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Preparation and Characterization of *Acaciamellifera*GumBased Hydrogels crosslinked with Citric Acid

تحضير وتشخيص هيدروجل صمغ الكتر المترابط بينيا بواسطة حمض الستريك

A Dissertation Submitted in Partial Fulfilment of the Requirements of Master degree in Chemistry

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Dedication

I dedicate my dissertation work to the soul of my beloved father. A special feeling of gratitude to my husband, whose words of encouragement and push for tenacity ring in my ears.

I also dedicate this work and give special thanks to my loving mother who has supported me throughout the process. To the most beautiful gift from Allah, my beloved son. To my brothers and my friends who have been affected in every way possible by this quest.

Thank you, my love for you all can never be quantified. Allah bless you.

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Abstract

Hydrogels obtained by chemical crosslinking of *Acacia mellifera*gum with citric acid were prepared and characterized in this study. Different amounts of citric acid, varying heating temperatures and heating times were carried out. The samples were characterized by the gel content, FT-IR, swelling behaviour and the variation of swelling behaviour with time and pH.

The results of gel content have shown that the amount of citric acid, temperature and time, have all influenced the crosslinking efficiency. Sample S7 (0.1g, 160^{o}C and 5 minutes) was found to have the best swelling characteristics (993.5%). The results of the variation of swelling behaviour with timehave shown an increase in water absorption with increasing time up to 36 hours; while the results of the variation of swelling characteristics with pH showed that the swelling ratio decreases in acidic medium and vice versa in alkaline medium. This is because COO^{-} - COO^{-} ion repulsionin acidic medium is screened by H^{+} ions which did not allowed the network to expand, but in alkaline medium the dominant species in the hydrogels are carboxylate anions and the hydrogels were swollen due to intra-ionic repulsion between carboxylate anions. The highest swelling ratio was found at pH 10 (11.52).

Finally, the FTIR results have displayed almost identical absorption bands for the crude gum and the hydrogel samples. However, the intensities of the stretching and the bending vibrations of –OH group were noticeably decreased for the hydrogel sample as result of crosslinking.

مستخلص البحث

في هذه الدراسة تم تحضير وتشخيص عينات الهيدروجل عن طريق الربط الكيميائي البيني لصمغ الكتر وحمض الستريك .تم إستخدام كميات مختلفة من حمض الستريك وكذلك أزمان تسخين ودرجات حرارة تسخين مختلفة.

شخصت العينات عن طريق محتوى الهلام وطيف الأشعة تحت الحمراء وكذلك سلوك الإنتفاخ وتباينه مع الزمن والأس الهيدروجيني . أوضحت نتائج محتوى الهلام أن كمية حمض الستريك ودرجة الحرارة والزمن جميعها تؤثر على كفاءة الربط البيني . وجد أن العينة رقم 7 (0.1 جم من حمض الستريك ، 160درجة مئوية، 5 دقائق) لها أفضل خصائص إنتفاخ (993.5%).أوضحت نتائج تباين سلوك الإنتفاخ مع الزمن زيادة في إمتصاص الماء مع زيادة الزمن حتى 36 ساعة بينما أوضحت نتائج خصائص الإنتفاخ مع الأس الهيدروجيني إنخفاض نسبة الإنتفاخ في الأوساط الحمضية والعكس في الأوساط القاعدية. وهذا لأن التنافر ين أيونات COO^- - COO^- في الأوساط الحمضية يحجب بأيونات الهيدروجين H^+ مما لايسمح بتوسع الهيدروجل، ولكن في الأوساط القاعدية نجد أن المجموعات الغالبة في الهيدروجل هي الأنيونات الكاربوكسيلية وإنتفاخ الهيدروجل ينتج عن التنافر داخل الأيوني بين الأنيونات الكاربوكسيلية. وجدت أعلى نسبة إنتفاخ عند أس هيدروجيني = 10 وهي(11.52).

أخيرا أوضحت نتائج طيف الأشعة تحت الحمراء تطابق شبه تام لقيم إمتصاص عينة الصمغ الخام وعينة الهيدروجل رقم (7). ولكن يوجد انخفاض ملاحظ في شدة ترددات الإستطالة والإنحناء لمجموعة الهيدروكسيل لعينة الهيدروجل نتيجة الربط البيني.

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pH ≡ potential of hydrogen	1
IPN ≡ interpenetrating polymeric hydrogel	3
NMR ≡ nuclear magnetic radiation	5
DSC ≡ differential scanning calorimetry	5
DMA ≡ dynamic mechanical analysis	7
MWs ≡ molecular weights	7
Micro-CT ≡ micro computed tomography	9
OM ≡ optical microscopy	9
SM ≡ stereo microscopy	9
SEM ≡ electron microscopy	9
TEM ≡ tunnelling microscopy	9
AFM ≡ atomic force microscopy	9
UV ≡ ultra violet	12
IR ≡ infrared	12
W/O ≡ water in oil	12
O/W ≡ oil in water	12
HLB ≡ hydrophilic–lipophilic- balance	12
PLGA ≡ poly lactic-co-glycolic acid	13
PEG ≡ poly ethylene glycol	13
PBT ≡ poly butylene terephthalate	13
W/O/W ≡ water in oil in water	14
W/W ≡ weight by weight	14
DMSO ≡ dimethyl sulfoxide	14
PBS ≡ phosphate-buffered-saline	14

BSA ≡ bovine serum albumin	14
PCL ≡ poly capro lactone	14
PNIPAAm ≡ poly(N-isopropylacrylamide)	14
PLLA ≡ poly L-lactic acid	14
PDLA ≡ poly D-lactic acid	14
Tm ≡ melting temperature	14
DNA ≡ deoxyribonucleic acid	15
IgG ≡ immunoglobulin G	15
PEG-Qa ≡ poly ethylene glycol-supported quaternary ammonium salt	17
AUL ≡ absorbency under load	18
GG ≡ guar gum	24
BTCA ≡ butane tetra carboxylic di anhydride	24
SR ≡ swelling ratio	24
Q ≡ swelling ratio	25
FTIR ≡ Fourier transform infrared	26
Q% ≡ swelling percentage	31

CHAPTER ONE

1. Introduction and Previous Studies

1.1 Introduction

Hydrogels are three-dimensional, hydrophilic, polymeric networks proficient in absorbing a great amount of water or biological fluids. Owing to their high water content, porosity and soft consistency, they intently simulate natural living tissue, more so than any other category of synthetic biomaterials. Hydrogels can either be chemically durable or they may eventually disintegrate and dissolve (Peppas, *et al.*, 2000).

Hydrogels are also known as 'reversible' or 'physical' gels if molecular entanglements and/or secondary forces such as ionic, hydrogen bonding or hydrophobic forces play the principal role in forming the linkage. Physical gels are often rescindable and it is achievable to dissolve them by altering the environmental conditions, such as potential of hydrogen (pH) and the ionic strength of solution or temperature. In 'permanent' or 'chemical' gels, the linkage of covalent bonds linking distinct macromolecular chains can be attained by crosslinking polymers in the dry state or in solution (Hoffman, 2012). These gels may be either charged or non-charged dependent on the behaviour of functional groups existing in their structure. The charged hydrogels typically display changes in swelling upon variations in pH and it is well known that they can undergo changes in shape when subjected to an electric field (Rosiak and Yoshii, 1999). Hydrogels can be manufactured practically from any water soluble polymer, including a wide range of chemical compositions and bulk physical properties (Hoare and Kohane, 2008). Additionally, hydrogels can also be formulated in a number of physical forms such as slabs, micro particles, nanoparticles, coatings or films. Accordingly, hydrogels are universally being employed in clinical practices and investigational medicine for a wide variety of applications, counting the tissue engineering and regenerative medicine,

diagnostic, cellular immobilization, separation of biomolecules or cells and barrier materials to control biological adhesions (Hoare and Kohane, 2008). Regardless of these beneficial properties, hydrogels have certain drawbacks. The low tensile strength of many hydrogels regulates their utilization in load-bearing applications and can lead to the untimely dissolution or falow away of the hydrogel from a targeted local site. This drawback may not be so significant in many conventional drug delivery applications (e.g. subcutaneous injection). Perhaps the crucial drawbacks which need a high attention are problems relating to the drug delivery properties of hydrogels. The quantity and homogeneity of drug loading into hydrogels may be restricted especially in the case of hydrophobic drugs. The high water content and large pore sizes of most hydrogels often cause relatively rapid drug release, over a few hours to a few days (Yao *et al.*, 2016).

Easiness of application can also be problematical; although some hydrogels are suitably deformable to be injectable, but many are not, obligating the surgical implantation. Each of these issues substantially confines the practical use of hydrogel-based drug delivery therapies in the treatment centres (Narayanaswamy and Torchilin, 2019). Hydrogels maybe chemically stable or they may eventually disintegrate and dissolve. They are formulated from materials such as gelatine, polysaccharides, cross-linked polyacrylamide polymer, polyelectrolyte complexes and polymers or copolymers originated from methacrylate esters. They are insoluble in water and exist in dry or hydrated sheets or as a hydrated gel in drug delivery systems intended for single use.

These exclusive physical properties of hydrogels have attracted a specific interest in their utilization in drug delivery applications. Their highly porous structure can be effortlessly altered by regulating the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also facilitates loading of drugs into the

gel matrix and successive drug release at a rate dependent on the diffusion coefficient of a small molecule or a macromolecule through the gel network (Hoare and Kohane, 2008). As the polymer cannot dissolve because of the covalent crosslinks, water uptakes far in excess of those feasible with hydrophilic linear polymers can be acquired.

1.2 Classification of hydrogel products

The hydrogel products can be categorized on different bases. These include source, polymeric composition, type of crosslinking and physical appearance. Based on source, hydrogels can be classified into two groups according to their natural or synthetic origins (Zhao *et al.*, 2013). Moreover, hydrogels can be classified according to polymeric composition into homo polymeric hydrogels, copolymeric hydrogels and multi-polymeric hydrogels.Homopolymeric hydrogels are referred to polymer network which are derived from a single species of monomer, which is the basic structural unit comprising of any polymer network (Iizawa et al., 2007). Homo polymers may have cross-linked skeletal structure dependent on the nature of the monomer and polymerization method.Copolymeric hydrogels in the other hand, are consisted of two or more distinct monomer species with at least one hydrophilic component, assembled in a random, block or alternating configuration along the chain of the polymernetwork (Yang et al., 2002). Finally, multi polymeric hydrogels which are also called as interpenetrating polymeric hydrogel (IPN), is an important class of hydrogels, which is made of two independent cross-linked synthetic and/or natural polymer component´s, confined in a network form. In semi-IPN hydrogel, one component is a cross linked polymer and the other component is a non-cross-linked polymer (Maolin et al., 2000). Hydrogels can also be classified based on configuration. This classification of hydrogels relies on their physical structure and chemical composition which can be illustrated as follows:

(i) Amorphous (non-crystalline).

(ii) Semi crystalline: A complex mixture of amorphous and crystalline phases.

(iii) Crystalline.

According to the type of cross-linkinghydrogels can be divided into two groups on the basis of their chemical or physical behaviour of the cross-link junctions. Chemically cross-linked networks have stable junctions, while physical networks have temporaryjunctions that results from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds or hydrophobic interactions (Hacker MC, 2011).

Based on physical appearance, hydrogels are classified as matrix, film or microsphere which is dependent on the procedure of polymerization employed in the formulation process. Hydrogels may be classified into four groups on the basis of presence or absence of electrical charge situated on the cross linked chains:

(i) Non-ionic (neutral).

(ii) Ionic (including anionic or cationic).

(iii) Amphoteric electrolyte (ampholytic) comprising both acidic and basic groups.

(iv) Zwitter ionic (poly betaines) consisting of both anionic and cationic groups in each structural repeating unit (Griffith, 2000).

1.3 Significant properties of hydrogels

1.3.1 Physical and chemical properties

In spite of so much advancement, a basic understanding of gel properties is not yet appropriate for a realistic design of novel gel systems. For such designs, it is imperative to know how solute molecules interact with the gel, particularly how they partition between the gel phase and the surrounding liquid phase. Partitioning majorly relies on two major effects i.e., size exclusion and molecular attraction/repulsion (Griffith, 2000).

Swelling

Hydrogels are cross-linked polymer networks swollen in a liquid medium. The absorbed liquid performs as a selective filter to permit free diffusion of some

solute molecules, while the polymer network acts as a matrix to hold the liquid together. Hydrogels may soak up from 10-20% (an arbitrary lower limit) up to thousands of times of their dry weight in water. The nature of the water in a hydrogel can ascertain the complete permeation of nutrients into and cellular products out of the gel. When a dry hydrogel starts to soak water, the initial water molecules moving into the matrix will hydrate the most polar and hydrophilic groups. As the primary bound water polar groups are hydrated, the hydrogel linkage swells and exposes hydrophobic groups, which also intermingle with water molecules, resulting in hydrophobically bound water or secondary bound water. Primary and secondary bound water are often merged and solely called as total bound water (Griffith, 2000). After the polar and hydrophobic sites have interacted with bound water molecules, the network will suck up additional water, because of osmotic driving force of the network chains towards infinite dilution. This surplus swelling is resisted by the covalent or physical crosslink, producing an elastic network retraction force. Consequently, the hydrogel will attain an equilibrium swelling level. The additional swelling water that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is termed as free water or bulk water and is presumed to fill the space between the network chains and/or the center of larger pores, macropores or voids. Progressively as the network swells, if the network chains or crosslink are degradable, the gel will initiate to disintegrate and dissolve, at a rate depending on its composition (Griffith, 2000). There are various methods employed by investigators to evaluate the relative amounts of free and bound water, as fractions of the total water content. All of them are contentious, since there is proton nuclear magnetic resonance (NMR) evidence that the exchange of water molecules between the so-called bound and free states is very prompt, perhaps as fast as one H_2O molecule every 10^{-9}s. The three main procedures exercised to characterize water in hydrogels are reliant on the use of small molecular probes, differential scanning calorimetry (DSC) and

NMR. When probe molecules are utilized, the labelled probe solution is equilibrated with the hydrogel, and the concentration of the probe molecule in the gel at equilibrium is determined. By presuming that only the free water in the gel can dissolve the probe solute, one can assess the free water content from the amount of the absorbed probe molecule and the known (measured) probe molecule concentration in the external solution. Then the bound water is determined by difference of the measured total water content of the hydrogel and the estimated free water content (Griffith, 2000). The use of DSC is based on the postulation that only the free water may be frozen, so it is believed that the endotherm measured when warming the frozen gel corresponds to the melting of the free water and that value will yield the amount of free water in the hydrogel sample being experimented. Then the bound water is achieved by difference of the measured total water content of the hydrogel test specimen, and the calculated free water content. In another formulation, swelling is the property to absorb water and retain it for a relative long time. It can be estimated by measuring the dry weight and the swollen state weight and calculating either a ponderal variation (water uptake) or a volume of adsorbed solvent (both the quantities are considered as percentages) (Griffith, 2000).

water uptake = swollen weight- dry weigh/dry weight×100

volume of adsorbed solvent = swollen weight- dry weigh/ water density ×100

The assessment of swelling is the crucial assay to be implemented on hydrogel samples, as it can be a measure for many of their properties i.e., crosslinking degree, mechanical properties, degradation rate and so on. For numerous gels, the estimation of swelling and swollen state stability is an easy, cheapest and assured way to differentiate between cross linked gels and the non-cross linked original polymer (Griffith, 2000).

1.3.2 Mechanical properties

The mechanical properties can fluctuate and be altered depending on the nature of the material. It is achievable to acquire a gel with superior stiffness by

increasing the crosslinking degree or lowering it by heating the material. The alterations in mechanical properties relate to a wide variety of variables. For instance, white gelatine shows a clear increase in Young Modulus through crosslinking, silk fibroin has a very high Young Modulus, but after the regeneration it will decrease (Byju and Kulkarni, 2013). These properties (Young modulus, Poisson modulus, storage and loss moduli, tanϴ) can be assessed by a Dynamic Mechanical Analysis (DMA) device or a rheometer, as stated by the thousands of techniques available on the market (Mondal et al., 2007). It's prominent to note that in a hydrogel, the Young Modulus is the outcome of the union between water and gel matrix. If we have to seeds osteoblast cells we will require a more rigid material than if we culture adipocyte, the same logic is valid for the advancement of a heterogeneous prosthetic device, e.g., substitute for the intervertebral disc.

1.3.3 Porosity and permeation

Pores may be created in hydrogels by the process of phase separation in the course of synthesis or they may present as smaller pores within the network. The average pore size, the pore size distribution and the pore interconnections are essential factors of a hydrogel matrix that are often challenging to compute, and are generally included together in the parameter called tortuosity. The effective diffusion path length across a hydrogel film barrier is evaluated by the film thickness times the ratio of the pore volume fraction divided by the tortuosity. These aspects, in turn, are most affected by the composition and crosslink density of the hydrogel polymer network. Labelled molecular probes of a range of molecular weights (MWs) or molecular sizes are used to probe pore sizes in hydrogels (Dong et al., 1994). Pore-size distributions of hydrogels are effectively influenced by three factors:

(i) Concentration of the chemical cross-links of the polymer strands. That concentration is calculated by the initial ratio of cross-linker to monomer.

(ii) Concentration of the physical entanglements of the polymer strands. That concentration is ascertained by the initial concentration of all polymerizable monomers in the aqueous solution (Dong et al., 1994).

iii) Net charge of the polyelectrolyte hydrogel. That charge is determined by the initial concentration of the cationic and/or anionic monomer.

These three factors can be calculated by using the composition of the hydrogel, that is, by the nominal concentrations of monomer and cross-linker.

The porous structure of a hydrogel is also influenced by the properties of the surrounding solution, principally by dissolved ionic solutes (Donnan effects) and by dissolved uncharged solutes which separate unevenly between the gel phase and the solution phase (Osmotic effects). For realistic design of hydrogels, it is advantageous to know the pore-size distribution which depends on the hydrogel characterization. Most procedures used to evaluate the porosity of hydrogels are restricted because they necessitate the pore solvent and/or temperature to be altered, which cause the gel to shrink, swell or require mathematical manipulation and postulation, which may set up unwanted artifacts. Porosity is a morphological characteristic of a material that can be illustrated as the presence of void cavity inside the bulk. It is worthwhile to control the porosity in many devices for a wide range of applications, such as optimal cell migration in hydrogel-based scaffolds or tunable lode/release of macromolecules (Dong et al., 1994).

In a sample, pores can display distinct morphologies, i.e., they can be closed, open as a blind end or interconnected, again divided in cavities and throats. These porosities can had been investigated and reported in papers in the past decades by employing a number of techniques. First of all, porosity can be estimated by theoretic procedures, such as unit cube analysis, mass technique, Archimedes method, liquid displacement method (Dong et al., 1994). These evaluations are generally coupled with optical and electronic microscopy. Other remarkable methods are the mercury porosimetry based on Washburn's

equation, gas pycnometry, gas adsorption (that can be issued by applying different procedures such as small quantity adsorption, monolayer and multilayer adsorption), liquid extrusion porosity, an assay that allows to assess sample's permeability too, capillary flow porosity, again a test based on Washburn's equation. Moreover, an alternate assay is the (Micro-CT), also called micro tomography or micro computed tomography, a relative new imaging technology, plainly expressed as non-destructive high resolution radiography, qualified for qualitative as well as quantitative assays on samples and estimation of their pore interconnections. Between the quantitative assays that can be executed, micro-CT can provide information on average pore size, pore size distribution, pore interconnection, struts/walls thickness and anisotropy/ isotropy of the sample (in the sense of presence/absence of preferential orientation of the pores). It is yet, nowadays, a costly procedure both in term of money and time (Dong et al., 1994). Microscopy techniques can be employed in a number of different assays including the hydrogels. They are engaged in both qualitative and quantitative tests, from simple morphological assessment of material's properties to more complex biocompatibility assays. Concisely, by microscopy techniques, topography and surface morphology can also be evaluated. These techniques can be categorized in many classes, by increasing magnification power: optical microscopy (OM), stereo microscopy (SM), electron microscopy (SEM), tunnelling microscopy (TEM) and atomic force microscopy (AFM). Crosslinking cannot be suitably defined as a property of hydrogels, while it is more of a cause of all the other properties of the material itself. The extent of the crosslinking can differ a lot (Gulrez et al., 2011).

Certainly, the hydrogel's network can be attained by many ways. The processes can be categorized into two big categories: first one is the physical crosslinking that occurs by hydrophobic interactions between chains, ionic interactions between a poly anion and a poly cation (complex coacervation) or ionic

interactions between a poly anion and multivalent cations (ionotropic hydrogel). The second category includes the chemical bound gels. The crosslinking can take place by ultraviolet irradiation, heating or chemical crosslinking via cross linker with a huge collaborative reactions, such as Michael's reaction, Michaelis-Arbuzov reaction, nucleophile addition and so on (Gulrez et al., 2011). By regulating the degree of crosslinking it is feasible to modify the property of the material and optimize it for many kinds of applications, in this way, a wide spectrum of applications starts from the same original polymer (Sung et al., 1999).

1.3.4 Biocompatible properties

It is imperative for the hydrogels to be biocompatible and nontoxic so as to make it pertinent in biomedical field. Most polymers used for this purpose must pass cytotoxicity and in vivo toxicity tests. Biocompatibility is the capability of a material to function with an appropriate host response in a specific application. Biocompatibility analysis consists of two parameters namely bio-safety and biofunctionality(Das, 2013):

i) Biosafety i.e. adequate host response not only systemic but also local as well (i.e. surrounding tissue), the absence of cytotoxicity, mutagenesis, and/or carcinogenesis.

ii) Biofunctionality i.e. the capacity of material to perform the specific task for which it is intended. This explanation is exceptionally applicable in tissue engineering since the nature of tissue construct is to constantly interact with the body through the healing and cellular regeneration process as well as during scaffold degradation (Das, 2013). Moreover, initiators, organic solvents, stabilizers, emulsifiers, unreacted monomers and crosslinkers utilized in polymerization and hydrogel synthesis may be toxic to host cells if they ooze out to tissues or encapsulated cells. To eradicate harmful chemicals from preformed gels, certain purification processes should be implemented such as solvent washing or dialysis (Das, 2013).

1.4 Technologies implemented in the preparation of hydrogels

On the whole, hydrogels can be formulated from either synthetic polymers or natural polymers. The synthetic polymers are hydrophobic in nature and chemically stronger in comparison to natural polymers. Their mechanical strength brings about slow degradation rate, but on the other hand, mechanical strength offers the sturdiness as well. These two opposite properties should be balanced through optimum design. Water soluble linear polymers of both natural and synthetic origin are cross-linked to form hydrogels in various ways (Ahmed, 2015):

(i) Linking polymer chains via chemical reaction.

(ii) Using ionizing radiation

(iii) Physical interactions such as entanglements, electrostatics and crystallite formation.

Generally, the three integral parts of the hydrogels preparation are monomer, initiator and crosslinker. To regulate the heat of polymerization and the final hydrogels properties, diluents can be employed in the formulation, such as water or other aqueous solutions. Hydrogels are normally prepared from polar monomers. According to their starting materials, they can be categorized into natural polymer, synthetic polymer and combinations of the two (Ahmed, 2015):

(a)Bulk polymerization:Many vinyl monomers can possibly be employed for the fabrication of hydrogels. Bulk hydrogels can be obtained with one or more types of monomers. Ordinarily, a small amount of cross-linking agent is supplemented for hydrogel formulation. The polymerization reaction is typically initiated with radiation, ultraviolet or chemical catalysts. The selection of an appropriate initiator relies upon the type of monomers and solvents being used. The polymerized hydrogel may be yielded in a wide range of forms counting the films and membranes, rods, particles and emulsions. Bulk

polymerization is the straightforward technique, which includes only monomer and monomer soluble

initiators. The viscosity of reaction enhances significantly with the conversion which generates the heat during polymerization. These problems can be prevented by regulating the reaction. The bulk polymerization of monomers to make a homogeneous hydrogel yields a glassy, transparent polymer matrix which is very tough. When placed in water, the glassy matrix swells to become soft and flexible (Ahmed, 2015).

(b) Solution polymerization/cross-linking: In solution copolymerization/cross-linking reactions, the ionic or neutral monomers are blended with the multifunctional cross-linking agent. The polymerization is instigated thermally by Ultra violet (UV) and Infrared (IR) radiation or by a redoxinitiator system. The prepared hydrogels require washing with distilled water to eliminate the monomers, oligomers, cross-linking agent, the initiator, the soluble and extractable polymer and other impurities. Phase separation takes place and the heterogeneous hydrogel is formed when the quantity of water during polymerization is more than the water content in proportion to the equilibrium swelling. Usual solvents utilized for solution polymerization of hydrogels include water, ethanol, water–ethanol mixtures and benzyl alcohol (Ahmed, 2015).

(c)Suspension polymerization or inverse-suspension polymerization: Dispersion polymerization is a worthwhile technique since the products are acquired as powder or microspheres (beads) and thus, grinding is not needed. Since water-in-oil (W/O) process is selected in preference to the more common oil-in-water (O/W), the polymerization is denoted as "inverse suspension". In this method, the monomers and initiator are distributed in the hydrocarbon phase as a homogenous mixture. The viscosity of the monomer solution, agitation speed, rotor design and dispersant type chiefly regulates the resin particle size and shape (Ogata et al., 2006). Several comprehensive discussions on hetero-phase

polymerizations have been published previously (Hunkeler, 1992). The dispersion is thermodynamically unsteady and necessitates both continuous agitation and addition of a low hydrophilic–lipophilic- balance (HLB) suspending agent.

1.5 Physically cross-linked gels

Increased interest in physically cross-linked hydrogels in current era is due to the absence of cross-linkers used for synthesis. Following are the different methods to synthesize physically cross-linked hydrogels.

1.5.1 By hydrogen bonding

Poly acrylic acid and poly methacrylic acid make complexes with poly ethylene glycol. These complexes have hydrogen bonding between the oxygen of the poly ethylene glycol and the carboxylic group of poly acrylic acid/poly methacrylic acid. Hydrogen bonding is found not only between poly methacrylic acid and poly ethylene glycol, but also in poly (meth acrylic acid-g-ethylene glycol) (Mathur et al., 1998). Hydrogen bonds are formed only if the protonation of carboxylic acid groups occurs which shows pH dependent swelling of the gels.

1.5.2 From amphiphilic graft and block polymers

Amphiphilic graft and block polymers have ability to self-assemble in aqueous media to form hydrogels and polymeric micelles, in which the polymers hydrophobic parts are self-assembled. Hydrophilic di block polymers produce lamellar phases, micelles etc(Förster and Antonietti, 1998). Multi block polymers may contain hydrophobic chains having hydrophilic grafts or a water-soluble polymer backbone to which hydrophobic segments are attached.

(i)Polymers of PLGA and PEG: The biodegradability of poly lactic-co-glycolic acid and biocompatibility of poly ethylene glycol motivated many researchers to create block polymers composed of these components, and for the purpose of

drug delivery, to construct hydrogels from them. Release of drug may be motivated by degradation phenomena and passive diffusion.

(ii)Polymers of PBT and PEG:Feijen and co-workers studied multi block polymers of PEG and a hydrophobic compound, poly (butylene terephthalate) (PBT) (Bezemer et al., 2000). Melt poly condensation of butane diol, PEG and dimethyl terephthalate was used to synthesize such biocompatible polymers. For drug loading, the solutions of polymers were made in a hexafluoro isopropanol and chloroform (1:6) mixture and then W/O emulsion was prepared having the protein 'lysozyme' in water phase. The abovementioned emulsions were cast, to produce a film, or microspheres were synthesized using water-in-oil-in-water (W/O/W) emulsification method.

(iii)Hydrophobized polysaccharides:By hydrophobic modification, physically cross-linked hydrogels can be made from polysaccharides such as dextran, chitosan, carboxymethylcurdlan and pullulan. Monodisperse hydrogel nanoparticles with high water constituent (typically 80% weight by weight (W/W) were produced from pullulan bearing cholesterol upon dialyzing a solution from dimethyl sulfoxide (DMSO) against phosphate-buffered-saline (PBS) buffer. Insulin, bovine serum albumin (BSA) and a-chymotrypsin have been loaded and a hydrophobic anticancer drug adriamycin was loaded by simply mixing Adriamycin and pullulan suspension (Akiyoshi, 1996).

(iv)Other graft and block polymers:Examples are: multi-block polymers of PEG-poly(c-benzyl Lglutamate), PEG-polyisobutylene , poly(2-ethyl-2-oxazoline)-polycaprolactone (PCL) which behaved like PEG–PCL hydrogels and thermosensitive hydrogels from PEG– poly(N-isopropylacrylamide)(PNIPAAm) (Lin and Cheng, 2001).

1.5.3 Cross-linking by crystallization

(i)Crystallization in homopolymer systems:When aqueous solutions of polyvinyl alcohol (a natural hydrophilic polymer) are stored at room temperature, a gel is created, but, with a little mechanical strength. A tough and

greatly elastic gel is produced when polyvinyl alcohol aqueous solution subjected to a freeze–thaw process (Yokoyama et al., 1986).

(ii)By stereocomplex formation:The homopolymers of L-lactic acid and D-lactic acid, respectively, are PLLA and PDLA (semi-crystalline substances). High molecular weight PLLA or PDLA, of either stereoisomer, has 170^{o}C Tm (melting temperature). In mixtures of high molecular weight PLLA and PDLA, a 230^{o}C Tm is observed, which is attributed to the stereocomplex formation.

1.5.4 Cross-linking by ionic interactions

Alginate may be cross-linked via calcium ions. Cross-linking is done at physiological pH and at room temperature. Alginate gels may be used as a matrix for protein release and for the living cells encapsulation (Goosen et al., 1985).

1.5.5 Cross-linking by protein interaction

(i) Genetically engineered proteins use Tirrell and Cappello pioneered a new field in materials chemistry i.e. protein engineering. The advantage of protein engineering is that the peptide sequence and therefore its physical and chemical properties may be controlled by rational design of the genetic code in synthetic deoxyribonucleic acid (DNA) sequences. In addition to natural amino acids, synthetic amino acids may also be used (Yoshikawa et al., 1994).

Cappello and colleagues synthesized polymers of sequential block containing silk-like and elastin-like blocks repetition, in which silk-like segments (insoluble) are associated with the shape of aligned hydrogen bonded beta sheets through genetic engineering (Cappello et al., 1998). These biocompatible ProLastins are solutions in water which may be mixed with drugs and due to crystallization of the silk like domains undergo an irreversible sol to gel transition (with time) in physiological conditions.

(ii)By antigen–antibody interactions:In the presence of an additional cross-linking agent i.e. antibody, rabbit immunoglobulin G (IgG) was grafted to chemically cross-linked polyacrylamide (Miyata et al., 1999). In the presence of free antigen, the hydrogel showed slight swelling due to the polymer bound antigen replacement, resulting in the antibodies release along with a decrease in the cross-linking density.

1.6 Chemically cross-linked gels

Increased interest in chemically cross-linked hydrogels in current era is due to the good mechanical strength of chemically cross-linked hydrogels. Following are the different methods to synthesize chemically cross-linked hydrogels.

1.6.1 Cross-linking by complementary groups chemical reaction

Hydrophilic polymers have certain hydrophilic groups namely NH_2, COOH, OH which may be used for the hydrogels development. The reactions such as an amine-carboxylic acid or an isocyanate-OH/NH_2 reaction or Schiff base formation, may be used to recognize covalent linkages between polymer chains.

(i)Cross-linking with aldehydes:Hydrophilic polymers having –OH groups e.g. polyvinyl alcohol may be cross-linked through glutaraldehyde (Zu et al., 2012). To establish cross-linking, tight conditions are applied (low pH, methanol added as a quencher, high temperature). Alternatively, polymers having amine-groups may be crosslinked by the use of same cross-linker under mild conditions in which Schiff bases are formed. It was specially designed for the cross-linked protein synthesis, for example gelatine, albumin and the amine containing polysaccharides (Jameela and Jayakrishnan, 1995).

(ii)By addition reactions:Bis or higher functional cross-linkers may be used to react with functional groups of hydrophilic polymers through addition reactions. Polysaccharides may be cross-linked by means of 1,6 hexamethylenediisocyanate , divinylsulfone , or 1,6-hexanedibromide (Coviello et al., 1999).

(iii)By condensation reactions:Polyesters and polyamides can be synthesized through condensationreactions among the –OH groups or $–NH_2$ with –COOH or derivatives, respectively. These reactions may be used for the hydrogel synthesis. A highly efficient reagent for cross-linking hydrophilic polymers having amide groups is N,N-(3-dimethylaminopropyl)-N-ethyl carbodiimide. To restrain any side reaction and to have a superior command on the hydrogel cross-linking density, N-hydroxysuccinimide was added during the reaction. Hydrogel was planned as a tool for antibacterial proteins release and was loaded into a prosthetic valve of Dacron. After synthesis, hydrogels were loaded with lysozyme and in vivo and in vitro lysozyme release was studied over a 2days period. For loading capacity improvement, anionic polysaccharide (chondroitin sulfate) was also loaded into hydrogel (Kuijpers et al., 2000).

1.6.2 Cross-linking by high energy radiation

High energy radiation e.g. gamma rays and electron beam may be used to polymerize unsaturated substances (Amin et al., 2012).

1.6.3 Cross-linking by free radical polymerization

Chemically cross-linked hydrogels may be produced by free radical polymerization of polymerizable group derivatized hydrophilic polymers, besides free radical polymerization of vinyl monomers mixtures. To synthesize gels via this route, natural, synthetic and semi-synthetic hydrophilic polymers were applied. Using enzymes as catalyst, methacrylic groups have been introduced into the mono and disaccharides, which may be used for the hydrogel synthesis. Moreover, by UV polymerization, the hydrogel synthesis may be done, the planned structures may be synthesized and photo-reversible systems are also possible, which means that after exposing to UV light, preformed hydrogels degrade and so a drug is released (Andreopoulos et al., 1998).

1.6.4 Cross-linking using enzymes

An attractive method was devised to create PEG-based gels via using an enzyme. They functionalized glutaminyl groups with tetrahydroxy PEG poly ethylene glycol-supported quaternary ammonium salt (PEG-Qa). To aqueous solutions of poly (lysine-co-phenylalanine) and PEG-Qa, addition of transglutaminase resulted in the formation of PEG networks. Transglutaminase catalyzed reaction between the c-carboxamide group of the PEG-Qa and the e-amine group of lysine resulted in the formation of an amide bond (Sperinde and Griffith, 1997).

1.7 Grafting to a support

Usually, hydrogels formulated by bulk polymerization have characteristic weak structure. To enhance the mechanical properties of a hydrogel, it can be attached on surface which is coated onto a sturdy support. This method that comprises the generation of free radicals onto a stronger support surface and then polymerizing monomers directly onto it, as a result of which, a chain of monomers are covalently bonded to the support. An assortment of polymeric supports have been employed for the synthesis of hydrogel by grafting techniques (Talaat et al., 2008). Ionizing high energy radiation, like gamma rays and electron beams, has been employed as an initiator to formulate the hydrogels of unsaturated compounds. The irradiation of aqueous polymer solution leads to the formation of radicals on the polymer chains. Also, radiolysis of water molecules brings about the formation of hydroxyl radicals, which also attack the polymer chains, leading to the formation of macro-radicals. Recombination of the macroradicals on different chains results in the formation of covalent bonds, so ultimately, a cross-linked structure is obtained. Examples of polymers crosslinked by the radiation method are poly (vinyl alcohol), poly (ethylene glycol), and poly (acrylic acid). The foremost benefit of the radiation initiation over the chemical initiation is the fabrication of relatively pure and initiator-free hydrogels (Talaat et al., 2008).

1.8 Hydrogel technical features

The purposeful characteristics of an ideal hydrogel material can be enumerated as below (Talaat et al., 2008):

i) The highest absorption capacity (maximum equilibrium swelling) in saline.

ii) Preferred rate of absorption (preferred particle size and porosity) depending on the application necessity.

iii) The topmost absorbency under load (AUL).

iv) The lowermost soluble content and residual monomer.

v) The lowest price.

vi) The utmost robustness and steadiness in the swelling environment as well as during the storage.

vii) The highest biodegradability without formation of toxic species following the degradation.

viii) pH-neutrality after swelling in water.

ix) Colorlessness, odorlessness and absolutely nontoxic.

x) Photo stability.

xi) Re-wetting competency (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it; dependent on the application requisite (e.g., in agricultural or hygienic applications).

Practically, it is unachievable that a hydrogel sample would concurrently fulfill all the above described required features. Actually, the synthetic components for acquiring the maximum level of some of these features will lead to inadequacy of the rest. Thus, in practice the production reaction variables must be adjusted such that an applicable balance between the properties can be attained. For instance, a hygienic products of hydrogels must retain the topmost absorption rate, the lowest re-wetting and the lowermost residual monomer and also the hydrogels employed in drug delivery must be porous and be responsive to either pH or temperature

(Talaat et al., 2008).

1.9 Applications of hydrogels

Hydrogels are widely used in various areas of tissue engineering, proteomic, bioseparations, electrophoresis and chromatography (as separation materials), foods, medicines, in diapers (as absorbents), in water purification (as filters), in controlled drug release (Wellner et al., 1998).

Some of the applications of hydrogels are:

1.9.1 Domestic applications

It contains diapers (In, 1986), cosmetics (An et al., 2014), perfume delivery (N.A. Peppas, 1997), water beeds for plants …etc. Water adsorption nature of hydrogels is used in diaper that they hold water, also used in creams and perfume.

1.9.2 Environmental applications

Different types of hydrogels are used for waste water treatment that can hold a lot of microorganism in their matrix. Currently, the biggest environmental problem is the loss of oil in seas or in other water sources. Many researchers and authors attempted to develop different types of hydrogels to retain water with oil-pollutant molecules (Zunan et al., 1995).

1.9.3 Bacteria culture

Bacteria can be cultured inside the matrix of hydrogels. Agar is main substrate for bacterial culture in biotechnological applications (Heginbothom et al., 1990).

1.9.4 Biosensor

Biosensors can be prepared by hydrogels, act as supports for immobilization of enzymes. The hydrogel Poly carbamoyl sulphonate is used for immobilization of the D-fructose dehydrogenase enzyme (Swarbrick and Zhong, 2013).

1.9.5 Sealant and adhesive

Hydrogels can adhere to various materials as plastics due to hydrophobic interactions and it can be used as sealant for vessels containing corrosive acids.

1.9.6 Contact lenses

It is the most widely used application of this polymer. For soft contact lenses, poly(2-hydroxyethyl methacrylate)-based hydrogels are used due to their extensive property (Phan et al., 2014).

1.9.7 Electronics

In electronics, hydrogels are used as matrixes which consider maximum tunability and precision of capacitors with hydrogel dielectrics for example, potassium poly(acrylate), poly(vinyl alcohol), poly(ethylene oxide), and gelatinetc(Choudhury et al., 2009).

1.9.8 Biomedical application

Hydrogels are widely used in biomedical applications such as, in Immunotherapy, Vaccine, Plastic surgery, Wound healing, Electrophoresis, Proteomic, Tissue engineering (Bone regeneration, Cardiac, Dental), Drug delivery, Wound dressing and so on.

1.10 *Acacia*

The genus *Acacia* is the second largest in the family *Leguminosae,* with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world, with the largest concentration of species in Australia (ca 957 species), and also with high numbers in the Americas (ca 185 species), Africa (144 species) and Asia (89 species). The genus has a long and convoluted history, with many genera being split or added to core *Acacia* over the last 250 years. The type of *Acacia* is generally considered to be *A. scorpioides*(L.) W. F. Wight (=*A. nilotica*(L.) Del., a species of tropical Africa and western Asia which is now naturalised in some other parts of the world (Orchard and Maslin, 2003). In the last 30 years considerable attention has been given to the phylogeny and the generic and infrageneric classification of *Acacia*, with the proposal by Pedley (1986) to subdivide *Acacia* into three genera having significant impact. Molecular and phylogenetic studies conducted since 1986

are lending considerable support for the fragmentation of *Acacia*, but not necessarily along the exact lines proposed by Pedley(Maslin et al., 2003).

Such actions to divide *Acacia*, while desirable from the point of view of presenting a more defensible phylogeny, will have considerable nomenclatural, economic and pragmatic repercussions. More than 75% of the species in this very large genus may require a new name or new combination. Many of these species are of considerable economic, ecological or iconic importance. It can be anticipated that name changes on this scale will elicit commentary in the scientific, popular and semi-popular press, and it is inevitable that some of this comment will question the rationality of the taxonomic process. Therefore, it is important that the taxonomic data be sufficiently robust to support such changes, and if they are, then ways to minimise the nomenclatural impact of the change should be sought (Maslin et al., 2003).

1.11 *Acacia mellifera*

Family: *Fabaceae (Leguminosae), Mimosiodeae*

Synonym: *Acacia detinens Burch.; Acacia senegal ssp. Mellifera (Vahl)*Roberty; *Mimosa melliferaVahl*.

Vernacular/Common names: Black thorn, hook thorn, wait-a-bit thorn (Engl.); kikwata (Swahili); bilel, lanen, laner (Somali); swartaak, swarthook (Afrikaans); kedad, kitir, kitr (Arabic).

*Acacia mellifera*has two separate distribution areas in Africa: Its largest distribution is in the Sahelian east Africa extending into the Arabic peninsula; another distribution area is dry southern Africa in Namibia and Botswana.

The species commonly occurs in dry savannah sites in western, eastern and southern Africa with mean annual rainfall 250-650 mm; sometimes extending up to 1500 masl on rocky hillsides. It thrives on a variety of soil types from sandy to heavy clay including *vertisols.* It can grow in mixed stands with e.g., *Commiphora, Salvadora, Balanitesaegyptiaca*and other *Acacias*. It is a strong

regenerator both by seed and root suckers and sometimes form large stands of 2-3 m high, dense, impenetrable thickets. Absence of grass fires tends to promote regeneration by *Acacia mellifera*(Schmidt and Mbora, 2008).

A low shrubby *Acacia* with a natural range in north and east Africa. Widespread in dry scrub with trees and in deciduous bushland. In Eritrea, it is common in the eastern and western lowlands, e.g. around Tokombia, Shambuko, Aderde, Hashishai, Mahmimet, Sheib, Ghedged, Ghahtelai and on the Buri Peninsula, 0-1,000 m.

*Acacia mellifera*is a multipurpose tree species of the dry and harsh environment. Wood is small and only applicable for small construction purposes e.g., native huts and fuel. Foliage and pods are eagerly browsed by camels and goats. Flowers are a good source of honey for bees. The plant has alleged medical properties as the bark is used for stomach-ache, sterility, pneumonia, malaria and syphilis. The plant has a shallow and aggressive root system, limiting its use in farms with crops (Schmidt and Mbora, 2008).

*Acacia mellifera*is a low branched tree or shrub, rarely more than 5-6 m. The bark is smooth and light brown, turning blackish with age. Thorns in pairs, small, black, sharp and hooked. Compound leaves with two pinnae, each with a single pair of leaflets. Leaflets elliptic 0.6-2 cm long and 0.6-1.2 cm wide, glabrous. Flowerbuds reddish, flowers white or cream coloured in elongated spikes, up to 3.5 cm long. Individual flowers small with ½-1½ mm pedicel, 1mm calyx and 2½-3½ mm corolla.

The fruit is a dehiscent pod containing 2-3-seeds. It is papery and reticulate, straw-coloured, flat, elongated, 2½-5½ cm long, 18-23 mm wide with pointed apex.

Seeds are hard-coated. The form is circular to lenticular, 7-9 mm long, 5-6 mm wide, compressed, 1.5-1.8 mm thick. Light brown-olive green, smooth. Pleurogram horse-shoe shaped. Funicle red brown. 1,000 seed weight, 42-47g; there are approximately 20000 seeds/kg. Reproduction may start after 3 years.

Flowering takes place in the dry season, usually before leaf flush, - in bimodal climates two flowering events may take place. Development from flowering to fruit takes about 3-4 months (Schmidt and Mbora, 2008).

Figure 1.1:*Acacia mellifera*tree (www.calphotos.berkeley.edu)

Figure 1.2: leaves of *Acacia mellifera*(www.commons.wikimedia.org)

1.12 Previous studies

Innovative hydrogels were obtained by chemical crosslinking of deacylatedGellan gum have been characterized in terms of water uptake and other properties. For the chemical cross-linked samples the water uptake is strongly dependent both on hydrogel and solution compositions. As expected, water uptake is inversely proportional to the crosslinking degree of the sample. When the hydrogels are swollen in water, their behaviour is quite similar to that of the super-absorbent materials (Matricardi et al., 2009).

In another study, guar gum hydrogels (GG) were prepared via esterification with 1,2,3,4-butanetetracarboxylicdianhydride (BTCA). The white coloured granular GG hydrogels absorbed water readily and formed transparent hydrogels upon soaking. The water absorbency of the GG hydrogels gradually increased over time and reached equilibrium within 12 h. Thus, the swelling ratio (SR) after 24 h of swelling was defined as the equilibrium SR. The SR of all hydrogels increased markedly with increased pH values because of the presence of carboxylate anions or carboxylic acids in the hydrogel structure. In acidic medium, the carboxyl groups of GG hydrogels were protonated yielding carboxylic acids, and the ionic repulsion disappeared, resulting in the shrinkage of the hydrogels. In neutral and alkaline media, the dominant species in the GG hydrogels were carboxylate anions, and the hydrogels were swollen due to intra-ionic repulsion between carboxylate anions (Kono et al., 2014).

Xanthan based hydrogels were prepared by crosslinking the Xanthan chains by esterification reaction at 165°C in the presence of citric acid. Higher crosslinking density was obtained using citric acid, as evidencedby its lower swelling degree. Hydrogels swellingdegree increased at high pH values, due to electrostatic repulsion and ester linkages rupture. Xanthan crosslinking by citric acid took place at 165°C by a condensation process, which involved dehydration and ester linkages formation between them. Xanthan–citric acid

hydrogels present more homogeneous porous structure and practically no Nano fibrils, indicating that

citric acid is an efficient cross-linking agent. These morphological features corroborated with the gel content and Q values.

In the hydrogel a crosslinking density is large, the free volume (bulk water) is small, decreasing the diffusion rate. A significant amount of water in a hydrogel is bulk water, which does not differ significantly from bulk water out of the gel. However, bulk water is not the only water structure present in hydrogels.

Swelling of hydrogels can be influenced by the medium pH, especially when there are ionizable groups present in their structure. Xanthan–citric acid hydrogels have acidic groups from citric acid, which can be completely deprotonated at pH > 6. Moreover, under basic medium, the unreacted hydroxyl groups are also deprotonated and ester bonds undertake alkaline hydrolysis. Thus, electrostatic repulsion and crosslink disruption favor the increase in swelling ratio (Q/Q0) observed for hydrogels in basic medium.

The hydrogels are more sensitive to pH variation, because they have more crosslinkings to be hydrolyzed. At pH 2 and pH 6.5 there is no significant effect of pH on the Q/Q0 values (Bueno et al., 2013).

(Rithe et al., 2014) were synthesized hydrogel samples using guar gum and chitosan with glutaraldehyde as the cross-linking agent, protonated with 98% conc. sulphuric acid. Concentration of chitosan was varied as 0, 12.5, 25, 37.5 and 50% (w/w) in guar gum, while that of glutaraldehyde was varied as 0, 1.5, 3 and 6% (w/w) of the total quantity of guar gum and chitosan. Prepared hydrogels were characterized for equilibrium water absorbency, swelling ability in acidic (pH = 3) and basic (pH = 11) pH distilled water and Fourier transform infrared spectroscopy.

It was determined that equilibrium water absorbency decreased with increased concentration of chitosan and glutaraldehyde, but they still had water swelling to

be classified as hydrogel. This, trend was observed regardless of addition of glutaraldehyde. However, equilibrium water absorbency decreased with increased concentration of glutaraldehyde in any of the guar gum/chitosan blend compositions. Prepared hydrogels maintained high water swelling in acidic pH distilled water as compared to basic pH distilled water. Nearly all samples got dissolved in acidic pH (pH = 3) distilled water, except the one containing highest quantity of chitosan; whereas, the water absorbency as well the number of samples getting dissolved were less for basic pH (pH = 11) distilled water. This shows that acidity has appreciable effect on the swelling behavior compared to basicity. In Fourier transform infrared (FTIR) spectra, broad band at 3600cm^{-1} was attributed to the stretching vibration of the -OH groups present due to water molecules. Sharp absorption peak at 1630cm^{-1} was due to the bending vibration of –OH groups present on guar gum and chitosan. These peaks disappeared or decreased in intensity on reacting with protonated glutaraldehyde giving rise to new peak at about 1555cm^{-1} belonging to the ester linkage. Whereas, the peaks at 2880, 1650, 1326 and 1080 were due to the stretching vibrations of aliphatic

C-H, amine (-NH2), amide II and amide III respectively. These are the characteristics peaks for chitosan. Intensity of this peaks also decreased on addition of glutaraldehyde (cross-linking agent), which on protonation with conc. sulphuric underwent reaction with them forming a cross-linked structure. Absorption peak at 1630 cm-1 was assigned to the C=O stretching of the amide linkage. Thus, it can be confirmed that guar gum and chitosan reacted with glutaraldehyde (on its protonation with sulphuric acid) to form a cross-linked structure (Rithe et al., 2014).

1.13 Objective

The objective of this study:

1. To optimize the chemical reaction conditions for preparing *Acacia mellifera*gum based hydrogels cross-linked with citric acid.
2. To characterize the hydrogel sample which produced under the optimum conditions.

CHAPTER TWO

2. Materials and methods

2.1 Sample collection and pretreatments

Authenticated *Acacia mellifera* gum sample was kindly supplied by Professor Mohammed Elmubarak. The gum sample was cleaned, crushed into a powder and stored in plastic bags.

2.2 Chemicals

Citric acid (99.5%), Hydrochloric acid (35%-38%), Sodium carbonate (99.5%) and Sodium hydroxide (97%). All chemicals were obtained from SD Fine Chem Limited (India) and were used as received.

2.3 Instrumentations

Analytical balance, oven, pH-meter and FT-IR.

2.4 Preparation of hydrogels samples

Acacia mellifera gum based hydrogel samples were prepared using varying amounts of citric acid as a crosslinker and varying heating times and temperatures. The detailed method of preparation of each sample is given in Table (2.1).

In a typical experiment, 5g of the *A. mellifera* gum were weighed and dissolved in 50 ml distilled water in a beaker. The desire amount of citric acid was weighed, dissolved in 5ml distilled water and mixed well with the gum solution in the beaker. The resultant solution was casted in a glass Petri-dish and allowed to dry at ambient conditions. The dried sample was transferred into an oven at 140^{o}C, heated for 5 minutes, removed out and left to cool. Then the sample was soaked in 100 ml of distilled water for overnight to get rid off the unreacted materials, washed several times with distilled water and left to dry. The dried hydrogel was weighed and the gel content of each sample was determined according to equation (2.1). Exactly typical steps were followed to prepare the

hydrogels of all the remaining samples Table (2.1). The percentage of the gel content was determined by the following equation:

Gel content (%) = $\frac{W1 \times 100}{Wo}$ ..... **(2.1)**

Where: W_1 represents the dry gel weight and W_o is the original weight of the gum sample.

Table (2.1): The detailed preparation conditions of the hydrogels samples.

Sample	**Wt of CA (g)**	**Temp (°C)**	**Time (minutes)**	**Sample**	**Wt of CA (g)**	**Temp (°c)**	**Time (minutes)**
S1	0.1	140	5	S15	0.17	150	15
S2	0.1	140	10	S16	0.17	160	5
S3	0.1	140	15	S17	0.17	160	10
S4	0.1	150	5	S18	0.17	160	15
S5	0.1	150	10	S19	0.5	140	5
S6	0.1	150	15	S20	0.5	140	10
S7	0.1	160	5	S21	0.5	140	15
S8	0.1	160	10	S22	0.5	150	5
S9	0.1	160	15	S23	0.5	150	10
S10	0.17	140	5	S24	0.5	150	15
S11	0.17	140	10	S25	0.5	160	5
S12	0.17	140	15	S26	0.5	160	10
S13	0.17	150	5	S27	0.5	160	15
S14	0.17	150	10	-	-	-	-

*CA = Citric acid, Wt = Weight

During the washing process of the produced hydrogels, the samples having characteristics swelling in the aqueous phase were noticed and selected.

Nineteen samples of the produced hydrogels have been selected (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S12, S13, S14, S15, S16, S17, S18, S19 and S20) based on the degree of swelling (samples that showed observable ability to absorb water).

2.5 Determination of hydrogels swelling behaviour

0.75 gram of each of the nineteen selected samples (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S12, S13, S14, S15, S16, S17, S18, S19 and S20) of hydrogels were immersed in a certain amount of distilled water (in pre-weighed containers) and left at same conditions for 24 hours. The unabsorbed water was removed by decantation and the contents were weighed again. The sample which has the highest swelling property was chosen (S7). The swelling behaviour property was expressed in term of swelling percentage (Q%).

$$\mathbf{Q\ \%} = \frac{(W_1 - W_0) \times 100}{W_0} \quad \textbf{(2.2)}$$

Where: Q is the swelling percentage, W_1 is the swelling gel weight and W_o is the dry gel weight.

2.6 Determination of the swelling ratio at different time intervals

5ml of water were added to 0.5g dried-best swelled sample (S7) and the sample was left for 12, 24, 36, 48, 60 and 72 hours. Then the swelling gel at each time intervals sample was weighed and the swelling ratio was determined by the following equation:

$$\mathbf{Q} = \frac{(W_1 - W_0)}{W_0} \quad \textbf{(2.3)}$$

Where: Q is the swelling ratio, W_1 is the swelling gel weight and W_o is the dried gel weight.

2.7 Determination of the swelling ratio in solutions of different pH

5ml of each an aqueous solution having pH of 2, 4, 6, 7, 8 and 10 (pH was adjusted using aqueous solutions of hydrochloric acid and sodium hydroxide with the aid of pH-meter) were added to 0.5g dried-best swelled sample (S7)

series and the samples was left for 12 hours. Then the swelling gel for each sample was weighed. The swelling ratio was determined by the equation 2.3.

2.8 FT-IR analysis of *Acacia mellifera* gum and the hydrogel sample

The infrared spectra of *A. mellifera* gum and citric acid cross-linked *A. mellifera* gum samples were recorded using a Shimadzu FTIR-8400S spectrophotometer in the range between 400 and 4000 cm^{-1}. Few milligrams of each sample were mixed thoroughly with few milligrams of spectroscopic grade KBr, pressed into pellets and the FT-IR spectrum of each sample was obtained.

CHAPTER THREE

3. Results and discussion

3.1 The gel content of the samples

Table (3.1) shows the gel content of the gum samples cross-linked with citric acid, the variation of the gel content with the amount of citric acid as well as with heating temperatures (140, 150 and 160^{o}C) and times (5, 10, and 15 minutes) is represented in Figure 3.1 to 3.3 respectively. As can be seen from the Table, sample S14 (0.17 citric acid /150^{o}C /10 minutes) has the highest gel content (82.07%) whereas the lowest gel content (38.41%) was obtained for sample S9 (0.1 citric acid/160^{o}C/15 minutes).

At high concentrations of citric acid, the hydrogel films formed were rigid, with reduced ability to absorb water. This rigidity and poor ability to absorb water might be due to the increase in crosslinking density, which in turn may have led to the reduction in the mobility of polymer chains and reduced the free volume of the hydrogel network. The heating temperature was varied from 140° to 160°. Crosslinking of the gum obviously influenced by the processing conditions as it is varied accordingly. It worth noting that there is no specific trend when these parameters are considered in many cases (e.g., S16, S17 and S18 as good examples). It is noted that at high temperatures, 160^{o}C, the gel content is decreased. The heating time was varied from 5 to 15 min. The 5 min heating time was found to be sufficient to form the citric acid crosslinked hydrogel film. However, higher heating times lead to formation of a film with insufficient swelling. This could be exhibited due to interaction between –OH groups of the gum and dehydrated citric acid leading to the formation of strong crosslinking.

Table (3.1): The gel content % of the samples

Sample number	Gel Content%	Sample number	Gel Content%
1	66.11	15	70.84
2	68.89	16	56.57
3	63.41	17	50.56
4	56.00	18	53.38
5	68.53	19	59.23
6	58.49	20	64.09
7	57.60	21	67.73
8	51.86	22	68.47
9	38.41	23	72.02
10	74.69	24	69.80
11	69.18	25	70.18
12	63.40	26	69.46
13	68.03	27	72.91
14	82.07	-	-

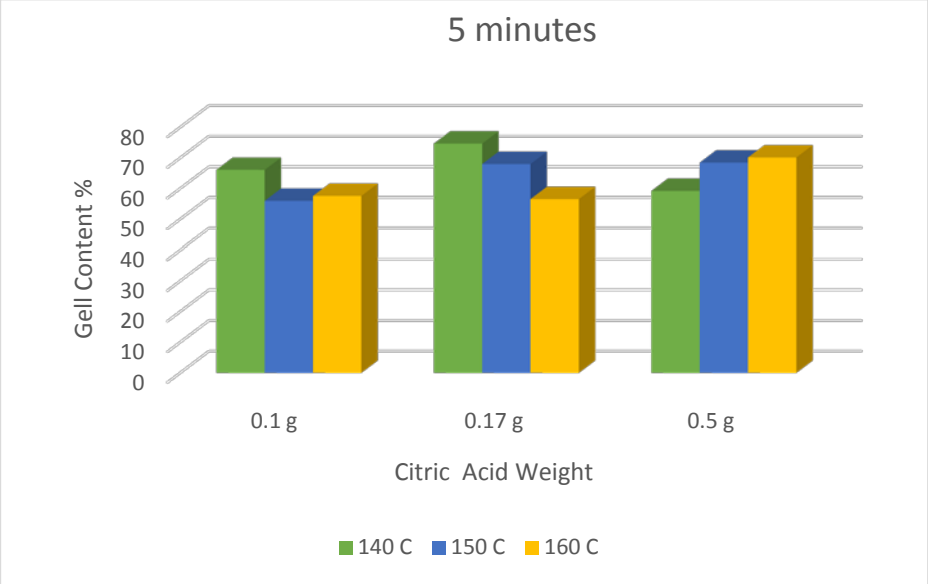

Figure (3.1): The gel content% of the samples at different temperatures and different amounts of citric acid at 5minutes.

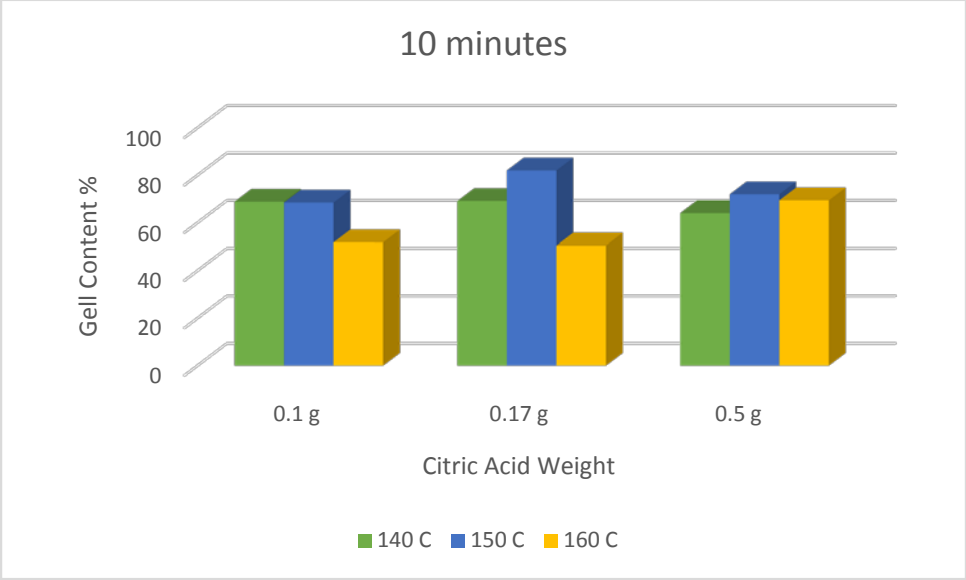

Figure (3.2): The gel content% of the samples at different temperatures and different amounts of citric acid at 10minutes.

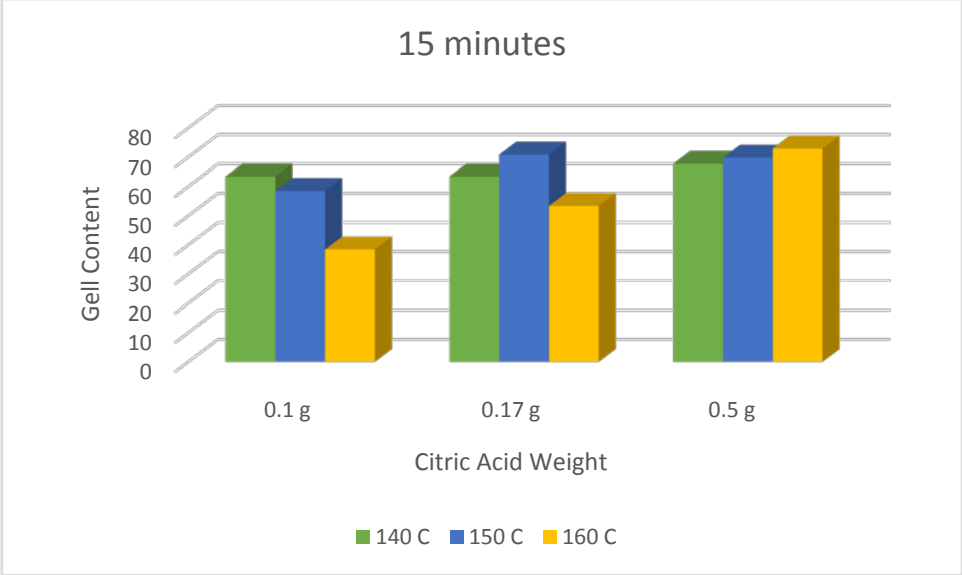

Figure (3.3): The gel content% of the samples at different temperatures and different amounts of citric acid at 15minutes

3.2 Swelling behaviour studies of hydrogels

The nineteen selected (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S12, S13, S14, S15, S16, S17, S18, S19 and S20) samples of hydrogels were examined for their ability to absorb water in 24 hours and the results are shown in Table (3.2). Sample S7 was found to have the highest absorption percentage (993.5%). The results show that the lowest cross-linked hydrogel produced the highest degree of swelling (S7), in this sample the amount of citric acid is 0.1g compared to 0.17g and 0.5g of citric acid in the other samples. In contrast, the samples prepared with higher concentrations of citric acid produced lower swelling degree like samples (S19 and S20). For the chemical cross-linked samples the water uptake is strongly dependent both on hydrogel and solution compositions. When the hydrogels are swollen in water, the behaviour is quite similar to that of the super-absorbent materials (Ambrosio *et al.*, 2008).

(Matricardi et al., 2009) have studied the water uptake property of hydrogels which obtained by physical and chemical crosslinking of deacylatedGellan gum.

Their results showed that water uptake is inversely proportional to the crosslinking degree of the sample (Matricardi et al., 2009).

Table (3.2): The water content % of hydrogels samples in 24 hours.

Hydrogels samples	**Water content%**	**Hydrogels samples**	**Water content%**
S1	826.3	S12	750.3
S2	731.0	S13	589.3
S3	717.5	S14	503.6
S4	814.5	S15	534.5
S5	848.9	S16	723.1
S6	780.7	S17	587.4
S7	993.5	S18	645.2
S8	778.3	S19	436.2
S9	709.2	S20	454.7
S10	826.7	-	-

3.3 Swelling behaviour studies of hydrogel of sample S7

The water absorption capacity of sample S7 was studied alone at different periods of time and the results were represented in Table (3.3) and Figure (3.4). The results show that an increase in water absorption with an increase in time up to 36 hours there after an increasing becomes not significant.

(Kono et al., 2014) have studied the swelling behaviour of hydrogels which prepared from guar gum via esterification with 1,2,3,4-butanetetracarboxylicdianhydride in an aqueous solution. Their results showed a gradually increased over time and reached equilibrium within 12 hours (Kono et al., 2014).

Table (3.3): The swelling ratios of sample S7 at different periods of time.

Time (hours)	Swelling gel weight (g)	Drying gel weight (g)	Swelling ratio
12	3.466	0.505	5.86
24	5.999	0.511	10.74
36	6.156	0.503	11.24
48	6.47	0.506	11.79
60	6.649	0.512	11.99
72	6.720	0.510	12.18

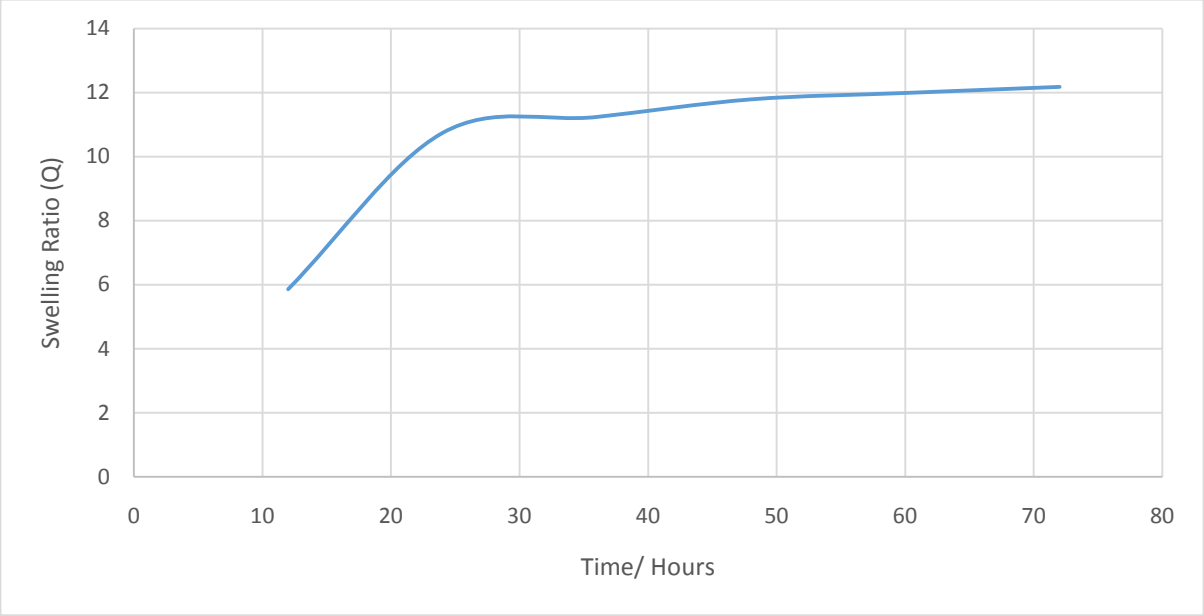

Figure (3.4): Variation of swelling ratio of sample S7 with time.

3.4 Effect of pH on the swelling behaviour of sample S7

The swelling behaviour of S7 was examined at different pH values (2, 4, 6, 7, 8, and 10). The results which displayed in Table (3.4) and Figure (3.5) show that the highest swelling ratio was obtained at pH 10 (11.52), while the lowest one was obtained at pH 2 (2.72). This displays that the ratio decreases in acidic medium and vice versa. In acidic medium, COO^- - COO^- ion repulsion is

screened by H^+ions which did not allowed the network to expand. Further increase in acidic character resulted in increased concentration of H^+ ions and increased screening effect and decreased COO^- - COO^- ion repulsion and hence a decreased swelling ratio. In neutral and alkaline media, the dominant species in the hydrogels were carboxylate anions, and the hydrogels were swollen due to intra-ionic repulsion between carboxylate anions.

(Kono et al., 2014) have studied the swelling behaviour of hydrogels which prepared from guar gum via esterification with 1,2,3,4-butanetetracarboxylicdianhydride in solutions at different pH values. Their results showed that the swelling ratio of hydrogels increased markedly with increased pH values because of the presence of carboxylate anions or carboxylic acids in the hydrogel structure (Kono et al., 2014).

Table (3.4): The swelling ratios of sample S7 in solutions of different pH

pH values	Swelling gel weight (g)	Drying gel weight (g)	Swelling ratio
2	1.869	0.502	2.72
4	2.887	0.508	4.68
6	3.762	0.511	6.36
7	4.158	0.512	7.12
8	4.781	0.507	8.43
10	6.386	0.510	11.52

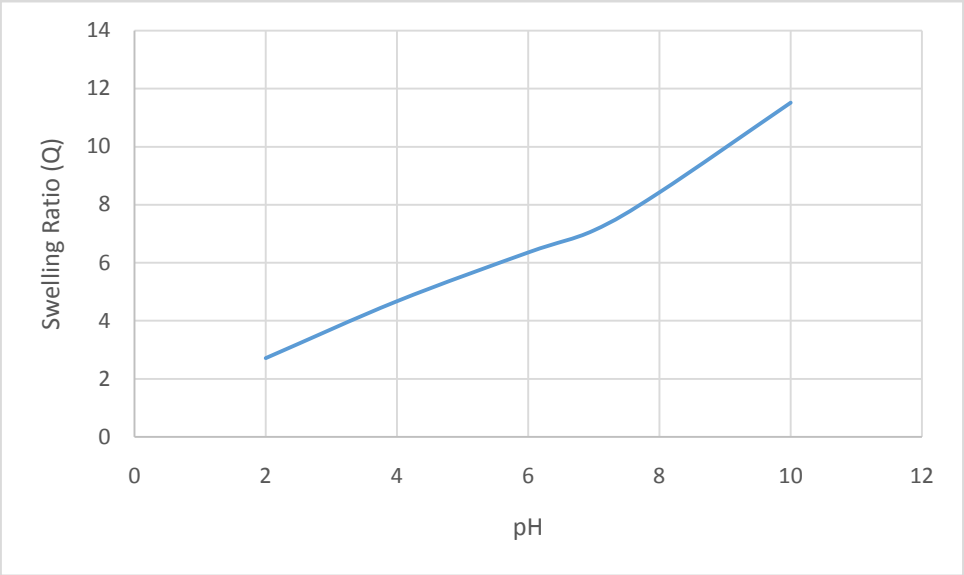

Figure (3.5): Variation of swelling ratio of sample S7 at different pH values.

3.5 FT-IR analysis

The FT-IR spectra of *A. mellifera* gum and citric acid cross-linked *A. mellifera* gum sample (S7) are given in Figures (3.6) and (3.7) respectively. From Figure (3.7) it can be noticed that the strong broad peak at 3438cm^{-1} represents the stretching vibration of –OH group. The sharp peak located at 2929cm^{-1} displays the stretching vibration of –CH group (sp^3 hybridized system). The absorption band appeared in 1425cm^{-1} is due to bending vibration of –CH group. The stretching vibration of C-O group has given bands at 1244cm^{-1} and 1149cm^{-1}. The FT-IR spectrum of the gum sample has shown almost identical absorption bands to the FT-IR spectrum of the hydrogel sample although there is a noticeable decrease in the stretching and bending vibrations of –OH group in case of hydrogel sample. This confirms that the ester linkage (cross-linking) was formed between the gum and citric acid in the hydrogel sample.

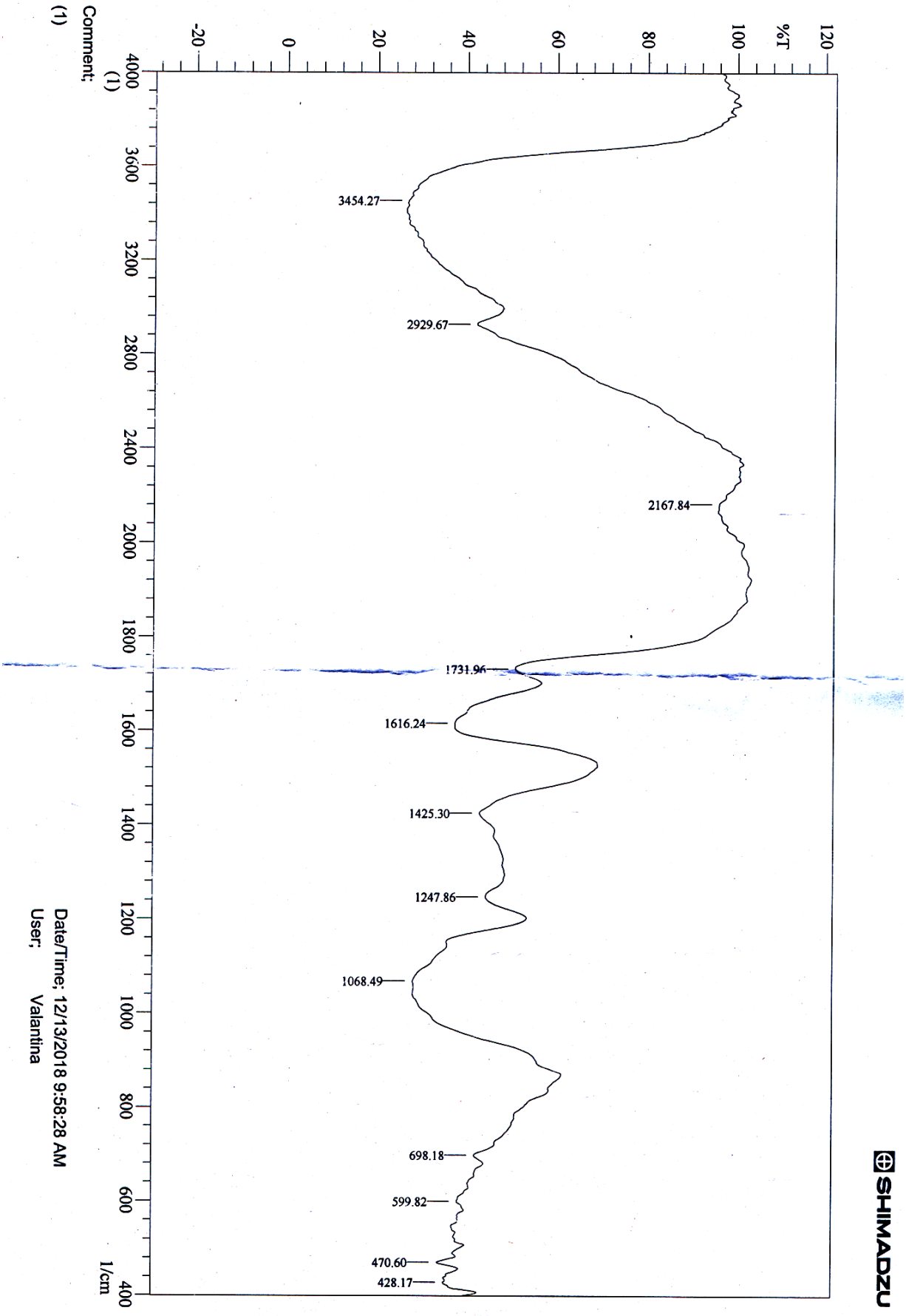

Figure (3.6): The FT-IR spectrum of *Acacia mellifera* gum

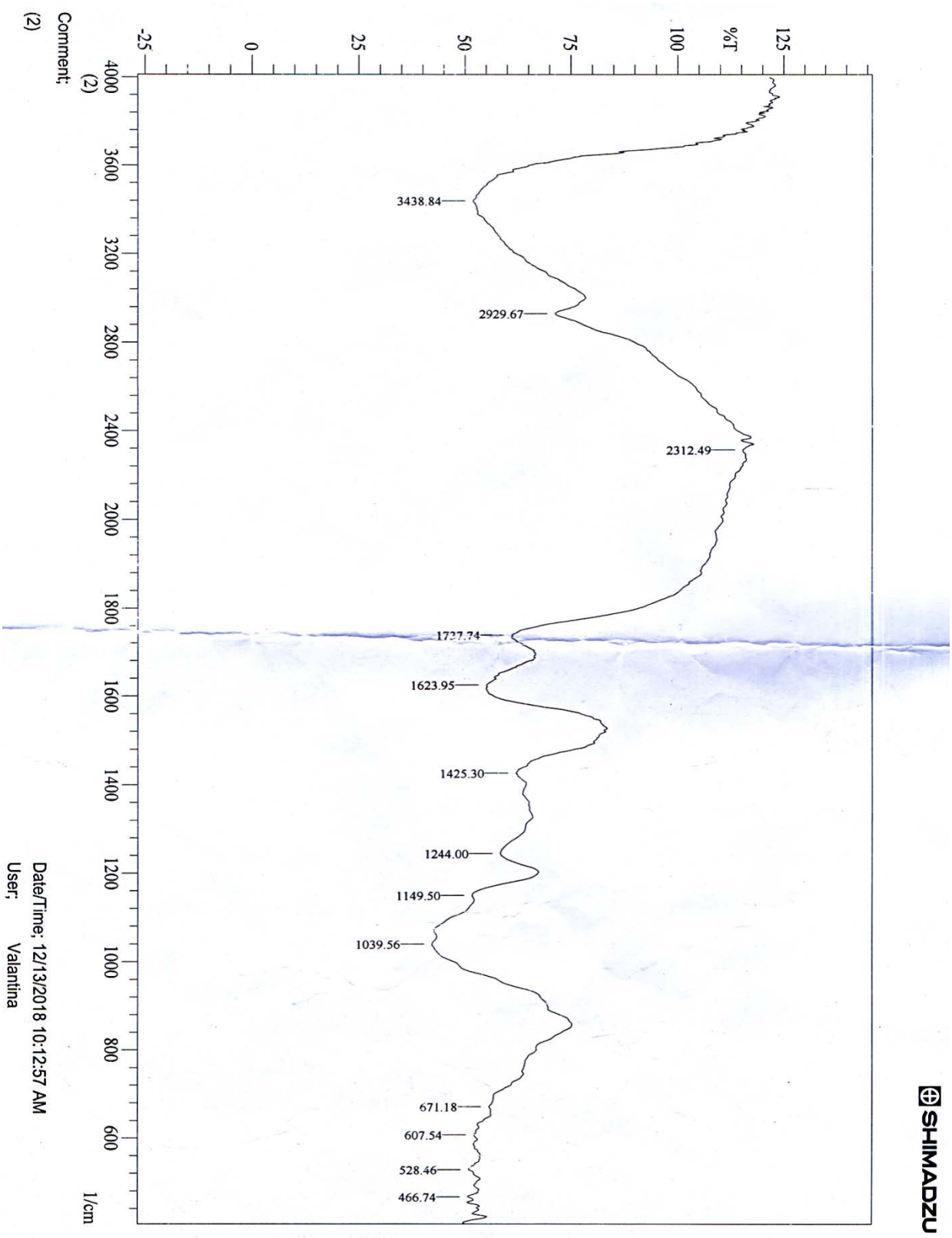

Figure (3.7): The FT-IR spectrum of citric acid crosslinked*Acacia mellifera* gum

CHAPTER FOUR

4. Conclusion and Recommendations

4.1 Conclusion

Hydrogels were prepared via esterification reaction using citric acid as a crosslinking agent. The crosslinking reaction was examined by FTIR analysis and the concentration of citric acid together with heating temperatures and times were found to influence the crosslinking efficiency observably. An optimal degree of swelling (993.5%) for practical applications was achieved using low citric acid concentrations.

4.2 Recommendations

I recommend to characterize the hydrogel samples by developed techniques like differential scanning calorimetry, x ray diffraction and thermogravimetric analysis to investigate the citric acid reactivity. Another technique can be used is tensiometry, which is a very precise and sensitive technique, was applied to study swelling rates and diffusion mechanisms of water.

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