



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**Sudan University of Science and Technology**

**College of Graduate Studies**

**preparation and Characterization of Succinic Acid-cross-linked *Acacia seyal* (Talha) Gum Hydrogel**

تحضير وتشخيص الهلام المائي لصمغ الطلح المربوط بينيا بحمض السكسينيك

**A Thesis Submitted in Partial Fulfillment for the Requirements of a Master Degree in Chemistry**

**By:**

**Esraa Hassan Alamin Alhossiun (BSc, honors Chemistry)**

**Supervisor:**

**Dr. Essa Esmail Mohammad Ahmad**

**August, 2019**

الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة (الآية 32)

## **Dedication**

To my parent, husband, sisters and daughters.

## **Acknowledgment**

First and foremost, I would like to thank Allah Almighty who has given me patience and blessing to complete this research.

With heartfelt appreciation, I express my sincere thanks to my supervisor Dr. Essa Esmail Mohammad for his guidance, support and cooperation.

I send my gratitude to my friends and colleagues for their moral support.

## Abstract

The aim of this study was to prepare and characterize a hydrogel samples based on *Acacia seyal var seyal* gum cross-linked with succinic acid. The samples were prepared using varying concentrations of the succinic acid (crosslinker), heating temperatures (130, 140 and 150 °C) and heating times (5, 15 and 20 minutes).

The gel content, FTIR, water uptake and the variation of the swelling behavior of the hydrogel samples at different PH were studied. The results of the gel content demonstrated that the main factors which influence the crosslinking efficiency the heating time and temperature. The highest gel content was found for sample S17 (86.4%) whereas the lowest was obtained for S13 (13. 57%). S25 was found to have the highest water holding capacity (1555%). Minor changes between the hydrogel and the crude gum were noticed in the FTIR, in the range 1700 to 1750  $\text{cm}^{-1}$  which is attributed to the characteristic carboxyl group stretching vibration derived from succinic acid cross-links.

The variation of swelling behavior with pH demonstrated high dependency of the hydrogel water holding capacity, on PH, sample S25 showed the highest water holding capacity at pH7.

## مستخلص البحث

هدفت هذه الدراسة لتحضير وتشخيص عينات من هيدروجل صمغ الطلح المرتبط بينيا بحمض السكسينيك. حضرت العينات بإستخدام تراكيز مختلفة من حمض السكسينيك ودرجات حرارية مختلفة وكذلك أزمان تسخين مختلفة.

تمت دراسة كل من محتوى الجل وطيف الأشعة تحت الحمراء وإمتصاص الماء والتغير في سلوك الانتفاخ عند أس هيدروجيني متباين. بينت نتائج محتوى الجل أن العوامل الأساسية التي تؤثر على كفاءة الربط البيني هي درجة الحرارة وزمن التسخين.

أعلى قيمه للجل تم الحصول عليها للعينة 17 (86.4%) بينما كانت أدني قيمه للعينه رقم 13 (13.57%) بالإضافة إلى ذلك فقد وجد أن العينة 25 لها أفضل قدره على امتصاص الماء والتي وصلت الي (1555%).

وقد لوحظ تغيرات طفيفة بين الهيدروجل والصمغ الخام بإستخدام مطيافية الأشعه تحت الحمراء في المدى (1750-1700) والذي يعزي لي تردد الاستطالة المميز لمجموعة الكاربوكسيل الموجودة في الروابط البينية.

دراسة التغير في قدره على امتصاص الماء مع تغيير الأس الهيدروجيني قد أوضح إعتقادا كبيرا للامتصاص على الأس الهيدروجيني وأوضحت النتائج أن العينة 25 لها أعلى قدره على امتصاص الماء عند الأس الهيدروجيني 7.

## List of Abbreviation

PVA	Poly vinyl acrylamide
EGDMA	Ethylene glycol di-meth acrylic acid
MAA	Meth acrylic acid
TMED	Tetra methyl ethylene diamine
PAA	Poly acrylic acid
PMA	Poly meth acrylic acid
PEG	Poly ethylene glycol
AUL	Absorbency under load
SIF	Simulated Intestinal Fluid
SGF	Simulated Gastric Fluid
XNT	xanthan hydrogels
XCA	xanthan–citric acid hydrogels
BTCA	Butane tetra carboxylic di anhydride
GG	Gellan gum
CMCNA	Sodium carboxyl methyl cellulose
HEC	Hydroxyl ethyl cellulose
CA	Citric acid
DSC	Differential scanning calorimeter
FTIR	Fourier transform infrared spectroscopy
JECFA	Joint FAO/WHO expert committee on food additives

## Table of Contents

Title	Pages
الآية	I
Dedication	Ii
Acknowledgement	Iii
Abstract	Iv
مستخلص البحث	V
List of abbreviations	Vi
Table of Contents	Vii
List of Tables	Ix
List of Figures	X
<b>Chapter one</b>	
<b>Introduction and literature review</b>	
1.1 Hydrogels	1
1.2 Crosslinking of Hydrogels	1
1.2.1 Chemically Crosslinked	2
1.2.2 Physically Crosslinked	3
1.3 Methods of crosslinking of hydrogels	4
1.4 Hydrogel technical features	5
1.5 Applications of Hydrogels	6
1.5.1 Drug delivery: Controlled release	6
1.5.2 Drug delivery: Targeted release	6
1.5.3 Tissue engineering	6
1.5.4 Wound dressing	6
1.5.5 Water purification	6



1.6 previous studies	7
1.7 <i>Acacia seyal</i> var., <i>seyal</i> (talha)	9
1.7.1 Classification and characteristics of <i>Acacia seyal</i> var., <i>seyal</i> (talha)	9
1.7.2 Physical and chemical properties of <i>Acacia seyal</i> gum	9
1.7.3 Chemical composition and structure of gums	11
1.7.4 Uses of <i>Acacia</i> Gums	12
1.8 objectives	13
<b>Chapter two</b>	
<b>Materials and methods</b>	
2.1 Sample collection and pretreatments	14
2.2. Preparation of the <i>A. seyal</i> gum based hydrogels	14
2.2.1 Determination of the gel fraction	15
2.2.2 Determination of the swelling percentages	16
2.3 Preparation of a hydrogel from sample 25 (S25)	16
2.4 Swelling of the hydrogel of S25 at different pH	16
2.5 Water holding capacity	17
2.6 FT IR Study	17
<b>Chapter three</b>	
<b>Results and discussion</b>	
3.1 Gel content	18
3.2 FT IR analysis	20
3.3 Swelling characteristics of hydrogel samples	23
3.4 effect of PH on swelling ratio	25
3.5 Conclusions	26

References	27
------------	----

### List of Tables

Table	Pages
<b>Table 1.1:</b> The physicochemical properties of <i>Acacia seyal</i> species	10
<b>Table 1.2:</b> Cationic composition of <i>Acacia seyal</i>	11
<b>Table 2.1:</b> Preparation of the <i>A. seyal</i> gum based hydrogels at succinic acid concentration of 0.3 g	14
<b>Table 2.2:</b> Preparation of the <i>A. seyal</i> gum based hydrogels at succinic acid concentration of 0.15 g	15
<b>Table 2.3:</b> Preparation of the <i>A. seyal</i> gum based hydrogels at succinic acid concentration of 0.10 g	15
<b>Table 3.1:</b> Gel content of <i>Acacia seyal</i> var. <i>seyal</i> gum cross-linked with succinic acid	18
<b>Table 3.2:</b> The swelling ratio of the hydrogel samples	23

## List of Figures

Figure	Page
<b>Figure 1.1:</b> Cross-linking in polymer	1
<b>Figure 1.2:</b> The structure of gum talha	12
<b>Figure 3.1:</b> Variation of gel content (%) with succinic acid concentration at 5 minutes	19
<b>Figure 3.2:</b> Variation of gel content (%) with succinic acid concentration at 15 minutes	20
<b>Figure 3.3:</b> Variation of gel content (%) with succinic acid concentration at 20 minutes	21
<b>Figure 3.4:</b> FTIR spectrum of the crude <i>Acacia seyal var. seyal</i> gum	22
<b>Figure 3.5:</b> FTIR spectrum of <i>Acacia seyal var. seyal</i> gum based hydrogel (S25)	23
<b>Figure 3.6:</b> variation of water absorption capacity with time intervals	24
<b>Figure 3.7:</b> variation of swelling with pH	25

# Chapter One

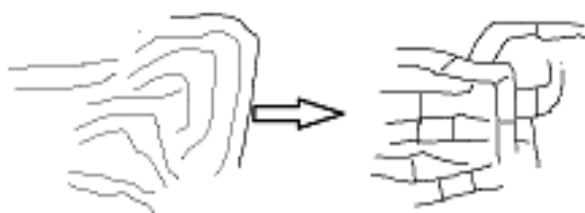
## Introduction and literature review

### 1.1 Hydrogels

Hydrogels are hydrophilic natured three-dimensional networks, held together by chemical or physical bonds. Water absorbed by hydrogel is not released under ordinary pressure. Hydrophilic groups such as hydroxyl (OH) and carboxyl (COOH) on the polymer chains absorb and store water. They are, widely, uses in various applications especially in controlled release for drug delivery. If sufficient interstitial space exists within the network, water molecules can become trapped and immobilized, filling the available free volume [laporte, 1997, Mack, *et al.*, 1988].

### 1.2 Crosslinking of Hydrogels

Hydrogels are synthesized by different polymerization methods using both chemical and physical crosslinking routes. Both natural polymers such as proteins or synthetic polymers like PVA with a high affinity for water can be crosslinked. Different crosslinking methods can be implemented for the design of a hydrogel. The following chemical and physical methods reflect the synthesis of hydrogels. PVA cross-linked membranes were synthesized using glutaraldehyde as cross-linking agent [Alemzadeh and Vossoughi, 2002].



**Figure (1.1): Cross-linking in polymer**

### **1.2.1 Chemical Crosslinking**

Hydrogels are synthesized by chain growth polymerization, addition and condensation polymerization and gamma and electron beam polymerization. Chain-growth polymerization includes free radical polymerization, controlled free radical polymerization, anionic and cationic polymerization. It is done in three steps, initiation, propagation, and termination. After initiation, a free radical active site is generated which adds monomers in a chain link-like fashion. Poly (*N*-isopropyl acrylamide) hydrogel are synthesized by typical free radical polymerization PVA based hydrogels are prepared by free radical copolymerization. PVA has been cross-linked chemically with monomer MAA in aqueous medium using EGDMA as cross-linking agent and benzoyl peroxide as reaction initiator. Monomer MAA is used to impart pH sensitive characteristics. This pH sensitive chemically cross-linked PVA hydrogels is a promising delivery system for colonic delivery of 5-fluorouracil in colorectal cancer [Minhas *et al.*, 2013].

Controlled living radical polymerizations offer the benefits of longer growing chain life compared to free radical polymerizations for macromolecular engineering. Anionic and Cationic polymerization methods suffer from extreme sensitivity toward aqueous environments and therefore, are not used in the synthesis of polymeric hydrogels.

Addition and condensation polymerization involves stepwise addition of Poly functional crosslinking agents with monomer functional groups. Water soluble monomers can be converted into hydrogels using crosslinking agents such as TMED. Polymer chains may be crosslinked in the presence of water to form a hydrogel. Water occupies voids in the network, giving the hydrogel its

characteristic surface properties. Polyurethanes, polyesters, or nylon polymers are most commonly synthesized for hydrogel applications [Hennink, 2002].

Gamma and electron beam polymerization involves high energy electromagnetic irradiation as crosslinker. These high energy radiations can crosslink water-soluble monomer or polymer chain ends without the addition of a crosslinker. During irradiation, using a gamma or electron beam, aqueous solutions of monomers are polymerized to form a hydrogel. Gamma and electron beam polymerizations also involves the initiation, propagation, and termination steps as in the free radical polymerization. Hydroxyl radicals are formed and initiate free radical polymerization among the vinyl monomers which propagate in a rapid chain addition fashion [Hennink, 2002].

The hydrogel is, finally, formed once the network reaches the critical gelation point. This process has an advantage over other crosslinking methods since it can be performed at room temperature and in physiological pH without using toxic and hard to remove crosslinking agents such as potassium persulfate [Hennink, 2002].

### **1.2.2 Physical Crosslinking**

Hydrogels are synthesized by ionic interaction, crystallization, stereo complex formation, hydrophobized polysaccharides, protein interaction and hydrogen bond. In ionic interactions, hydrogels can be crosslinked under mild conditions, at room temperature and physiological pH. This process of cross-linking does not require presence of ionic groups in the polymer. The use of metallic ions yields stronger hydrogel.

For stereo complex formation, a hydrogel is formed through crosslinking that is formed between lactic acid oligomers of opposite chirality. Hydrophobic interactions result in polymer swelling and water uptake that forms the hydrogel. Polysaccharides such as chitosan, dextran, pullulan and carboxy methyl curdlan are reported in literature for the preparation of physically crosslinked hydrogels by

hydrophobic modification [Hennink, 2002]. Protein interaction involves block copolymers that contains repetition of silk-like and elastin-like blocks called ProLastins. ProLastins are fluid solutions in water and can undergo a transformation from solution to gel under physiological conditions because of the crystallization of the silk-like domains [Hennink, 2002].

PAA and PMA form complexes with PEG from the hydrogen bonds between the oxygen of the PEG and carboxylic group of PMA [Hennink, 2002].

This interaction allows for the complex to absorb liquids and swell at low pH which transforms the system into a gel. Crystallization involves freezing-thawing process and creates a strong and highly elastic gel.

PVA hydrogels can be formed by physical crosslinking through repeated freezing/thawing methods, or chemically crosslinked with glutaraldehyde or epichlorohydrin.

### **1.3 Methods of hydrogels crosslinking**

Depending upon the nature of the polymer, different techniques may be used to cause cross linking. Cross linking may occur through polymerization of monomers having functional groups more than two or by covalent bonding between polymeric chain through irradiation, subpar vulcanization or chemical reaction by adding different chemicals in conjunction with heating and sometimes, pressure, in all cases, the chemical structure of polymer is altered through the cross linking process. Cross-linking by irradiation is done by using high energy ionization, irradiation, like electron beam, gamma or X-ray. Gamma irradiation is, usually, most economical at lower doses (80 KGY and below) and for large, high density parts. Electron beam is, commonly, used for small parts, particularly low density parts and in liner product processed reel to reel (e.g., cable, wire, tubing) [Nagasawa *et al.*, 2004].

## 1.4 Hydrogel functional features

The functional features of an ideal hydrogel material can be listed as follows:

- The highest absorption capacity (maximum equilibrium swelling) in saline.
- Desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
- The highest absorbency under load (AUL).
- The lowest soluble content and residual monomer.
- The lowest price.
- The highest durability and stability in the swelling environment and during storage.
- The highest biodegradability without formation of toxic species following degradation.
- pH-neutrality after swelling in water.
- Colorlessness, odorlessness, and absolute non-toxicity.
- Photo stability.
- Re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it; depending on the application requirement (e.g., in agricultural or hygienic applications).

Obviously, it is impossible that a hydrogel sample would simultaneously, fulfill, all the above mentioned, required, features. In fact, the synthetic components for achieving the maximum level of some of these features will lead to inefficiency of the rest. Therefore, in practice, the production reaction variables must be optimized such that an appropriate balance between the properties is achieved. For example, a hygienic products of hydrogels must possess the highest absorption rate, the lowest re-wetting, and the lowest residual monomer, hydrogels used in drug delivery must be porous and their response to either pH or temperature [Zohuriaan-Mehr, 2006].



## **1.5 Applications of Hydrogels**

**1.5.1. Drug delivery: Controlled release.** Incorporation of a second component into the hydrogel system at different dosages will change the structure and morphology of the network, subsequently controlling their diffusion properties [Chang, *et al.*, 2010].

**1.5.2 Drug delivery: Targeted release.** It would be most desirable if drug release could be administered in a manner that, precisely, matches the physiological needs at specific sites [Bhattacharai, *et al.*, 2010].

### **1.5.3 Tissue engineering**

Tissue engineering is a most recent application of hydrogels, in which they are used as scaffolds to mimic many roles of extracellular matrixes and to engineer new tissues, i.e., these scaffolds provide space and nutrients for new tissue formation, and, potentially, control the structure and function of the engineered tissue in situ or in vitro [Marler, *et al.*, 1998].

Currently, hydrogel scaffolds are at or near clinical uses in engineering many tissues in the body including cartilage, bone, muscle, skin, fat, artery, ligament, tendon, liver, bladder, and neurons [Lee and Mooney, 2001].

### **1.5.4. Wound dressing**

An ideal wound gel dressing should allow gaseous exchange, maintain the proper moisture level and constant temperature of the wound bed, remove excess exudates, protect the wound against bacteria and contamination, accelerate healing, and alleviate pain [Jayakumar, *et al.*, 2011].

### **1.5.5. Water purification**

Many liquid- and solid-phase extraction techniques have been used to remove toxic pollutants from water such as chemical precipitation, flocculation, flotation, coagulation, membrane filtration, ion exchange, adsorption, and electrochemical treatment. Hydrogels have attracted special attention for water purification through

adsorption, due to their high absorbency, porous structure, rich functional groups, and relatively low crystallinity [Fu, *et al.*, 2011].

## 1.6 Previous studies

Different hydrogel samples of gellan gum were prepared using physical and chemical methods crosslinking methods. Water uptake, rheological properties and compressibility were studied. The release from the various gels of loaded model molecules of different steric hindrance was also investigated and the trend of the release profiles has been related to the structures proposed for the physical and the chemical hydrogel. The results of water uptake at different medium (H<sub>2</sub>O, NaCl 0.9 % w/v, SIF, SGF) have shown that the physical hydrogels have a similar behaviour in the different media, meaning that their different composition does not affect their water uptake: only the physical hydrogel [Gellan 2%] show slightly lower values of the parameters S and wR, especially in SGF (Simulated Gastric Fluid, HCl 0.1 M, pH = 1) solution. This behaviour is probably due to the low pH environmental conditions. For the chemical crosslinked samples the water uptake is strongly dependent both on hydrogel and solution compositions [Matricardi *et al.*, 2009].

Bueno *et al.*, [2013] prepared different hydrogels of xanthan gum which were cross linked using physical and chemical methods, by esterification reaction at 165 °C either in the absence or in the presence of citric acid. Higher crosslinking density was obtained using citric acid, as evidenced by its lower swelling degree. Hydrogels swelling degree increased at high pH values, due to electrostatic repulsion and ester linkages rupture.

The results in the presence of citric acid led to network with higher crosslinking density, XNT and XCA hydrogels presented high acid resistance, but under alkaline conditions swelling degree increased, especially for XCA samples, due to ester linkages hydrolysis. In the absence of citric acid, esterification between

xanthan acid groups (pyruvyl or acetyl) and OH groups can also occur upon heating the xanthan film.

Different hydrogels of guar gum which were cross linked using esterification with BTCA were prepared by Kono, *et al.*, [2014]. Detailed spectroscopic analysis using FTIR and solid-state NMR revealed that an increase in the BTCA feed amount in the preparation mixture led to an increased degree of cross linking, which affected the swelling behavior and rheological properties of the hydrogels.

The results of hydrogels exhibited good adsorption of BSA and lysozyme, and the protein-adsorbed GG hydrogels released the proteins in buffer solutions. The adsorbed proteins exhibited favorable drug release profiles; after an initial burst, a slow and steady release occurred. Based on these unique properties, it was concluded that the GG hydrogels have potential abilities as drug delivery carriers for protein-based drugs, although the biological safety of the GG hydrogels will need to be verified.

Similarly, Demitri *et al.*, [2008] have prepared different hydrogels of cellulose which were cross linked using two cellulose derivatives, CMCNa and HEC. CA, a crosslinking agent able to overcome toxicity and costs associated with other cross linking reagents was selected in a heat activated reaction DSC, FTIR, and swelling measurements were performed during the reaction progress to investigate the CA reactivity with each of the polymers. Also, CMCNa/HEC polymer mixtures (3/1 w/w) cross linked with CA were investigated and compared with previous results.

The results mixture of two polysaccharides was addressed. An esterification mechanism based on an anhydride intermediate formation was proposed to explain the reaction of cellulose polymers with CA. The crosslinking reaction for CMCNa/HEC system was observed either by DSC or by FTIR analysis. The evolution of the different crosslinking reactions was monitored by means of FTIR spectra collected at different reaction times using an excess of CA.

## **1.7 *Acacia seyal* var., *seyal* (talha)**

### **1.7.1 Classification and characteristics of *Acacia seyal* var., *seyal* (talha)**

Family:	<i>Leguminous</i>
Sub Family:	<i>Mimosoideae</i>
Genus:	<i>Acacia</i>
Species:	<i>seyal</i> var. <i>fistula</i> , <i>seyal</i> var. <i>seyal</i>
Vercular surnames:	<i>talha</i>

*Acacia seyal* trees are up to 17 m tall, with a flat top crown. It has a distinctive smooth powdery bark, from white to greenish yellow or orange red, with a green layer beneath. In some population both red and yellow barked trees can be found. There are two varieties, differing primarily in whether or not pseudo-galls ("ant galls") develop and in bark colour. In *A. 95 seyal* var. *seyal*, there are no pseudo-galls and a reddish bark color prevails, although periodic bark exfoliation exposes a pale powdery surface which darkens slowly. In *A. seyal* var. *Fistula* pseudo-galls are present and the powdery bark typically remains whitish or greenish-yellow. In general, there are two main varieties of *A. seyal*; variety *seyal* and variety *fistula*. Variety *seyal* is found in both western and eastern Africa and also on the Arabian Peninsula; while variety *fistula* is found in the eastern parts of Africa indicate that variety *seyal* is native to northern-tropical Africa and Egypt. The two varieties can be easily distinguished; variety *seyal* has a greenish-yellow to reddish-brown bark, while variety *fistula* has white to greenish-yellow bark. However, *A. seyal* trees are naturally grown in the Sudan till yet no reforestation done by human being [Mohammed, 2011].

### **1.7.2 Physical and chemical properties of *Acacia seyal* gum**

Chemical and physical properties of gum are considered as important characters for the article of commerce, to ensure the identity and purity of gum and avoiding

mixing and adulteration. Moisture content gives an indicator for both purity and a real weight. All results fall within limit specified by JECFA (1990). The Specific Optical rotation (S.O.R.) the range of the mean value vary from (+ 51.4 °) to (+ 62.8°). The range of the mean value of Protein content varies from 0.69 to 0.96 %. The pH ranged from 4.02 to 4.77 for *Acacia seyal*.

**Table (1.1): The physicochemical properties of *acacia seyal* species[obied,2012]**

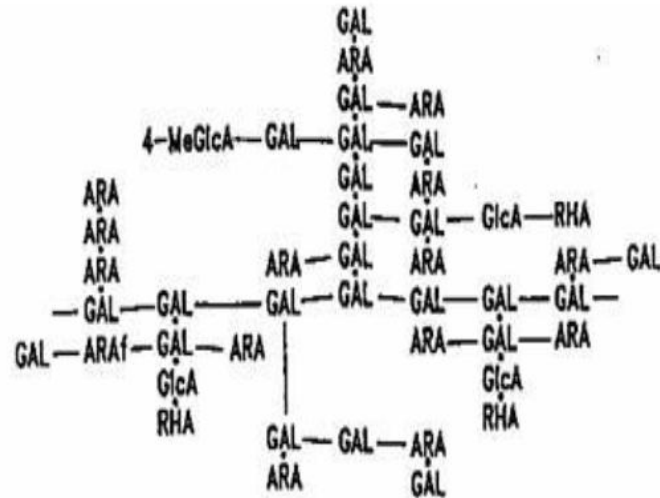
NO	Moisture	Ash	pH	S.O.R.	Protein content
1	10.04	3.03	4.77	+51.4	0.96
2	10.08	2.12	4.27	+60.5	0.69
3	9.63	1.92	4.31	+62.8	0.83
4	10.04	1.88	4.43	+57.8	0.85
5	9.63	2.1	4.12	+53.9	0.88
6	9.49	2.5	4.31	+59	0.96
7	9.26	2.35	4.02	+56	0.77
8	10.88	2.17	4.46	+62	0.91

**Table (1.2): Cationic composition of *Acacia seyal* [Buffo, 2001; Obied, 2012]**

<b>Element</b>	<b><i>A. seyal</i> 1 (ppm)</b>	<b><i>A. seyal</i> 2 (mg/g)</b>	<b><i>A. seyal</i> 3 (w/w %)</b>	<b><i>A. seyal</i> 3 (mg/g)</b>
Na	6.54-49.55	111.054	00048-0.03986	4.8125-398.6
K	2400-3558	2802.803	0.1808-0.4605	1807.93-4605.01
Ca	9453-10145	9417.1973	0..60994-0.67025	6093.81-9165.63
Mg	1224-1295	1229.0424	0.136415-0.2159	1364.15-2159
Pb	<0.84	7.5757	0.000228-0.008261	2.275-82.6125
Cr	0.30-0.32	-	0.006449-0.032218	64.4875-378.15
Cu	0.21-2.49	-	0.00008-0.000415	0.8-3.9875
Fe	6.51-17.06	43.9815	-	-

### **1.7.3 Chemical composition and structure of gums**

The uncrystallizable properties of gums, rendering their purification difficult and uncertain, that has been the cause of their chemistry being uncertain for so many years. Nitrogen is not regarded as an essential constituent, considered as impurity. Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucocidic bonds. Until the last century, gums were simply considered as carbohydrate, substances similar to sugar, starch and cellulose, and the formula  $C_{12}H_{22}O_{11}$  or  $(C_6H_{10}O_5)_n$  assigned to them. **(Figure1.2)**



[GAL = Galactose, ARA = Arabinose, GlcA = Glucuronic Acid

RHA = Rhamnose, 4-MeGlcA = 4-O-methylglucuronic Acid]

**Figure (1.2):** The structure of gum Arabic [Chikamai, 1996].

#### 1.7.4 Uses of Acacia Gums

Gums are widely, used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry, and to a lesser extent in textiles, ceramics, lithography, cosmetic, and pharmaceutical industry [Verbeken *et al.*, 2003]. In the food industry, Gum Arabic is primarily used in confectionery, bakery, dairy, beverage, and as a microencapsulating agent.

## **1.8 Objectives**

The objective of the present study was to optimize the preparation conditions of *Acacia seyal* (Talha) gum based hydrogels cross-linked with succinic acid and prepared hydrogels.



# Chapter Two

## Materials and methods

### 2.1 materials

Authenticated gum sample was kindly supplied by Professor Mohamed Elmubarak. The gum sample was cleaned from any extraneous materials, ground into a powder using a mortar and pestle and stored in plastic bags for the subsequent use.

### 2.2. Preparation of the *A. seyal* gum based hydrogels

*A. seyal* gum -based hydrogels were prepared as follows: *A. seyal* gum films were produced by casting an aqueous solution which contains 3 g of the gum in the presence of different amounts of succinic acid in each experiment (0.3, 0.15, and 0.10 g). Crosslinking was achieved by heating the dried films at 130, 140, and 150 °C for 5, 15, and 20 min in each separate experimental condition. Twenty-seven samples were prepared by using fixed amount of the gum and varying the heating temperatures and heating times. The obtained hydrogel samples were washed three times by using distilled water and then dried at 100 °C. The following tables (Tables 2.1, 2.2 and 2.3) show the detailed methods of preparation.

**Table 2.1: Preparation of the *A. seyal* gum based hydrogels at succinic acid concentration of 0.3 g**

Sample	<i>A. Seyal</i> gum (g)	Time (min)	Temperature (°C)
S1	3	5	130
S2	3	5	140
S3	3	5	150
S4	3	15	130
S5	3	15	140
S6	3	15	150
S7	3	20	130
S8	3	20	140
S9	3	20	150

**Table 2.2: Preparation of the *A. seyal* gum based hydrogels at succinic acid concentration of 0.15 g**

Sample	<i>A. Seyal</i> gum (g)	Time (min)	Temperature(°C)
S10	3	5	130
S11	3	5	140
S12	3	5	150
S13	3	15	130
S14	3	15	140
S15	3	15	150
S16	3	20	130
S17	3	20	140
S18	3	20	150

**Table 2.3: Preparation of the *A. seyal* gum based hydrogels at succinic acid concentration of 0.10 g**

Sample	<i>A. Seyal</i> gum (g)	Time (min)	Temperature (°C)
S19	3	5	130
S20	3	5	140
S21	3	5	150
S22	3	15	130
S23	3	15	140
S24	3	15	150
S25	3	20	130
S26	3	20	140
S27	3	20	150

### 2.2.1 Determination of the gel fraction

The gel content for each sample was calculated according to the following equation:

$$[\text{Gel fraction (\%)} = (W_1/W_0) \times 100] \dots \dots \dots 2.1$$

Where,  $W_1$  and  $W_0$  are the weights of the sample before and after washing respectively.

16 samples of the produced hydrogels have been selected (**S2, S3, S4, S5, S6, S8, S9, S12, S14, S16, S17, S18, S21, S24, S25, and S27**) based on the degree of swelling (samples that showed observable ability to absorb water).

### **2.2.2 Determination of the swelling percentages**

0.2 gram of each of the sixteen selected (**S2, S3, S4, S5, S6, S8, S9, S12, S14, S16, S17, S18, S21, S24, S25, and S27**) samples of hydrogels was immersed in a suitable amount of distilled water (in pre-weighed container) and left in ambient conditions for 48 hours. The unabsorbed water was got rid off and the contents were reweighed again. The swelling percentage was determined for each sample and the highest swelling percentage was found for sample (**S25, 2382.5%**).

### **2.3 Preparation of a hydrogel from sample 25 (S25)**

Exactly, identical conditions were followed to prepare enough quantity of hydrogel from sample **S25**. In a typical experiment, 20g of the gum sample and 0.67g of succinic acid (Identical ratio of the gum and succinic acid as well as the heating temperature and time were kept as shown in **Table 2.3 (S25)**) were dissolved in a suitable amount of distilled water in a beaker. The solution was casted on a Petri dish and left to dry in room temperature. Dried sample was heated in an Oven at 130°C for 20 minutes, left to cool and soaked in distilled water to remove unreacted materials. Several steps of washing were performed and finally the obtained gel was left to dry first at room temperature and then at 100 °C for complete dryness at one hour.

### **2.4 Swelling of the hydrogel of S25 at different pH**

Aqueous solutions having different pH values (2, 4, 7, 9, and 12) were prepared using hydrochloric acid and sodium hydroxide solutions with the aid of pH-meter. 0.2 g of S25 was accurately weighed and transferred to each of the containers containing the solutions with the specified pH value and left for 48 hours. The

unabsorbed solution in each container was removed and the contents were reweighed. The swelling ratio (Q) was calculated according to the following equation and plotted against the pH values.

$$[\text{Swelling ratio (Q)} = \frac{W_{\text{swollen gel}} - W_{\text{dried gel}}}{W_{\text{dried gel}}}] \dots \dots \dots 2.2$$

Where  $W_{\text{dried gel}}$  is the mass of dried hydrogel and  $W_{\text{swollen gel}}$  is the mass of swollen hydrogel.

### **2.5 Water absorption capacity**

0.2g of gel of S25 was taken into a pre-weighed container and a suitable amount of distilled water was added. The sample was left for 24 hours. The remaining water was removed and the contents were reweighed again. Fresh amount of water was added and left for 48 hours and the same process was repeated again. Exactly identical steps were done at 72, 96 and 120 hours.

The swelling ratio (Q) was calculated at each time interval according to equation below:

$$\text{Swelling ratio (Q)} = \frac{W_{\text{swollen gel}} - W_{\text{dried gel}}}{W_{\text{dried gel}}} \dots \dots \dots 2.3$$

Where  $W_{\text{dried gel}}$  is the mass of dried hydrogel and  $W_{\text{swollen gel}}$  is the mass of swollen hydrogel.

### **2.6 FT IR Study**

The infrared spectra of the crude and hydrogel gum samples were recorded using a Shimadzu-Fourier transform infrared spectrometer in the range between 4000 and 400  $\text{Cm}^{-1}$ . Few milligrams (2mg) of each sample were mixed thoroughly with 200 mg of spectroscopic grade KBr, pressed into pellets and the FTIR spectra of the crude and hydrogel gum samples were obtained.

# Chapter Three

## Results and Discussion

### 3.1 Gel content

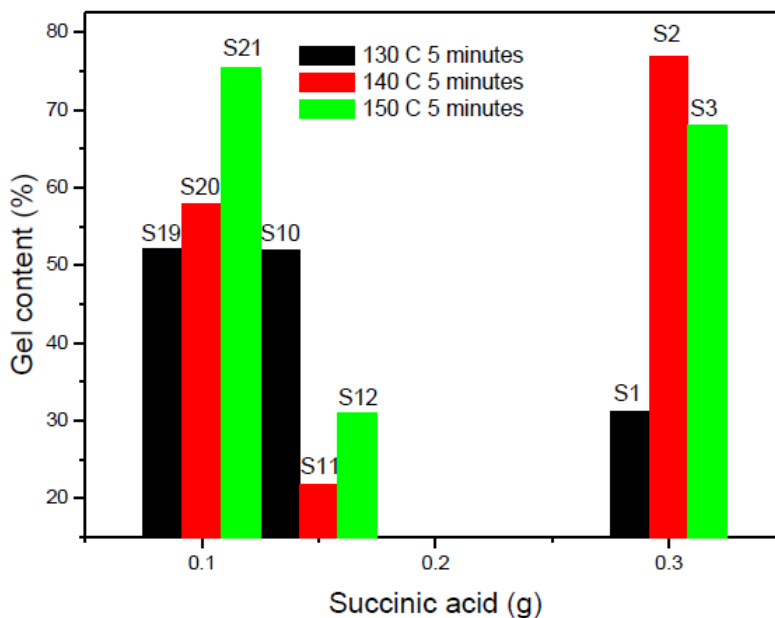
The gel content of the gum samples cross-linked with succinic acid is shown in Table 3.1 and the variation of the gel content with the amounts of succinic acid as well as with the heating temperatures are represented in Figures 3.1, 3.2 and 3.3 respectively.

**Table 3.1:** Gel content of *Acacia seyal var. seyal* gum cross-linked with succinic acid

Sample	Gel content (%)	Sample	Gel content (%)
S1	31.1	S15	47.43
S2	76.93	S16	64.67
S3	68.03	S17	86.4
S4	52.33	S18	74.8
S5	77.77	S19	52.07
S6	72.67	S20	57.97
S7	34.55	S21	75.47
S8	68.73	S22	45.7
S9	68.6	S23	20.16
S10	51.97	S24	79.53
S11	21.76	S25	60.10
S12	31	S26	68.67
S13	13.57	S27	77.53
S14	79.8		

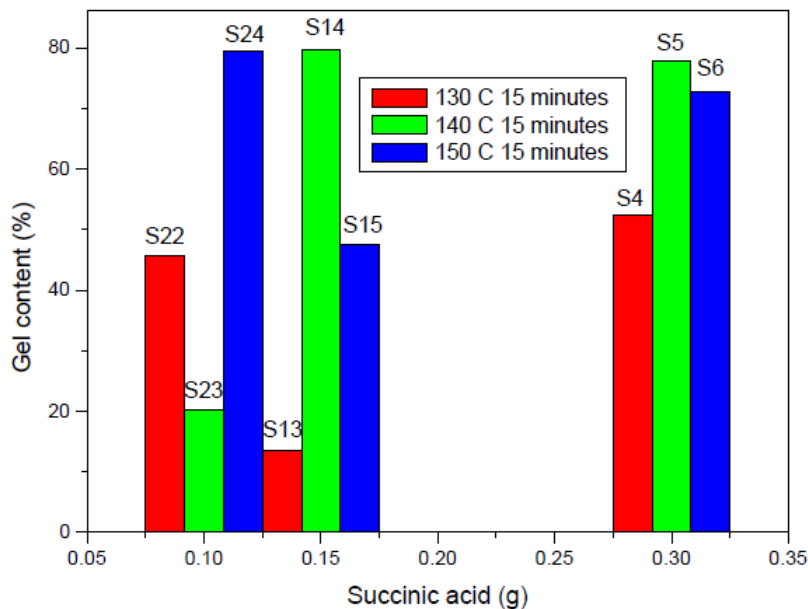
sample S17 (0.15 succinic acid/140°C/20 minutes) has the highest gel content (86.4%) and S13 has the lowest gel content (13.57%) (0.15 succinic acid/130°C/15 minutes). Comparing the conditions of preparation of S17 and S13 it is clear that the most influencing factors which determine the crosslinking efficiency are the temperature and time. Similarly, samples S14, S24, S5, S27 and S2 have shown a

similar trend as S17 and the values of gel content of these samples are 79.8, 79.53, 77.77, 77.53 and 76.93% respectively.



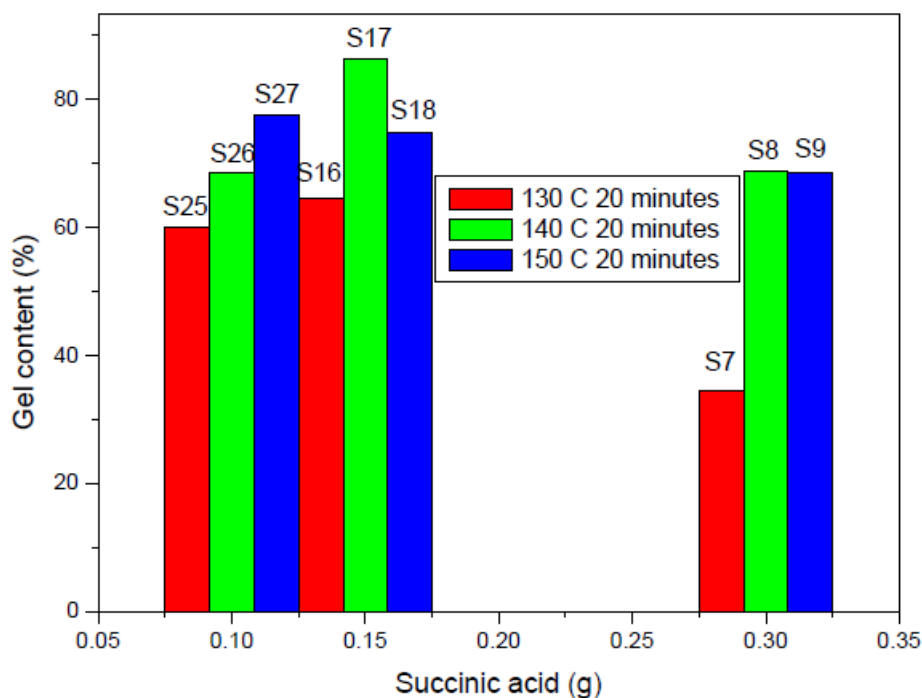
**Figure 3.1:** Variation of gel content (%) with succinic acid concentration at 5 minutes

samples S23, S11, S12, S1 and S7 have revealed similar trend to sample S13 and their gel contents are 20.16, 21.76, 31, 31.1 and 34.55% respectively shown in Table 2.1.



**Figure 3.2:** Variation of gel content (%) with succinic acid concentration at 15 minutes

The high percentages of gel contents of S13 and the other similar samples could be due to better conditions of reactivity of succinic acid with the gum (higher crosslinking density) under high temperature and reaction time. The influence of the reaction conditions on the crosslinking of polysaccharides was investigated by Patel and Pandya [2013]. The results showed that the degree of crosslinking was affected by heating temperature and time as well as with the succinic acid concentration.



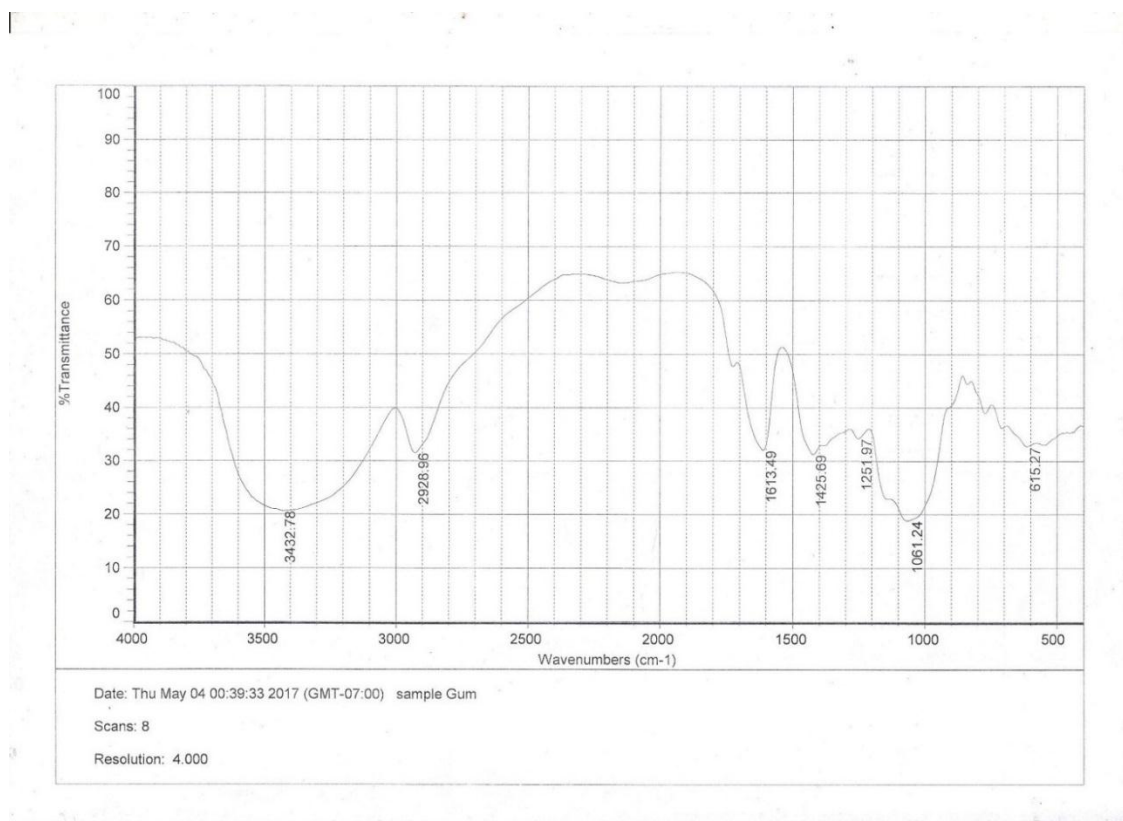
**Figure 3.3:** Variation of gel content (%) with the amount of succinic acid concentration at 20 minutes

### 3.2 FT-IR analysis

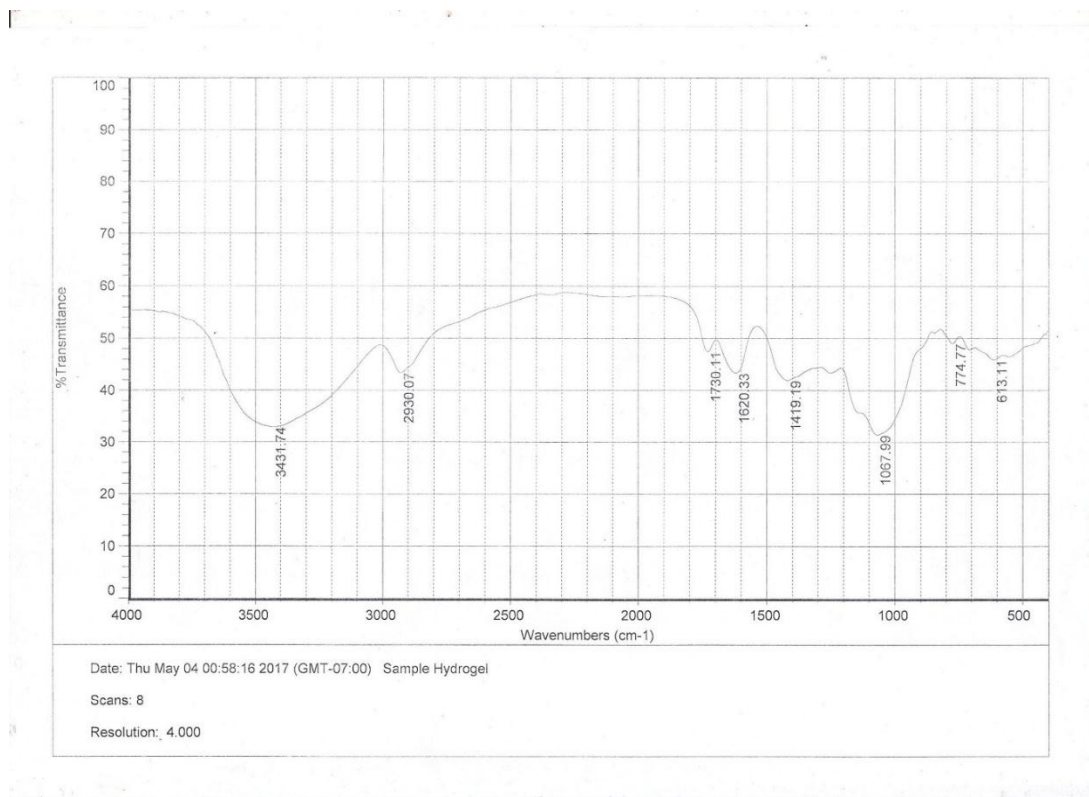
The FTIR spectrum of the crude *A. seyal* and the crosslinked sample (S25) are shown in Figures 3.4 and 3.5 respectively. It can be seen from Figure 3.4 that the intense-broad peak at  $3432\text{ cm}^{-1}$  represents the stretching vibration of -OH. In addition, the sharp peak located at  $2930\text{ cm}^{-1}$  displays the stretching vibration of -CH bond ( $\text{sp}^3$  hybridized carbon). The intense absorption band at  $1630\text{ cm}^{-1}$  is due to bending vibration of -OH group [Tsuboi, 1957]. The bending vibration of -CH bond, the stretching vibration of -C-O and -C-C (all  $\text{sp}^3$  hybridized) as well as the bending vibration of C-O-C have given intense and overlapped bands at  $1500\text{-}1000\text{ cm}^{-1}$  respectively [Li, *et al.*, 2015]. The FTIR spectrum of the hydrogel, has shown almost identical absorption bands to the ones of the crude gum and the only difference is the increase in the intensity and separation of the absorption band at



1730  $\text{cm}^{-1}$  which is due to carbonyl stretching vibration of the ester bonds (succinate cross-links).



**Figure 3.4:** FTIR spectrum of the crude *Acacia seyal* var. *seyal* gum



**Figure 3.5:** FTIR spectrum of *Acacia seyal var. seyal* gum based hydrogel (S25)

### 3.3 Swelling characteristics of hydrogel samples

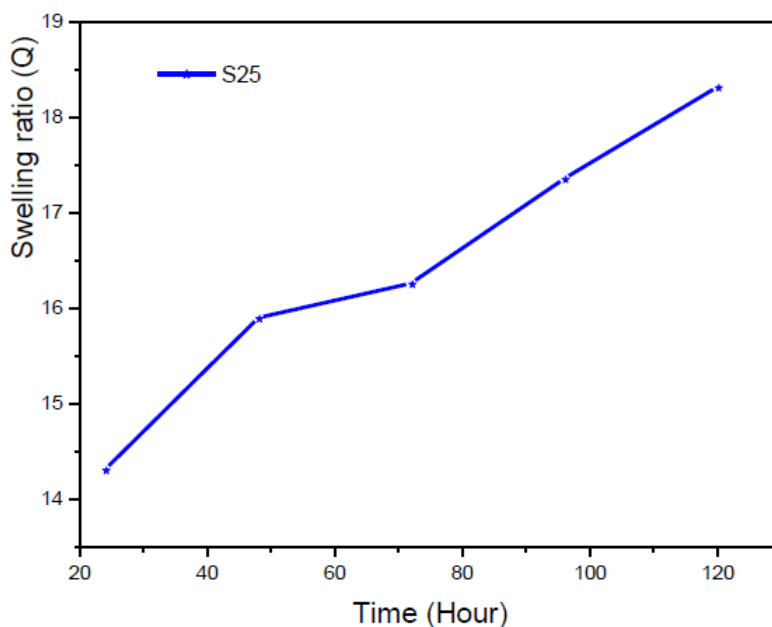
Table 3.2 displays the swelling characteristics of some of the succinic acid crosslinked samples. The selection was based on the ability of the sample to swell during immersion on water for 48 hours.

**Table 3.2: The swelling ratio of the hydrogel samples**

Sample	Swelling ratio (Q)	Sample	Swelling ratio (Q)
S4	9.74	S17	12.37
S2	8.77	S18	12.08
S9	11.96	S12	11.28
S6	8.81	S14	12.09
S3	13.44	S27	9.75
S8	9.51	S24	11.49
S5	9.18	S21	7.33
S16	10.35	S25	15.55

Sixteen samples were identified as having the highest ability to swell. Sample 25 (130°C/20 minutes/0.1g) was found to have the highest swelling behaviour (15.55). The swelling characteristics of sample S25 was investigated at different time intervals and the results are shown in Figure 3.6. It is clear that the value of swelling ratio (Q) increases as time increases for S25. It is important to mention that the swelling behaviour of the hydrogels depends on the crosslink density and it decreases with the increase of the cross-links due to restriction of the movement of the polymer chains [Wong *et al.*, 2015].

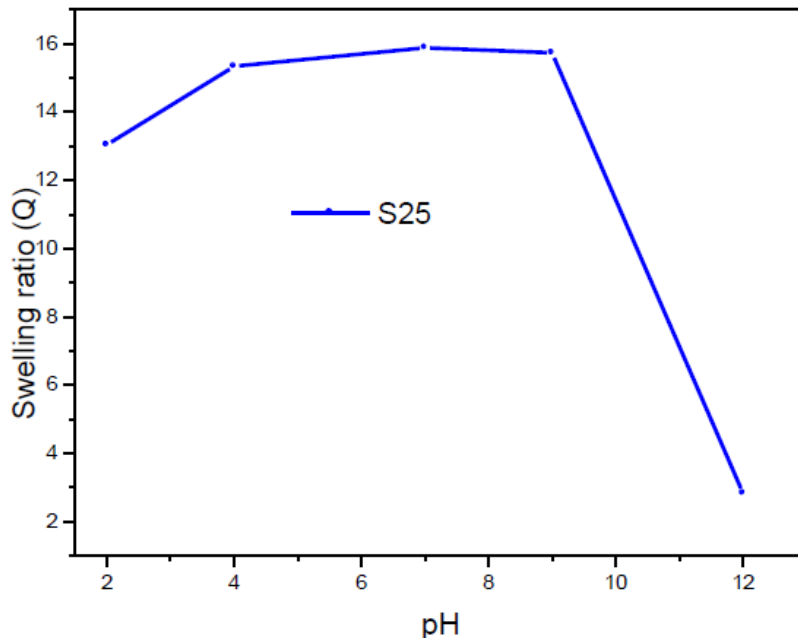
Examining the preparation conditions of S25 one could anticipate a lower crosslink density because of the mild conditions of both the temperature, 130°C, and the amount of the crosslinker, 0.1g, although the gel content was found to be 60.1%. Other samples such as S2, S4, S6, S21 and S27 which have significantly lower swelling ratio (7.32 (S21) – 9.74 (S4)) compared to S25 are all resemble each other in terms of severity of the reaction conditions (longer heating time or high heating temperature or high amount of crosslinker-(Table 2.1).



**Figure 3.6:** variation of water holding capacity and time

### 3.4 effect of PH on swelling ratio

The value of the swelling ratio (Q) in the acidic medium increases as the value of pH increases up to it is highest value at pH 7 while it is decreased in alkaline medium. In the acidic medium, the gel absorbs water and preserves its properties until it reaches the highest absorption in the neutral medium and at the basic medium the absorption is reduced due to dissolution o the gel and rupture of the ester bonds (alkaline hydrolysis) until the absorption reaches zero (complete dissolution of the gel and lose absorption properties). On the other hand, in acidic pH values the conversion of free hydroxyl groups enhances its size and thereby reduces the voids, which is responsible for the lower absorbing capability [Thakur *et al.*, 2011].



**Figure 3.7:** variation of the swelling ratio at with pH.

## **Conclusions**

The results have demonstrated that the crosslinking efficiency depends greatly on the heating temperatures and times.

The swelling behavior of the hydrogel sample has shown significant pH dependency.

## References

- Alemzadeh, I., Vossoughi, M.Y., (2002).** Controlled release of paraquat from poly vinyl alcohol hydrogel. *Chem Eng Process*; 41, pp.707-710.
- Bhattacharai, N., Gunn, J., and Zhang, M. Q., (2010).** *Adv. Drug Delivery Rev.*, 62, pp.83–99.
- Bueno, V.B., Bentini, R., Catalani, L.H. and Petri, D.F.S., (2013).** Synthesis and swelling behavior of xanthan-based hydrogels. *Carbohydrate polymers*, 92(2), pp.1091-109.
- Buffo, R.A., Reineccius, G.A., Oehlert, G.W., (2001).** Factors affecting the emulsifying and rheological properties of gum Acacia in beverage emulsions. *Food Hydrocolloids*, 15, pp.53-66 [8].
- Chang, C. Y., Duan, B., Cai, J., and Zhang, L. N., (2010).** *Eur. Polym. J.*, 46, pp.92–100.
- Chikamai, B.N., Banks, W.B., Anderson, D.M.W., and Weiping, W., (1996).** Processing of gum Arabic and some new opportunities. *Food Hydrocolloids*, vol.10 no3, pp.309- 31.
- Demitri, C., Del Sole, R., Scalera, F., Sannino, A., Vasapollo, G., Maffezzoli, A., Ambrosio, L., and Nicolais, L., (2008).** Novel superabsorbent cellulose-based hydrogels crosslinked with citric acid. *Journal of Applied Polymer Science*, 110(4), pp.2453-2460.
- Fu, F. L., Wang, Q., and Environ, J., Manage, (2011).** 92, pp.407–418.
- Hennink, W.E., Nostrum, C.F., van, (2002).** Department of Pharmaceutics, Utrecht University. *Advanced Drug Deliveries Review*. Volume 54, January, pp.13-36.

- Jayakumar, R., Prabakaran, M., Kumar, P.T. S., Nair, S. V., and TamuraH., (2011).** *Biotechnol. Adv*, 29, pp.322–337.
- Kono, H., Otaka, F., and Ozaki, M., (2014).** Preparation and characterization of guar gum hydrogels as carrier materials for controlled protein drug delivery. *Carbohydrate polymers*, 111, pp.830-840.
- LaPorte, R.J., (1997).** Hydrophilic polymer coating for medical devices. *Lancaster, USA: Techonomic Publishing Co*, pp.19-50.
- Lee, K.Y., and Mooney, D. J., (2001).** *Chem. Rev*, 101, pp.1869–1879.
- Li, M.X., Wang, X.W., Yang, Y.Q., Chang, Z., Wu, Y.P., Holze, R. A., (2015).** dense cellulose-based membrane as a renewable host for gel polymer electrolyte of lithium ion batteries. *J. Membr. Sci*, 476, pp.1
- Mack, E.J., Okano, T., and Kim, S.W., Peppas, N.A., (1988).** Editor, Hydrogels in medicine and pharmacy-polymers vol. II. *CRC Press, Boca Raton, USA*, pp. 65.
- Marler, J. J., Upton, J., Langer, R., and Vacanti, J. P., (1998).** *Adv. Drug Delivery Rev*, 33, pp.165–182.
- Matricardi, P., Cencetti, C., Ria, R., Alhaique, F., and Coviello, T., (2009).** Preparation and characterization of novel gellan gum hydrogels suitable for modified drug release. *Molecules*, 14(9), pp.3376-3391.
- Minhas, M., Ahmad, M., Ali, L., and Sohail, M., (2013).** Synthesis of chemically cross-linked polyvinyl alcohol-co-poly (methacrylic acid) hydrogels by copolymerization; a potential graft-polymeric carrier for oral delivery of 5-fluorouracil DARU. *Journal of Pharmaceutical Sciences*, 21,pp.44.

- Mohammed, M.H., (2011).** Management of Natural Stands of *Acacia seyal* Del. variety *seyal* (Brenan) for Production of Gum Talha, South Kordofan, Sudan, MSc. *Thesis in Forestry, University of Khartoum.*
- Nagasawa, Naotsugu, Toshiaki, Yagi, Tamikazu, Kume, and Fumio Yoshii, (2004).** “Radiation Crosslinking of Carboxymethyl Starch.” *Carbohydrate Polymers* 58(2): pp.109–13.
- Obied, I.S., (2012).** Evaluation of Physicochemical, Microbiological and Toxicological Aspects of *Acacia seyal* Gum. *PhD thesis in Chemistry, Sudan University of Sciences and Technology, Society*; pp. 2–4 [in Persian].
- Thakur, A., Wanchoo, R. K., & Singh, P., (2011).** Structural parameters and swelling behavior of pH sensitive poly (acrylamide-co-acrylic acid) hydrogels. *Chemical and Biochemical Engineering Quarterly*, 25, pp.181–194.
- Tsuboi, M., (1957).** Infrared spectrum and crystal structure of cellulose. *Polym. Sci*, 25, pp.159–171.
- Verbeken, D., Dierckx, S., and Dewettinck, (2003).** Exudate gums: Occurrence, production, and applications. *Applied Microbiology and Biotechnology*, 63(1): pp.10–21.
- Wong, R.S.H., Ashton, M., and Dodou, K., (2015).** Effect of crosslinking agent concentration on the properties of unmedicated hydrogels. *Pharmaceutics*, 7(3), pp.305-319.
- Zohuriaan-Mehr, M.J., (2006).** Super-absorbents. *Tehran: Iran Polymer.*