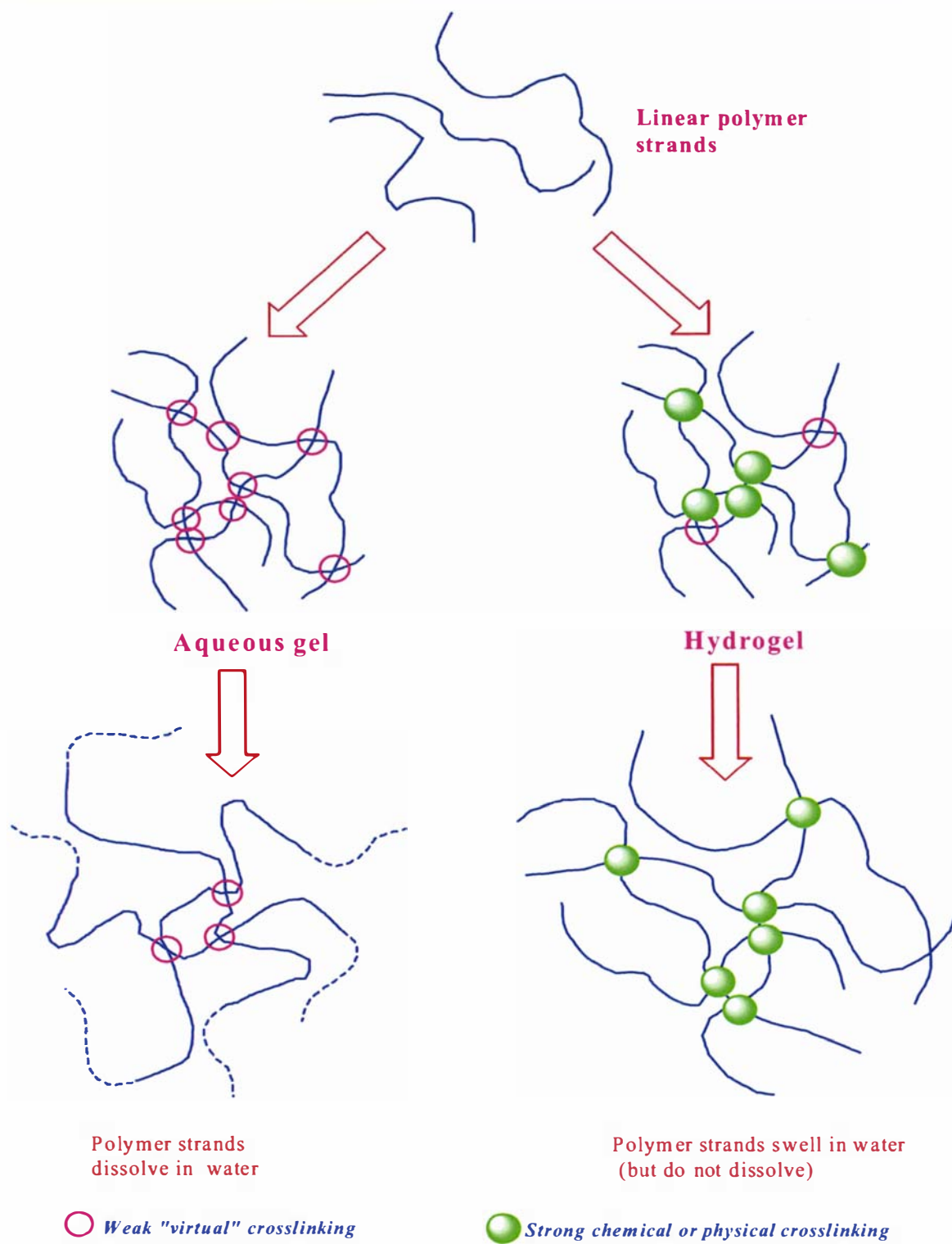


Figure 1.3. The distinct behaviors of gels and hydrogels in aqueous environment [22].

## 1.2.2. Classifications of Hydrogels

Numerous classifications have been applied to hydrogels; some of these classifications are discussed below [23].

### 1.2.2.1. According to the hydrogel origin

Hydrogels can be classified into *natural*, *synthetic* and *semi-synthetic* according to their origin. Most of the synthetic hydrogels are synthesized by traditional polymerization of vinyl-activated monomers. The equilibrium swelling values of these synthetic hydrogels vary widely depending on the hydrophilicity of the monomers and the crosslinking extent. A bi-functional monomer is usually added to carry out an *in-situ* crosslinking reaction. The natural hydrogels are based on natural polymers including polypeptides, polynucleotides and polysaccharides. These natural polymers are derived from different natural sources. For instance, collagen is derived from mammals whereas chitin and chitosan (*Ch*) are obtained from the exoskeleton of shellfish. **Table 1.2 [9]** shows some examples of different types of polymers used in the preparation of hydrogels.

**Table 1.2.** Some examples of different classes of polymers used in the preparation of hydrogels [9].

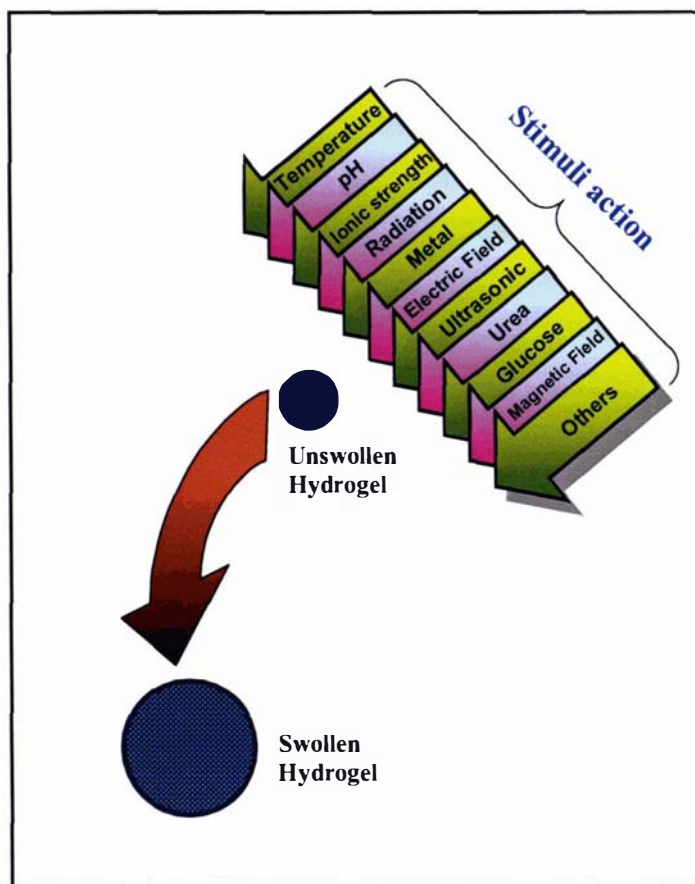
<i>Polymer category</i>	<i>Examples</i>	<i>Ref.</i>	
<i>Natural polymers</i>	<i>Neutral</i>	Agarose	[24]
		Dextran	[25]
	<i>Cationic</i>	<b>Ch</b>	[26]
	<i>Anionic</i>	Alginic acid	[27]
		Pectin	[28]
		Carrageenan	[29]
	<i>Amphipathic</i>	Collagen	[30]
		Gelatin	[31]
		Carboxymethyl chitin	[32]
	<i>Synthetic polymers</i>	<i>Polyesters</i>	<b>PEG-fumarate</b>
<b>PHB</b>			[34]
<i>Others</i>		<b>PBCPP</b>	[35]
		<b>PET</b>	[36]
		<b>PVA</b>	[37]
		<b>PEO</b>	[38]
		<b>PAAm</b>	[39]
<b>P(MAA-g-EG)</b>	[40]		
<i>Semi-synthetic polymers</i>	Collagen-acrylate	[41]	
	<b>P(NIPA-co-EG)-co-peptides</b>	[42]	

### 1.2.2.2. According to the hydrogel durability

Hydrogels can be classified into *durable* or *biodegradable* depending on their stability characteristics in a physiological environment. Recently, a significant body of research has focused on the preparation and utilization of new biodegradable hydrogels. The applications of these biodegradable hydrogels now cover many areas, including both biomedical and non-biomedical uses [43]. The degradable polymers inside the hydrogel undergo chain scission to form low molecular weight oligomers. Ultimately, the resulting oligomers are either eliminated by the body or undergo further degradation.

### 1.2.2.3. According to the hydrogel response to the environmental factors

The past few years have witnessed enormous advances in developing and investigating a unique class of hydrogels. This class is called “*intelligent*” or “*smart hydrogels*”. The smart hydrogels are, in most respects, similar to the conventional hydrogels. Both of them are prepared using similar techniques and they are similar in their general characteristics. However, smart hydrogels can exhibit unusual changes in their swelling behavior, network structure and/or mechanical properties in response to different stimuli such as temperature, pH, light, ionic strength or electric field [44-46]. **Figure 1.4** shows the significant variation in the swelling extent of a hydrogel in response to various stimuli.



**Figure 1.4.** Stimuli-responsive swelling of hydrogels.

In general, the changes occur to the smart hydrogels in response to any of the stimuli and disappear upon removal of the stimulus. Consequently the hydrogels return to their original state in a reversible manner.

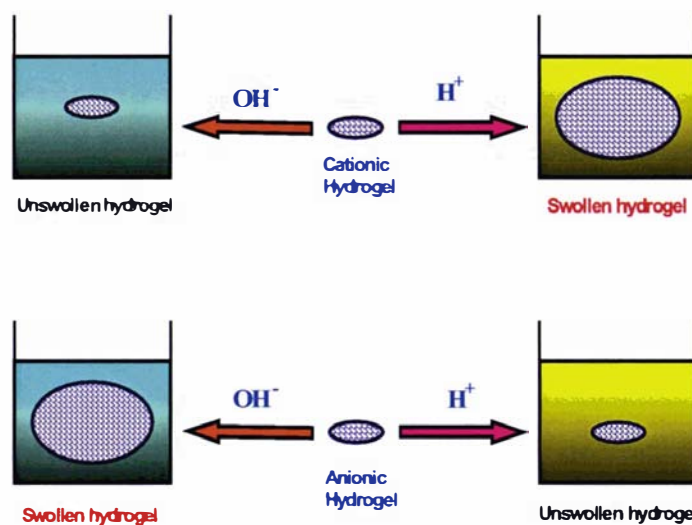
The applications of the smart hydrogels are now attracting the interest of a large number of researchers [44-46]. Most of these applications are directed towards the stimuli-responsive drug delivery. The main reason for the interest in using smart hydrogels in the drug delivery is that, a good drug delivery system should respond to the physiological requirements, sense the changes and accordingly amend the drug release pattern. In other words, the smart hydrogels optimize the drug delivery profiles for pulsed or self-regulated mechanisms [22]. **Table 1.3** shows some of the recent potential applications of these stimuli-responsive controlled drug release systems.

**Table 1.3.** Examples of utilization of some stimuli-responsive hydrogels in controlled drug release [22].

<i>Stimuli</i>	<i>Smart hydrogel</i>	<i>Uploaded drug</i>	<i>Ref.</i>
<i>Temperature</i>	<i>PNIPA</i>	Heparin	[44]
	<i>PHEMA</i>	Salicylic acid	[45]
<i>pH</i>	<i>PAA-PEO</i>	Salicylamide, Prednisolone, Nicotinamide	[47]
	<i>Gelatin-PEO</i>	Riboflavin	[48]
	<i>(PNVP-PEG diacrylate)-Ch</i>	Theophylline, 5-fluorouracil	[49]
	<i>Ch-PEO</i>	Amoxicillin, Metronidazole	[50]
	<i>Temperature and pH</i>	<i>P(NIPA-co-BMA-co-AA)</i>	Calcitonin
<i>Electric field</i>	<i>PHEMA</i>	Propranolol hydrochloride	[46]
<i>Ultrasonic radiation</i>	<i>E-co-VAc</i>	Insulin,	[52]
	<i>E-co-VA</i>	Zinc bovine insulin	
<i>Glucose</i>	<i>E-co-VAc</i>	Insulin	[53]
<i>Magnetic field</i>	<i>E-co-VAc</i>	Insulin	[54]
<i>Urea</i>	<i>MVE-co-maleic anhydride</i>	Hydrocortisone	[55]
<i>Morphine</i>	<i>MVE-co-maleic anhydride</i>	Naltrexone	[56]

### *pH-responsive hydrogels*

Within the class of smart hydrogels, the preparation and utilization of pH-responsive hydrogels have been extensively investigated for stimuli-responsive drug delivery. The importance of this category of smart hydrogels is due to the various pHs of the internal organs of the body such as the gastrointestinal tract, GIT [57]. pH-responsive smart hydrogels are mainly based on polymers with ionic pendant groups distributed along their backbones such as poly(acrylic acid) (*PAA*), poly(methacrylic acid) (*PMAA*) and poly(diethyl-aminoethyl methacrylate) (*PDEAEMA*) [15]. These pendant ionic groups are responsible for the pH-sensitivity of the hydrogels. At certain pH values, the pendant groups ionize and generate fixed charges on the polymer network that results in developing electrostatic repulsive forces. These repulsive forces are responsible for the pH-dependent swelling/deswelling of the hydrogels [52]. In the case of hydrogels based on cationic polymers such as *Ch*, the pendant groups of the cationic hydrogel are ionized at lower pH and non-ionized at neutral and higher pH values. In a contrary situation, the pendant groups of anionic hydrogels are non-ionized at low pHs and ionized at pHs above the pKa of the polymeric network respectively [22]. Figure 1.5 shows a schematic representation of the pH-dependent swelling of hydrogels.



**Figure 1.5.** Schematic representation of pH-dependent swelling of a smart hydrogel [22].

### 1.2.3. Methods of preparation of hydrogels

Hydrogels can be prepared by various routes depending on the designed structure and the desired application. These methods are discussed below [23]:

#### 1.2.3.1. Free radical polymerization

Conventional free radical polymerization is the preferred method for the preparation of hydrogels based on some monomers such as acrylates, vinyl lactams and amides [58,59]. Also, it can be applied to the preparation of hydrogels based on natural polymers provided that these polymers have suitable functional groups or have been functionalized with radically polymerizable groups [60,61]. Preparation of hydrogels by this method involves the chemistry of typical free-radical polymerizations, which includes initiation, propagation, chain transfer, and termination steps. In the initiation step a wide variety of thermal, visible, ultraviolet, and redox initiators can be utilized for the radical generation. Then the radicals react with the monomers converting them into active forms. These active monomers react with more monomers and so on in the propagation step. The resulting long chain radicals undergo termination either through chain transfer or through radical combination forming a polymeric matrix. The conditions of polymerization reactions vary widely depending on the monomer solubility and reactivity and also on the nature of the initiator used.

Free radical polymerization reactions can be performed either in solution or neat. Solution polymerizations are favored during the synthesis of large quantities of hydrogels. In this method, water is the most common solvent. However, a wide variety of other polar solvents can be used provided that they can be exchanged by water in the hydration step. Neat (bulk) polymerizations are very fast and have the advantage over solution polymerizations, as there is no need for solvent removal. This removal of solvent is time-consuming in many cases. Hydrogels can be prepared also via free radical emulsion and suspension polymerizations [62]. This route of free radical polymerization is the superior one for the preparation of the hydrogels in the form of beads and microspheres. Thereby, this way is extremely good for developing hydrogels used as matrices for drug delivery purposes. In this method, a predetermined amount of a suspension agent and/or emulsifier is placed together with the monomer, the solvent, and the initiator. The procedure of the free radical emulsion and suspension

polymerization is quite simple. However, it is very difficult to get rid off the emulsifier and/or the suspension agent in many cases.

During the synthesis of a hydrogel by free radical polymerization, a predetermined amount of a bi-functional agent should be added to achieve *in situ* crosslinking of the polymer chains. The main point to be taken into consideration, if this method is used for hydrogel preparation, is the selection of the appropriate crosslinking agent. Using a crosslinking agent of reactivity highly different from that of the monomer(s) used for the hydrogel preparation may produce a problem. This reactivity difference can lead to formation of a heterogeneously crosslinked hydrogel matrix. Methylene bisacrylamide (**BIS**) is one of the most commonly used bi-functional crosslinking agents especially for hydrogels based on acrylamide monomers [58].

### 1.2.3.2. Irradiation crosslinking of polymers

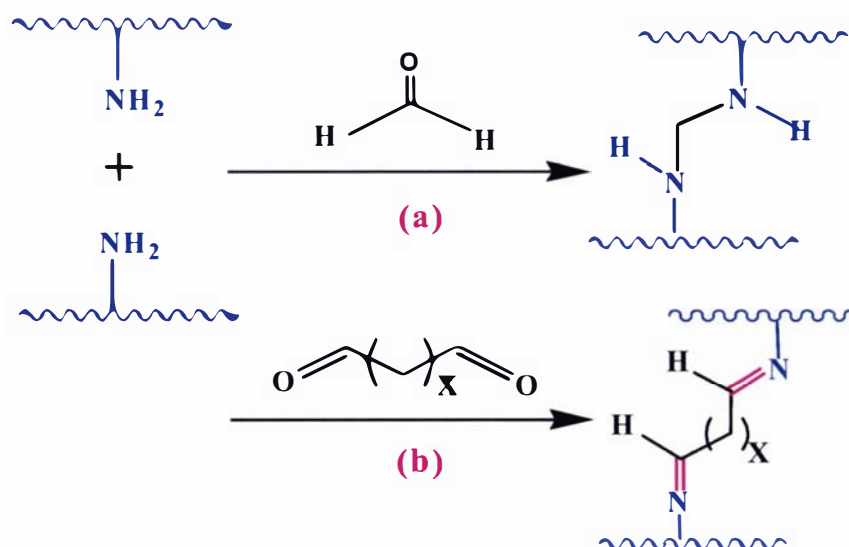
Ionizing-radiation techniques, especially if combined with simultaneous sterilization of the product, are very convenient tools for the synthesis of hydrogels. The ionizing radiation possesses a high energy enough to ionize simple molecules either in air or water [23]. This type of irradiation includes both electron beam and  $\gamma$ -irradiation. During irradiation of a polymer solution, many reactive sites are generated along the polymer backbone. Then upon combination of these radicals, a large number of crosslinks are formed. The formation of hydrogels by this technique can be performed by irradiation of the polymers either in bulk or in solution. However, irradiation of a polymer solution is the preferred method due to the less energy required for the formation of macroradicals. Also, in solution the efficiency of the radicals is high due to the reduced viscosity of the reaction mixture.

Applying irradiation to hydrogel preparation has many advantages over other conventional methods [63]. During the irradiation process, no catalysts or additives are needed to initiate the reaction. Also, irradiation methods are relatively simple, and the degree of crosslinking, which strongly determines the extent of swelling of hydrogels, can be controlled easily by varying the irradiation dose [64, 65]. Due to these advantages, this technique has found great utilization in preparing hydrogels for medical and pharmaceutical applications, where even the slightest contamination is undesirable [66]. However, irradiation is not recommended for preparation of hydrogels from some

polymers that can degrade under the ionizing irradiation [67]. In other words, each polymer system is unique and so its appropriate irradiation conditions should be determined experimentally to minimize its chain degradation and maximize its crosslinking extent [68].

### 1.2.3.3. Chemical crosslinking of polymers

The chemical crosslinking of hydrophilic polymers [69] is one of the fundamental methods of hydrogel preparation. This method is based on addition of a bi-functional crosslinking agent to dilute solution of the hydrophilic polymer(s). The hydrophilic polymer should have a suitable functionality that is able to react with the crosslinking agent. The gelation reaction is typically performed in solution, but may also be carried out through a suspension reaction if the desired hydrogel is required to be in the form of beads, spheres or microparticles. This method is suitable for preparation of hydrogels based on either naturally occurring or synthetic hydrophilic polymers. In case of naturally occurring polymers, most of them can be crosslinked by this method. For instance, albumin and gelatin can be crosslinked with either a difunctional dialdehyde or formaldehyde [70,71]. In this example, the gelation involves the reaction of the aldehyde groups with an amino group along the albumin polymer backbone. Using an analogous approach, diaminododecane catalyzed by dicyclohexycarbodiimide can be employed to crosslink chondroitin sulfate [72]. In this example, the degree of crosslinking increased with increasing the concentration of the diaminododecane. Also, cross-linking of functionalized *PEG* and a lysine-containing polypeptide by this method have been reported [73]. Transparent hydrogels of high water content have been also prepared by this method [73]. **Figure 1.6** shows an example of hydrogel preparation by chemical crosslinking of an amino-bearing polymer using formaldehyde or a dialdehyde crosslinking agent.



**Figure 1.6.** General examples of hydrogels prepared by chemical crosslinking of an amino-bearing polymer using (a) formaldehyde and (b) dialdehyde crosslinking agents.

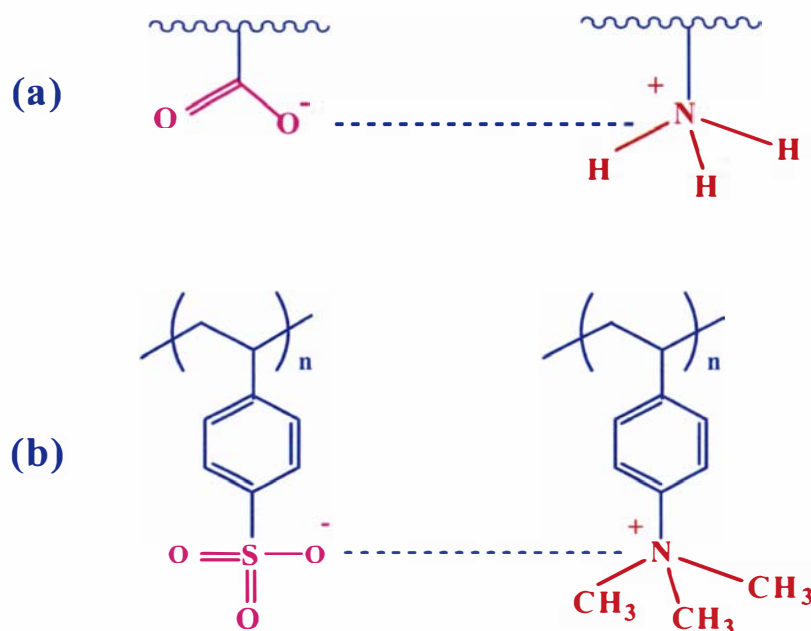
#### 1.2.3.4. Physical crosslinking of polymers

Crosslinking of polymers through physical interactions is one of the most common and easy routes for hydrogel formation. This physical crosslinking includes interactions such as polyelectrolyte complexation, hydrogen bonding and hydrophobic association [23]. The hydrogels obtained by this technique are usually prepared under mild conditions. In the case of the preparation of hydrogel films based on physical interactions, the typical procedures involve either solvent casting or precipitation techniques.

##### □ *Polyelectrolyte Complexation (Ionic interactions)*

In this method, hydrogels can be easily prepared through the formation of polyelectrolyte complexes [74]. In polyelectrolyte complexation, the links are formed between pairs of charged sites along the polymer backbones. The hydrogels obtained through polyelectrolyte complexation are insoluble in water and the formed electrolytic links vary in their stabilities depending on the pH of the system. Polyelectrolyte complexed hydrogels may be divided into four subclasses depending on the acidity and basicity of the polyelectrolytes (polymers). These subclasses involve strong acid–strong

base, strong acid–weak base, weak acid–strong base and weak acid–weak base complexes. As an example of hydrogels prepared by this method, the hydrogels resulting from the polyelectrolyte complexation of the carboxylate groups of sodium alginate with the amino groups distributed along *Ch* backbone (Figure 1.7a) [75]. A mixture of sodium alginate and carboxymethyl chitosan (*CMCh*) also form a strong complex through the association with the calcium ions [76]. Another example is the complexation of poly(sodium styrene sulfonate) and poly(4-vinylbenzyltrimethylammonium chloride) (Figure 1.7b) [77]. In the last example, the hydrogel was formed immediately by mixing the water-based solutions of the two polymers at equimolar concentration.

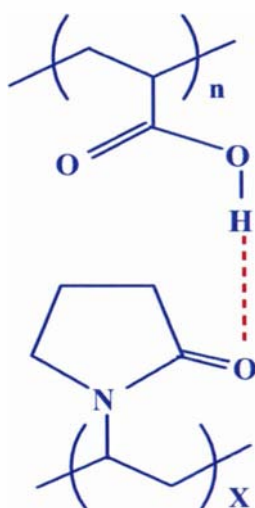


**Figure 1.7.** Examples of hydrogels prepared from the polyelectrolyte complexation of (a) sodium alginate with *Ch* acidic solution [75] and (b) poly(sodium styrene sulfonate) with poly(4-vinylbenzyltrimethylammonium chloride) [77].

### □ Hydrogen Bonding

Hydrogen bonding between macromolecular chains can also participate in the hydrogel formations. A hydrogen bond is formed through the association of electron deficient hydrogen atom and a functional group of high electron density. Similar to the polyelectrolyte complexes described earlier, hydrogen-bonded polymeric hydrogels

occur in many biological systems. **Figure 1.8** shows a recent example of the hydrogen-bonded hydrogels. In this example a hydrogel can result from hydrogen bond formation between *PAA* and *PNVP* [78]. This hydrogel is affected by a variety of factors, such as the polymer concentration, the molar ratio of each polymer, the type of solvent, the solution temperature, and the polymer structure (degree of association between the polymer functionalities).

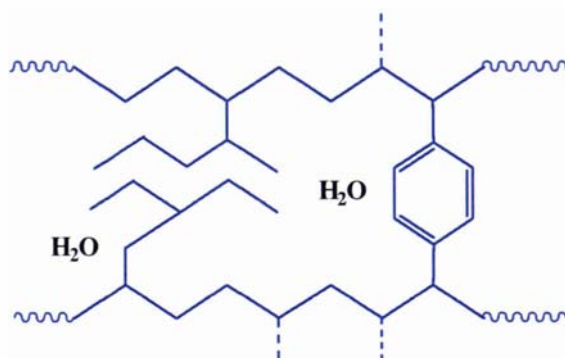


**Figure 1.8.** Recent example of a hydrogel resulting from the hydrogen bond formation between *PAA* and *PNVP* [78].

#### □ *Hydrophobic Association*

Hydrogels can also be formed through hydrophobic interactions [79–81]. Polymer systems such as graft copolymers, block copolymers and polymer blends usually form structures separated by hydrophobic microphases/microdomains. These hydrophobic domains act as associated crosslinking sites in the whole polymeric structure. Also, these hydrophobic phases are surrounded by hydrophilic water absorbing regions. **Figure 1.9** shows a general example of the hydrophobic association arises between polymers. In general, the mechanical properties of these hydrophobically combined polymers are poor due to the poor interfacial adhesion. Besides, the hydrogel films prepared by this route are opaque as a result of the macrophase polymer separation.

This approach for hydrogel preparation has numerous advantages. These advantages include the low cost of the system where the commercially available polymers can be applied to develop a wide range of low cost, high strength hydrogels. Another advantage of this approach is that the resulting hydrogels are soluble in organic solvents and can also flow at elevated temperatures. These characteristics facilitate the processing of these hydrophobic hydrogels using injection molding techniques. In contrast, covalently crosslinked hydrogel systems are generally insoluble in organic solvents and can not flow even at elevated temperatures [82].



**Figure 1.9.** General example of a hydrogel in which polymeric chains are held together via crosslinking with divinyl benzene in addition to the hydrophobic association.

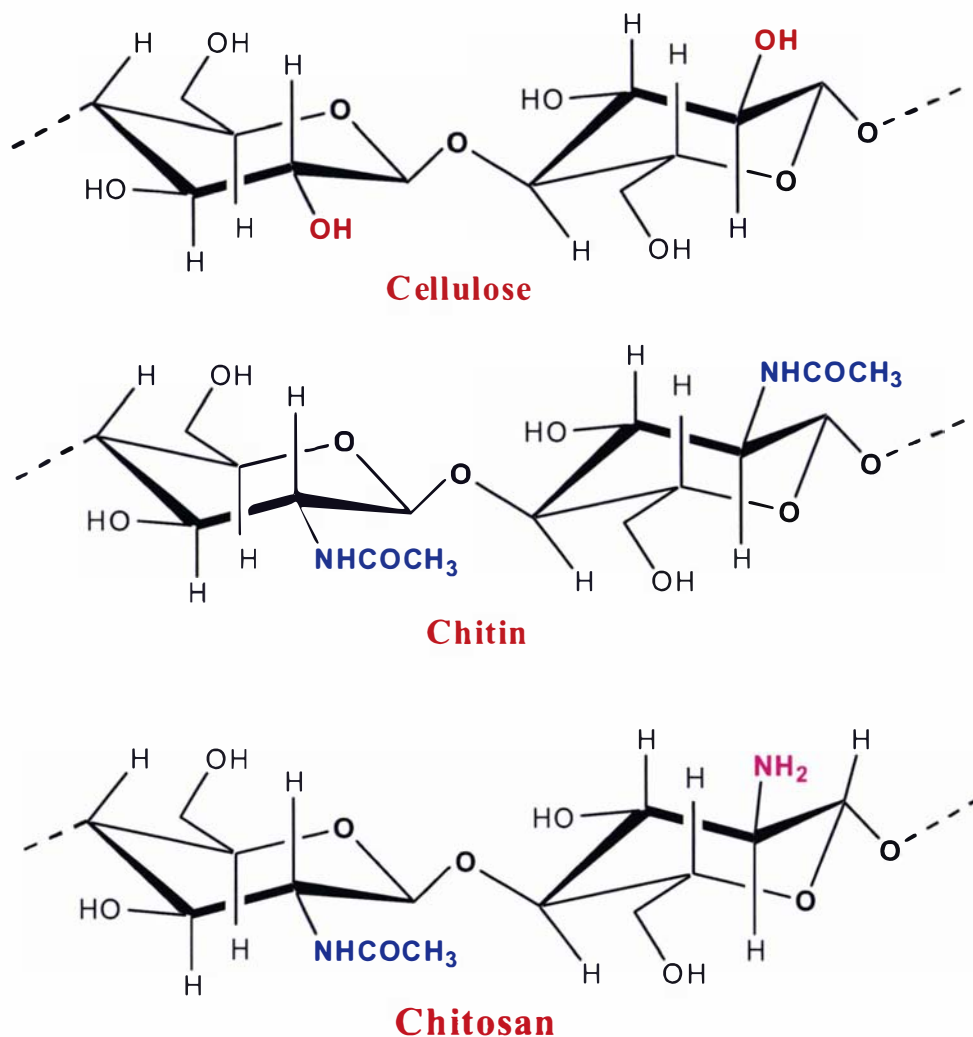
#### 1.2.4. Applications of hydrogels in controlled drug release

A significant body of research has focused on the preparation and investigation of different types of hydrogels based on various polymers for drug delivery purposes [83,84]. Also, hydrogels that swell and contract in response to external pH [85,86] have been investigated. These pH-sensitive hydrogels possess potential application in the site-specific delivery of drugs to specific regions of the gastrointestinal tract [87]. The drug release from hydrogels depends on many factors. The main one is based on the chemical structure of the polymers used in the synthesis of these hydrogels. For this reason, it is important to select the appropriate polymers upon designing a hydrogel for drug delivery applications. These polymeric materials should be non-toxic and biodegradable. Unfortunately, the number of polymers suitable for the controlled release of viable therapeutics is quite limited compared to the number of available synthetic polymers because of the inherent toxicity or the lack of certain properties such as

biodegradability and swelling ability in specific environments. Therefore, in the current work, *Ch* was chosen as the base polymer for the development of new hydrogels that could be tailored for use in the site-specific delivery of drugs to the gastrointestinal tract.

#### 1.2.4.1. *Ch* and *Ch*-based hydrogels for controlled drug release

*Ch* [a (1→4) 2-amino-2-deoxy-β-D-glucan] is a cationic biopolymer obtained through the alkaline *N*-deacetylation of the naturally occurring polymer, chitin. Chitin, the supporting material of crustaceans, insects and fungal mycelia is well known to consist of 2-acetamido-2-deoxy-β-D-glucose through a β (1→4) linkage. Chitin can be degraded by chitinase. It is a highly insoluble, white, hard and inelastic nitrogenous polysaccharide resembling cellulose in its solubility and low chemical reactivity [88]. Chitin may be regarded as cellulose with the hydroxyl at position C-2 replaced by an acetamido group. The *N*-deacetylation process of chitin to produce *Ch* is almost never complete and no sharp nomenclature based on the degree of *N*-deacetylation has been defined between chitin and *Ch* [88,89]. Both chitin and *Ch* are of commercial significance due to their high percentage of nitrogen as compared to synthetically substituted cellulose. This makes both chitin and *Ch* useful polymers with many applications [88]. **Figure 1.10** shows the structures of cellulose, chitin and *Ch*.



**Figure 1.10.** Structure of *Ch* as compared with that of chitin and cellulose.

### ▣ Production of *Ch*

The production of *Ch* from chitin involves alkali treatment of chitin yielding *Ch*-glucan complexes. The alkali simultaneously deacetylates chitin and removes any protein, which may contaminate the *Ch* [90]. The deacetylation process is normally carried out in 40% NaOH solution at 120 °C for 1–3 h. This treatment usually produces 70% deacetylated *Ch*. Repeating the alkali treatment many times increases the deacetylation extent. **Figure 1.11** shows the deacetylation process.

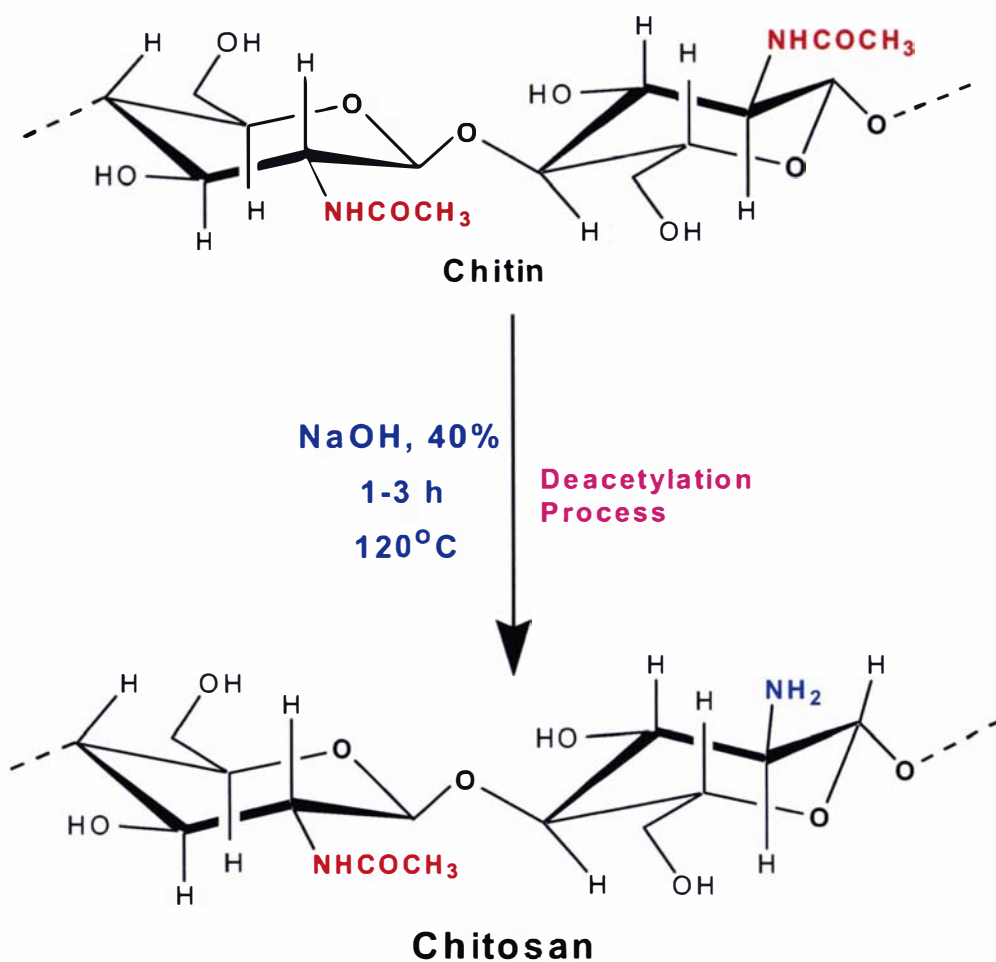


Figure 1.11. The alkaline deacetylation of chitin to *Ch*.

#### □ *Properties of Ch*

##### (A) Physical properties

*Ch* is a basic polysaccharide whereas most of the other naturally occurring polysaccharides such as pectin, agarose, agar, cellulose, dextran, carrageenan and alginic acid are either neutral or acidic in nature. *Ch* has many unique characteristics such as the ability to form films and polyoxysalts. Also, *Ch* has good optical properties [91]. The N-deacetylation degree of *Ch* is one of the main parameters that affect its properties. This degree of N-deacetylation is the ratio of the 2-acetamido-2-deoxy-D-glucopyranose to the 2-amino-2-deoxy-D-glucopyranose structural units. This ratio has a remarkable effect on *Ch* solubility and its other physical properties. Several analytical

techniques such as FTIR spectroscopy,  $^1\text{H}$ -NMR spectroscopy,  $^{13}\text{C}$  solid state NMR, HPLC and various titration techniques have been applied to estimate this ratio [92-101]. Both chitin and *Ch* are highly hydrophobic and are insoluble in water and most organic solvents. *Ch* is soluble in dilute acids such as acetic acid, formic acid and hydrochloric acid. Also, both chitin and *Ch* degrade before melting, which is typical for polysaccharides with extensive hydrogen bonding. For this reason, to impart functionality, it is necessary to dissolve them in an appropriate solvent system. For each solvent system, the effect of some parameters such as pH, temperature and polymer concentration on the solution viscosity must be known [102].

### (B) Chemical properties

The nitrogen percent of *Ch* varies depending on its degree of deacetylation. This nitrogen content presents mostly in the form of primary aliphatic amino groups. For this reason, *Ch* can be considered as a reactive polymer. It easily undergoes the typical reactions of amines such as N-acylation and Schiff's base (imine) formation. Also, it shows a good chelating ability towards metal ions. In addition, due to this reactivity, *Ch* derivatives can be obtained under mild conditions [103]. Both the physical and chemical characteristics of *Ch* can be summarized as follow:

- It is a cationic polyamine.
- It has high positive charge density at lower pHs.
- It adheres to negatively charged surfaces.
- It forms hydrogels with polyanions.
- It is highly hydrophobic at neutral and higher pHs.
- It is insoluble in water and most organic solvents.
- It is soluble in dilute acids.
- It forms high viscous solutions.
- It degrades before melting.
- It is a high molecular weight linear polyelectrolyte.
- It has good optical properties.
- It forms films and polyoxysalts.
- It has reactive amino/hydroxyl groups.
- It readily undergoes chemical modification.
- It can chelate transition metals.

A significant body of interest has recently been paid to *Ch* as a potential polysaccharide resource [104]. Several studies have discussed the preparation of a wide variety of functional derivatives of *Ch* through its chemical modifications [105–110]. Due to the presence of reactive amino groups, *Ch* can easily form imines with aldehydes and ketones at room temperature. N-alkyl *Ch* can be produced by hydrogenation of the *Ch* imines. In addition, *Ch* can be carboxymethylated by reaction with chloroacetic acid [76]. Some of the *Ch* derivatives and their potential applications are shown in Table 1.4.

Table 1.4. *Ch* derivatives and their proposed uses [2].

<i>Derivative</i>	<i>Examples</i>	<i>Potential applications</i>
<i>N-Acyl Ch</i>	Formyl, acetyl, propionyl, butyryl, hexanoyl, octanoyl, decanoyl, dodecanoyl, tetradecanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, benzoyl.	Textiles, medical aids and membranes
<i>N-Carboxyalkyl (aryl) Ch</i>	<i>N</i> -Carboxybenzyl, glycine-glucan ( <i>N</i> -carboxymethyl <i>Ch</i> ), alanine glucan, phenylalanine glucan.	Chromatography and metal ion capture
<i>N-Carboxyacyl Ch</i>	From anhydrides such as maleic, itaconic, acetyl-thiosuccinic, glutaric, cyclohexane 1,2-dicarboxylic, phthalic, <i>cis</i> -tetrahydrophthalic.	?
<i>O-Carboxyalkyl Ch</i>	<i>O</i> -Carboxymethyl, crosslinked <i>o</i> -carboxymethyl	Molecular sieves, viscosity builders and metal ion collection
<i>Metal ion chelates</i>	Palladium, copper, silver, iodine	Catalyst, photography, health products, and insecticides

Continued

<i>Derivative</i>	<i>Examples</i>	<i>Potential applications</i>
<i>Semi-synthetic Ch resins</i>	Copolymer of <i>Ch</i> with <i>MMA</i> , polyurea-urethane, poly (amide-ester), acrylamide-maleic anhydride	Textiles
<i>Natural polysaccharide complexes, (Miscellaneous)</i>	<i>Ch</i> -glucans from various organisms	Flocculation and metal ion chelation
	Cyanoethyl <i>Ch</i>	Desalting filtration, dialysis and insulating papers
	Hydroxy ethyl glycol <i>Ch</i>	Enzymology, dialysis and special papers
	Glutaraldehyde <i>Ch</i>	Enzyme immobilization
	Linoelic acid- <i>Ch</i> complex	Food additive and anticholesterolemic

### (C) Biological properties

*Ch* has many desirable biological properties, being biodegradable, biocompatible, non-toxic, bioabsorbable [111] and having a hydrogel-forming ability at low pH. Moreover, *Ch* itself has antacid and antiulcer activities, which can prevent or weaken drug-induced irritation in the stomach [112,113]. Some of the main biological characteristics of *Ch* are summarized as follow:

- Natural polymer.
- Biocompatible.
- Biodegradable to normal body constituents.
- Safe and non-toxic.
- Bioabsorbable.
- Hemostatic.
- Bacteriostatic.
- Fungistatic.
- Spermicidal.
- Anticancerogen.
- Anticholesteremic.
- Antiulcer and antiacid.

#### ▣ *General applications of Ch*

*Ch* has a wide range of applications. These applications cover many areas, mainly the biomedical and the environmental areas. The following are some examples on some recent applications of *Ch* and its derivatives:

##### (a) *Ch* as heparinoid (blood anti-coagulant)

*Ch* sulfates have been found to have blood anti-coagulant and lipoprotein lipase (LPL)-releasing activities. These *Ch* derivatives showed about two-fold anti-coagulant activity and 0.1fold LPL-releasing activity over those of heparin. Therefore, these sulfated derivatives might be beneficial as heparinoids for artificial blood dialysis [114].

##### (b) *Ch* as antibacterial agent

*Ch* was found to inhibit the growth of *Fusarium*, *Alternaria* and *Helminthosporium*. Also, the growth of *Escherichia coli* was inhibited in the presence of more than 0.025%

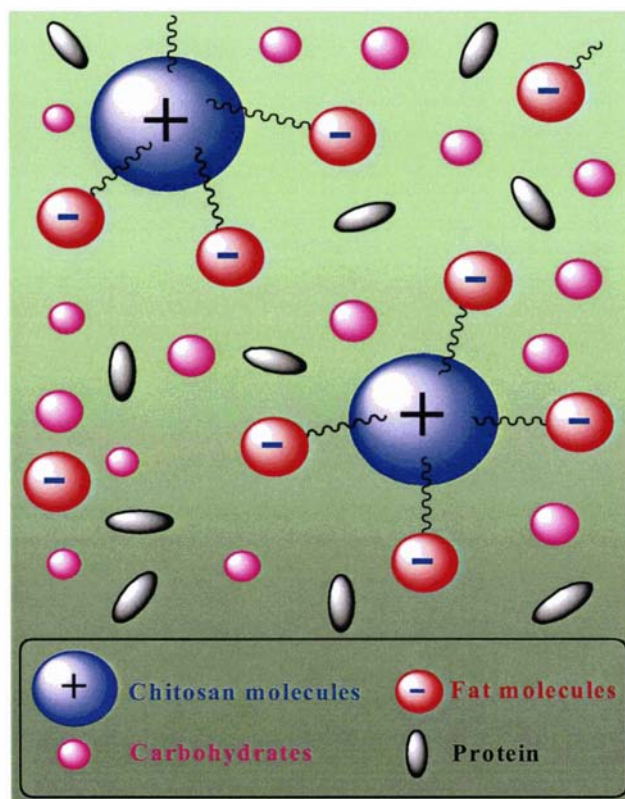
**Ch.** This antibacterial activity may be attributed to the binding of the cationic amino groups of **Ch** with the anionic groups of the microorganisms, leading to growth inhibition [115].

**(c) Ch as anti-thrombogenic and haemostatic material**

**Ch** fibres were found to act as thrombogenic and haemostatic agents in an *in-vitro* test. N-hexanoyl and N-octanoyl **Ch** fibres were found to be anti-thrombogenic [116].

**(d) Ch as fat trapper**

In the stomach, **Ch** fibres are positively charged therefore, they have the ability to bind chemically with the negatively charged lipids, fats and bile acids [117-118]. This binding takes place before the digestion of the fats and bile acids, thus entrapping them and consequently preventing their absorption by the digestive tract. Beside, the combination of **Ch** fibres and fats forms a mass, which can not be readily absorbed and thus can be easily eliminated by the body. **Figure 1.12** shows a schematic diagram for the role of **Ch** in trapping the fats.



**Figure 1.12.** Schematic representation for the role of *Ch* as a fat trapper.

### (e) In contact lenses

The properties of *Ch* enable it to be a good candidate in developing the ideal contact lens. These characteristics include mechanical stability, wet-ability, optical clarity, immunological compatibility and oxygen permeability. *Ch*-based contact lenses are made by spin casting technology from partially depolymerized and purified squid pen *Ch*. These contact lenses possess good characteristics. For instance, they are tough, clear and hold other required physical characteristics such as modulus, tensile strength, tear strength, elongation, suitable water content and oxygen permeability [12].

### (f) In wound healing products

Although *Ch* has many unique biomedical characteristics, most of the *Ch*-based wound healing products are still at the early stages of development. For instance, an attempt has been reported [119] to synthesize and evaluate a wound-covering material from

copoly(*Ch*-g-N-carbobenzoxy-L-lysine). The biocompatibility was evaluated by studying human skin fibroblast cell culture on the membrane surface made of the *Ch* copolymer.

#### **(g) In enzyme immobilization**

Use of many enzymes is accompanied by many practical problems. These problems include the enzymes instability once they are isolated from their natural environments, the high cost of their isolation and purification, and also their sensitivity either to any trace amounts of inhibitors or to any medium conditions rather than the optimal ones. In addition, it is not often easy to recover the enzyme at the end of the reaction. Several methods have been suggested to overcome these limitations, one of the most successful being the enzyme immobilization on or within solid supports [120,121]. In some trials, chitin- and *Ch*-based materials have been used as supports for enzymes [122,123]. These chitinous supports vary in their shape to include powders, flakes and gels.

#### **(h) In food and nutrition**

Several animal nutritional studies have reported that the utilization of whey may be improved if the diet contains small amounts (0.5-2%) of chitinous material. This improvement is explained by the change in the intestinal microflora brought about by the chitinous supplement [124].

#### **(i) In cosmetic products**

*Ch* has fungicidal and fungistatic properties and it is a natural cationic polymer that becomes viscous upon neutralization with acids. These good properties enable *Ch* and some of its derivatives to be used in creams, nail lacquers and permanent waving lotions [125].

#### **(j) In photography**

The unique characteristics of *Ch* such as the film forming ability, the good resistance to abrasion and the promising optical characteristics mean it can have important applications in photography. Moreover, silver complexes are not appreciably retained by *Ch* and hence can easily be penetrated from one layer to another of a film by diffusion. This has serious implications for photography [126].

**(k) In the paper industry**

Hydroxymethyl *Ch* and some other water-soluble *Ch* derivatives are found to be useful end additives in the paper industry. Also, *Ch* itself has been reported to impart wet strength to paper and offer the paper better finish properties [126]. However, to date, *Ch* is still non-commercially significant component in the paper industries.

**(l) In batteries**

*Ch* can supply ionic conductivity when dissolved in acetic acid. This conductivity is attributed to the presence of protons from the acetic acid solution. The transfer of these protons is thought to occur through many microvoids in the polymer since the dielectric constants from piezoelectric studies are small [126,127].

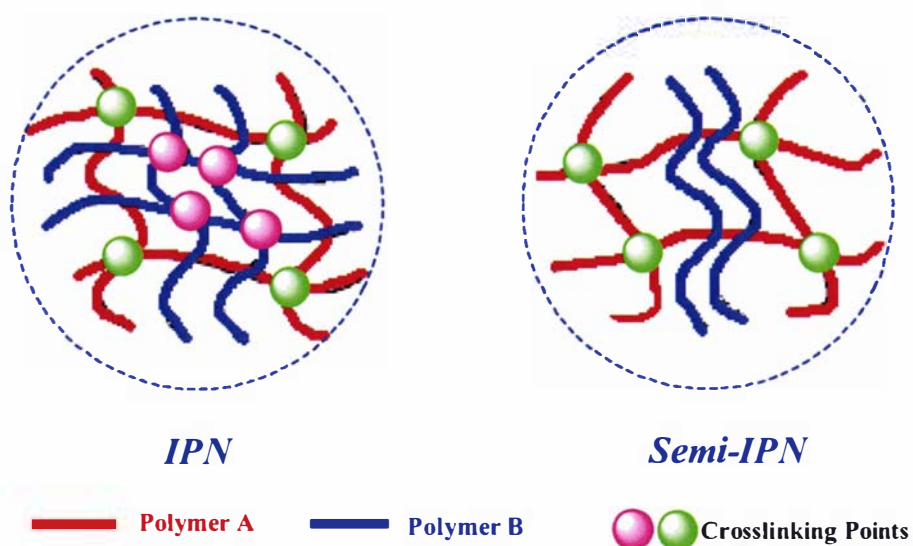
**(m) In metal capture and treatment of wastewater**

Several investigations clearly indicated that *Ch* has a natural selectivity for some heavy metal ions and thereby it can be exploited in the treatment of wastewater. For instance, some studies [128,129] have shown that *Ch* can be used for the removal of mercury from solutions. The results indicated that the efficiency of adsorption of  $\text{Hg}^{2+}$  by *Ch* depends mainly on the quantity of *Ch*, the period of treatment, the initial concentration of  $\text{Hg}^{2+}$  and the particle size. Also, several *Ch* derivatives have been prepared and investigated [130] for adsorbing different metal ions. Most of these derivatives were obtained by grafting new functional groups onto the *Ch* backbone. These function groups work to increase the number of sorption sites, to change the optimum pH range for the metal sorption and to change the sorption sites in order to increase the sorption selectivity for the target metal.

**(n) In controlled drug release formulations**

The interesting properties of *Ch* make it an ideal candidate in developing hydrogels for use in controlled drug release formulations. However, this naturally abundant material also shows some limitations such as hydrophobicity and a high pH-dependency of its physical properties. Therefore, it is not easy to control drug release with *Ch* itself because of the various pHs of the internal organs of the human body. This may negatively reflect on the human body because of drug over-release [131]. The most commonly used strategy to overcome these limitations is the incorporation of *Ch* in an interpenetrating polymeric network (*IPN*) hydrogel. In these *IPNs*, *Ch* is incorporated

with either hydrophilic polymers or with hydrophilic monomer(s) treated to bring about in situ copolymerization in the presence of *Ch* and a suitable crosslinking agent. An *IPN* can be defined as a combination of two or more chemically distinct polymers held together ideally and solely by their permanent mutual entanglements. The *IPNs* have two basic characteristics: first, one or more of the polymers must be synthesized and/or crosslinked, in the immediate presence of the other(s), and second, the combination produces an advanced multicomponent polymeric system, with new property profiles [132,133]. In general, when only one polymer undergoes crosslinking, the resulted matrix is called semi-*IPN*. **Figure 1.13** shows a schematic representation of both *IPN* and semi-*IPN* hydrogels. The following section discusses the results from a literature survey of some *Ch*-based hydrogels and *IPN* hydrogels developed and investigated for the controlled release studies.



**Figure 1.13.** Schematic representation of *IPN* and Semi-*IPN* hydrogels [134].

***Ch-based hydrogels and IPN hydrogels (films and beads)***

Kim et al. [135] have prepared an *IPN* hydrogel composed of *Ch* and *PVA* by UV irradiation. This *IPN* hydrogel exhibited a relatively high swelling ratio, in the range of 210–350%, at 35 °C and its swelling was both pH- and temperature-dependent. Differential scanning calorimetry (DSC) was used for the quantitative determination of the amounts of freezing and non-freezing water. The electric properties and the stimuli-response of the same *IPN* hydrogel in electric fields have also been investigated [136,137]. When a swollen *Ch/PVA IPN* was placed between a pair of electrodes, the *IPN* exhibited bending behavior in response to the applied electric field. Both bending angle and bending speed of the *Ch/PVA IPN* increased with increasing the applied voltage. Moreover, the water sorption behavior of the *IPN* was measured at various temperatures and humidity levels. With increasing the temperature, the vapour sorption behavior was found to be affected more by the density of water vapour, than by the hydrophilic properties. In addition, the equilibrium water uptake increased with increasing humidity.

Wang et al. [138] prepared a semi-*IPN* from *Ch* and *PVA* with glutaraldehyde as the crosslinking agent. The chemical bonds formed through the crosslinking reaction and transitions of these bonds in different pH media were investigated. The gelation property of the *Ch/PVA* solution and the mechanical properties of the hydrogel were also studied. FTIR spectra of the hydrogel before and after swelling at pH 3 and pH 7 indicated the formation of Schiff's base (C=N) and  $\text{—NH}_3^+$ . They also showed a pH-induced transition of C=N to C—N, and  $\text{—NH}_3^+$  to  $\text{—NH}_2$ , as well as the instability of the Schiff's base. The addition of *PVA* improved the mechanical properties of the hydrogel. However, *PVA* tends to leach out at longer swelling times in the acidic medium due to hydrolysis of the Schiff's base of the hydrogel network.

Gupta et al. [139] prepared and studied a semi-*IPN* hydrogel of *Ch*/glycine crosslinked with different concentrations of glutaraldehyde for controlled release of chlorphenamine maleate (*antihistamine*). The structural and morphological studies of the hydrogel were carried out using FTIR and SEM techniques. The swelling behavior at different time intervals was monitored in solutions of different pHs. Moreover, the structural changes of the hydrogel in response to solution pH were put forward using the data obtained from the FTIR/UV spectral analysis. The results indicated that this *Ch*-based semi-*IPN*

hydrogel might be useful as a vehicle for controlled release of drugs.

In their studies on *Ch*-based hydrogels for biomedical and pharmaceutical applications, Shantha et al. [140] reported the preparation of hydrogels based on *Ch* grafted with poly(ethylene glycol) diacrylate (*PEGDA*) crosslinked with glutaraldehyde. The graft copolymer was prepared thermally in presence of ceric ammonium nitrate as initiator. Both thermal and morphological properties of the prepared hydrogel were investigated. Also, a procedure for preparing semi-*IPN* hydrogels based on *Ch* and *PEGDA* has been reported by Lee et al. [141]. The hydrogels were prepared by dissolving a mixture of *Ch* and *PEGDA* in 2% w/w aqueous acetic acid. The dried films produced from casting the *Ch/PEGDA* solutions were then crosslinked with the aid of 2,2-dimethoxy-2-phenylacetophenone, as a non-toxic photo initiator, by UV irradiation (450 watt UV lamp). The crystallinity, thermal and mechanical properties of the prepared semi-*IPN* hydrogels were also studied.

A semi-*IPN* hydrogel based on glutaraldehyde-crosslinked *Ch* with an interpenetrating polyether (*PEt*) polymer network has been prepared [142]. Also, the swelling, the release kinetics and the structural changes of the hydrogel were investigated in different buffer solutions [143,144]. It is known that the physico-chemical properties of a hydrogel depend on many parameters such as the hydrogel structure, the extent of crosslinking and also on both the content and state of the water in the hydrogel. Determination of the physical state of water in the hydrogels is of great importance to understand the nature of interactions between the polymers and the absorbed water. For this reason, Yao et al. [144] studied the state of water, the dynamic water absorption characteristics and the relationship between the state of water and the swelling profiles of the *Ch/PEt* semi-*IPN* hydrogels. The authors have applied various techniques including positron annihilation (PAS) lifetime spectroscopy and DSC. It has been found that the inclusion of water significantly affects the performance of the hydrogels.

In another study [86], Yao et al. investigated the pH-dependent hydrolysis of the *Ch/PEt* semi-*IPN* hydrogel. A rapid hydrolysis of the hydrogel was noticed with the decrease in the ionic strength. Also, the hydrolysis of the semi-*IPN* hydrogel was controlled by the amount of the applied crosslinking agent. The more crosslinking agent added, the higher the crosslinking density of the hydrogel, which leads to a lower

degree of swelling and subsequently slower hydrolysis [86]. Cimetidine (*anti-ulcer*) and chlorhexidini acetate (*anti-septic*) were used by Yao et al. [85] as model drugs for the drug release studies from the same type of *IPN*. It has been found that, a fast swelling of the hydrogels leads to higher extent of drug release at  $\text{pH} < 6$  in comparison to that at higher  $\text{pH}$  values. Recently, Wu et al. [145,146] have investigated the effect of temperature, mass ratio of *PEt* and *Ch* and the concentration of the crosslinking agent on the swelling ratio of *PEt/Ch* hydrogels. The result showed that the semi-*IPN* attained its maximum swelling ratio when the temperature was  $45\text{ }^{\circ}\text{C}$ , *PEt:Ch* was 0.4:1.0 and the concentration of the crosslinking agent was  $0.05\text{ mol/L}$ .

A salt and  $\text{pH}$ -sensitive *Ch/PAA* semi-*IPN* hydrogel membrane has been prepared and investigated [147]. Evidence from FTIR spectra proved the formation of polyelectrolyte complex through the electrostatic interaction between the  $\text{NH}_3^+$  groups, from *Ch* and the  $\text{COO}^-$  groups from *PAA*. The semi-*IPN* membrane exhibited a typical  $\text{pH}$ -sensitivity. The swelling degrees of the semi-*IPN* in different salt solutions with the same ionic valences and equal ionic strengths were on similar levels. Furthermore, the elongation of the semi-*IPN* membrane was found to vary reversibly by immersion into  $\text{CaCl}_2$  and  $\text{KCl}$  solutions alternately.

Lee et al. [148] have prepared a series of hydrogels based on *PAA*, *PNVP* and *Ch* by photopolymerization. The swelling behavior, gel strength, and drug release profile of the *PAA/PNVP* copolymeric hydrogels and the corresponding *IPN* hydrogels were investigated. Results showed that the swelling ratios decreased with the increasing of *NVP* content in the hydrogel. Increasing the *NVP* content also results in increasing the gel strength. Results also showed that the drug-release behavior from the hydrogels is related to both the ionic nature of the drug and the swelling ratio of the hydrogel. A  $\text{pH}$ -sensitive freeze-dried *Ch/PNVP* hydrogel, as a controlled release carrier for antibiotic delivery, was also developed in another study [149]. The hydrogels were synthesized by the crosslinking of *Ch* and *PNVP* blend with glutaraldehyde to form a semi-*IPN*. The semi-*IPN* formation was confirmed by FTIR analysis. The surface morphology, wettability, swelling properties and the  $\text{pH}$ -dependent swelling of both the air and the freeze-dried hydrogel membranes were compared. It was suggested that the increased swelling of the hydrogels, under acidic conditions, was due to the protonation of the primary amino groups on *Ch*, as confirmed by FTIR analysis. Also, it was reported that

the freeze-dried membranes released around 73% of the amoxicillin (33% if air-dried) in 3 h at pH 1.0 and thus had superior drug-release properties to the air-dried hydrogels. From the results obtained, it was concluded that the freeze-dried membranes based on *Ch/PNVP* semi-*IPN* hydrogels could serve as potent candidates for antibiotic delivery in an acidic environment.

A homogeneous *Ch*/amine oxide (*AO*) hydrogel has been prepared by Dutta et al. [150-152]. The swelling behavior of the hydrogel and its drug release profile in a buffer solution at pH 7.4, corresponding to that of the intestine, at room temperature were also investigated. Homogenous erosion of the matrix and a near zero-order release of ampicillin trihydrate were observed. In another study, the authors investigated the thermal properties of the same *Ch/AO* hydrogel [151].

A hydrogel based on chemically crosslinked *Ch*/gelatin with a glutaraldehyde hybrid polymer network was reported [153]. A drastic swelling of the hydrogel was noticed at acidic pHs in comparison to basic solutions. Some model drugs such as chloramphenicol (*anti-bacterial*), cimetidine (*anti-ulcer*) and levamisole (*anti-cancer*), were uploaded to the hydrogel for the controlled release study. The swelling extent of these hydrogels was investigated in different buffer solutions. The study showed a pH-dependent release of cimetidine, levamisole and chloramphenicol from the hydrogel.

An approach for the preparation of *Ch*-based hydrogel nanobeads has been reported [154]. The ionotropic gelation process used for the preparation of the beads was carried out in mild aqueous conditions at room temperature. The procedures involved a mixture of two aqueous solutions of the *Ch* and the diblock copolymer of ethylene oxide (*EO*) and sodium tripolyphosphate (*TPP*). Bovine serum albumin (*BSA*) was entrapped, as a model protein, inside the hydrogel beads. Release data showed that the beads have high protein entrapment efficiency (up to 80% of the *BSA*). Moreover, it has been found that these nanobeads can provide continuous release of the entrapped protein for up to 1 week.

Huguet et al. [155] reported two procedures for the encapsulation of haemoglobin into *Ch*-calcium alginate hydrogel beads. Both procedures lead to beads with great loading capacity of haemoglobin (more than 90% of the initial concentration, 150 g/l) provided

the *Ch* concentration is sufficient. It has been noticed that the release of haemoglobin in water during the bead storage depends on the molecular weight of *Ch*. Also, other reports [156-158,76] have discussed the swelling behavior and the encapsulation of some molecules of different molecular weights in the calcium alginate beads coated with *Ch*.

Production of multiporous *Ch* beads via simple coacervation of *Ch* solutions was extensively investigated [159,160]. The technique, in general, depends on blowing the aqueous acidic solution of *Ch* into a solution of coacervation agent such as NaOH, NaOH-methanol, or ethylenediamine solution to form coacervate drops. Several factors were found to control the diameter of the droplets including the nozzle diameter and the exclusion rate of the *Ch* solution. In addition, it has been noticed that, both the strength and the porosity of the beads are dependent on the N-deacetylation degree of *Ch* and also on the concentration of both *Ch* and coacervation agent solutions [160].

## THESIS OUTLINE

*Ch* possesses many superior properties such as biodegradability, biocompatibility and non-toxicity. These interesting properties make *Ch* an ideal candidate for the preparation of hydrogels for controlled drug release. However, as mentioned earlier, *Ch* exhibits some shortcomings including the hydrophobicity and the high pH-dependency of its physical properties. As a consequent, it is very difficult to control drug release with *Ch* itself because of the various pHs of the internal organs of the human body. This may negatively affect the human body because of drug over-release [131]. For this reason, in this thesis, three main approaches have been applied to overcome the shortcomings of *Ch*. The first approach was the incorporation of *Ch* in an *IPN* hydrogel with either hydrophilic polymers of good mechanical properties (such as *PVA*, **Chapter 2**) or with hydrophilic monomers (such as N-acryloylglycine, *NAGly* and/or *PEGDA*, **Chapters 3 and 4**) treated to bring about in situ copolymerization in the presence of *Ch* and a suitable crosslinking agent. The second approach was the chemical modification of *Ch* by grafting of a suitable vinyl monomer (such as *PEGDA*) onto the *Ch* backbone, then crosslinking this modified *Ch* (**Chapter 5**). All the hydrogels developed using these two approaches (**Chapter 2 to Chapter 5**) were pH-responsive and swelled faster and higher at pH 2.1 (simulated gastric fluid, *SGF*) than at pH 7.4 (simulated intestinal fluid, *SIF*). 5-Fluorouracil (*5-FU*), an anti cancer drug, was entrapped as a model drug in all the hydrogels prepared using these two approaches. The *in-vitro* drug release studies were carried out at 37 °C in *SGF* and *SIF*. From these preliminary investigations, these hydrogels may be tailored and exploited to expand the utilization of these systems in drug delivery applications. In the third approach an attempt was carried out to modify *Ch* in such a fashion that the hydrogels produced were also pH-responsive but swelled higher in *SIF* than in *SGF* (**Chapter 6**). Hence, the resulting hydrogels could be tailored for utilization for intestine-targeted delivery of protein drugs. In this third approach *Ch* was chemically modified via carboxymethylation. Then, ionotropic gelation was carried out for this modified *Ch* with another suitable natural polymer (alginate) in the presence of a divalent ion (**Chapter 6**). Bovine serum albumin (*BSA*), a model protein drug was entrapped in the hydrogels prepared using the third approach and the *in-vitro* drug release profiles were established at 37 °C in *SGF* and *SIF*.

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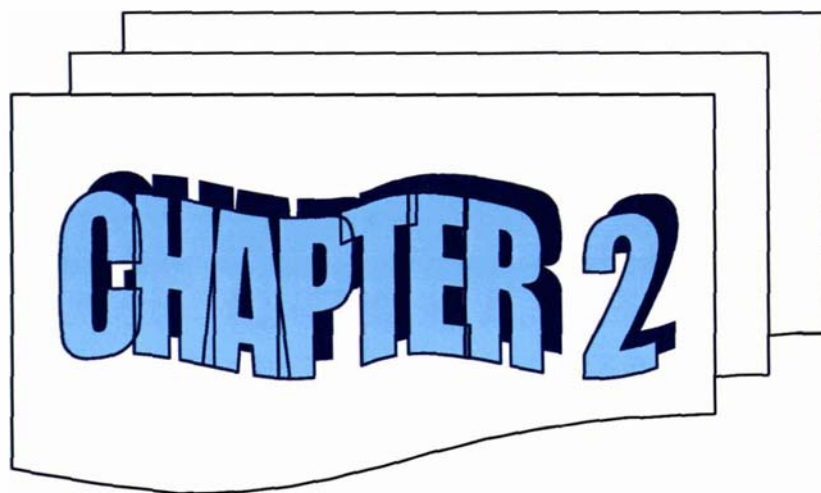
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**CHAPTER 2**

## CHAPTER 2

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### pH-RESPONSIVE SEMI-INTERPENETRATING POLYMERIC HYDROGELS BASED ON CHITOSAN AND POLY(VINYL ALCOHOL) FOR *IN-VITRO* CONTROLLED RELEASE STUDY.<sup>a-c</sup>

#### ABSTRACT

Two series of pH-responsive biodegradable semi-interpenetrating polymeric (semi-*IPN*) hydrogels based on chitosan (*Ch*) and poly(vinyl alcohol) (*PVA*) were prepared and characterized for controlled drug release investigations. The first series was chemically crosslinked with different concentrations of glutaraldehyde and the second was crosslinked by different doses of  $\gamma$ -irradiation. The equilibrium swelling characteristics were investigated for the hydrogels at 37 °C in buffer solutions of pH 2.1 (simulated gastric fluid, *SGF*) and pH 7.4 (simulated intestinal fluid, *SIF*). 5-Fluorouracil (*5-FU*) was entrapped in the hydrogels, as a model therapeutic agent, and the *in-vitro* release profiles of the drug were established at 37 °C at pH 2.1 and 7.4. FTIR, SEM and 2D-XRD were used to characterize and investigate the structural changes of the hydrogels with the variation of the hydrogel composition and the content of the crosslinking agent before and after the drug loading.

**Keywords:** hydrogel, controlled drug release, gelation, irradiation

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## 2.1. INTRODUCTION

Characteristics of interpenetrating polymeric (*IPN*) hydrogels such as degree of swelling, soluble fraction and cumulative release profile of an entrapped bioactive material depend mainly on the type and the amount of monomers used for the preparation of these *IPNs*. Also, these characteristics depend on the type and the degree of crosslinking [1,2]. Different hydrogel matrices of precisely defined structure have been prepared and used as drug delivery systems [3, 4].

Chitosan (*Ch*) is a cellulose-like polymer that is produced by the alkaline deacetylation of natural chitin [5-9]. *Ch* may refer to a large number of polymers with different degrees of N-deacetylation (40-98%) and molecular weights ( $5 \times 10^4$ – $2 \times 10^6$  Da). These two characteristics are very important and strongly affect the physico-chemical properties and consequently the biological properties of *Ch* [10]. *Ch* exhibits many desirable biological properties such as biocompatibility, biodegradability and non-toxicity [9]. Moreover, *Ch* has good film forming ability from dilute acetic acid solutions using a casting technique. However, *Ch* exhibits limitations in its reactivity and processing ability. For this reason, many attempts have been reported to overcome such limitations by chemical or physical modification through incorporation of *Ch* with different polymers in the *IPNs*. From a number of polymers, *PVA* which has many superior properties such as water solubility, high hydrophilicity and good film forming ability by solution casting [11] was chosen for the study discussed in this chapter.

Glutaraldehyde is a common crosslinking agent used in crosslinking polypeptides, proteins, *Ch* and some polysaccharides such as heparin and hyaluronic acid [12]. In spite of the many appealing properties of both *Ch* and *PVA* especially their good film forming ability, little work has been reported to date regarding *IPN* hydrogel films based on *Ch* and *PVA* combinations as drug delivery matrices [13].

Irradiation, especially if combined with simultaneous sterilization of the product, is a very convenient tool for the synthesis of hydrogels. Radiation processing has several advantages over other conventional methods [14]. In radiation processing, no catalysts or additives are needed to initiate the reaction. Also, radiation methods are relatively simple and the degree of crosslinking, which strongly determines the swelling extent of

hydrogels, can be controlled easily by varying the radiation dose [15, 16]. Therefore, these methods are found to be very useful in preparing hydrogels for medical applications, where even the slightest contamination is undesirable [17]. There are several published articles discussing the  $\gamma$ -irradiation of wide spectrum of polymers including *PVA* [18].

In this chapter, different semi-*IPN* hydrogel films based on *Ch* and *PVA* crosslinked either chemically with glutaraldehyde or by  $\gamma$ -irradiation were prepared and characterized. Then the equilibrium swelling and the *in-vitro* cumulative release of *5-FU*, as a model drug, were investigated.

## **2.2. MATERIALS AND METHODS**

### **2.2.1. MATERIALS**

*Ch*, 98% hydrolyzed *PVA* and *5-FU* were purchased from Aldrich (Milwaukee, WI, USA). *Ch* was further purified by dissolving it in 2% acetic acid solution to make a solution of 1% (w/v) final concentration then filtering under suction. The homogeneous clear viscous solution was then re-precipitated by the addition of 1M NaOH solution. The precipitated *Ch* was repeatedly washed with hot distilled water and then dried in a vacuum oven at 25 °C for two days. Glutaraldehyde (25% aqueous solution) was obtained from Sigma Chemical Co. (St. Louis, USA). Acetic acid and all other reagents were of analytical grade and used as received.

### **2.2.2 METHODS**

#### **2.2.2.1 Characterization of *Ch***

The % N-deacetylation of the *Ch* under investigation was determined with aid of FTIR analysis using the following relationship [19]:

$$\% \text{ N-deacetylation} = 100 [1 - (A_{1655}/A_{3340}) (1/1.33)]$$

where,  $A$  is the absorbance at the given wave number. These two absorption signals (1655 and 3340  $\text{cm}^{-1}$ ) correspond to the amide and the primary amino groups of *Ch* respectively. The factor (1.33) represents the value of the ratio of  $A_{1655} / A_{3340}$  for the fully N-acetylated *Ch*. The sample was prepared for the FTIR scanning by grinding 2 mg of *Ch* into fine powder together with 200 mg of analytical grade KBr in a glass mortar. The powder was then loaded into an evacuable die and compressed to 10 MPa for 1 min. to obtain a thin transparent disk. Elemental analysis was also used to determine the degree of N-deacetylation of *Ch* based on the mole fraction concept [20].

The average molecular weight ( $M_w$ ) of *Ch* was determined using the Mark-Houwink viscometry method [21]. The *Ch* (100 mg) was dissolved in 100 ml of mixed solvent (0.1 M acetic acid/0.2 M NaCl) to give a 0.1% (w/v) final concentration. The solvent was prepared by addition of glacial acetic acid (571  $\mu\text{l}$ , 17.5 M) to a solution of NaCl (1.17 g in 100 ml distilled water). After maintaining the *Ch* solution overnight at room temperature, it was filtered through glass wool to remove any insoluble residuals. Then, five diluted *Ch* concentrations, 0.02%, 0.04%, 0.06%, 0.08% and 0.10% (w/v) were prepared by transferring 5, 10, 15, 20 and 25 ml of the master *Ch* solution into 25 ml volumetric flasks and completing to the mark with the solvent. The solutions were maintained at 25 °C by using a water bath (Grant Instruments, Cambridge Ltd, Shepreth Cambridgeshire, SG8 6GB, England). The Cannon-Fenske Routine Viscometer (Cannon Instrument Co, State College, PA. 16801, USA) was used for measuring the efflux times of 3 ml aliquots of the solvent and the *Ch* solutions. Each sample was measured three times. The viscosities were calculated using the following relationships:

$$\eta_r = t/t_0$$

$$\eta_{sp} = \eta_r - 1$$

$$\eta_{red} = \eta_{sp} / C$$

where,  $t_0$  and  $t$  are the efflux times for the solvent and the *Ch* solution respectively.  $\eta_r$ ,  $\eta_{sp}$  and  $\eta_{red}$  are the relative, specific and reduced viscosities respectively.  $C$  is the concentration of *Ch* solution. The Y-intercept obtained upon plotting the  $\eta_{red}$  values *versus* the corresponding solution concentrations represents the value of the intrinsic viscosity  $[\eta]$  of *Ch*, from which the molecular weight can be estimated [21].

### **2.2.2.2 Film preparation (Casting technique)**

#### **(A) Preparation of *Ch* and *Ch/PVA* films**

For preparation of the *Ch/PVA* thin films (ChVA1-ChVA3, **Table 2.1**), a predetermined amount of *PVA* was dissolved in deionized water by heating to 80 °C with stirring for 30 min. until a 10% *PVA* solution was obtained. The weighed *Ch* sample was dissolved in 2% acetic acid solution to prepare 1.5% *Ch* solution. The *Ch* solution was then added to the *PVA* solution and the mixture was stirred for 10 min. Then the mixture was poured into a Petri dish and left to dry for two days at room temperature then completely dried in a vacuum oven at 20 °C. In the case of preparations of the *Ch* hydrogel films (CCh1-CCh4, **Table 2.1**), a 5 ml of distilled water containing the predetermined volumes of glutaraldehyde (25% aqueous solution) were added to the 1.5% *Ch* solution. Then the mixture was stirred for 2 min. and then poured into a Petri dish to undergo gelation at room temperature. The thickness of the obtained films was in the range of 0.2-0.6 mm. The compositions of the prepared films are shown in **Table 2.1**.

#### **(B) Preparation of *Ch/PVA IPN* hydrogel films**

##### **▣ *Using glutaraldehyde as a crosslinking agent***

The chemically crosslinked *Ch/PVA* hydrogel films (CVA1-CVA6, **Table 2.1**) were prepared by the same manner as ChVA1-ChVA3 but with addition of 5 ml of distilled water containing the appropriate volumes of the glutaraldehyde (25% aqueous solution) to the *Ch/PVA* mixture and continuing the stirring for further 2 min. before pouring the whole mixture into the Petri dish. The mixture was left to undergo gelation at room temperature. The gelation time ranged from 1 h to 4 h depending on the amount of glutaraldehyde.

##### **▣ *Using $\gamma$ -irradiation***

After the preparation of *Ch/PVA* films with the predetermined blend ratios using casting technique as described before, the films were  $\gamma$ -irradiated to the desired doses (10 or 20 KGy). The strips of the dried films were placed in the  $\gamma$ -irradiation chamber in

such a way that each one was exposed to the same dose. Irradiation was carried out under air atmosphere at a dose rate of 6.92 KGy/h. The compositions of all the prepared films are represented in **Table 2.1**.

### **2.2.2.3 Determination of the gel fraction of the irradiated Ch/PVA films**

After irradiation, a weighed piece of the film was soaked in 2% aqueous acetic acid solution at room temperature for 48 h and then washed with water for 10 min. After washing, the sample was put into distilled water for 24 h at 50 °C. Then, the remaining gel portion was dried to constant weight at 20 °C under vacuum. The gel fraction was determined gravimetrically using the relation;

$$\text{Gel Fraction (\%)} = 100 (W_g / W_o)$$

where  $W_o$  and  $W_g$  are the weights of the initial film sample and that of the dried gel, after the extraction of the soluble part, respectively. The data represents the mean values of gel fraction of 3 pieces of each hydrogel film with  $\pm$ SD values.

### **2.2.2.4 Characterizations**

The FTIR of the dried hydrogel films was recorded with a Perkin Elmer Paragon 1000 FTIR spectrometer in the range 4000-400  $\text{cm}^{-1}$ . The elemental analysis was performed with Carlo Erba Elemental Analyser EA 1108 using a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand). The  $\gamma$ -irradiation of the polymer films was carried out in a  $^{60}\text{Co}$  gamma cell (made in Russia) at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The surface morphology of the films before and after 24 h of *in-vitro* release of **5-FU** in buffer of pH 7.4 was investigated by scanning electron microscope (Cambridge Stereoscan S-250 mk 3 SEM, Hort Research, Palmerston North, New Zealand). Samples were placed on an aluminium mount, sputtered with gold using Baltec. scd. 050 sputter coater, and then scanned at an accelerating voltage of 20 KV. The

**Table 2.1.** Compositions of *Ch* and *Ch/PVA* blend films.

Type	Sample Code	<i>Ch</i>		<i>PVA</i>		Glutaraldehyde		$\gamma$ -irradiation dose (KGy)
		<i>g</i>	%	<i>g</i>	%	<i>ml</i>	%	
<i>Ch/PVA</i> Blend films	Ch	2.0	100	-	-	-	-	-
	ChVA1	1.0	50	1.0	50	-	-	-
	ChVA2	0.8	40	1.2	60	-	-	-
	ChVA3	0.5	25	1.5	75	-	-	-
Chemically crosslinked <i>Ch</i> films	CCh1	2.0	100	-	-	0.4	5	-
	CCh2	2.0	100	-	-	0.8	10	-
	CCh3	2.0	100	-	-	2.0	25	-
	CCh4	2.0	100	-	-	2.8	35	-
Chemically crosslinked <i>Ch/PVA</i> films	CVA1	1.0	50	1.0	50	0.4	5	-
	CVA2	1.0	50	1.0	50	0.8	10	-
	CVA3	1.0	50	1.0	50	2.0	25	-
	CVA4	1.0	50	1.0	50	2.8	35	-
	CVA5	0.8	40	1.2	60	2.0	25	-
	CVA6	0.5	25	1.5	75	2.0	25	-
Irradiation crosslinked <i>Ch/PVA</i> films	GCV1	1.0	50	1.0	50	-	-	10
	GCV2	0.8	40	1.2	60	-	-	10
	GCV3	0.5	25	1.5	75	-	-	10
	RCV1	1.0	50	1.0	50	-	-	20
	RCV2	0.8	40	1.2	60	-	-	20
	RCV3	0.5	25	1.5	75	-	-	20

crystallography patterns of the polymer films were investigated using 2D-XRD equipment (Rigaku Micro Max 007 microfocus imitating anode X-ray generator (Cu K $\alpha$ ) coupled with Osmic “Blue” confocal optics and a Rigaku RAxis (VI++) image-plate detector (Massey University, New Zealand). Images were recorded and analysed with Crystal Clear (1.3.6-SPI, Pflugrath, JW, 1999, Acta Crystallogr. D50 1718-1725). The film specimens were mounted in such a way that the X-ray beam was directed to the flat surface.

#### **2.2.2.5 Entrapment of a model drug**

The films of *Ch* and *Ch/PVA* loaded with *5-FU*, as a model drug, were prepared in the same manner as discussed above. Known amounts of the drug (24 mg g<sup>-1</sup> matrix) were added to the reaction mixture, stirred vigorously and then the whole mixture was poured into a Petri dish until complete gelation. In the case of films crosslinked using  $\gamma$ -irradiation, the dry drug-loaded films were irradiated at the required dose. The drug-loaded films were then washed with distilled water, dried under vacuum at 20 °C and stored until further use.

#### **2.2.2.6 Determination of the amount of drug entrapped**

The amount of drug entrapped in the prepared films was determined by an indirect method [22]. After the film preparation, the washings were collected, filtered with a 0.45 mm Millipore filter (Bedford, MA) and tested at  $\lambda_{\text{max}}$  of 268 nm corresponding to *5-FU* using Hewlett Packard UV-VIS. spectrophotometer. Both the entrapped and the free drug exhibited the same  $\lambda_{\text{max}}$ . This tends to indicate that the entrapped drug has not undergone chemical reactions during the hydrogel films formation. The difference between the amount of drug initially used (24 mg g<sup>-1</sup> matrix) and the drug content in the washings was taken as a measure of the amount of drug entrapped.

#### **2.2.2.7 Equilibrium swelling studies**

The swelling behavior of *Ch* and *Ch/PVA* films was measured at 37 °C in two buffer solutions of pH 2.1 (*SGF*) and 7.4 (*SIF*). The buffer solutions were prepared from a mixture of phosphoric acid (54 mmol), boric acid (40 mmol) and acetic acid (42 mmol) then adjusting the pH to the required value by the dropwise addition of 0.2 N NaOH solution. The pH values were precisely checked by a pH-meter (PHM82/ STANDARD,

accuracy  $\pm 0.1$ ). The swollen weights, after removal of the surface liquid with a tissue paper, were determined at certain time intervals until equilibrium swelling was attained. The data represents mean  $\pm$ SD from three independent experiments. The percent swelling was calculated by the following equation:

$$\% \text{ Swelling} = 100 [(W_t - W_0)/W_0]$$

where,  $W_0$  is the initial weight and  $W_t$  is the final weight of the swelled hydrogel films at time  $t$ .

### 2.2.2.8 *In-vitro* cumulative release studies

The *in-vitro* release of the entrapped *5-FU* drug was carried out by placing a pre-weighed piece of the hydrogel film loaded with the drug in a buffer solution of pH 2.1 (*SGF*) and pH 7.4 (*SIF*) at 37 °C. The study was carried out in a Grant shaking water-bath (Grant Instruments, Cambridge Ltd, Shepreth Cambridgeshire, SG8 6GB, England). At periodic intervals 3 ml sample was withdrawn and measured at  $\lambda_{\text{max}}$  of 268 nm corresponding to *5-FU* using Hewlett Packard UV-VIS spectrophotometer. The withdrawn 3 ml aliquot was replaced each time with an equal volume of fresh buffer of the same pH to keep the volume of release medium constant. The data represents mean  $\pm$ SD from three independent experiments.

## 2.3. RESULTS AND DISCUSSION

### 2.3.1. *Structure investigations*

#### 2.3.1.1. Degree of N-deacetylation of *Ch*

The degree of N-deacetylation of *Ch* refers to the percentage of primary amino groups in the total glycosaminoglycans after the chemical or enzymatic N-deacetylation of natural chitin. It is one of the most significant characteristics that affect the functional properties of a particular *Ch*. As the N-deacetylation % increases, the acid solubility, the metal ion uptake capacity and the over all reactivity of *Ch* increases. This is due to the

higher reactivity of the primary amino groups. FTIR is one of the best tools to quantify the N-deacetylation % of **Ch**. From FTIR, the N-deacetylation % of the **Ch**, used in this study, was found to be 67.2 % which is nearly similar to that estimated from the elemental analysis data of **Ch** (C%: 44.80; N%: 7.86; H%: 7.02) based on the mole fraction concept [20].

### 2.3.1.2. Average molecular weight of Ch

Molecular weight is also one of the significant characteristics that control the functional properties of **Ch**. Viscosity is one of the simple techniques that is widely used for estimation of the molecular weights of polymers. For a linear chain polymer the relationship between the viscosity average molecular weight,  $M_w$  and the intrinsic viscosity,  $[\eta]$  is given by the Mark-Houwink equation [23,24] as follows:

$$[\eta] = k M_w^a$$

where,  $k$  and  $a$  are constants independent of  $M_w$  over a wide range. They are affected by the solvent conditions such as pH, ionic strength and temperature. In case of **Ch**, dissolved in (0.1 M acetic acid/0.2 M NaCl), these constants were determined at 25 °C to be  $k = 1.81 \times 10^{-3}$  and  $a = 0.93$  [21,24]. For the **Ch** used in this study,  $[\eta]$  was experimentally determined, as in **Figure 2.1**, from the Y-intercept of the plot of  $\eta_{red}$  versus the concentration of the different **Ch** solutions. From **Figure 2.1**,  $[\eta]$  is 355.8 ml g<sup>-1</sup> and by applying the Mark-Houwink equation, the estimated  $M_w$  was found to be 492 kDa.

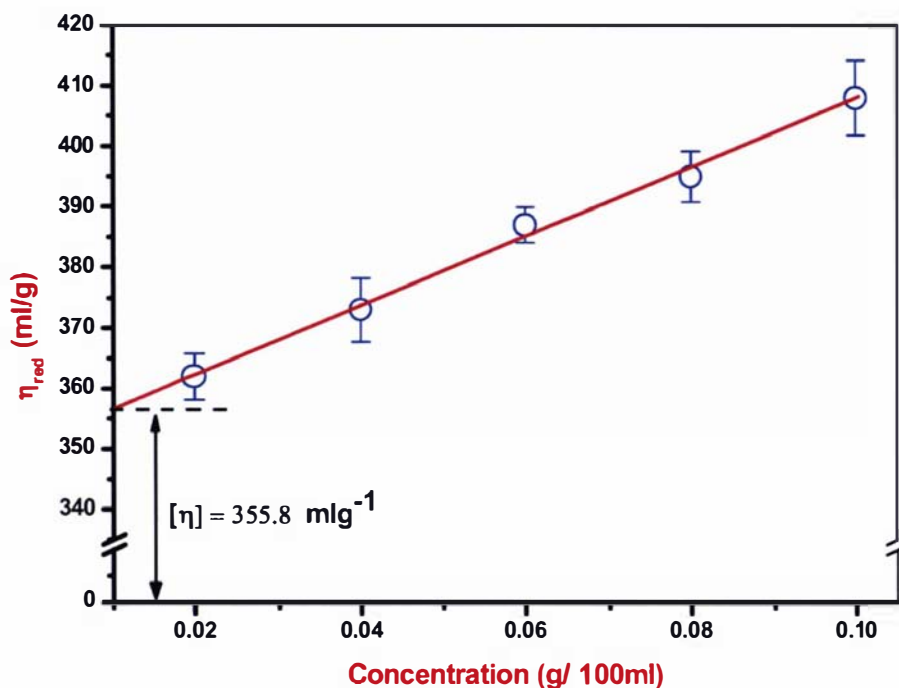


Figure 2.1. Determination of  $[\eta]$  and  $M_w$  of *Ch*.

### 2.3.1.3. FTIR investigation

Figure 2.2 shows the FTIR spectrum of *Ch*. From this figure, the signals appearing at  $907\text{ cm}^{-1}$  and  $1156\text{ cm}^{-1}$  are related to the saccharide structure [25]. The strong absorption peaks at  $1654\text{ cm}^{-1}$  and  $1321\text{ cm}^{-1}$ , which are characteristic of chitin and *Ch* correspond to both the amide C=O stretching vibration and the amide C-H deformation respectively [25]. The sharp absorption signal appearing at  $1383\text{ cm}^{-1}$  is assigned to the  $\text{CH}_3$  symmetrical deformation mode. The strong peak at  $1026\text{ cm}^{-1}$  belongs to the C-O stretching vibration. The absorption peaks at around  $2854\text{ cm}^{-1}$  and  $2923\text{ cm}^{-1}$  are caused by C-H stretching vibrations while the broad signal appeared at about  $3426\text{ cm}^{-1}$  is typical for both N-H and O-H stretching vibrations.

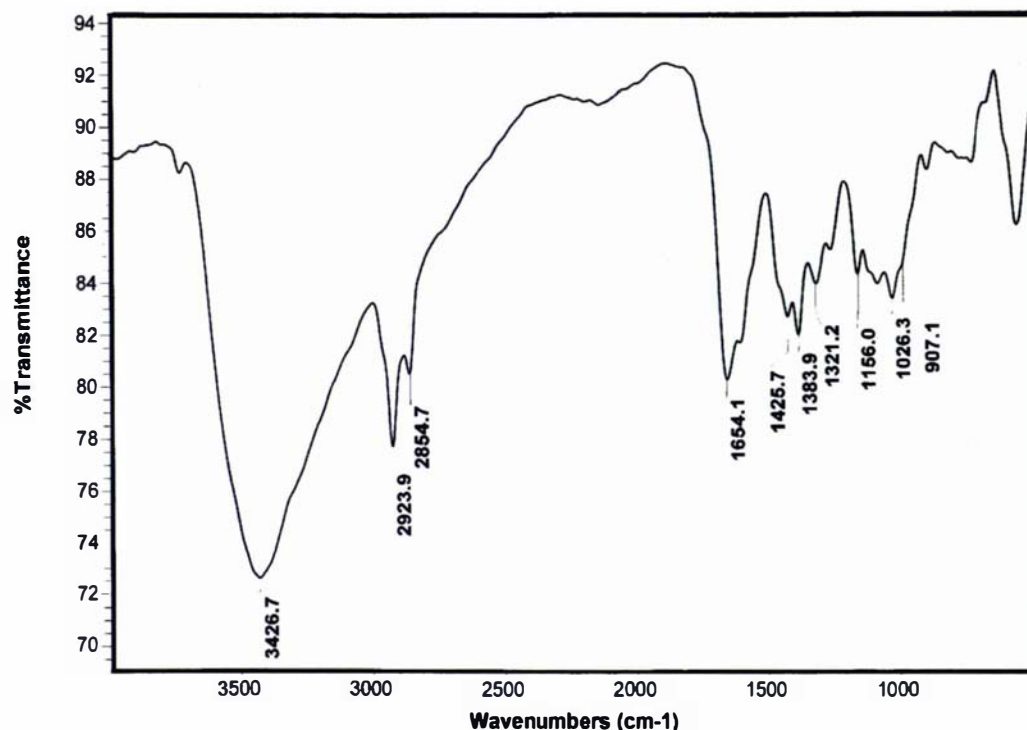
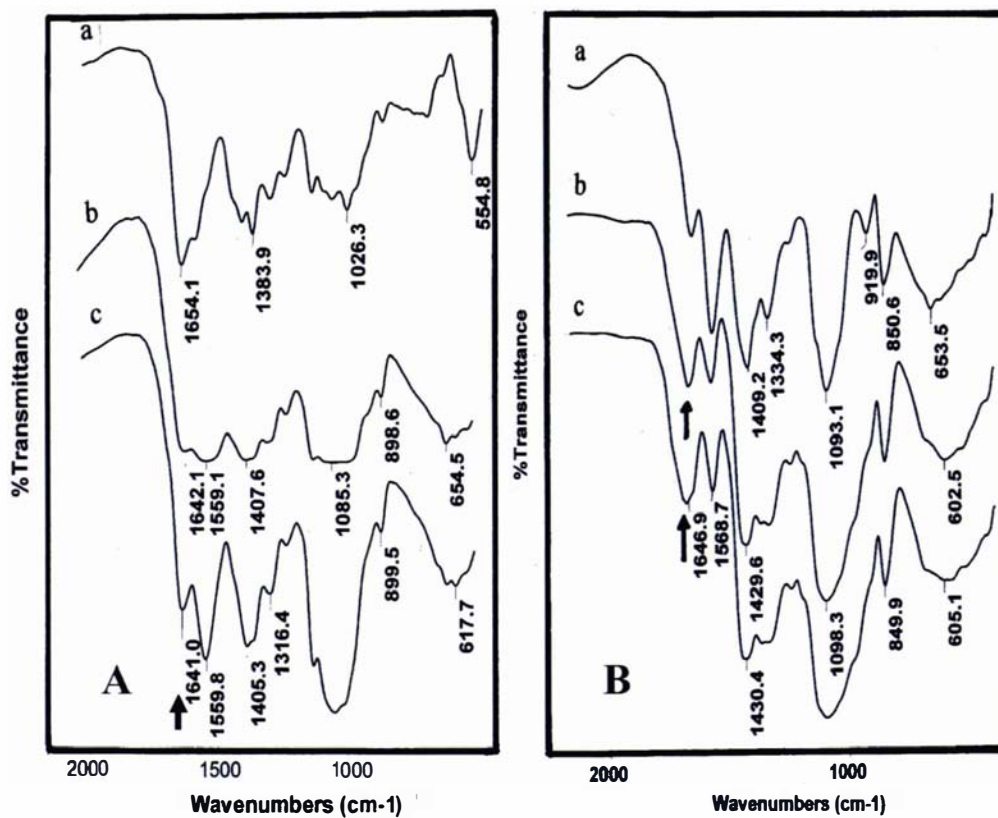


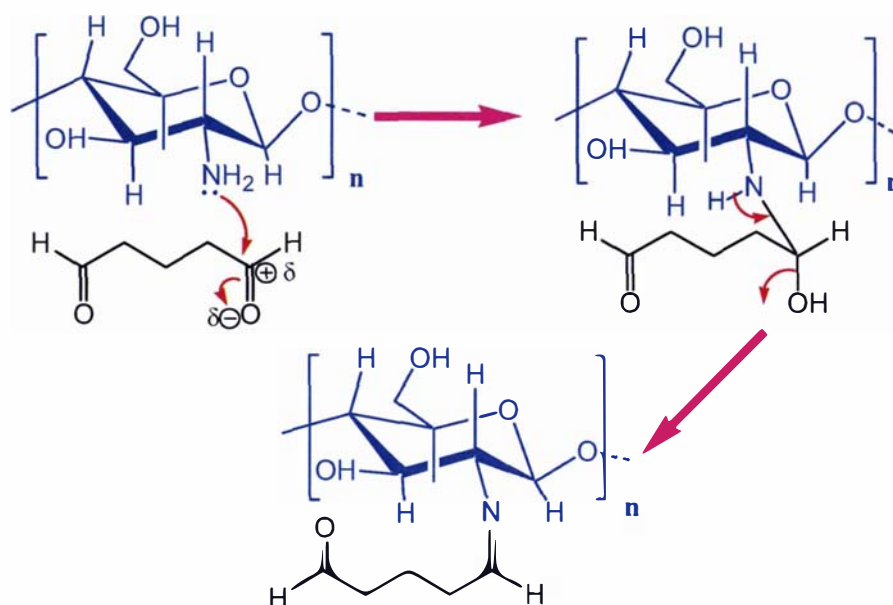
Figure 2.2. FTIR spectrum of *Ch*.

Glutaraldehyde-crosslinked *Ch* (CCh1-CCh4, see Table 2.1) showed a new peak at about  $1642\text{ cm}^{-1}$  due to the formation of an imine bond ( $\text{C}=\text{N}$ ) upon the reaction of the amino groups of *Ch* with the aldehyde groups of glutaraldehyde. This peak becomes stronger and sharper as the glutaraldehyde content increases. Figure 2.3A shows FTIR spectra of *Ch* in comparison with that of *Ch*-crosslinked with 5% (CCh1) and 25% (CCh3) glutaraldehyde as examples. This holds also true for *Ch/PVA* films (ChVA1) compared with that chemically crosslinked with 5% (CVA1) and 25% (CVA3) of glutaraldehyde. As shown in Figure 2.3B, a new significant signal appeared at  $1646\text{ cm}^{-1}$  assigned for  $\text{C}=\text{N}$  bonding formed upon condensation of amino groups of *Ch* with glutaraldehyde which strengthened as glutaraldehyde content increases.



**Figure 2.3.** FTIR spectra of chemically crosslinked films (A) *Ch*: a) *Ch*, b) CChI and c) CCh3; (B) *Ch/PVA*: a) ChVA1, b) CVA1 and c) CVA3.

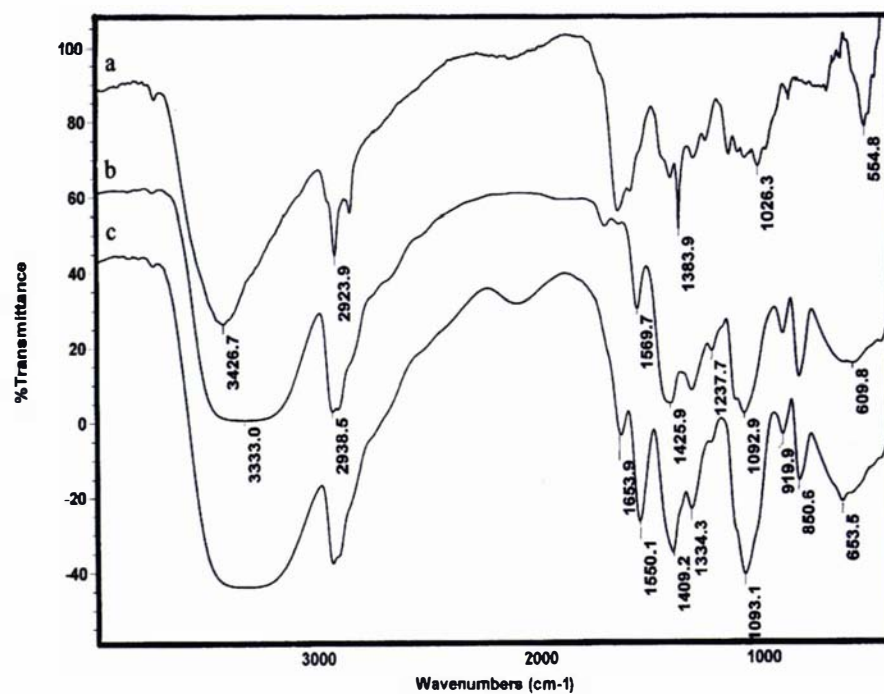
It has been found [25], upon investigation of the *Ch/PVA* blend crosslinked with glutaraldehyde, that almost no acetal or hemiacetal products of possible crosslinking reaction between the *PVA* and glutaraldehyde were formed. This also can be confirmed by the absence of acetal and hemiacetal characteristic peaks (Figure 2.3B) located at 1140-1190 cm<sup>-1</sup> and 1035-1060 cm<sup>-1</sup>. This tends to suggest that the crosslinking reaction might occur only between glutaraldehyde and the *Ch* molecules. The mechanism of this crosslinking is illustrated in Scheme 2.1. According to this mechanism, a nucleophilic attack of the nitrogen atom of -NH<sub>2</sub> group occurs onto the electron deficient carbon atom of the aldehydic C=O group and results in the loss of a water molecule leading to formation of the imine bond (C=N, Schiff's base).



**Scheme 2.1.** Mechanism of formation of the Schiff's base (C=N) between the amino groups of *Ch* and the aldehyde groups of glutaraldehyde.

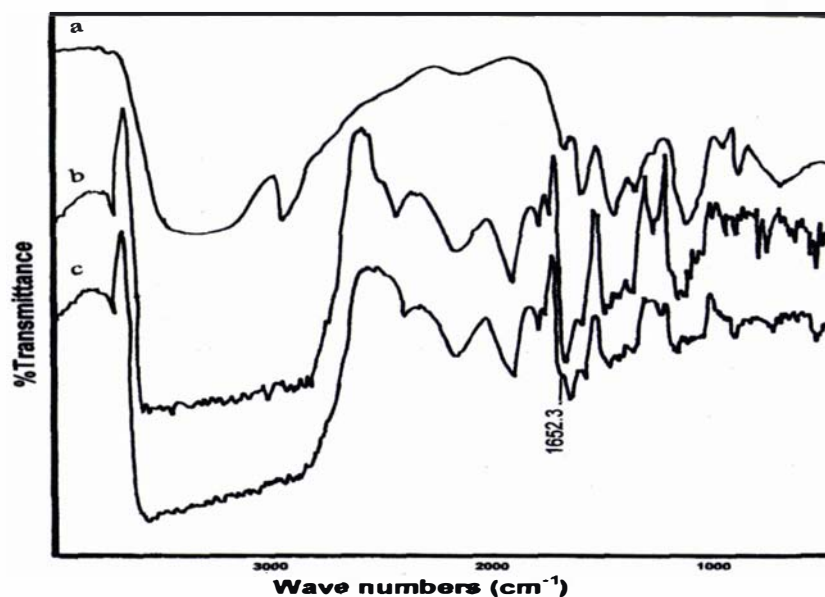
The FTIR spectrum of ChVA1 film in comparison with that of *Ch* and *PVA* is shown in **Figure 2.4**. The differences that appear between the blend spectrum and the spectra of the single polymers can be attributed to the ionization of the primary amino groups in the *Ch/PVA* (ChVA1) films during the film preparation as *Ch* was dissolved in acetic acid solution [25]. This ionization of the amino groups ( $-\text{NH}_3^+$ ), usually appears in the range  $1548\text{--}1560\text{ cm}^{-1}$ , representing the symmetrical deformation of  $-\text{NH}_3^+$ . It is known that the existence of hydrogen bonding changes the position of the absorption band due to O-H stretching from  $3600\text{ cm}^{-1}$  for compounds of no or less extensive hydrogen bonding to lower values (close to  $3330\text{ cm}^{-1}$ ) for those with extensive hydrogen bonding [26]. As shown in **Figure 2.4**, it can be noted that both FTIR spectra for pure *Ch* and *PVA* show the existence of extensive hydrogen bonding in the range of  $3300\text{--}3500\text{ cm}^{-1}$ . However, it seems that the *Ch/PVA* polymer blend (ChVA1) possess relatively higher extents of hydrogen bonding than the *Ch* and *PVA* polymers alone.

The intensity of the OH band decreased upon irradiation of the *Ch/PVA* blend films up to 20 KGy compared with the non-irradiated films and it is inversely related to the irradiation dose as shown in **Figure 2.5** which is in agreement with the literature [26].



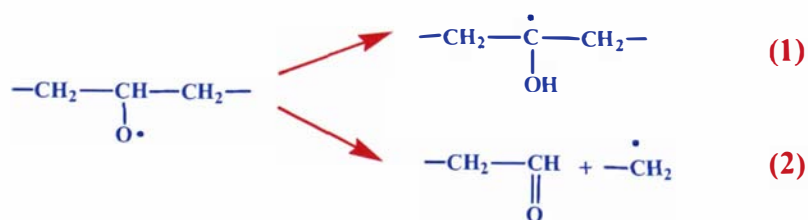
**Figure 2.4.** FTIR spectrum of a) *Ch*, b) *PVA* and c) *ChVAI* films.

During irradiation, radicals are formed on the macromolecules leading to a type of self-crosslinking for *PVA* chains [27] accompanied by degradation of *Ch* chains. The extent of these effects depends mainly on the irradiation dose.



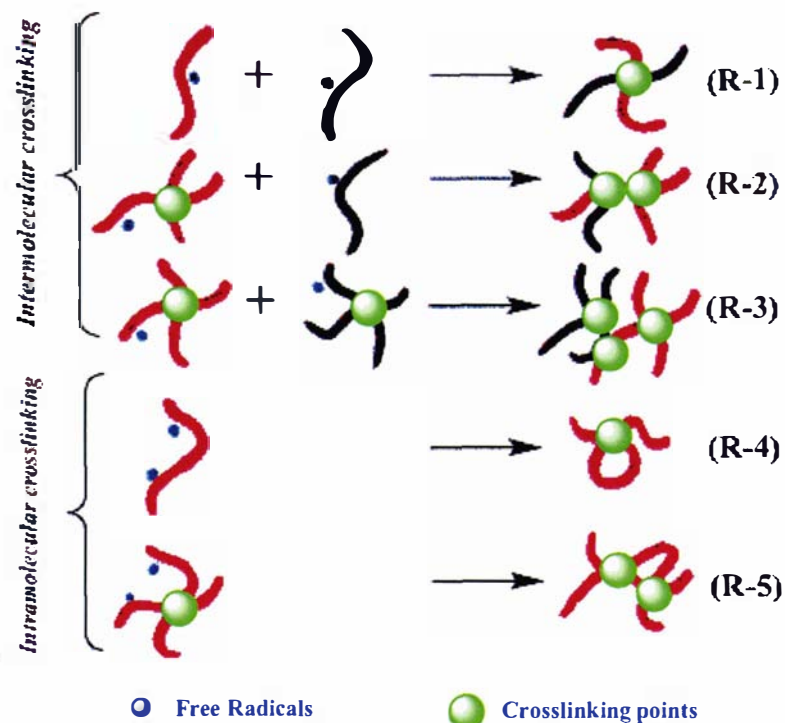
**Figure 2.5.** FTIR spectrum of ChVA1 films a) non-irradiated b) irradiated (10KGy) and c) irradiated (20KGy).

It has been suggested [28] that the alkoxy radicals resulting from  $\gamma$ -irradiation of *PVA* decay mainly by one of two routes, the first leads to formation of  $\alpha$ -carbon radicals and the second leads to the formation of alkyl radicals and an aldehyde as shown in **Scheme 2.2**.



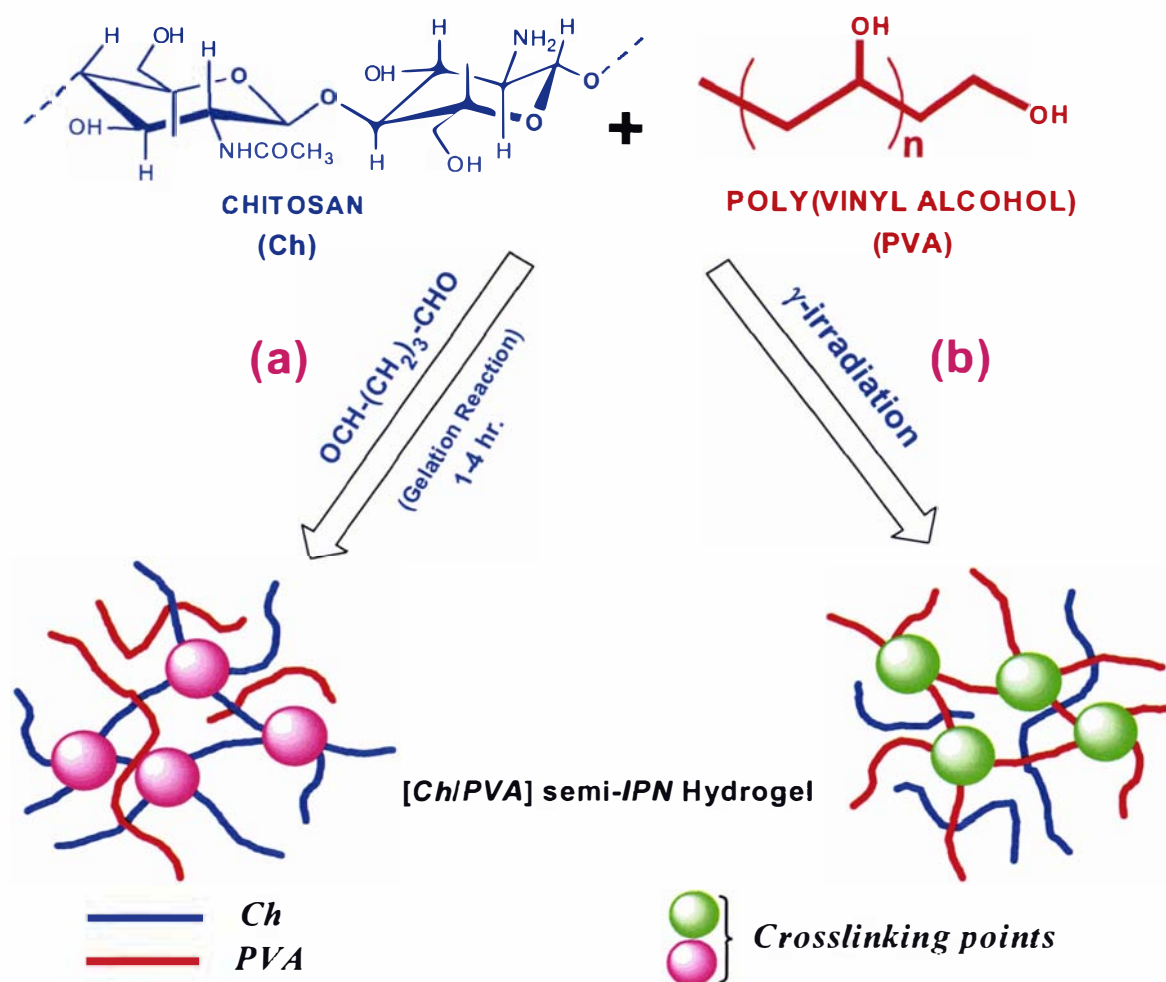
**Scheme 2.2.** The decay products of the alkoxy radicals resulted from  $\gamma$ -irradiation of *PVA*.

The chain end alkyl radical formed in equation (2) can readily undergo an abstraction reaction to form the  $\alpha$ -carbon radical. Also it has been proposed that the networks (hydrogels) resulted from  $\gamma$ -irradiation of *PVA* and other polymers are yielded via the formation of branched polymers as the precursor. In principle, five crosslinking reactions were predicted as shown in **Scheme 2.3** [29].



**Scheme 2.3.** Schematic representation of possible crosslinking reactions occurring for *PVA* polymer upon  $\gamma$ -irradiation [29].

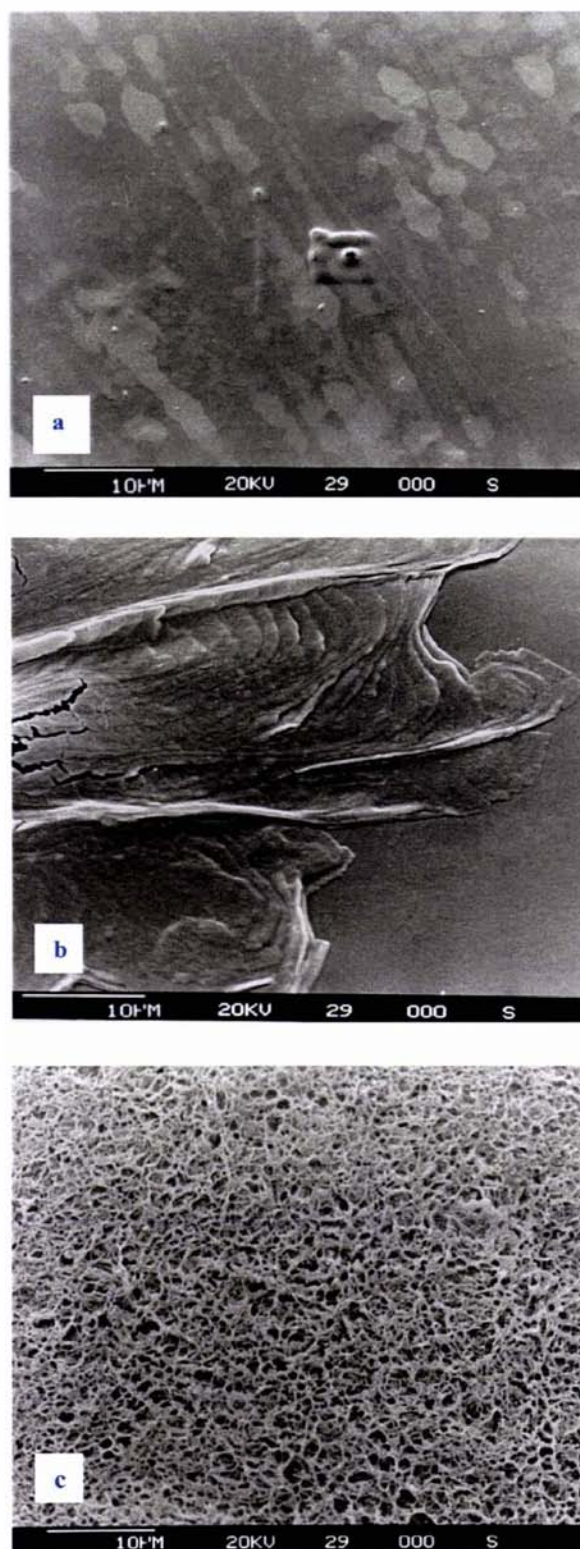
The reactions (R-1) to (R-3) [29] represent the intermolecular crosslinking by which both molar mass and size of the resulting polymer matrix will increase, while (R-4) and (R-5) are intramolecular crosslinking causing the contraction of a linear or branched polymer chain. The overall schematic representation of the semi-*IPN* hydrogels resulting from chemical crosslinking and  $\gamma$ -irradiation of *Ch/PVA* films are shown in **Scheme 2.4**.



**Scheme 2.4.** Schematic representation of *Ch/PVA* semi-IPN hydrogels resulting from (a) chemical crosslinking and (b)  $\gamma$ -irradiation.

#### 2.3.1.4. The SEM investigation

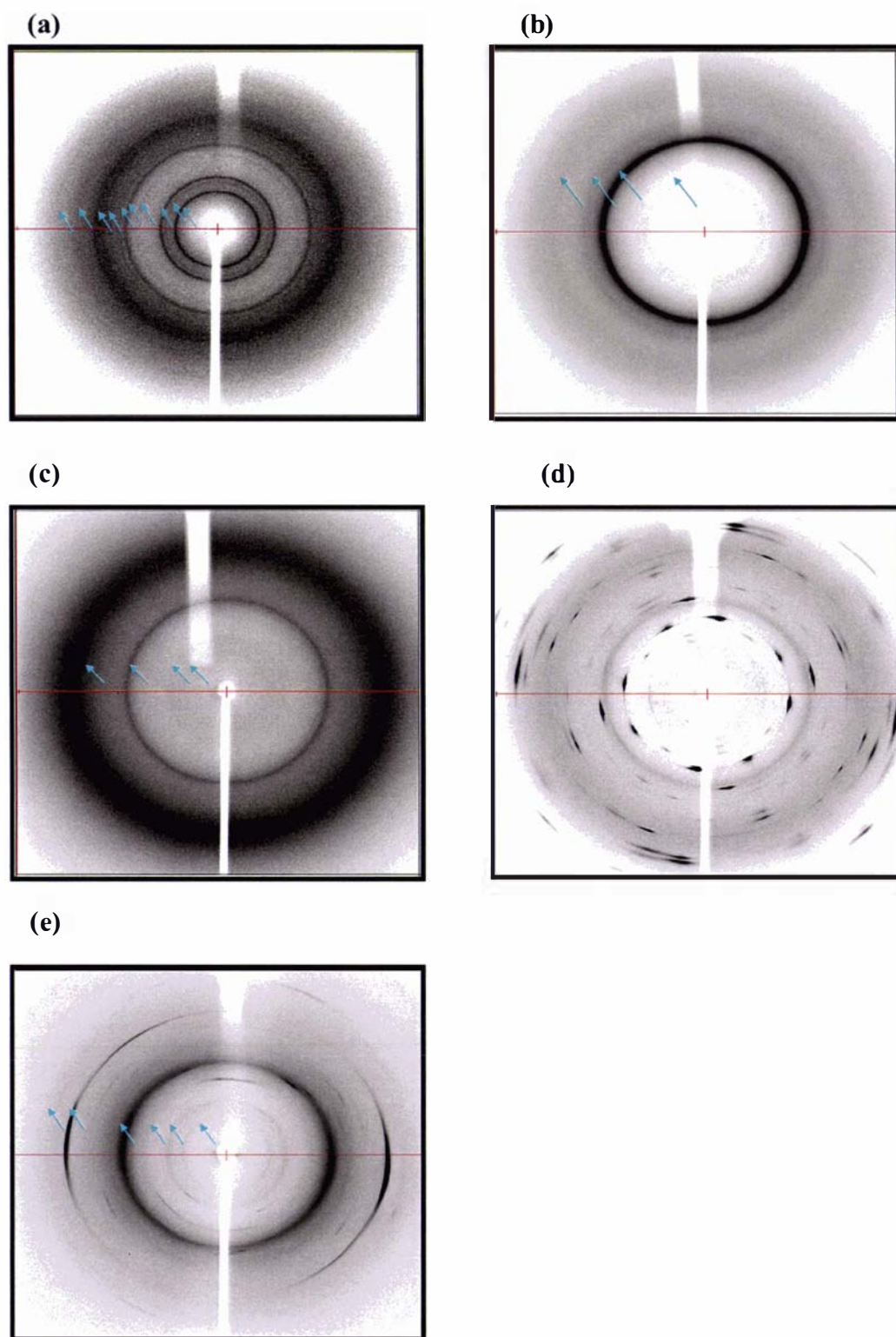
The surface morphologies of *Ch/PVA* film (ChVA1), ChVA1 loaded with 24 mg g<sup>-1</sup> matrix of *5-FU* and ChVA1 film after 24 h of drug release at pH 7.4 at 37 °C are shown in **Figure 2.6**. As noted from this figure, the surface of (1:1) *Ch/PVA* blend (**Figure 2.6a**) is integrated, dense and very smooth. However, loading with *5-FU* leads to a noticeable roughness and many deficiencies over most of the film surface (**Figure 2.6b**). After 24 h of drug release at pH 7.4 at 37 °C the film has been converted to now show a highly porous surface (**Figure 2.6c**).



**Figure 2.6.** Scanning electron micrograph of the surface of (a) ChVAI film, (b) ChVAI film loaded with 5-FU (24 mg g<sup>-1</sup> matrix) and (c) ChVAI film after 24 h of 5-FU release at pH 7.4 at 37 °C.

### 2.3.1.5. The X-ray diffraction patterns

**Figure 2.7** shows the 2D-XRD patterns of ChVA1 with and without *5-FU* entrapped inside in comparison to that of *Ch* and *PVA*. The diffractogram of *Ch* film (**Figure 2.7a**) shows three major crystalline peaks at  $2\theta$  values of 8.38, 11.49 and 18.25° in addition to a lot of weak and broad crystalline peaks (see **Table 2.2**). This diffraction pattern reflects a high degree of crystallinity for the *Ch* under investigation. **Figure 2.7b** shows the diffractogram of the *PVA* film. Here, *PVA* has two major crystalline peaks at  $2\theta$  values of 11.49 and 19.55° plus two other broad bands correspond to  $2\theta$  values of about 22.80 and 29.78°. The diffractogram of 1:1 *Ch/PVA* blend (ChVA1, **Figure 2.7c**) seems to keep the characteristic peaks of *Ch* and *PVA* but the peaks became weaker and broader. This broadness may be attributed to the formation of H-bonding between the amino and hydroxyl groups of *Ch* with the hydroxyl groups of *PVA* leading to a kind of compatible blend. Upon loading of 5 w% of *5-FU* onto this compatible and transparent blend, the resulted film of FU-loaded ChVA1 still keeping some transparency, as the drug-free ChVA1, but with appearance of a type of uniform feathery or dendritic aggregations. These uniform distributed dendritic aggregations tend to refer to a possibility of H-bonds formation between the drug molecules and the polymer chains. The X-ray diffractograms of both the feathery and the transparent parts are shown in **Figure 2.7d** and **Figure 2.7e** respectively. These diffraction patterns tend to reflect the microcrystalline nature of the entrapped drug [13].



**Figure 2.7.** The 2D-XR diffraction patterns of (a) *Ch* (b) *PVA* (c) ChVAI (d) ChVAI loaded with 5% of *5-FU* (feathery part of the film) (e) ChVAI loaded with 5% of *5-FU* (non-feathery part of the film).

**Table 2.2.** The 2D-XR diffraction data for *Ch*, *PVA*, drug-free and drug-loaded ChVA1.

<i>Sample Code</i>	<i>d</i> (°Å) (from low to high resolution)	$2\theta^\circ$
<i>Ch</i>	10.55, 8.50, 7.70, ~5.50, 4.86, ~4.60, ~4.20, 3.84 (broad), ~3.30, ~3.00	8.38, 10.40, 11.49, ~16.10, 18.25, ~19.30, ~21.20, 23.16, ~27.02, ~29.78
<i>PVA</i>	~7.70, 4.54, ~3.90, ~3.00 (v. broad)	~11.49, 19.55, ~22.80, ~29.78
ChVA1	10.40, 7.70, 4.61, ~3.00 (v. broad)	8.50, 11.49, 19.25, ~29.78
ChVA1-FU	10.80, 7.90, 5.70, 4.45, ~4.00, ~3.50, 3.10, 2.88	8.19, 11.20, 15.55, 19.95, ~22.22, ~25.45, 28.80, 31.05

### 2.3.2. Equilibrium swelling studies

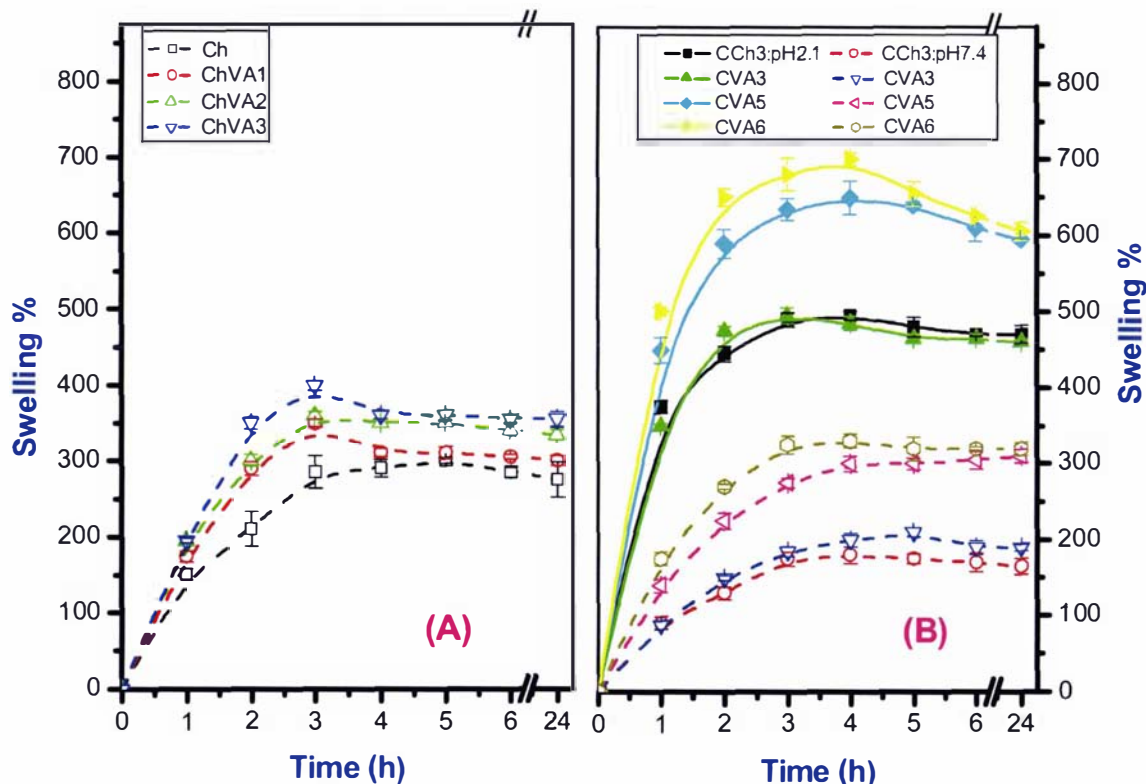
Swelling is the most significant characteristic of hydrogels and it reflects the affinity of the chemical structure of hydrogels for water and other surrounding fluids. The swelling of *Ch* is normally investigated in neutral medium and in slightly higher pH values. In acidic medium, however, *Ch* usually dissolves. For this reason, it is essential to obtain *Ch* in a crosslinked form. There are many factors affecting the equilibrium swelling profiles of hydrogels, for instance, the hydrogel composition and the mobility restrictions of the polymer chains between crosslinks [1]. The following section reports and discusses some of the factors affecting the swelling values of the hydrogels prepared in this study.

#### 2.3.2.1. Effect of *PVA* content

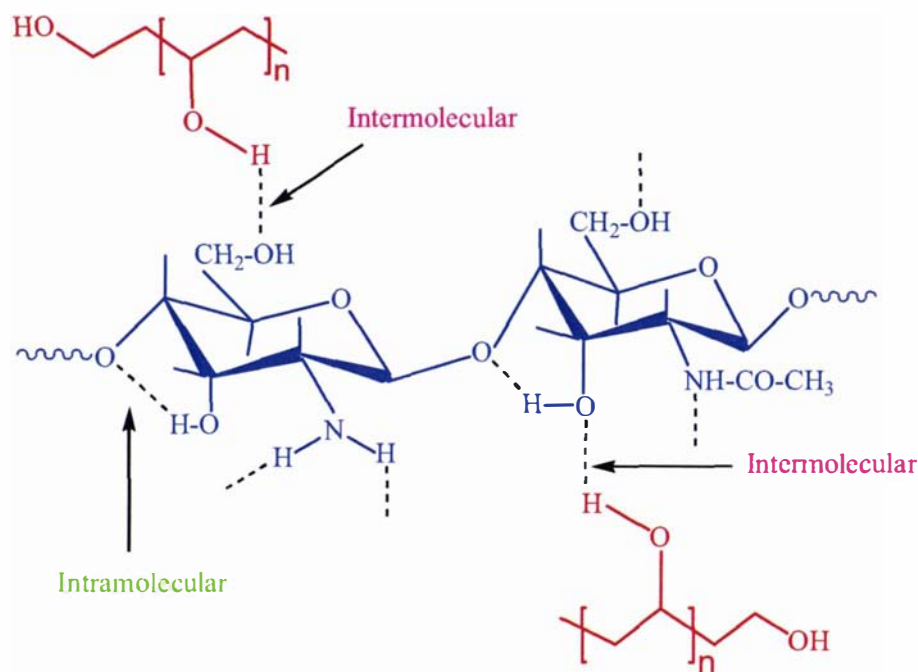
Figure 2.8A shows the swelling behavior of the non-crosslinked films prepared from *Ch* and *Ch* blended with *PVA* at different blend ratios (ChVA1-ChVA3). The swelling measurements for these samples were carried out only at 37 °C at pH 7.4 (*SIF*) to avoid

the dissolution of non-crosslinked *Ch* at lower pH values. From this figure, *Ch* film attained about 270% swelling at equilibrium. This swelling value increased to 305%, 340% and 360% upon blending with an increasing amount of *PVA*, 50%, 60% and 75% respectively. This swelling behavior is expected, as *PVA* is a highly hydrophilic water-soluble polymer. As a consequence, this hydrophilicity facilitates the entrance of swelling fluid into the film to attain higher values of swelling. The physical nature of the *Ch/PVA* blend and the hydrogen bond formation (Scheme 2.5) restrict the dissolution of the *PVA* during swelling investigation.

The same behavior was also noticed in the case of crosslinked *Ch* and *Ch/PVA* films (Figure 2.8B). From this figure, with 25% glutaraldehyde as crosslinker, the % equilibrium swelling was directly related to the *PVA*% in the hydrogel film. For instance, at pH 7.4, CCh3 attained about 170% swelling at equilibrium. This value of swelling increased to 205%, 308% and 325% in case of CVA3, CVA5 and CVA6 which contain 50%, 60% and 75% of *PVA* respectively.



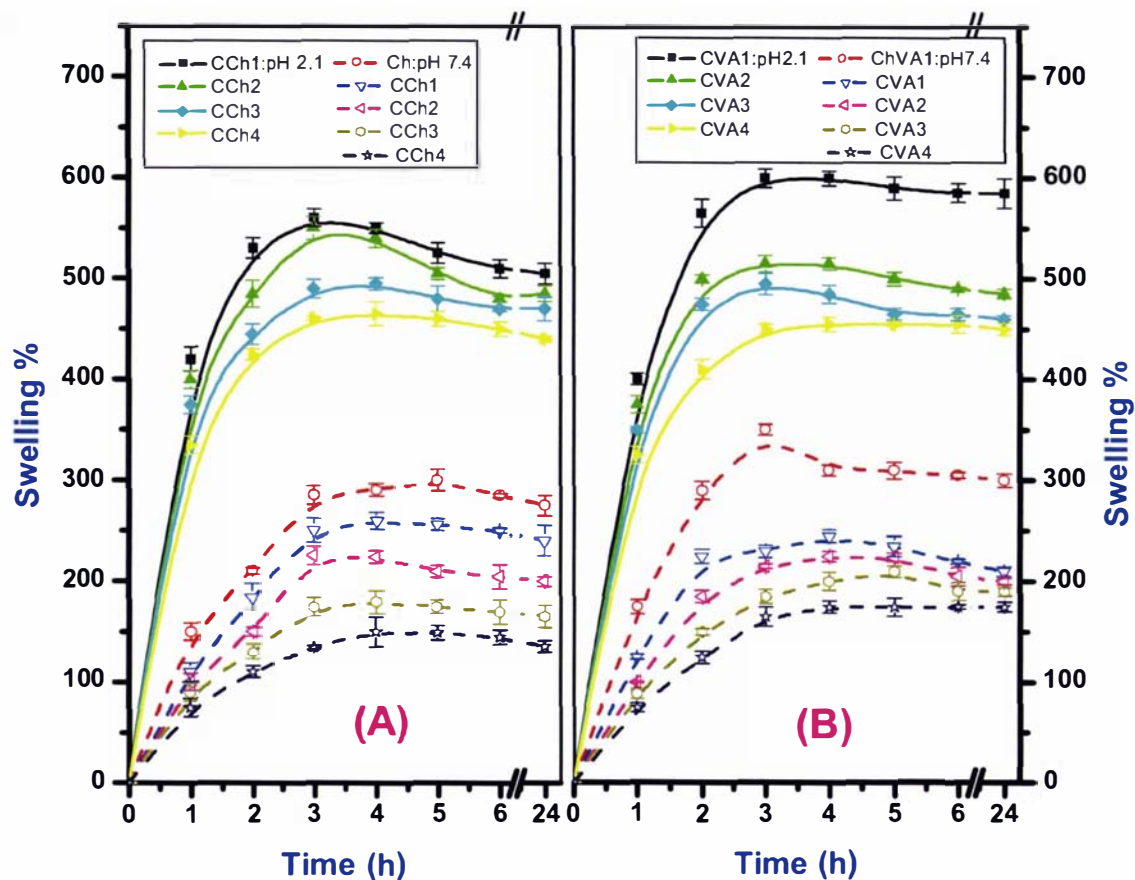
**Figure 2.8.** The swelling behavior of (A) non-crosslinked and (B) crosslinked *Ch* and *Ch/PVA* films with different blend ratios at pH 7.4 and 37 °C.



**Scheme 2.5.** Schematic representation of the proposed structure of *Ch/PVA* blend.

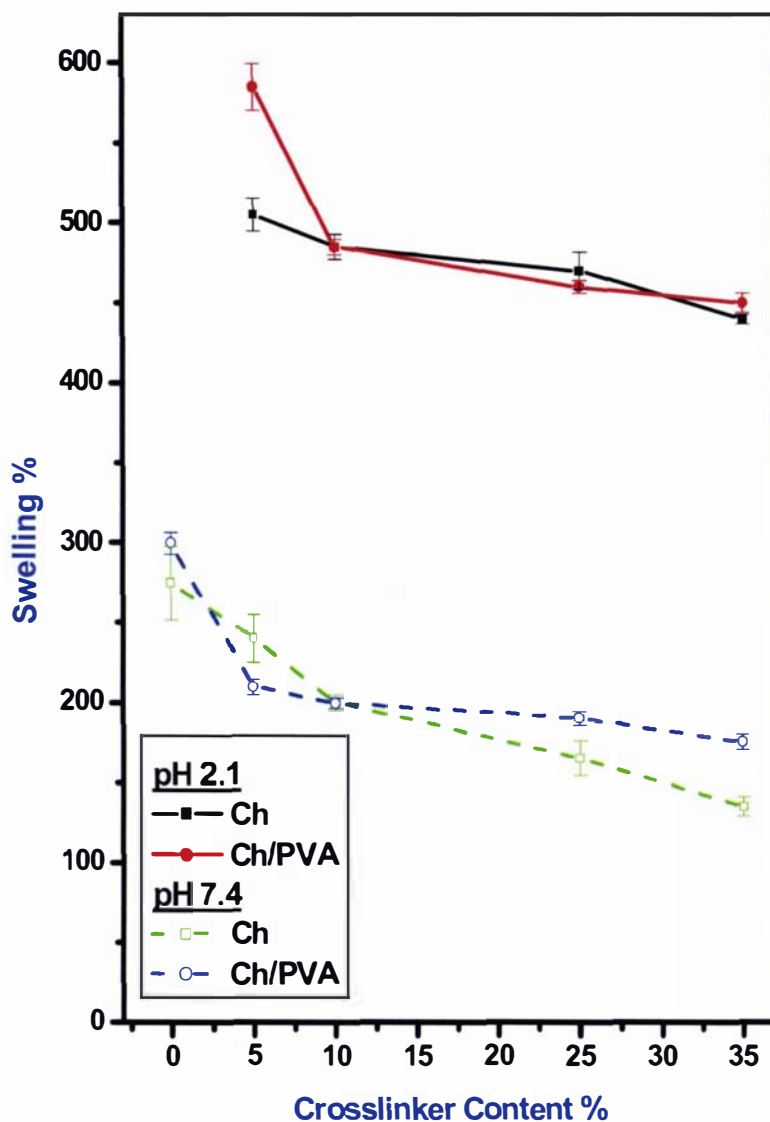
### 2.3.2.2. Effect of glutaraldehyde content

Introduction of glutaraldehyde as a crosslinking agent affected the swelling behavior of both *Ch* and *Ch/PVA* films. **Figure 2.9A** shows the differences in swelling at equilibrium at pH 2.1 and pH 7.4 at 37 °C for crosslinked *Ch* films (CCh1-CCh4) prepared with varying amounts of glutaraldehyde. From this figure, it can be seen that, as the glutaraldehyde content increases, the extent of crosslinking increases and consequently the % equilibrium swelling decreases. For instance, at pH 2.1, the % equilibrium swelling decreases from about 520% to 350% upon increasing the glutaraldehyde % from 5% to 35%. At pH 7.4, increasing the glutaraldehyde % from 5% to 35% decreased the % equilibrium swelling from about 250% to 125%. **Figure 2.9B** represents also a similar behavior for 1:1 *Ch/PVA* films (CVA3, CVA5 and CVA6) crosslinked with varying amounts of glutaraldehyde at both pH 2.1 and pH 7.4.



**Figure 2.9.** The swelling behavior of crosslinked (A) *Ch* and (B) 1:1 *Ch/PVA* films at pH 2.1 and pH 7.4 at 37°C.

The inverse dependence of the swelling % of both *Ch* and *Ch/PVA* films on the glutaraldehyde content is shown in **Figure 2.10**. From this figure, there is a considerable dependence of the swelling % on the glutaraldehyde content especially in case of *Ch/PVA* films, up to 10% glutaraldehyde. Beyond this concentration, the dependence of the swelling % on glutaraldehyde content tends to be similar for both *Ch* and *Ch/PVA* films.

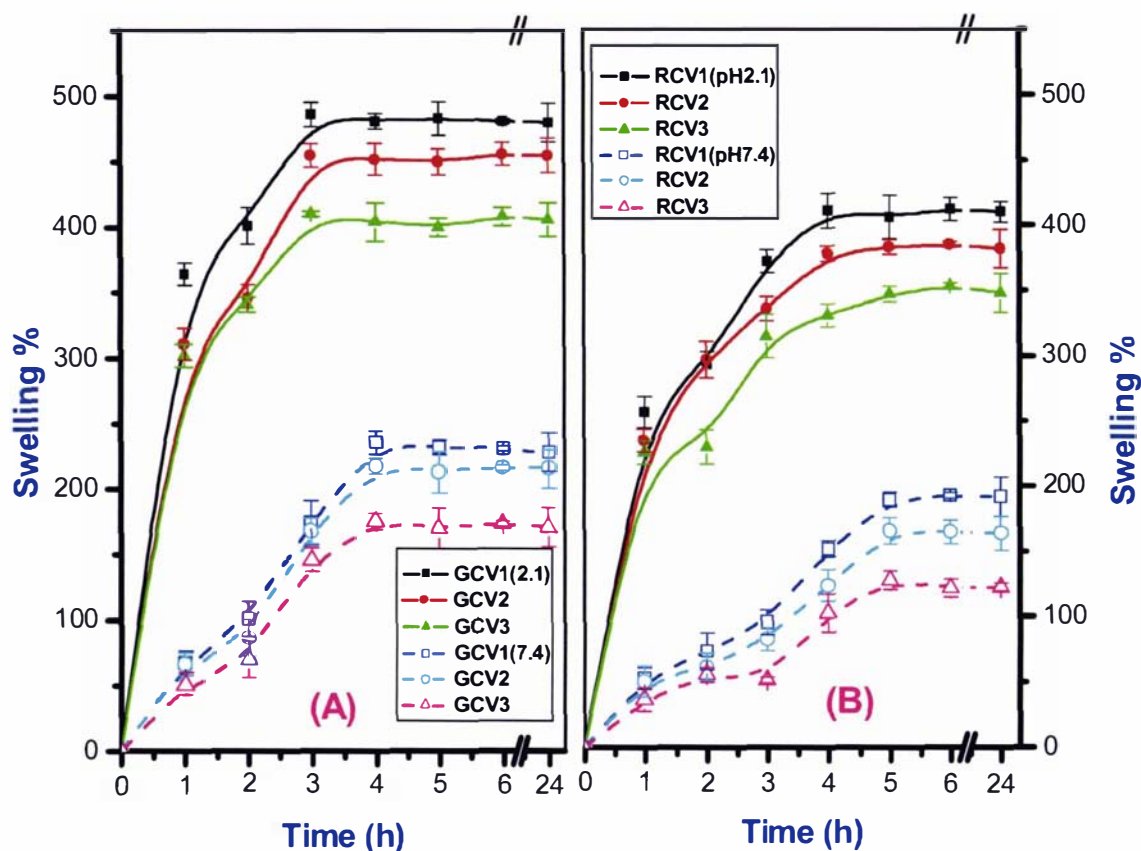


**Figure 2.10.** Influence of glutaraldehyde content on the swelling % of *Ch* and *Ch/PVA* films after 24 h at pH 2.1 and 7.4 at 37 °C.

### 2.3.2.3. Effect of irradiation dose

**Figure 2.11A** shows the swelling patterns of *Ch/PVA* films of different blend ratios crosslinked by exposure to a 10 KGy dose of  $\gamma$ -irradiation. From the figure, at the same pH, increasing the *PVA* content increases the crosslinking extent leading to a noticeable decrease in the swelling values at equilibrium. Also, the films attained their equilibrium

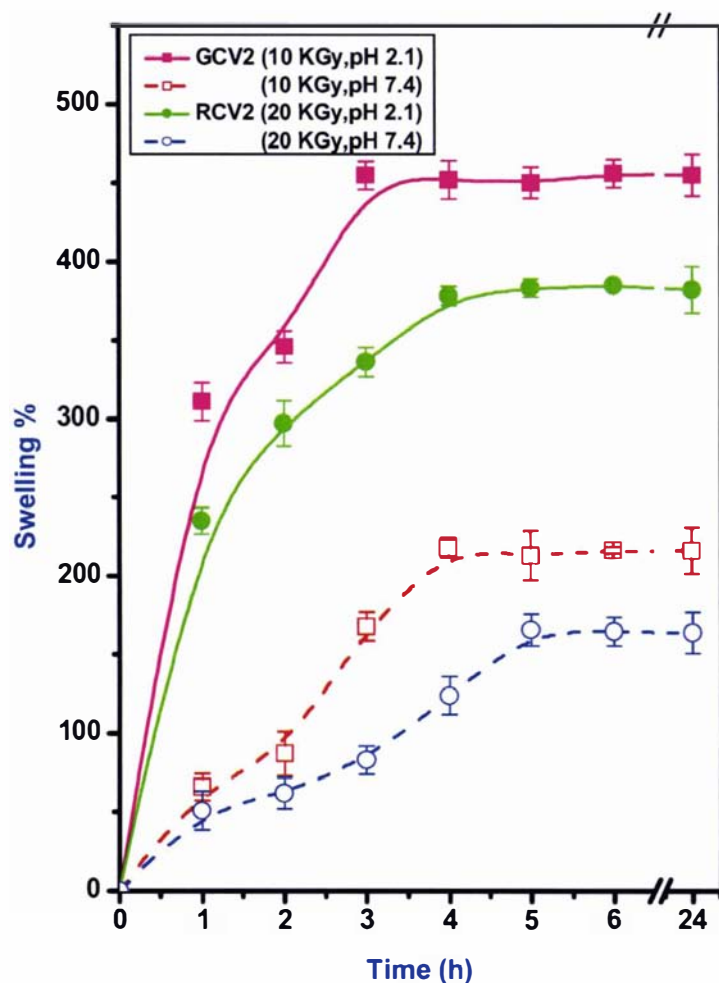
swelling after about 3 h at pH 2.1. This swelling time increased up to 4 h at pH 7.4. The same swelling behavior can be recognized for the films of the same composition subjected to 20 KGy dose (**Figure 2.11B**). The films in this case reached their equilibrium swelling after 4 and 5 h at pH 2.1 and 7.4 respectively.



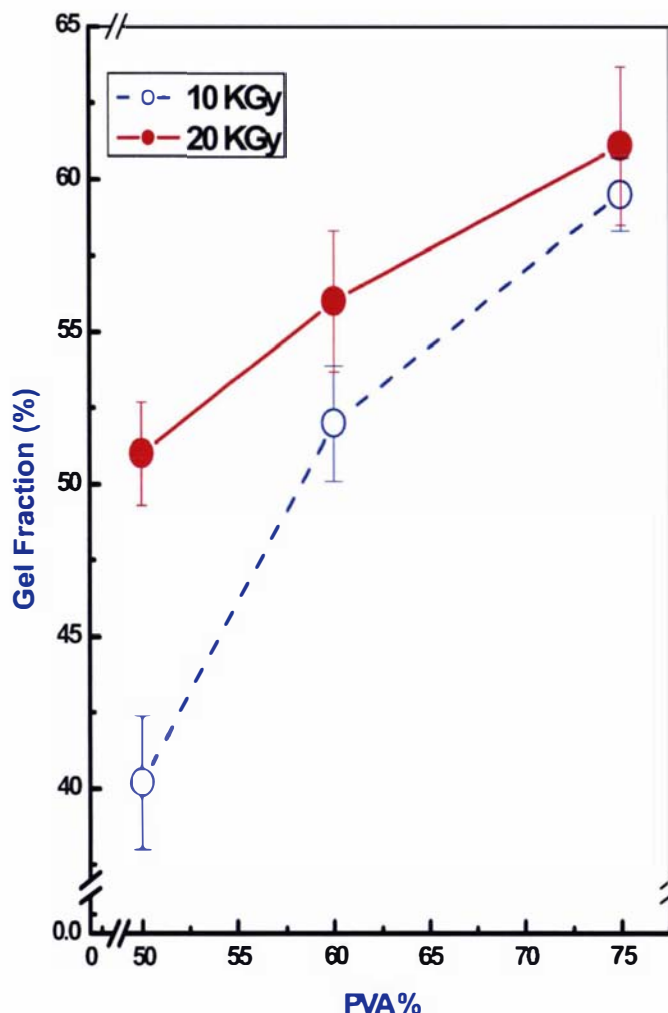
**Figure 2.11.** The swelling behavior of (A) 10 KGy and (B) 20 KGy  $\gamma$ -irradiated *Ch/PVA* films of different blend ratios in pH 2.1 and 7.4 at 37 °C.

The swelling behavior of 1:1.5 *Ch/PVA* films exposed to a 10 KGy dose of  $\gamma$ -irradiation (GCV2) in comparison with that exposed to 20 KGy (RCV2) is shown in **Figure 2.12**. As expected, at the same pH value, increasing the irradiation dose from 10 KGy to 20 KGy leads to an increase in the crosslinking extent of *PVA* and consequently the % equilibrium swelling decreased. For instance, at pH 2.1, GCV2 attained 458% swelling at equilibrium while RCV2 attained only 380% of equilibrium swelling. The effect of the  $\gamma$ -irradiation dose onto the crosslinking extent of *PVA* can be also confirmed from

**Figure 2.13** which shows the gel % values of the  $\gamma$ -irradiated *Ch/PVA* films. From this figure, it appeared that within the applied small range of  $\gamma$ -irradiation doses (10-20 KGy), the gel % was directly related to both the irradiation dose and the *PVA* content.



**Figure 2.12.** The swelling behavior of  $\gamma$ -irradiated 1:1.5 *Ch/PVA* films at pH 2.1 and 7.4 at 37°C.



**Figure 2.13.** The effect of irradiation dose and the *PVA* content on the gel % of the crosslinked *Ch/PVA* films.

The swelling % values attained at equilibrium were higher in the acidic buffer (pH 2.1) than in the slightly alkaline (pH 7.4) buffer. This pH sensitivity can be attributed to the chemical structure of *Ch* where, in the acidic medium, protonation can occur at the free  $\text{NH}_2$  groups of *Ch* leading to dissociation of the hydrogen bonding involving these groups and consequently facilitates the entrance of swelling fluid into the film to attain higher values of swelling. In addition, the swelling process will increase the ionization of the amino groups in the acidic buffer solution. The acid would then be attracted to these positively charged amino groups leading to an increase in the weight of the film in

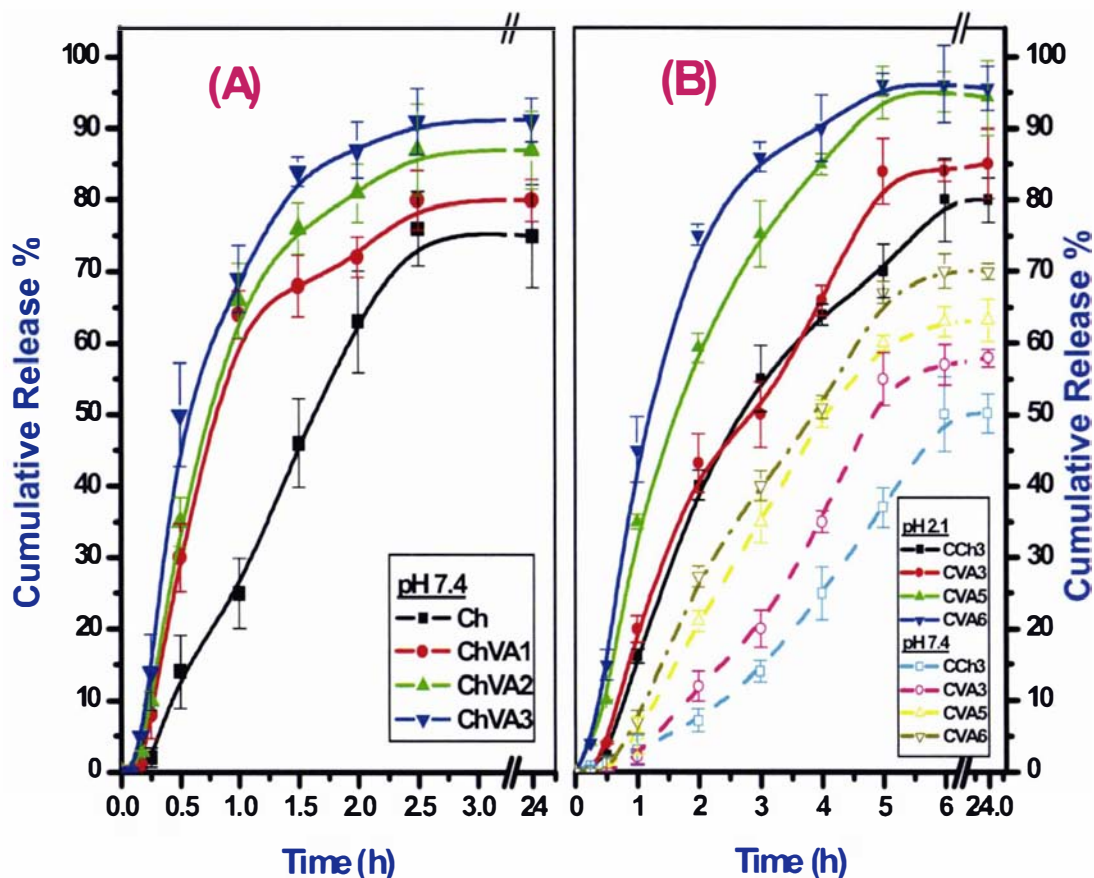
the acidic buffer [22,30]. Moreover, the swelling values at pH 7.4 will be lower than the values at pH 2.1 due to the increased hydrophobicity of the **Ch**-based hydrogel films dominating at higher pH values, thus preventing more swelling in neutral and alkaline media [31].

### **2.3.3. Cumulative release measurements**

The release patterns of a drug from polymeric matrices depend mainly on the swelling behavior of these matrices. The following section discusses some of the factors affecting the release profiles of **5-FU**, as a model drug, from the prepared **Ch** and **Ch/PVA** hydrogel films.

#### **2.3.3.1. Effect of PVA content**

**Figure 2.14A** shows the cumulative release profiles of **5-FU** from the non-crosslinked **Ch** and **Ch/PVA** films at pH 7.4 at 37 °C. This figure demonstrates the variation in release at equilibrium for films prepared with varying contents of **PVA**. In the case of **ChVA1-3**, there is an initial burst release in the first 1.5 h followed by an almost constant release rate of **5-FU**. Also, it can be noted from **Figure 2.14A** that as the **PVA** content increases in the blend, the release of **5-FU** becomes faster and attains higher values of release at equilibrium. The figure also shows that most of the accessible **5-FU** was released within 2.5 h from these non-crosslinked films. **Figure 2.14B** shows a similar effect of **PVA** content on the release pattern of **5-FU** from the crosslinked **Ch** and **Ch/PVA** hydrogel films. From **Figure 2.14B**, at the same pH, increasing the **PVA** percent in the drug-loaded hydrogel films from 0 % (**CCh3**) up to 75 % (**CVA6**) increased the gel hydrophilicity leading to an increase in the equilibrium swelling and consequently higher values of drug release were attained.

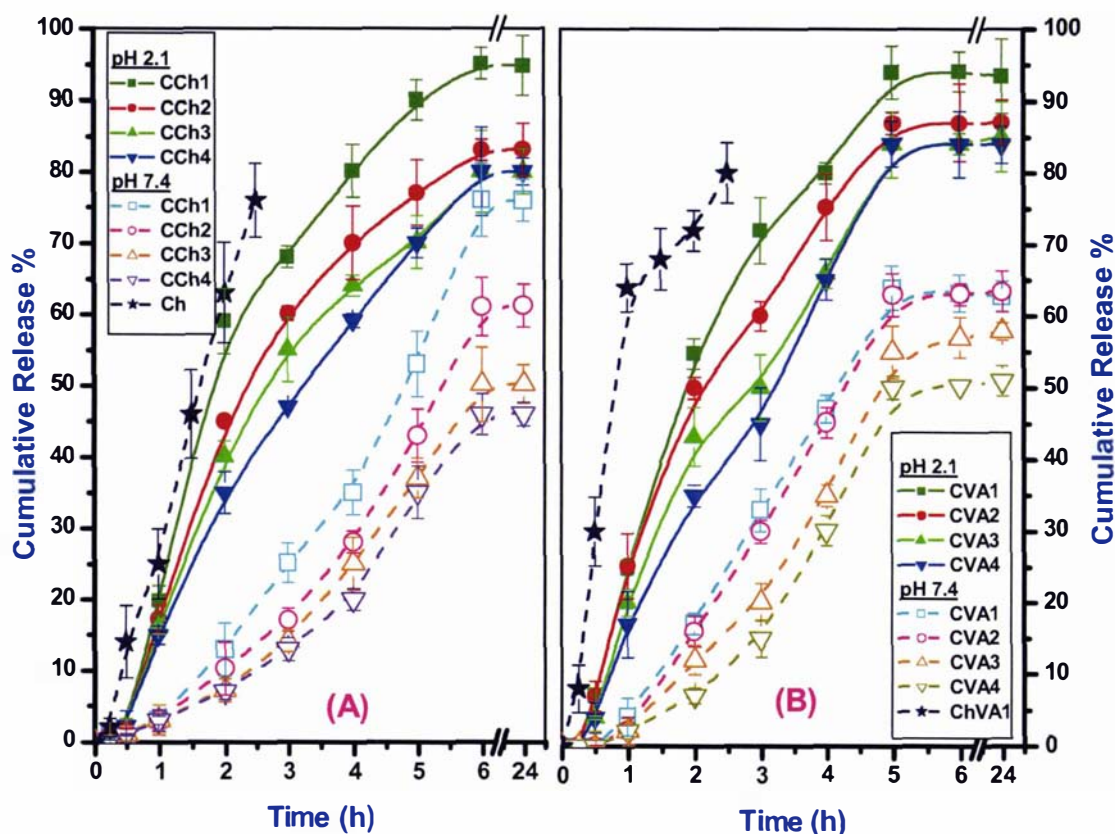


**Figure 2.14.** The cumulative release of 5-FU from (A) non-crosslinked (pH 7.4) and (B) crosslinked *Ch* and *Ch/PVA* films of different blend ratio at pH 2.1 and 7.4 at 37 °C.

### 2.3.3.2. Effect of glutaraldehyde content

The dependence of the amount of 5-FU released on the glutaraldehyde % in the *Ch* and *Ch/PVA* hydrogel films is shown in Figure 2.15. From Figure 2.15A, the amounts of drug released from CCh1-4 clearly confirm that the extent of release at equilibrium is inversely related to the degree of crosslinking. For instance, at pH 2.1, about 95% of 5-FU were released at equilibrium from CCh1 (5% glutaraldehyde). This value was lowered to 81% in case of CCh4 (35% glutaraldehyde). Approximately, no difference was observed in the percent of drug released at equilibrium from CCh3 and CCh4. A similar behavior was observed for the drug-loaded samples CCh1-4 at pH 7.4 at 37 °C.

Also, **Figure 2.15A** shows that the crosslinked *Ch* films attained equilibrium release later (6 h) than the non-crosslinked films (less than 2.5 h).



**Figure 2.15.** The cumulative release behavior of 5-FU from (A) *Ch* and (B) 1:1 *Ch/PVA* crosslinked films with varying amounts of glutaraldehyde at pH 2.1 and 7.4 at 37 °C.

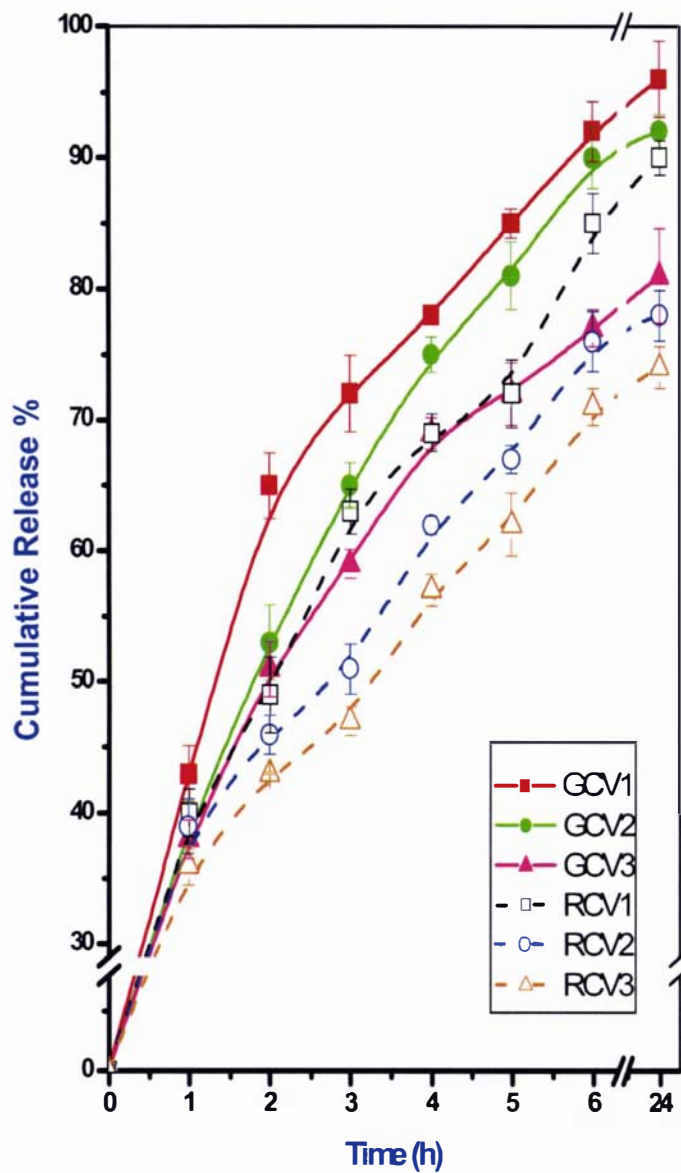
**Figure 2.15B** shows the cumulative release of 5-FU from 1:1 *Ch/PVA* films crosslinked with different amounts of glutaraldehyde. From this figure, at the same pH as the glutaraldehyde amount increased from 5% (CVA1) to 35% (CVA4), the extent of crosslinking increased and consequently the amount of drug released at equilibrium decreased. In both media (pH 2.1 and 7.4), the release from the drug loaded (CVA1-4) samples attained its equilibrium value at approximately 5 h.

The extent of drug release from all the gel films irrespective of their composition was much higher in the acidic buffer (pH 2.1) than in the weakly alkaline one (pH 7.4),

because the release rate depends on the swelling degree of the hydrogel. The principle mechanism that explains such release is based on the diffusion through the swollen gels. As discussed before, these hydrogel films attained higher values of swelling at equilibrium at pH 2.1 than pH 7.4. Comparing the release behavior at pH 2.1 with that at pH 7.4 reveals that the pH of the release medium has a greater effect on the release values than does the effect of the gel composition. This may be attributed to the chemical structure of *Ch* where its amino groups are the responsible for such pH-sensitivity.

### **2.3.3.3. Effect of irradiation dose**

**Figure 2.16** shows the cumulative release of *5-FU* at pH 2.1 and 37 °C from *Ch/PVA* films crosslinked by  $\gamma$ -irradiation. From this figure, the amount of *5-FU* released at equilibrium is inversely related to the *PVA* % in the hydrogel films where with increasing the *PVA* content, more crosslinking occurs for the *PVA* leading to decreasing of swelling and consequently a decreasing of the release extent. Also the figure shows that increasing the irradiation dose (from 10 to 20 KGy) leads to increasing the crosslinking extent and consequently decreases the amounts of the drug released. **Table 2.3** shows the cumulative release of *5-FU* from  $\gamma$ -irradiated *Ch/PVA* films at pH 7.4 and 37 °C. The results shown in the table confirm the effects discussed above of both the *PVA*% and the irradiation dose on the release behavior of the *5-FU*.



**Figure 2.16.** The cumulative release of 5-FU from  $\gamma$ -irradiated *Ch/PVA* films of different *PVA* contents at pH 2.1 and 37 °C.

**Table 2.3.** The cumulative release % of 5-FU from  $\gamma$ -irradiated Ch/PVA films with different PVA contents at pH 7.4 and 37 °C.

Time (h)	Average cumulative release % (pH 7.4)											
	(10 KGy)						(20 KGy)					
	GCV1	$\pm$ SD	GCV2	$\pm$ SD	GCV3	$\pm$ SD	RCV1	$\pm$ SD	RCV2	$\pm$ SD	RCV3	$\pm$ SD
1	33.1	1.4	34.2	2.2	32.1	1.8	34.9	1.7	31.4	2.1	29.2	2.8
2	48.3	1.1	35.8	2.1	36.0	1.6	37.7	2.5	32.3	2.1	33.1	1.9
3	56.0	2.1	53.2	1.7	36.6	1.8	46.5	1.4	37.4	1.3	34.1	2.4
4	61.4	1.9	56.2	2.2	48.5	2.1	49.8	1.9	41.1	1.7	42.3	1.3
5	69.2	1.8	63.0	1.9	54.9	2.1	61.0	2.1	50.0	1.2	48.7	2.0
6	74.0	2.4	67.3	1.9	56.1	1.5	63.1	1.5	56.9	1.9	51.5	1.6
24	77.4	1.9	71.6	1.3	60.2	1.9	67.1	3.1	59.0	1.4	55.4	1.6

$\pm$ SD: Standard deviation values

The release patterns from similar *Ch* and *Ch/PVA* hydrogel films but loaded with two different concentrations of *5-FU* (14 and 40 mg g<sup>-1</sup> matrix) were also investigated in comparison with the release profiles discussed earlier (24 mg g<sup>-1</sup> matrix). The equilibrium release data of these different concentrations are represented in **Tables 2.4-2.6**. From these tables, it can be noted that the amount of *5-FU* released was directly related to the concentration of the drug initially loaded in the matrix. The percent of drug released at equilibrium was also increased with increasing the drug loading, which tends to refer to the role played by the hydrophilicity of *5-FU* in increasing the rate of release. Also, these variations in the release percents upon increasing the drug loading tends to confirm that the mechanism of drug release from these types of matrices is due to the diffusion through the swollen gels, in agreement with that found and discussed earlier in the swelling measurements section.

**Table 2.4.** The cumulative release data from *Ch* and *Ch/PVA* films loaded with different concentrations of *5-FU* at pH 7.4 and 37 °C.

Sample Code	Ei (mg g <sup>-1</sup> )	Ea (mg g <sup>-1</sup> )	E %	pH 7.4 ( <i>SIF</i> )		
				Ra (mg g <sup>-1</sup> )	R%	±SD
<i>Ch</i>	14	10.92	78.0	7.92	72.5	2.1
	24	17.02	70.9	12.94	76.0	2.7
	40	29.28	73.2	22.78	77.8	1.1
ChVA1	14	10.29	73.5	7.91	76.9	1.5
	24	16.32	68.0	13.12	80.4	2.3
	40	26.56	66.4	21.81	82.1	1.6
ChVA2	14	11.52	82.3	9.79	85.0	3.3
	24	16.73	69.7	14.56	87.0	2.6
	40	28.64	71.6	25.26	88.2	1.9
ChVA3	14	9.72	69.4	8.55	88.0	2.7
	24	16.27	67.8	14.90	91.6	3.5
	40	29.88	74.7	27.13	90.8	4.1

**Ei:** the initially loaded amount of *5-FU* (mg g<sup>-1</sup> matrix), **Ea:** the entrapped amount of *5-FU* (mg g<sup>-1</sup>) after matrix washing, **E %:** the percent of *5-FU* entrapped, **Ra:** the average released amount of *5-FU* (mg g<sup>-1</sup>) at equilibrium, **R %:** the mean values of the released percents of the drug at equilibrium and **±SD:** Standard deviation.

**Table 2.5.** The cumulative release data from chemically crosslinked *Ch* films loaded with different concentrations of *5-FU* at pH 2.1 and 7.4 at 37 °C.

Sample Code	E <sub>i</sub> (mg g <sup>-1</sup> )	E <sub>a</sub> (mg g <sup>-1</sup> )	E %	pH 2.1 ( <i>SGF</i> )			pH 7.4 ( <i>SIF</i> )		
				R <sub>a</sub> (mg g <sup>-1</sup> )	R %	±SD	R <sub>a</sub> (mg g <sup>-1</sup> )	R %	±SD
CCh1	14	11.62	83.0	10.70	92.1	4.1	8.84	76.1	2.1
	24	19.70	82.1	18.72	95	2.8	14.97	76.0	3.8
	40	33.32	83.3	32.25	96.8	2.1	25.59	76.8	1.1
CCh2	14	12.14	86.7	9.87	81.3	4.3	7.31	60.2	4.2
	24	20.86	86.9	17.36	83.2	2.2	12.72	61.0	2.6
	40	34.04	85.1	28.49	83.7	1.9	21.00	61.7	1.7
CCh3	14	12.09	86.4	9.20	76.1	2.3	5.85	48.4	1.9
	24	20.42	85.1	16.34	80.0	2.2	10.25	50.2	2.1
	40	35.00	87.5	29.58	84.5	1.8	17.50	50.0	3.8
CCh4	14	12.84	91.7	10.36	80.7	3.7	5.82	45.3	2.6
	24	22.18	92.4	17.74	80.0	2.5	10.20	46.0	1.5
	40	36.32	90.8	29.89	82.3	2.1	16.74	46.1	1.3

E<sub>i</sub>: the initially loaded amount of *5-FU* (mg g<sup>-1</sup>matrix), E<sub>a</sub>: the entrapped amount of *5-FU* (mg g<sup>-1</sup>) after matrix washing, E %: the percent of *5-FU* entrapped, R<sub>a</sub>: the average released amount of *5-FU* (mg g<sup>-1</sup>) at equilibrium, R %: the mean values of the released percents of the drug at equilibrium and ±SD: Standard deviation.

**Table 2.6.** The cumulative release data from chemically crosslinked *Ch/PVA* hydrogel films loaded with different concentrations of *5-FU* at pH 2.1 and 7.4 at 37 °C.

Sample Code	Ei (mg g <sup>-1</sup> )	Ea (mg g <sup>-1</sup> )	E %	pH 2.1 ( <i>SGF</i> )			pH 7.4 ( <i>SIF</i> )		
				Ra (mg g <sup>-1</sup> )	R %	±SD	Ra (mg g <sup>-1</sup> )	R %	±SD
CVA1	14	11.91	85.1	11.03	92.6	3.3	7.48	62.8	3.1
	24	20.64	86.0	19.40	94.0	2.9	13.00	63.0	2.6
	40	34.12	85.3	32.52	95.3	2.4	21.87	64.1	2.2
CVA2	14	12.24	87.4	10.66	87.1	1.3	7.82	63.9	3.2
	24	21.17	88.2	18.42	87.0	2.2	13.44	63.5	1.2
	40	35.56	88.9	31.65	89.0	1.5	23.19	65.2	1.9
CVA3	14	12.22	87.3	10.17	83.2	2.8	6.68	54.7	3.3
	24	21.12	88.0	17.74	84.0	2.5	12.04	57.0	1.2
	40	34.84	87.1	29.47	84.6	1.7	20.66	59.3	1.8
CVA4	14	12.39	88.5	10.09	81.4	1.7	6.20	50.0	3.5
	24	20.93	87.2	17.37	83.0	2.6	10.72	51.2	1.5
	40	34.32	85.8	29.24	85.2	2.4	18.19	53.0	2.1
CVA5	14	12.92	92.3	12.27	95.0	1.8	7.89	61.1	2.9
	24	21.82	90.9	20.73	95.0	4.3	13.75	63.0	2.1
	40	36.28	90.7	35.05	96.6	2.8	23.65	65.2	2.4
CVA6	14	13.19	94.2	12.78	96.9	1.6	10.13	76.8	2.1
	24	22.32	93.0	21.43	96.0	2.3	15.62	70.0	1.4
	40	37.92	94.8	37.24	98.2	1.8	28.29	74.6	1.6

**Ei**: the initially loaded amount of *5-FU* (mg g<sup>-1</sup>matrix), **Ea**: the entrapped amount of *5-FU* (mg g<sup>-1</sup>) after matrix washing, **E %**: the percent of *5-FU* entrapped, **Ra**: the average released amount of *5-FU* (mg g<sup>-1</sup>) at equilibrium, **R %**: the mean values of the released percents of the drug at equilibrium and **±SD**: Standard deviation.

## **CONCLUSIONS**

Two groups of pH-dependent semi-*IPN* hydrogels based on *Ch* and *PVA* were prepared and investigated for controlled drug release studies. The first group was chemically crosslinked with different concentrations of glutaraldehyde and the second was crosslinked by different doses of  $\gamma$ -irradiation. FTIR, SEM and 2D-XRD were used to characterize and investigate the structural changes of the prepared hydrogels with the variation of the hydrogel composition and the content of the crosslinker before and after drug loading. The pH-responsive behavior of these hydrogels was observed through studying their equilibrium swelling at 37 °C in simulated body fluids (pH 2.1 and 7.4). The equilibrium swelling of the chemically crosslinked *Ch/PVA* hydrogel films was found to be directly dependent on the content of *PVA* and inversely related to the crosslinker content. Upon irradiation, more crosslinking occurs as the content of *PVA* increases leading to a remarkable decrease in the swelling % at equilibrium. The *in-vitro* release profiles of *5-FU*, as a model therapeutic agent, from the hydrogels were also estimated at the same pH values (pH 2.1 and 7.4). The amounts of drug released at equilibrium were found to be dependent on many factors such as *PVA*%, crosslinker %,  $\gamma$ -irradiation dose and the pH of the medium.

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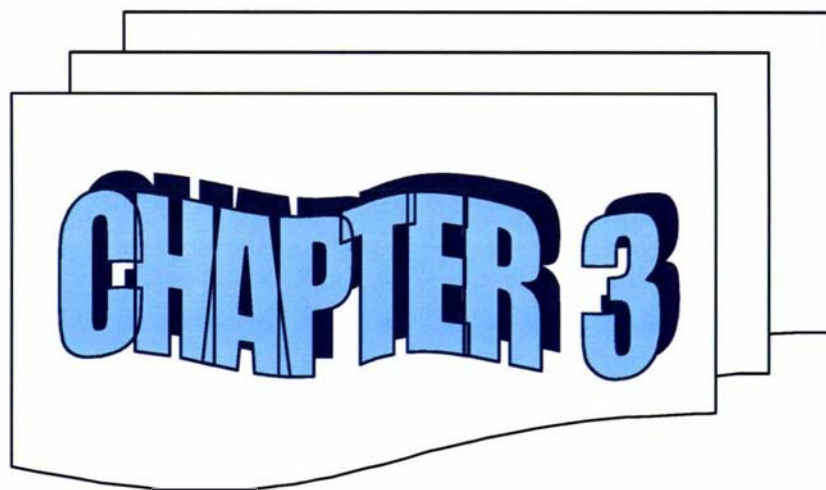
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**CHAPTER 3**

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## CHAPTER 3

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### pH AND THERMALLY RESPONSIVE SEMI-INTERPENETRATING POLYMERIC HYDROGELS BASED ON CHITOSAN AND POLY(*N*-ACRYLOYLGLYCINE) FOR *IN-VITRO* CONTROLLED RELEASE STUDY.\*

#### ABSTRACT

New biodegradable pH- and thermal-responsive semi-interpenetrating polymeric (semi-*IPN*) hydrogels were prepared for controlled drug delivery studies. The *IPN* hydrogels were obtained in mild aqueous acid media by irradiation of solutions of *N*-acryloyl glycine (*NAGly*) mixed with chitosan (*Ch*), in the presence of glutaraldehyde as a crosslinking agent and using 2,2-dimethoxy-2-phenyl acetophenone as photoinitiator. These hydrogels were subjected to equilibrium swelling studies at different temperatures (25 °C, 37 °C and 45 °C) in buffer solutions of pH 2.1 (*SGF*) and 7.4 (*SIF*). 5-Fluorouracil (*5-FU*) was entrapped in the hydrogels, and the *in-vitro* drug release studies were carried out at 37 °C in buffer solutions at pH 2.1 and 7.4.

**Keywords:** hydrogel, biodegradable, chitosan, *in-vitro* release

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\***El-Sherbiny, I.M.**, Lins, R.J., Abdel-Bary, E.M., Harding, D.R.K. Preparation, characterization, swelling and in-vitro drug release behavior of poly [N-acryloyl glycine-chitosan] interpolymeric pH and thermally-responsive hydrogels. *Eur. Polym. J.* **41** (2005) 2584. (One of top 25 in *Eur. Polym. J.*, 2005)

### 3.1. INTRODUCTION

Hydrogels are crosslinked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids. In the swollen state they are soft and rubbery, resembling living tissue. Some of the hydrogels also possess excellent biocompatibility [1,2]. The response of hydrogels to environmental changes that simulate changes in biological systems, such as pH, temperature, electric field and ionic strength, is an active area of research [3-9]. The pH-sensitive hydrogels have potential use in site-specific delivery of drugs to the gastrointestinal tract [10].

Chitosan (*Ch*) obtained through the alkaline deacetylation of chitin, is a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine [11-13]. *Ch* has desirable biological properties, being biodegradable, non-toxic, bioabsorbable [14] and having gel-forming ability at low pH. Moreover, *Ch* itself has antacid and antiulcer activities, which can prevent or weaken drug-induced irritation in the stomach [15,16]. These interesting properties make *Ch* an ideal candidate for use in controlled drug release formulations. However, this naturally abundant material also exhibits limitations in its reactivity and processability. One strategy to overcome these shortcomings is to incorporate *Ch* in interpenetrating polymer network (*IPN*) hydrogels. An *IPN* can be defined as a combination of two chemically distinct polymers that have two basic characteristics: first, one of the polymers must be synthesized, or crosslinked, in the immediate presence of the other, and second, the combination produces an advanced multicomponent polymeric system, with new property profiles [4,17,18].

In the previous chapter, *Ch* was incorporated into different pH sensitive semi-*IPN* hydrogels with *PVA*, as a highly hydrophilic polymer. This chapter discusses the synthesis, characterization and the *in-vitro* evaluation of a new biodegradable, pH and thermo-responsive semi-*IPN* hydrogel. In this semi-*IPN*, *Ch* was incorporated with the hydrophilic monomer (*NAGly*) and treated to bring about in situ copolymerization in the presence of glutaraldehyde as the crosslinker.

## 3.2. MATERIALS AND METHODS

### 3.2.1. MATERIALS

*Ch*, *5-FU* and 2,2 dimethoxy-2-phenyl acetophenone were purchased from Acros Organics (New Jersey, USA). Acryloyl chloride was supplied by Merck (Schuchardt OHG, Hohenbrunn, Germany). Glutaraldehyde (25% aqueous solution) was obtained from Sigma Chemical Co. (St. Louis, USA). Glycine, acetic acid, phosphoric acid, boric acid and all other reagents were of analytical grade and used as received.

### 3.2.2. METHODS

#### 3.2.2.1. Synthesis of NAGly

*NAGly* was prepared by a modified method to that described by Bentolila et al. [19]. Glycine (2 g, 26.7 mmol) was dissolved in NaOH solution (3 M, 20 mL) and the solution cooled in an ice bath for 10 min. with stirring. A solution of acryloyl chloride (2.2 ml, 27.2 mmol) dissolved in 1,4-dioxan (10 ml) was added dropwise with vigorous stirring, and the solution stirred at 0 °C for 1.5 h. The reaction mixture was washed with ether (2 x 25 ml), then the aqueous layer was acidified to pH 2 (4 M HCl), saturated with NaCl, and extracted with ethyl acetate (5 x 20 ml). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered, concentrated under reduced pressure, and the residue freeze-dried. The weight of crude product was 2.7 g (78%). A sample was recrystallised (1:1 ethyl acetate:diethyl ether) for analysis; m.p. 132-132.5 °C (lit. 132°C [20]).

$\delta_{\text{H}}$  4.03 (2H, s, NHCH<sub>2</sub>), 5.78 (1H, dd  $J$  10.0, 1.5 Hz, COCHCH<sub>trans</sub>), 6.21 (1H, dd  $J$  17.2, 1.5 Hz, COCHCH<sub>trans</sub>), 6.30 (1H, dd  $J$  17.2, 10.0 Hz, COCHCH<sub>2</sub>) ppm;  
 $\nu_{\text{max}}$  3314, 1720, 1648, 1609, 1548, 1405, 1345, 1222, 982, 683 cm<sup>-1</sup>;  
 $m/z$  (ES<sup>+</sup>) 130 (45 %, M+1), 112, 100, 96, 84, 55;  
 $m/z$  (ES<sup>-</sup>) 128 (100 %, M-1), 113, 84, 82, 69.

### 3.2.2.2. Preparation of the Ch/Poly(NAGly) semi-IPN hydrogel

The appropriate amount of *NAGly* (see **Table 3.1**) dissolved in 20 ml of 1,4-dioxan was added to solution of *Ch* (2.5% w/v) in dilute acetic acid (2% w/v). To this mixture was added a solution of 2,2-dimethoxy-2-phenyl acetophenone (2% based on the *NAGly*) in THF (5 mL). The appropriate volume of glutaraldehyde (25% aqueous solution) was added, with agitation. The reaction mixture was poured into a glass Petri dish and was maintained at room temperature. The polymerization was initiated by irradiation with an incandescent broad-spectrum lamp (Philips Comptalux, 150 W), positioned 25 cm above the Petri dish. Irradiation was continued for 2 h until gelation was complete. The hydrogels obtained were extensively washed with distilled water to remove any residual monomer, then freeze-dried and stored until further use. The compositions leading to the semi-IPNs prepared in this study are listed in **Table 3.1**.

**Table 3.1.** Composition of the prepared semi-IPNs.

Sample Code	<i>Ch</i> (g)	<i>NAGly</i> (g)	Glutaraldehyde solution (25% <i>aq.</i> ) ( $\mu$ l)
AGC-1	0.80	0.80	160
AGC-2	0.80	0.80	320
AGC-3	0.80	0.80	800
AGC-4	0.80	1.20	800

### 3.2.2.3. Characterizations

*NAGly* was characterized by FTIR (Perkin Elmer Paragon 1000 FTIR spectrometer), MS (Micro Mass ZMD ES-MS spectrometer), and  $^1\text{H-NMR}$  (JEOL-GX 270 FT-NMR spectrometer, deuterium oxide solvent) spectroscopy. It was also characterized qualitatively by simple chemical tests using ninhydrin and picrylsulphonic acid reagents. FTIR was used for characterization of the prepared *Ch*/Poly(*NAGly*) semi-IPN hydrogels. Also, the surface morphology of the hydrogel films before and after 24 h of *in-vitro* release of *5-FU* at pH 7.4 was investigated by scanning electron microscope (Cambridge Stereoscan S-250 mk 3 SEM).

#### 3.2.2.4. Entrapment of a model drug

Hydrogels loaded with an anti cancer drug (*5-FU*) as a model drug were prepared in the same manner reported in section 3.2.2.2. Known amounts of the drug were added to the reaction mixture, stirred vigorously and then the polymerization reaction was carried out. The resulting hydrogels were washed with distilled water, freeze-dried and stored until further use.

#### 3.2.2.5. Determination of the amount of drug entrapped

The quantity of drug entrapped in the *Ch/Poly(NAGly)* semi-*IPN* hydrogels was determined by an indirect method. After the hydrogel preparation the washings were collected, filtered with a 0.45 mm Millipore filter, and the amount of *5-FU* present was estimated from the absorption at  $\lambda_{\max}$  268 nm. The difference between the amount of drug initially added to the hydrogel and that estimated in the washings was taken as a measure of the amount of drug entrapped.

#### 3.2.2.6. Equilibrium swelling studies

The swelling behavior of the *Ch/Poly(NAGly)* semi-*IPN* hydrogels was measured at different temperatures (25 °C, 37 °C, and 45 °C) in two buffer solutions of pH 2.1 (*SGF*) and pH 7.4 (*SIF*) (see buffer preparation in Chapter 2). The swollen weights of the hydrogels were determined at intervals, after removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by the following equation:

$$\% \text{ Swelling} = 100 [(W_t - W_0)/W_0]$$

where  $W_0$  is the initial weight and  $W_t$  the final weight of the hydrogel at time  $t$ . Data points are means of three determinations.

#### 3.2.2.7. In-vitro cumulative release studies

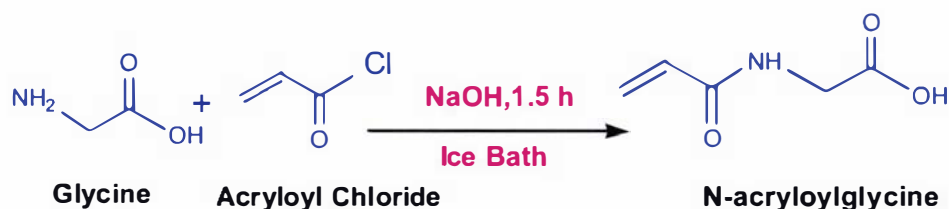
The *in-vitro* release of entrapped *5-FU* was determined by placing the pre-weighed hydrogel film loaded with drug in a buffer solution of pH 2.1 (*SGF*) or pH 7.4 (*SIF*) at 37 °C. At intervals, a 3 ml aliquot was withdrawn and its absorbance at  $\lambda_{\max}$  268 nm was measured. The withdrawn sample was replaced with an equal volume of fresh

buffer, to keep the volume of release medium constant. Data points are means of three determinations.

### 3.3. RESULTS AND DISCUSSION

#### 3.3.1. Synthesis and characterization of NAGly

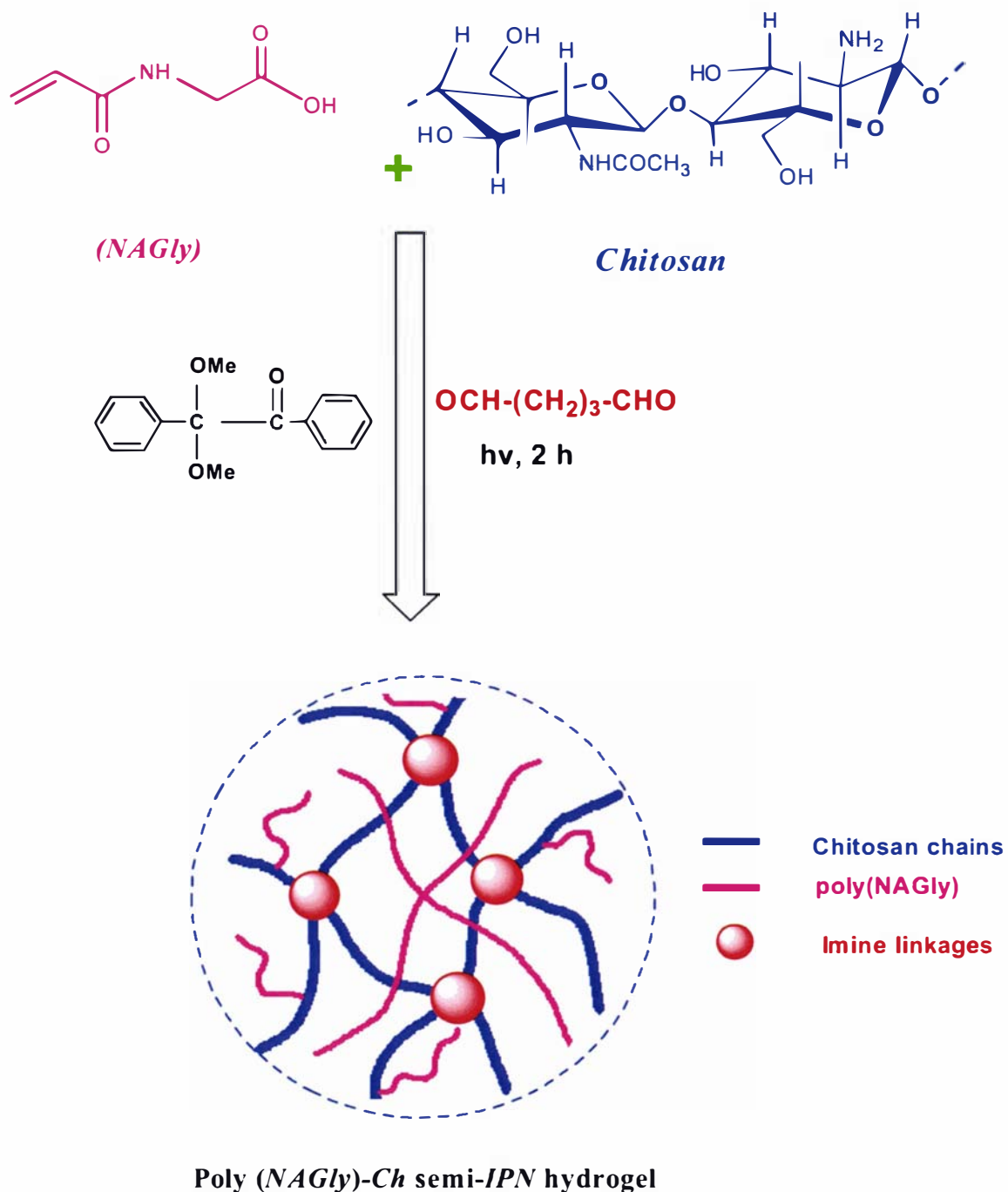
NAGly was prepared in good yield by a method adapted from Bentolila et al. (Scheme 3.1) [19]. The FTIR spectrum of NAGly displayed a peak at  $1609\text{ cm}^{-1}$  that was assigned to the acryloyl C=C stretching. Peaks at  $1648$  and  $1720\text{ cm}^{-1}$  were assigned to C=O stretching for the amide and carboxyl groups respectively, while signals due to N-H bending and stretching appeared at  $1548$  and  $3314\text{ cm}^{-1}$  respectively. The mass spectrum of NAGly (ES+) showed a strong peak at  $m/z$  129.85 corresponding to (M+1) for NAGly, while no peaks were observed at  $m/z$  76 (glycine, M+1) or 91.5 (acryloyl chloride, M+1). In the negative mode spectrum (ES-), a strong peak appeared at  $m/z$  127.8 (M-1), and no peak was observed at  $m/z$  72 (acrylic acid, M-1). The  $^1\text{H-NMR}$  spectrum for NAGly showed characteristic vinylic proton signals at 5.78, 6.21 and 6.30 ppm, together with a signal at 4.03 ppm that was assigned to the methylene group of glycine. NAGly did not respond to ninhydrin or picrylsulphonic reagents (as compared with the deep violet and orange colors respectively obtained upon reaction of the same reagents with glycine itself), indicating the absence of a free amino group.



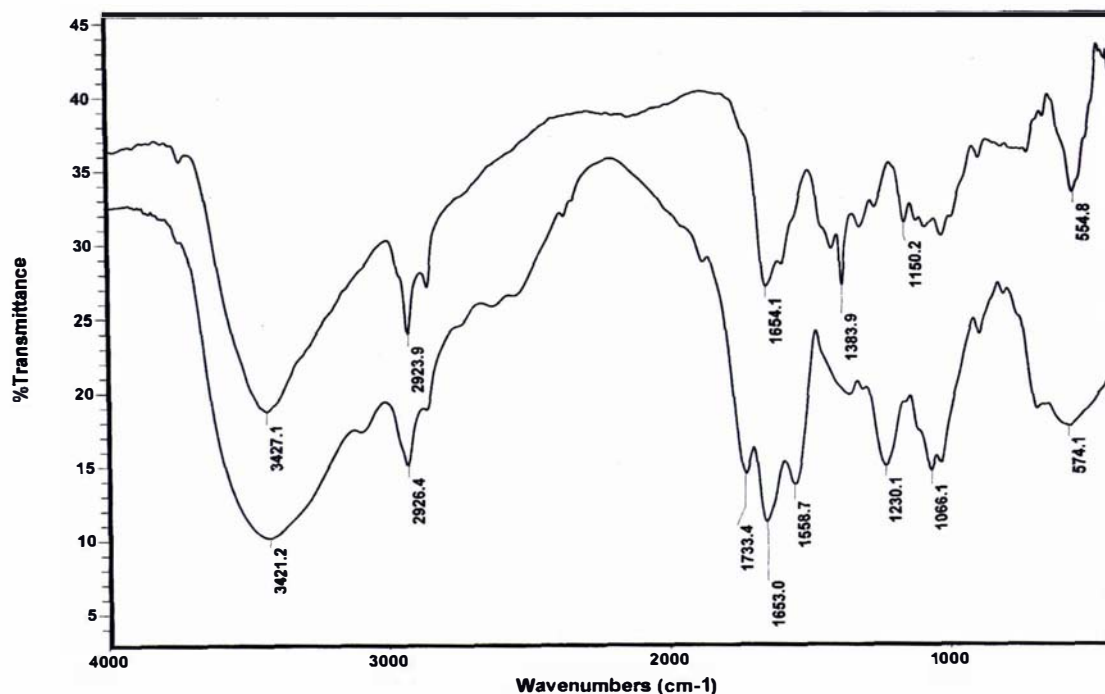
Scheme 3.1. Preparation of NAGly.

### 3.3.2. Preparation and characterization of the semi-IPN hydrogel

The preparation of *Ch*/Poly(*NAGly*) semi-IPN hydrogels is shown in **Scheme 3.2**. An intermixing in the form of entrapped poly(*NAGly*) chains is expected to occur within the crosslinked *Ch* matrix. However, after rigorous washing the weight of the semi-IPN remains slightly higher than that of the constituent *Ch* and glutaraldehyde components. This tends to indicate that some grafting of poly(*NAGly*) onto the *Ch* backbone may also occur. An attempt was carried out to check the grafting possibility of *NAGly* onto the *Ch* backbone at the same conditions used for the preparation of the semi-IPN hydrogel. The results confirmed the occurrence of some grafting. However, the grafting yield did not exceed 6.5% under the used reaction conditions. The FTIR spectra for *Ch* and the *Ch*/Poly(*NAGly*) semi-IPN hydrogels are compared in **Figure 3.1**. The peak at  $1733\text{ cm}^{-1}$  was assigned to carboxylate (*NAGly*) C=O stretching, whereas the peak at  $1653\text{ cm}^{-1}$  was assigned to both amide C=O and imine C=N stretching. No clear peaks appeared in the range of  $1408\text{-}1420\text{ cm}^{-1}$ . This tends to indicate the complete absence of the vinylic double bond of the *NAGly* monomer.



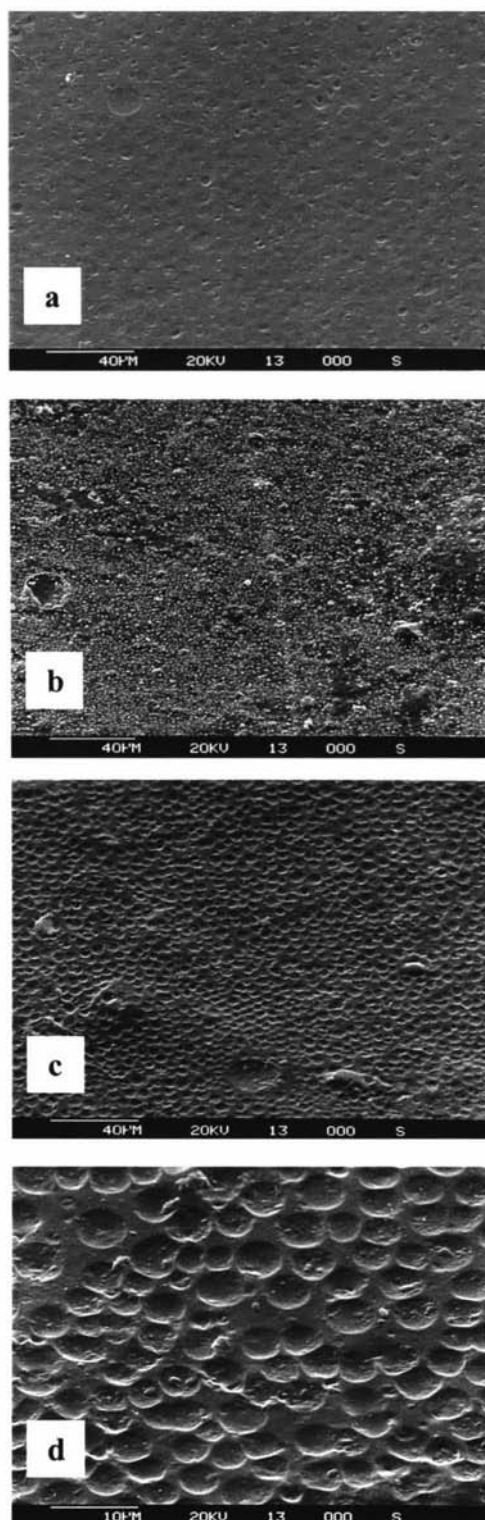
**Scheme 3.2.** Preparation of *Ch*/Poly(*NAGly*) semi-*IPN* hydrogels.



**Figure 3.1.** FTIR spectra for *Ch* (upper trace) and *Ch/Poly(NAGly)* semi-IPN hydrogels (lower trace).

### 3.3.3. The SEM investigation

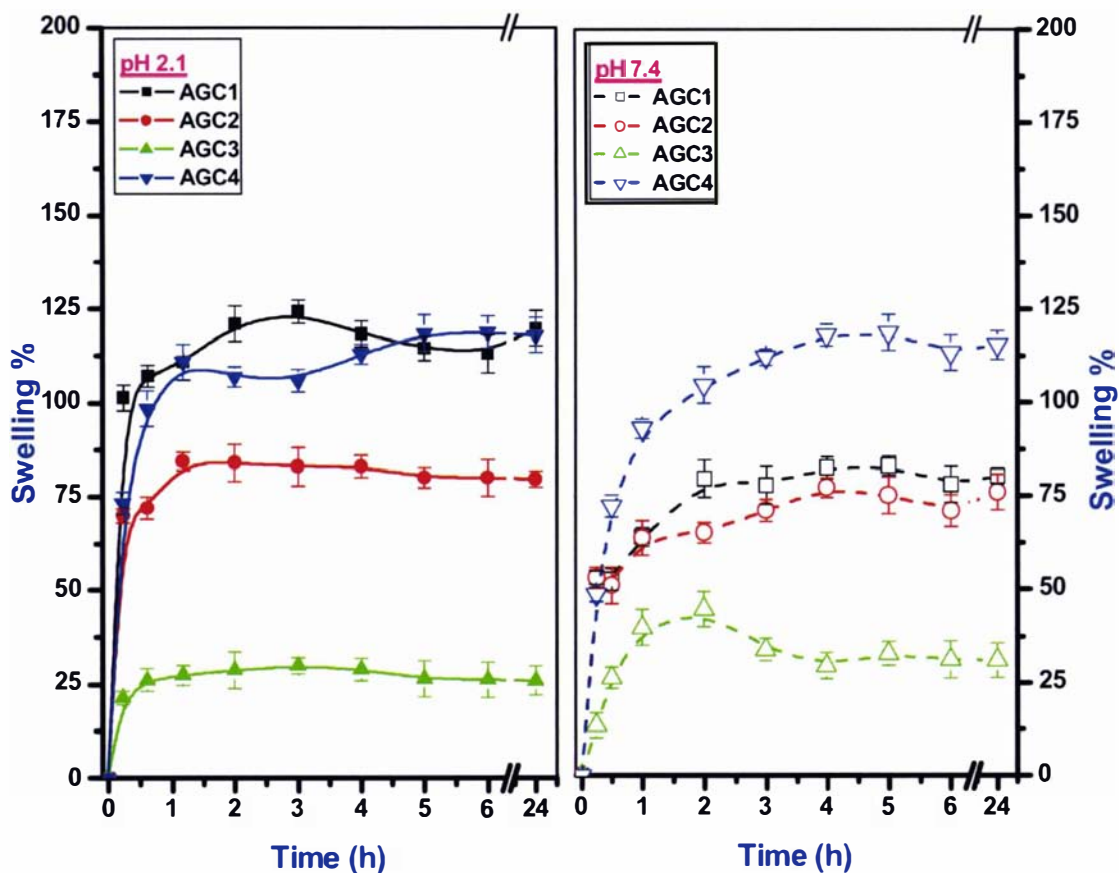
The surface morphologies of *Ch/poly(NAGly)* semi-IPN hydrogel film, *Ch/poly(NAGly)* film loaded with 5% *5-FU* and *Ch/poly(NAGly)* film after 24 h of drug release at pH 7.4 at 37 °C are shown in **Figure 3.2**. As noted from this figure, the surface of *Ch/poly(NAGly)* semi-IPN hydrogel film (**Figure 3.2a**) is smooth, obviously dense and integrated. However, loading with *5-FU* leads to a noticeable increase in roughness with appearance of many irregularities over most of the film surface (**Figure 3.2b**). After the drug release the surface of the film is now seen as a more regular and highly porous (**Figure 3.2c**). This surface pattern of the film after drug release is particularly obvious in **Figure 3.2d**, at high magnification.



**Figure 3.2.** Scanning electron micrograph of the surface of *Ch*/poly(*NAGly*) hydrogel film (a) drug-free, (b) drug-loaded, and (c,d) after 24 h of *5-FU* release at pH 7.4 at 37 °C.

### 3.3.4. Equilibrium swelling studies

The % equilibrium swelling values of the prepared *Ch*/Poly(*NAGly*) semi-IPN hydrogels at 37°C at pH 2.1 and 7.4 are shown in **Figure 3.3**. The % equilibrium swelling of AGC-1 hydrogels were 121% and 83% at pH 2.1 and 7.4 respectively. The AGC-2 hydrogels were swollen to 84% and 75% at equilibrium and the AGC-3 hydrogels attained 30% and 31% swelling. In the case of AGC-4, the % equilibrium swelling values were 119% and 116% at pH 2.1 and 7.4 respectively. These swelling measurements demonstrate the difference in swelling at equilibrium for hydrogels prepared with varying amounts of glutaraldehyde and *NAGly*.

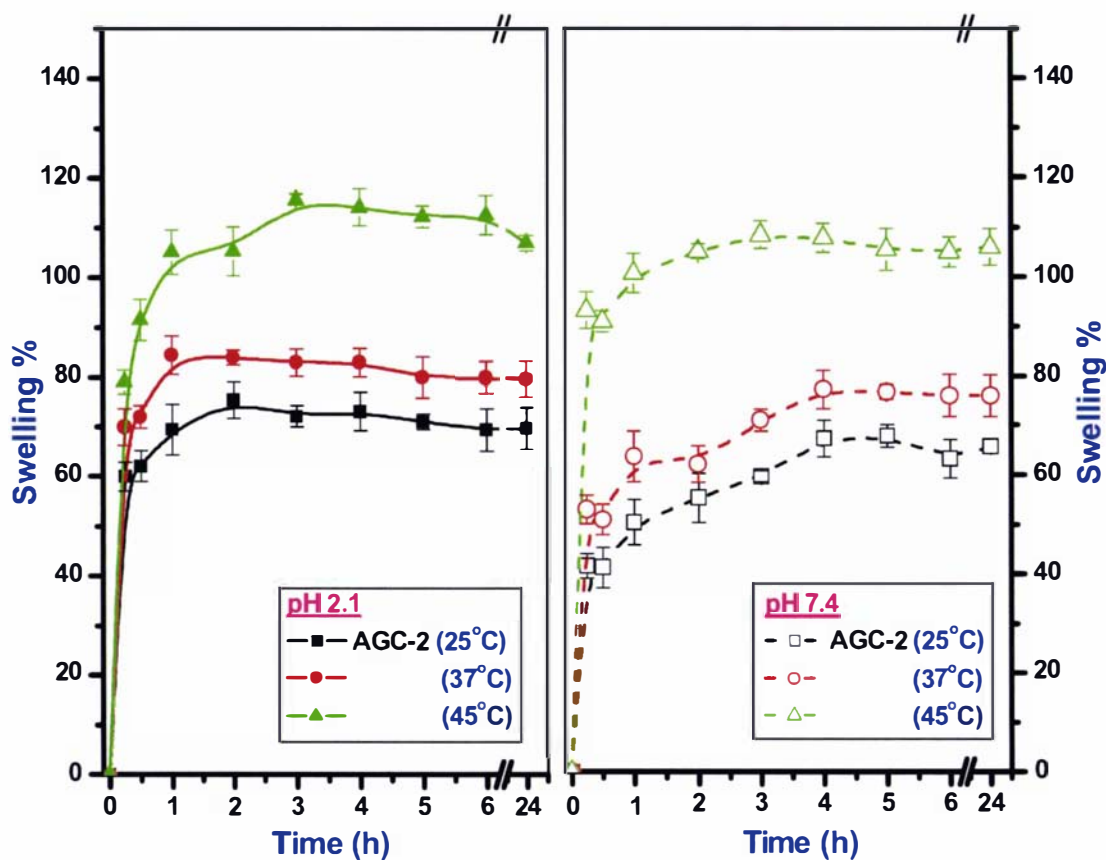


**Figure 3.3.** Swelling behaviour of *Ch*/Poly(*NAGly*) semi-IPN hydrogels at pH 2.1 and pH 7.4 at 37 °C.

Comparing the swelling values of AGC-1, AGC-2 and AGC-3 at the same pH clearly confirms again (see Chapter 2) that as the % of glutaraldehyde increases, the extent of crosslinking increases and consequently the % equilibrium swelling decreases. The high swelling of AGC-4 as compared with AGC-3, even though both have the same glutaraldehyde content (25 wt% based on *Ch*), is due to its higher content of *NAGly*, which is hydrophilic and so should attract water toward the core of the hydrogel. Also the water solubility of the poly(*NAGly*) chains entrapped inside the hydrogel matrices may enable their replacement by the solvent molecules.

The values of the equilibrium swelling % were found to be higher at pH 2.1 than at pH 7.4. As discussed earlier in Chapter 2, this behaviour can be explained by protonation of the unreacted NH<sub>2</sub> groups of *Ch* at acidic pH, leading to dissociation of the hydrogen bonding involving the amino groups, and consequent facilitation of the entrance of solvent into the hydrogel matrix. The swelling process of hydrogels involves the ionization of the unreacted amino groups in the acidic buffer solution. The acid would be attracted to the hydrogels therefore, the weight of the hydrogels increased in the acidic buffer [21]. The swelling values at pH 7.4 will be lower than the values at pH 2.1 due to the increased hydrophobicity of the *Ch*-based hydrogels dominating at higher pH values, thus preventing more swelling in neutral and alkaline media [22]. Both AGC-3 and AGC-4 hydrogels show low pH sensitivity because both of them are highly crosslinked (high percent of glutaraldehyde) and in the case of AGC-4 hydrogel the amount of *Ch* was reduced relative to the *NAGly*.

**Figure 3.4** shows the temperature responsive swelling of AGC-2 (moderately crosslinked), as an example for other hydrogels at pHs 2.1 and 7.4. The % equilibrium swelling increased with increasing temperature of the swelling media. This temperature dependence of swelling may be due to the dissociation of the hydrogen bonding in the *Ch* within the hydrogel matrices [21].



**Figure 3.4.** Swelling behaviour of AGC-2 hydrogel at pH 2.1 and pH 7.4 at 25 °C, 37 °C and 45 °C.

The percent equilibrium swelling of AGC-2 and AGC-2 loaded with *5-FU* (AGC-2FU, 50 mg g<sup>-1</sup> matrix) at pH 2.1 and 7.4 are shown in **Figure 3.5**. At pH 2.1, AGC-2 attained about 80% swelling at equilibrium after 1 h whereas under the same conditions AGC-2FU reached equilibrium at 140% after about 4.5 h. Similar behaviour was noted at pH 7.4. This increase in % swelling upon loading *5-FU* can be attributed to both the expected higher hydrophilicity and small size of the *5-FU* molecules. These two characteristics facilitate the diffusion of the swelling fluids into the partially swollen hydrogels.

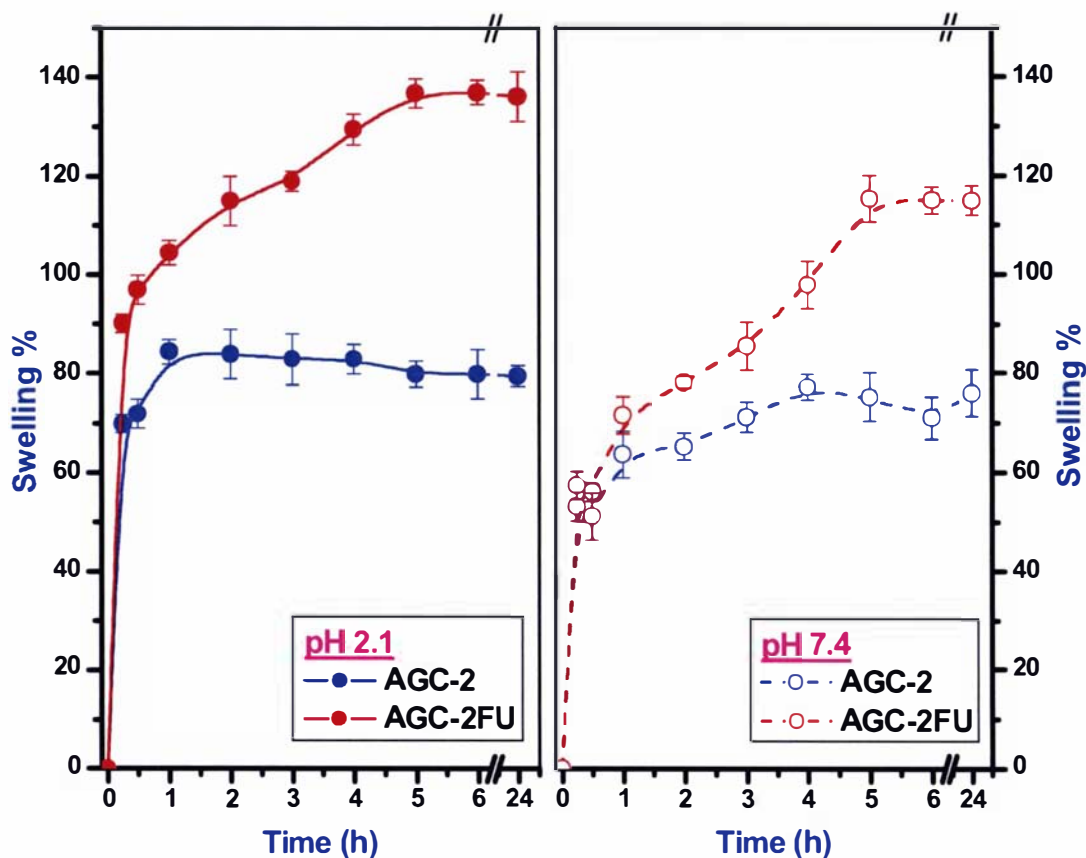


Figure 3.5. Swelling behaviour of AGC-2 and AGC-2FU at pH 2.1 and pH 7.4 at 37 °C.

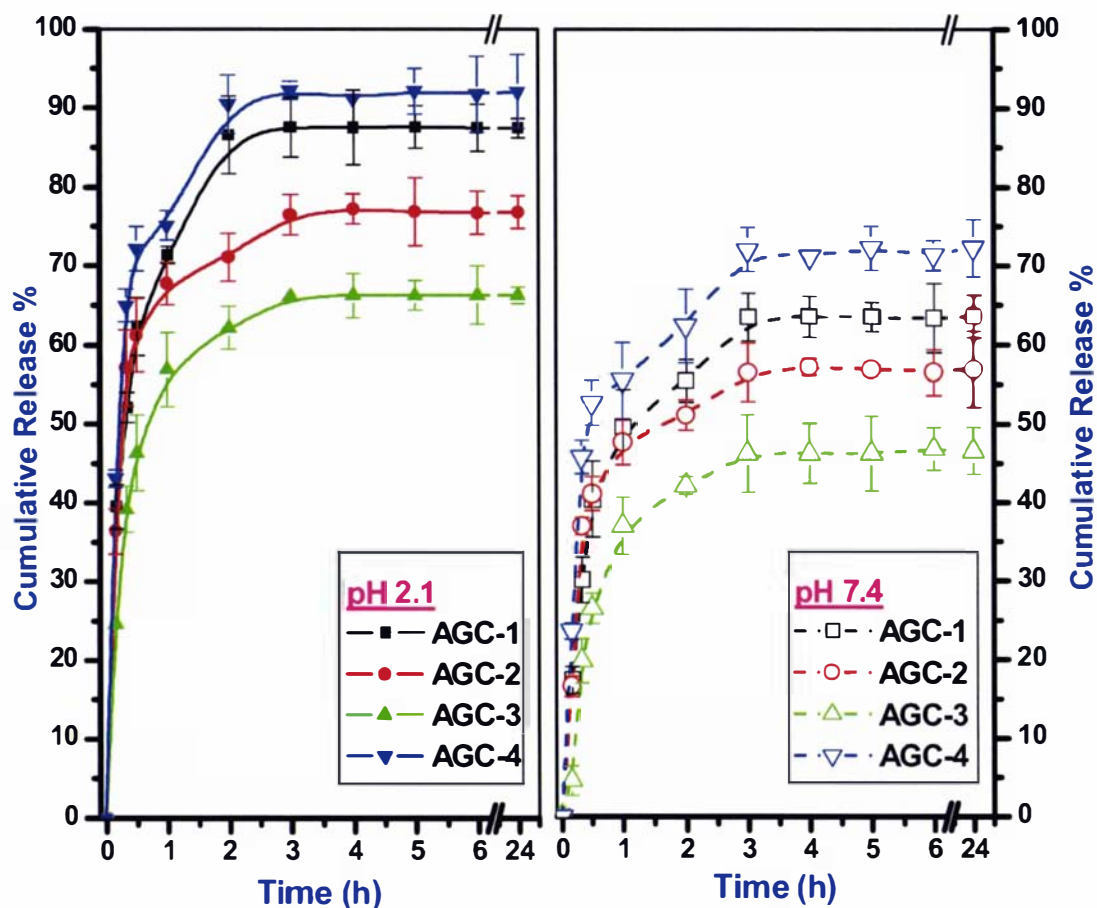
### 3.3.5. Amount of drug entrapped

The calculated amounts of drug entrapped inside the hydrogel, after excluding the amounts released in the washing water at room temperature, were found to be dependent on the composition of the hydrogel. The amounts of drug entrapped were approximately the same for AGC 1-3 (about 95 %) while AGC-4 entrapped slightly less drug (about 90 %).

### 3.3.6. In-vitro cumulative release studies

Figure 3.6 shows the cumulative release profiles from the hydrogels at 37 °C at pH 2.1 and 7.4. The percent of drug released was much higher in acidic buffer than in basic buffer, because the release percent depends on the swelling of the hydrogel where the mechanism of drug release may be due to diffusion through the swollen hydrogels. As

discussed above, the swelling of the prepared hydrogels in acidic medium is greater than in basic medium. Comparing the amounts of drug released from AGC1-3 in the same pH clearly shows that the extent of release at equilibrium was inversely related to the degree of crosslinking. For instance, AGC-1 hydrogel released at equilibrium 87% and 62% of the drug at pH 2.1 and 7.4 respectively. The percent of *5-FU* released from AGC-2 was 77% and 55%. In the case of the AGC-3 hydrogel, the percent of drug released at equilibrium was 65% and 43% at pH 2.1 and 7.4 respectively. With increased hydrophilicity, AGC-4 (higher *NAGly* content) exhibited a particularly high degree of release. In all cases the hydrogels reached equilibrium after about 2.5-3 h, which seems to be short for some applications but this time of release can be controlled by varying both the extent of crosslinking of *Ch* and the content of poly *NAGly*. Besides, this preliminary study was carried out using a single model drug (small and highly hydrophilic) and applying it for entrapment of other drugs may be more useful.



**Figure 3.6.** *In-vitro* cumulative release measurements of 5-FU loaded Ch/Poly(NAGly) semi-IPN hydrogels at pH 2.1 and 7.4 at 37 °C.

It was noticed also from **Figure 3.6** that the drug was not quantitatively released from the hydrogels and this is the expected behaviour in such cases where, some drug molecules will be deeply buried in the *IPN* matrix and are slower to be released or indeed may never be released into the surrounding medium until the matrix starts to break down. In other words, the quantitative release of 100% of drug tends to be unlikely unless the carrier matrix has been completely dissolved.

## **CONCLUSIONS**

The equilibrium swelling measurements of the prepared *Ch*/Poly(*NAGly*) semi-*IPN* hydrogels carried out at different temperatures in simulated body fluids (pH 2.1 and 7.4) clearly showed the pH and thermal-responsive nature of them. The *in-vitro* release profiles of *5-FU*, as a model drug, from the hydrogels were also estimated at pH 2.1 and 7.4. This preliminary investigation of *Ch*-based interpolymeric hydrogels showed that they may be exploited to expand the utilization of these systems in drug delivery applications.

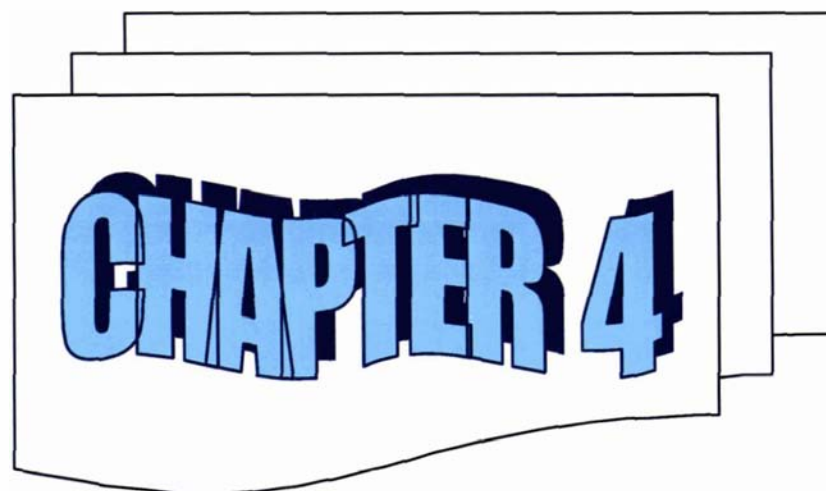
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**CHAPTER 4**

## CHAPTER 4

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### **pH RESPONSIVE INTERPENETRATING POLYMERIC HYDROGELS BASED ON CHITOSAN, POLY(N-ACRYLOYL GLYCINE) AND POLY(ETHYLENE GLYCOL) FOR *IN-VITRO* CONTROLLED RELEASE STUDY.\***

#### **ABSTRACT**

New pH-dependent, biodegradable interpenetrating polymeric network (*IPN*) hydrogels were prepared for controlled drug release investigations. The *IPN* hydrogels were prepared by irradiation of solutions of *N*-acryloylglycine (*NAGly*), polyethylene glycol diacrylate (*PEGDA*) mixed with chitosan (*Ch*), in the presence of a smaller amount of glutaraldehyde (than previous studies, Chapter 3) as the crosslinker and using 2,2-dimethoxy-2-phenyl acetophenone as the photo-initiator. The equilibrium swelling studies were carried out for the hydrogels at 37 °C in buffer solutions of pH 2.1 (*SGF*) and 7.4 (*SIF*). 5-Fluorouracil (*5-FU*), an anticancer drug was entrapped as a model therapeutic agent in the hydrogels and equilibrium swelling studies were carried out for the drug-loaded hydrogels at 37 °C. The *in-vitro* release profiles of the drug were established at 37 °C at pH 2.1 and 7.4.

**Keywords:** swelling, hydrogel, chitosan, crosslinking, biodegradable

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\***El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. Preparation and swelling study of a pH-dependent interpolymeric hydrogel based on chitosan for controlled drug release. *Int. J. Polym. Mater.* **55** (2006) 789.

## **4.1. INTRODUCTION**

A significant body of research has focused on the preparation and characterization of hydrogels for their use in controlled release of drugs, biotechnology and many other fields of life [1-8]. However, the number of polymers suitable for the controlled release of viable therapeutics is quite limited compared to the total available synthetic polymers because of inherent toxicity or lack of certain properties such as biodegradability and swellability in specific environments.

Chitosan (*Ch*) is a cationic biopolymer obtained through alkaline *N*-deacetylation of natural chitin. *Ch* has been considered as a biodegradable, non-toxic and biocompatible polymer with many superior properties [9-12]. These interesting properties make *Ch* an ideal candidate in the preparation of hydrogels for controlled drug release. However, *Ch* exhibits some shortcomings such as hydrophobicity and a high pH-dependence of its physical properties. Therefore, it is very difficult to control drug release with *Ch* itself because of the various pHs of the internal organs of the human body. This may negatively reflect on the human body because of drug under- or over-release [13].

To improve the hydrophilic nature of *Ch*, some trials have been done by blending *Ch* with hydrophilic polymers such as *PVA* [14], *PEG* [13-15] or with poly(*NAGly*) [16].

*PEG* is a highly water soluble polymer widely used in pharmacological products due to its high hydrophilicity, biocompatibility and lack of toxicity. The chemical modification of *Ch* with *PEG* is considered to be a convenient route to synthesize drug carriers [15].

Glutaraldehyde is a common crosslinker used in the crosslinking of polypeptides, proteins and *Ch* due to the high reactivity of its aldehyde groups, which can easily form Schiff's base with the amino groups of polymer [17]. However, from the health point of view, use of a high percent of glutaraldehyde as a crosslinker in a matrix for a drug delivery is not recommended [18].

In the previous chapter, a study was reported to enhance the hydrophilicity of *Ch* by incorporation of it into a semi-*IPN* with a hydrophilic monomer (*NAGly*) in presence of

glutaraldehyde (5-25% based on *Ch* weight). The study reported in this chapter has developed, characterized and studied a new pH-sensitive *IPN* hydrogel for controlled drug release investigations. This *IPN* attempted to improve the *Ch* hydrophilicity through the in situ copolymerization of two hydrophilic monomers, *NAGly* and *PEG*-diacrylate (*PEGDA*) in the presence of *Ch* and a lower amount of glutaraldehyde (2-4% based on total matrix weight). The diacrylate derivative of *PEG* was used to also act as a co-crosslinker with glutaraldehyde in the matrix.

## **4.2. MATERIALS AND METHODS**

### **4.2.1. MATERIALS**

*Ch*, *5-FU* and 2,2 dimethoxy-2-phenyl acetophenone were obtained from Acros Organics (New Jersey, USA). *PEGDA* of average  $M_n$  ca. 575 was supplied by Aldrich (Milwaukee, USA). Acryloyl chloride was purchased from Merck (Schuchardt OHG, Hohenbrunn, Germany). Glutaraldehyde (25% aqueous solution) was obtained from Sigma Chemical Co. (St. Louis, USA). Glycine and all other reagents and solvents were of analytical grade and used as received.

### **4.2.2. METHODS**

#### **4.2.2.1 Characterization of *Ch***

The % N-deacetylation of the *Ch*, used in this study, was determined (see Chapter 2) to be 67% with the aid of FTIR analysis using the following relationship [19]:

$$\% \text{ N-deacetylation} = 100 [1 - (A_{1655}/A_{3340}) (1/1.33)]$$

where,  $A$  is the absorbance at the given wave number. A similar value of *N*-deacetylation of *Ch* was estimated from the elemental analysis based on the mole fraction concept [20].

The molecular weight ( $M_w$ ) of **Ch** was determined to be  $4.92 \times 10^5$  Da using the Mark-Houwink viscometry method [21], in a solvent of 0.1 M acetic acid/0.2 M NaCl maintained at 25 °C (see Chapter 2).

#### 4.2.2.2. Synthesis of (NAGly) monomer

The modified method for the synthesis of **NAGly** has been described in detail in Chapter 3 [16].

#### 4.2.2.3. Preparation of the IPN hydrogel

Pre-determined quantities of **NAGly** (see Table 4.1) dissolved in 30 ml of 1,4-dioxan were added to a solution of **Ch** (3% w/v) in dilute acetic acid (2% w/v). The appropriate weight of **PEGDA** dissolved in 10 ml of 1,4-dioxan was added to this mixture then a solution of 2,2-dimethoxy-2-phenyl acetophenone (2% based on the total weight of **NAGly** and **PEGDA**) in THF (5 mL) was added dropwise. The calculated volume of glutaraldehyde (25% aqueous solution) was added with agitation. The reaction mixture was then poured into a glass Petri dish and the polymerization was initiated by irradiation with an incandescent broad spectrum lamp (Philips Comptalux, 150 W), positioned 25 cm above the Petri dish. Irradiation was continued for 2 h until complete gelation had occurred. The hydrogels produced were extensively washed with distilled water to remove any residual monomers, freeze-dried and stored until further use. The compositions of the prepared **IPNs** are listed in Table 4.1.

**Table 4.1.** Composition of the prepared poly[**NAGly-PEGDA**]-**Ch** IPN hydrogels.

Sample Code	<b>Ch</b> (g)	<b>NAGly</b> (g)	<b>PEGDA</b> (g)	Glutaraldehyde solution (25% aq.)	
				( $\mu$ l)	% <sup>a</sup>
GEC-1	2.50	----	----	500	5
GEC-2	2.50	----	----	1000	10
GEC-3	1.00	0.75	0.75	200	5
GEC-4	1.00	0.75	0.75	400	10
GEC-5	1.00	1.40	1.60	400	10

<sup>a</sup> : based on **Ch** weight.

#### 4.2.2.4. Characterizations

The FTIR spectra of the dried poly[NAGly-PEGDA]-Ch IPN hydrogels were recorded with a Perkin Elmer Paragon 1000 FTIR spectrometer in the range 4000-400  $\text{cm}^{-1}$ . The surface morphology of the hydrogel films before and after 24 h of *in-vitro* release of 5-FU at pH 7.4 was investigated by scanning electron microscope (Cambridge Stereoscan S-250 mk 3 SEM). NAGly was characterized using different tools (see Chapter 3).

#### 4.2.2.5. Entrapment of a model drug

The poly[NAGly-PEGDA]-Ch IPN hydrogels loaded with 5-FU as a model therapeutic agent were prepared in the same manner described in section 4.2.2.3. A predetermined amount of the drug was added to the reaction mixture, stirred vigorously and then the polymerization reaction was carried out. The resulting hydrogels were washed with distilled water and freeze-dried.

#### 4.2.2.6. Determination of the amount of 5-FU entrapped

The amount of 5-FU entrapped in the hydrogels was evaluated by the indirect method (see Chapter 3) [16].

#### 4.2.2.7. Equilibrium swelling measurements

The swelling behavior of the prepared poly[NAGly-PEGDA]-Ch IPN hydrogels was studied at 37 °C at pH 2.1 (*SGF*) and 7.4 (*SIF*). The weights of the swollen hydrogels were measured at intervals, after removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by the following equation:

$$\% \text{ Swelling} = 100 [(W_t - W_0)/W_0]$$

where,  $W_0$  is the initial weight and  $W_t$ , the final weight of the swelled hydrogel at time  $t$ . The data represents mean  $\pm$ SD from three independent experiments.

For studying the effect of 5-FU entrapped (up to 5%) in the hydrogel on the hydrogel swelling behavior, a cyclic swelling procedure was carried out. Known weights of the hydrogel and the drug-loaded hydrogel were left in the swelling medium until maximum equilibrium swelling was attained. The swelled sample was weighed after

removal of surface liquid, completely freeze-dried and reweighed. This swelling-deswelling process was repeated three times for the same sample.

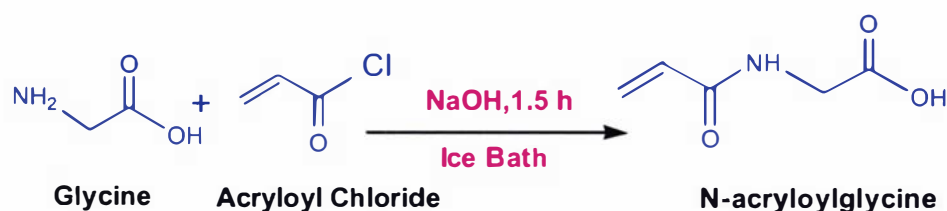
#### 4.2.2.8. *In-vitro* cumulative release studies

The *in-vitro* release profiles of entrapped *5-FU* were determined by placing the pre-weighed hydrogel loaded with the drug in a buffer solution of pH 2.1 (*SGF*) or pH 7.4 (*SIF*) at 37 °C. At periodic intervals a 3 ml aliquot was withdrawn and its absorbance at  $\lambda_{\text{max}}$  268 nm was measured. The withdrawn sample was replaced with an equal volume of fresh buffer, to keep the volume of release media constant. The data represents mean  $\pm$ SD from three independent experiments.

### 4.3. RESULTS AND DISCUSSION

#### 4.3.1. Synthesis and characterization of NAGly

The preparation of *NAGly* in good yield (78%) by a modified method and its complete characterization were reported earlier in Chapter 3 [16] (Scheme 4.1).

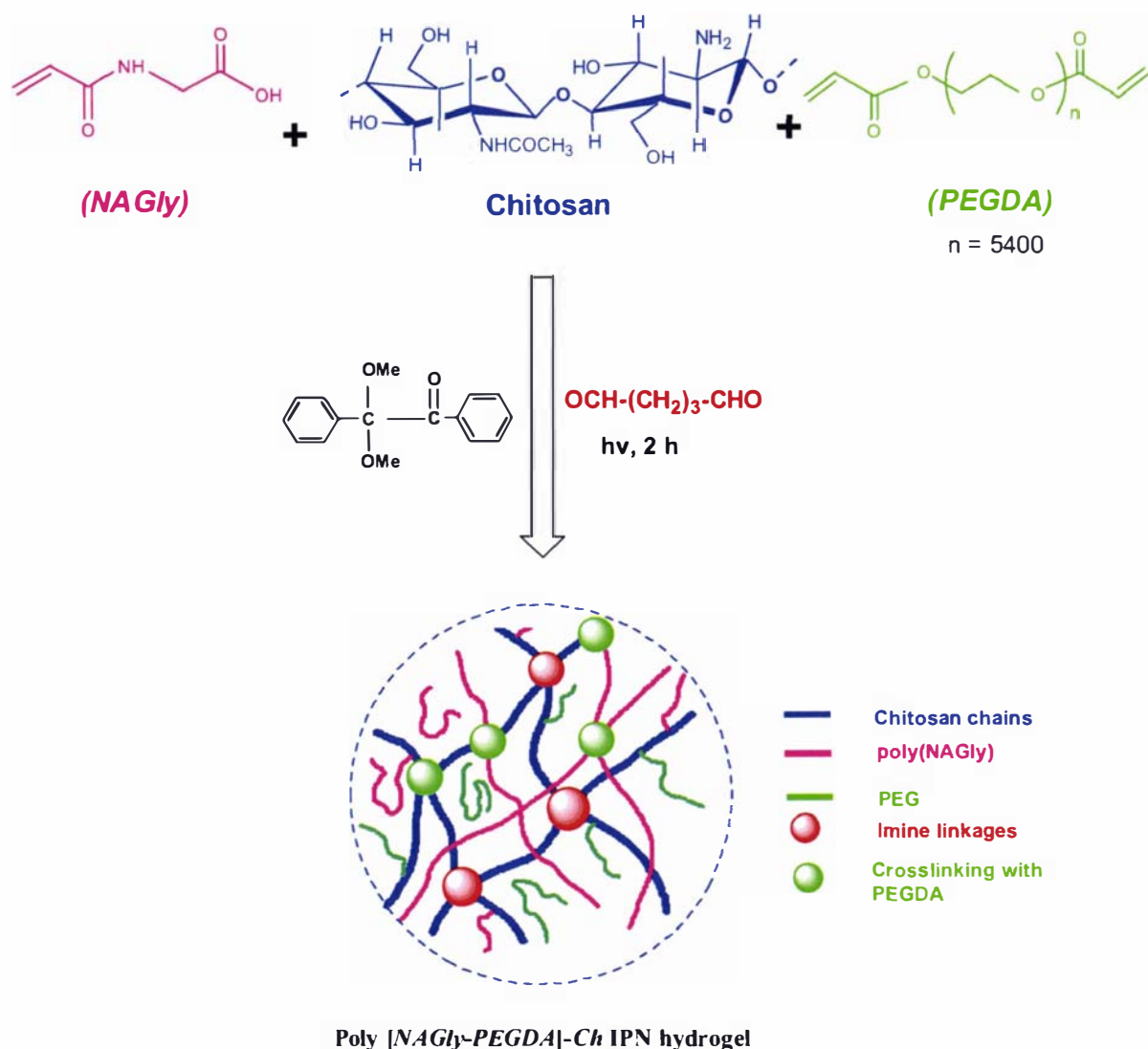


Scheme 4.1. Preparation of *NAGly*.

#### 4.3.2. Preparation and characterization of the IPN hydrogel

Scheme 4.2 shows the preparation of poly[*NAGly*-*PEGDA*]-*Ch* IPN hydrogels. Inside these crosslinked *Ch* matrices, a type of intermixing in the form of graft copolymerized and entrapped poly(*NAGly*) and *PEG* chains might occur. As reported earlier in Chapter 3, grafting of *NAGly* can occur onto *Ch* backbone during the hydrogel preparation. An attempt was also carried out to check the grafting possibility of *PEGDA* onto *Ch* using

2,2 dimethoxy-2-phenyl acetophenone as the initiator and under the same reaction conditions used for the hydrogel preparation. The results showed that some *PEGDA* had been grafted onto *Ch* but the graft yield was relatively low (maximum 9.8% under the conditions used). Also, the diacrylate groups of *PEGDA* can participate during the hydrogel preparation in the crosslinking process of both *Ch* and poly(*NAGly*).



**Scheme 4.2.** Preparation of poly[*NAGly-PEGDA*]-*Ch* IPN hydrogels.

Figure 4.1 shows the FTIR spectra for the poly[*NAGly-PEGDA*]-*Ch* hydrogel as compared to that of *Ch*. The signal appeared at  $1730\text{ cm}^{-1}$  was assigned to the C=O stretching of both carboxylate (*NAGly*) and ester (*PEGDA*), whereas the absorption peak at  $1654\text{ cm}^{-1}$  was attributed to both amide C=O and imine C=N stretching. No clear peaks appeared in the range of  $1408\text{--}1425\text{ cm}^{-1}$ , which tends to indicate the absence of the vinylic double bond of both *NAGly* and *PEGDA* monomers.

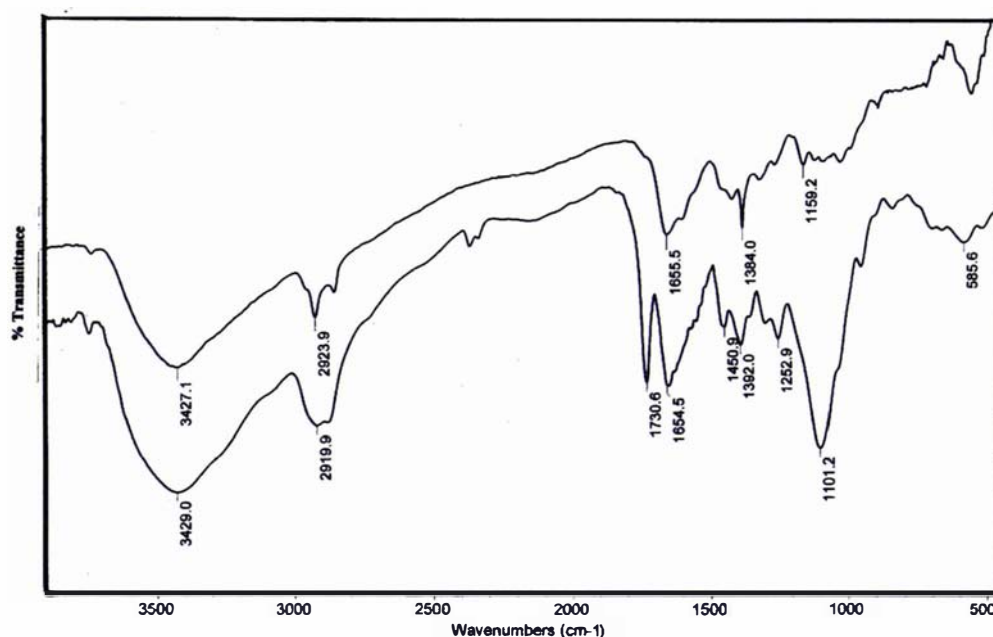
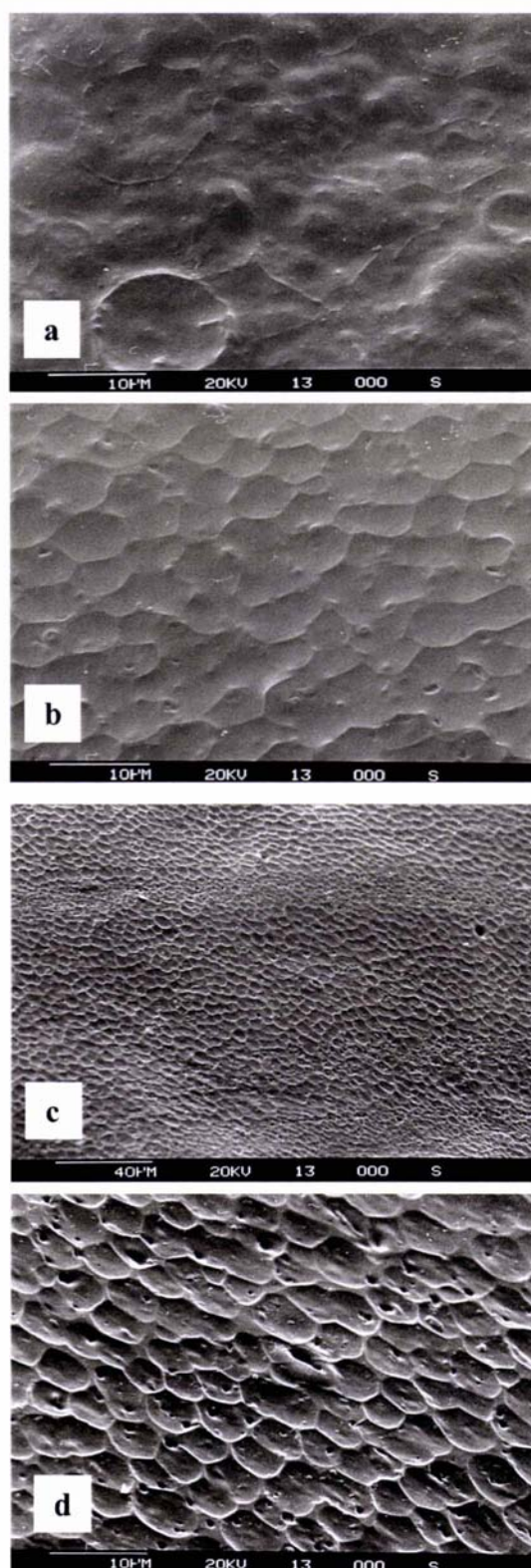


Figure 4.1. FTIR spectra for *Ch* (upper trace) and the poly[*NAGly-PEGDA*]-*Ch* IPN hydrogel (lower trace).

### **4.3.3. The SEM investigation**

The surface morphologies of poly[*NAGly-PEGDA*]-*Ch* IPN hydrogel film, poly[*NAGly-PEGDA*]-*Ch* film loaded with 5% *5-FU* and poly[*NAGly-PEGDA*]-*Ch* film after 24 h of drug release at pH 7.4 at 37 °C are shown in **Figure 4.2**. As noted from this figure, the surface of poly[*NAGly-PEGDA*]-*Ch* IPN hydrogel film (**Figure 4.2a**) is dense and relatively smooth with many irregular lumps over most of the film surface. The fine distribution of *5-FU* introduced into the hydrogel (**Figure 4.2b**) renders the surface pattern smoother and regular. After the drug release the film has been converted to now show a highly porous surface (**Figure 4.2c**). This surface pattern, after the drug release, is particularly obvious in **Figure 4.2d**, at the high magnification.



**Figure 4.2.** Scanning electron micrograph of the surface of poly[NAGly-PEGDA]-Ch IPN hydrogel film (a) drug-free, (b) drug-loaded, and (c,d) after 24 h of 5-FU release at pH 7.4 at 37 °C.

#### 4.3.4. Equilibrium swelling measurements

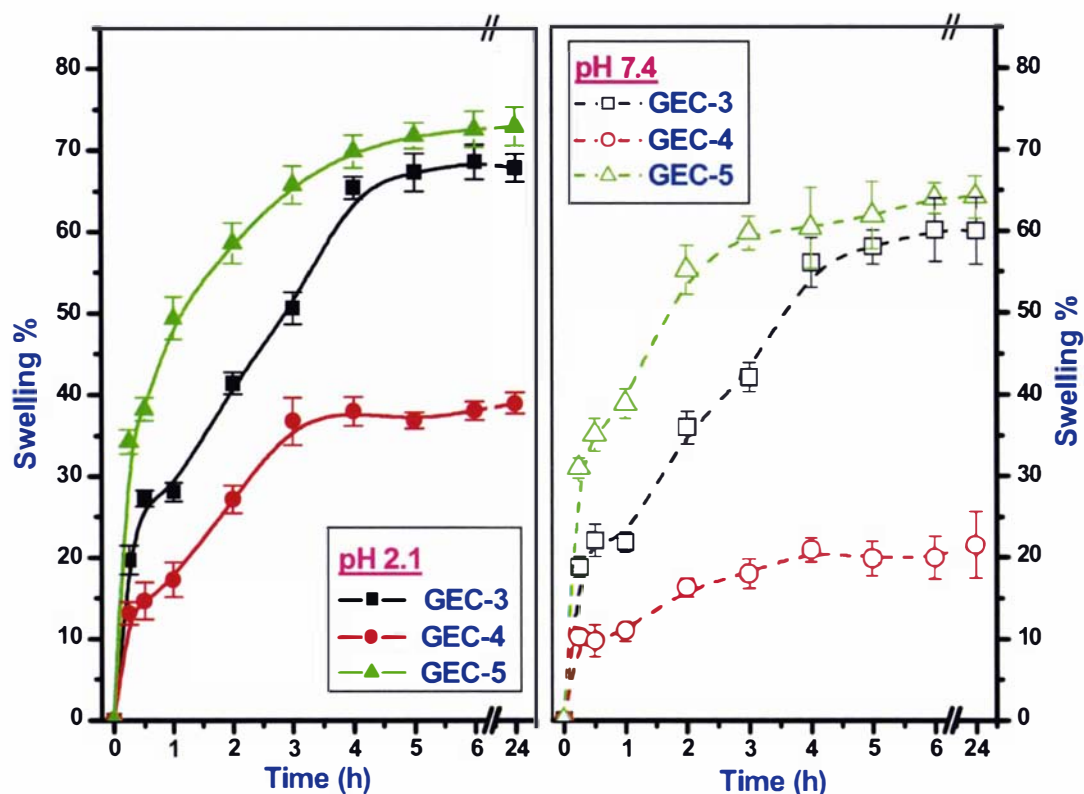
A preliminary study was carried out to detect the effect of the *IPN* composition on the swelling characteristics of *Ch*. As shown in **Table 4.2**, *Ch* itself has a high degree of swelling depending on the concentration of the crosslinking agent. For instance, the maximum equilibrium swelling attained about 240% (GEC-1) and 200% (GEC-2) for *Ch* crosslinked with 5% and 10% of glutaraldehyde, respectively. In contrast, GEC-3 and GEC-4 attained a maximum equilibrium swelling of 60% and 21%, respectively. This indicates that the swelling of crosslinked *Ch* alone is very high which could be negatively reflected in the controlled drug release pattern. The decrease in the maximum equilibrium swelling for the *IPN* system is expected and can be controlled by changing the composition of the components as seen in **Table 4.1** and discussed in this chapter.

**Table 4.2.** Swelling values of poly[*NAGly-PEGDA*]-*Ch* *IPN* hydrogels (GEC 3-4) at pH 7.4 at 37 °C as compared to crosslinked *Ch* (GEC 1-2).

Time (h)	Swelling %							
	GEC-1	±SD	GEC-2	±SD	GEC-3	±SD	GEC-4	±SD
0.25	45.8	2.1	40.1	4.9	18.7	5.8	10.16	4.5
0.5	59.2	3.8	55.3	5.1	22	7.5	9.7	3.7
1	110	4.5	100	2.1	21.8	3.7	10.9	5.9
2	185	7.1	150	6.8	36	5.9	16.3	2.1
3	250	5.1	215	9.5	42	2.1	17.9	2.1
4	246	4.8	204	6.1	56.1	6.1	20.9	4.1
5	245	10.5	210	8.8	58	9.8	19.3	3.7
6	241	6.1	205	4.5	60.1	2.5	19.9	6.8
24	240	3.9	200	3.7	60	6.1	21.5	3.4

±SD: Standard deviation

The equilibrium swelling behavior of the hydrogels GEC 3-5, measured at 37 °C at pH 2.1 and 7.4 are shown in **Figure 4.3**. The GEC-3 hydrogels attained 68% and 60% swelling at equilibrium at pH 2.1 and 7.4 respectively. The % equilibrium swelling of GEC-4 hydrogels were 38% and 21% whereas GEC-5 hydrogels swollen to 72% and 62% at pH 2.1 and 7.4 respectively. These swelling values demonstrate the differences that can be achieved in swelling at equilibrium for hydrogels prepared with varying amounts of *PEGDA*, *NAGly* and glutaraldehyde.

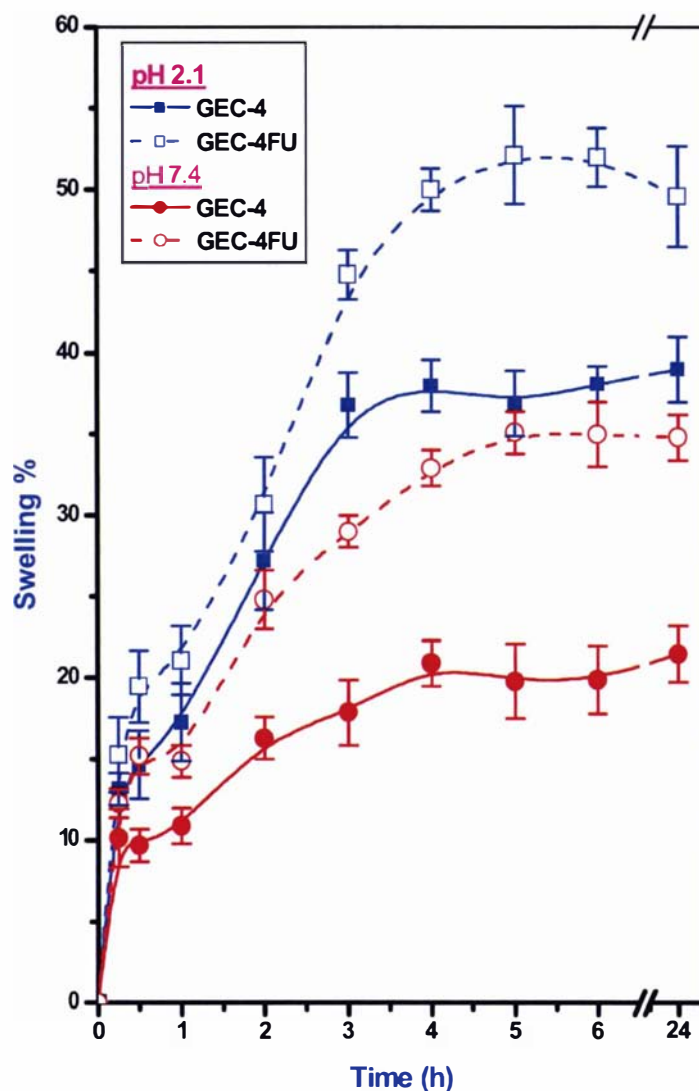


**Figure 4.3.** Swelling behavior of poly[*NAGly-PEGDA*]-*Ch* IPN hydrogels at pH 2.1 and 7.4 at 37 °C.

Comparing the swelling values of GEC-3 and GEC-4 at the same pH implies that as the percent of glutaraldehyde increases, the extent of crosslinking increases leading to a decrease of the equilibrium swelling. The % equilibrium swelling values attained at the same pH for GEC-4 and GEC-5 showed that, increasing the amounts of both *NAGly* and *PEGDA* increases the hydrophilicity of the hydrogel and consequently the % equilibrium swelling increases. During the hydrogel preparation, some of *PEGDA* may act as a co-crosslinker for *Ch* in addition to glutaraldehyde and so it may work to decrease the swelling extent of the hydrogel but from the swelling data obtained this factor seems to be less effective than the increasing of the hydrogel hydrophilicity. Also, decreasing the percent of *Ch*, relative to *NAGly* and *PEGDA*, in GEC-5 may lead to decreasing of the total crosslinking extent of the matrix.

The swelling values attained at equilibrium for poly[*NAGly-PEGDA*]-*Ch* hydrogels were found to be higher at pH 2.1 than at pH 7.4. As discussed in Chapter 2, this pH-responsive character of the hydrogels can be attributed to chemical structure of *Ch* where, in the acidic pH, protonation can occur at both of unreacted NH<sub>2</sub> groups of *Ch* and the imine (C=N) groups leading to dissociation of the hydrogen bonding involving amino/imine groups. Moreover, the acidic medium can hydrolyse the imine bond leading to facilitation of the entrance of solvent into the hydrogel to attain higher values of swelling. Also, the swelling process of hydrogels involves the ionization of the unreacted amino groups in the acidic buffer solution, then the acid would be attracted to the hydrogels leading to increasing the weight of the hydrogels in the acidic buffer [16, 22]. Again, the equilibrium swelling values at pH 7.4 will be lower than the values in pH 2.1 due to the increased hydrophobicity of the *Ch*-based hydrogels dominating at higher pH values, thus preventing more swelling in neutral and alkaline media [23].

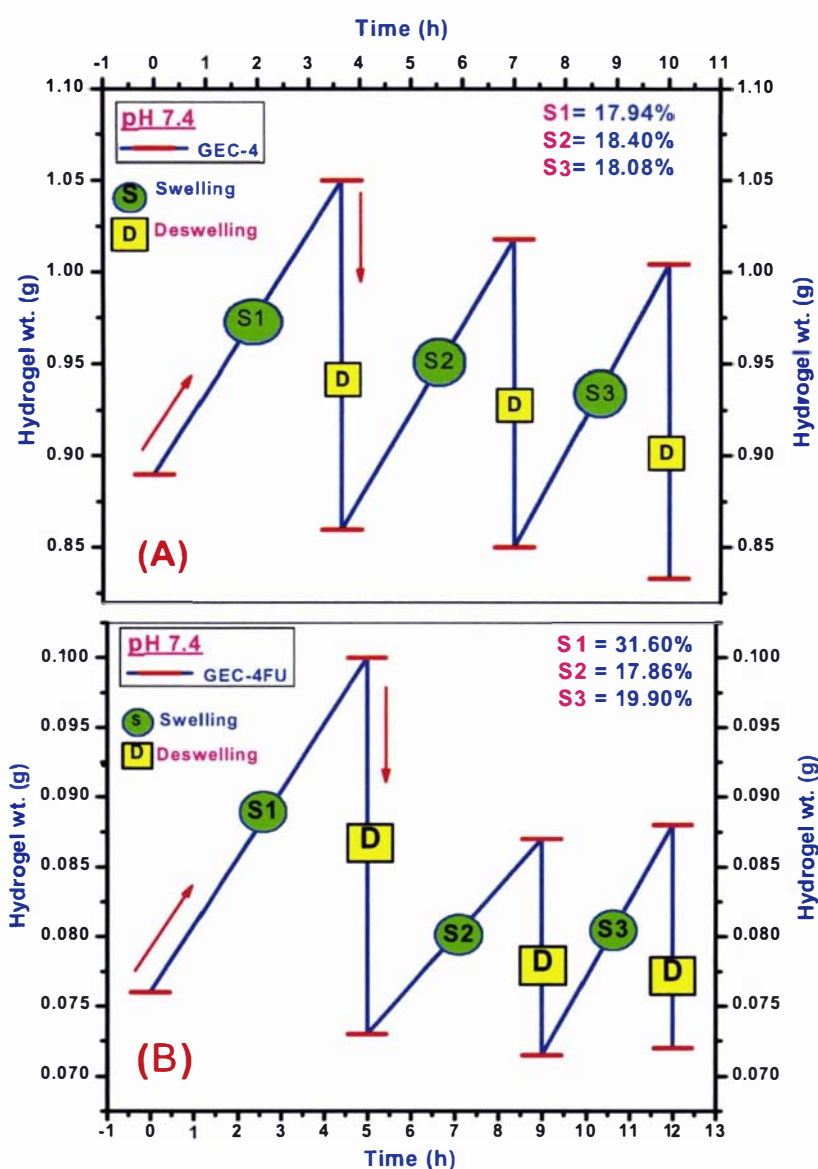
**Figure 4.4** shows the % equilibrium swelling of GEC-4 and GEC-4 loaded with *5-FU* (GEC-4FU, 5% uploading) at pH 2.1 and 7.4. At pH 2.1, GEC-4 reached equilibrium at about 38% swelling after 3 h whereas under the same conditions GEC-4FU attained equilibrium at 53% after approximately 4.5 h. Similar behavior was observed at pH 7.4. This increase in % swelling upon loading *5-FU* can be attributed to the hydrophilic nature of the *5-FU* molecules, which facilitates the diffusion of the swelling fluids into the partially swollen hydrogels. These results also confirm, as discussed earlier in Chapter 2, the role of the *5-FU* hydrophilicity in the swelling process.



**Figure 4.4.** Swelling behavior of GEC-4 and GEC-4FU at pH 2.1 and 7.4 at 37 °C.

The change in the swelling behavior of the hydrogel upon loading the drug was also confirmed by carrying out the cyclic swelling for both the drug-loaded (GEC-4FU) and the drug-free (GEC-4) samples as shown in **Figure 4.5**. From this figure, GEC-4 hydrogel (**Figure 4.5A**) attained about 18% equilibrium swelling in the first cycle. Little change was noticed in the other two cycles except a slight decrease in the weight of the dried hydrogel. In case of the drug-loaded hydrogel (**Figure 4.5B**), the equilibrium swelling for the first cycle attained about 31%. Deswelling of the sample

and repeating the experiment shows that the equilibrium swelling decreases and achieved its maximum value after a shorter time. The third cycle shows almost no change in the maximum equilibrium swelling with a slight decrease in the time of achieving this equilibrium. These results confirm that *5-FU* is responsible for the increase of equilibrium swelling value due to its hydrophilic nature. **Figure 4.5B** shows also that all the accessible *5-FU* is released from the matrix in the first cycle. The lack of change seen in the other two cycles implies the role played by *5-FU* and tends to indicate that little or no readily soluble fraction remains in the matrix after 5 h of initial swelling.



**Figure 4.5.** The cyclic swelling for (A) GEC-4 and (B) GEC-4FU at pH 7.4 at 37 °C.

#### 4.3.5. In-vitro cumulative release studies

The *in-vitro* cumulative release profiles of 5-FU from the poly[NAGly-PEGDA]-Ch IPN hydrogels at pH 2.1 at 37 °C are shown in Figure 4.6A. Comparing the extent of release from GEC-3 and GEC-4 clearly confirms that the amount of drug released at equilibrium is inversely related to the degree of crosslinking. GEC-5 (higher NAGly and PEGDA contents) exhibits a particularly high extent of release compared to GEC-4. The same behavior of drug release from the hydrogels was noted at pH 7.4 (Figure 4.6B).

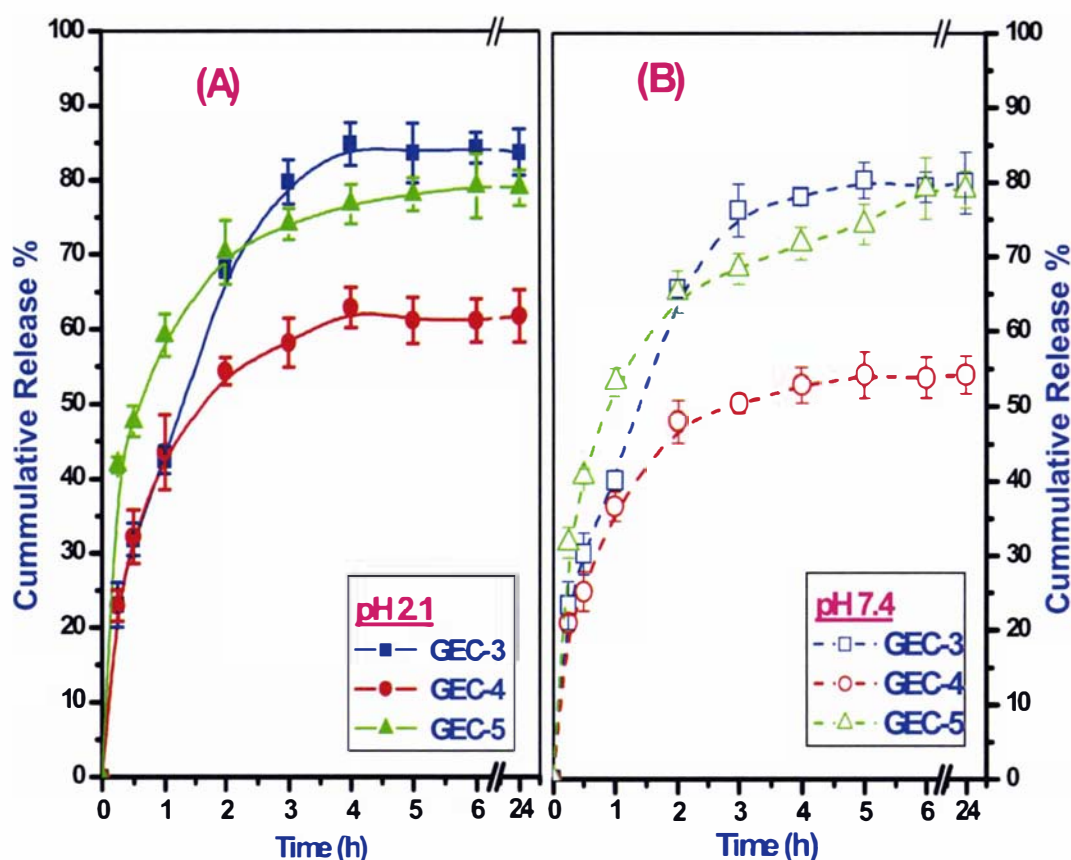


Figure 4.6. *In-vitro* cumulative release measurements of 5-FU loaded poly[NAGly-PEGDA]-Ch IPN hydrogels at pH 2.1 and 37 °C.

The hydrogels exhibited a higher percent of drug release at pH 2.1 than at pH 7.4. This difference in the release rate can be attributed to the difference in swelling behavior of the hydrogels where the drug release may be attributed to the diffusion-dissolution

mechanism through the swollen gels. As discussed earlier, the swelling of the prepared poly[*NAGly-PEGDA-Ch*] IPN hydrogels in the acidic medium is higher than in weakly basic medium. In the case of GEC-5, the extent of drug release shows little pH sensitivity. This may be due to the reduced amount of *Ch* relative to *NAGly* and *PEGDA*. This again confirms the role of the chemical structure of *Ch* in the pH sensitivity of the prepared hydrogels.

As shown in **Figure 4.6**, the hydrogels attained equilibrium after about 4-6 h and 5-6 h at pH 2.1 and 7.4, respectively. Varying the crosslinking density and/or the percent of the *IPN* components can modulate this release time. Also, repeating this preliminary study using other model drugs may change both the time and the rate of release.

## CONCLUSIONS

New biodegradable pH-dependent IPN hydrogels of poly[*NAGly-PEGDA-Ch*] were prepared by irradiation for controlled drug release studies. The pH-responsive behavior of these hydrogels was observed through studying their equilibrium swelling at 37 °C in simulated body fluids (pH 2.1 and 7.4). The *in-vitro* release profiles of *5-FU*, as a model therapeutic agent, from the hydrogels were also estimated at the same pH values. In comparison with the results obtained from poly[*NAGly-Ch*] semi-IPN hydrogels (Chapter 3), the results obtained in this study showed that the inclusion of *PEGDA* in the matrix assembly was beneficial. In the presence of *PEGDA*, longer release times were achieved than our previous study [16] with reduced use of the relatively toxic crosslinker, glutaraldehyde. This preliminary study points to these *Ch* based interpolymeric hydrogels also being good candidate matrices that may be tailored for utilization in drug delivery applications.

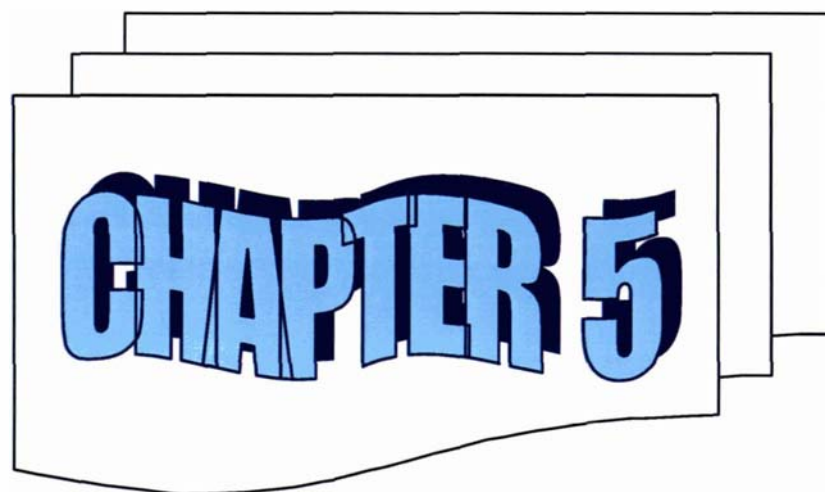
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**CHAPTER 5**

## CHAPTER 5

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### pH AND THERMALLY RESPONSIVE HYDROGELS BASED ON CHITOSAN-g-POLY(ETHYLENE GLYCOL) FOR *IN-VITRO* CONTROLLED RELEASE STUDY.\*

#### ABSTRACT

The grafting of poly(ethylene glycol) diacrylate macromer (*PEGDA*) onto a chitosan (*Ch*) backbone was carried out with different macromer concentrations. The grafting was achieved by  $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ -induced free radical polymerisation. Biodegradable, pH and thermally responsive hydrogels of *Ch-g-PEGDA* crosslinked with a relatively low amount of glutaraldehyde were prepared for controlled drug release studies. Both the graft copolymers and the hydrogels were characterised by FTIR, elemental analysis and scanning electron microscopy (SEM). The hydrogels obtained were subjected to equilibrium swelling studies at different temperatures (25 °C, 37 °C and 45 °C) in buffer solutions of pH 2.1 (*SGF*) and 7.4 (*SIF*). 5-Fluorouracil (*5-FU*), an anti cancer drug, was entrapped in these hydrogels and the equilibrium swelling studies were carried out for the drug-loaded hydrogels at pH 2.1 and 7.4 at 37 °C. The *in-vitro* release profile of the drug was established at 37 °C at pH 2.1 and 7.4.

**Key Words:** hydrogel, biodegradable, chitosan, graft copolymers, *in-vitro* release

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\***El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. Swelling characteristics and in-vitro drug release study with pH and thermo-sensitive hydrogels based on modified chitosan. *J. Appl. Polym. Sci.* 102 (2006) 977.

## 5.1. INTRODUCTION

Considerable interest has been focused on the preparation and characterization of hydrogels for their use in controlled release of drugs and many other fields of life because of their high water content, softness and pliability [1-4].

Chitosan (*Ch*) [a (1→4) 2-amino-2-deoxy-β-D-glucan] is a cationic biopolymer obtained through alkaline *N*-deacetylation of natural chitin. *Ch* has many superior properties such as biodegradability, non-toxicity and biocompatibility [5-7]. These interesting properties make *Ch* an ideal candidate in the preparation of hydrogels for controlled drug release. However, *Ch* exhibits some shortcomings such as hydrophobicity, poor mechanical properties and a high pH-dependence of its physical properties. Therefore, it is very difficult to control drug release with *Ch* itself where this may negatively reflect on the human body because of drug under- or over-release [8].

To overcome these shortcomings of *Ch*, two main approaches have been applied. The first approach was the incorporation of *Ch* in an interpolymeric hydrogel (*IPN*) [1-4]. The second was the chemical modification by the grafting of a suitable vinyl monomer(s) onto *Ch* backbone then crosslinking this modified *Ch* [3,9].

Poly(ethylene glycol) (*PEG*) is a highly water soluble polymer. Due to the high hydrophilicity, lack of toxicity and biocompatibility of *PEG*, grafting of it onto *Ch* is considered to be a convenient route to synthesize drug carriers [10]. *PEG* and some of its derivatives have been used for the modification of *Ch* via grafting: by *PEG* alone [11-14], by *PEG*-aldehyde [15-17] and by *PEG*-ester [18-19]. However, one can find little reported work on exploiting these *Ch*-g-*PEG* copolymers in the preparation of hydrogels for controlled release studies [9,10] as compared with the number of *IPN* systems based on *Ch* with *PEG* used for the same purpose [20-22]. Therefore, in this study, an attempt was carried out to improve the hydrophilicity of *Ch* via grafting the diacrylate derivative of *PEG* (*PEGDA*) onto it. The grafting was carried out in acidic medium with ceric ammonium nitrate (*CAN*) as the initiator. The hydrogels obtained from these graft copolymers crosslinked with a lower amount of glutaraldehyde were characterized and their swelling characteristics and drug release profile were estimated.

## 5.2. MATERIALS AND METHODS

### 5.2.1. MATERIALS

*Ch* and *5-FU* were purchased from Acros Organics (Morris Plains, New Jersey, USA). *PEGDA* of average  $M_n$  ca. 575 was supplied by Aldrich (Milwaukee, USA). *CAN* was obtained from Ajax Chemicals (Sydney, Australia). Glutaraldehyde (25% aqueous solution) was obtained from Sigma Chemical Co (St Louis, USA). Acetic acid and all other reagents were of analytical grade and used as received.

### 5.2.2. METHODS

#### 5.2.2.1 Characterization of *Ch*

The % N-deacetylation of the *Ch*, used in this study, was determined (see Chapter 2) to be 67% with the aid of FTIR analysis using the following relationship [23]:

$$\% \text{ N-deacetylation} = 100 [1 - (A_{1655}/A_{3340}) (1/1.33)]$$

where,  $A$  is the absorbance at the given wave number. A similar value of N-deacetylation of *Ch* was estimated from the elemental analysis based on the mole fraction concept [24].

The molecular weight ( $M_w$ ) of *Ch* was determined to be  $4.92 \times 10^5$  Da using the Mark-Houwink viscometry method [25], in a solvent of 0.1 M acetic acid/0.2 M NaCl maintained at 25 °C (see Chapter 2).

#### 5.2.2.2. Preparation of *Ch-g-PEGDA*

*Ch-g-PEGDA* was prepared by a modified method to that described by Shantha and Harding [26]. In a typical experiment, a solution of 1.5 g *Ch* dissolved in 300 ml of 1 M hydrochloric acid was placed into a flask fitted with a condenser. *CAN* (0.12 g) dissolved in 40 ml of 1 M nitric acid was added to the *Ch* solution and the flask was flushed with nitrogen for 30 min. The appropriate amount of *PEGDA* (see Table 5.1) was added dropwise and the reaction mixture was stirred for 6 h at 80 °C under

nitrogen. The reaction mixture was filtered through wet cheesecloth. The product was then precipitated with 5% NaOH, collected by centrifugation and extensively washed free of alkali with distilled water. The homopolymer formed was removed by extensive extraction with methanol. The residual graft copolymer obtained was washed with distilled water, freeze-dried and weighed.

FTIR (KBr): 3415 (NH<sub>2</sub>, OH), 2881 (ν<sub>s</sub> CH<sub>2</sub>), 1728 (ester C=O), 1654 (amide C=O), 1384 (δ CH<sub>2</sub>), 1078 cm<sup>-1</sup> (twisting vibration of CH<sub>2</sub>). Elemental analysis data for (a) the copolymer (CC): C%, 42.32; N%, 7.19; H%, 6.81.

The percent grafting (G %) and grafting efficiency (GE %) of the copolymers were calculated as follow:

$$G \% = 100 [(W_g - W_d)/W_c]$$

$$GE \% = 100 [W_g/(W_g + W_h)]$$

Where  $W_g$ ,  $W_h$  and  $W_c$  are the weights of graft copolymer, homopolymer and *Ch* respectively.

### **5.2.2.3. Preparation of (Ch-g-PEGDA) hydrogels**

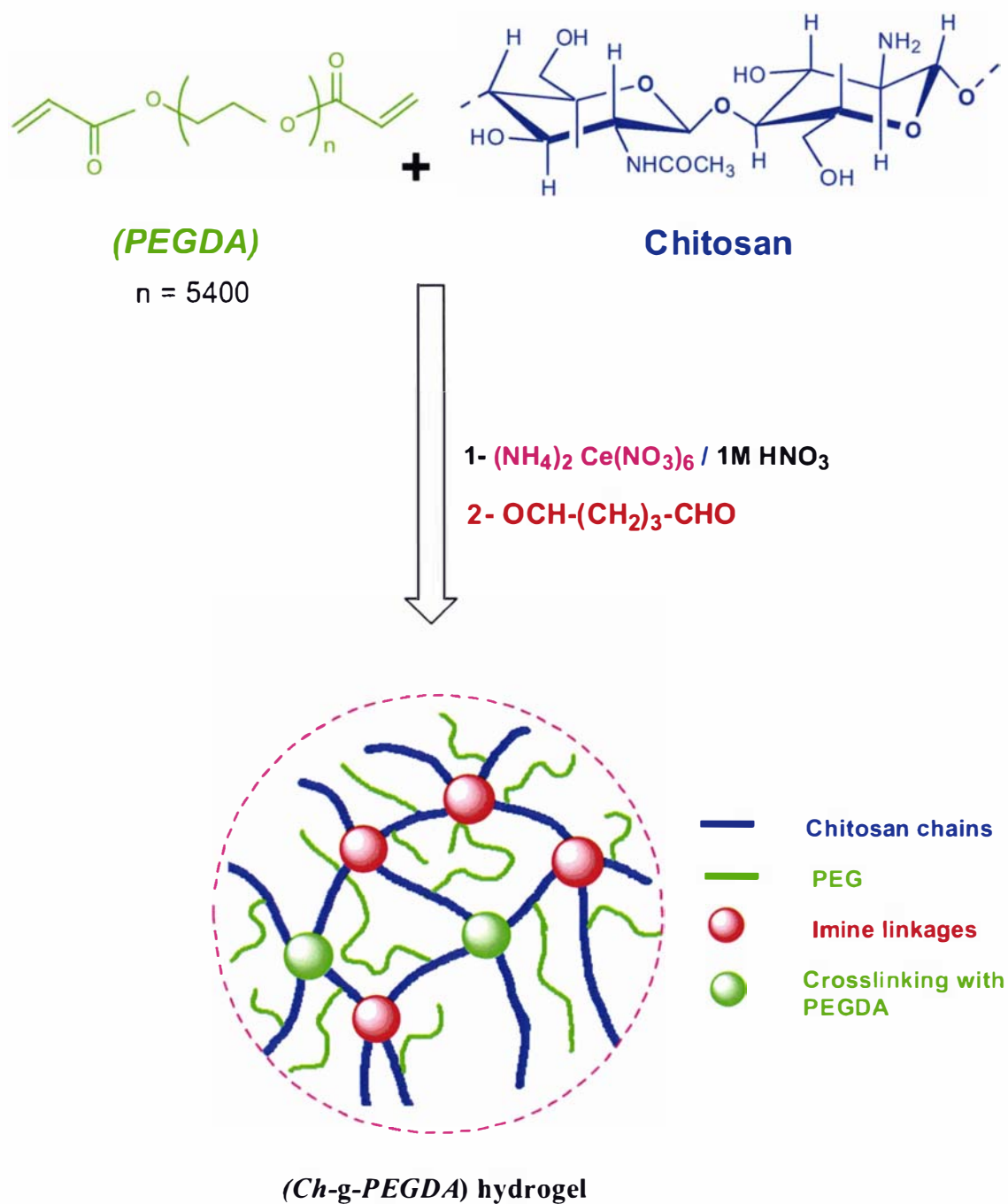
A solution of 2 g of *Ch-g-PEGDA* in 60 ml of 2% aqueous acetic acid was prepared. To this solution was added a pre-determined volume of the glutaraldehyde solution (see **Table 5.1**) with stirring for 10 minutes. The mixture was left to undergo gelation at room temperature. The gelation time was varied depending on the amount of glutaraldehyde and the grafting degree of the copolymer. The hydrogels produced were extensively washed with distilled water, freeze-dried and stored until further use. **Scheme 5.1** shows the synthesis of the *Ch-g-PEGDA* hydrogel.

FTIR (KBr): 3363 (NH<sub>2</sub>, OH), 2929 (ν<sub>s</sub> CH<sub>2</sub>), 1724 (ester C=O), 1651 (amide C=O and C=N), 1377 cm<sup>-1</sup> (δ CH<sub>2</sub>).

Table 5.1. Preparation details of the *Ch-g-PEGDA* copolymers and their hydrogels.

Graft copolymer ( <i>Ch-g-PEGDA</i> )	<i>Ch</i> (g)	<i>PEGDA</i> (g)	G %	GE %	Hydrogel	Glutaraldehyde (25% aqueous)	
						$\mu\text{L}$	W% <sup>a</sup>
<b>CA</b>	1.5	1.5	<b>26</b>	56	CA-1	0	0
					CA-2	400	5
					CA-3	800	10
---	1.5	1.8	35	59	---	---	---
<b>CB</b>	1.5	2.25	<b>59</b>	71	CB-1	0	0
					CB-2	400	5
					CB-3	800	10
---	1.5	3	47	58	---	---	---
<b>CC</b>	1.5	4.5	<b>44</b>	59	CC-1	0	0
					CC-2	400	5
					CC-3	800	10

*G* %: Grafting %, *GE* %: Grafting efficiency %: *a*: based on the weight of graft copolymer (2 g).



Scheme 5.1. Preparation of (Ch-g-PEGDA) hydrogel.

#### **5.2.2.4. Characterizations**

FTIR of the prepared *Ch-g-PEGDA* copolymer and its hydrogels was recorded with a Perkin Elmer Paragon 1000 FTIR spectrometer in the range 4000-400  $\text{cm}^{-1}$  using KBr pellets. The elemental analysis was performed with Carlo Erba EA 1108 elemental analyser (now CE Instruments, Wigan, UK) with a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand). The surface morphology of the freeze-dried copolymer and its hydrogels was investigated by scanning electron microscope, Cambridge Stereoscan S-250 mk 3 SEM, Cambridge Instruments Ltd., Cambridge, UK (Hort Research, Palmerston North, New Zealand). The samples were placed on an aluminium mount, sputtered with gold using Baltec scd 050 sputter coater, and then scanned at an accelerating voltage of 20 KV.

#### **5.2.2.5. Entrapment of anticancer drug**

*Ch-g-PEGDA* hydrogels loaded with *5-FU* as a model drug were prepared in the same manner described in section 5.2.2.3 above. Known amounts of the drug were added to the reaction mixture, stirred vigorously and then the gelation reaction carried out. The resulting hydrogels were washed with distilled water, freeze-dried and stored until further use.

#### **5.2.2.6. Determination of the amount of drug entrapped**

The quantity of drug entrapped in the *Ch-g-PEGDA* hydrogels was determined by the indirect method [27] (Chapter 2).

#### **5.2.2.7. Equilibrium swelling studies**

The swelling behavior of the *Ch-g-PEGDA* hydrogels was measured at different temperatures (25 °C, 37 °C, and 45 °C) in two buffer solutions of pH 2.1 (*SGF*) and 7.4 (*SIF*). The swollen weights of the hydrogels were determined at intervals, after the removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by the following equation:

$$\% \text{ Swelling} = 100 [(W_t - W_0)/W_0]$$

where  $W_0$  is the initial weight and  $W_t$  the final weight of the hydrogel at time  $t$ . The data points represent the mean  $\pm$ SD from three independent experiments.

For studying the effect of *5-FU* entrapped (up to 5%) in the hydrogel on the hydrogel swelling behavior, a cyclic swelling procedure was carried out. Known weights of the drug-free and the drug-loaded hydrogels were left in the swelling medium until maximum equilibrium swelling was attained. The swelled samples were weighed after removal of surface liquid, completely freeze-dried and reweighed. This swelling-deswelling process was repeated three times for each sample.

#### **5.2.2.8. *In-vitro* cumulative release studies**

The release of entrapped *5-FU* *in-vitro* was determined by placing the pre-weighed hydrogel loaded with the drug in a buffer solution of pH 2.1 (*SGF*) or pH 7.4 (*SIF*) at 37 °C. At intervals, a 3 ml aliquot was withdrawn and its absorbance at  $\lambda_{\text{max}}$  268 nm was measured. The withdrawn sample was replaced with an equal volume of fresh buffer, to keep the volume of the release medium constant. The data points represent the mean  $\pm$ SD from three independent experiments.

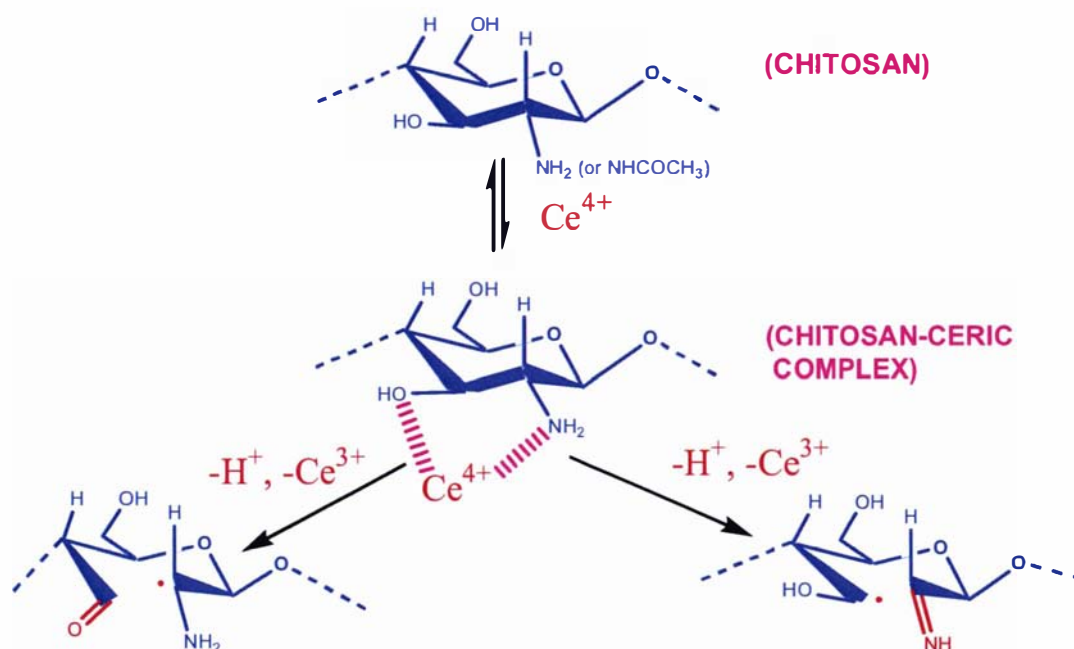
### **5.3. RESULTS AND DISCUSSION**

#### **5.3.1. *Preparation and characterization of Ch-g-PEGDA and its hydrogels***

As described earlier in Chapter 4, an attempt has been carried out to graft *PEGDA* onto *Ch* backbone using 2,2-dimethoxy-2-phenyl acetophenone as photo-initiator in presence of broad-spectrum lamp (Philips Comptalux, 150 W). Although the results have showed the occurrence of grafting, the grafting yield (G %) was relatively low (less than 10%). Also, the resulting (more crosslinked) graft copolymer was sparingly soluble in dilute acetic acid. That made the preparation of the hydrogels very difficult from the

copolymer solution using glutaraldehyde as a crosslinker. For this reason, *CAN* was used as initiator instead of 2,2-dimethoxy-2-phenyl acetophenone to graft *PEGDA* onto the *Ch* backbone through a modified procedure to that described by Shantha and Harding [26].

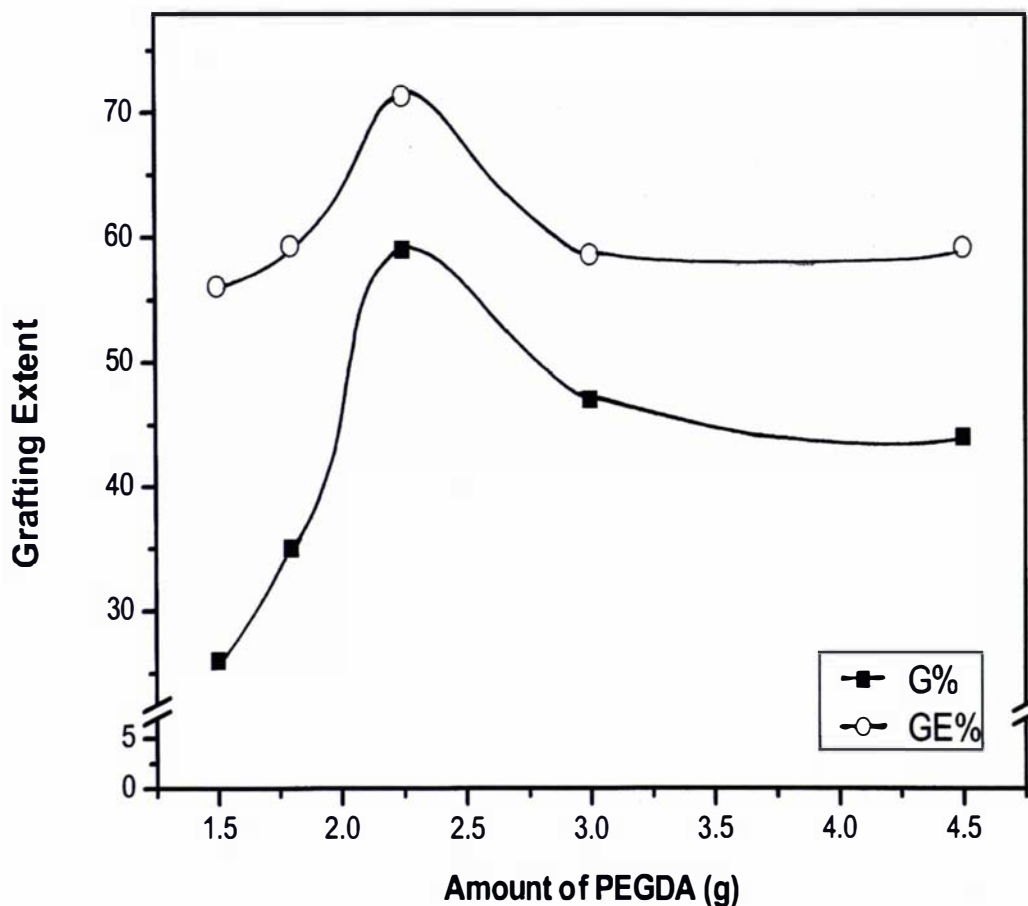
Doba et al. [28] have shown that the mechanism of graft copolymerization induced by  $Ce^{4+}$  ions onto polysaccharides involves predominantly oxidation of the anhydroglucose units through C<sub>2</sub>-C<sub>3</sub> bond cleavage. Therefore, Pourjavadi et al [29] suggested a general reaction mechanism for grafting onto *Ch*, analogous to that previously reported [28] for polysaccharides. According to this suggested mechanism,  $Ce^{4+}$  reacts reversibly with the nucleophilic groups,  $NH_2$  and  $OH$  on C<sub>2</sub> and C<sub>3</sub> to form a complex with *Ch*. Subsequently, the C-C bond cleaves to yield an aldehyde and a free radical or an imine and a free radical. Once the initiation reaction has started, the grafting of monomer onto *Ch* chains occurs immediately through traditional chain polymerization. The role of  $Ce^{4+}$  in the generation of free radicals onto the *Ch* backbone is shown in **Scheme 5.2**.



**Scheme 5.2.** The role of  $Ce^{4+}$  in the initiation of graft copolymerization onto *Ch*.

**Figure 5.1** shows the influence of the amount of macromer, *PEGDA* on the graft copolymerization. It is apparent that there is an increase in both grafting factors (G % and GE %) upon increasing the *PEGDA* amount up to a certain extent, and then both decrease. The higher G % was attained when *Ch* and *PEGDA* were reacted in a 1:1.5 ratio (CB graft copolymer, **Table 5.1**). This phenomenon can be attributed to the limited number of active centres available for grafting onto the *Ch* backbone. Then upon increasing the amount of *PEGDA*, more competition occurs between the *PEGDA* units for the same sites leading to an increase in the grafting extent until saturation of the backbone is achieved.

At higher amounts of *PEGDA*, however, an excess of *PEGDA* units could induce steric hindrance to the  $Ce^{4+}$  attack. Also, increasing the amounts of *PEGDA* may promote many more chain-transfer and termination reactions. These two factors may lead to more homopolymerization instead of grafting [30]. Moreover, the increase in homopolymer formation may lead to an increase in the viscosity of reaction medium. Accordingly, the mobility of the macromer, *PEGDA*, decreases, and this leads to a decrease in the grafting yield.



**Figure 5.1.** Effect of the amount of the macromer, *PEGDA* on the grafting parameters (*Ch*, 1.5 g; CAN, 0.12 g; time, 6 h; temperature, 80 °C).

*PEGDA* has two terminal diacrylate groups and both of them can participate in the graft copolymerization leading to the possibility of crosslinking. Within the range shown in **Table 5.1**, (*Ch:PEGDA*, 1:1 to 1:3) there was no noticeable crosslinking because all the graft copolymers prepared in this range were completely soluble in 2% acetic acid. First with the ratio of 1:4, incomplete dissolution was observed upon the dissolution of the graft copolymer in 2% acetic acid. The amount of the insoluble gel fraction increased with increasing the quantity of *PEGDA*.

**Figure 5.2** shows the FTIR spectra of the graft copolymer as compared to *Ch*. In the copolymer spectrum, the medium new peak appearing at  $1728\text{ cm}^{-1}$  was assigned to the

ester C=O stretching of *PEGDA* side chains. As expected, the FTIR charts for both graft copolymer and its corresponding hydrogel are very similar except the absorption peaks at  $1654\text{ cm}^{-1}$  and  $1651\text{ cm}^{-1}$  are assigned to amide C=O and both amide C=O and imine C=N stretching in the graft copolymer and the hydrogel respectively.

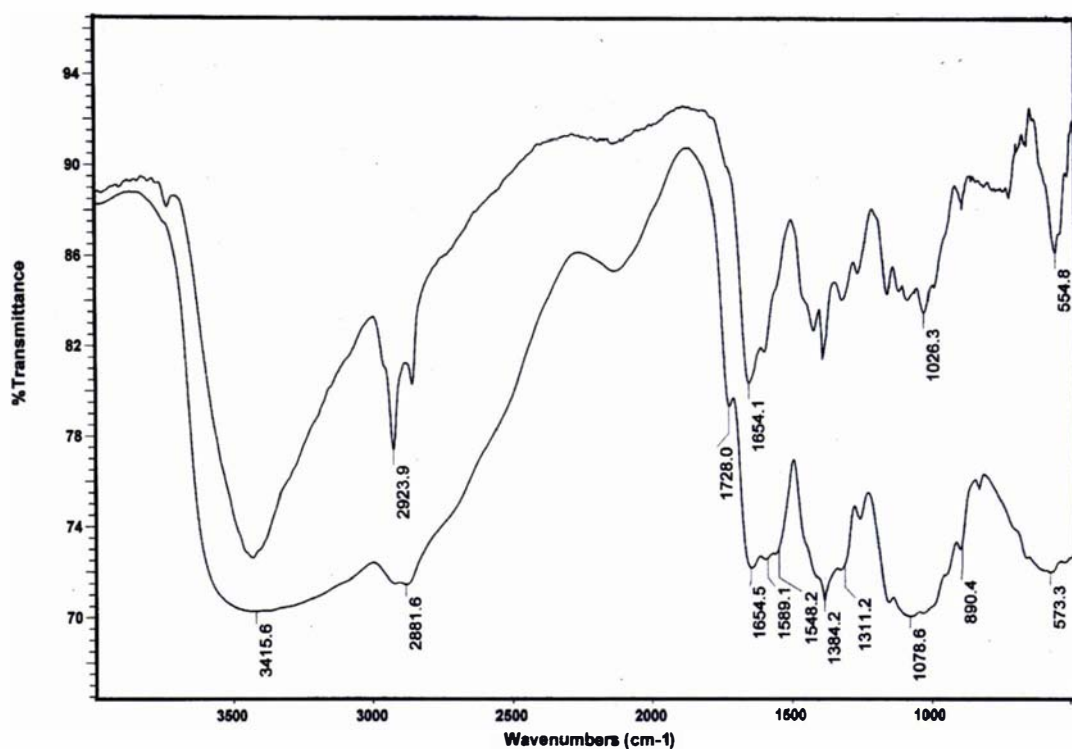
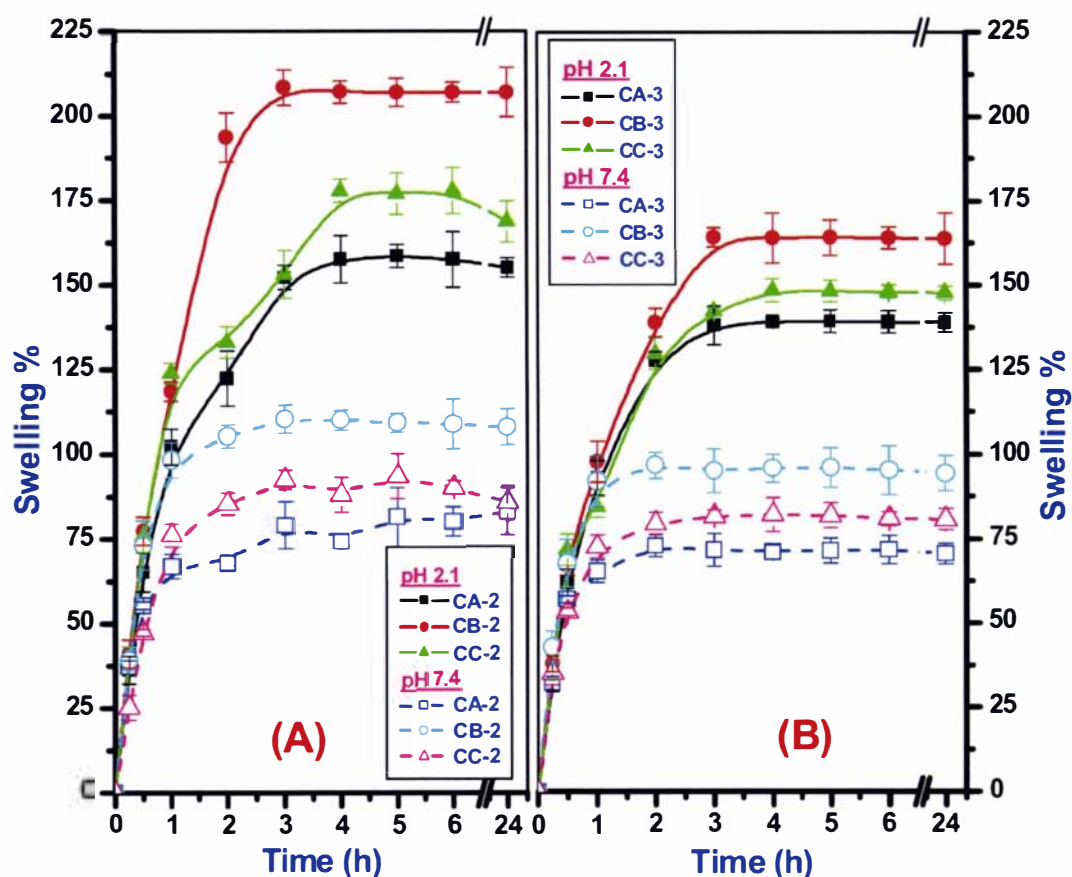


Figure 5.2. FTIR spectra for; *Ch* (upper trace) and *Ch-g-PEGDA* (lower trace).

### 5.3.2. Equilibrium swelling studies

Figure 5.3 shows the swelling % values at  $37\text{ }^{\circ}\text{C}$  at pH 2.1 and 7.4 of hydrogels prepared from *Ch-g-PEGDA* with different G %. For instance, at the same % of glutaraldehyde, 10%, Figure 5.3B), the % equilibrium swelling of CA-3 hydrogels was 138% and 73% at pH 2.1 and 7.4 respectively. The CB-3 hydrogels were swollen to 164% and 96% at equilibrium and in case of CC-3, the % equilibrium swelling values were 148% and 81% at pH 2.1 and 7.4 respectively. The equilibrium swelling was reached after 3-4 and 2 h at pH 2.1 and 7.4 respectively. Comparing the swelling values of CA-3, CB-3 and CC-3 at the same pH shows that increasing the grafting extent of

*PEGDA* onto *Ch* backbone increases the hydrophilicity of the hydrogel and consequently the % equilibrium swelling increases. The same swelling behavior can be noted in case of CA-2, CB-2 and CC-2 (5% glutaraldehyde, **Figure 5.3A**). A similar swelling pattern was also observed upon investigation of the non-crosslinked graft copolymers, CA-1, CB-1 and CC-1 at pH 7.4 (the non-crosslinked graft copolymers, CA-1, CB-1 and CC-1 are readily soluble in acidic buffer).



**Figure 5.3.** Swelling behavior of hydrogels prepared from *Ch-g-PEGDA* with different G % at 37 °C at pH 2.1 and 7.4, (A): 5% glutaraldehyde and (B): 10% glutaraldehyde.

**Figure 5.4** shows the difference in swelling at equilibrium at pH 2.1 and 7.4 at 37 °C for hydrogels prepared with varying amounts of glutaraldehyde. For instance, comparing the swelling values of CB-2 and CB-3 (**Figure 5.4B**) at the same pH confirms that as the % of glutaraldehyde increases, the extent of crosslinking increases and consequently the % equilibrium swelling decreases. The same behavior can be noted upon comparing the swelling values of CA-2 with CA-3 (**Figure 5.4A**) and CC-2 with CC-3 (**Figure 5.4C**).

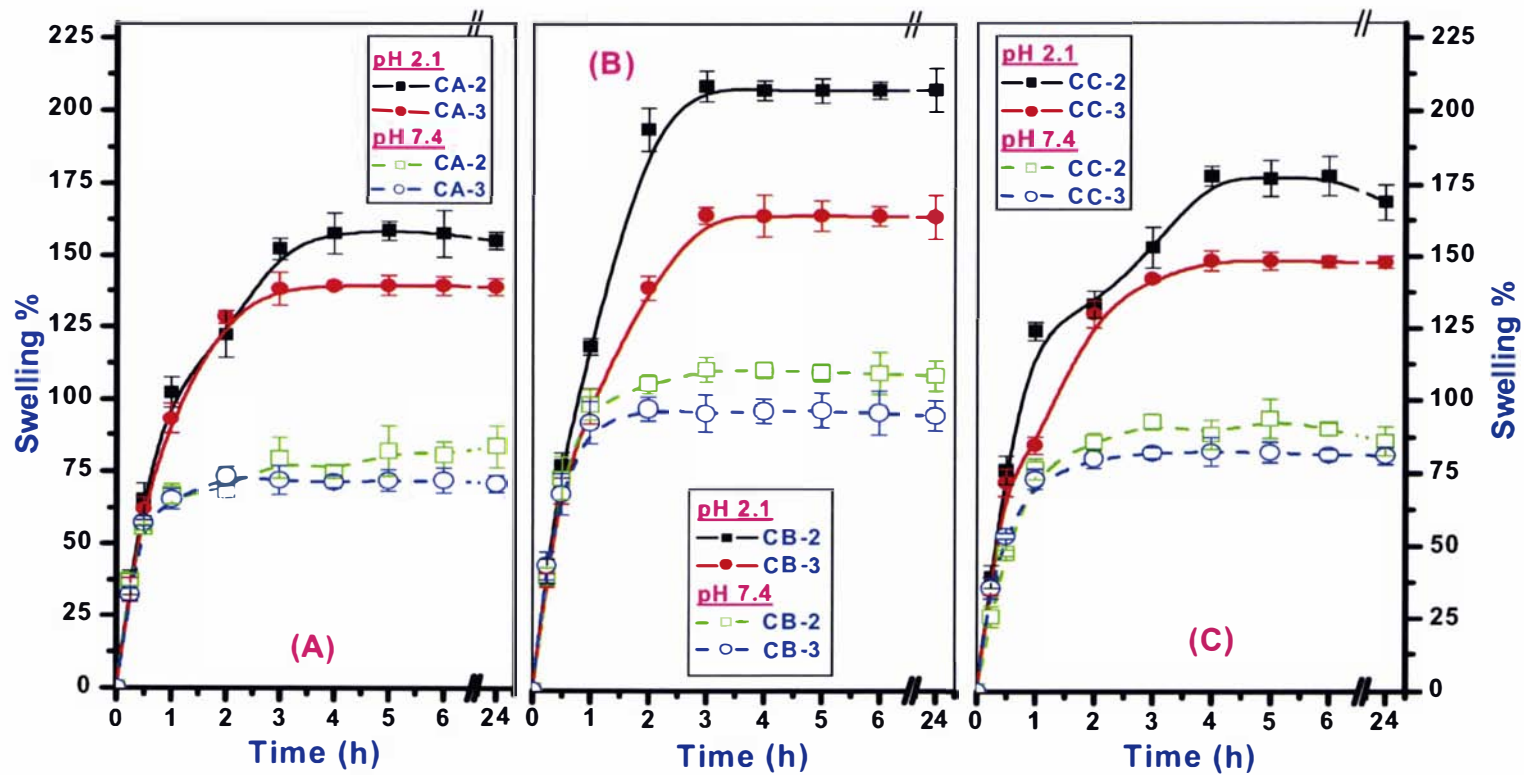


Figure 5.4. Swelling behavior of hydrogels prepared with varying amounts of glutaraldehyde (5% and 10%) at 37 °C at pH 2.1 and 7.4.

Some of the hydrogels reported in the literature showed a pH sensitivity in such a way that they swelled higher at neutral and alkaline pH values than at acidic pH values. For instance, the gelatin-based hydrogels prepared and investigated by Vazquez et al [31]. In case of *Ch-g-PEGDA* hydrogels, investigated in this chapter, the equilibrium swelling values were higher at pH 2.1 than at pH 7.4. This contrary pH sensitivity of *Ch-g-PEGDA* hydrogels can be, as discussed in Chapter 2, attributed to the chemical structure of *Ch* where, in the acidic medium, protonation can occur at the unreacted  $\text{NH}_2$  groups of *Ch* leading to dissociation of the hydrogen bonding involving amino groups [32]. The acidic medium can also hydrolyse the imine bond and consequently higher values of swelling could be obtained. Also, the swelling values at pH 7.4 will be lower than the values in pH 2.1 due to the increased hydrophobicity of the *Ch*-based hydrogels dominating at higher pH values, thus preventing more swelling in neutral and alkaline media [27].

As an example for the other hydrogels in this study, the equilibrium swelling values of CB-3 and CB-3 loaded with *5-FU* (CB-3FU, 50 mg g<sup>-1</sup> matrix) at pH 2.1 and 7.4 are shown in **Figure 5.5**. At pH 2.1, CB-3 attained about 164% swelling at equilibrium after 3 h whereas under the same conditions, CB-3FU reached equilibrium at 208% after approximately 4.5 h. Similar behavior was observed at pH 7.4, at which CB-3 and CB-3FU attained 95% and 136% after 1.5 and 4 h, respectively. This increase in the swelling upon the loading of *5-FU* confirms, as reported earlier in Chapters 3 and 4, the role of the higher hydrophilicity of the *5-FU* molecules, which facilitates the diffusion of the swelling fluids into the partially swollen hydrogels.

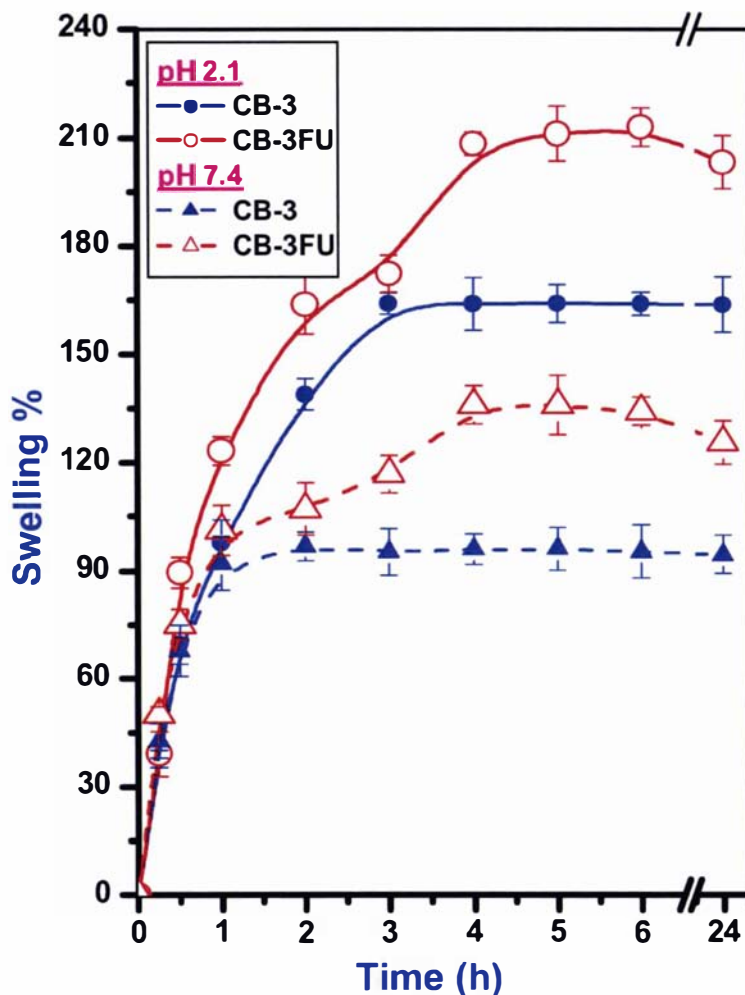
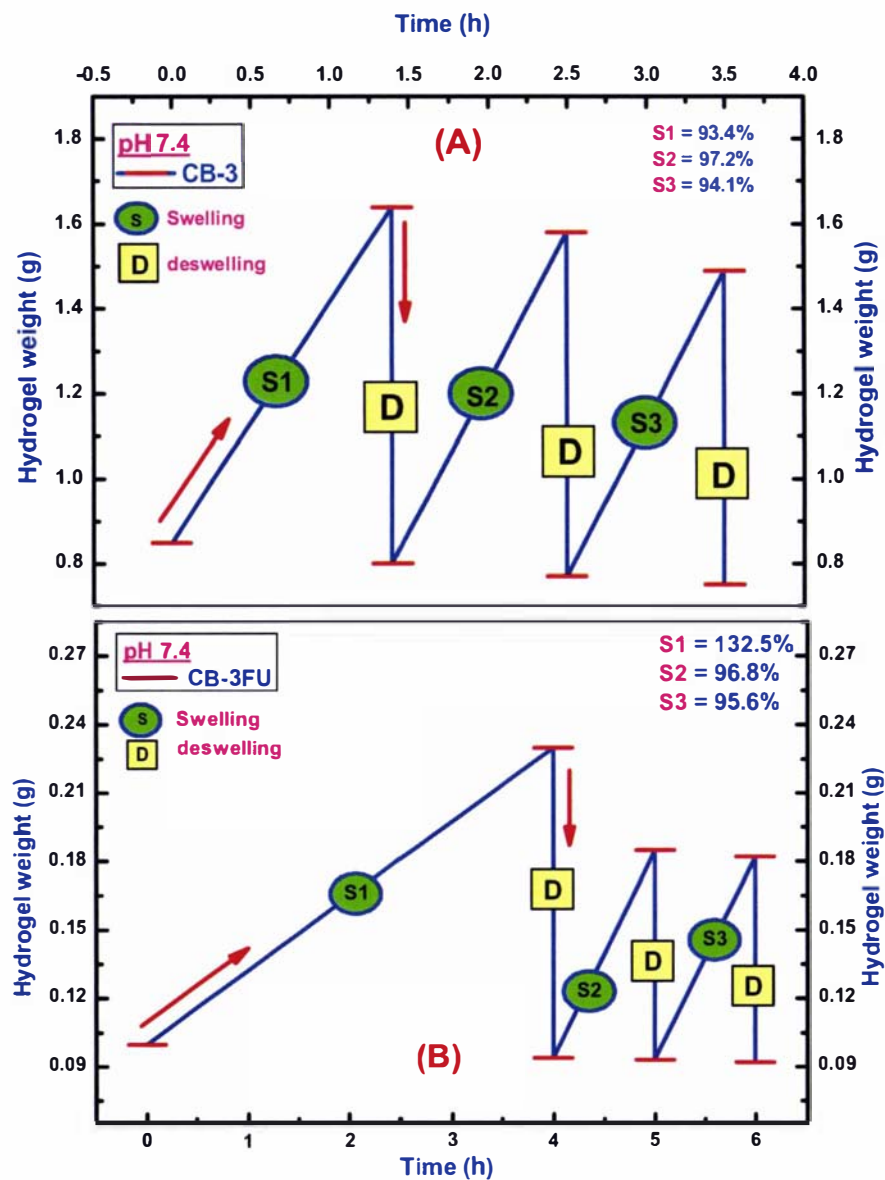


Figure 5.5. Swelling behavior of CB-3 and CB-3FU at 37 °C at pH 2.1 and 7.4.

The difference in the swelling behavior of both drug-loaded (CB-3FU) and drug-free (CB-3) hydrogels was also confirmed by studying their cyclic swelling. Figure 5.6 illustrates that the CB-3 hydrogel (Figure 5.6A) attained about 93% equilibrium swelling in the first cycle. Only small changes were noted for the other two cycles plus a slight decrease in the weight of the dried hydrogel. In the case of the drug-loaded hydrogel (Figure 5.6B), the equilibrium swelling for the first cycle attained about 133%. Deswelling of the sample and repetition of the experiment showed that the equilibrium swelling decreased and achieved its maximum value after a shorter time.

The third cycle showed little change in both the maximum equilibrium swelling and the time of achieving this equilibrium. These results confirm that *5-FU* is responsible for the increase in the equilibrium swelling value because of its hydrophilic nature. **Figure 5.6B** shows also that all the accessible *5-FU* was released from the matrix in the first cycle. The lack of change seen in the other two cycles implies the role played by *5-FU* and tends to indicate that little or no soluble fraction remains in the matrix after 4 h of initial swelling.



**Figure 5.6.** Cyclic swelling profile of CB-3 and CB-3FU at 37 °C and pH 7.4.

Figure 5.7 shows the temperature-responsive swelling of CB-3, as an example for all the other prepared hydrogels, at pH 2.1 and 7.4. The equilibrium swelling increased with increasing the temperature of the swelling medium. This temperature responsive swelling behavior is due to the dissociation of the hydrogen bonding in *Ch* within the hydrogel matrices [32].

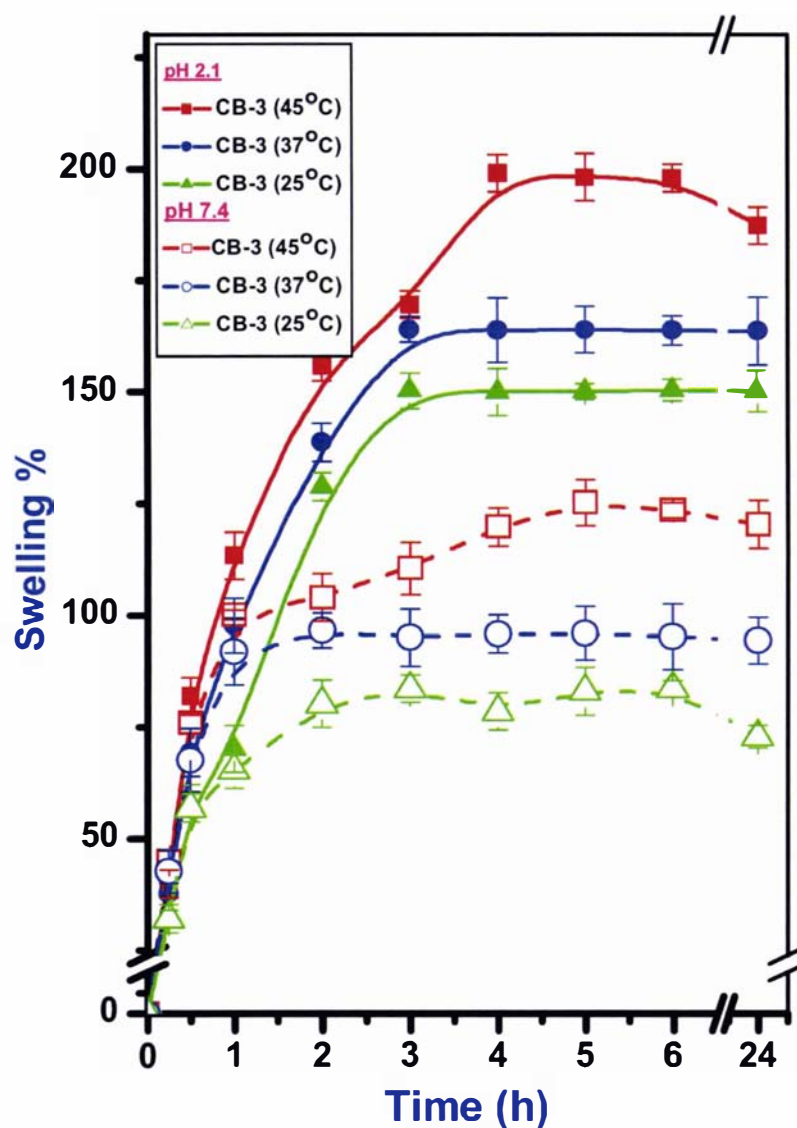
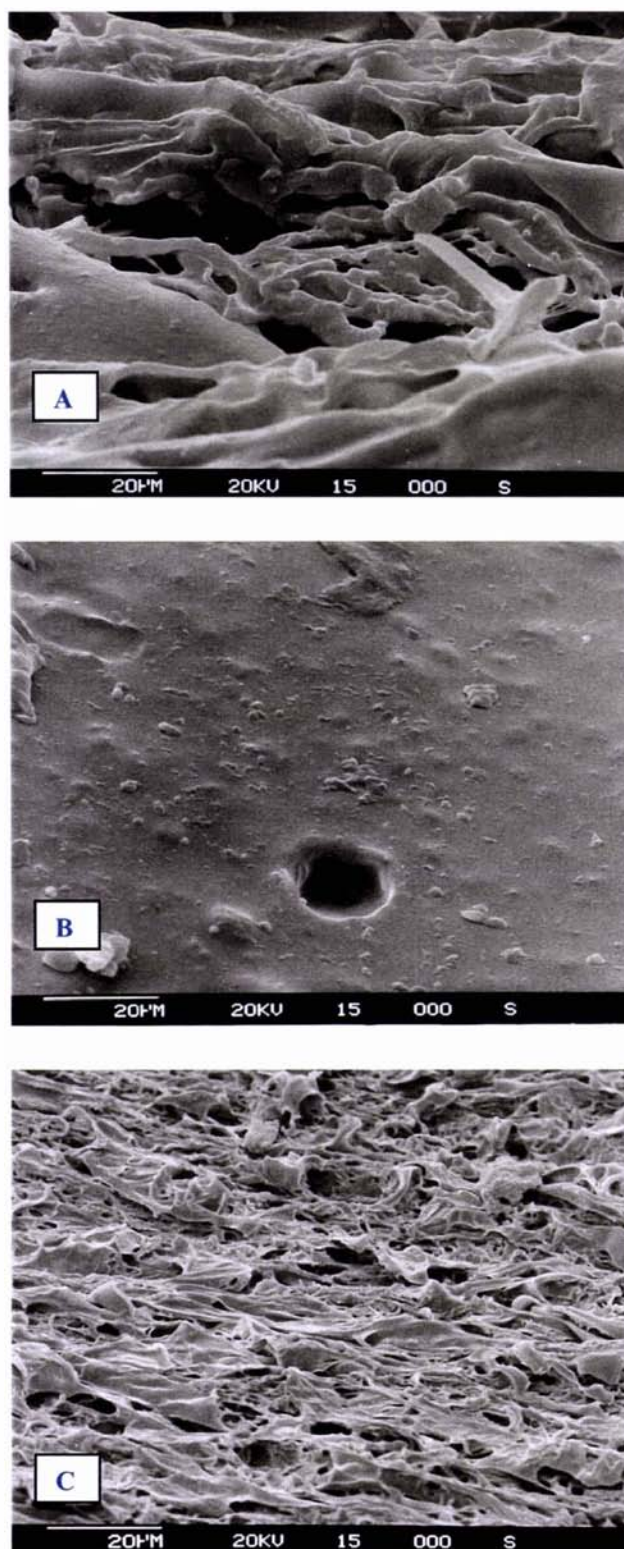


Figure 5.7. Swelling behavior of CB-3 at 25 °C, 37 °C and 45 °C at pH 2.1 and 7.4.

The surface morphology of the freeze-dried **Ch-g-PEGDA** copolymer (**Figure 5.8A**) shows grafted **PEGDA** onto the **Ch** backbone rendering the surface rough. The fine distribution of **5-FU** introduced into the hydrogel (**Figure 5.8B**) renders the surface smoother. After the release of **5-FU** for 24 h at 37 °C at pH 2.1 (**Figure 5.8C**) very fine holes appeared indicating the release process.



**Figure 5.8.** Scanning electron micrographs of (A) the freeze-dried *Ch-g-PEGDA* copolymer, (B) the freeze-dried hydrogel before the drug release, and (C) the freeze-dried hydrogel after 24 h of drug release at 37 °C at pH 2.1.

### 5.3.3. In-vitro cumulative release studies

Figure 5.9 shows the cumulative release profiles from the hydrogels CA-3, CB-3 and CC-3, as examples for other hydrogels, at pH 7.4. These release values demonstrate the variation in release at equilibrium for hydrogels prepared from *Ch* with different grafting extents of *PEGDA*. Comparing these release values showed that the % release at equilibrium was directly proportional to the % grafting of *PEGDA* onto *Ch* backbone.

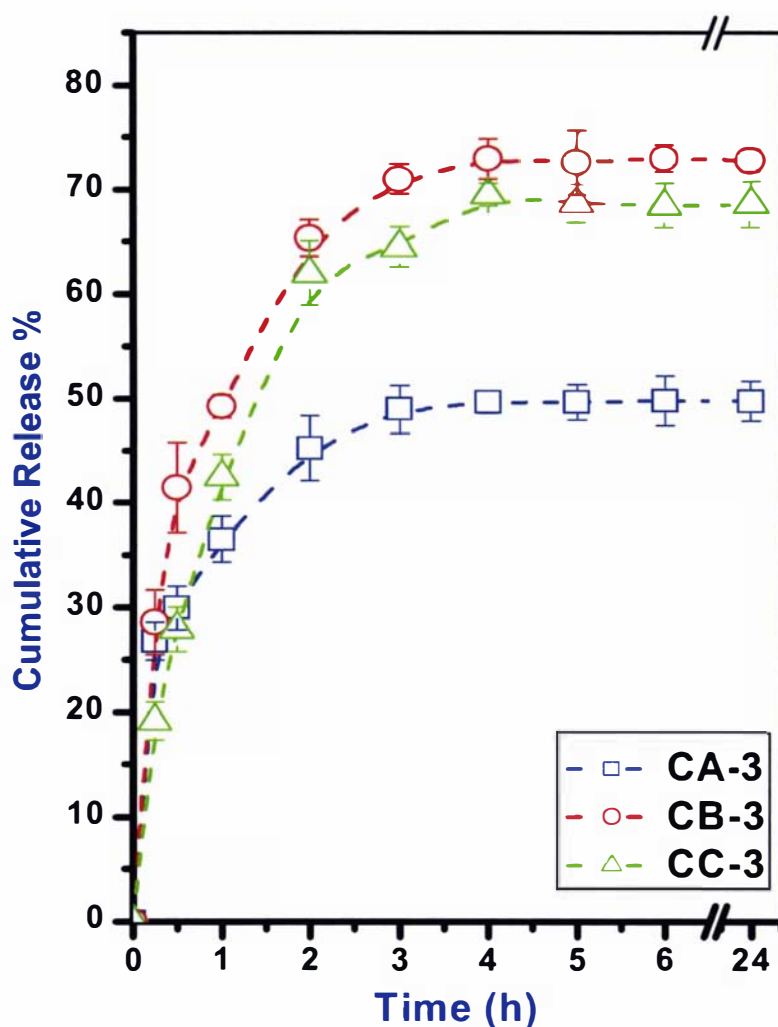
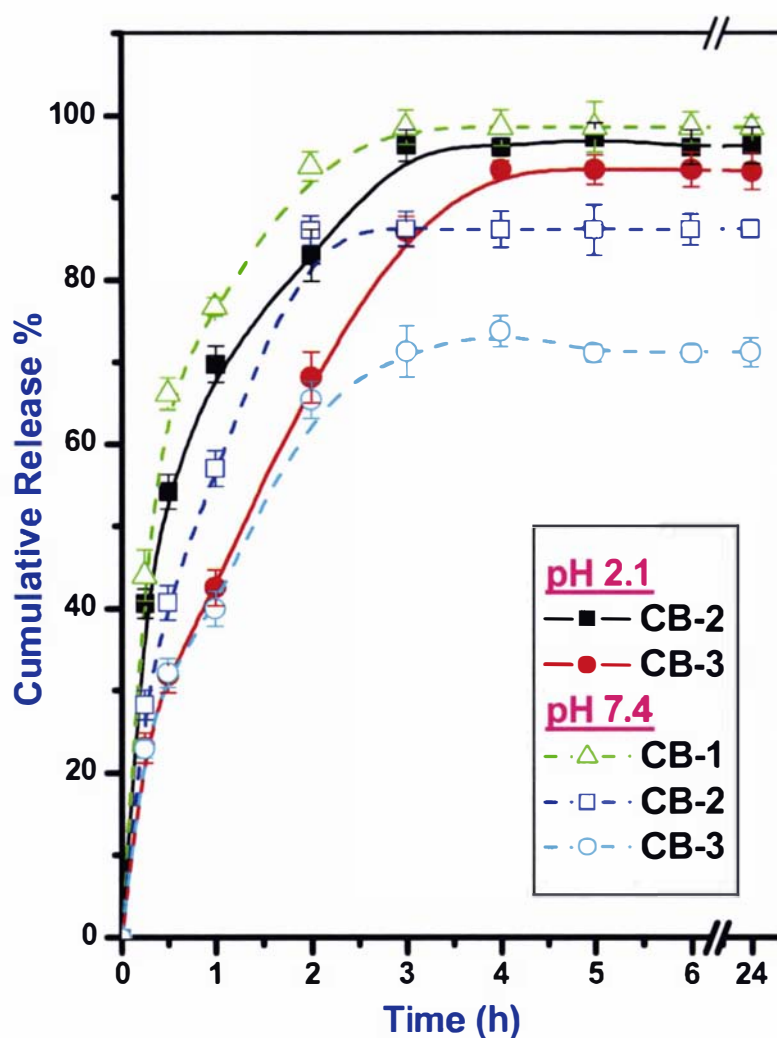


Figure 5.9. In-vitro cumulative release measurements for 5-FU loaded CA-3, CB-3 and CC-3 hydrogels at 37 °C and pH 7.4.

The cumulative release values from the CB-1 – CB-3, as an example for the other hydrogels, at 37 °C for pHs 2.1 and 7.4 are shown in **Figure 5.10**. These release measurements estimate the difference in release at equilibrium for hydrogels prepared with varying amounts of glutaraldehyde. Comparing the amounts of drug released from CB-1 – CB-3 at pH 7.4 clearly confirmed yet again that the extent of drug release at equilibrium was inversely related to the degree of crosslinking. Similar results were obtained from the comparison of the drug release patterns of CB-2 and CB-3 hydrogels at pH 2.1 (CB-1 was completely dissolved at pH 2.1 within 10 min).



**Figure 5.10.** *In-vitro* cumulative release measurements of 5-FU-loaded CB-1 – CB-3 hydrogels at 37 °C at pHs 2.1 and 7.4.

At the same extent of crosslinking, the percent of drug released was much higher in an acidic buffer (pH 2.1) than in a weakly basic buffer (pH 7.4). As discussed Chapter 2, this behavior is due to the fact that the release depends on the swelling rate of the hydrogel where the drug release may be due to the diffusion-dissolution mechanism through swollen hydrogels. As reported previously, the swelling of the prepared hydrogels in acidic medium is greater than in weakly basic medium.

Most of the hydrogels reached equilibrium after about 3-4.5 h. This time of release may seem short for some applications but it can be controlled by the variation of both the extent of crosslinking and the degree of grafting onto the *Ch* backbone. Besides, this preliminary study was carried out with a single model drug (small and highly hydrophilic) and applying the results of this study directly to the entrapment of other drugs may be more useful. It was noticed also from the release data that the drug was not quantitatively released from the hydrogels. Some drug molecules may be deeply buried in the hydrogel matrix and are slower to be released or indeed may never be released into the surrounding media as long as the hydrogel remains undissolved.

## CONCLUSION

Biodegradable, pH and thermally responsive hydrogels based on *Ch-g-PEGDA* were prepared for controlled drug release studies. The equilibrium swelling measurements of the hydrogels carried out at different temperatures in simulated body fluids (pH 2.1 and 7.4) clearly showed their pH and thermally responsive nature. The *in-vitro* release profiles of *5-FU* from the hydrogels were also estimated at pH 2.1 and 7.4. The results obtained illustrated that the grafting of *PEGDA* in the matrix assembly was beneficial. With the increase in the *PEGDA* grafting extent, higher percents of the drug were released over longer release times with a reduced use of the crosslinker, glutaraldehyde. This preliminary investigation of *Ch*-based hydrogels showed that they may be used to expand the utilization of these systems in controlled release applications.

In all the studies reported in Chapters 2 to 5, *Ch* was either incorporated into an *IPN* hydrogel or chemically modified before preparation of the hydrogel. All the *Ch*-based hydrogels prepared in these chapters were pH-responsive. These hydrogels swelled

faster and higher at acidic pH values than at neutral and slightly alkaline pH values. In all these studies (Chapters 2-5), 5-FU was used as a model drug. In the next chapter, an attempt was carried out to modify *Ch* by such away that the hydrogels produced from this modified *Ch* attain limited swelling at lower pH values and high swelling at neutral and higher pH values. Therefore, the resulting hydrogels could be tailored for utilization for intestine-targeted delivery of protein drugs. Hence, in the next chapter, another model drug, Bovine serum albumin (*BSA*) was used, as a model protein drug, to check the ability of the produced hydrogels to control the release of protein drugs into the intestinal with a potential drug protection from the harsh acidity of stomach.

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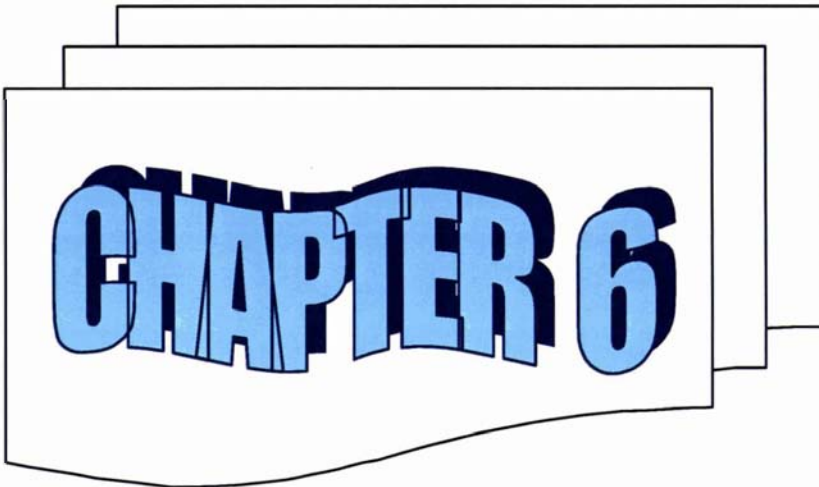
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**CHAPTER 6**

## CHAPTER 6

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### PREPARATION AND *IN-VITRO* EVALUATION OF NEW pH-SENSITIVE GEL BEADS BASED ON CHEMICALLY MODIFIED CHITOSAN FOR ORAL DELIVERY OF PROTEIN DRUGS.<sup>a,b</sup>

#### ABSTRACT

The oral route is one of the most convenient and comfortable ways for drug administration. However, in the case of peptide and protein drugs, they are readily degraded in the harsh high acidity of stomach. In this study, carboxymethyl chitosan (*CMCh*) was prepared and characterized by elemental analysis, FTIR and 2D-XRD. Grafting of methacrylic acid sodium salt (*MAA*) onto *CMCh* backbone was carried out with different monomer concentrations. The grafting was achieved by ammonium persulfate (*APS*)-induced free radical polymerization. The structure changes of chitosan (*Ch*), *CMCh* and *CMCh-g-MAA* were investigated by FTIR and 2D-XRD. Biodegradable pH-responsive hydrogel beads based on *CMCh-g-MAA* and alginic acid sodium salt were also prepared and characterized for the controlled release study of protein drugs in the small intestine. The ionotropic gelation reaction was carried out under mild aqueous conditions, which should be appropriate for retention of the biological activity of an uploaded protein drug. The equilibrium swelling studies were carried out for the gel beads at 37 °C in buffer solutions of pH 2.1 (*SGF*) and pH 7.4 (*SIF*). Bovine serum albumin (*BSA*), a model protein drug was entrapped in the hydrogels and the *in-vitro* drug release profiles were established at 37 °C in *SGF* and *SIF*.

**Key words:** carboxymethyl chitosan, grafting, alginate, protein drugs

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## 6.1. INTRODUCTION

Controlled drug release enhances the safety, efficacy and reliability of drug therapy. The oral route is one of the most convenient and comfortable ways for drug administration. However, in the case of peptide and protein drugs, the oral route exhibits many shortcomings. For instance, these types of drugs are readily degraded, if taken orally, due to the enzymatic attack in the upper small intestinal tract and the harsh high acidity of stomach. Also, the short half-life of the protein drugs and their limited transit time in the gastrointestinal tract represent major challenges [1,2]. Several attempts have been reported [3-5] to overcome these shortcomings and to formulate protein drugs with maximum oral bioavailability.

Hydrogels are crosslinked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids. Hydrogels are soft and rubbery, in the swollen state, resembling living tissue [6,7]. The pH-sensitive hydrogels, a class of the smart hydrogels, have potential use in the site-specific delivery of drugs to the gastrointestinal tract. [8]. Polymers suitable for preparing hydrogels for the controlled release of drugs are quite limited, as compared to the total available synthetic polymers, because of inherent toxicity or lack of certain properties such as biodegradability and swellability in a specific environment.

Alginate is a non-toxic biodegradable polyanionic copolymer. It consists of 1, 4-linked  $\beta$ -D-mannuronic acid (*M*) and  $\alpha$ -L-guluronic acid (*G*) residues arranged either as consecutive blocks or in a random distribution [9]. Hydrogels based on calcium-crosslinked alginate has been widely investigated for protein drug delivery [10,11]. However, the swelling of the calcium-crosslinked alginate beads at pH 7.4 is minimal [12] due to the relatively strong ionic interaction between the carboxylic groups on alginate and  $\text{Ca}^{2+}$ . This may limit the drug release at the intestinal tract. Various trials have been reported to overcome this disadvantage by preparing hydrogels based on alginate with other polymers such as chitosan (*Ch*) and its derivatives [10-12].

*Ch* is a cationic biopolymer obtained through alkaline N-deacetylation of natural chitin. *Ch* has been considered as a biodegradable, non-toxic, biocompatible and

environmentally friendly material with many superior properties [13-16]. Carboxymethyl chitosan (*CMCh*) is a water soluble derivative of *Ch*. Due to the unique chemical, physical and biological properties of *CMCh*, particularly its low toxicity, biocompatibility and its ability to form hydrogels [17-20], it has been extensively used in many biomedical fields. For instance it has been used as a moisture-retention agent, as a bactericide, as wound dressings, as artificial bone and skin, in blood anticoagulants and as a component in drug delivery matrices.

Considerable interest has been focused on chemical modification by grafting synthetic polymers onto *Ch* [21-27] whereas to date, very little reported work has discussed the graft copolymerization of vinyl monomers onto *CMCh* [28-32]. In this study, *Ch* was carboxymethylated and then graft copolymerized with *MAA* in a mild aqueous medium using *APS* as initiator. Biodegradable pH-responsive hydrogels based on *CMCh-g-MAA* with alginic acid sodium salt were then prepared and characterized for the *in-vitro* controlled intestinal release studies using *BSA* as a model protein drug. The hydrogels investigated in this chapter have been prepared in the form of beads where the preparation method used in this chapter (ionotropic gelation) involves the dropwise addition of a polymer solution onto the crosslinker solution ( $\text{CaCl}_2$ ). In addition to carrying out a brief study of alternate synthesis (physical) and form (bead) of hydrogels it was decided to investigate the reverse of Chapters 2-5, namely preferred controlled release at pH 7.4. In other words an attempt was made to produce hydrogels that would survive the harsh pH of the stomach and preferably release the drug in the intestine.

## **6.2. MATERIALS AND METHODS**

### **6.2.1. MATERIALS**

*Ch* and *BSA* were purchased from Acros Organics (New Jersey, USA). Alginic acid sodium salt of high viscosity (approx. 14,000 cps for a 2% solution at 25 °C) and Bradford reagent were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). *MAA* and monochloroacetic acid were supplied by Riedel-De Haenag Seelze (Hanover, Germany). *APS* was obtained from AJAX Chemicals, Clyde Industries Ltd (Auburn,

N.S.W, Australia). Isopropyl alcohol, acetone, methanol, acetic acid, calcium chloride and all other reagents were of analytical grade and used as received.

## **6.2.2. METHODS**

### **6.2.2.1. Characterization of Ch**

The % N-deacetylation of the *Ch*, used in this study, was determined (see Chapter 2) to be 67% with the aid of FTIR analysis using the following relationship [33]:

$$\% \text{ N-deacetylation} = 100 [1 - (A_{1655}/A_{3340}) (1/1.33)]$$

where, *A* is the absorbance at the given wave number. A similar value of *N*-deacetylation of *Ch* was estimated from the elemental analysis based on the mole fraction concept [34].

The molecular weight ( $M_w$ ) of *Ch* was determined to be  $4.92 \times 10^5$  Da using the Mark-Houwink viscometry method [35], in a solvent of 0.1M acetic acid/0.2M NaCl maintained at 25 °C (see Chapter 2).

### **6.2.2.2. Preparation of CMCh**

Water-soluble *CMCh* was prepared by a modified method to that described by Xie et al [32]. In a typical procedure, 2 g of *Ch* were put in 500 ml reactor and suspended in 60 ml of isopropyl alcohol at room temperature for 5 h. To the swollen *Ch* suspension, 75 ml of aqueous NaOH solution (60% w/v) were added and then the whole mixture was refluxed at 85 °C for 2 h. Then, 100 ml of aqueous monochloroacetic acid solution (60% w/v) were added over a period of 15 min. The mixture was heated, with stirring, at 65 °C for a further 4 h. The reaction mixture was then neutralized using HCl solution (5 M). After removal of the undissolved residue by filtration, *CMCh* was precipitated by adding methanol. The product was filtered, washed several times with a mixture of CH<sub>3</sub>OH / H<sub>2</sub>O (1:1) and freeze-dried. The weight of crude product was 2.4 g.

### 6.2.2.3. Characterization of CMCh

The intrinsic viscosity,  $[\eta]$  and the average molecular weight,  $M_r$  of CMCh dissolved in 0.1 M aqueous NaCl were estimated at 30 °C using the following relationships; [36].

$$\begin{aligned}\eta_r &= t/t_o \\ \eta_{sp} &= \eta_r - 1 \\ [\eta] &= (4 \eta_{sp}^{1.02} \times \text{Ln } \eta_r) / C^{1.01} (3 \eta_{sp} + \text{Ln } \eta_r) \\ [\eta] &= 7.92 \times 10^{-5} M_r^{1.00}\end{aligned}$$

where  $t$  and  $t_o$  are the delivery times of CMCh solution and the solvent,  $C$  is the concentration of CMCh (g/ml).  $\eta_r$  and  $\eta_{sp}$  are the relative and specific viscosities respectively.

### 6.2.2.4. Graft copolymerization

Graft copolymerization reactions were carried out through a modified procedure to that reported by Sun et al. [28]. The reactions were performed in a 250 ml two-necked flask using 0.1 g CMCh. Before addition of the predetermined amount of monomer, MAA, the monomer was neutralized using NaOH (4 M) and then made up to the desired volume with deionized water. The monomer concentrations used were in the range of (0.5-3 M). The components were mixed and stirred for 30 min with bubbling of a slow stream of nitrogen gas. The flask was then placed in a thermostated oil bath at 70 °C. Finally, the predetermined concentration of the initiator, APS (8 mM based on the total volume of reaction mixture) dissolved in 10 ml of deionized water was added dropwise with stirring. The graft copolymerization was carried out for 2 h at 70 °C. The reaction was stopped by letting air into the flask and rapidly cooling down the reactor. The products were precipitated by pouring the reaction mixture into acetone. The precipitate was filtered off, washed with acetone and the crude product was freeze-dried and weighed. The homopolymer formed (PMAA) was extensively extracted in a Soxhlet apparatus with methanol for 24 h. The residual graft copolymer obtained was washed with methanol, freeze-dried and weighed. The percent grafting (G %) and the grafting efficiency (GE %) of the copolymers were calculated as follow:

$$G \% = 100 [(W_g - W_o) / W_o]$$

$$GE \% = 100 [W_g / (W_g + W_h)]$$

where  $W_g$ ,  $W_h$  and  $W_o$  are the weights of graft copolymer, homopolymer and *CMCh* respectively.

#### 6.2.2.5. Preparation of *CMCh-g-MAA/sodium alginate hydrogel beads*

The *CMCh-g-MAA/sodium alginate hydrogel beads* were prepared via the ionotropic gelation technique by using calcium chloride solution as the coagulation fluid. Homogenous aqueous *CMCh-g-MAA/sodium alginate* solutions (20 ml) with predetermined compositions (see **Table 6.1**) were prepared and left overnight to degas. Then, these prepared aqueous polymer solutions were used as dope and dropped into 80 ml of gently stirred (200 rpm) calcium chloride solution (0.1–0.3 M) through a tip of micropipette (1000  $\mu$ l). Ionotropic gelation reaction occurred instantaneously to form hydrogel beads. The beads that formed were allowed to crosslink with  $Ca^{2+}$  in solution for 30 min. Then, the resulting calcium-crosslinked *CMCh-g-MAA/alginate* beads were collected and washed with distilled water to remove the unreacted calcium chloride. The beads were then freeze-dried and stored until further use.

**Table 6.1.** Different compositions of the prepared CMCh-g-MAA/alginate hydrogel beads and their characteristics.

Formulation	CMCh-g-MAA		Alginate (%)	CaCl <sub>2</sub> (M)	Bead Size		Equilibrium Swelling (%)				Entrapment Efficiency (%)
	G %	(%)			Mean ( $\mu\text{m}$ )	$\pm$ SD	SGF (3 h)	$\pm$ SD	SIF (8 h)	$\pm$ SD	
<b>A1</b>		1.0	1.0	0.1	873	84	239.0	19.9	4019.3	25.9	29.25
<b>A2</b>		1.0	1.0	0.2	823	59	201.0	48.8	3827.3	193.7	48.25
<b>A3</b>		1.0	1.0	0.3	780	85	194.3	32.3	3721.7	122.0	37.50
<b>A4</b>		2.5	2.5	0.1	1430	62	190.7	18.5	3514.7	15.8	82.80
<b>A5</b>		2.5	2.5	0.2	1380	53	184.3	60.8	2978.0	117.5	95.10
<b>A6</b>	<b>570</b>	2.5	2.5	0.3	1307	25	183.7	16.1	2982.7	22.5	96.30
<b>A7</b>		2.5	1.0	0.1	1617	12	244.0	40.5	4116.7	102.0	69.43
<b>A8</b>		2.5	1.0	0.2	1273	38	211.3	63.8	3500.1	261.0	87.57
<b>A9</b>		2.5	1.0	0.3	1380	52	217.0	39.0	3478.7	151.0	87.00
<b>A10</b>		1.0	2.5	0.1	1293	21	209.7	45.1	3873.0	111.0	55.57
<b>A11</b>		1.0	2.5	0.2	1070	26	187.0	10.1	3432.3	96.6	74.57
<b>A12</b>		1.0	2.5	0.3	883	84	186.0	23.8	3422.3	61.7	73.14
<b>B1</b>		1.0	1.0	0.1	1063	21	206.0	11.7	4223.3	109.0	37.50
<b>B2</b>		1.0	1.0	0.2	853	17	172.0	18.8	4196.7	163.0	44.00
<b>B3</b>		1.0	1.0	0.3	797	26	173.0	30.0	3909.7	212.3	43.50
<b>B4</b>		2.5	2.5	0.1	1543	59	111.0	70.5	3728.3	93.0	87.10
<b>B5</b>		2.5	2.5	0.2	1447	60	166.0	49.5	3719.5	126.1	96.28
<b>B6</b>	<b>1615</b>	2.5	2.5	0.3	1380	52	83.0	34.6	3295.0	93.5	97.40
<b>B7</b>		2.5	1.0	0.1	1619	25	327.0	47.9	4530.0	7.2	72.43
<b>B8</b>		2.5	1.0	0.2	1537	40	378.7	61.0	3495.0	178.0	83.29
<b>B9</b>		2.5	1.0	0.3	1497	15	276.7	27.0	4094.0	42.0	82.76
<b>B10</b>		1.0	2.5	0.1	1440	56	201.3	12.5	3691.0	180.0	70.00
<b>B11</b>		1.0	2.5	0.2	1297	15	169.3	60.0	3442.6	68.0	77.86
<b>B12</b>		1.0	2.5	0.3	1070	30	147.3	37.0	3399.0	160.5	79.86

Continued.

Formulation	CMCh-g-MAA		Alginate (%)	CaCl <sub>2</sub> (M)	Bead Size		Equilibrium Swelling (%)				Entrapment Efficiency (%)
	G %	(%)			Mean (μm)	±SD	SGF (3 h)	±SD	SIF (8 h)	±SD	
<b>C1</b>		1.0	1.0	0.1	850	19	209.3	60.0	4211.0	86.0	31.00
<b>C2</b>		1.0	1.0	0.2	790	51	219.5	75.2	4100.0	173.0	37.00
<b>C3</b>		1.0	1.0	0.3	747	91	162.0	24.1	3919.2	21.1	44.00
<b>C4</b>		2.5	2.5	0.1	1427	60	134.0	90.8	3723.0	95.3	76.20
<b>C5</b>		2.5	2.5	0.2	1370	52	133.8	60.3	3714.0	133.0	91.30
<b>C6</b>	<b>1930</b>	2.5	2.5	0.3	1363	38	99.0	36.1	3286.3	97.0	94.14
<b>C7</b>		2.5	1.0	0.1	1527	40	499.0	87.4	4533.1	75.0	71.43
<b>C8</b>		2.5	1.0	0.2	1494	13	505.8	32.4	3913.3	68.3	78.43
<b>C9</b>		2.5	1.0	0.3	1483	21	253.7	61.0	3908.0	79.1	97.20
<b>C10</b>		1.0	2.5	0.1	1298	19	205.0	82.2	3699.0	127.0	76.43
<b>C11</b>		1.0	2.5	0.2	1070	40	136.5	60.0	3313.7	98.0	82.86
<b>C12</b>		1.0	2.5	0.3	1050	44	133.4	33.0	3412.0	107.0	77.00
<b>D1</b>		-	2.5	0.1	767	81	149.3	36.0	757.7	26.1	38.20
<b>D2</b>	---	-	2.5	0.2	733	74	109.0	23.1	547.3	77.0	43.00
<b>D3</b>		-	2.5	0.3	727	75	100.0	26.0	530.7	21.5	37.20

#### **6.2.2.6. Characterizations**

The elemental analysis for *Ch*, *CMCh* and the *CMCh-g-MAA* copolymers was performed with Carlo Erba EA 1108 elemental analyser (now CE Instruments, Wigan, UK) with a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand). Also, *Ch*, *CMCh* and *CMCh-g-MAA* were characterized by FTIR (Perkin Elmer Paragon 1000 FTIR spectrometer). The crystallography patterns of *Ch*, *CMCh*, and *CMCh-g-MAA* copolymer were investigated using 2D-XRD equipment (Rigaku Micro Max 007 microfocus imitating anode X-ray generator (Cu K $\alpha$ ) coupled with Osmic “Blue” confocal optics and a Rigaku RAxis (VI++) image-plate detector. Images were recorded and analysed with Crystal Clear (1.3.6-SPI, Pflugrath, JW, 1999, Acta Crystallogr. D50 1718-1725). The morphology of the prepared hydrogel beads was examined using an optical microscope (Zoom Stereo LEICA MZ12, Leica Microsystems GmbH). The size of three beads from each formulation was measured using a micrometer (MOORE & WRIGHT, Sheffield, England) and averaged.

#### **6.2.2.7. Entrapment of a model protein drug**

Hydrogel beads loaded with *BSA* as a model protein drug were prepared in the same manner described in section 6.2.2.5. Known amounts of the *BSA* (15% w/w, based on the total weight of the polymer mixture) were added to the aqueous polymer mixture, stirred vigorously and then the gelation reaction was carried out by dropping this drug-containing solution onto the CaCl<sub>2</sub> solution. The resulting hydrogel beads were collected, rinsed with 20 ml of distilled water, freeze-dried and stored until further investigation.

#### **6.2.2.8. Determination of the entrapment efficiency of BSA**

The quantity of *BSA* entrapped in the *CMCh-g-MAA*/alginate beads was determined by the indirect method. After the preparation of the beads, both the washings (20 ml) and the CaCl<sub>2</sub> solution (80 ml) were collected, filtered and the amount of *BSA* present was estimated, using Bradford method [37], from the absorption at  $\lambda_{\max}$  595 nm with the aid of a Varian Inc. Corg 50 scan (Palo Alto, CA) UV-VIS. spectrophotometer. The difference between the amount of *BSA* initially added to the hydrogel beads and that

estimated in the 100 ml (washings plus CaCl<sub>2</sub>) was taken as a measure of the amount of *BSA* entrapped. The entrapment efficiency (*EE* %) of *BSA* was calculated as follow:

$$EE \% = 100 (m_r / m_i)$$

where  $m_i$  and  $m_r$  are the amounts (mg) of the *BSA* initially uploaded and remain after beads washing respectively.

### 6.2.2.9. Swelling studies

The maximum swelling values of the *CMCh-g-MAA*/alginate beads (Table 6.1) were determined by immersing a predetermined weight of the sample in 20 ml buffer solution of pH 2.1 (*SGF*) or pH 7.4 (*SIF*) at 37 °C until the equilibrium was attained. Then the weights of the swollen samples were determined after removal of the surface water using tissue paper. The swelling profiles of the hydrogels prepared using 0.2 M CaCl<sub>2</sub> solution, as an example of other formulations, were also determined. In a typical procedure, a certain weight of the dried beads was immersed in 20 ml buffer of pH 2.1 (*SGF*) at 37 °C for 3 h and subsequently transferred into another 20 ml buffer of pH 7.4 (*SIF*) at 37 °C for 8 h. The swollen weights of the hydrogel beads were determined at intervals, after removal of the surface liquid using tissue paper. The percent swelling was calculated by the following equation:

$$\% \text{ Swelling} = 100 [(W_t - W_0)/W_0]$$

where  $W_0$  is the initial weight and  $W_t$  the final weight of the beads at time  $t$ . The data points represent mean  $\pm$ SD from three independent experiments.

### 6.2.2.10. In-vitro cumulative release studies

The *in-vitro* cumulative release patterns of the entrapped *BSA* were determined by placing the pre-weighed hydrogel beads loaded with *BSA* in 10 ml of buffer at pH 2.1 (*SGF*) at 37 °C for 3 h and subsequently in 10 ml buffer of pH 7.4 (*SIF*) at 37 °C for 8 h. At intervals, 100  $\mu$ l aliquot was withdrawn and analyzed by the Bradford method at  $\lambda_{\text{max}}$  595 nm using a UV-VIS. Spectrophotometer [37]. The withdrawn sample was replaced with an equal volume of fresh buffer, to keep the volume of the release

medium constant. The data points represent mean  $\pm$ SD from three independent experiments.

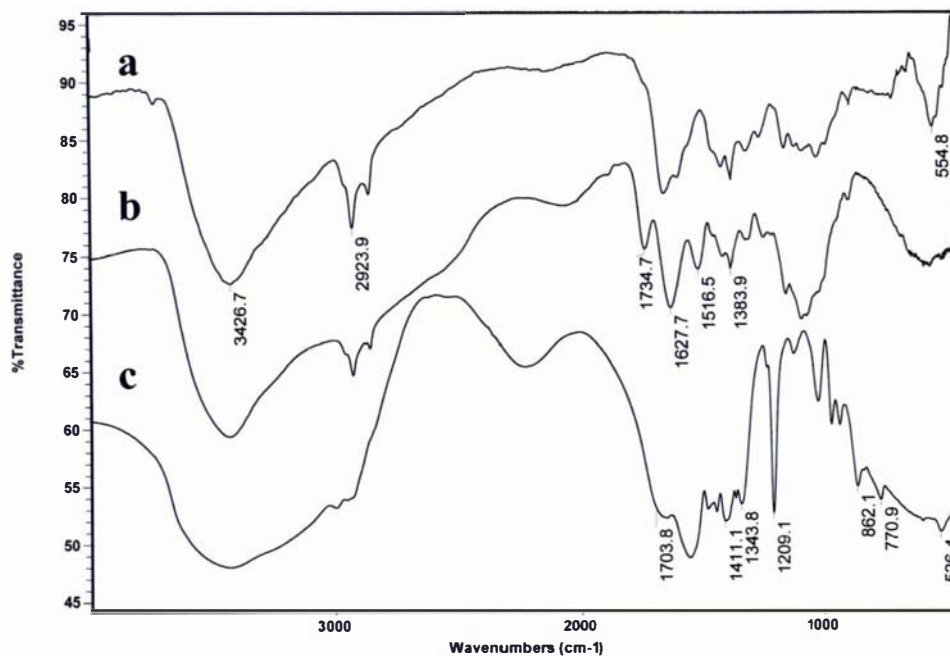
### 6.2.2.11. Statistical analysis

The results were analyzed and expressed as mean  $\pm$ SD. Statistical analysis was performed by using factorial design for characterization of CMCh-g-MAA/alginate hydrogel beads. Effects of various parameters on the properties of the gel beads were statistically analyzed by two-way analysis of variance (ANOVA) using the General Linear Models procedures of the SAS (SAS Institute (1997) SAS/STAT User's Guide: Statistics. Version 6.12. Cary, NC:SAS Institute Inc.). Differences were considered significant at the level of  $p < 0.05$ .

## 6.3. RESULTS AND DISCUSSION

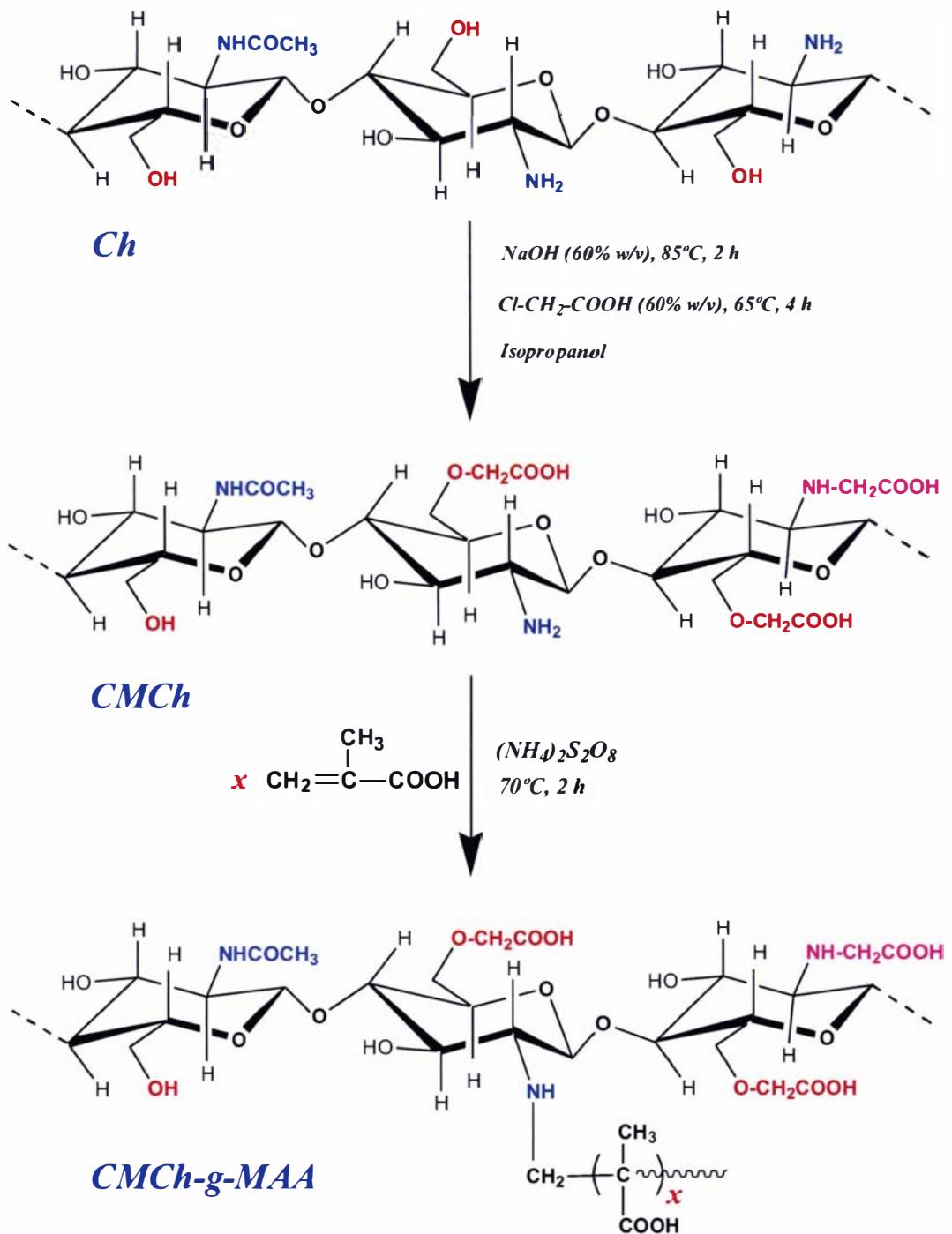
### 6.3.1. Preparation of CMCh and CMCh-g-MAA copolymer

CMCh was prepared by a modified method to that adapted from Xie et al [32]. The intrinsic viscosity of the prepared CMCh in 0.1 M aqueous NaCl at 30 °C was approximately 5.1 dL/g. The structural changes of Ch and its derivatives (CMCh and CMCh-g-MAA) were confirmed by FTIR (Figure 6.1). In the FTIR spectrum of Ch (Figure 6.1a), the strong peak appeared at 3427  $\text{cm}^{-1}$  was assigned to the N-H extension vibration, O-H stretching vibration, and the intermolecular H-bonds of the polysaccharide moieties. The weak peak at 1655  $\text{cm}^{-1}$  is due to the amide C=O stretching. The FTIR spectrum of CMCh (Figure 6.1b) shows a strong new peak at 1735  $\text{cm}^{-1}$  representing the carboxylate C=O asymmetric stretching. The signal at 1384  $\text{cm}^{-1}$  could be assigned to the symmetric stretching vibration of carboxylate C=O. In case of FTIR spectrum of CMCh-g-MAA (Figure 6.1c), the asymmetric stretching vibration of the carboxylate C=O of both CMCh backbone and poly(methacrylic acid), PMAA side chains (1550-1910  $\text{cm}^{-1}$ ) has overlapped with the amide C=O stretching (1655  $\text{cm}^{-1}$ ) to produce a strong peak at 1704  $\text{cm}^{-1}$ . Also, the FTIR spectrum of CMCh-g-MAA shows some new absorption peaks at 1411, 1209 and 1160  $\text{cm}^{-1}$  which are characteristic for PMAA [28].



**Figure 6.1.** FTIR spectra of (a) *Ch*, (b) *CMCh* and (c) *CMCh-g-MAA* (G %=1930%).

In addition to the FTIR spectrum of *CMCh-g-MAA*, which had the characteristic peaks of both *CMCh* and *PMAA*, the higher weight of the graft products over that of the starting *CMCh* after the extensive removal of the homopolymer can be taken as an experimental evidence of grafting. Also, the occurrence of grafting can be deduced from the decreasing of *N%* upon comparing the elemental analysis data of *CMCh* (C%: 37.21; N%: 5.11; H%: 5.85) and the *CMCh-g-MAA* copolymers (C%: 33.62; N%: 0.65; H%: 4.77 for copolymer of G%=1615). The preparation of *CMCh* and *CMCh-g-MAA* from *Ch* are shown in **Scheme 6.1**.

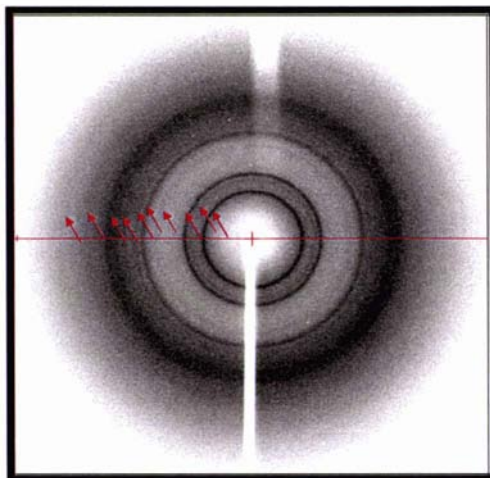
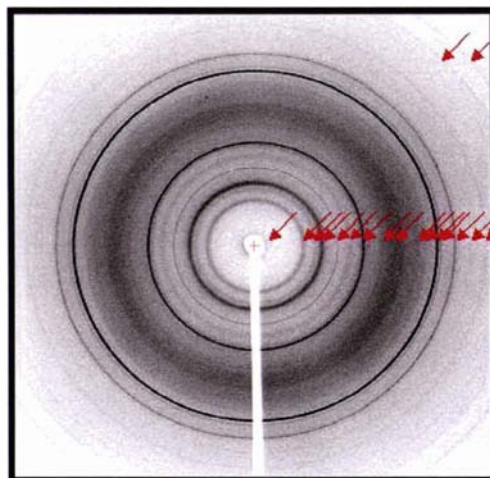
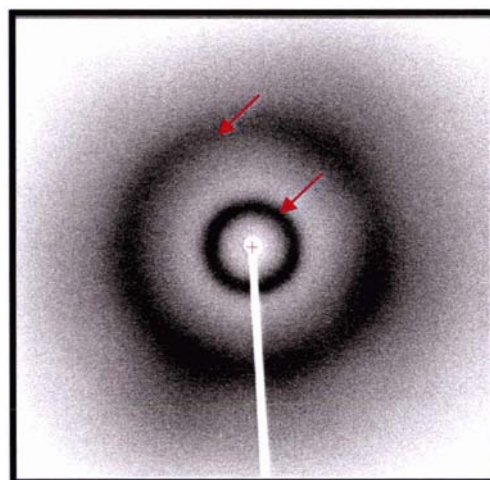


Scheme 6.1. The preparation of CMCh and CMCh-g-MAA from Ch.

**Figure 6.2** shows the 2D-XRD patterns of *Ch* and its derivatives, *CMCh* and *CMCh-g-MAA*. The diffractogram of *Ch*, **Figure 6.2a**, shows three major crystalline peaks at  $2\theta$  values of 8.38, 11.49 and 18.25° in addition to a lot of weak and broad crystalline peaks (see **Table 6.2**). This diffraction pattern reflects a high degree of crystallinity for the *Ch* under investigation. **Figure 6.2b** shows the diffractogram of *CMCh*, from which, *CMCh* has many crystalline peaks (see **Table 6.2**) plus two broad and weak bands corresponding to  $2\theta$  values of about 21.43 and 26.33°. The diffractogram of *CMCh* (**Figure 6.2b**) seems to keep some of the characteristic peaks of *Ch*. For instance, *CMCh* still have the bands at  $2\theta$  values of 10.40, 11.49, 16.10, ~18.46 and ~21.43°. The grafting of *MAA* onto the *CMCh* backbone turned the resulting copolymer, *CMCh-g-MAA*, into an amorphous material. The diffractogram of *CMCh-g-MAA* copolymer (**Figure 6.2c**) shows two peaks, the first is corresponding to a  $2\theta$  value of 7.75° and the other one is patchy appeared at  $2\theta$  of ~21.65°. This resulting amorphous structure of *CMCh-g-MAA* may be attributed to the occurrence of the grafting in a random manner along the *CMCh* backbone and consequently destroying the regularity of the packing of the original *CMCh* chains leading to the formation of amorphous structure.

**Table 6.2.** The 2D-XRD data of *Ch*, *CMCh* and *CMCh-g-MAA* copolymer.

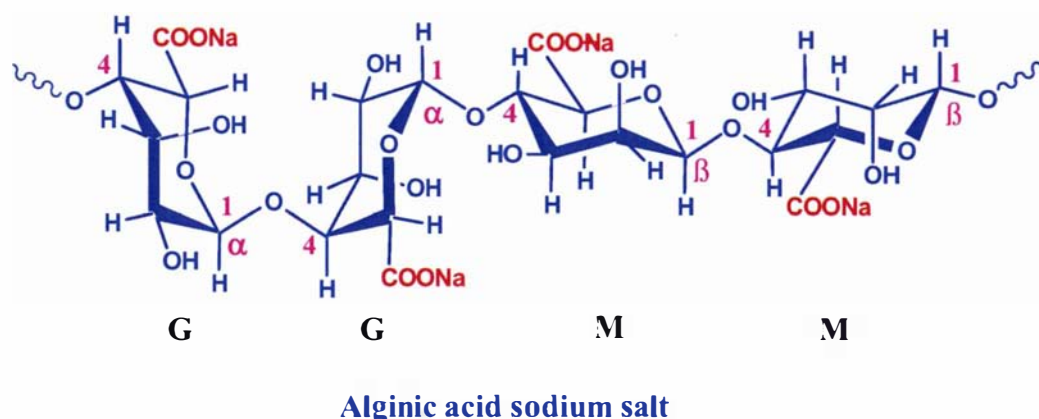
Sample Code	$d$ (°Å) (from low to high resolution)	$2\theta$ °
<i>Ch</i>	10.55, 8.50, 7.70, ~5.50, 4.86, ~4.60, ~4.20, 3.84 (broad), ~3.30, ~3.00	8.38, 10.40, 11.49, ~16.10, 18.25, ~19.30, ~21.20, 23.16, ~27.02, ~29.78
<i>CMCh</i>	46, 32.50, 8.50, 7.68, 6.44, ~5.5, 4.80 (sharp, strong), 4.14 (weak), ~3.38 (weak), 3.21, 3.04 (sharp, strong), 2.98, 2.82, 2.66, 2.53, 2.26, 2.16	1.92, 2.72, 10.40, 11.49, 13.73, ~16.10, 18.46, 21.43, ~26.33, 27.76, 29.34, 29.95, 31.69, 33.65, 35.44, 39.84, 41.77
<i>CMCh-g-MAA</i>	11.4, 4.1 (patchy)	7.75, 21.65

(a) *Ch*(b) *CMCh*(c) *CMCh-g-MAA***Figure 6.2** . 2D-XRD patterns of (a) *Ch*, (b) *CMCh* and (c) *CMCh-g-MAA*.

### 6.3.2. Preparation of CMCh-g-MAA/sodium alginate hydrogel beads

The resulting water-soluble *CMCh-g-MAA* was used in this study to develop pH-sensitive hydrogel beads for the delivery of a model protein drug (*BSA*). Three *CMCh-g-MAA* copolymers of different grafting percents (570%, 1615 % and 1930%) were selected for the hydrogel preparation (see **Table 6.1**). The developed hydrogel is based on a combination of the *CMCh-g-MAA* copolymer blended with sodium alginate and ionotropically crosslinked by dropping this aqueous mixture into a  $\text{Ca}^{2+}$  solution.

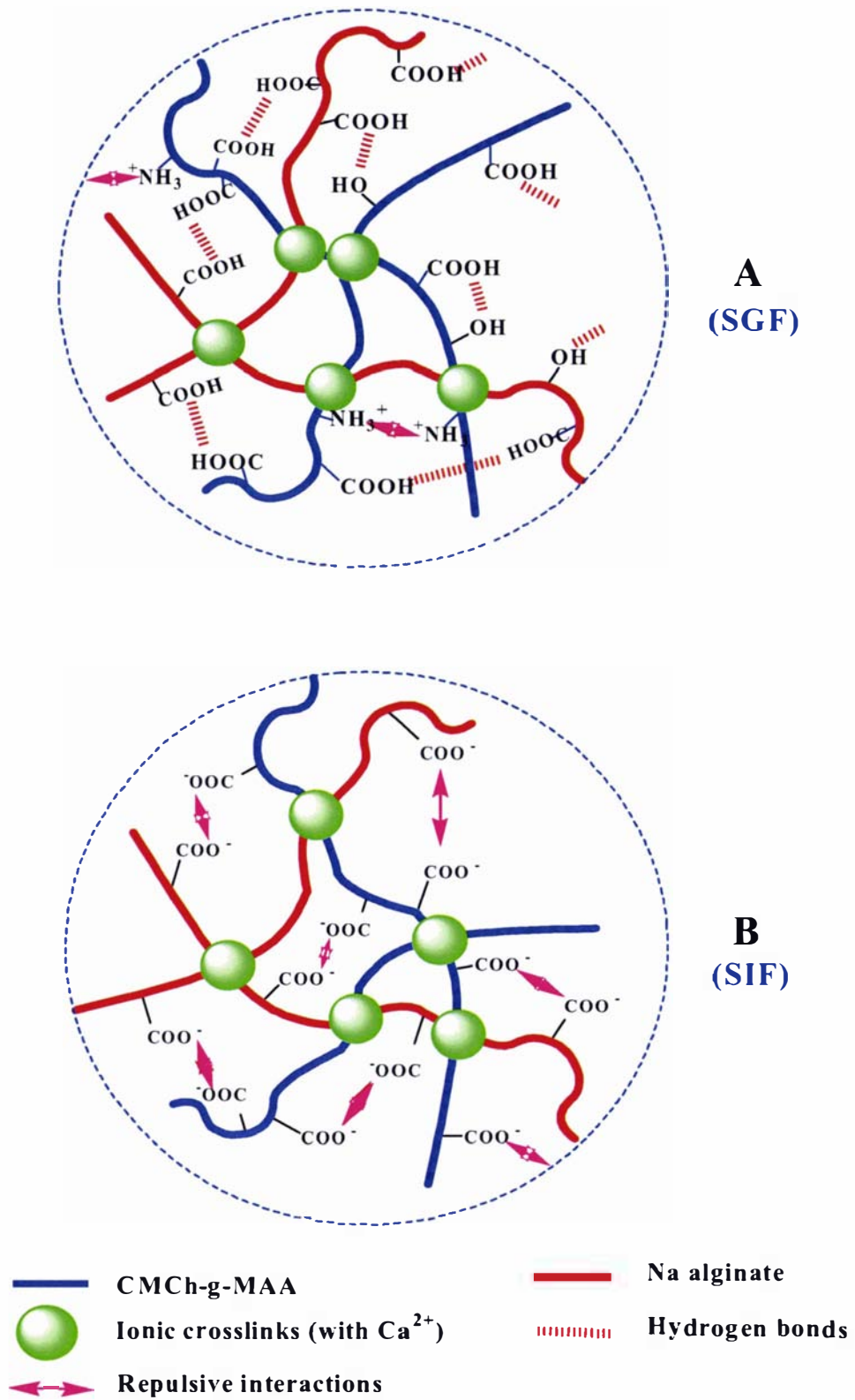
Alginate is a linear copolymer which has 1, 4-linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) residues arranged either as consecutive blocks or in a random distribution [9]. It has been reported [10] that the poly(guluronic acid) blocks bind more strongly with  $\text{Ca}^{2+}$  than the poly(mannuronic acid) blocks. Therefore, alginates with the higher guluronic acid levels are normally the ones that show the strongest interaction with  $\text{Ca}^{2+}$  and hence the strongest hydrogel strength [10]. The structure of sodium alginate is shown in **Figure 6.3**.



**Figure 6.3.** Structure of sodium alginate.

Hydrogels based on calcium-crosslinked alginate has been widely investigated as drug delivery matrices [10,11]. However, it has been found [12] that the swelling of the calcium-crosslinked alginate beads at pH 7.4 was minimal due to the relatively strong ionic interaction between the alginate and  $\text{Ca}^{2+}$ . This may limit the drug release in the intestinal tract. In an attempt to overcome this shortcoming, Lin et al. [12] developed a

hydrogel complex composed of alginate blended with **CMCh** by dropping an aqueous mixture of the two polymers onto a  $\text{Ca}^{2+}$  solution. The resulting hydrogel beads showed enhanced swelling behavior in intestine as compared to that of the hydrogels based on alginate alone. However, the swelling extent of these gel beads, prepared by Lin et al. [12], was relatively higher than alginate in the stomach. This may lead to the loss of some of the uploaded protein drug in the stomach. Therefore, in this study, **CMCh** was prepared and further modified via grafting of **MAA** onto its backbone. The main purpose of this modification is to increase the number of the carboxylic groups in the **CMCh**. Thus by developing a hydrogel based on alginate with this modified **CMCh**, these carboxylic groups are expected to participate in minimizing the swelling at pH 2.1 (**SGF**) and maximizing it at pH 7.4 (**SIF**). This expected role of the carboxylic groups in controlling the swelling extent of the prepared **CMCh-g-MAA**/alginate hydrogel is illustrated in **Scheme 6.2**.



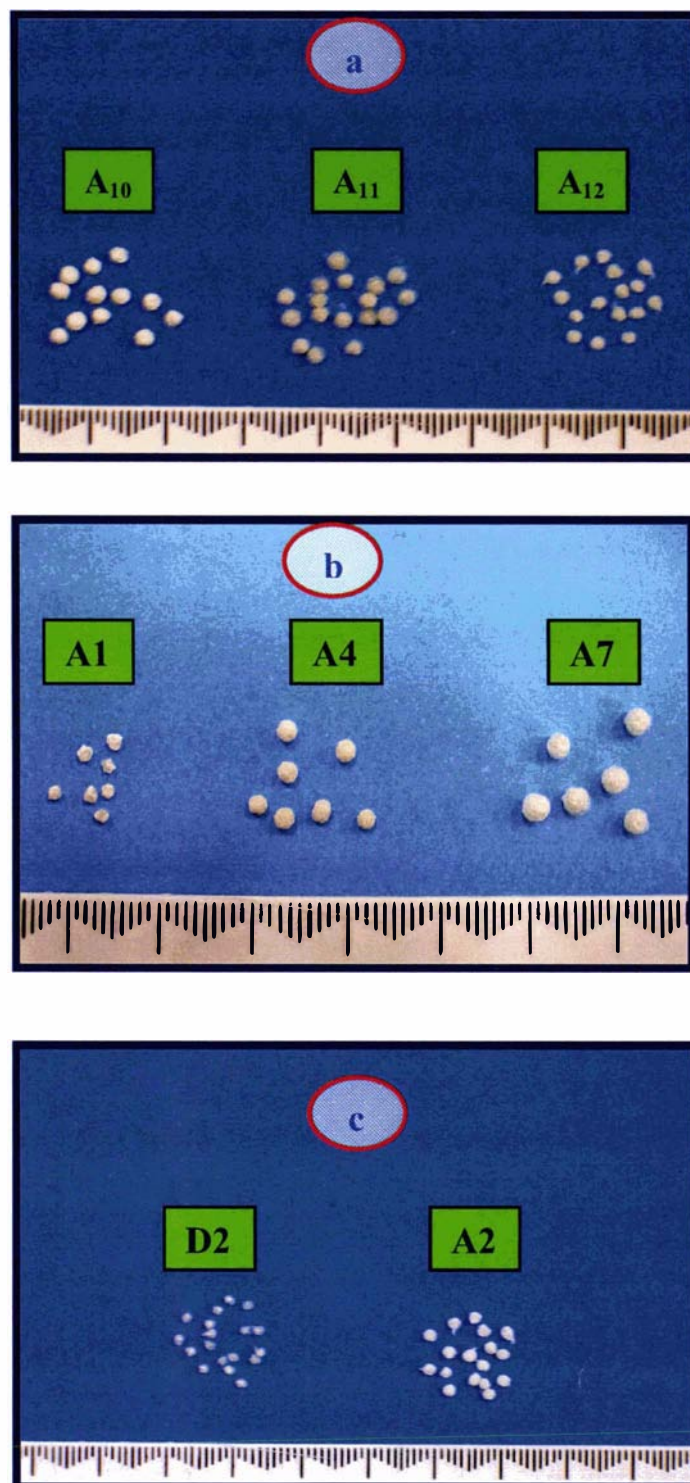
**Scheme 6.2.** A schematic representation of the structure of *CMCh-g-MAA*/alginate hydrogel beads at both pH 2.1 (*SGF*) and pH 7.4 (*SIF*).

During the early part of this study, it was observed that the aqueous solution of *CMCh-g-MAA* can form hydrogels when dropped onto a  $\text{Ca}^{2+}$  solution. However, the resulting hydrogels had random shapes and were not symmetrical beads. Therefore, the hydrogel formulations based on *CMCh-g-MAA* alone were not investigated further in this study. In combination with alginate, as represented in **Scheme 6.2**, the hydrogel can form strong ionic crosslinks with  $\text{Ca}^{2+}$  at both pHs, 2.1 and 7.4. At pH 2.1 (**Scheme 6.2A**), the free carboxylic groups, which are not involved in the ionic crosslinking with  $\text{Ca}^{2+}$ , tend to form hydrogen bonds with each other and with the OH groups of the sugar moieties. Both of the ionic crosslinks and the hydrogen bonds act to minimize the swelling at pH 2.1. The repulsive interactions that may occur between the protonated  $\text{NH}_2$  groups work to increase the gel swelling. However, these repulsive forces are expected to be few, as the number of  $\text{NH}_2$  groups remaining free, after the carboxymethylation of *Ch* and its further modification via grafting, should be quite limited. In contrast, at pH 7.4 (**Scheme 6.2B**), most of the free carboxylic groups would be ionized. Hence, strong repulsive forces are created by the electrostatic repulsion between these ionized carboxylate groups ( $\text{COO}^-$ ). These repulsive forces are thus responsible for attaining the hydrogels higher values of swelling at pH 7.4.

### 6.3.3. Bead size measurements

The shape of all the prepared alginate and *CMCh-g-MAA*/alginate hydrogel beads is spherical. The average bead size of the different formulations is between  $727 \pm 75 \mu\text{m}$  and  $1619 \pm 25 \mu\text{m}$ . As shown in **Table 6.1**, in most cases, for a certain bead composition, increasing the concentration of  $\text{Ca}^{2+}$  used in the hydrogel preparation led to a significant decrease ( $p < 0.05$ ) in the size of the beads. For instance, according to the size,  $\text{A10} > \text{A11} > \text{A12}$  (**Figure 6.4a**). For the majority of formulations, there is a slight difference ( $p > 0.05$ ) in size between the beads prepared by 0.2 M and 0.3 M  $\text{Ca}^{2+}$ . Increasing the concentration of *CMCh-g-MAA* in the beads and/or the concentration of the whole polymer mixture forming the beads led to a significant increase ( $p < 0.05$ ) in the size. From the results, it seems that, increasing the concentration of the *CMCh-g-MAA* in the hydrogel affects the bead size more than increasing the concentration of both the components of the mixture. For example, according to the size,  $\text{A7} > \text{A4} > \text{A1}$  (**Figure 6.4b**). Increasing the G % of the *CMCh-g-MAA* from 570% (formulations A) to 1615% (formulations B) led to increasing the bead size. Further increase in the G % to 1930%

(formulations C) led to a decrease in the bead size. The size of the gel beads based on alginate alone are less than that of the *CMCh-g-MAA*/alginate hydrogel beads (Figure 6.4c).



**Figure 6.4.** Photographs illustrate the differences in size of some dry drug-free *CMCh-g-MAA*/alginate beads.

**Figure 6.5** illustrates microscopic images of some of the prepared *CMCh-g-MAA*/alginate hydrogel beads. From this figure, with increasing the  $\text{Ca}^{2+}$  concentration, from A4 to A6 and from C4 to C6, the beads became smaller and more spherical with a smoother surface. This can be attributed to the increase in the extent of crosslinking. The same behavior can be noted also in A7-A9 and in D1-D3. Also, this figure confirmed that the difference in size between the beads prepared using 0.2 M and 0.3 M  $\text{Ca}^{2+}$  is non significant ( $p > 0.05$ ) than that between the beads prepared using 0.1 M and 0.2 M.

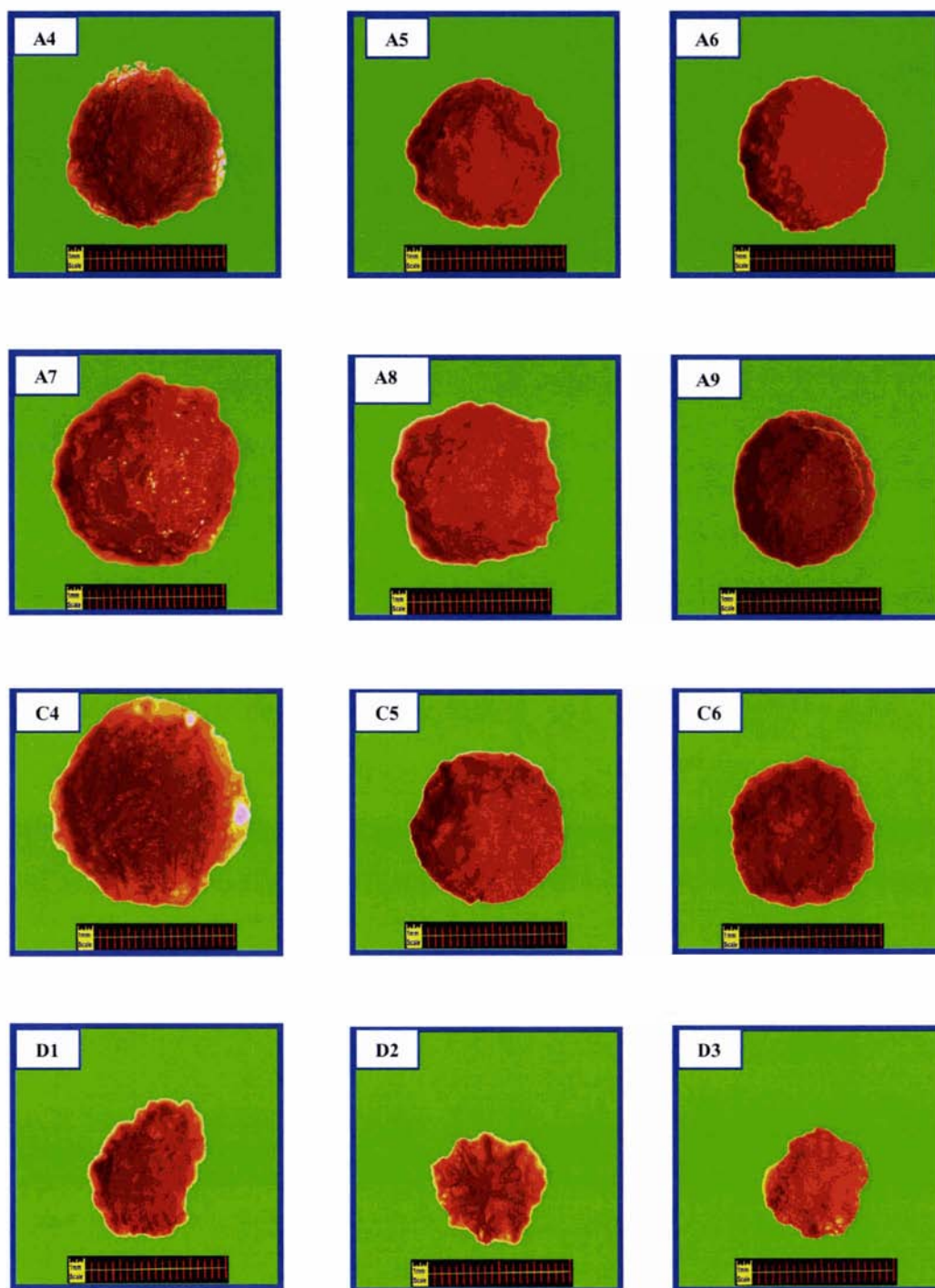


Figure 6.5. Microscopic photographs of *CMCh-g-MAA*/alginate hydrogel beads, (Scale bar: 1 mm scale).

### 6.3.4. Swelling characteristics

The swelling pattern of a hydrogel is one of the most significant characteristics that control the rate of drug release from this hydrogel. The swelling measurements of the prepared CMCh-g-MAA/alginate hydrogel beads were carried out for 3 h at pH 2.1 (*SGF*) followed by 8 h at pH 7.4 (*SIF*) at 37 °C. From the swelling data shown in **Table 6.1**, at both pHs 2.1 and 7.4, increasing the concentration of CaCl<sub>2</sub> increased the crosslinking extent and consequently the equilibrium swelling % of the hydrogel beads decreased. It has been found that the difference in swelling at equilibrium between the beads prepared using 0.2 M and 0.3 M CaCl<sub>2</sub> is non significant ( $p > 0.05$ ) than the swelling difference between the beads prepared using 0.1 M and 0.2 M CaCl<sub>2</sub> ( $p < 0.05$ ). The hydrogel formulations prepared using 0.2 M CaCl<sub>2</sub> were selected, as an example for the other formulations, for the investigation of their swelling profiles. As shown in **Figure 6.6**, increasing the total % of all the polymer mixtures used in the preparation of the hydrogel beads make the beads more compact and consequently decreases their swelling values. For instance, in **Figure 6.6A**, A5 (2.5% CMCh-g-MAA:2.5% alginate) attained swelling of 184% and 2978% at 37°C after 3 h and 8 h in *SGF* and *SIF* respectively, whereas A2 (1% CMCh-g-MAA:1% alginate) attained 201% and 3827% in *SGF* and *SIF* respectively. The same behavior can be noted by comparing the swelling % of B5 and B2 (**Figure 6.6B**). Increasing the percent of the CMCh-g-MAA in the hydrogel beads relative to alginate led to increasing the swelling values in both *SGF* and *SIF*. For example, A8 (2.5% CMCh-g-MAA:1% alginate) attained swelling of 211% and 3500% at 37°C in *SGF* and *SIF* respectively, whereas, A11 (1% CMCh-g-MAA:2.5% alginate) achieved swelling of 187% and 3432% in *SGF* and *SIF* respectively. The same result can be also noted by comparing the swelling % values attained in both *SGF* and *SIF* for B8 and B11 (**Figure 6.6B**).

From **Table 6.1** and **Figure 6.6A**, it can be seen that the gel beads prepared from alginate only (D1-D3) attained relatively low swelling values in *SGF* as compared to the CMCh-g-MAA/alginate hydrogel beads. However these alginate beads have limited swelling in the *SIF* which limit the drug release from them in the *SIF*. Therefore, it seems that, the CMCh-g-MAA/alginate beads, developed in this study, are more appropriate for the delivery of protein drugs to the intestine region than the gel beads prepared from alginate only. It seemed also, from the swelling results, that these

CMCh-g-MAA/alginate gel beads prepared in this study achieved enhanced swellings (lower in *SGF* and higher in *SIF*) over those of the CMCh/alginate gel beads investigated recently [12]. For instance, in the majority of the prepared formulations, the swelling % values attained at 37 °C after 3 h in *SGF* by CMCh-g-MAA/alginate beads were in the range between  $83\pm 34.6$  and  $253.7\pm 61$  whereas in case of CMCh/alginate beads [12], the swelling % at 37 °C after 2 h in *SGF* were relatively higher (up to 800% in some formulations). Also, the CMCh-g-MAA/alginate beads, prepared in this study, achieved enhanced (higher) swelling (up to  $4533\pm 75$  as in C7) in *SIF* than that achieved by CMCh/alginate beads [12].

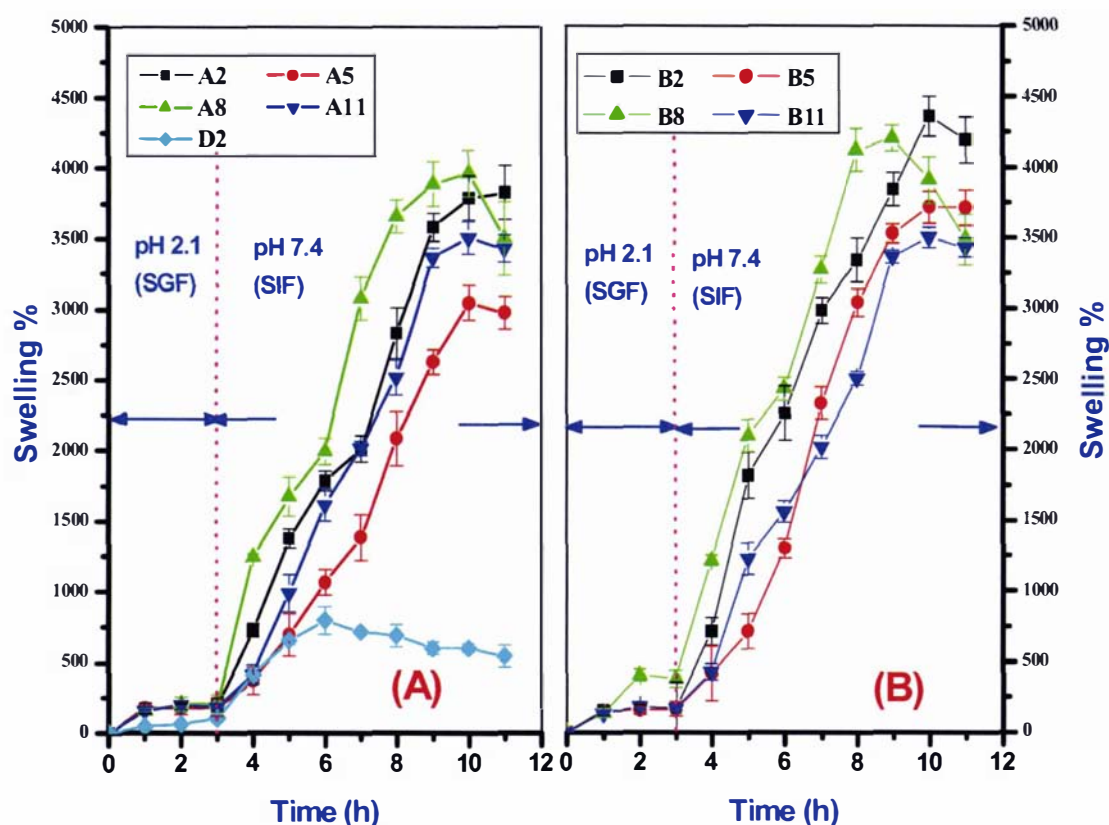
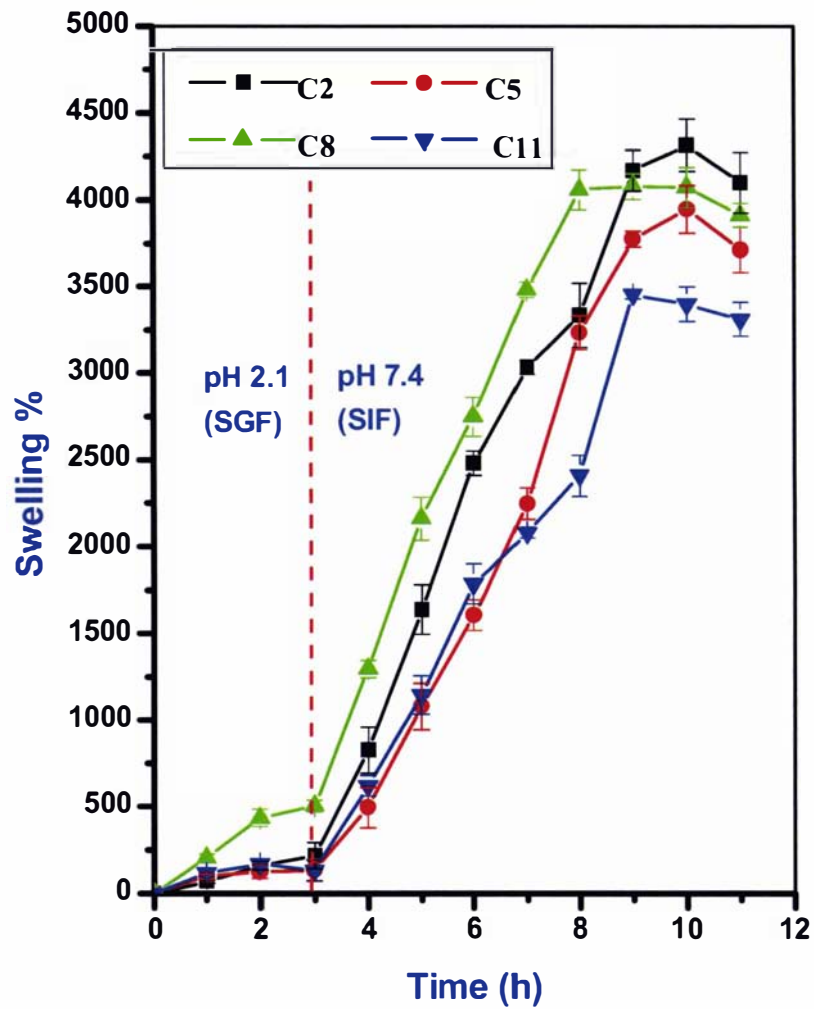
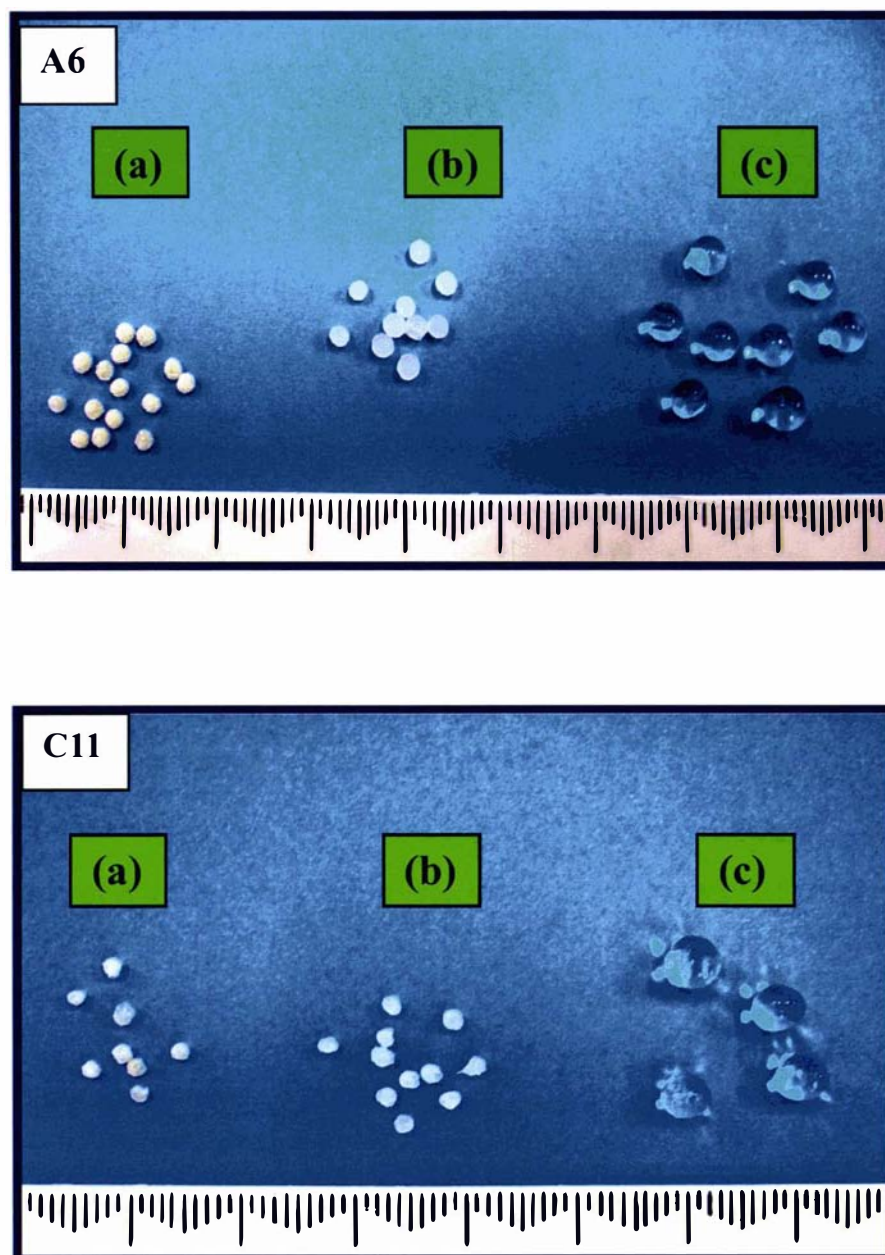


Figure 6.6. Swelling behaviour of some formulations of CMCh-g-MAA/alginate beads in *SGF* for 3 h, followed by 8 h in *SIF*.

From the results in **Figures 6.6A** and **6.6B**, it can be noted that, increasing the grafting percent (G %) of *MAA* onto *CMCh* and consequently increasing the number of the carboxylic groups led to improving (lower swelling % in *SGF* and higher swelling % in *SIF*, respectively) the swelling of *CMCh-g-MAA*/alginate beads. This behavior confirms the role played by the carboxylic groups in encouraging bead contraction in *SGF* by formation of H-bonds and the bead expansion in *SIF* due to the repulsion (**Scheme 6.2**). Almost no difference ( $p > 0.05$ ) in the swelling profiles was noted between the formulations B (G % = 1615%) and the formulations C (G % = 1930%, **Figure 6.7**). Some photographs that illustrate the difference in size between the dry and the swollen states of some drug-free *CMCh-g-MAA*/alginate beads are shown in **Figure 6.8**.



**Figure 6.7.** Swelling % of some *CMCh-g-MAA* ( $G\%= 1930$ )/alginate hydrogel beads in *SGF* for 3 h followed by 8 h in *SIF*.



**Figure 6.8.** Illustrations of the difference in size between the dry and the swollen states of some drug-free *CMCh-g-MAA*/alginate beads, a) dry b) after 3 h in *SGF* c) after 8 h in *SIF*.

### 6.3.5. Entrapment efficiency

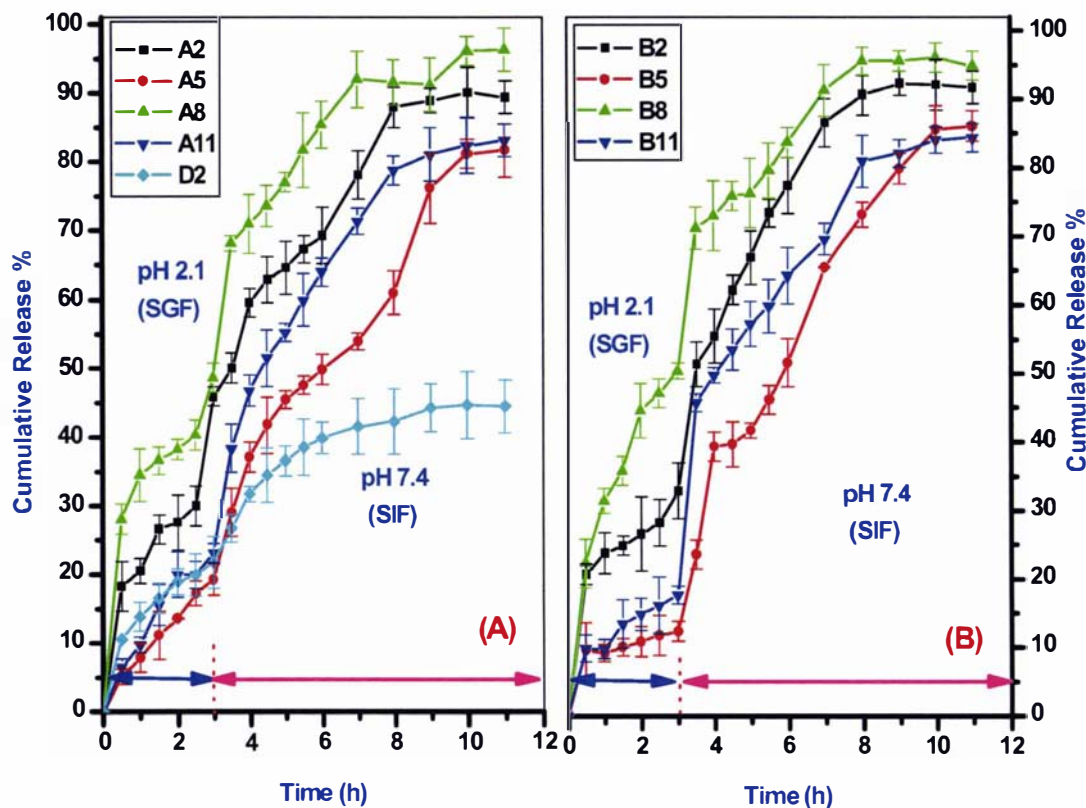
From the results of the drug loading efficiency (Table 6.1), it can be seen, in general, that the amount of the *BSA* loaded in the beads seems to increase with increasing the concentration of  $\text{Ca}^{2+}$  in solution. However, in most cases, the maximum efficiency of drug loading was attained when the *CMCh-g-MAA*/alginate mixture was crosslinked using 0.2 M  $\text{Ca}^{2+}$ . For this reason the hydrogel beads prepared using 0.2 M  $\text{Ca}^{2+}$  were selected for studying their release profiles.

### 6.3.6. In-vitro cumulative release studies

Figure 6.9 shows the cumulative release profiles of *BSA* from the *CMCh-g-MAA*/alginate hydrogel beads at 37 °C for 3 h in *SGF* followed by 8 h in *SIF*. The percent of drug released was much higher in *SIF* than in *SGF*, because the release rate depends on the swelling of the hydrogel where the mechanism of drug release may be due to the diffusion through the swollen hydrogels. As discussed earlier, the swelling of the prepared hydrogel beads in *SIF* was greater than in *SGF*. From Figure 6.9A, various amounts of *BSA* were released (lost) at 37 °C within 3 h in the *SGF* depending on the hydrogel composition. For instance, A8 (2.5% *CMCh-g-MAA*:1% alginate) released (lost) about 49% of the uploaded amount of *BSA* at 37 °C after 3 h in *SGF*. Another high amount of *BSA* (46%) was also released from A2 (1% *CMCh-g-MAA*:1% alginate). In case of A11 (1% *CMCh-g-MAA*:2.5% alginate) and D2 (2.5% alginate) only 23.2% and 21.8% *BSA* were released in *SGF* respectively. In the case of A5 (2.5% *CMCh-g-MAA*:2.5% alginate), only 19.3% of *BSA* was lost in *SGF* at 37 °C. From these results, increasing the total % of the polymers used in the preparation of the gel beads makes the beads more compact and consequently decreases their swelling in *SGF* and thus, it limits the drug loss in *SGF*. Whereas, increasing the % of *CMCh-g-MAA* in the hydrogel increased the swelling and consequently increased the loss of *BSA* in *SGF*.

In *SIF*, A2, A5, A8 and A11 released 89.4%, 81.7%, 96.3% and 83.1% of *BSA* at 37 °C after 8 h in *SIF*, respectively. These values are much better than the amount of *BSA* released from alginate alone, D2, (44.5%). The same release behavior was noted in case of the hydrogel formulations B and C. For instance, Figure 6.9B shows the release patterns of *BSA* from different hydrogels of formulations B. From this figure, the

formulation B5 achieved the best release pattern (limited loss of *BSA* in *SGF* with a reasonable maximum release after 8 h in *SIF*).



**Figure 6.9.** Cumulative release profiles of *BSA* from the *CMCh-g-MAA*/alginate hydrogel beads at 37 °C for 3 h in *SGF* followed by 8 h in *SIF*.

## CONCLUSIONS

The equilibrium swelling measurements of the prepared *CMCh-g-MAA*/alginate hydrogel beads at 37 °C in *SGF* and *SIF* clearly showed the pH-responsive nature of them. The *in-vitro* release profiles of *BSA* from the hydrogel beads were also estimated at 37 °C in *SGF* and *SIF*. From this preliminary investigation, the *CMCh-g-MAA*/alginate hydrogel beads prepared in this study, showed promising release profiles of *BSA*, as a model protein drug. However, this hydrogel bead study requires more effort to limit the swelling and consequently the loss of drug in the *SGF*, to act as an excellent candidate for intestine-specific delivery of peptide and protein drugs.

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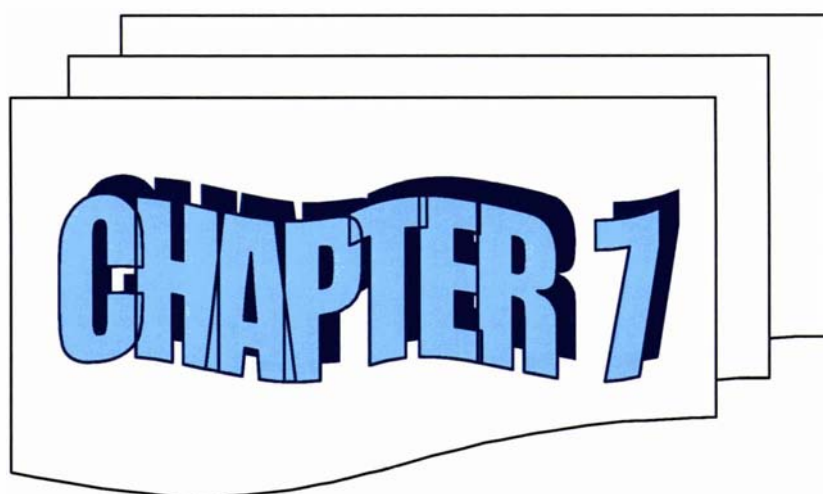
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**CHAPTER 7**

## CHAPTER 7

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### SUMMARY AND FUTURE WORK

#### SUMMARY

Controlled drug release enhances the safety and reliability of drug therapy. Regulation of the drug release rate results in a reduction in the frequency of drug administration and should encourage patients to comply with dosing instructions. Conventional dosage forms often lead to broad fluctuations in serum drug concentrations. These fluctuations often cause unacceptable side effects.

Hydrogels are crosslinked, three-dimensional hydrophilic polymers, which swell without dissolving when brought into contact with water or other biological fluids. In the swollen state they are soft and rubbery, resembling living tissue. Hydrogels that respond to the environmental stimuli such as pH, temperature, electric field and ionic strength have a wide range of applications, particularly in the controlled release of drugs. However, the number of polymers appropriate for the preparation of matrices for the controlled release of drugs is quite limited compared to the number of available synthetic polymers. This shortage in polymers is due to the inherent toxicity or lack of certain properties such as biodegradability and swellability in particular media. Hence, for this thesis, chitosan (*Ch*) was selected as the base polymer for the development of new hydrogels that can be tailored for use in the site-specific delivery of drugs to the gastrointestinal tract.

*Ch*, a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine, is obtained through the alkaline deacetylation of natural chitin. Its structure is very similar to that of cellulose, except that amino or acetylamino group replaces the hydroxyl group on the C-2 position of cellulose. *Ch* has many attractive biological properties, being non-toxic, biodegradable and bioabsorbable. It also has antacid and antiulcer activities, which can inhibit or deteriorate drug-induced irritation in the stomach. These remarkable characteristics make chitosan an ideal candidate for use in controlled drug release formulations. However, *Ch* also exhibits some shortcomings such as hydrophobicity and a high pH-dependency for its physical properties. Hence, it is very difficult to

control drug release with chitosan itself because of the various pHs of the internal organs of the human body. This may negatively reflect on the human body because of drug under- or over-release.

In a structured programme, some new *Ch*-based hydrogels have been prepared for the controlled drug release investigations. During the preparation of these *Ch*-based hydrogels, three main approaches have been applied to overcome the shortcomings of *Ch*. These approaches are discussed in the following sections:

The first approach was the incorporation of *Ch* in a semi-*IPN* or *IPN* hydrogels with either a hydrophilic polymer of good mechanical properties (such as *PVA*, Chapter 2) or with hydrophilic monomers (such as *NAGly* and/or *PEGDA*, Chapters 3 and 4) treated to bring about in situ copolymerization in the presence of *Ch* and a suitable crosslinking agent.

Initially, using this first approach, two series of pH-dependent semi-*IPN* hydrogels based on *Ch* and *PVA* were prepared and studied (Chapter 2) as a basis for investigations into hydrogel construction. The first series was chemically crosslinked with different concentrations of glutaraldehyde and the second was crosslinked by different doses of  $\gamma$ -irradiation. The structural changes of the prepared hydrogels with the variation of the hydrogel composition and the content of the crosslinker before and after drug loading were investigated using FTIR, SEM and 2D-XRD. The pH-responsive nature of these hydrogels was noted through studying their equilibrium swelling at 37 °C in simulated body fluids (pH 2.1 and 7.4). This preliminary study showed that the equilibrium swelling of the chemically crosslinked *Ch/PVA* hydrogel films is directly related to the content of *PVA* and inversely dependent on the crosslinker content. Upon irradiation, more crosslinking occurs as the content of *PVA* increases leading to a noticeable decrease in the swelling % at equilibrium. The *in-vitro* release profiles of *5-FU*, as a model therapeutic agent, from the hydrogels were also studied in *SGF* and *SIF*. The amounts of drug released at equilibrium were found to be dependent on many factors such as *PVA* %, crosslinker %,  $\gamma$ -irradiation dose and the pH of the medium. The rate of drug release was much slower from the hydrogels prepared via  $\gamma$ -irradiation than from the chemically crosslinked hydrogels using glutaraldehyde.

The studies continued (Chapter 3) to include another pH-responsive, thermosensitive semi-*IPN* based on *Ch* crosslinked with glutaraldehyde (2.5-12.5% based on total matrix weight) with in situ copolymerization of the hydrophilic *NAGly* monomer. Promising pH-responsive behavior was noted for these matrices that pointed toward them being good candidates for use in drug delivery. However, relatively short drug release times (2.5-3 h) were noted from these hydrogels, at least in the case of *5-FU*. Although glutaraldehyde is commonly used in the crosslinking of many polymers due to its high reactivity, use of a high percent of glutaraldehyde in a hydrogel matrix for a drug delivery is not preferred. Therefore, the study was extended to the synthesis and characterization of a pH-sensitive full *IPN* hydrogels (Chapter 4). In these *IPNs*, attempts were made to enhance the hydrophilicity of *Ch* through the in situ copolymerization of two hydrophilic monomers, *NAGly* and *PEG*-diacrylate (*PEGDA*) in the presence of a lower amount of glutaraldehyde (2-4% based on total matrix weight). The diacrylate derivative of *PEG* was also used to act as a co-crosslinker with glutaraldehyde in the matrix. The results obtained in this study (Chapter 4) showed that the inclusion of *PEGDA* in the matrix assembly was beneficial. In the presence of *PEGDA*, longer release times were achieved than the *Ch/poly(NAGly)* semi-*IPN*, reported in Chapter 3, with reduced use of the relatively toxic crosslinker, glutaraldehyde.

*PEG*, a highly water-soluble polymer is widely used in pharmacological products due to its high hydrophilicity, biocompatibility and lack of toxicity. Some of *PEG* derivatives such as *PEG*-aldehyde and *PEG*-ester have been used for the modification of *Ch* via grafting. However, little work has been reported exploiting these *Ch-g-PEG* copolymers in the preparation of hydrogels for controlled release studies. Therefore the next stage of the development of *Ch*-based hydrogels involved a second approach. In this approach (Chapter 5) attempts were made to enhance the hydrophilicity of *Ch* by grafting *PEGDA* onto *Ch* backbone in an acidic medium using ceric ammonium nitrate (*CAN*) as the initiator. The parameters affecting the grafting yield were studied and the optimum grafting conditions were estimated. Then, the hydrogels obtained from these graft copolymers, crosslinked with a reduced amount of glutaraldehyde, were prepared and characterized. Also the swelling characteristics of the prepared hydrogels and their drug release profiles were investigated. The results obtained from this preliminary investigation of the modified *Ch*-based hydrogels (Chapter 5) illustrated that the

grafting of *PEGDA* in the hydrogel matrix was helpful. With the increase in the *PEGDA* grafting extent, higher percents of the drug were released over longer release times with a reduced use of the crosslinker, glutaraldehyde.

All the hydrogels developed using the *first* and *second* approaches (Chapter 2 to Chapter 5) are pH-responsive and swelled faster and to a greater extent at pH 2.1 (*SGF*) than at pH 7.4 (*SIF*). From these preliminary investigations, these *Ch*-based hydrogels may be further tailored and exploited to expand the utilization of these systems in drug delivery applications.

In the *third* approach, an attempt was carried out to modify *Ch* in such a fashion that the hydrogels produced were also pH-responsive but swelled higher in *SIF* than in *SGF* (Chapter 6). Hence, the resulting hydrogels could be tailored for example, for utilization for intestine-targeted delivery of protein drugs. In this third approach *Ch* was chemically modified into *CMCh* via carboxymethylation followed by grafting of *MAA* onto its backbone. Then, ionotropic gelation was carried out for this modified *Ch* with another suitable natural polymer (alginate) in the presence of a divalent ion,  $\text{Ca}^{2+}$  (Chapter 6). Bovine serum albumin (*BSA*) was used, as a model protein drug, to check the ability of the prepared hydrogels to control the release of protein drugs into the intestine with a potential drug protection from the harsh acidity of stomach. From this preliminary investigation, the *CMCh-g-MAA*/alginate hydrogels reported in this study (Chapter 6) showed promising release profiles for *BSA*, as a model protein drug. However, this hydrogel bead study requires more effort to completely limit the swelling and consequently the loss of drug in the *SGF*, to act as an excellent candidate for intestine-specific delivery of peptide and protein drugs.

In conclusion, this investigation of the *Ch*-based hydrogels reported in this thesis showed their pH-responsive nature and illustrated that they may be further tailored to expand the utilization of these systems in controlled drug release applications. However, as with many PhD studies, the research reported in this thesis might be said to have just “scratched the surface”. There is still much to do to explore this highly attractive and exciting area of research.

## FUTURE WORK

In the near future, the author hopes to carry out the following:

- 1- Study extensively the graft copolymerization of *NAGly* and *PEGDA* onto *Ch* with different reaction conditions and using different initiators for determination of the optimum conditions that produce the highest grafting yield.
- 2- Repeat some of the studies reported in this thesis using different drugs (hydrophobic and/or of higher molecular weight).
- 3- Additional studies to minimize the swelling of the *CMCh-g-MAA*/alginate gel beads (and consequently reduce loss of drug) in the *SGF* to act as an excellent candidate for intestine-specific delivery of peptide and protein drugs.
- 4- Preparation of some new hydrogels based on modified *Ch* in combination with some other natural polymers to be used for enzyme immobilization.



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**The work reported and published from the thesis to date:**

- [1] **El-Sherbiny, I.M.**, Lins, R.J., Abdel-Bary, E.M., Harding, D.R.K. Preparation, characterization, swelling and in vitro drug release behaviour of poly[N-acryloylglycine-chitosan] interpolymeric pH and thermally-responsive hydrogels. *Eur. Polym. J.* **41** (2005) 2584. (*One of Top 25 articles in EPJ, 2005*)
- [2] **El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. Preparation and swelling study of a pH-dependent interpolymeric hydrogel based on chitosan for controlled drug release. *Int. J. Polym. Mater.* **55** (2006) 789.
- [3] **El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. Swelling characteristics and in-vitro drug release study with pH and thermo-sensitive hydrogels based on modified chitosan. *J. Appl. Polym. Sci.* **102** (2006) 977.
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- [5] **El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. In-vitro evaluation of new biodegradable pH-responsive hydrogel beads for oral delivery of protein drugs to the small intestine. (2006) *In preparation*.
- [6] Abdelaal, M.Y., Abdel-Razik, E.A., Abdel-Bary, E.M., **El-Sherbiny, I.M.** Some factors affecting the delivery of some bioactive materials from chitosan. *7th Arab International Conference on Polymer Science & Technology, and 3rd Arab Conference on Materials Science, (ACMS-III)*. (2003) Cairo-Hurghada, Egypt, October 5-9 (B-P9).
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- [8] **El-Sherbiny, I.M.**, Abdel-Bary, E.M., Harding, D.R.K. Preparation, characterization, swelling kinetics and in-vitro evaluation of new pH-sensitive gel beads based on chemically modified chitosan for oral delivery of protein drugs. *The International Conference on Polymer Synthesis, Warwick Polymer 06.* (2006) Warwick University, Warwick, United Kingdom, 31 July-3 August, (P52).
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