MODIFIED GUAR GUM-BASED HYDROGELS: SYNTHESIS, CHARACTERISATIONS AND SWELLING BEHAVIOUR

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MODIFIED GUAR GUM-BASED HYROGELS: SYNTHESIS, CHARACTERISATIONS AND SWELLING BEHAVIOUR

ABSTRACT

The growing concern for synthetic materials that are primarily derived from petroleum and coal as raw materials for the manufacture of hydrogel has opened a field of research focused on nature-based products. Massive efforts are being taken on biopolymer-based hydrogels due to their abundance, renewability, biodegradability and biocompatibility. Modification of biopolymer by grafting as alternative hydrogels is a promising strategy for imparting beneficial properties on them. Guar gum has solubility in water, possesses uncontrolled and enormous swelling that restricts its potential to be used in diverse applications. Thus, in this study, native guar gum (Native GG) was modified into guar gum acetate (GGA) and phthaloyl guar gum (PHGG) by transesterification and phthaloylation, respectively. These two modified guar gums have better solubility in organic solvents such as dimethylsulfoxide and dimethylformamide. The success of these modifications was confirmed through Fourier-transform infrared spectroscopy with new characteristic peaks at 1733 cm^{-1} for GGA and 1709 cm^{-1} for PHGG. The degree of substitution of GGA was 5.65 meanwhile PHGG was 1.04 as determined from proton nuclear magnetic resonance spectroscopy. X-ray diffractometry results revealed that both GGA and PHGG are less crystalline compared to Native GG. GGA was found to be thermally more stable than Native GG while PHGG was slightly less thermally stable in comparison with Native GG. The swelling behaviour revealed that Native GG had the highest swelling equilibrium with 918.4 ± 46.6%, followed by PHGG with 537.0 ± 2.9% and GGA with 393.0 ± 13.4% in distilled water. These hydrogels (Native GG, GGA and PHGG) were found to be stimuli sensitive towards pH and ionic salt solution. The samples responded to simulated normal saline isotonic solution, showing lower swelling

compared to swelling in distilled water. For simulated gastric fluid (SGF) medium, GGA showed higher swelling while Native GG and PHGG showed higher swelling in simulated intestinal fluid (SIF) medium. Therefore, these guar gum hydrogels have potential to be used in biomedical fields such as tissue engineering and drug-delivery.

Keywords: Guar gum acetate, Guar gum, Hydrogel, Phthaloyl guar gum, Swelling study

HIDROGEL BERASASKAN GAM GUAR TERUBAH SUAI: SINTESIS, PENCIRIAN DAN SIFAT PEMBENGKAKAN

ABSTRAK

Peningkatan permintaan terhadap bahan sintetik berasaskan petroleum dan arang batu sebagai bahan mentah untuk pembuatan hidrogel telah membuka bidang penyelidikan yang tertumpu kepada produk berasaskan alam semula jadi. Pelbagai kaedah telah dijalankan pada hidrogel yang berasaskan biopolimer kerana kepelbagaian sifatnya, boleh diperbaharui, bersifat biodegradasi dan biokeserasian. Modifikasi biopolimer secara cantuman sebagai hidrogel alternatif adalah strategi yang berkesan. Gam guar, menunjukkan sifat keterlarutan dalam air, memiliki sifat pembengkakan yang tidak terkawal dan sangat besar sehingga menyekat potensinya untuk digunakan dalam pelbagai aplikasi. Oleh itu, dalam kajian ini, gam guar asal (Native GG) dimodifikasikan menjadi gam guar asetat (GGA) dan gam guar phthaloyl (PHGG) masing-masing dengan kaedah transesterifikasi dan kaedah phthaloyl. Kedua-dua gam guar yang dimodifikasi ini mempunyai keterlarutan yang baik dalam pelarut organik seperti dimetilsulfoksida dan dimetilformamida. Kejayaan pengubahsuaian ini disahkan melalui spektroskopi Fourier transformasi inframerah dengan puncak baru pada 1733 cm^{-1} untuk GGA dan 1709 cm^{-1} untuk PHGG. Nilai penggantian GGA adalah 5.65 sementara PHGG adalah 1.04, ditentukan dari spektroskopi proton resonans magnetik nuklear. Hasil pembelauan sinar-X menunjukkan bahawa kedua-dua GGA dan PHGG adalah kurang hablur berbanding dengan Native GG. GGA didapati lebih stabil secara termal daripada Native GG sementara PHGG adalah sedikit kurang stabil secara termal berbanding dengan Native GG. Sifat pembengkakan menunjukkan bahawa Native GG mempunyai peratusan pembengkakan tertinggi dengan 918.4 ± 46.6%, diikuti oleh PHGG dengan 537.0 ± 2.9% dan GGA dengan 393.0 ± 13.4% dalam air suling. Hidrogel ini juga sensitif terhadap

rangsangan seperti pH dan larutan garam ionik. Sampel bertindak balas terhadap larutan simulasi isotonik garam normal, menunjukkan sifat pembengkakan yang lebih rendah berbanding dengan pembengkakan dalam air suling. Bagi media simulasi cecair gastrik (SGF), GGA telah menunjukkan pembengkakan yang lebih tinggi sementara Native GG dan PHGG menunjukkan pembengkakan yang lebih tinggi dalam media simulasi cecair usus (SIF). Oleh itu, hidrogel gam guar ini berpotensi untuk digunakan dalam bidang bioperubatan seperti kejuruteraan tisu dan penghantaran ubat.

Kata kunci: Gam guar asetat, Gam guar, Gam guar phthaloyl, Hidrogel, Kajian bengkak

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Yours sincerely,

Mazrina

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LIST OF SYMBOLS AND ABBREVIATIONS

δ_d	:	the energy from dispersion forces between molecules
δ_p	:	the energy from dipolar intermolecular forces between molecules
δ_h	:	the energy from hydrogen bonds between molecules
δ_t	:	total solubility parameter
δ^2_t	:	total energy forces between molecules
ρ	:	true density
χ_p	:	fractional polarity
V_m	:	molar volume
^{1}H NMR	:	Proton nuclear magnetic resonance
DCM	:	Dichloromethane
DMA	:	Dimethylacetamide
DMF	:	*N,N*-dimethylformamide
DMSO	:	Dimethylsulfoxide
DS	:	degree of substitution
ECM	:	extracellular matrix
FTIR	:	Fourier-transform infrared spectroscopy
gal	:	galactose
GIT	:	gastrointestinal tract
GGA	:	guar gum acetate
HCl	:	hydrochloric acid
man	:	mannose
NaCl	:	sodium chloride
PHGG	:	phthaloyl guar gum

SGF	:	simulated gastric fluid
SIF	:	simulated intestinal fluid
TGA	:	Thermogravimetric analysis
THF	:	tetrahydrofuran
XRD	:	X-ray diffractometry

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Research Background

Many researchers have synthesised hydrogels by blending of native biopolymers with a cross-linker for example the blending of guar gum (GG) with chitosan using glutaraldehyde as the cross-linker (Rithe *et al.*, 2014), but chemical modification towards biopolymer has not being explored widely in comparison to modifications through blending and cross-linking (Giri *et al.*, 2020; Sionkowska, 2011). One of the most promising potential biopolymers that can be chemically modified includes GG. The high hydrophilic nature of GG results in excessive swelling and susceptibility towards microbial contamination, hindering its potential as a hydrogel for biomedical application as this induces burst release of entrapped drug before reaching the action site (Rana *et al.*, 2011). The significance of chemical modification helps to overcome GG limitation by reducing swelling thus controlling drug release. GG originated from the endosperm of the seeds of *Cyamopsis tetragonoloba* (cluster bean) (Whistler *et al.*, 1979). Chemical modification substitutes some of the hydroxyl groups with hydrophobic moieties, reducing the interaction between its molecules and becoming more hydrophobic as compared to its native form (Hongbo *et al.*, 2017). This improves organosolubility so that the swelling of modified GG in water occurs in a controlled manner which will translate into sustained release of drugs from the GG-based hydrogel. Some of the chemical modifications that have been done previously include carboxymethylation, benzoylation, polyacrylamide grafting, succinylation and propionate linkage (Giri *et al.*, 2020).

This work is a pioneer attempt of synthesising two types of hydrogels by chemically modifiying the native GG (Native GG) into GG acetate (GGA) through transesterification using vinyl acetate alone and phthaloylation of GG. To the best of our knowledge, phthaloylation of GG is the first to be done. Secondly, this is the first attempt to modify

GG alone without using cross-linker. Functional groups and degree of substitution were determined from Fourier-transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy respectively. Apart from that, the crystallinity of the samples was determined by X-ray diffractometry (XRD) and thermal stability was evaluated by Thermogravimetric analysis (TGA). The swelling behaviour of the modified GG-based hydrogels in distilled water, buffer solution and ionic salt solution were also being investigated.

1.2 Problem Statement

Synthetic polymers are more popular to be used in many synthesis applications of hydrogels, however problems occur when they are non-biodegradable and their degradation products are hazardous towards environment. Biopolymers can be used as alternative materials to synthetic polymers due to their non-toxicity, biodegradability varying solubility, film forming ability, controlled release property and susceptibility to microbial degradation in environment (Barth *et al.*, 1981). However, hydrogels from biopolymers such as GG are prone to have uncontrolled swelling ratio and high water uptake which are, amongst others, the undesirable properties of hydrogels. Thus, modifications of biopolymers are essential to form a more stable hydrogels for applications such as in agriculture, biomedical, nanocomposites, and tissue engineering.

1.3 Objectives

This study is guided by three main objectives:

i. To impart organosolubility to GG by esterification process (chemical modification).

ii. To characterise the Native GG, GGA and PHGG.

iii. To evaluate the performance (swelling behaviour) of the Native GG, GGA and PHGG.

1.4 Scope of Research Work

Hydrogel has been used as an emerging and promising tool in drug delivery and other biomedical applications. The literature review on synthetic and biopolymer hydrogels, chemical modifications and applications of GG are reviewed in Chapter 2. Chapter 3 will discuss the experimental procedures for the chemical modification, characterisation and swelling behaviour of Native GG, GGA and PHGG in distilled water, buffer solution and ionic salt solution. Chapter 4 presents the results obtained to study the effect of substitution of two hydrophobic moieties (acetate linear chain versus bulky phthaloyl) on the properties of GG-based hydrogels. The work will be concluded in Chapter 5 whereby suggestions for future work are included.

1.5 Research Outline

In the current research, two GG derivatives have been synthesised through transesterification to produce GGA and phthaloylation to produce PHGG. The outline for research work is summarized in the following chart (**Figure 1.1**).

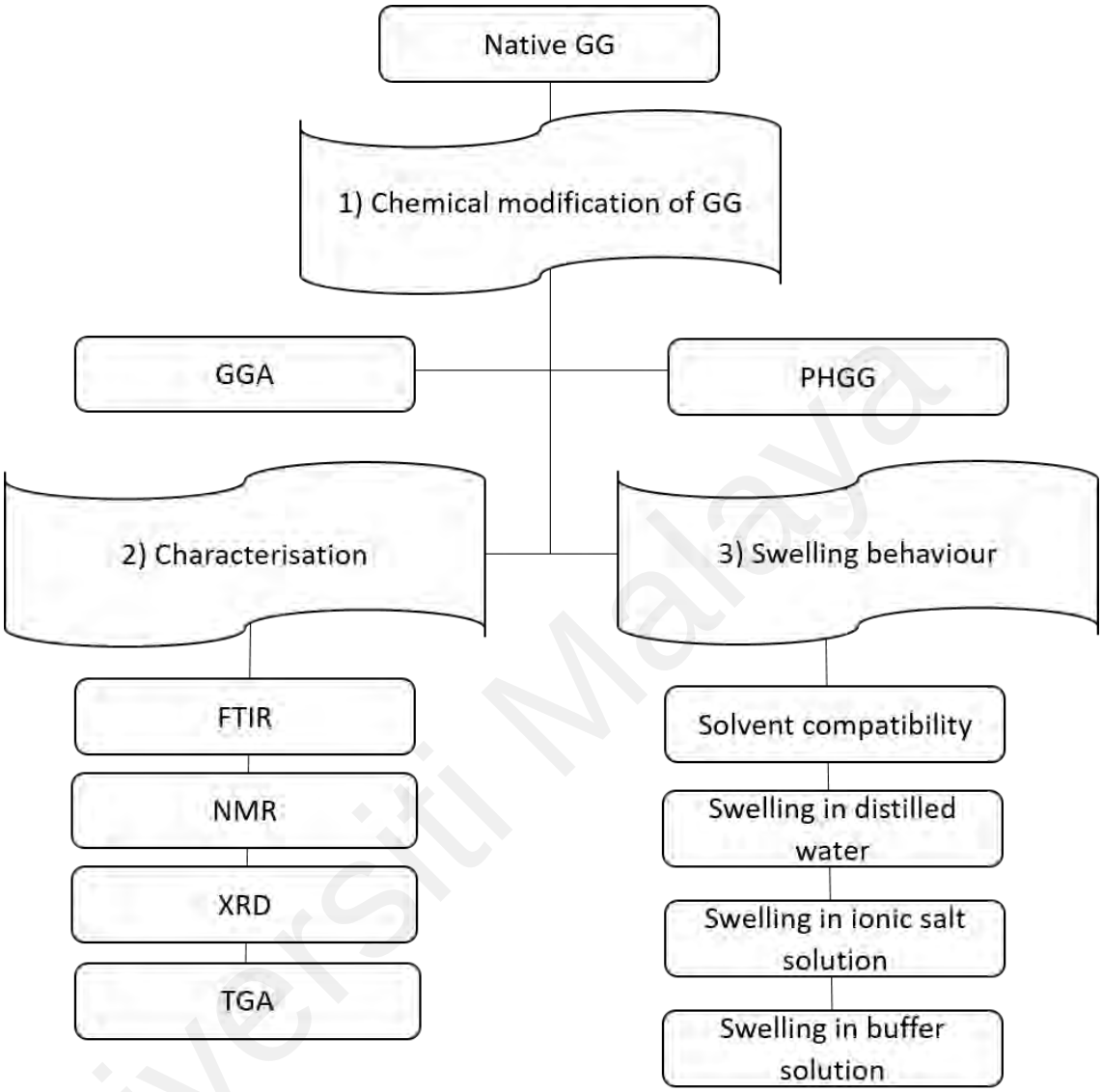

Figure 1.1: Outline of research work

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction of Hydrogels

Hydrogel is a three-dimensional cross-linked, viscoelastic polymeric network which can absorb large amount of water or biological fluid (Shetye *et al.*, 2015). By definition, water must represent at least 10% of a material's total weight (or volume) to be considered hydrogel. Its water absorption ability is due to the presence of hydrophilic groups such as hydroxyl (–OH), secondary amide (–CONH–), primary amide (–$CONH_2$), carboxyl (–COOH) and sulphonic (–SO_3H) along the polymer chains as pendant groups which forms hydrogel structure as shown in **Figure 2.1** (Hamidi *et al.*, 2008).

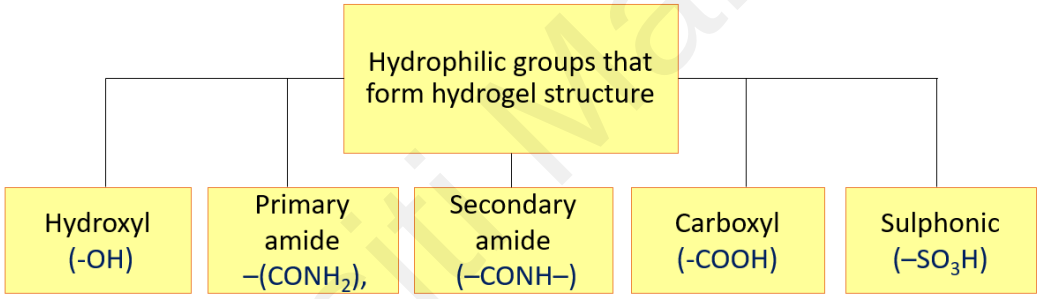

Figure 2.1: Hydrophilic groups that form hydrogel structure

Hydrogel posseses physicochemical properties that mimick natural extracellular matrix (ECM) (Kakkar *et al.*, 2016). The research in polymer chemistry has increased since the discovery of hydrogel, namely poly(2-hydroxyethyl methacrylate) (pHEMA) for contact lens application which was pioneered by Witcherle and Lim in 1960, (Wichterle *et al.*, 1960). Until now, hydrogel is still an interesting material extensively studied by scientists and biomedical researchers in order to broaden its formulations and applications ranging from industrial to biological and medical.

The history of hydrogels comprises of three main blocks (Buwalda *et al.*, 2014). First generation of hydrogels is comprised of simple, cross-linking procedures involving the chemical modifications of a monomer or polymer with an initiator for synthetic polymers.

The general purpose is to produce material with high swelling and strong mechanical properties. Beginning in the seventies, second generation of hydrogel materials became capable of reacting to changes in environmental conditions, which are also known as "intelligent" or "smart" hydrogel, such as fluctuations in temperature, pH, light, magnetic field, ion or concentration of biomolecules in solution (Chirani *et al.*, 2015). Such particular factors may be used to induce specific events, for example the material polymerisation, gel formation, drug delivery or an *in situ* pore formation (Abebe *et al.*, 2012; Buwalda *et al.*, 2014). Later, in the mid-1990s, the third generation of hydrogels focuses on the development of stereo-complex development (e.g. PEG-PLA interaction) (Yom-Tov *et al.*, 2014) where the hydrogel materials were cross-linked by other physical interactions (e.g. cyclodextrines) (Chung *et al.*, 2008; Kirakci *et al.*, 2014).

2.2 Synthetic Hydrogels

The polymer hydrogels have been an area of intensive study for the last four decades. Synthetic hydrogels contain synthetic polymers which offer additional flexibility in tuning chemical and mechanical properties of the hydrogels. Poly(acrylamide) and its derivatives, poly(ethylene glycol) and poly(vinyl alcohol) to name a few, are examples of synthetic hydrogels. Synthetic polymers are hydrophobic, and are chemically and mechanically stronger than biopolymers (Ahmed, 2015; Gyles *et al.*, 2017). These hydrogels are more reproducible but their final composition is influenced by the conditions of polymerisation. As such, it is important to track the preparation process carefully, including temperature and environmental monitoring. The concentration or molecular weight of the precursor can differ, and the concentration of cross-linkers may be altered (Chirani *et al.*, 2015).

Filipovic *et al.* exploited free radical crosslinking copolymerisation with lipase derived from *Candida rugosa* to synthesise novel temperature and pH-sensitive hydrogels

based on N-isopropylacrylamide and itaconic acid. Such hydrogels were used for drug delivery purposes in a pH-responsive system. The hydrogels were found to be highly sensitive to temperature and pH, while retaining constant ionic strength (Milašinović *et al.*, 2010).

Synthetic hydrogels, however, suffer from certain limitations as they have poor biological activity compared with biopolymer hydrogels. The main limitation of synthetic hydrogels is their non-biodegradability. Biopolymer hydrogels have been a better choice in terms of degradability and its non-toxicity, replacing the synthetic material (Ali *et al.*, 2018).

2.3 Biopolymer Hydrogels

Biopolymers are polymeric biomolecules, comprising covalently bound monomeric groups, which form larger molecules (Mohan *et al.*, 2016). Examples of biopolymer hydrogels are illustrated in **Figure 2.2**. Biopolymer hydrogels, imitating the ECM *in situ*, are mostly the physiological hydrogels.

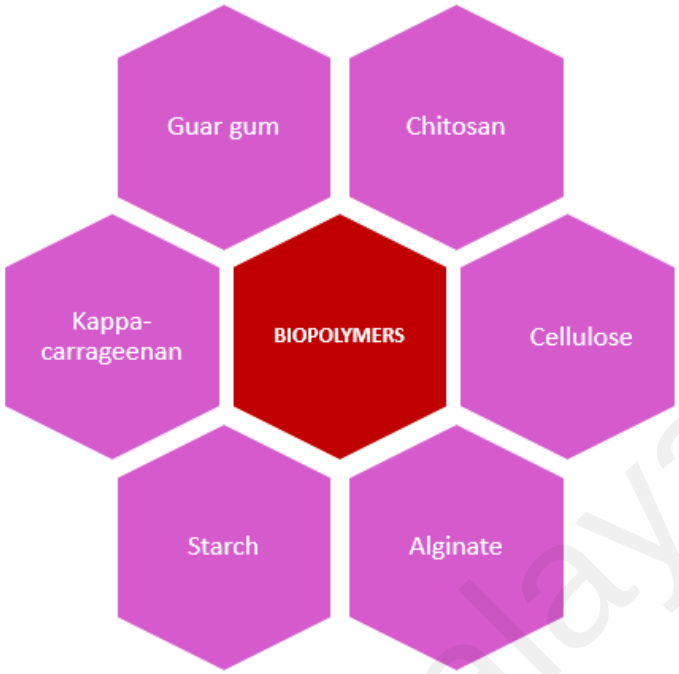

Figure 2.2: Examples of biopolymer hydrogels

Examples of biopolymer hydrogels are starch (Hashem *et al.*, 2008), cellulose (Senna *et al.*, 2014), chitosan (Islam *et al.*, 2013) and GG (George *et al.*, 2007; Huang *et al.*, 2007). Starch has poor mechanical behaviour, high permeability of water vapour and is vulnerable to retrogradation which hinders its use in biomedical applications (Ribba *et al.*, 2017). Cellulose is insoluble in water and most organic solvents due to the extensive intra- and inter-molecular hydrogen bonding which restricts its use in biomedical applications (Mohd *et al.*, 2017). Chitosan, on the other hand, has limited use in neutral or alkaline pH media, owing to its poor mechanical strength and insolubility. Biopolymer-based hydrogel suffers from two significant drawbacks, i.e the challenge involves controlling their final microstructures and properties from batch to batch experiments in a reproducible way (Caló *et al.*, 2015). It undergoes deformation under extreme pressure, leading to substantial swelling in acidic aqueous solutions (Cravotto *et al.*, 2005; Rizwan *et al.*, 2017). GG is one of the most promising biopolymers for biomedical applications

because of its interesting attributes: it is found abundantly in nature, non-toxic, biodegradable, capable of forming hydrogen bonding with water molecules, and more importantly, because it undergoes microbial degradation in intestinal fluids and can be chemically modified by grafting, derivatization and network formation to improve its physicochemical properties such as solubility, viscosity and swelling index to suit a wide spectrum of biomedical applications (Mudgil *et al.*, 2014; Prabaharan, 2011; Rizwan *et al.*, 2017; Thakur *et al.*, 2015).

2.4 Hydrogel Formation

Hydrogel is basically synthesised by cross-linking networks which prevent its dissolution despite in large amounts of water or poor physiological absorption (Bhattarai *et al.*, 2010). Hence, based on the cross-linking, hydrogels can be classified into two categories: (a) physical hydrogel or self-assembled hydrogel and (b) chemical hydrogel (Chung *et al.*, 2009; Slaughter *et al.*, 2009). Hydrogel can also be prepared by incorporating hydrophobic groups into the polymer chains (Boucard *et al.*, 2005).

2.4.1 Physical hydrogels

Owing to the fast development and absence of cross-linking agents during their synthesis, mechanically cross-linking hydrogels or named as reversible hydrogels have gained importance. Physical dissolution of cross-linked gels is prevented by physical interactions (Varaprasad *et al.*, 2017). Physical hydrogels show reversible response to stimuli in which they are disordered and frail due to their weak secondary interactions between the polymer chains (Boucard *et al.*, 2005). Some of the methods to synthesise physical hydrogels include:

a. Freeze- thawing

Repetitive freeze-thaw cycles should be used to obtain physical cross-linking. This mechanism allows microcrystals forming (Zhang *et al.*, 2013).

b. Stereocomplex formation

The major advantage of hydrogel forming a stereocomplex is that can be formed easily by dissolving each product in water and mixing the solution. Poly(lactic acid) is one of the best examples which gives excellent stereocomplex properties (Tsuji *et al.*, 1992).

c. Ionic polymer formation

This ionic hydrogel method results in an ionic cross-linking by the addition of di- or trivalent counter ions. This approach follows the concept of gelling up a polyelectrolyte solution of opposite charge multivalent ions. For instance, synthesised porous hydrogel films of chitosan-glycerol phosphate salt (Zhao *et al.*, 2009) and poly[di(carboxylate phenoxy) phosphazene] calcium salt (Ebara *et al.*, 2014).

d. Physically cross-linked gel-like structures can be prepared via hydrogen bonding interactions. The best example of such hydrogel is the forming of hydrogen-bond carboxymethyl cellulose network by dispersing it in 0.1 M HCl (Takigami *et al.*, 2007).

2.4.2 Chemical hydrogels

'Chemical' or 'permanent' hydrogels are formed from covalent bonds within the matrix by regulating the degree of hydrogel swelling depending on the interactions between polymer and water and the degree of reticulation (Gyles *et al.*, 2017).

2.4.2.1 Chemical cross-linking

In chemical cross-linked hydrogels, cross-linkers, such as glutaraldehyde, epichlorohydrin, borax and polyaldehydes, are widely used to obtain cross-linked hydrogel networks of synthetic and natural polymers as illustrated in **Figure 2.3**.

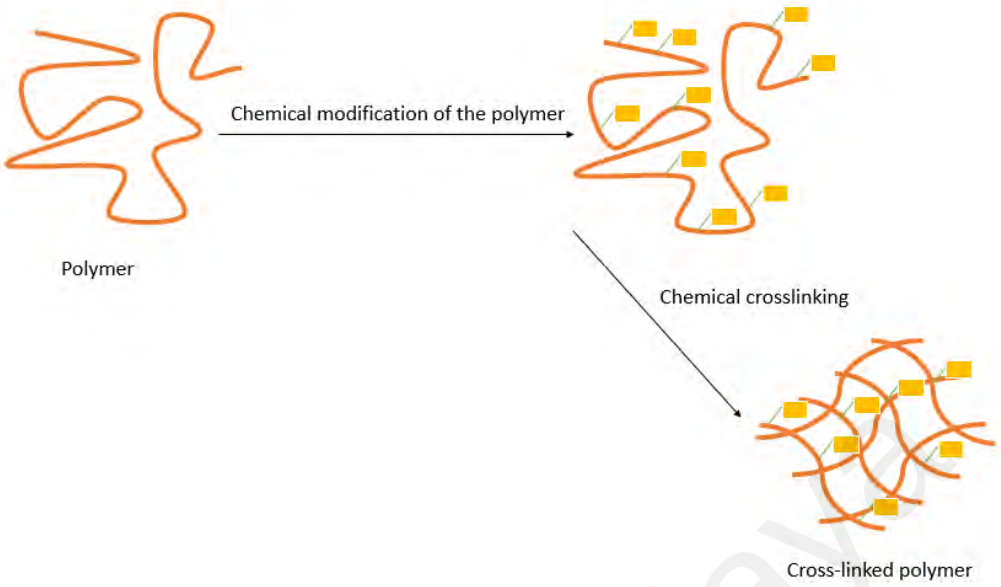

Figure 2.3: Chemical cross-linking to form hydrogels

Besides, covalent linkages between polymer chains can be formed by the reaction of functional groups, such as an amine/carboxylic acid or an isocyanate-OH/NH_2, or by Schiff base formation, with complementary reactivity (Hennink *et al.*, 2012). Some methods to synthesise chemical hydrogels include:

a. Free radical mechanism

Polymerisation of oligomers and cross-linkers by free radical process helps in the development of chemical cross-linked hydrogels. The reactions begin by using initiator such as potassium persulphate, azobisisobutyronitrile, ammonium persulphate (KPS) and benzoyl peroxide. Kumar *et al.* studied psyllium and acrylic acid based polymeric networks synthesized under either air or inert conditions, via γ-radiations and using KPS and hexamethylenetetramine as the initiator crosslinker system (Kumar *et al.*, 2017).

b. Ionising radiation

The polymerisation of hydrogel is done by electron beam irradiation and high energy. These radiations generate radicals on the backbone of polymer, interacting with other polymer chains, ending up recombining forming as cross-linked structure. Radiation method is superior over chemical method as it leaves no residue (Ahmed, 2015).

c. Complementary functional group

By direct cross-linking of polymers, *in situ* forming hydrogels may be prepared by chemical reactions with complementary functional groups. The complementary functional groups of biopolymers are mainly amino, hydroxyl and carboxylic. Some of the suitable reactions include click chemistry, Michael addition, thiol-ene/yne coupling, Diels-Alder reaction, disulfide formation, Schiff-base formation, and epoxide reactions (Bi *et al.*, 2016).

d. Aldehyde as cross-linker

Biopolymers, which consist of the polar functional groups like –OH and $–NH_2$, can be functionalised by cross-linking with glutaraldehyde to synthesise hydrogel. For example, for the controlled delivery of protein drugs, George *et al.* synthesise pH sensitive alginate–GG hydrogel cross-linked with glutaraldehyde (George *et al.*, 2007). This cross-linker however has a downside in which it is toxic and can impede the cell growth (Fürst *et al.*, 2005).

e. Condensation reaction

Condensation reactions can also be used to cross-link the biopolymers with the polar functional groups –OH and $–NH_2$ with –COOH or derivatives, for example for the synthesis of polyester and polyamide. This method is used to cross-link the hydrophilic

polymers such as poly(vinyl alcohol), carboxymethyl cellulose, carrageenan and alginates (Akhtar *et al.*, 2016).

f. Enzyme as cross-linker

Cross-linking by enzymes is a new enhanced method compared to 'traditional' photo cross-linking by irradiation, chemical and physical cross-linkings. Different enzymes are used for cross-linking the hydrogels. A study conducted by Chen *et al.* used microbial trans-glutaminase and mushroom tyrosinase to catalyse the gel formation by blending of gelatin and chitosan (Chen *et al.*, 2003).

g. Esterification

Esterification occurs in –OH or –COOH groups of polymers. High temperature and a cross-linker agent are required to form hydrogel. For instance, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, citric acid, and fumaric acid are some of the commonly used in an esterification reaction to form a hydrogel (Kayra *et al.*, 2019). Preparation of superabsorbent hydrogels by Kono *et al.* dissolved native cellulose in mixture of lithium chloride and N-methyl-2-pyrrolidinone (LiCl/NMP) esterified with 1,2,3,4-butanetetracarboxylic dianhydride (BTCA) (Kono *et al.*, 2014).

2.4.2.2 Grafting

Hydrogel preparation based on grafting, involves the polymerisation of a monomer on the backbone of a preformed polymer (Varaprasad *et al.*, 2017). This method can impart various functional groups to a polymer. Graft polymers are also called as graft copolymer which contain at least two different types of monomer units such as the grafted side chains that are different structurally from the main chain. The monomer to be grafted may be of one or more types; therefore, the graft chains in grafted copolymer may either be homopolymers or copolymers (Sherazi, 2016).

2.5 Uses of Hydrogel

Smart hydrogels have been used in diverse applications, such as in making artificial muscles chemical valves, immobilisation of enzymes and cells, and concentrating dilute solutions in bioseparation (Qiu *et al.*, 2001). Some of the common biomedical applications of hydrogels are biosensors, bioadhesives, contact lenses, delivery of drugs, proteins and genes, therapeutical implants and dressing (Gyles *et al.*, 2017). Hydrogels can also be used as a biomaterial and coating for medical devices, which are used in the hygiene products and tissue engineering scaffolds (Caló *et al.*, 2015).

2.6 Biomedical Applications of Hydrogel

Hydrogels are one of the most popular polymer groups often used in numerous biological and biomedical applications due to their ideal properties of biocompatibility and similarity to living tissue (Kabir *et al.*, 2018). Some of the more common biomedical applications resulting from these studies include contact lenses, drug delivery applications, conductive biomedical applications and tissue engineering applications.

2.6.1 Hydrogels in Contact Lens

After the synthesis of poly(2-hydroxyethyl methacrylate) (pHEMA) by Witcherle and Lim for contact lens application in 1960, hydrogels are extensively studied till today. Contact lenses are generally classified as either ‘hard’ or ‘soft’ depending on their elasticity. Hard lenses are longer lasting compared to soft lenses and are based mainly on hydrophobic materials such as poly(methyl methacrylate) (PMMA) or poly(hexa-fluoroisopropyl methacrylate) (HFIM); however they require a longer tolerance time for the wearers (Caló *et al.*, 2015).

2.6.2 Hydrogels in Drug Delivery

Controlled drug delivery systems are used to release correct dose of drugs at certain rates during required periods of time. This allows maximizing their efficacy of the therapeutic while minimising the possible side effects. The stunning properties of hydrogels make them a great choice in drug delivery applications. The possibility of releasing pharmaceuticals for extended period of time (sustained release) is the main benefit obtained from hydrogels in drug delivery investigations, resulting in the supply of long period of time of a high concentration of drug to a specific location (Bahram *et al.*, 2016). There are different ways in which drug can enter the body, such as oral implants, parental, subcutaneous, nasal, ocular, epidermal, transdermal, injectable form, and wound dressing (Peppas *et al.*, 2000).

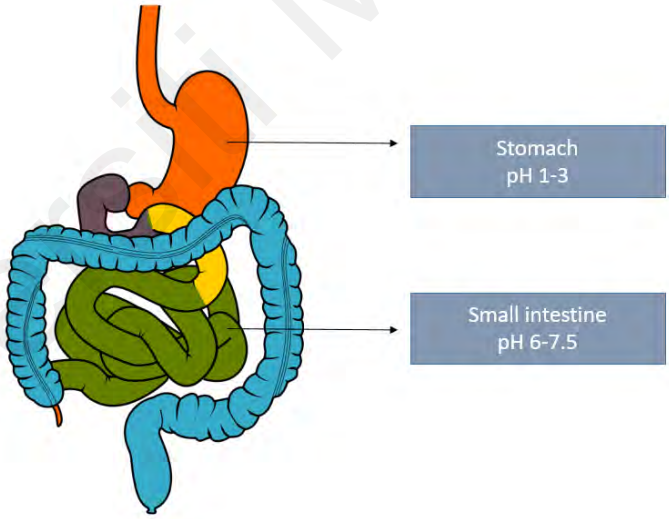

Figure 2.4: pH in GIT tract of human body (Sharpe *et al.*, 2014)

A wide range of pH variations along the gastrointestinal tract (GIT) is illustrated in **Figure 2.4** is used for biodegradable stimuli sensitive hydrogels for targeted drug delivery application. Stomach and intestine have different pH levels in which hydrogels produced for the controlled release of drug to intestine must swell a bit in acidic condition (pH 1.2) and swell the most (burst of drug release) in slightly alkaline environment (pH 7.4) as in

Figure 2.4. Subrasheema Das *et al.* has synthesised pH-responsive GG hydrogels for controlled delivery of dexamethasone to the intestine (Das *et al.*, 2015). Bashir *et al.* synthesised N-succinyl chitosan-g-poly(acrylamide-co-acrylic acid) hydrogels for *in vitro* drug release studies. This hydrogel displayed that swelling is higher in pH 1.2 compared to pH 7.4 (Bashir *et al.*, 2017).

2.6.3 Hydrogels in Conductive Biomedical Applications

Biopolymer-based polyelectrolyte hydrogels are ion conductive and are electrochemically stable and hence can be used to design electrochemical devices for biomedical applications such as electrode coating, bio-electrodes, biosensors, and gas sensors. These electrical devices are pH sensitive, low manufacturing cost, solvent resistant and excellent in mechanical properties (Guimard *et al.*, 2007). Biocompatible characteristics of polymer hydrogels are benefits of conducting polymer hydrogels relative to traditonal metal or semiconductor materials (Yi *et al.*, 2016). Lira *et al.* synthesised polyaniline–polyacrylamide composites by electropolymerisation of the conducting polymer inside an insulating hydrogel matrix with different pore sizes. This resulting new material was electroactive due to the presence of polyaniline inside the pores and were applied to electrochemically controlled drug delivery devices (Lira *et al.*, 2005).

2.6.4 Hydrogels in Tissue Engineering

Millions of patients suffer from the loss or malfunction of an organ or a tissue caused by an accident or a disease every year (Lee *et al.*, 2001). Hydrogels work as scaffolds to mimic cellular functions of ECM, producing new tissues (Radhakrishnan *et al.*, 2015). These scaffolds provide space and nutrients for a desired new tissue generation and regulate the structure and function of the engineered tissue (Shen *et al.*, 2016). Han *et al.* prepared an injectable calcium silicate/sodium alginate hybrid hydrogel; within 10 min,

this hydrogel formed internal *in situ* gelling due to calcium ions being released from calcium silicate in the presence of D-gluconic acid *δ*-lactone. This hydrogel was shown to promote the proliferation and differentiation of osteogenic and angiogenic cells (Han *et al.*, 2013).

2.7 Guar Gum

GG is a non-ionic polysaccharide, composed of galactose (Gal) and mannose (Man) units from the seed of *Cyamopsis tetragonoloba L*. The backbone of GG is a linear β-D-(1–4) linked mannose units having α-D-galactopyranose units connected by (1–6) linkages (**Figure 2.5**).

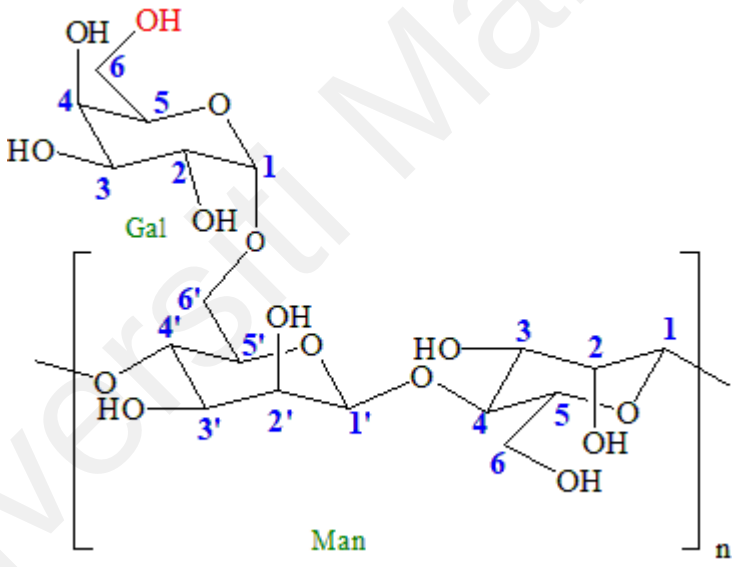

Figure 2.5: Chemical structure of guar gum

It is a white to creamish amorphous powder, nearly odourless, dispersible in cold and hot water to form a translucent and colourless to whitish colloidal solution but insoluble in most organic solvents (Nemade *et al.*, 2015). India is the major producer and exporter of GG, contributing around 80% to the world production. Rajasthan and Haryana States produce 85% of the total production of GG in India (Nemade *et al.*, 2015).

GG has important properties such as biodegradability, non-toxicity and easily available. Other than acting as excellent thickener and stabiliser, GG is also resistant to oils, chemicals and greases as well as having gel and film forming ability. GG has stability in wide pH range (pH 4.5-10) and can be degraded in colon and thus making it suitable for wide pharmaceutical applications (Hasan *et al.*, 2018; Seeli *et al.*, 2016). However, in its native form, GG is highly hydrophilic due to the presence of many hydroxyl groups enabling it to form hydrogen bonds, thus exhibiting enormous swelling in water and can induce a burst release of drugs (Rana *et al.*, 2011). GG has high ability for chemical modification and cross-linking, changing its swelling power and rheology to diversify its application (Hasan *et al.*, 2018). GG has been proven particularly useful for colon delivery as it can be degradable by specific enzymes in the intestinal tract (Sinha *et al.*, 2001).

2.7.1 Guar Gum-Based Hydrogels

GG-based hydrogels have drawn significant interest in research and industry for exploring miscellaneous applications such as biomedical, agriculture, food, textile, explosives and cosmetics (Thakur *et al.*, 2018). GG has the ability to form hydrogels. The properties of GG based hydrogels are governed mainly by the primary interaction with water molecules via hydrogen-bond formation, thus has a direct influence on swelling, retention of water, and moisture sorption (Raouf, 2019). To control the enormous swelling of GG in water which will induce a burst release of drugs (Rana *et al.*, 2011), modification of GG is needed to substitute some of the hydroxyl group with hydrophobic moieties and at the same time improving its organosolubility property. Thus, the swelling of GG can be reduced, resulting in sustained release of drugs from the hydrogel. Like other polymers used for colon targeting, GG-based hydrogels can protect the drug from bursting in the stomach and small intestine environment, while delivering the drug to the colon. Here, it

undergoes assimilation by specific microorganisms or degradation by the enzymes, leading to the final drug delivery. Hence, it can be used either to form prodrugs, as a coating material or as a hydrogel entrapping drugs inside its network (Thakur *et al.*, 2015).

2.7.2 Limitations of Guar Gum

The exploitation of GG in various applications is often inhibited by few downsides. First, GG is susceptible to microbial contamination. The equilibrium moisture content present in GG is normally around 10% and in terms of structure, they are carbohydrates. Microbial contamination may occur as GG is exposed to the external environment during production, limiting its long term application. GG also encounters uncontrolled rate of hydration which reduces its viscosity for long term storage (Thombare *et al.*, 2016). Unlike synthetic polymer having controlled procedure with fixed ingredient quantities, GG as a biopolymer has batch to batch variation depending on environmental and seasonal factors. There are inconsistencies in the collection of natural materials at different times, as well as differences in region, species, and climate conditions that affect the percentage of chemical constituents present in GG.

2.7.3 Chemical Modifications of Guar Gum

The limitations of GG as mentioned above have driven researchers to further design modifications of GG. GG can be chemically modified into various derivatives by substitution of the reactive hydrogen from free hydroxyl groups along the macromolecular backbone with different reactive functional groups. These modifications can overcome its inherent difficulties such as the pH-dependent solubility, viscosity reduction due to uncontrolled rate of hydration, turbidity in aqueous dispersion and high susceptibility to microbial attack which limit its long term application and improve its solubility and hydrophobicity (Hasan *et al.*, 2018). The chemical modification of GG involves oxidation, etherification, and esterification (Hasan *et al.*, 2018).

Chemical oxidation of GG is the substitution of the hydroxyl groups of GG through oxidation with formation of aldehyde or carboxyl groups and /or substitutive mechanisms which typically involve a series of reaction (Lewicka *et al.*, 2015).

Esterification of GG involves the conversion of the available hydroxyl groups to alkyl or aryl derivatives. The most common is acetylation where the hydroxyl group is substituted with ester, takes place under the influence of organic and inorganic acids and their derivatives such as acid anhydrides, oxychlorides, and chlorides. These GG esters are widely used in food, biotechnology and textile industries (Lewicka *et al.*, 2015).

Etherification is a modification where hydroxyl groups of GG are substituted with either carboxymethyl, hydroxypropyl, and/or hydroxyethyl groups through formation of an ether link (R-OR). Typically, etherification requires alkaline catalyst. Sodium hydroxide is commonly used to initiate chemical substitutions. The summarised chemical modifications of GG are shown in **Figure 2.6**.

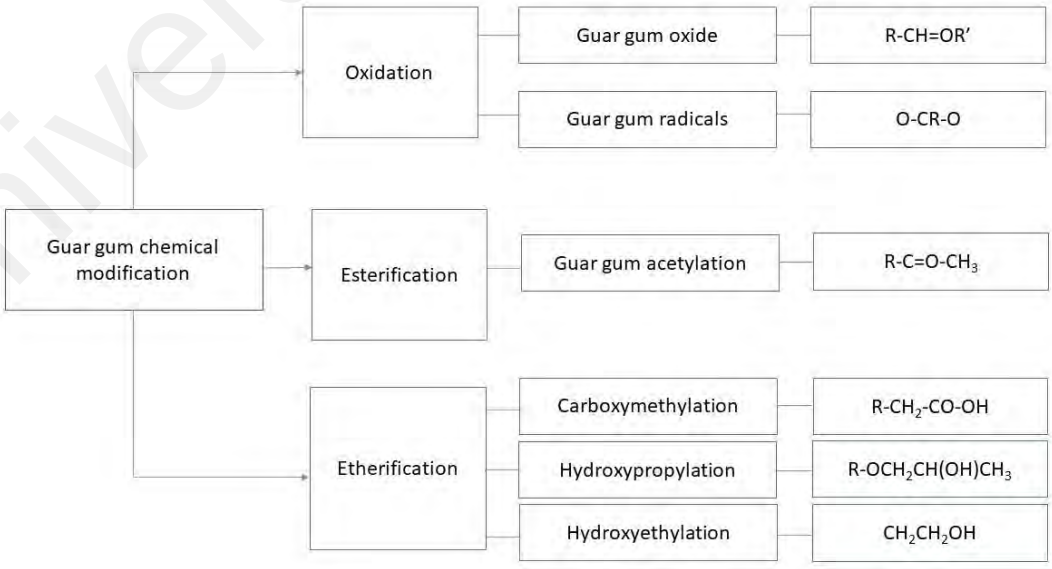

Figure 2.6: Chemical modifications of guar gum with their functional groups

Some examples of modifications of GG are listed in **Table 2.1**.

Table 2.1: Some of chemical modifications of guar gum from literature review

Chemical modification	Example	Reference
Oxidation	C-6 hydroxyl groups of galactose unit side chains were oxidised first by D-Galactose oxidase to aldehyde groups and then to carboxylic groups by halogen oxidation.	(Frollini *et al.*, 1995).
	Reacting GG with hydrogen peroxide and a small amount of solvent for reactive dry printing.	(Gong *et al.*, 2011)
Etherification	Carboxymethylation	
	Reaction of GG and monochloroacetic acid in the presence of NaOH.	(Gong *et al.*, 2011)
	Carboxymethyl GG nanoparticles-based conductive polyaniline/carboxymethyl GG nanocomposites was synthesised by chemical oxidative method by potassium dichromate as an oxidant.	(Gupta *et al.*, 2015; Verma *et al.*, 2014)
	Hydroxypropylation	
	The partially hydrolysed hydroxypropylated GG was prepared using epoxy propane as the etherifying agent and HCl as the hydrolysis agent.	(Hongbo *et al.*, 2014)
	Hydroxyethylation	
	Hydroxyethyl GG has been synthesised and its rheological properties was investigated.	(Patel *et al.*, 1987)
	Reaction of GG with ethylene oxide in the presence of NaOH as catalyst.	(Lapasin *et al.*, 1991)
Esterification	Esterification of GG using acetic anhydride as acetylating agent.	(Hongbo *et al.*, 2017)
	GG with succinic anhydride as reactant.	(Fujioka *et al.*, 2009)

2.7.4 Applications of Guar Gum

The potential of GG has been broadened in numerous applications such as biomedical, textile, agriculture and food as listed in **Table 2.2**. The GG properties can be further tailored and tuning the physical and chemical composition to make it best fit for any application either by grafting, blending and chemical modification with both synthetic and biopolymers. Hence, GG is a versatile raw material that can exploited in new arenas of human interests (Thombare *et al.*, 2016).

Table 2.2: Applications of guar gum and their examples

Application	Example	Reference
Biomedical	Antibacterial properties of GG-polyacrylic acid-polyaniline.	(Kaith *et al.*, 2015)
	Carboxymethyl GG for tissue engineering.	(Kundu *et al.*, 2018)
	In-vitro drug release of 5-fluorouracil of GG compression-coated tablet.	(Krishnaiah *et al.*, 2002)
	GG grafted with poly(epsilon-caprolactone) for *in vitro* controlled drug delivery of ketoprofen.	(Tiwari *et al.*, 2010)
Textile	Carboxymethyl GG as thickener in textile printing technology.	(Dure Najaf *et al.*, 2020; Schneider *et al.*, 2003)
Agriculture	Polyacrylamide grafted carboxymethyl GG for waste water treatment.	(Prabaharan, 2011; Tiwari *et al.*, 2010)
	GG grafted with acrylic acid and cross-linking with ethylene glycol dimethacrylic acid (EGDMA) for agriculture sector.	(Thombare *et al.*, 2018)
Food	GG improve rheological characteristics of dough suitable for chapatti making.	(Arya, 2009)
	GG improve rheological, physical and sensory characteristics of low fat ice cream.	(Javidi *et al.*, 2016)

CHAPTER 3: MATERIALS AND METHOD

3.1 Materials

The chemicals, solvents and reagents and the corresponding suppliers used in the synthesis processes during experimental work are listed in **Table 3.1**. The chemicals have been used as received without further purification.

Table 3.1: List of chemicals and their uses in the synthesis

Material /Chemical	Purity (%)	Manufacturer
Guar gum	-	Sigma-Aldrich
Acetone	-	Chemiz
Acetonitrile	99.0	Chemiz
Ethanol	95.0	Chemiz
Isopropanol	-	Chemiz
Chloroform	-	Merck
Dichloromethane	-	Merck
N,N-dimethylacetamide	99.9	Merck
N,N-dimethylformamide	99.8	Merck
dimethylsulfoxide	99.9	Merck
Phthalic anhydride	-	Merck
Pyridine	99.8	Merck
Sodium hydroxide	-	Merck
Tetrahydrofuran	99.9	Merck
Vinyl acetate	99.0	Tokyo Chemical Industry Co.

3.2 Chemical Modifications of Guar Gum

3.2.1 Synthesis of GGA

The transesterification of GG was adopted from literature where cellulose was transesterified using vinyl acetate (Cao *et al.*, 2014). An amount of 1.0 g of Native GG powder was mixed with 50 mL dimethylsulfoxide (DMSO). Then, 1.25 mL of 10 M NaOH was added dropwise to the mixture and stirred for 15 min at room temperature to activate the hydroxyl groups. The mixture was heated to 100°C for 5 min followed by the addition of 3.18 g of vinyl acetate with vigorous stirring and continuous heating for another 2 h. Later, the mixture was precipitated and washed in ethanol thrice. The off-white product was dried in vacuum oven at 60°C until constant weight.

Yield (%) of GGA was obtained from Equation (3.1) (Chen *et al.*, 2016):

$$\text{Yield} = \frac{W_1}{1.519 \times W_0} \times 100\% \quad (3.1)$$

where, W_1 is the weight of GGA sample obtained whereas W_0 is the weight of Native GG powder used and 1.519 is the molecular weight ratio of GGA (M = 738) to the original anhydroglucose unit (AGU) (M = 486).

3.2.2 Synthesis of PHGG

The synthesis of PHGG was adopted from literature where starch was esterified using phthalic anhydride (Selvanathan *et al.*, 2017). An amount of 1.0 g of Native GG powder was dispersed in 15 mL pyridine and 30 mL DMSO. The temperature was raised to 90°C and 5.32 g of phthalic anhydride was gradually added. Reaction was continued for 6 h, and the mixture was precipitated in acetone and later washed with isopropanol thrice. The off-white coloured product was dried in vacuum oven at 60°C until constant weight.

Yield (%) of PHGG obtained was calculated as in Equation (3.2) (Chen *et al.*, 2016):

$$\text{Yield} = \frac{W_1}{1.305 \text{ x } W_0} \text{ x } 100\% \quad (3.2)$$

where, W_1 is the weight of PHGG sample obtained whereas W_0 is the weight of Native GG powder used and 1.305 is the molecular weight ratio of PHGG (M = 634) to the original anhydroglucose unit (AGU) (M = 486).

3.3 Characterisation of the Modified Guar Gum

3.3.1 Fourier-Transform Infrared

The Fourier-transformed infrared (FTIR) used in the qualitative and quantitative determination of polymers composition and is a fast and well recognized fingerprinting method to determine functional groups. FTIR analysis was performed via the ATR technique on a PerkinElmer Spectrum 400 instrument, with acquisition parameters of 32 scans and a resolution of 4 cm^{-1} in the range of 650–4000 cm^{-1} at room temperature to identify carboxyl, ester, hydroxyl, unsaturation and aromatic ring which are present in GGA and PHGG. The relative percentage of –OH left after both modifications with Native GG are compared after normalization the appropriate peaks (refer **Appendix B**) (Selvanathan *et al.*, 2017).

3.3.2 Proton Nuclear Magnetic Resonance

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is an analytical technique to determine the molecular structure of sample. It can identify the carbon-hydrogen framework of e.g. an organic compound and the functionality at a specific carbon and its neighbouring carbons. ^{1}H NMR was taken at 400 MHz with an accumulation of 32 scans using a JEOL ECA 400 spectrometer to verify the chemical modifications of Native GG of about 0.72% w/v. The solvent used for Native GG was D_2O whereas DMSO-d_6 was

used for GGA and PHGG with sample concentration of about 2.15% w/v. All chemical shifts were reported in parts per million (ppm) (Li *et al.*, 2017).

3.3.3 X-Ray Diffraction

X-Ray diffraction (XRD) spectra of the samples were recorded on a PANalytical EMPYREAN Diffractometer. The samples were scanned at 2θ angles between 5-60°. The degree of crystallinity x_c values of the samples were calculated using the Equation (3.3) (Yusof *et al.*, 2017):

$$x_c = \frac{A_c}{A_t} \text{ x } 100\% \tag{3.3}$$

where A_c and A_t are the areas of crystalline and total hump, respectively. The percentage of crystallinity of samples were determined by High Score Plus (version 3.0.4) software.

3.3.4 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) is the changes in the mass of material during a controlled temperature ramp which can be used to determine the thermal stability of polymers by comparing the degradation curves. The derivative curve of TGA (dTG) can be used to improve the determination of onset and end point of decomposition in multi-polymer systems. The degradation profiles were used as guideline to later run differential scanning calorimetry (DSC) analysis. A PerkinElmer TGA 4000 instrument was used. An amount of 3 mg of samples were placed in a platinum pan and heated from 30°C to 800°C under inert Nitrogen atmosphere at a heating rate of 10 cm^{-1} min^{-1} (Cao *et al.*, 2014).

3.4 Preparation of Hydrogels

Native GG (0.4 g) was dissolved in 40 mL deionised water and stirred for 24 h at room temperature to form 1% (w/v) solution. The solution was poured into a mould with

dimension of 4 cm x 4 cm, dried at 45°C for 1 day and vacuum dried for 6 h. GGA and PHGG (0.4 g each) on the other hand were dissolved in DMSO (10 mL) for 2 h at 60°C to form 4% (w/v) solution. These solutions were poured into a mould with dimension 4 cm x 4 cm and dried at 60°C for 4 days. Both GGA and PHGG samples were rinsed with distilled water (to remove the excess trapped DMSO), dried again at 60°C for 1 day and then vacuum dried for 6 h at same temperature. The dried samples were stored in vacuum desiccator till further use.

3.5 Swelling Behaviour

3.5.1 Solvent Compatibility

The solvent compatibility was performed by immersing 2.5 mg of the solid samples (Native GG, GGA and PHGG) in 1.0 mL of the test solvents with varying polarity indices and stirred for 30 min. Observations were made for conditions at room temperature, 50, 60, 70 and 80 °C. The density of each sample was determined by toluene displacement method using a pycnometer and Hansen Solubility Parameter (HSP) was used to determine the solvent compatibility by using Excel's Solver of Microsoft. The score of "1" are given to those solvents evaluated as good (swell) and "0" for bad solvents (not soluble nor swell); the cells that were not in use for the solvents in the evaluation are left blank (**Appendix F**) (de los Ríos *et al.*, 2020).

3.5.2 Swelling in Distilled Water

Experiments for the swelling behaviours of Native GG, GGA and PHGG hydrogels were carried out in triplicates (Rasool *et al.*, 2019). An amount of 0.4 g of solid was immersed in 50 mL distilled water at ambient temperature. At different time intervals, the weight of the swollen sample was determined after carefully wiping off the excess solution on the surface until equilibrium point was reached (Mahdavinia *et al.*, 2014). The

swelling percentage of the sample was determined gravimetrically using the Equation (3.4) (Panariello *et al.*, 2008):

$$\text{Swelling percentage (\%)} = \frac{W_{s-}\, W_d}{W_d} \text{ x } 100\% \quad (3.4)$$

where W_d is the dry weight and W_s is the swollen weight of the sample at time t.

3.5.3 Swelling in Ionic Salt Solution

Ionic salt solution (simulated normal saline isotonic solution) consisting of 0.9% of sodium chloride (NaCl) was prepared by dissolving 9 g of NaCl in 100 mL of deionised water. Experiments for the swelling behaviours of Native GG, GGA and PHGG hydrogels were carried out in triplicates (Pourjavadi *et al.*, 2008). An amount of 0.4 g solid sample was immersed in 50 mL 0.9% NaCl at 37°C. The weight of the swollen sample was determined after carefully wiping off the excess solution on the surface at swelling equilibrium point (determined from Section 3.5.1). The swelling percentage was then calculated using Equation (3.4) .

3.5.4 Swelling in Buffer Solutions

Buffer solutions were used to simulate gastrointestinal tract (GIT) simulated gastric fluid (SGF) (pH 1.2) was prepared by mixing 50 mL of 0.2 M solution of potassium chloride (KCl) and 64.5 mL of 0.2 M HCl, adjusting the pH and diluted up to 200 mL with deionised water. Simulated intestinal fluid (SIF) (pH 7.4) was prepared by dissolving each phosphate buffer saline tablet supplied by Sigma Aldrich and diluted up to 200 mL with deionised water. Experiments for the swelling percentage of Native GG, GGA and PHGG hydrogels were carried out in triplicates (Minhas *et al.*, 2013). 0.4 g of solid was immersed in 50 mL of buffer at pH 1.2 and pH 7.4 at 37°C. The weight of the swollen sample was then determined after carefully wiping off the excess solution on the surface

at swelling equilibrium point (determined from Section 3.5.1). The swelling percentage was calculated using Equation (3.4).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Synthesis of GGA and PHGG

Studies on acetylated GG is still scarce. Although modifications of GG into GGA has been done previously, it uses an unpleasant, highly corrosive acetic anhydride as acetylating reagent, catalysed by acetic acid and pyridine as solvent (Hongbo *et al.*, 2017). The replacement of acetic anhydride with vinyl esters such as vinyl acetate to GGA is very crucial in order to avoid the acidic environment. In 2016, Singh *et al.* has employed vinyl acetate to modify GG, however it was applied in a binary mixture system together with ethyl acrylate with potassium persulfate/ascorbic acid redox initiator system (Singh *et al.*, 2016a). In another study, GGA has been synthesized by using poly(vinyl acetate) through grafting method using the same redox initiator (Singh *et al.*, 2016b). As mentioned earlier in Section 1.1, the usage of vinyl acetate alone in guar gum by transesterification has not been reported. Some of the other polysaccharides that have been modified using vinyl acetate include cellulose and chitin (Chen *et al.*, 2016; Yu *et al.*, 2019). On the other hand, phthaloyl guar gum (PHGG) modification has also never been published yet. Some of the natural polymers that have been successfully modified using phthalic anhydride are chitosan (Aziz *et al.*, 2012; Karuna *et al.*, 2018), starch (Selvanathan *et al.*, 2017) and chitin (Uzun *et al.*, 2012).

Figure 4.1 shows the plausible reaction scheme for the formation of GGA and PHGG. Generally transesterification occurs at the Native GG Gal C6 hydroxyl group with the carboxyl group of the acid as it is more susceptible for the reaction to take place due to greater reactivity of primary hydroxyl group (Shenoy *et al.*, 2010) compared to other secondary hydroxyl group in GG chemical structure (Sittikijyothin *et al.*, 2005). However, it should be noted that transesterification could also possibly occur at other hydroxyl positions of Native GG.

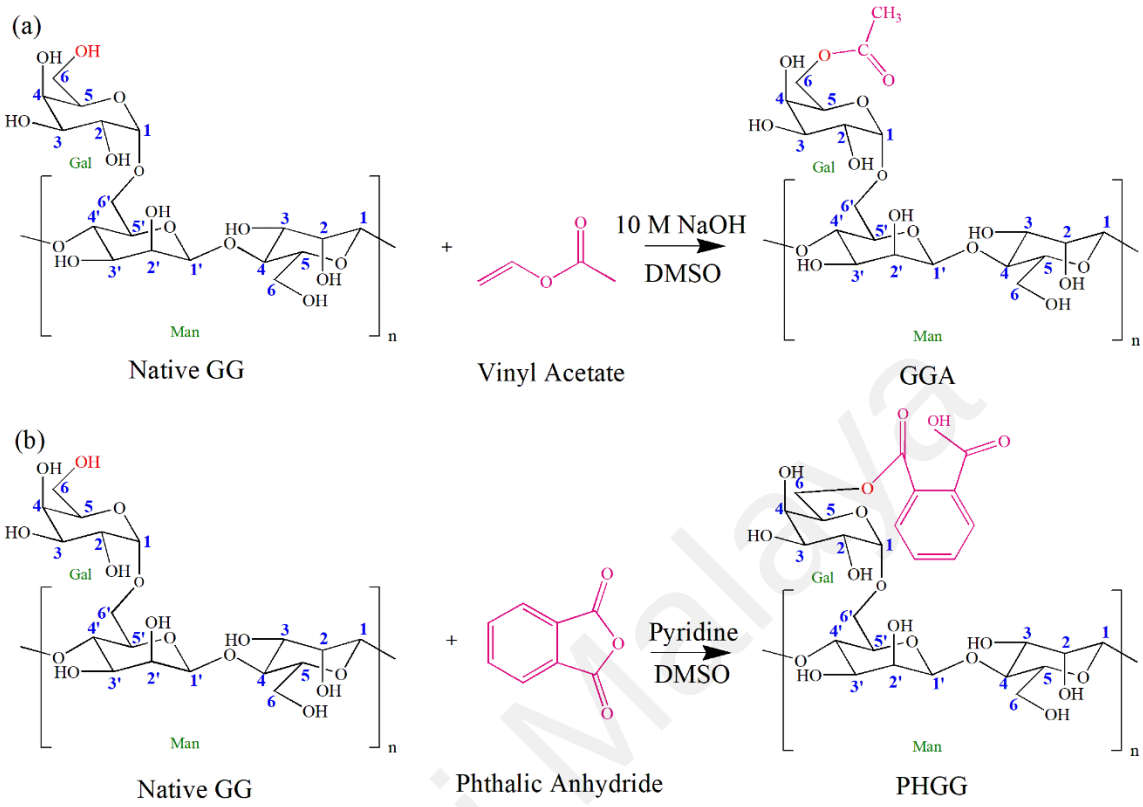

Figure 4.1: Plausible reaction scheme for the formation of (a) GGA and (b) PHGG

The synthesis of GGA and PHGG involved continuous stirring using magnetic bead. For synthesis of GGA, it took 2 h of reaction after addition of vinyl acetate at 100°C, while PHGG took 6 h at 90°C. Although GGA took shorter time of reaction, the DS of GGA was much higher which could be attributed to easier attachment of smaller acetate group compared to bulky phthaloyl group which will be discussed in Section 4.3. The product yield of GGA and PHGG are 95.46% and 91.95% respectively (See **Appendix A** for the calculation).

From physical appearance, Native GG, initially slightly yellowish powder turned to a bit more tense yellowish for GGA but more whitish for PHGG (**Figure 4.2**).

Figure 4.2: From left: GGA, Native GG and PHGG

The physical properties and chemical structures of GGA and PHGG are further discussed in Section 4.2 onwards.

4.2 FTIR Analysis

The FTIR spectra of Native GG, GGA and PHGG are displayed (**Figure 4.3**) to compare the changes in their chemical structures. The common feature of these spectra is the fingerprint region between 862 to 1145 cm^{-1} which corresponds to the stretching modes of the C-O-C in the saccharide ring (Rathore *et al.*, 2017). The other prominent peaks are at 1640 and 2910 cm^{-1} corresponding to –C-O– and $–CH_2–$ stretchings in the saccharide ring respectively (Mudgil *et al.*, 2012; Rathore *et al.*, 2017). The broad peak at 3310 cm^{-1} is assigned to the stretching vibration of –OH bond due to the presence of large number of free hydroxyl groups in the GG backbone (Rathore *et al.*, 2017).

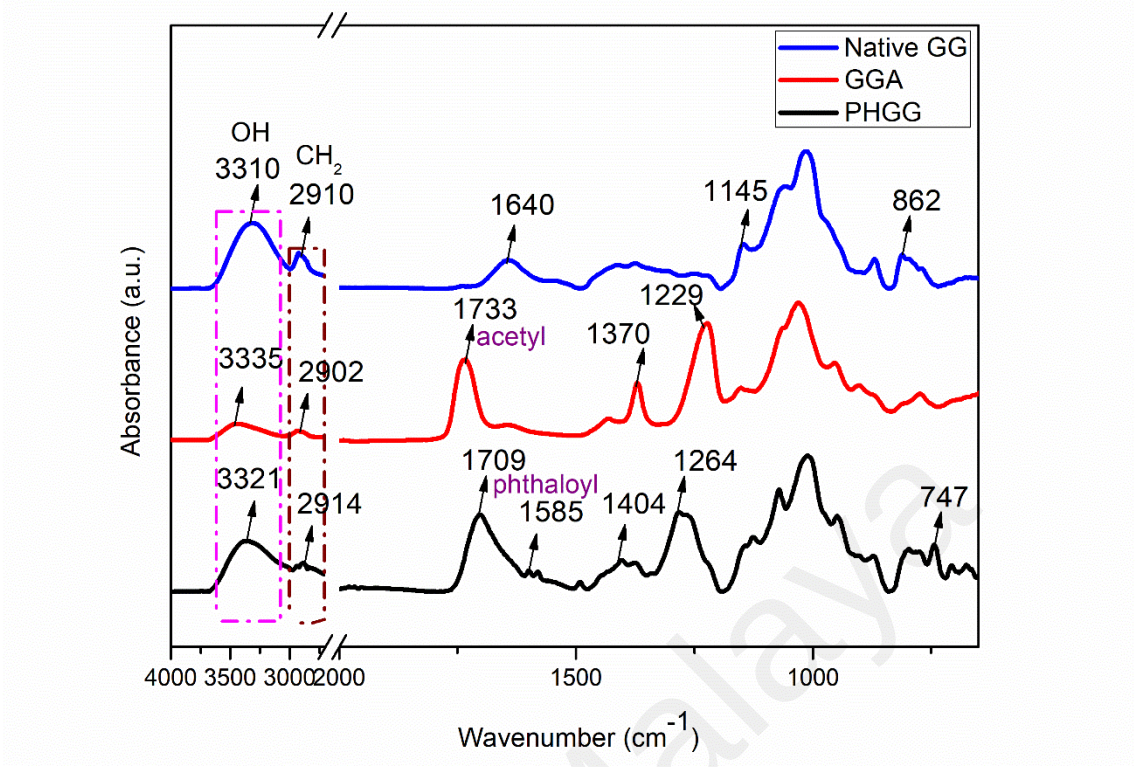

Figure 4.3: FTIR spectra of Native GG, GGA and PHGG

In the case of GGA, the peak intensities of –OH and –CH_2– has diminished slightly compared to those in Native GG, which indicate that some of the hydroxyl groups (26.50% of -OH left after modification, **Appendix B**) have been successfully replaced with the acetyl groups during the transesterification, (**Figure 4.3**). This is further confirmed by appearance of a new sharp peak at 1733 cm^{-1} that can be attributed to the presence of C=O stretching absorption of the carboxylic ester from the acetate moiety (Cao *et al.*, 2014; Schilling *et al.*, 2010) and a new absorption peak at 1370 cm^{-1} ascribed to the symmetric bending of C-CH_3 and the peak at 1229 cm^{-1} assigned to the asymmetric stretching of the carboxylate group (Ali *et al.*, 2014; Chen *et al.*, 2016).

However, in the case of PHGG, phthaloylation of Native GG into PHGG is by substituting the hydroxyl group with the bulky phthaloyl group (84.81% of -OH left after modification, **Appendix B**). The peak intensities of –OH and –CH_2- are comparably similar to those of Native GG as shown in the **Figure 4.3**. This suggests that during the

modification reaction, phthalic anhydride undergoes ring opening where half of the anhydride ring forms a new ester linkage with the hydroxyl group on Native GG and the other half forms a new –OH moiety, as shown in **Figure 4.3**, therefore preserving the quantity of the –OH groups in the system. The ensemble of many –OH groups in this new environment may explain the slight shift to a higher wavenumber than 3310 cm^{-1} when compared against Native GG. Due to the steric hindrance from phthalate in the new GG derivative, the inter/intra-molecular hydrogen bondings between –OH groups have become disrupted. C-H stretch of CH_2 group has also shifted from 2910 cm^{-1} to 2914 cm^{-1}. The attachment of phthalate group is further confirmed by the presence of a strong C=O peak at 1709 cm^{-1} due to the formation of links between (–OH–) of GG and (−COOH−) of phthalate groups (Selvanathan *et al.*, 2017) together with other noticeable new peaks at 1585 cm^{-1}, 1404 cm^{-1} and 747 cm^{-1} which correspond to the aromatic ring vibration present in phthalate group, C-O stretching in carboxyl, and out-of-plane C-H bending of the benzene ring (Wang *et al.*, 2016). The additional peak at 1264 cm^{-1} is assigned to esteric C-O stretch. These results confirm the successful esterification and attachment of the phthaloyl moieties to the GG backbone chain and are in agreement with previous literature (Selvanathan *et al.*, 2017; Ubaidulla *et al.*, 2007).

4.3 ^{1}H NMR Analysis

^{1}H NMR spectrum for Native GG is shown in **Figure 4.4(a)**. All of the C-H protons on the anhydroglucose unit (AGU) of H2-6 Gal and H2-H6’ Man GG appear very closely together between δ = 3.40 to 5.50 ppm. The most distinct of these peaks are at δ = 5.20 ppm (Dodi *et al.*, 2011) and 5.49 ppm (Kono *et al.*, 2014), which correspond to H1 resonance of Man ring and H1 Gal residue, respectively.

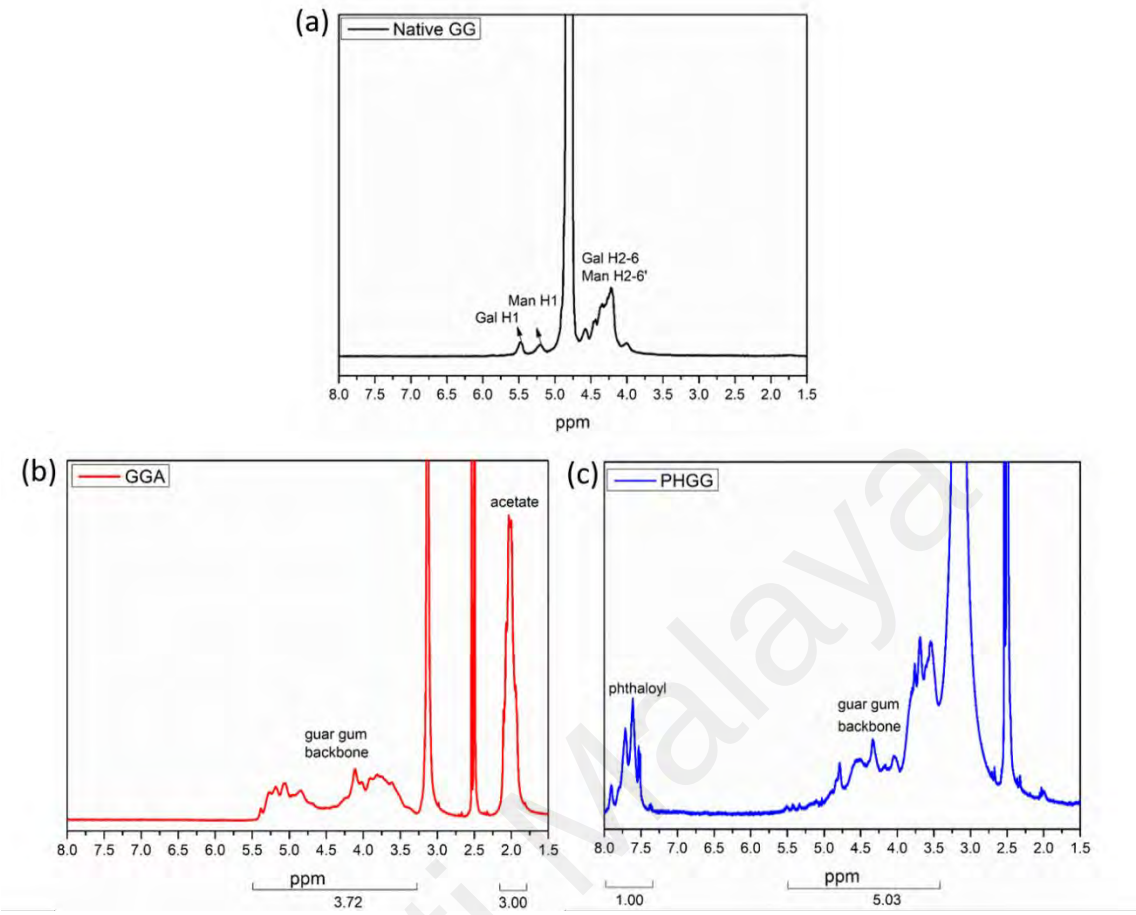

Figure 4.4: ^{1}H NMR spectra of (a) Native GG in D_2O-d_1, (b) GGA in DMSO-d_6 and (c) PHGG in DMSO-d_6

For GGA (**Figure 4.4 (b)**), a new set of peaks between 1.80 to 2.20 ppm, belongs to the methyl protons of the acetate group (Ass *et al.*, 2006; Chen *et al.*, 2016) and the peak at 3.30 ppm is assigned to the peak absorption of water. The integral area in this region is designated as I_A. In pristine vinyl acetate, the peaks at 4.56, 4.87 and 7.26 ppm confirm the success of the washing procedure to obtain the isolated GGA product. The integral area for the protons belonging to the GGA AGU unit in the region between δ = 3.40 to 5.50 ppm was designated as I_x. The degree of substitution (DS) for GGA was then calculated as in Equation (4.1) (Ifuku *et al.*, 2011; Sun *et al.*, 2013):

$$\mathrm{DS} = \frac{21 \times I_A}{3 \times I_x} \tag{4.1}$$

*$I_{x=}I_{AGU\ GGA}$ for GGA and $I_{x=}I_{AGU\ PHGG}$ for PHGG

From the spectrum, $I_{AGU\ GGA}$ = 3.72, I_A = 3.00, therefore DS of GGA was 5.65 suggesting that almost two thirds of the available reactive hydroxyl sites on the AGU unit have been substituted. This corroborates the observation for (Section 4.6.1) where it was found that GGA had greater compatibility with organic solvents, due to greater disruption of inter- and intra-molecular hydrogen bondings by the acetyl moieties.

In the case of PHGG (**Figure 4.4 (c)**), the new set of peaks between 7.40 to 7.90 ppm (integral area I_{PH}), is due to the four aromatic protons. These peaks appearing broad, indicate the successful attachment of the bulky phthaloyl group (as opposed to unreacted phthalic anhydride which has distinct narrow peaks at 7.60 and 8.00 ppm). The integral area for the protons belonging to the PHGG AGU unit in the region between δ = 3.40 to 5.50 ppm is designated as I_x. The peak at 3.30 ppm is assigned to the peak of absorption of water.

From the spectrum, $I_{AGU\ PHGG}$ = 5.03, I_{PH} = 1.00, therefore DS of PHGG was 1.04. It can be inferred that the substitution reaction favors only one phthaloyl group for every repeating unit of the GG backbone since the phthaloyl group is much larger than the acetyl group. This would also explain why there were fewer organic solvents that were compatible with PHGG as seen in Section 4.6.1 and **Appendix D**.

In comparison, GGA had a higher degree of substitution than PHGG. This could be due to the effect of steric hindrance imposed by the bulky phthaloyl groups. Being less

bulky, the attachment of acetate groups is expected to occur more easily on the polymer backbone.

It is noteworthy to mention ^{1}H NMR has been adopted as a fast and convenient way to determine DS of polymers. Some of earlier works have reported DS calculated through ^{1}H NMR include Cao *et al.* who synthesized cellulose acetate, cellulose propionate and cellulose butyrate (Cao *et al.*, 2014) and Chen *et al.* synthesized cellulose acetate made from microcrystalline cellulose and various plant sources (Chen *et al.*, 2016). The ratio of the area of acetyl group to the proton of AGU of biopolymer has been compared to calculate the DS after modification has taken place.

4.4 XRD Analysis

The XRD patterns of Native GG, GGA and PHGG are shown in **Figure 4.5**. The high intensity of the peaks at 17.2° and 20.4° is attributed to the predominantly crystalline component in Native GG (Rathore *et al.*, 2017). The broadening of the peak in the range of 2θ=10.0°–25.8◦ shows that it also possesses an amorphous structure to a lesser extent (Kumar *et al.*, 2015).

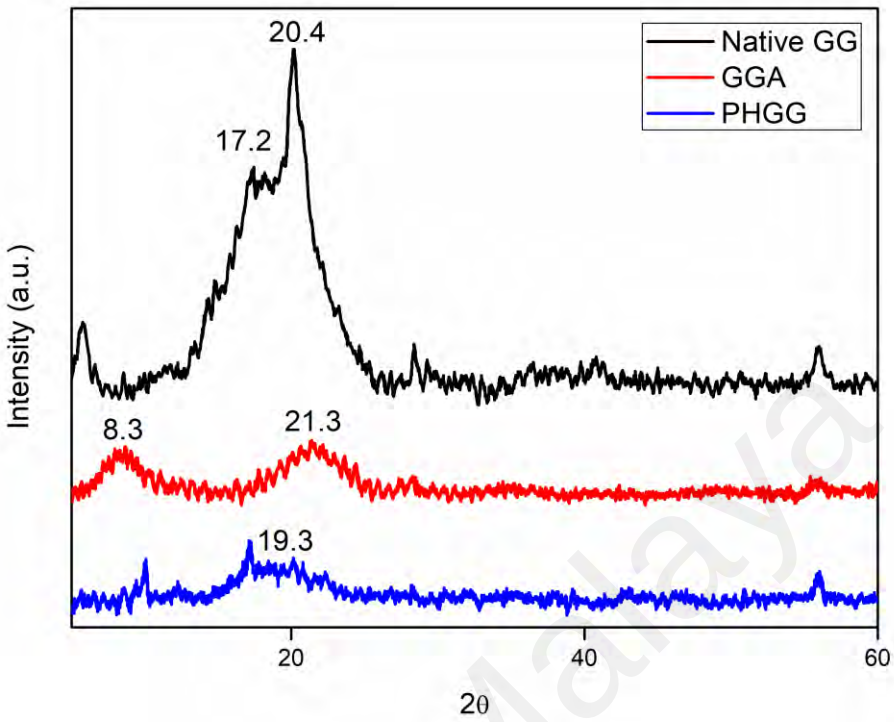

Figure 4.5: XRD of Native GG, GGA and PHGG

Upon chemical modification, both GGA and PHGG showed a drastic suppression of crystallinity indices, (GGA = 27.07% and PHGG 19.22%) compared to Native GG (31.45%). Steric hindrance from the acetyl or phthaloyl moieties would cause the breaking of inter- and intra-molecular hydrogen bonds of Native GG. With the introduction of new pendant groups along the GG backbone, the ordered crystalline structure becomes disrupted and in turn causes the incident X-rays to scatter in a wider range which explains the broad humps that are observed in the XRD pattern (**Figure 4.5**) resulting in increase amorphousness. This can also be related to the improved solubility in organic solvents such as DMSO and DMF in both GGA and PHGG as shown earlier (Section 4.6.1 and **Appendix D**) and earlier results reported by Nishimura (Nishimura *et al.*, 1991). In the case of GGA, the peak at $2\theta = 8.3°$ is assigned to principal characteristic of semi-crystalline region of acetylation (Sun *et al.*, 2013). For GGA, the residual crystallinity has shifted towards 21.3° whereas for PHGG, it has moved to 19.2° but still

possessing the native peak at 17.2° with decreased intensity. Relatively, PHGG depicts a much declined crystallinity than GGA despite the lower DS discussed earlier. Hence it is deduced that although lesser substitution of phthaloyl group took place, the bulky nature of the substituent plays a bigger role in manipulating the crystallinity of GG compared to higher substitution of linear acetate on GG (Selvanathan *et al.*, 2017).

4.5 TGA Analysis

The thermal profiles for Native GG, GGA and PHGG are illustrated in **Figure 4.6** and the quantitative comparisons are shown in **Table 4.1**. The samples underwent two stages of weight losses. The first stage was a minor weight loss which is due to adhered moisture, and the second major weight loss is attributed to degradation of the polymer backbone (Singh *et al.*, 2016b). Native GG exhibited initial weight loss at 65.0-133.0°C (Δwt = 1.23%) due to the removal of moisture. The major decomposition was observed with a maximum derivative peak temperature, D_{Tp} at 340.2°C. This second stage represented the thermal degradation of the polymer backbone (Hongbo *et al.*, 2017) with Δwt = 92.97%. For GGA, the evaporation of water occurred at similar region as Native GG of around 55.0-130.0°C (Δwt = 0.62%). The loss of water was less in comparison with Native GG, which suggests that GGA is more hydrophobic. On the other hand, desorption of water for PHGG occurred at around 55.0-137.0°C (Δwt = 2.16%), which suggests that it may have better water absorption capability.

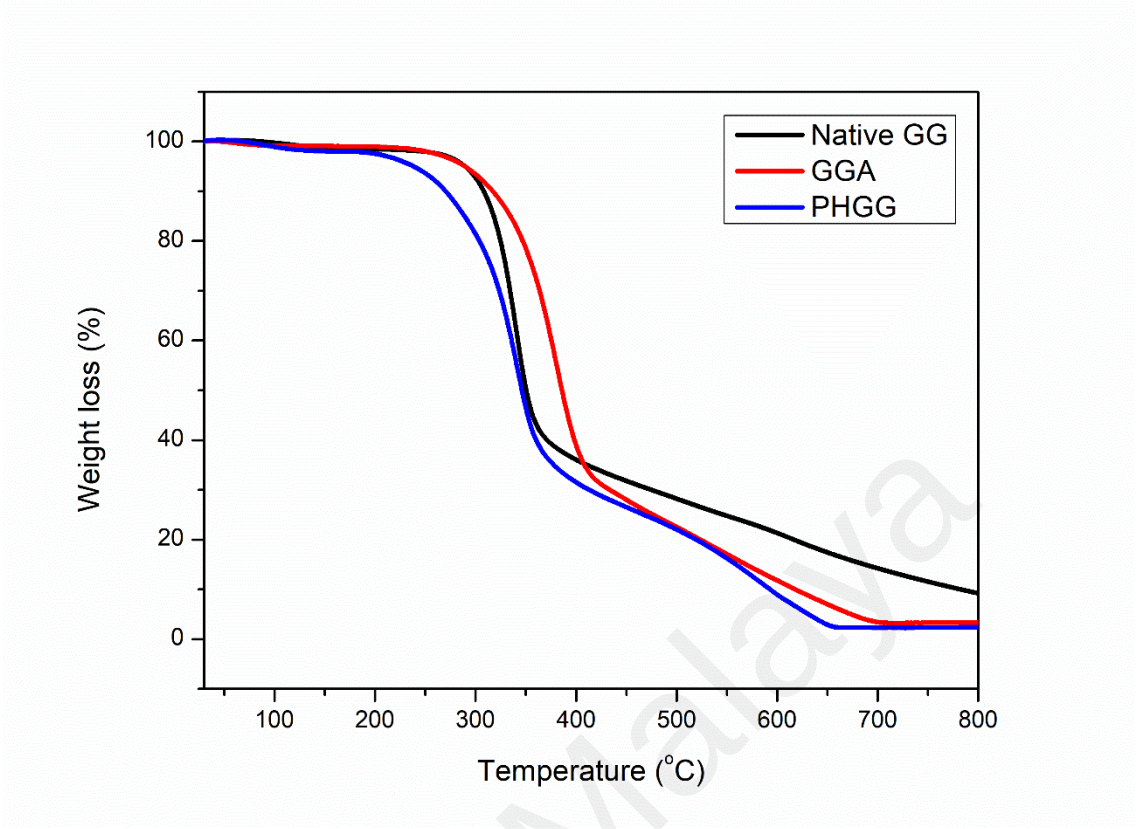

Figure 4.6: TGA curves of Native GG, GGA and PHGG

Table 4.1: TGA data of Native GG, GGA and PHGG

Sample	T_{onset} (°C)	D_{Tp} (°C)	$T_{50\%}$ (°C)	wt. loss (%)
Native GG	312.2	340.2	350.3	92.97
GGA	350.0	378.4	399.6	95.62
PHGG	294.2	340.7	346.0	95.51

The thermal stability for GGA was noticeably better compared to its parent material, with significantly higher values for onset of degradation temperature (T_{onset}=350.0°C), temperature at 50% wt loss ($T_{50\%}$=399.6°C) and D_{Tp} (378.4°C). Meanwhile as for PHGG, the observed data indicate a slight decrease in thermal stability as compared to Native GG.

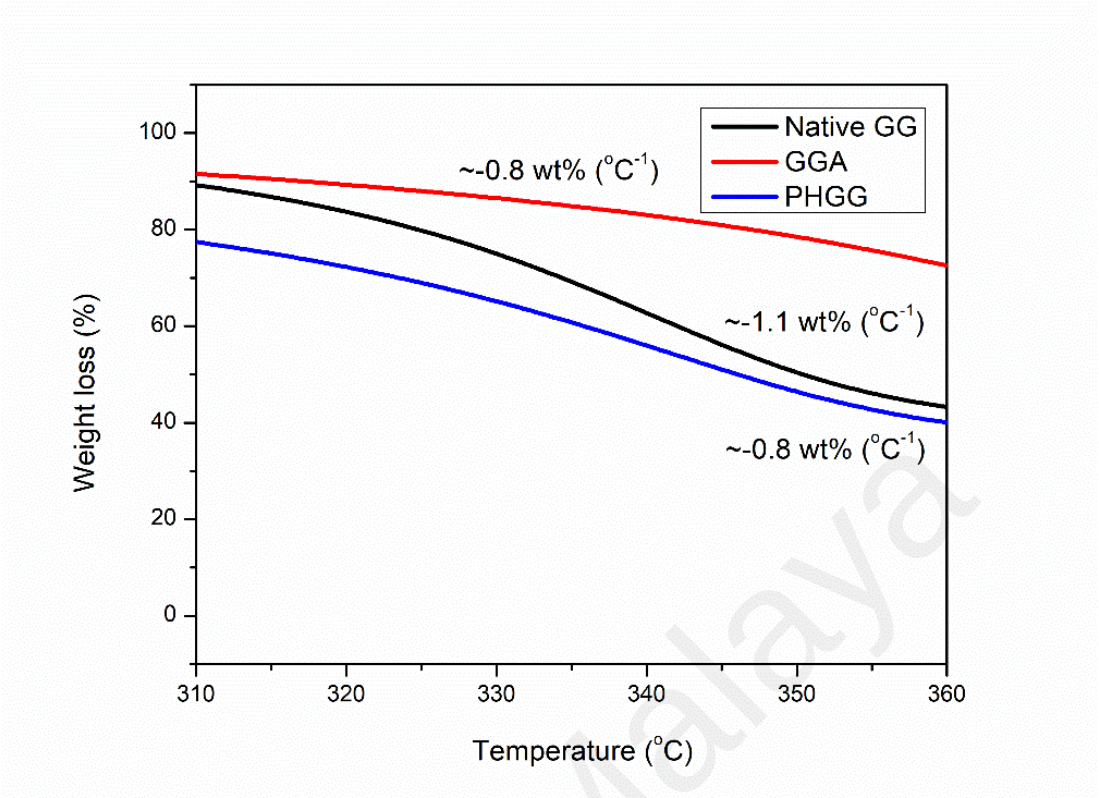

Figure 4.7: TGA curves of Native GG, GGA and PHGG from 310°C until 360°C

It should be noted that there is another aspect of thermal stability which can be deduced from the gradient of the thermal curves (Aziz *et al.*, 2012); both GGA and PHGG showed lower gradient of thermal curves (~ -0.8 wt% °C $^{-1}$) compared to Native GG which was steeper (~ -1.1 wt% °C $^{-1}$) (**Figure 4.7**). This would imply that in the presence of a pendant substituent the decomposition occurred more gradually over a wider range of temperatures. The total weight losses for both GGA and PHGG were also very similar (Δwt ≈ 95%). The final stage of decomposition began at around 409.5, 444.5 and 395.9°C for Native GG, GGA and PHGG, respectively. Hence, from the data it can be concluded that the grafting of linear acetate group into Native GG increases its thermal stability whereas attachment of bulky phthaloyl group decreases the thermal stability. In comparison to Native GG which left a char residue, GGA and PHGG were completely decomposed. In biomedical applications, the GGA and PHGG would be better than Native GG because they are able to completely degrade (Filip *et al.*, 2011; Torgbo *et al.*,

2020). Hence, from the data it can be concluded that the grafting of linear acetate group into Native GG increases its thermal stability whereas attachment of bulky phthaloyl group decreases the thermal stability. The TGA results corroborated with the XRD results discussed earlier (Section 4.4) whereby GGA exhibited higher crystallinity index than PHGG whereby a more ordered structure would lead to higher thermal stability (Santmartí *et al.*, 2018).

4.6 Swelling Behaviour

4.6.1 Solvent Compatibility

Amorphous polymer contains a network of entangled, flexible chains in a continuous motion. When there is presence of solvent, the polymer network will swell from osmotic activity of the solvent and increase its freedom to move sufficiently to allow transitional movement to the chains thereafter the substances will separate out to form a solution. Further dilution will result in the intermolecular forces that exist between the polymers chains to become weak and decrease, and finally the solution properties will exhibit polymer-solvent interaction forces.

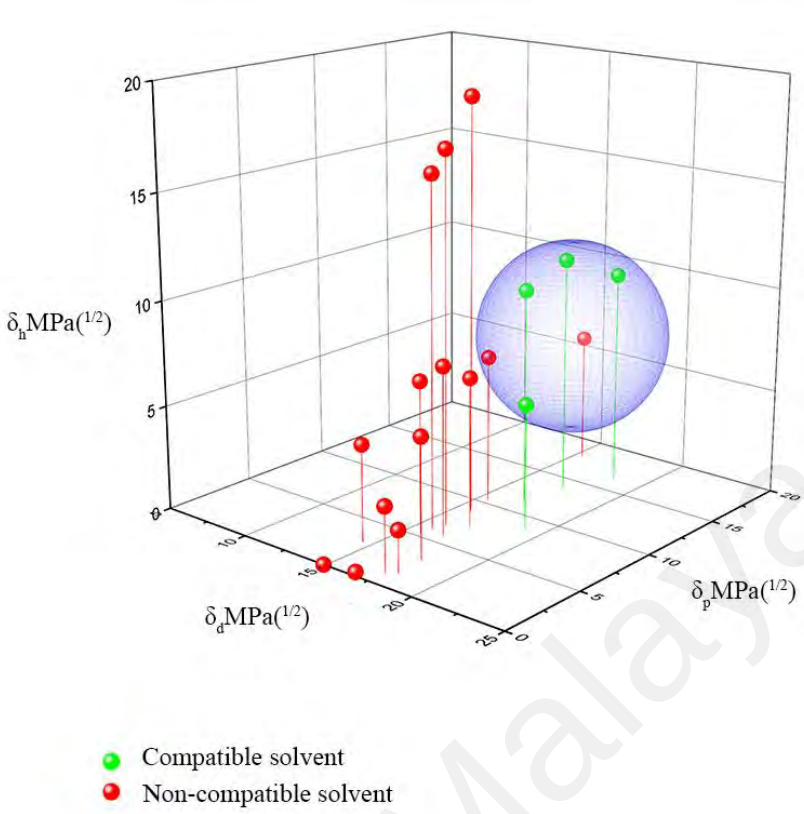

Figure 4.8: Hansen sphere for solvent interaction with GGA and PHGG

Figure 4.8 illustrates the Hansen sphere for solvent interaction with GGA and PHGG in which the results of solvent compatibility are provided in **Appendix D**. From **Appendix D**, Native GG exhibited water solubility only, however, GGA and PHGG exhibited reduced water solubility but improved compatibility with polar aprotic solvents. In general, the affinity for the solvents was DMSO>DMF>DMA>pyridine with GGA showing greater solvent uptake than PHGG.

A method (de los Ríos *et al.*, 2020) was adopted to determine the Hansen sphere radius for the solvents that are compatible with GGA and PHGG. The equation of Hansen Solubility Parameter (HSP) is given in Equation (4.2):

$$\delta^2_T = \delta^2_d + \delta^2_p + \delta^2_h \quad (4.2)$$

*δ^2_T total energy forces between molecules
δ_d -The energy from dispersion forces between molecules,

δ_p – The energy from dipolar intermolecular forces between molecules,
δ_h - The energy from hydrogen bonds between molecules,

Each sample is given three Hansen parameters (δ_d, δ_p, δ_h) that is plotted in a tridimensional plot (x,y,z), each generally measured in $MPa^{1/2}$.

The interaction radius for both the polymer systems was found to be 4.4 $MPa^{1/2}$ centered at (δ_d, δ_p, δ_h) = (18.7, 12.5, 8.2) $MPa^{1/2}$ (determined from solver add-in on Microsoft excel (**Appendix F**) (de los Ríos *et al.*, 2020) as shown by the blue sphere in **Figure 4.8**. Although none of the solvents tested showed 100% solubility (**Appendix D**), any solvent system coordinated within the interior of this sphere showed at least 50% swelling at elevated temperatures.

The HSP theoretically obtained (Brandrup *et al.*, 1999) based on component group contributions of the biopolymer are given in **Appendix E**. Upon modification of GG as seen in the DS values (**Table 4.2**), δ_d increases whereas δ_p and δ_h decreases, which would explain why GGA and PHGG had lower affinity for water but more for polar aprotic solvents. The fractional polarity, χ_p of Native GG is larger than that of GGA and PHGG (**Table 4.2**). This is attributed to the fact that intermolecular hydrogen bonding was disrupted by the hydrophobic acetyl and phthaloyl groups (Xu *et al.*, 2010). However, true organosolubility was not achieved for GGA and PHGG. This is because the calculated δ_d, δ_p, δ_h of GGA and PHGG as shown in **Table 4.2** do not meet the centre of interaction radius (refer **Figure 4.8)** and a solvent system that simultaneously satisfies all three HSP values was difficult to find from a list of common solvents.

Table 4.2: Degree of substitution (DS), Hansen Solubility Parameters (HSP) (δ_d, δ_p, δ_h) and fractional polarity (χ_p) of GGA and PHGG

Sample	DS	δ_d ($MPa^{1/2}$)	δ_p ($MPa^{1/2}$)	δ_h ($MPa^{1/2}$)	χ_p
Native GG	0.00	16.12	5.70	25.09	0.72
GGA	5.65	16.20	3.40	15.45	0.49
PHGG	1.04	20.85	5.39	23.87	0.58

4.6.2 Swelling in Distilled Water

The swelling response of a hydrogel in water is attributed to the absorption mechanism caused by diffusion of solvent into the hydrogel network (Islam *et al.*, 2012b; Jabeen *et al.*, 2017). The capability of absorbing and retaining water is one of the most important parameters to evaluate the potential of hydrogels (Che *et al.*, 2018; Islam *et al.*, 2012a). Generally the swelling ratio increases with time at the beginning due to the expansion of the hydrogel network caused by the penetration of water into the hydrogel, forming hydrogen bonds (**Figure 4.10**) (Islam *et al.*, 2012b; Ullah *et al.*, 2018). The time-dependent swelling capability in distilled water was evaluated to see the difference in swelling percentage of Native GG with its two derivatives, GGA and PHGG, as shown in **Figure 4.9**. Each sample showed a different level of continuous increase in the swelling percentage.

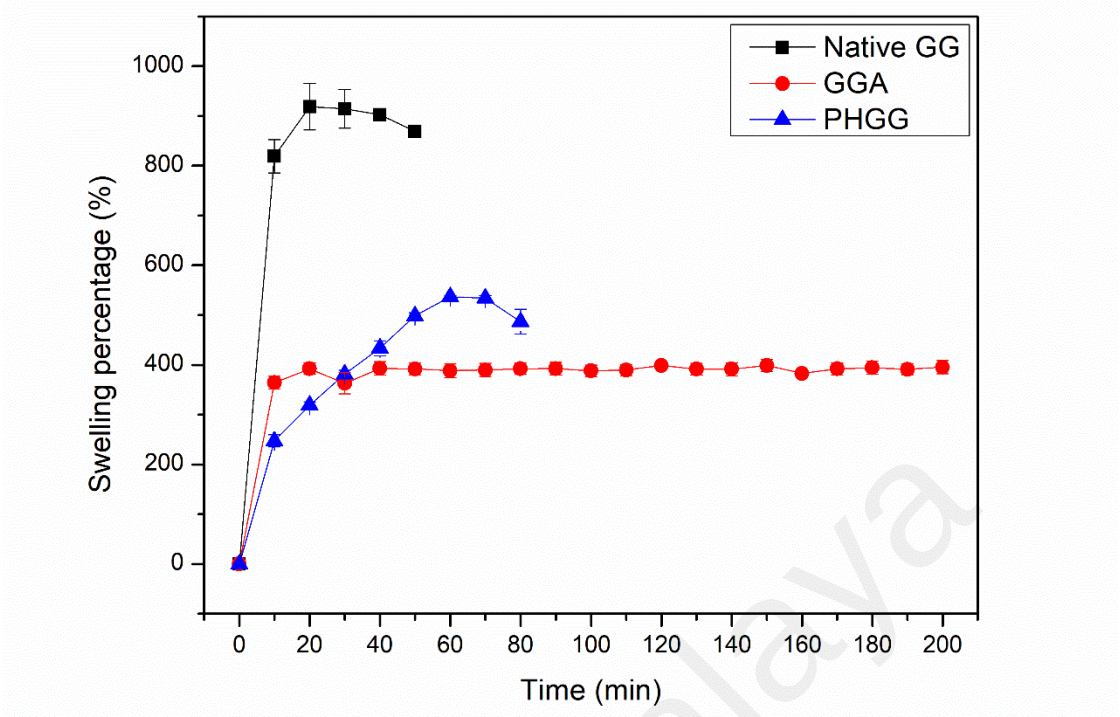

Figure 4.9: Time-dependent swelling behaviours of Native GG, GGA and PHGG in distilled water

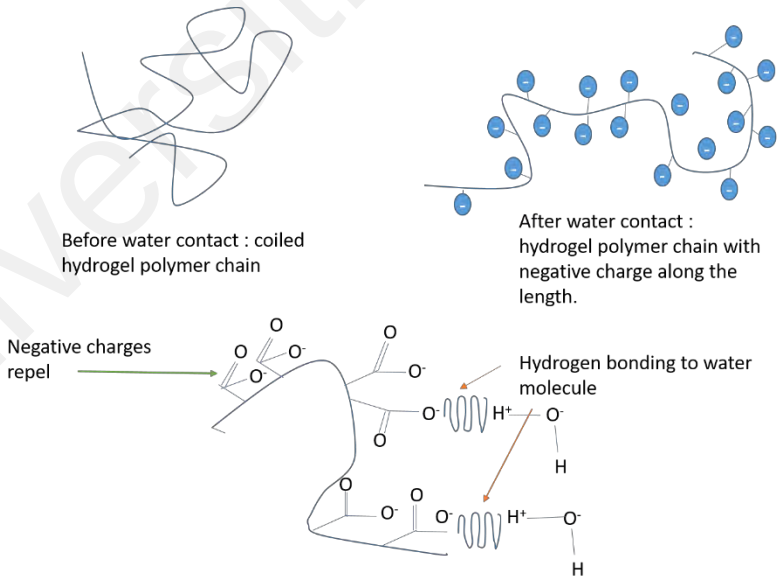

Figure 4.10: Mechanism of water absorption (Bhatnagar *et al.*, 2016)

Native GG had swelling equilibrium of 918.4 ± 46.6% at 20 min and thereafter the swelling value could not be measured as it started to dissolve in water. After reaching the equilibrium point, the Native GG would have start to rupture and break into smaller pieces

and consequently dissolves in the water. As the Native GG contains nine hydroxyl groups, it can form hydrogen bonding the most with water molecules as compared to GGA and PHGG.

Meanwhile for PHGG, it had swelling equilibrium of 537.0 ± 2.9% at 60 min. Beyond 60 min, the PHGG started to dissolve. However, it took longer for the percentage to drop and that its swelling has decreased compared to Native GG. This is due to the presence of hydrophobic bulky phthaloyl moiety that causes network contraction and decelerates water diffusion. The attachment of phthaloyl moeity also prevents formation of inter- and intramolecular hydrogen bonds (Kalia *et al.*, 2013). Therefore, this result shows that PHGG could potentially be used as a material for drug release retardant. The lower and slower water uptake of PHGG could potentially prevent the burst of drug (Mughal *et al.*, 2011).

As for GGA, its swelling equilibrium was at 393.0 ± 13.4% at 40 min, which is the lowest among the other samples (Native GG and PHGG), indicating that it is the least hydrophilic, therefore GGA exposes more polar, hydrophobic acetyl groups and lesser hydrophilic groups (-OH) compared to its parent Native GG. At this point of time, GGA had reached its swelling equilibrium state and no further water uptake was observed up to 200 min. Compared to Native GG and PHGG, GGA is the most stable and it may be more suitable to be used for the slow and gradual release of an absorbed substance (Mateescu *et al.*, 2014).

4.6.3 Swelling in Ionic Salt Solution

Electrolyte has a well-known effect on the swelling behaviour of the hydrogels. The swelling responses of Native GG, GGA and PHGG in ionic salt solution (simulated normal saline isotonic solution, i.e. 0.9% of NaCl in deionised water are shown in

(**Figure 4.11**) and the presence of cation (Na^+) and anion (Cl^-) had adversedly affected the swelling.

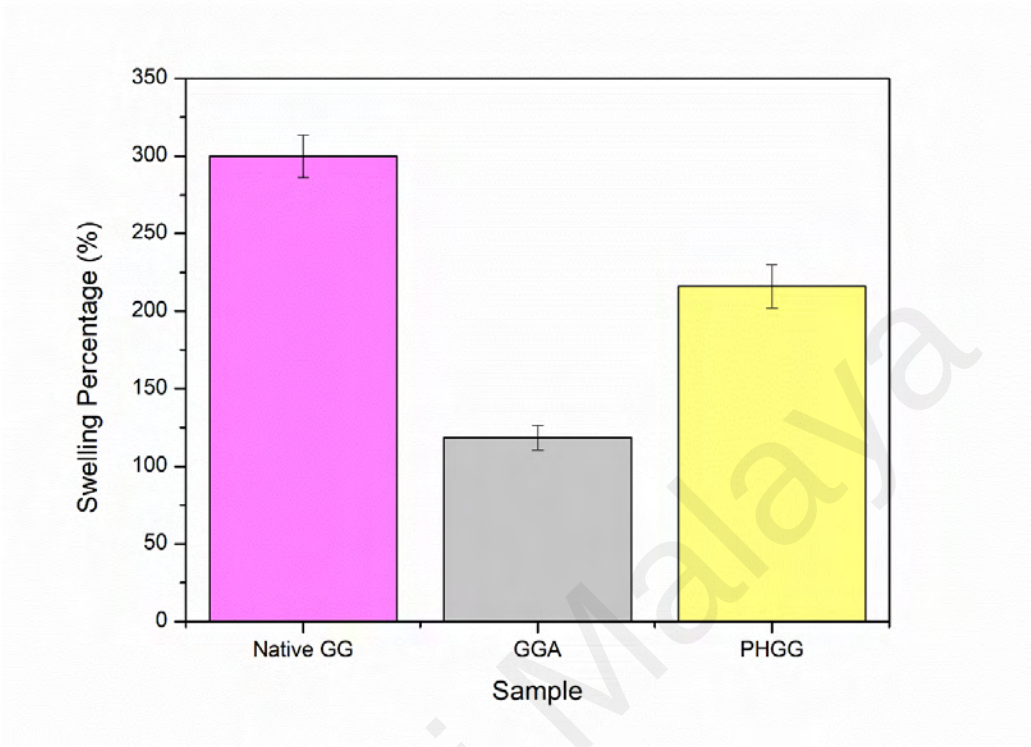

Figure 4.11: Native GG, GGA and PHGG equilibrium swelling properties in 0.9% NaCl solution
*Results reported as mean ± standard error (n=3) value of each plot.

Swelling ability of Native GG, GGA and PHGG in 0.9% NaCl solution appreciably decreased compared to the swelling values in distilled water (**Figure 4.11**), with swelling values of 299.8 ± 13.7%, 118.4 ± 7.8% and 216.0 ± 13.9% for Native GG, GGA and PHGG respectively. This undesired swelling-loss (300% times weight loss for Native GG,GGA and 250% times for PHGG) is often attributed from the presence of sodium ion (Na^+) and chloride ion (Cl^-) to a "charge screening effect" of the additional cation (Na^+) causing a non-perfect anion-anion (Cl^-) electrostatic repulsion for Native GG and PHGG, while for GGA additional anion (Cl^-) cause the cation-cation (Na+) electrostatic repulsion (**Figure 4.12 (b)**) (Flory, 1953; Kazemzadeh *et al.*, 2015).

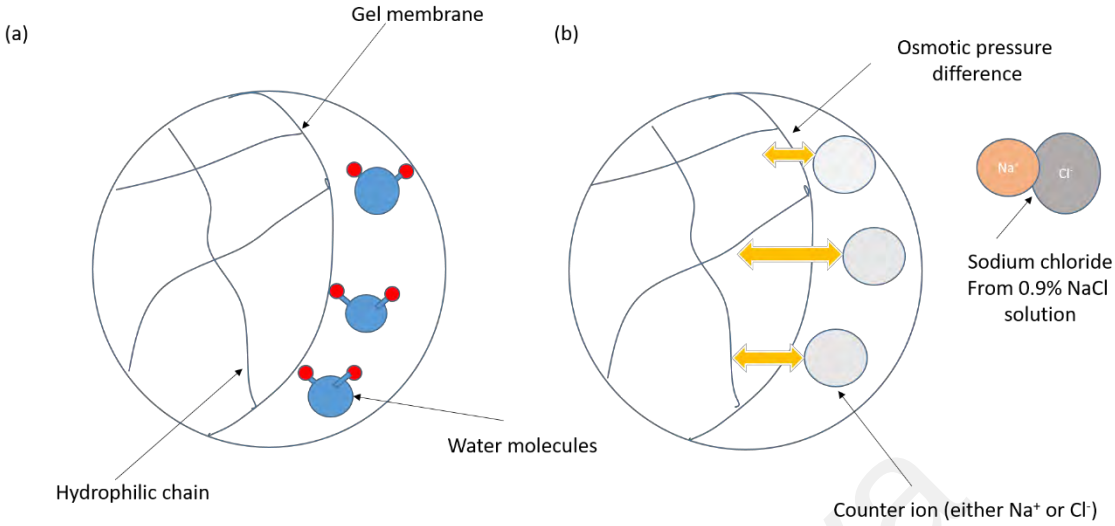

Figure 4.12: (a) Attraction of solvent (water) molecules from external solution towards the hydrophilic polymer chains causing fluid to enter the hydrogel. (b) Counter ions from external solution into the hydrogel causing osmotic pressure gradient. (Fennell *et al.*, 2020)

The osmotic pressure as a result of the difference of mobile ion concentration between the hydrogels and aqueous phases decreased hence the absorbency amounts reduced (Pourjavadi *et al.*, 2006). GGA has the lowest swelling reading as it possesses the highest hydrophobic acetyl group substitution, and the space for ion entry into the hydrogel is the lowest.

4.6.4 Swelling in Buffer Solution

Swelling in buffer solutions (simulated GIT) of pH 1.2 and pH 7.4 are crucial parameters to assess a hydrogel for its drug delivery potential in GIT. The swelling responses of the Native GG, GGA and PHGG in SGF (pH 1.2) and SIF (pH 7.4) at 37°C mimicking GIT of human body were evaluated and the data have been depicted in **Figure 4.13**.

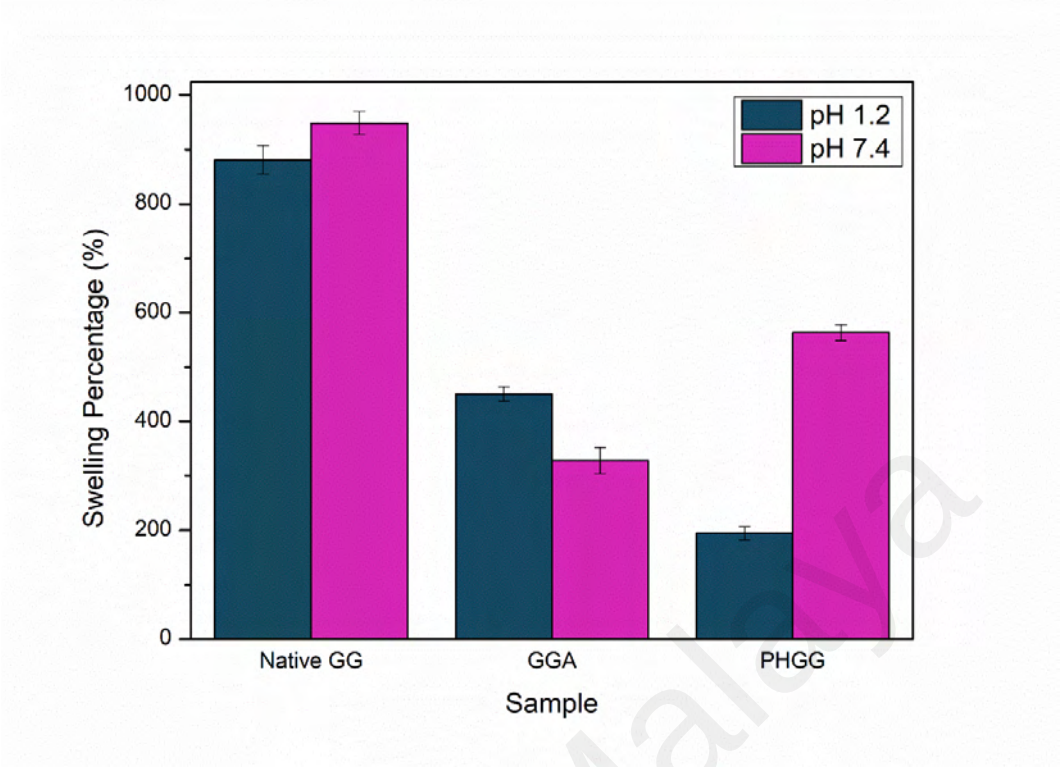

Figure 4.13: Native GG, GGA and PHGG equilibrium swelling properties in pH 1.2 and pH 7.4
*Results reported as mean ± standard error (n=3) value of each plot.

The Native GG, GGA and PHGG hydrogels displayed swelling in both buffer solutions (**Figure 4.13**) reflecting pH-responsive swelling characteristics. The swelling percentage of Native GG and PHGG were found to be higher (in pH 7.4 while GGA showed higher swelling in pH 1.2. During swelling experiment, -OH functional group of Native GG at pH 1.2 protonates, together with formation of intermolecular hydrogen bonding among the polymer chains which results in shrinkage of the polymer, decreasing water absorption and hence lower swelling (Jalababu *et al.*, 2019; Purkait *et al.*, 2018). PHGG possessed lower swelling in pH 1.2 (194.6 ± 11.8%) compared to Native GG (880.7 ± 26.2%). The presence of phthaloyl group might have hindered the water penetration in PHGG in comparison with Native GG (Surini, 2014). It can be observed that the higher swelling in pH 1.2 for GGA (450.7 ± 12.6%) might be due to higher hydrophilicity and strong repulsion between acetate group. The acetate group protonates

at lower pH which results in an increase charge of GGA and increases repulsion among the group (cationic hydrogel) (**Figure 4.14(a)).** In contrast at high pH (pH 7.4), fewer protons are available to generate positive charge, and this charge-based repulsion among the groups decline due to the decrease of ionisation of these groups, thus resulting in more interaction, and hence shrinking of GGA, and lower swelling (328.0 ± 24.3%) (Purkait *et al.*, 2018).

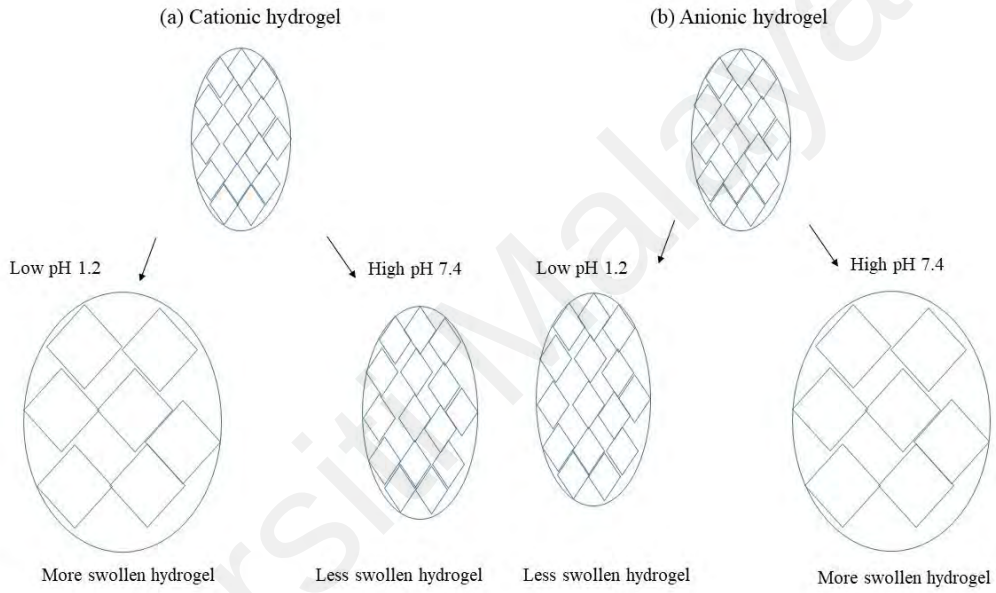

Figure 4.14: pH-dependent swelling of (a) Cationic hydrogel and (b) Anionic hydrogel (Rizwan *et al.*, 2017)

The stimuli responsive performances of these hydrogels show their potential to be used in drug delivery especially for GI tract of the human body. Native GG and PHGG might be used for sustained drug release at intestinal tract (anionic hydrogel) while GGA might be used for drug release in the stomach (cationic hydrogel). PHGG may be used as hydrogel material to protect drugs from gastric degradation and denaturation at low pH (pH 1.2) and release drugs in specific locations, such as the upper small intestine and colon, further in the GIT (anionic hydrogel) (**Figure 4.14 (b)**) (Sharpe *et al.*, 2014). As GGA imparts higher swelling at acidic pH than at neutral pH (pH 7.4), GGA may provide

protection of the drug in the oral cavity (pH 5.8 – 7.4), while releasing the drug in the stomach (pH 1 – 3.5) (Sharpe *et al.*, 2014).

CHAPTER 5: CONCLUSION

5.1 Conclusion

Biopolymer-based GGA and PHGG hydrogels were successfully modified by substitution with linear acetate moiety and bulky phthaloyl respectively. Both the modified GGA and PHGG were observed to have better solubility in organic solvents such as DMSO and DMF, compared to Native GG which was initially only soluble in water. However, there was no true organosolubility achieved for both GGA and PHGG. XRD and TGA revealed that modifications of Native GG led to less crystalline products and that GGA was thermally more stable than PHGG respectively. The swelling behaviour revealed that Native GG had the highest swelling equilibrium with 918.4 ± 46.6%, followed by PHGG with 537.0 ± 2.9% and GGA with 393.0 ± 13.4% in distilled water. These hydrogels also responded to stimuli such as pH and ionic salt solutions. In simulated normal saline isotonic solution these hydrogels showed lower swelling compared to in distilled water. GGA showed higher swelling in SGF medium while Native GG and PHGG showed higher swelling in SIF medium. Based on their swelling behaviour, these hydrogels are expected to be useful for biomedical fields such as tissue engineering and drug-delivery.

5.2 Suggestions for Future Studies

The present work highlights the potential of GGA and PHGG for biomedical applications. Further studies need to be carried out to assess the potential of the GG in terms of its pH-dependent, biodegradable, its potential in drug release and in-vitro cytotoxicity. These criteria are important to prove the usability of these hydrogels as good retardants of drug release (sustained release) for biomedical applications. The aforementioned approaches are swelling of the Native GG, GGA and PHGG based hydrogels in non-buffer media, biodegradability and their drug release.

REFERENCES

Abebe, D. G., & Fujiwara, T. (2012). Controlled thermoresponsive hydrogels by stereocomplexed PLA-PEG-PLA prepared via hybrid micelles of pre-mixed copolymers with different PEG lengths. *Biomacromolecules, 13*(6), 1828-1836.

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research, 6*(2), 105-121.

Akhtar, M. F., Hanif, M., & Ranjha, N. M. (2016). Methods of synthesis of hydrogels … A review. *Saudi Pharmaceutical Journal, 24*(5), 554-559.

Ali, A., & Ahmed, S. (2018). Recent advances in edible polymer based hydrogels as a sustainable alternative to conventional polymers. *Journal of Agricultural and Food Chemistry, 66*(27), 6940-6967.

Ali, S., Khatri, Z., Oh, K. W., Kim, I.-S., & Kim, S. H. (2014). Zein/cellulose acetate hybrid nanofibers: Electrospinning and characterization. *Macromolecular Research, 22*(9), 971-977.

Arya, S. (2009). Effect of guar gum on dough stickiness and staling in chapatti-An Indian unleavened flat bread. *International Journal of Food Engineering, 5*(3).

Ass, B. A., Ciacco, G. T., & Frollini, E. (2006). Cellulose acetates from linters and sisal: Correlation between synthesis conditions in DMAc/LiCl and product properties. *Bioresource technology, 97*(14), 1696-1702.

Aziz, N., Majid, S., & Arof, A. K. (2012). Synthesis and characterizations of phthaloyl chitosan-based polymer electrolytes. *Journal of Non-Crystalline Solids, 358*(12-13), 1581-1590.

Bahram, M., Mohseni, N., & Moghtader, M. (2016). An introduction to hydrogels and some recent applications. In *Emerging Concepts in Analysis and Applications of Hydrogels*.

Barth, H. G., & Smith, D. A. (1981). High-performance size-exclusion chromatography of guar gum. *Journal of Chromatography A, 206*(2), 410-415.

Bashir, S., Teo, Y. Y., Ramesh, S., & Ramesh, K. (2017). Physico-chemical characterization of pH-sensitive N-Succinyl chitosan-g-poly (acrylamide-co-acrylic acid) hydrogels and in vitro drug release studies. *Polymer Degradation and Stability, 139*, 38-54.

Bhatnagar, A., Kumar, R., Singh, V., & Pandey, D. S. (2016). Hydrogels: A boon for increasing agricultural productivity in water-stressed environment. *Current Science, 111*(11), 1773-1779.

Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. *Advanced Drug Delivery Reviews, 62*(1), 83-99.

Bi, X., & Liang, A. (2016). In situ‐forming cross‐linking hydrogel systems: Chemistry and biomedical applications. *Emerging Concepts in Analysis and Applications of Hydrogels, 86*, 541-547.

Boucard, N., Viton, C., & Domard, A. (2005). New aspects of the formation of physical hydrogels of chitosan in a hydroalcoholic medium. *Biomacromolecules, 6*(6), 3227-3237.

Brandrup, J., Immergut, E. H., Grulke, E. A., Abe, A., & Bloch, D. R. (1999). *Polymer handbook (4th ed.)* (Vol. 89): New York, America : Wiley Interscience.

Buwalda, S. J., Boere, K. W., Dijkstra, P. J., Feijen, J., Vermonden, T., & Hennink, W. E. (2014). Hydrogels in a historical perspective: From simple networks to smart materials. *Journal of Controlled Release, 190*, 254-273.

Caló, E., & Khutoryanskiy, V. V. (2015). Biomedical applications of hydrogels: A review of patents and commercial products. *European Polymer Journal, 65*, 252-267.

Cao, X., Peng, X., Zhong, L., Sun, S., Yang, D., Zhang, X., & Sun, R. (2014). A novel transesterification system to rapidly synthesize cellulose aliphatic esters. *Cellulose, 21*(1), 581-594.

Che, Y., Li, D., Liu, Y., Yue, Z., Zhao, J., Ma, Q., Zhang, Q., Tan, Y., Yue, Q., & Meng, F. (2018). Design and fabrication of a triple-responsive chitosan-based hydrogel with excellent mechanical properties for controlled drug delivery. *Journal of Polymer Research, 25*(8), 169.

Chen, J., Xu, J., Wang, K., Cao, X., & Sun, R. (2016). Cellulose acetate fibers prepared from different raw materials with rapid synthesis method. *Carbohydrate Polymers, 137*, 685-692.

Chen, T., Embree, H. D., Brown, E. M., Taylor, M. M., & Payne, G. F. (2003). Enzyme-catalyzed gel formation of gelatin and chitosan: potential for in situ applications. *Biomaterials, 24*(17), 2831-2841.

Chirani, N., Yahia, L. H., Gritsch, L., Soumiachirani, Motta, F. L., & Fare, S. (2015). History and applications of hydrogels. *Journal of Biomedical Sciences, 4*(2).

Chung, H. J., Lee, Y., & Park, T. G. (2008). Thermo-sensitive and biodegradable hydrogels based on stereocomplexed Pluronic multi-block copolymers for controlled protein delivery. *Journal of Controlled Release, 127*(1), 22-30.

Chung, H. J., & Park, T. G. (2009). Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. *Nano Today, 4*(5), 429-437.

Cravotto, G., Tagliapietra, S., Robaldo, B., & Trotta, M. (2005). Chemical modification of chitosan under high-intensity ultrasound. *Ultrasonics Sonochemistry, 12*(1-2), 95-98.

Das, S., & Subuddhi, U. (2015). pH-Responsive guar gum hydrogels for controlled delivery of dexamethasone to the intestine. *International Journal of Biological Macromolecules, 79*, 856-863.

de los Ríos, M. D., & Ramos, E. H. (2020). Determination of the Hansen solubility parameters and the Hansen sphere radius with the aid of the solver add-in of Microsoft Excel. *SN Applied Sciences, 2*(4), 1-7.

Dodi, G., Hritcu, D., & Popa, I. M. (2011). Carboxymethylation of guar gum: Synthesis and characterization. *Cellulose Chemistry and Technology, 45*, 171-176.

Dure Najaf, I., Arif, N., Munawar, I., & Muhammad, Y. (2020). Green synthesis and characterization of carboxymethyl guar gum: Application in textile printing technology. *Green Processing and Synthesis, 9*(1), 212-218.

Ebara, M., Kotsuchibashi, Y., Narain, R., Idota, N., Kim, Y.-J., Hoffman, J. M., Uto, K., & Aoyagi, T. (2014). *Smart biomaterials*. Japan: Springer.

Fennell, E., Kamphus, J., & Huyghe, J. M. (2020). The importance of the mixing energy in ionized superabsorbent polymer swelling models. *Polymers, 12*(3), 609.

Filip, D., Macocinschi, D., & Vlad, S. (2011). Thermogravimetric study for polyurethane materials for biomedical applications. *Composites Part B: Engineering, 42*(6), 1474-1479.

Flory, P. J. (1953). *Principles of polymer chemistry*: Cornell University Press.

Frollini, E., Reed, W. F., Milas, M., & Rinaudo, M. (1995). Polyelectrolytes from polysaccharides: Selective oxidation of guar gum — a revisited reaction. *Carbohydrate Polymers, 27*(2), 129-135.

Fujioka, R., Tanaka, Y., & Yoshimura, T. (2009). Synthesis and properties of superabsorbent hydrogels based on guar gum and succinic anhydride. *Journal of Applied Polymer Science, 114*(1), 612-616.

Fürst, W., & Banerjee, A. (2005). Release of glutaraldehyde from an albumin-glutaraldehyde tissue adhesive causes significant in vitro and in vivo toxicity. *The Annals of Thoracic Surgery, 79*(5), 1522-1528.

George, M., & Abraham, T. E. (2007). pH sensitive alginate–guar gum hydrogel for the controlled delivery of protein drugs. *International Journal of Pharmaceutics, 335*(1), 123-129.

Giri, P., Zandu, S., & Singh, I. (2020). Chemical modifications of guar gum for drug delivery applications: A review. *Asian Journal of Chemistry, 32*, 1259-1265.

Gong, H., Liu, M., Zhang, B., Cui, D., Gao, C., Ni, B., & Chen, J. (2011). Synthesis of oxidized guar gum by dry method and its application in reactive dye printing. *International Journal of Biological Macromolecules, 49*(5), 1083-1091.

Guimard, N. K., Gomez, N., & Schmidt, C. E. (2007). Conducting polymers in biomedical engineering. *Progress in Polymer Science, 32*(8-9), 876-921.

Gupta, A. P., & Verma, D. K. (2015). Synthesis and characterization of carboxymethyl guar gum nanoparticles stabilized polyaniline/carboxymethyl guar gum nanocomposites. *Journal of Nanostructure in Chemistry, 5*(4), 405-412.

Gyles, D. A., Castro, L. D., Silva, J. O. C., & Ribeiro-Costa, R. M. (2017). A review of the designs and prominent biomedical advances of natural and synthetic hydrogel formulations. *European Polymer Journal, 88*, 373-392.

Hamidi, M., Azadi, A., & Rafiei, P. (2008). Hydrogel nanoparticles in drug delivery. *Advanced Drug Delivery Reviews, 60*(15), 1638-1649.

Han, Y., Zeng, Q., Li, H., & Chang, J. (2013). The calcium silicate/alginate composite: Preparation and evaluation of its behavior as bioactive injectable hydrogels. *Acta Biomaterialia, 9*(11), 9107-9117.

Hasan, A. M. A., & Abdel-Raouf, M. E. (2018). Applications of guar gum and its derivatives in petroleum industry: A review. *Egyptian Journal of Petroleum, 27*(4), 1043-1050.

Hashem, A., Ahmad, F., & Fahad, R. (2008). Application of some starch hydrogels for the removal of mercury (II) ions from aqueous solutions. *Adsorption Science & Technology, 26*(8), 563-579.

Hennink, W. E., & Van Nostrum, C. F. (2012). Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews, 64*, 223-236.

Hongbo, T., Shiqi, G., Yanping, L., & Siqing, D. (2017). Synthesis, characterization and properties of acetylated guar gum. *Cellulose Chemistry, 51*(3/4), 237-244.

Hongbo, T., Yanping, L., Siqing, D., & Min, S. (2014). Etherification optimization for preparing partially hydrolized hydroxypropylated guar gum and its properties. *Journal of Applied Polymer Science, 131*(13).

Huang, Y., Yu, H., & Xiao, C. (2007). pH-sensitive cationic guar gum/poly (acrylic acid) polyelectrolyte hydrogels: swelling and in vitro drug release. *Carbohydrate Polymers, 69*(4), 774-783.

Ifuku, S., Miwa, T., Morimoto, M., & Saimoto, H. (2011). Preparation of highly chemoselective N-phthaloyl chitosan in aqueous media. *Green Chemistry, 13*(6), 1499-1502.

Islam, A., Riaz, M., & Yasin, T. (2013). Structural and viscoelastic properties of chitosan-based hydrogel and its drug delivery application. *International Journal of Biological Macromolecules, 59*, 119-124.

Islam, A., & Yasin, T. (2012a). Controlled delivery of drug from pH sensitive chitosan/poly (vinyl alcohol) blend. *Carbohydrate Polymers, 88*(3), 1055-1060.

Islam, A., Yasin, T., Bano, I., & Riaz, M. (2012b). Controlled release of aspirin from pH‐sensitive chitosan/poly (vinyl alcohol) hydrogel. *Journal of Applied Polymer Science, 124*(5), 4184-4192.

Jabeen, S., Islam, A., Ghaffar, A., Gull, N., Hameed, A., Bashir, A., Jamil, T., & Hussain, T. (2017). Development of a novel pH sensitive silane crosslinked injectable hydrogel for controlled release of neomycin sulfate. *International Journal of Biological Macromolecules, 97*, 218-227.

Jalababu, R., Satya Veni, S., & Reddy, K. V. N. S. (2019). Development, characterization, swelling, and network parameters of amino acid grafted guar gum based pH responsive polymeric hydrogels. *International Journal of Polymer Analysis and Characterization, 24*(4), 304-312.

Javidi, F., Razavi, S. M. A., Behrouzian, F., & Alghooneh, A. (2016). The influence of basil seed gum, guar gum and their blend on the rheological, physical and sensory properties of low fat ice cream. *Food Hydrocolloids, 52*, 625-633.

Kabir, S. M. F., Sikdar, P. P., Haque, B., Bhuiyan, M. A. R., Ali, A., & Islam, M. N. (2018). Cellulose-based hydrogel materials: chemistry, properties and their prospective applications. *Progress in Biomaterials, 7*(3), 153-174.

Kaith, B. S., Sharma, R., & Kalia, S. (2015). Guar gum based biodegradable, antibacterial and electrically conductive hydrogels. *International Journal of Biological Macromolecules, 75*, 266-275.

Kakkar, P., & Madhan, B. (2016). Fabrication of keratin-silica hydrogel for biomedical applications. *Materials Science and Engineering, 66*, 178-184.

Kalia, S., & Sabaa, M. (2013). *Polysaccharide based graft copolymers* (Vol. 22): Springer.

Karuna, D. S., Rathnam, G., Ubaidulla, U., Ganesh, M., & Jang, H. T. (2018). Chitosan phthalate: A novel polymer for the multiparticulate drug delivery system for diclofenac sodium. *Advances in Polymer Technology, 37*(6), 2013-2020.

Kayra, N., & Aytekin, A. Ö. (2019). Synthesis of cellulose-based hydrogels: Preparation, formation, mixture, and modification. *Cellulose-Based Superabsorbent Hydrogels*, 407-434.

Kazemzadeh, B., Hosseinzadeh, H., & Babazadeh, M. (2015). Synthesis of a novel pectin-based superabsorbent hydrogel with salt and pH-responsiveness properties. *Biomedical and Pharmacology Journal, 6*(1), 41-48.

Kirakc ícha, V. c., Holub, J t, P., & Lang, K. (2014). Luminescent hydrogel particles prepared by self-assembly of β-cyclodextrin polymer and octahedral molybdenum cluster complexes. *Inorganic Chemistry, 53*(24), 13012-13018.

Kono, H., Otaka, F., & Ozaki, M. (2014). Preparation and characterization of guar gum hydrogels as carrier materials for controlled protein drug delivery. *Carbohydrate Polymers, 111*, 830-840.

Krishnaiah, Y. S. R., Bhaskar Reddy, P. R., Satyanarayana, V., & Karthikeyan, R. S. (2002). Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis. *International Journal of Pharmaceutics, 236*(1), 43-55.

Kumar, A., De, A., & Mozumdar, S. (2015). Synthesis of acrylate guar-gum for delivery of bio-active molecules. *Bulletin of Materials Science, 38*(4), 1025-1032.

Kumar, D., Pandey, J., Raj, V., & Kumar, P. (2017). A review on the modification of polysaccharide through graft copolymerization for various potential applications. *The Open Medicinal Chemistry Journal, 11*, 109-126.

Kundu, S., Das, A., Basu, A., Ghosh, D., Datta, P., & Mukherjee, A. (2018). Carboxymethyl guar gum synthesis in homogeneous phase and macroporous 3D scaffolds design for tissue engineering. *Carbohydrate Polymers, 191*, 71-78.

Lapasin, R., Pricl, S., & Tracanelli, P. (1991). Rheology of hydroxyethyl guar gum derivatives. *Carbohydrate Polymers, 14*(4), 411-427.

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

Lewicka, K., Siemion, P., & Kurcok, P. (2015). Chemical modifications of starch: Microwave effect. *International Journal of Polymer Science, 2015*, 867697.

Li, Q., Wang, W., Jia, H., & Zhang, Y. (2017). Molecular structural properties of a polysaccharide isolated and purified from Sophora japonica pods and its relationship to their rheology. *International Journal of Food Properties, 20*(11), 2844-2854.

Lira, L. M., & Torresi, C. r. d. (2005). Conducting polymer–hydrogel composites for electrochemical release devices: Synthesis and characterization of semi-interpenetrating polyaniline–polyacrylamide networks. *Electrochemistry Communications, 7*(7), 717-723.

Mahdavinia, G. R., Rahmani, Z., Karami, S., & Pourjavadi, A. (2014). Magnetic/pH-sensitive κ-carrageenan/sodium alginate hydrogel nanocomposite beads: preparation, swelling behavior, and drug delivery. *Journal of Biomaterials Science, 25*(17), 1891-1906.

Mateescu, M. A., Ispas-Szabo, P., & Assaad, E. (2014). *Controlled drug delivery: The role of self-assembling multi-task excipients*. United Kingdom: Elsevier.

Milašinović, N., Krušić, M. K., Knežević-Jugović, Z., & Filipović, J. (2010). Hydrogels of N-isopropylacrylamide copolymers with controlled release of a model protein. *International Journal of Pharmaceutics, 383*(1-2), 53-61.

Minhas, M. U., Ahmad, M., Ali, L., & 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*(1), 44.

Mohan, S., Oluwafemi, O. S., Kalarikkal, N., Thomas, S., & Songca, S. P. (2016). Biopolymers–application in nanoscience and nanotechnology. *Recent Advances in Biopolymers, 1*(1), 47-66.

Mohd, N., Draman, S., Salleh, M., & Yusof, N. (2017). *Dissolution of cellulose in ionic liquid: A review.* Paper presented at the AIP conference proceedings.

Mudgil, D., Barak, S., & Khatkar, B. S. (2012). X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *International Journal of Biological Macromolecules, 50*(4), 1035-1039.

Mudgil, D., Barak, S., & Khatkar, B. S. (2014). Guar gum: processing, properties and food applications—A Review. *Journal of Food Science and Technology, 51*(3), 409-418.

Mughal, M. A., Iqbal, Z., & Neau, S. H. (2011). Guar gum, xanthan gum, and HPMC can define release mechanisms and sustain release of propranolol hydrochloride. *AAPS PharmSciTech, 12*(1), 77-87.

Nemade, S., & Sawarkar, S. (2015). Recovery and synthesis of guar gum and its derivatives. *International Journal of Advanced Research in Chemical Science, 2*, 33-40.

Nishimura, S., Kohgo, O., Kurita, K., & Kuzuhara, H. (1991). Chemospecific manipulations of a rigid polysaccharide: syntheses of novel chitosan derivatives with excellent solubility in common organic solvents by regioselective chemical modifications. *Macromolecules, 24*(17), 4745-4748.

Panariello, G., Favaloro, R., Forbicioni, M., Caputo, E., & Barbucci, R. (2008). *Synthesis of a new hydrogel, based on guar gum, for controlled drug release.* Paper presented at the Macromolecular symposia.

Patel, S. P., Ranjan, G., & Patel, V. S. (1987). Rheological properties of guar gum and hydroxyethyl guar gum in aqueous solution. *International Journal of Biological Macromolecules, 9*(6), 314-320.

Peppas, N. A., Bures, P., Leobandung, W., & Ichikawa, H. (2000). Hydrogels in pharmaceutical formulations. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 27-46.

Pourjavadi, A., Kurdtabar, M., & Ghasemzadeh, H. (2008). Salt-and pH-resisting collagen-based highly porous hydrogel. *Polymer Journal, 40*(2), 94-103.

Pourjavadi, A., Sadeghi, M., Hashemi, M., & Hosseinzadeh, H. (2006). Synthesis and absorbency of gelatin-graft-poly(sodium acrylate-*co*-acrylamide) superabsorbent hydrogel with salt and pH-responsiveness properties. *e-Polymers, 6*.

Prabaharan, M. (2011). Prospective of guar gum and its derivatives as controlled drug delivery systems. *International Journal of Biological Macromolecules, 49*(2), 117-124.

Purkait, M., Sinha, M. K., Mondal, P., & Singh, R. (2018). Stimuli responsive polymeric membranes. *Smart Polymeric Membranes, 25*, 40.

Qiu, Y., & Park, K. (2001). Environment-sensitive hydrogels for drug delivery. *Advanced Drug Delivery Reviews, 53*(3), 321-339.

Radhakrishnan, A., Jose, G. M., & Kurup, M. (2015). PEG-penetrated chitosan–alginate co-polysaccharide-based partially and fully cross-linked hydrogels as ECM mimic for tissue engineering applications. *Progress in Biomaterials, 4*(2), 101-112.

Rana, V., Rai, P., Tiwary, A. K., Singh, R. S., Kennedy, J. F., & Knill, C. J. (2011). Modified gums: Approaches and applications in drug delivery. *Carbohydrate Polymers, 83*(3), 1031-1047.

Raouf, M. E. A. (2019). Guar gum based hydrogels for sustained water release applications in agriculture, a review. *Current Research in Biopolymers*.

Rasool, A., Ata, S., Islam, A., & Khan, R. U. (2019). Fabrication of novel carrageenan based stimuli responsive injectable hydrogels for controlled release of cephradine. *RSC Advances, 9*(22), 12282-12290.

Rathore, K., & Loonker, S. (2017). Synthesis, characterization and swelling behaviour of guar gum-g-poly (methyl methacrylate) superabsorbent nanocomposite. *Asian Journal of Chemical Sciences, 2*(1), 1-13.

Ribba, L., Garcia, N. L., D'Accorso, N., & Goyanes, S. (2017). Chapter 3 - Disadvantages of starch-based materials, feasible alternatives in order to overcome these limitations. In M. A. Villar, S. E. Barbosa, M. A. García, L. A. Castillo, & O. V. López (Eds.), *Starch-Based Materials in Food Packaging* (pp. 37-76): Academic Press.

Rithe, S. S., Kadam, P. G., & Mhaske, S. T. (2014). Preparation and analysis of novel hydrogels prepared from the blend of guar gum and chitosan: Cross-linked with glutaraldehyde. *Advances in Materials Science and Engineering, 1*(2).

Rizwan, M., Yahya, R., Hassan, A., Yar, M., Azzahari, A., Selvanathan, V., Sonsudin, F., & Abouloula, C. (2017). pH sensitive hydrogels in drug delivery: Brief history, properties, swelling, and release mechanism, material selection and applications. *Polymers, 9*(4), 137.

Santmartí, A., & Lee, K.-Y. (2018). Crystallinity and thermal stability of nanocellulose. In *Nanocellulose and sustainability* (pp. 67-86). United States: CRC Press.

Schilling, M., Bouchard, M., Khanjian, H., Learner, T., Phenix, A., & Rivenc, R. (2010). Application of chemical and thermal analysis methods for studying cellulose ester plastics. *Accounts of Chemical Research, 43*(6), 888-896.

Schneider, R., & ostar-Turk, S. (2003). Good quality printing with reactive dyes using guar gum and biodegradable additives. *Dyes and Pigments, 57*(1), 7-14.

Seeli, D. S., & Prabaharan, M. (2016). Guar gum succinate as a carrier for colon-specific drug delivery. *International Journal of Biological Macromolecules, 84*, 10-15.

Selvanathan, V., Azzahari, A. D., Abd. Halim, A. A., & Yahya, R. (2017). Ternary natural deep eutectic solvent (NADES) infused phthaloyl starch as cost efficient quasi-solid gel polymer electrolyte. *Carbohydrate Polymers, 167*, 210-218.

Senna, A. M., Novack, K. M., & Botaro, V. R. (2014). Synthesis and characterization of hydrogels from cellulose acetate by esterification crosslinking with EDTA dianhydride. *Carbohydrate Polymers, 114*, 260-268.

Sharpe, L. A., Daily, A. M., Horava, S. D., & Peppas, N. A. (2014). Therapeutic applications of hydrogels in oral drug delivery. *Expert Opinion on Drug Delivery, 11*(6), 901-915.

Shen, X., Shamshina, J. L., Berton, P., Gurau, G., & Rogers, R. D. (2016). Hydrogels based on cellulose and chitin: fabrication, properties, and applications. *Green Chemistry, 18*(1), 53-75.

Shenoy, M. A., & D'Melo, D. J. (2010). Synthesis and characterization of acryloyloxy guar gum. *Journal of Applied Polymer Science, 117*(1), 148-154.

Sherazi, T. A. (2016). Graft Polymerization. In E. Drioli & L. Giorno (Eds.), *Encyclopedia of Membranes* (pp. 886-887). Berlin, Heidelberg: Springer Berlin Heidelberg.

Shetye, S. P., Godbole, A., Bhilegaokar, S., & Gajare, P. (2015). Hydrogels: Introduction, preparation, characterization and applications. *Human Journals, 1*, 47-71.

Singh, V., Singh, A., Joshi, S., & Malviya, T. (2016a). Grafting of vinyl acetate-ethylacrylate binary monomer mixture onto guar gum. *International Journal of Biological Macromolecules, 84*, 442-447.

Singh, V., Singh, A., Preeti, S., Joshi, S., & Malviya, T. (2016b). Synthesis, characterization and mercury (II) removal using poly (vinylacetate) grafted guar gum. *Advanced Materials Letters, 7*(7), 573-578.

Sinha, V. R., & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. *International Journal of Pharmaceutics, 224*(1-2), 19-38.

Sionkowska, A. (2011). Current research on the blends of natural and synthetic polymers as new biomaterials. *Progress in Polymer Science, 36*(9), 1254-1276.

Sittikijyothin, W., Torres, D., & Gonçalves, M. (2005). Modelling the rheological behaviour of galactomannan aqueous solutions. *Carbohydrate Polymers, 59*(3), 339-350.

Slaughter, B. V., Khurshid, S. S., Fisher, O. Z., Khademhosseini, A., & Peppas, N. A. (2009). Hydrogels in regenerative medicine. *Advanced Material, 21*(32 - 33), 3307-3329.

Sun, X., Lu, C., Zhang, W., Tian, D., & Zhang, X. (2013). Acetone-soluble cellulose acetate extracted from waste blended fabrics via ionic liquid catalyzed acetylation. *Carbohydrate Polymers, 98*(1), 405-411.

Surini, S. (2014). Preparation and characterization of pragelatinized cassava starch phthalate as pH-Sensitive polymer for enteric coated tablet formulation. *International Journal of Pharmacy and Pharmaceutical Sciences, Volume 6*.

Takigami, M., Amada, H., Nagasawa, N., Yagi, T., Kasahara, T., Takigami, S., & Tamada, M. (2007). Preparation and properties of CMC gel. *Transactions of the Materials Research Society of Japan, 32*(3), 713.

Thakur, S., Sharma, B., Verma, A., Chaudhary, J., Tamulevicius, S., & Thakur, V. K. (2018). Recent approaches in guar gum hydrogel synthesis for water purification. *International Journal of Polymer Analysis and Characterization, 23*(7), 621-632.

Thakur, V. K., & Thakur, M. K. (2015). *Handbook of Polymers for Pharmaceutical Technologies*: Wiley Online Library.

Thombare, N., Jha, U., Mishra, S., & Siddiqui, M. (2016). Guar gum as a promising starting material for diverse applications: A review. *International Journal of Biological Macromolecules, 88*, 361-372.

Thombare, N., Mishra, S., Siddiqui, M. Z., Jha, U., Singh, D., & Mahajan, G. R. (2018). Design and development of guar gum based novel, superabsorbent and moisture retaining hydrogels for agricultural applications. *Carbohydrate Polymers, 185*, 169-178.

Tiwari, A., & Prabaharan, M. (2010). An amphiphilic nanocarrier based on guar gum-graft-poly(ε-caprolactone) for potential drug-delivery applications. *Journal of Biomaterials science, Polymer edition, 21*(6-7), 937-949.

Torgbo, S., & Sukyai, P. (2020). Biodegradation and thermal stability of bacterial cellulose as biomaterial: The relevance in biomedical applications. *Polymer Degradation and Stability, 179*, 109232.

Tsuji, H., Horii, F., Nakagawa, M., Ikada, Y., Odani, H., & Kitamaru, R. (1992). Stereocomplex formation between enantiomeric poly (lactic acid) s. 7. Phase structure of the stereocomplex crystallized from a dilute acetonitrile solution as studied by high-resolution solid-state carbon-13 NMR spectroscopy. *Macromolecules, 25*(16), 4114-4118.

Ubaidulla, U., Sultana, Y., Ahmed, F., Khar, R., & Panda, A. (2007). Chitosan phthalate microspheres for oral delivery of insulin: preparation, characterization, and in vitro evaluation. *Drug Delivery, 14*(1), 19-23.

Ullah, F., Javed, F., Othman, M., Khan, A., Gul, R., Ahmad, Z., & Md. Akil1, H. (2018). Synthesis and functionalization of chitosan built hydrogel with induced hydrophilicity for extended release of sparingly soluble drugs. *Journal of Biomaterials science, Polymer edition, 29*(4), 376-396.

Uzun, İ., & Topal, G. (2012). Synthesis and physicochemical characterization of chitin derivatives. *Journal of Chemistry, 2013*.

Varaprasad, K., Raghavendra, G. M., Jayaramudu, T., Yallapu, M. M., & Sadiku, R. (2017). A mini review on hydrogels classification and recent developments in miscellaneous applications. *Materials Science Engineering: C, 79*, 958-971.

Verma, S., Kumar, N., & Sharma, P. K. (2014). Buccal film: an advance technology for oral drug delivery. *Advances in Biomedical Research, 8*, 260-267.

Wang, H.-H., Zhang, X.-Q., Long, P., Zhang, A.-P., Liu, C.-F., & Sun, R.-C. (2016). Reaction behavior of cellulose in the homogeneous esterification of bagasse modified with phthalic anhydride in ionic liquid 1-allyl-3-methylimidazium chloride. *International Journal of Polymer Science, 2016*.

Whistler, R. L., & Hymowitz, T. (1979). Guar: Agronomy, production, industrial use, and nutrition. In. United States: Purdue University Press.

Wichterle, O., & Lim, D. (1960). Hydrophilic gels for biological use. *Nature, 185*(4706), 117.

Xu, Y., Ding, W., Liu, J., Li, Y., Kennedy, J. F., Gu, Q., & Shao, S. (2010). Preparation and characterization of organic-soluble acetylated starch nanocrystals. *Carbohydrate Polymers, 80*(4), 1078-1084.

Yi, N., & Abidian, M. R. (2016). 10 - Conducting polymers and their biomedical applications. In L. Poole-Warren, P. Martens, & R. Green (Eds.), *Biosynthetic Polymers for Medical Applications* (pp. 243-276). England: Woodhead Publishing.

Yom-Tov, O., Neufeld, L., Seliktar, D., & Bianco-Peled, H. (2014). A novel design of injectable porous hydrogels with in situ pore formation. *Acta Biomaterialia, 10*(10), 4236-4246.

Yu, W., Zhang, D., Liu, X., Wang, Y., Tong, J., Zhang, M., & Ma, X. (2019). Amphiphilic sodium alginate-vinyl acetate microparticles for drug delivery. *Journal of Oceanology and Limnology, 37*(3), 855-862.

Yusof, Y. M., Illias, H. A., Shukur, M. F., & Kadir, M. F. Z. (2017). Characterization of starch-chitosan blend-based electrolyte doped with ammonium iodide for application in proton batteries. *Ionics, 23*(3), 681-697.

Zhang, H., Zhang, F., & Wu, J. (2013). Physically crosslinked hydrogels from polysaccharides prepared by freeze–thaw technique. *Reactive and Functional Polymer, 73*(7), 923-928.

Zhao, Q. S., Ji, Q. X., Xing, K., Li, X. Y., Liu, C. S., & Chen, X. G. (2009). Preparation and characteristics of novel porous hydrogel films based on chitosan and glycerophosphate. *Carbohydrate Polymers, 76*(3), 410-416.