BRNO UNIVERSITY OF TECHNOLOGY

VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

FACULTY OF CHEMISTRY

FAKULTA CHEMICKÁ

INSTITUTE OF MATERIALS SCIENCE

ÚSTAV CHEMIE MATERIÁLŮ

PREPARATION OF PHOTOSENSITIVE POLYSACCHARIDES FOR WOUND HEALING

PŘÍPRAVA CITLIVÝCH POLYSACHARIDŮ PRO HOJENÍ RAN

BACHELOR'S THESIS

BAKALÁŘSKÁ PRÁCE

AUTHOR — Bc. Erik Kalla

AUTOR PRÁCE

SUPERVISOR — Ing. Lenka Michlovská, Ph.D.

VEDOUCÍ PRÁCE

BRNO 2021

BRNO UNIVERSITY OF TECHNOLOGY
FACULTY OF CHEMISTRY

Specification Bachelor's Thesis

Project no.:	FCH-BAK1598/2020	Academic year:	2020/21
Department:	Institute of Materials Science		
Student:	**Bc. Erik Kalla**		
Study programme:	Chemistry and Chemical Technologies		
Study field:	Chemistry, Technology and Properties of Materials		
Head of thesis:	**Ing. Lenka Michlovská, Ph.D.**		

Title of Bachelor's Thesis:

Preparation of photosensitive polysaccharides for wound healing

Bachelor's Thesis:

1) Literature searching on light–crosslinked polysaccharides, their modifications and possibilities of synthesis
2) Modification of natural resin and preparation of macromonomers
3) Chemical analysis
4) Evaluation of results and interpretation of data
5) Conclusion

Deadline for Bachelor's Thesis delivery: 30.7.2021:

Bachelor's Thesis should be submitted to the institute's secretariat in a number of copies as set by the dean This specification is part of Bachelor's Thesis

Bc. Erik Kalla Student	Ing. Lenka Michlovská, Ph.D. Head of thesis	doc. Ing. František Šoukal, Ph.D. Head of department
		prof. Ing. Martin Weiter, Ph.D. Dean

In Brno dated 1.2.2021

ABSTRACT

This bachelor thesis deals with the preparation of a modified natural polysaccharide with subsequent potential use in medicine as a sprayable hydrogel for wound healing.
Gum Karaya is a natural polysaccharide with healing and antibacterial effects, which can be employed in the form of films to cover burns. However, some complicated and deep skin defects should not be healed by the films. Sprayable hydrogel does not limit the applicability in relation to the depth or shape of the defect.
The theoretical part of the thesis summarizes the basic physical and chemical properties of hydrogels and the possibilities of their modification, especially methacrylation. Double bond attachment is a suitable method for simple light cross-linking to form a hydrogel.
The experimental part of the work describes a synthetic strategy for the preparation of methacrylated polymers. It further presents common instrumental analytical methods, such as infrared spectroscopy, Raman spectroscopy, or nuclear magnetic resonance spectroscopy to verify whether the synthesis was successfully carried out. The mentioned nuclear magnetic resonance spectroscopy represents the key analysis to provide information about the quality and molar amount of bound double bonds of the prepared polysaccharide. The characteristic peaks in the spectra correspond to the chemical shift of double bonds. Nuclear magnetic resonance analysis clearly confirms that the polymer was modified to form a methacrylated polysaccharide. Part of the experimental section represents a test of the solubility of the prepared modified polymer in two solvents, water and chloroform. Due to these facts, it is possible to continue the preparation of hydrogels and offer a new alternative method for wound healing in medicine.

KEY WORDS

Methacrylation, gum Karaya, hydrogel, glycidyl methacrylate

ABSTRAKT

Tato bakalářská práce se zabývá přípravou modifikovaného přírodního polysacharidu s následným potenciálním využitím v medicíně jako sprejovatelného hydrogelu pro hojení ran. Pryskyřice gum Karaya je přírodním polysacharidem s hojivými a antibakteriálními účinky, která může být využita ve formě filmů ke krytí popálenin. Některé komplikované a hluboké kožní defekty však není vhodné hojit pomocí filmů. Alternativní možností je využití sprejovatelného hydrogelu, který neomezuje použitelnost v souvislosti s hloubkou či tvarem defektu.

Teoretická část práce shrnuje základní fyzikální a chemické vlastnosti hydrogelů a možnosti jejich modifikace zejména pomocí methakrylace. Navázání dvojných vazeb představuje vhodnou metodu k jednoduchému zesítění polysacharidu pomocí světla za tvorby hydrogelu.

Experimentální část práce popisuje syntetickou strategii pro přípravu methakrylovaného polymeru. Dále představuje základní instrumentální analýzy jako infračervenou spektroskopii, Ramanovu spektroskopii či nukleární magnetickou rezonanční spektroskopii k ověření, zda byla daná syntéza úspěšně provedena. Stěžejní analýzu představuje zmíněná nukleární magnetická rezonance, která poskytuje informace o kvalitě i množství navázaných dvojných vazeb připraveného polysacharidu. Charakteristické píky ve spektrech odpovídají chemickým posunem navázaným dvojným vazbám. Analýza nukleární magnetickou rezonancí jednoznačně potvrzuje, že došlo k modifikaci polymeru za tvorby methakrylovaného polysacharidu. Součástí experimentální části práce je také zkouška rozpustnosti připraveného modifikovaného polymeru ve dvou rozpouštědlech, vodě a chloroformu. Díky tomu lze pokračovat v přípravě hydrogelů a nabídnout novou alternativní metodu k hojení ran v oblasti medicíny.

KLÍČOVÁ SLOVA

Methakrylace, gum Karaya, hydrogel, glycidyl methakrylát

KALLA, Erik. *Preparation of photosensitive polysaccharides for wound healing*. Brno, 2021. Bachelor's thesis. Brno University of Technology, Faculty of Chemistry, Institute of material chemistry. 40p. Supervised by Ing. Lenka Michlovská, Ph.D.

DECLARATION

I declare that the bachelor's thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the bachelor's thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

……..

Student's signature

ACKNOWLEDGEMENT

I would like to thank my supervisor Ing. Lenka Michlovská, Ph.D., and my consultant doc. Ing. Lucy Vojtová, Ph.D. for valuable advice and the possibility to work in the laboratory. Furthermore, I would like to thank Ing. Eva Černá for advice in the laboratory, Ing. Jana Dorazilová for ATR-FTIR training, Ing. Jakub Palovčík for Raman spectroscopy measurement, and Ing. Pawel Jewula, Ph.D. for NMR measurement. Finally, I also thank my friends and family and all the people who support me during my studies and convinced me to not give up. Thank you so much!

TABLE OF CONTENTS

1 INTRODUCTION

Natural products are ubiquitous chemical substances created by living organisms. Polysaccharides are one of the common natural product groups. A polysaccharide might be defined as a natural macromolecule (polymer) which consists of many units of monosaccharides linked via broad spectrum of bonds. On the other hand, monosaccharide is a sugar unit that is able to link by glycoside bonds with other monosaccharides.[1,2] The most common and readily abundant sugar unit is D-glucose.[3] Due to the variability of monosaccharides, they can be separated into two main groups – homopolysaccharides (only one sugar unit is present in a macromolecule) and heteropolysaccharides (more than one sugar unit is observed).[1] Polysaccharides might occur in cell walls (*e.g.* in starch, cellulose), animal tissue (*e.g.* chitosan), tree exudates (*e.g.* gum Karaya and gum arabic) and bacteria (*e.g.* gellan gum, dextran).[4] Except for obvious biological functions of these compounds, for example for energy storage and structural support, some of them could play a crucial role in medicine (*e.g.* application as hydrogels).[5]

Unlike natural polymers, hydrogels are mainly prepared artificially from synthetic materials. However, it does not mean that natural hydrogels do not exist. Although hydrogel biocompatibility is not perfect compared to natural polymers, some of them are biocompatible as well as natural polymers. On the other hand, synthetic hydrogels provide preferable mechanical properties. More biocompatible hydrogels with good mechanical properties could be produced by using natural polymer modified by synthetic routes.[4] Possible application and synthesis of modified natural product from gum Karaya as a hydrogel in medicine is described and discussed below.

The aim of this thesis is to carry out a literary research connected to cross-linked polysaccharides and to prepare a methacrylated gum Karaya that is capable of cross-linking after irradiation using and to provide a hydrogel for wound healing. After that, the hydrogel could be employed in medicine.

2 THEORETICAL PART

2.1 Hydrogels

Hydrogels are cross-linked hydrophilic polymers composed of three-dimensional (3D) networks with an application in drug delivery systems[6], biosensors[7], implants[8], and other branches. The network chains are usually formed by covalent or ionic bonds, although physical cross-links, such as hydrogen bonds, hydrophobic, and van der Waals interactions can provide a network with different characteristics and properties. The general hydrogel network structure is depicted below (*Figure **1***). Hydrogel is composed of mesh size that define the molecular structure at molecular level. Cross-linking of hydrogels can be obtained by chemical (covalent) and physical (entanglement/hydrogen bonding/junctions) crosslinks.[9]

Hydrogels are made up of natural or synthetic polymers (or their combination) and they could be classified as chemical and physical gels according to the nature of the cross-linked network. Physical gels (called reversible) consist of physically cross-linked networks and they might be disrupted under specific physical conditions (pH, temperature, application of stress). On the contrary, chemical gels (called permanent/non-reversible) are composed of a covalently cross-linked network.[4,10]

Highly hydrated 3D hydrogel network permits to bind several times higher amount of water compared to their dry weight (up to thousands of times). The process including water absorption is called swelling. Different size and shape of hydrogel are caused by exclusive physical properties. Due to their transparency, hydrogels are utilized in the biomedical field as wound healing cover recently.[11] Moreover, healing is accelerated by possible oxygen diffusion owing to the hydrophilic structure of the hydrogel.[12]

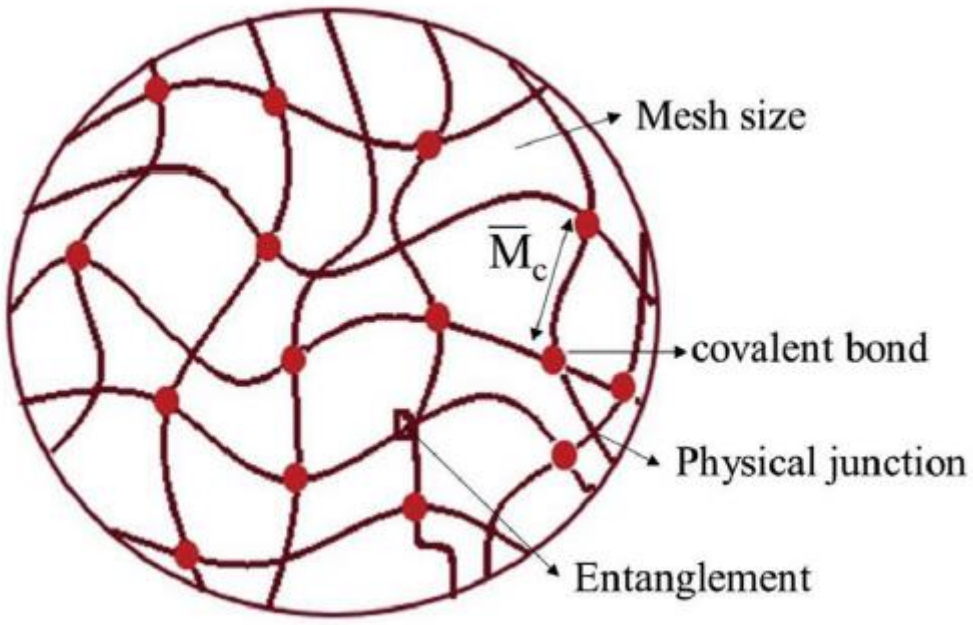

*Figure **1**. Hydrogel network structure at molecular level.*[9]

2.2 Hydrogel Cross-linking Methods

As mentioned above, there are two types of hydrogels – physical and chemical cross-linked. Chitosan-based hydrogels are examples of physical hydrogels which are biodegradable.[13] Whereas physical hydrogels are predominantly thermo-reversible, chemical cross-linking progresses on the certain temperature range. Chemical hydrogels are produced by many chemical reactions, such as polymerization, condensation, and cross-linked radiation. Furthermore, they might be prepared from hydrophilic polymers or hydrophobic polymer can be converted into hydrophilic or amphiphilic one.[12]

If an organic molecule (including macromolecules) is exposed to ionizing radiation, ionization and excitation of the molecule occurs. The excited high energy state can relax by energy emission or undergo homolysis to form free radicals. Formation of radicals is the main chemical reaction leading to subsequent cross-linking. Furthermore, macromolecules can withstand the radiation effect (they are not degraded) unlike viruses or bacteria. Moreover, a submission of the radiation can lead to chain scission, therefore cross-linking and chain scission are two main competitive reactions (*Figure **2***). In case of chain scission, the macromolecule degrades strongly with the evolution of low molecular weight fragments.[4,14,15]

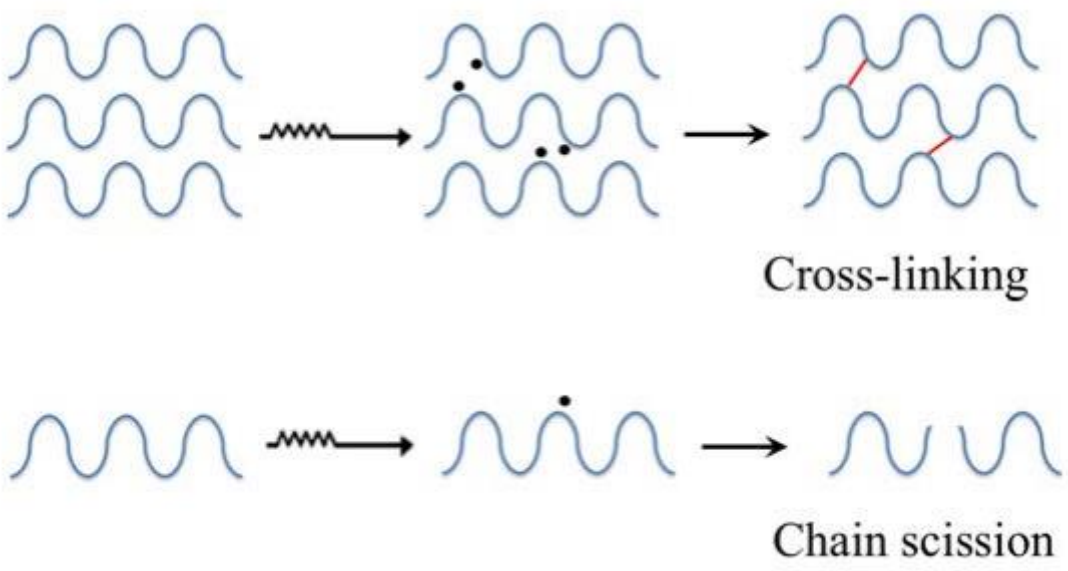

*Figure **2**. Process of cross-linking and chain scission. The upper part of the figure demonstrates required cross-linking, the lower part indicates chain scission.[4]*

2.3 Photopolymerization

Ionizing radiation represents an efficient method to modify polysaccharides without any chemicals *(e.g.,* initiators). On the other hand, photopolymerization (a type of radical polymerization) is commonly used in hydrogel preparation. This method is based on the presence of photoinitiator and ultraviolet (UV) or visible (VIS) light in aqueous solution. After a short exposure of radiation, hydrogels with low or no cytotoxicity are provided by this reaction. The photo-cross-linked hydrogels are generated at room temperature with minimal heat production. There are two possible processes of hydrogel application based on

photopolymerization. One of them may be carried out *in vitro* before the hydrogel is being implanted into the body. On the contrary, the second one uses an injected aqueous solution of mixture into the body and the hydrogel is formed by applying light through the skin, consequently.[16,17]

2.3.1 Photoinitiators

Photoinitiator (PI) is an essential chemical compound in photopolymerization, that is capable of absorbing specific light (each PI is efficient in specific range of wavelengths). Owing to the absorption, the PI breaks down to form free radicals and the radicals initiate the polymerization. Radical photoinitiating systems can be classified into two categories according to the radical generation mechanism. The first one is when the PI is dissociated into two free radicals (cleavage , *e.g.* lithium phenyl-2,4,6-trimethylbenzoylphosphinate, Li-TPO) or the second option is, when PI abstracts a hydrogen atom from co-initiatior to form another free radical ("abstract type", for instance 3-(4-benzoylphenoxy)-2-hydroxy-*N*,*N*,*N*-trimethyl-1-propanaminium chloride, Quantacure).[17,18]
Recently, water-soluble and biocompatible PIs containing acylphosphinoxide group are widely used.[18–20] Some of the commercially available acylphosphineoxide salts (*Figure **3***) and Li-TPO dissociation (*Figure **4***) are depicted below. These PIs are efficient in UV and VIS range of spectrum.

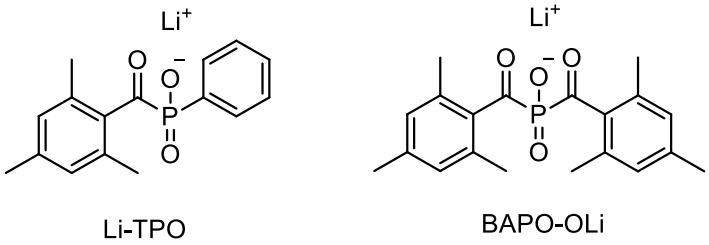

*Figure **3**. Commercially available mono/bisacylphosphineoxide salts.*

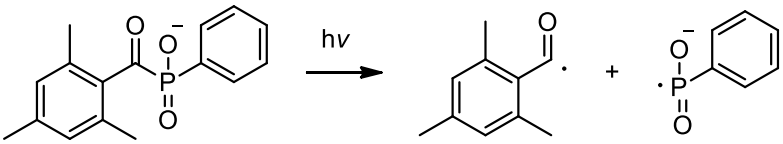

*Figure **4**. Li-TPO photodissociation.*

2.3.2 Ultraviolet and Visible Light

Electromagnetic radiation refers to the waves of the electromagnetic field radiating through space. Electromagnetic radiation is represented by a wide set of wavelengths and energies, which is known as the electromagnetic spectrum (*Figure **5***). This spectrum is divided into many parts/categories, containing also electromagnetic radiation defined as light, which can be, in specific interval of wavelengths (ca. 400 – 780 nm), visible for human eye. Generally, the higher the wavelength, the less energy of radiation is present. Although the ultraviolet and infrared (IR) radiation is not visible for the human eye, it is called (UV/IR) light, too.[21,22]

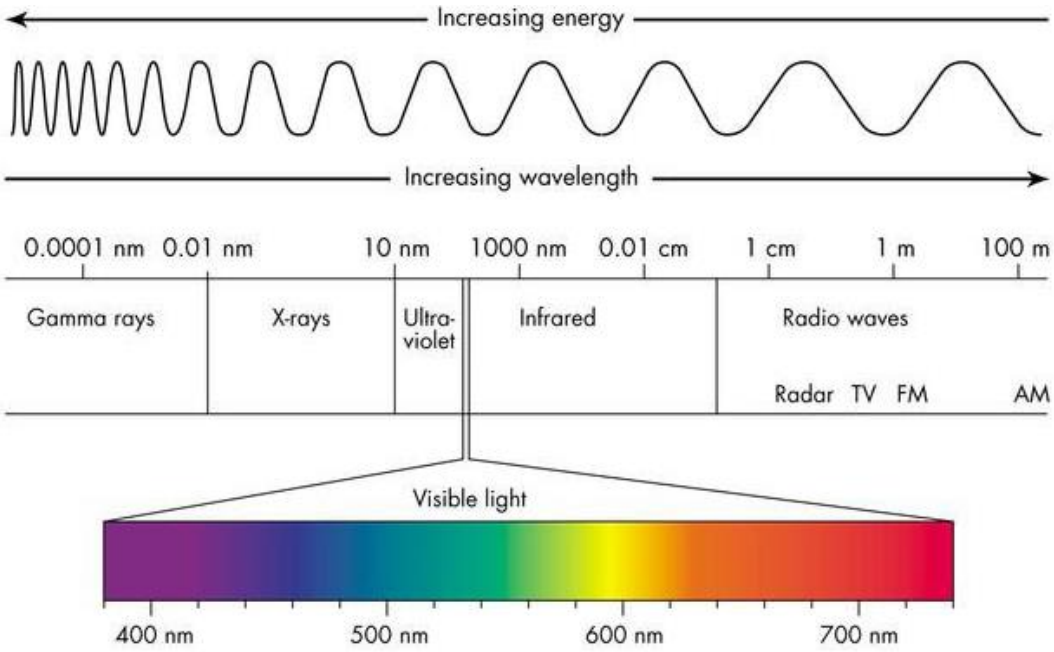

Figure 5. Electromagnetic spectrum.[22]

UV light (with higher energy than VIS light) can be classified into several subcategories depending on their wavelength. Harmless UV-A (315 – 400 nm) is a major part of UV radiation from the sun, which is often used in dermatology. On the contrary, a dangerous part of sunlight, the minor part – UV-B (280 – 315 nm), causes skin cancer and skin aging. Finally, UV-C (200 – 280 nm) with germicidal effect (chemical bond destroyer, microorganism killer) is absorbed in the ozone layer and therefore it is impossible to measure it at the Earth's surface area.[23,24]
During the photopolymerization, UV light was found to be carcinogenic light, thereby the blue light started to be applied in medicine.[25] Above that, shorter wavelengths of UV light do not transmit through the skin as well as the blue one.[23]
Blue light (visible light with short wavelength) with antibacterial effect (405 – 470 nm) is able to destroy bacterial cells due to reactive oxygen species (ROS), which are created during the photoexcitation of endogenous porphyrins.[26] Commercially available lamps (400 – 500 nm) could be employed for polysaccharides cross-linking. Other possible lamp utilization can be 3D printing photosensitive resin system.[20,27] Blue light is also currently used in treatments in dental clinics (*e.g.* dental bleaching method).[28]

2.4 Polysaccharide Modifications

Impurities (organic or inorganic) are often closely related to struggles during the synthesis in the laboratory. Except for the final hydrogel, the cross-linking provides undesirable impurities (unreacted agent, monomers) which should be removed owing to the residue toxicity. However, the problem with purification can be solved with water-soluble and nontoxic macromonomers, which are utilized during cross-linking.[29] Currently, most of the hydrogels are prepared by radical polymerization of water-soluble polysaccharide with vinyl groups.[30] In view of the fact that the vinyl groups are not always present, the synthetic routes leading towards the modified

polymer are essential. The substituted vinyl group introducing into the polysaccharide is called methacrylation.

2.4.1 Methacrylation

Methacrylates can be defined as a class of chemical compounds that are derived from methacrylic acid (MMA). Due to the presence of reactive double bonds in the structure, methacrylates are frequently used in macromolecular chemistry (cross-linking). They are used as building blocks to make a wide range of polymers, especially when durability and stability are needed. Additionally, they are currently utilized in medicinal (*e.g.*, medical equipment such as heart valves or intravenous tubing collectors) and dental (*e.g.*, white dental fillings, artificial teeth) applications.[31,32]

However, methacrylates are widely used during the hydrogels preparation. Hydroxyethyl methacrylate (HEMA) was primarily used in a polymer form as a component of contact lenses. HEMA was firstly discovered by Otto Wichterle and Drahoslav Lím. National Patent Development Corporation bought the licence for the technology in 1965.[28] Hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA) was based on HEMA cross-linked with ethylene glycol dimethacrylate as a cross-linking agent.[4,32] After that, other polysaccharides (such as dextran, gelatin, gellan gum or GK) undergo the methacrylation to form hydrogels employed in biomedical applications.

2.4.1.1 Dextran

Dextran is a bacterial polysaccharide (*Figure* ***6***) which is composed of α-1,6 linked D-glucopyranose with a few percent side chains (*e.g.* α-1,2). Owing to the satisfactory biocompatibility, dextran is also used for the hydrogels preparation, which could be achieved by cross-linking dextran in dimethylsulphoxide (DMSO) with 1,6-hexanediisocyanate.[33]

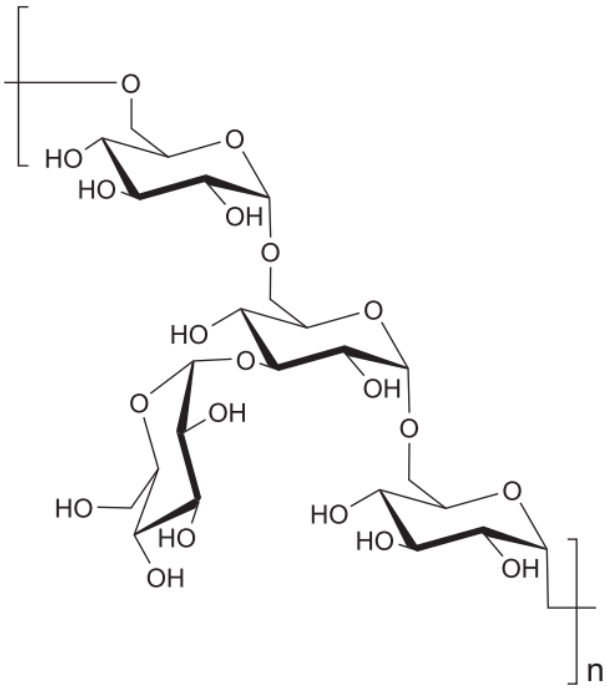

*Figure **6**. Structure of dextran.*[34]

Nevertheless, the reaction does not utilize methacrylate for the subsequent cross-linking.[33] After the unsuccessful attempt to prepare methacrylated dextran by Edman's et al. [35], other studies achieved better results. Möller et al. utilized coupling of glycidyl methacrylate (GMA) to dextran in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) using DMSO as an aprotic solvent (*Figure **7***).[36] Additionally, in 2006, Klemm et al. prepared methacrylated dextran in other way. They employed methacrylic anhydride as a coupling reagent in the presence of triethylamine (TEA) using dimethylformamide (DMF) as a solvent. The reaction provides the methacrylated dextran after 10 h at 80 °C (*Figure **8***).[37]

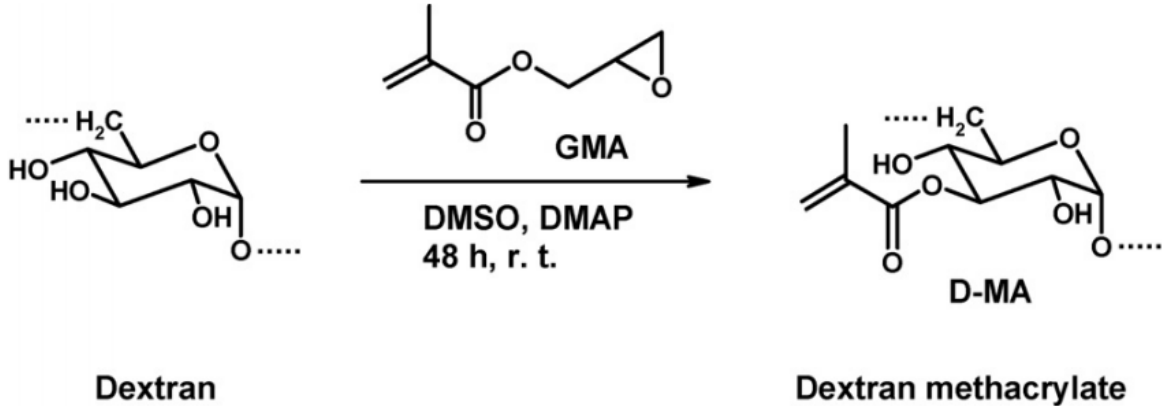

*Figure **7**. Synthesis of dextran methacrylate (Möller).*[36]

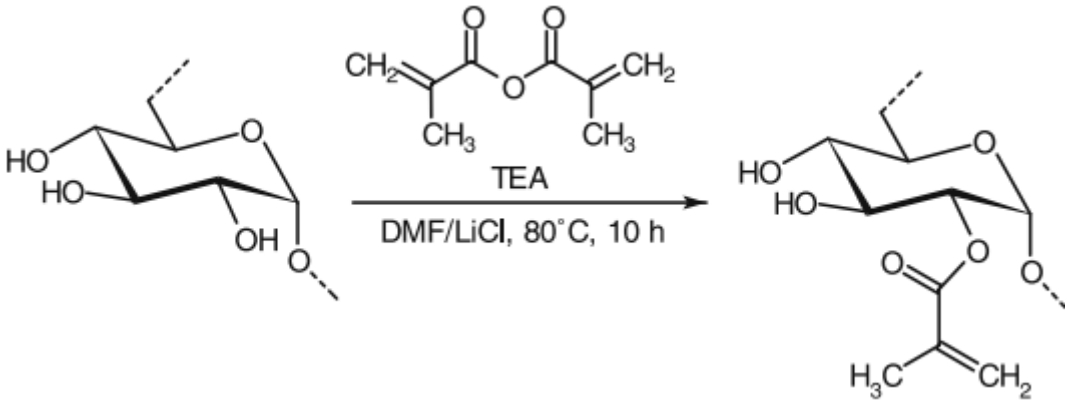

*Figure **8**. Synthesis of dextran methacrylate (Klemm).*[37]

Furthermore, in 2007, the developed synthesis of dextran methacrylate was used for carboxymethyl dextran methacrylate and hyaluron methacrylate preparation.[36]

2.4.1.2 Gelatin

Gelatin is a natural commercially available polysaccharide that was recognized as a suitable material for engineering applications. In 2018, Monteiro et al.[38] described the application of gelatin-based hydrogels in regenerative dentistry (*Figure **9***). Methacrylated gelatin (gelatin methacryloyl, GelMA) was synthesized via gelatin (10% w/v) from porcine skin which was dissolved in Dulbecco's phosphate buffered saline at 50 °C. After that, methacrylic anhydride was added and the mixture was stirred for 1 hour (*Figure **10***).

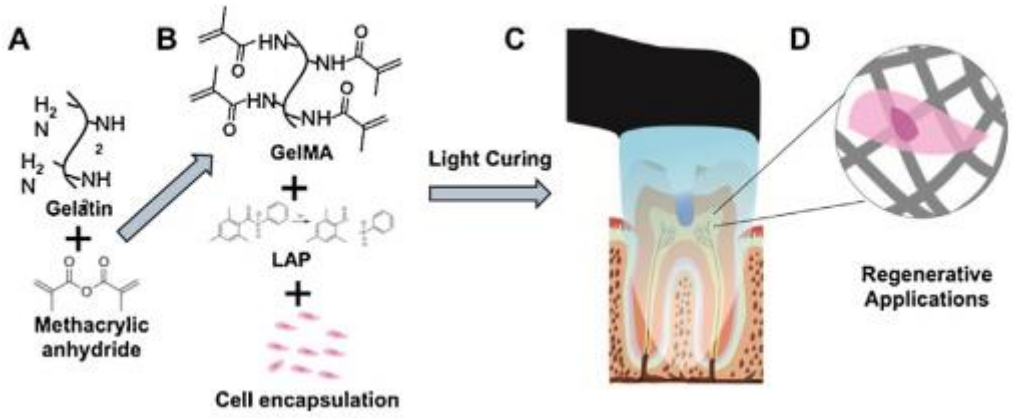

*Figure **9**. Application of GelMA hydrogel. A – starting materials for preparation; B – cell encapsulation; C – example intracanal hydrogel loading and photopolymerization; D – the resulting cell-laden hydrogel material.*[38]

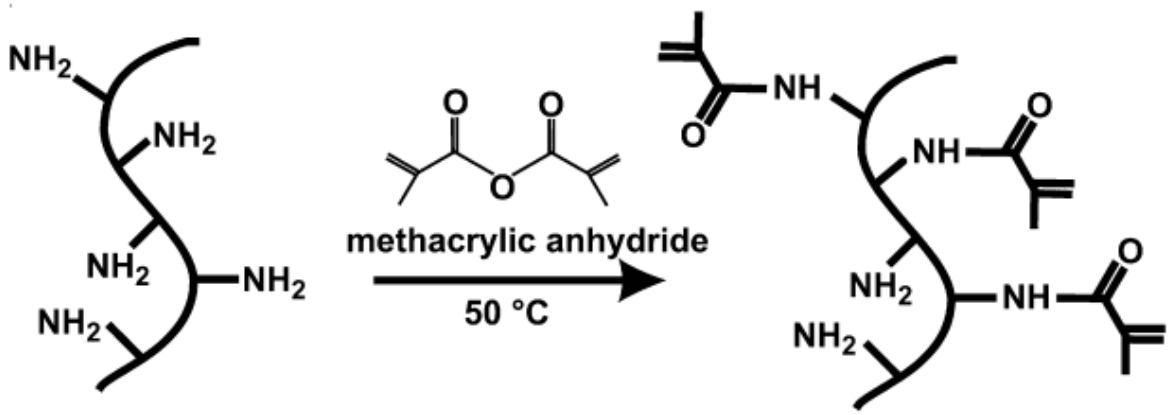

*Figure **10**. Synthesis of gelatin methacryloyl.*[39]

2.4.1.3 Gellan Gum

Another bacterial water-soluble polysaccharide – gellan gum (GG) – is currently exploited in many pharmaceutical applications. Native GG is composed of a repeating unit of β-1,3-D-glucose, β-1,4-D-glucoronic acid, α-1,4-L-rhamnose and two acyl groups. Those acyl groups must be removed in the presence of alkali due to the consequent methacrylation. There are two types of deacetylated GG depending on the degree of deacetylation – high (with limited deacetylation) and low (with almost completed deacetylation) acyl GG.[40]
Coutinho et al.[41] and Pacelli et al.[42] incorporated methacrylate groups into low acyl GG via methacrylic anhydride in the presence of DMAP and TEA using DMSO as an aprotic solvent (*Figure **11***). Methacrylated GG was employed as injectable and photocross-linkable gel in biomedicine.[41,42]

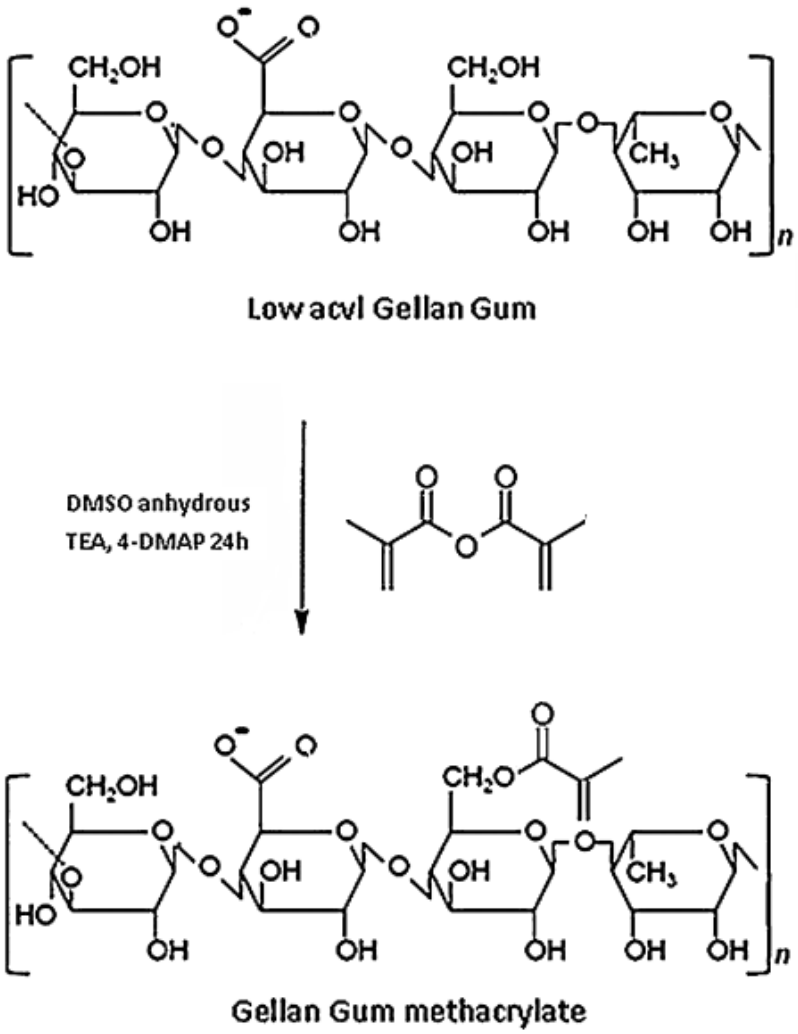

*Figure **11**. General preparation of gellan gum methacrylate; modified.*[42]

2.4.1.4 Gum Karaya

Gum Karaya (GK) is a commercially available heteropolysaccharide from large and bushy tree, *Sterculia urens,* grown in India[43] with really high molecular weight (9 500 000 $g \cdot mol^{-1}$).[44] GK has several industrial applications in pharmaceutical industry, food and environment fields due to its hydrophilic, anionic and biocompatible nature.[45] GK is a highly viscous exudate containing neutral monosaccharide units (galactose and rhamnose), acid residues (galacturonic and glucuronic acid) and acetyl groups.[46] Despite the fact that the chemical structure was already published,[46,47] accurate position of acetyl groups is not completely defined yet. The chemical structure of GK is depicted in *Figure **12***.[45] Moreover, in 2017 Bie et al. described the GK 3D structure, unfortunately, neither this depicted chemical structure contains the precise placement of acetyl groups.[48]

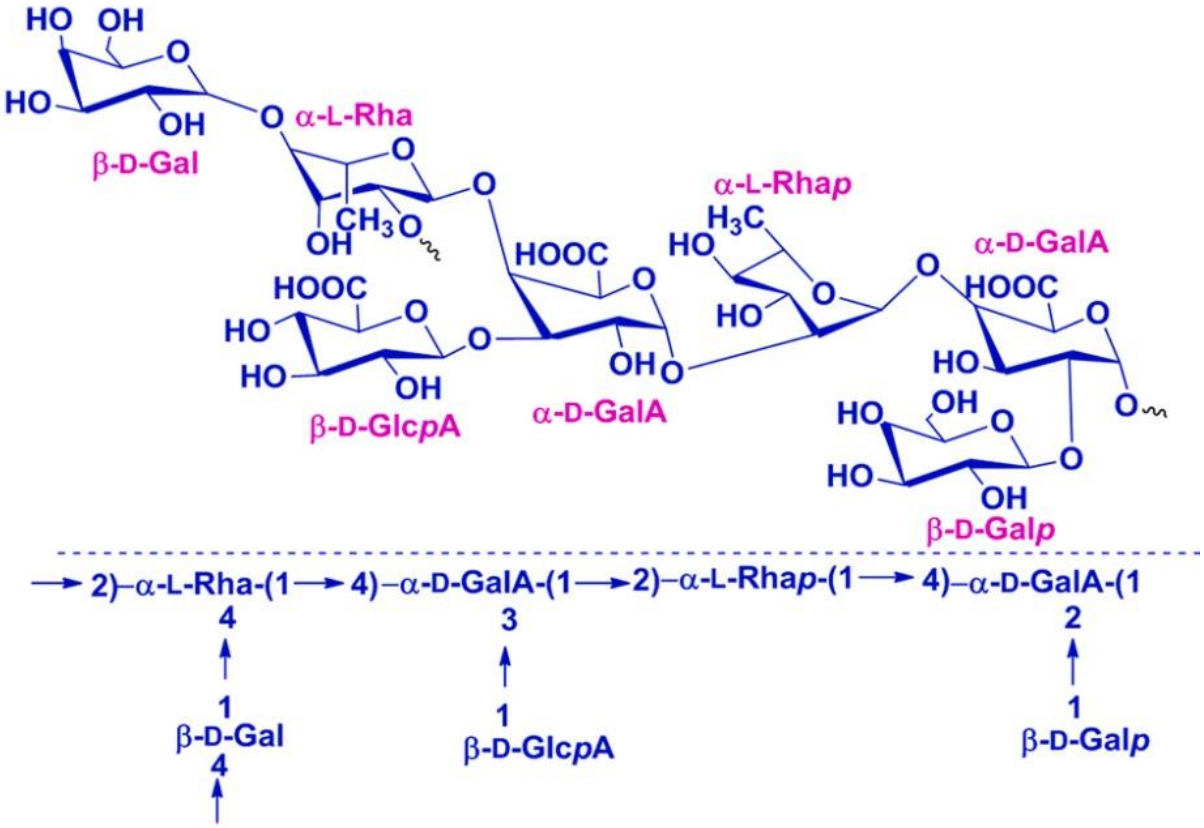

*Figure **12**. Gum Karaya structure (β-D-Galp = β-D-galactose; β-D-GlcpA = β-D-glucoronic acid; α-L-Rhap = α-L-rhamnose; α-D-GalpA = α-D-galacturonic acid).*[45]

Original gum Karaya (OGK) is insoluble in water and organic solvents. That is the reason why the subsequent processing and broader utilization of gray-pink GK in medicine is limited. Natural GK only swells in water due to the presence of acetyl groups and gets sixty times higher volume. This complication was solved by Postulkova et al.[49] Deacetylation of commercially available GK provides water-soluble product – deacetylated gum Karaya (DGK) via hydrolysis of 2 wt. % dispersion of GK in solution of sodium hydroxide. The process of deacetylation (pH = 12) is depicted below. However, electrostatic interactions between anionic groups (carboxyl – carboxyl; carboxyl – hydroxyl) and bivalent ions (*e.g.* Ca^{2+}) can play a crucial role in the aspect of GK solubility. At least partial replacement of bivalent or multivalent ions by monovalent ion (*e.g.* Na^{+}) leads to higher GK solubility in water. This replacement was carried out via strong alkali – sodium hydroxide (*Figure **13***).[49] Deacetylation causes lower GK hydrophobicity owing to the methyl groups removing.[50]

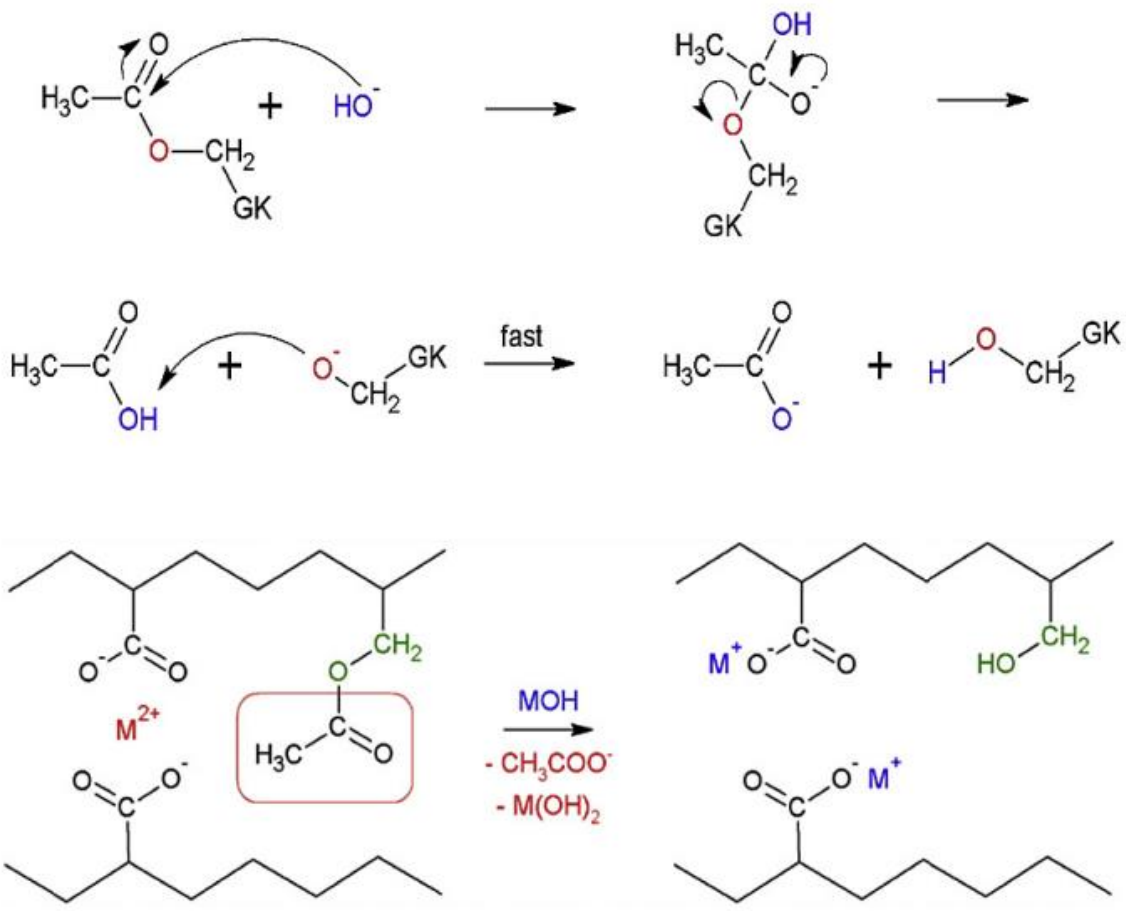

*Figure **13**. OGK deacetylation.*[49]

Methacrylation does not represent the only one possible modification of GK. Other modification was described in 2011 by Singh et al. Solution of GK and polyvinyl alcohol (PVA) was irradiated by gamma rays for a specific time interval to form a crosslinked polymer. Then, the polymer was stirred for 3 hours in 1 : 1 distilled water : ethanol to remove the soluble fraction left in the polymer matrix to obtain GK-cl-PVA hydrogel for biomedical applications.[51] Another GK derivative with antimicrobial activity based on maleic anhydride was prepared in 2020 by Silva et al. To GK was added maleic anhydride in the ratio of 1:2 w/w under stirring at 53 °C followed by *N,N*-dimethylacetamide (DMA) addition at this temperature as a solvent (*Figure **14***). After that, the obtained derivative was washed with distilled water to remove the reaction by-products.[47]

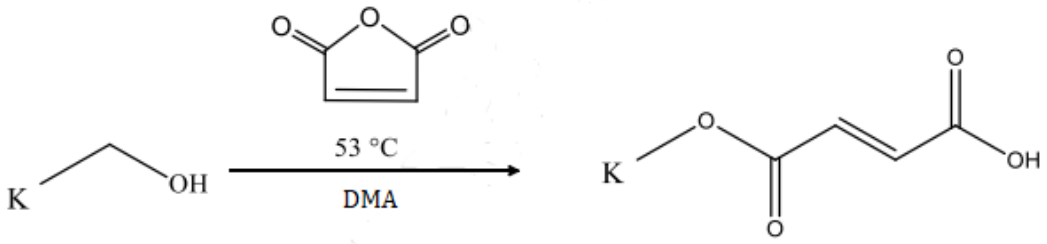

*Figure **14**. Preparation of modified GK via maleic anhydride; modified. K represents the rest of GK chain.*[47]

Despite the fact that methacrylation is not the only way to form GK derivatives with biomedical applications, other synthetic routes are not a point of broad scientific interest. There are many types of methacrylates which enable cross-linking and hydrogel forming. Methacrylates provide diverse types of polysaccharide derivatives which can be employed in biomedicine and other applications. Although the maleic anhydride modification according to Silva et al. provides modified GK, it does not provide a modification than undergoes cross-linking readily and thus is not suitable for hydrogel preparation. There have been described several ways of GK modification, however, for further processing of such modifications by cross-linking, only methacrylates provide the potential of simple and readily available ways of such postprocessing. Moreover, the broad variety of possible methacrylation methods allow additional further control of structure, characteristics and thus final properties of cross-linked modifications of gum Karaya. This determines the methacrylation modifications are the most studied and utilized modifications of GK.[45]

Low price and great availability of GK induce its combination with other polymers and combinations provided remarkable results. Some groups of organic dyes that might be accumulated in water were found to be carcinogenic, mutagenic, and harmful to life. Owing to their photosensitivity and persistence in the environment, generally, they must be eliminated from the water system. Grafted GK via 2-(dimethylamino)ethyl methacrylate might be employed as an efficient adsorbent for the removal of dyes from water.[52] Raizaday et al. developed a synthesis of pH-sensitive microparticles of GK by spray drying technology. This spray can be used for threatening various diseases such as chronic hypertension and diverticulitis.[53] Vinod et al. explored the nanofibres of GK with PVA along with silver nanoparticles and proved their antibacterial activity against specific bacteria (*e.g. Escherichia Coli*).[54] Kankeu et al. investigated the utilization of a hydrogel nanocomposite made of grafted GK with iron oxide for the removal of heavy metal ions from mine effluents.[55] Furthermore, natural GK was utilized as a binder for silicon-based anodes in batteries.[48]

3 RESEARCH OBJECTIVES

The aim of experimental part is to modify natural polysaccharide to form soluble polymer. After that, it is necessary to prepare macromonomers by methacrylation to form a polymer ready to use as a sprayable hydrogel for wound healing. Eventually, to make subsequent chemical analysis to confirm presence of double bonds and choose the specific conditions for the methacrylation.

The work included following steps

- Modification of natural resin and preparation of macromonomers
- Chemical analysis of macromonomers
- Evaluation of results and interpretation of data
- Conclusion

4 EXPERIMENTAL PART

4.1 Chemicals

- Karaya gum, Powder, supplier Sigma-Aldrich, Germany
- Sodium hydroxide, Pearls, g.r., supplier Lach-Ner, Czech Republic
- Hydrochloric acid, 35 %, a.g., supplier Penta, Czech Republic
- Ethanol absolute, a.g., supplier Penta, Czech Republic
- Ethanol, 96 %, supplier VWR International, Czech Republic
- Glycidyl methacrylate, ≥97.0%, supplier Sigma-Aldrich, Germany
- Ultrapure water (Type 1), system Milipore Direct-Q® 3 UV, CEITEC BUT
- Nitrogen (liquid), supplier Linde Gas, Czech Republic
- Chloroform-d 99.8 Atom % D for NMR spectroscopy, supplier Sigma-Aldrich, Germany
- Chloroform, min. 99.5 %, supplier Lach-Ner, Czech Republic

4.2 Equipment

- All glass high-vacuum line, CEITEC BUT
- Centrifuge 5804 R, Eppendorf, USA
- Freeze dryer Epsilon 2-10D LSCplus, Martin Christ, Germany
- Multipoint stirrer Cimarec, Thermo Scientific, USA
- FTIR spectrometer with attenuated total reflectance (ATR) Vertec 70/70v, Bruker, USA
- Raman confocal microscope SOL Nanofinder II, 633 nm laser, RMI, Czech Republic
- NMR Avance NEO, 700 MHz, Bruker, USA

4.3 Syntheses

4.3.1 Deacetylation of Gum Karaya

Original gum Karaya (OGK, 14.4429 g) was mixed with ultrapure water (705.6 g) and the mixture (2 wt. %) was stirred at a speed of 300 rpm at room temperature for 24 h. After that, sodium hydroxide (NaOH, 1 mol dm^{-3}, 240 cm^3) was added to the dispersion of the OGK to adjust pH to 12. The mixture was stirred at room temperature for 7 min. Subsequently, hydrochloric acid (HCl, 0.3 mol dm^{-3}, ca. 720 cm^3) was added slowly until the pH of the reaction mixture reached 7. The neutralized solution was centrifuged at 4200 rpm for 20 min at 25 °C and filtered using the Büchner funnel to remove undesired residues. Eventually, the filtrate was dropped into absolute ethanol in a ratio 2 : 1 (ethanol : solution), and the precipitated product was freeze-dried, crushed into the powder, and stored in a desiccator at room temperature. The reaction provided brown DGK (8.9270 g).

4.3.2 Methacrylation of Gum Karaya

The whole synthesis was performed at a vacuum line under an inert atmosphere (nitrogen) in two-neck reaction flask (*Figure **15***).
Deacetylated gum Karaya (DGK, 0.4013 g) was put into a nitrogenized flask and the flask with DGK was evacuated subsequently (3× vacuo, 3× nitrogen; 10 min for each vacuuming). After that, stripped ultrapure water (39.6 g) (*Figure **16***) was mixed with DGK under inert atmosphere and stirred by magnetic stirrer in the flask using a rod with a ferromagnetic tail at a speed of 700 rpm at 90 °C until DGK was dissolved (approximately 1 h). Then, the solution (1 wt. %) was cooled to 50 °C and sodium hydroxide (NaOH, 0.1 mol dm^{-3}, 0.2 cm^3) was added to reach pH equal to 10 – 11 (measured by litmus paper) against the nitrogen flow. Glycidyl methacrylate (GMA, 1.71 cm^3, 1.0 equiv.) was added dropwise into the solution afterward, and the reaction mixture was stirred at 50 °C for 24 h. The apparatus was wrapped with aluminium foil currently. Subsequently, the unprecipated product was centrifuged with 4200 rpm for 15 min at 25 °C and the solution was dropped into liquid nitrogen pre-cooled ethanol (400 cm^3). The precipitated product was freeze-dried, crushed into the powder, and stored in a desiccator at room temperature. The reaction provided pink-brown methacrylated gum Karaya (MGK, 0.2798 g). The procedure was performed based on Petra Waclawiková master's thesis[56] and the methacrylation was repeated in 4 times under procedure changes (*Table **1***).

*Table **1**. Screening of conditions for the methacrylation.*

Synthesis[a]	GMA : GK units	GMA equiv.	GMA [cm^3]	Time [h]
MGK_1	1 : 2	1.0	1.71	24
MGK_2	1 : 2	1.2	2.05	24
MGK_3	1 : 2	1.2	2.05	48
MGK_4	1 : 1	1.2	4.10	24

[a] All reactions were carried out on a ca. 0.4 g GK scale.

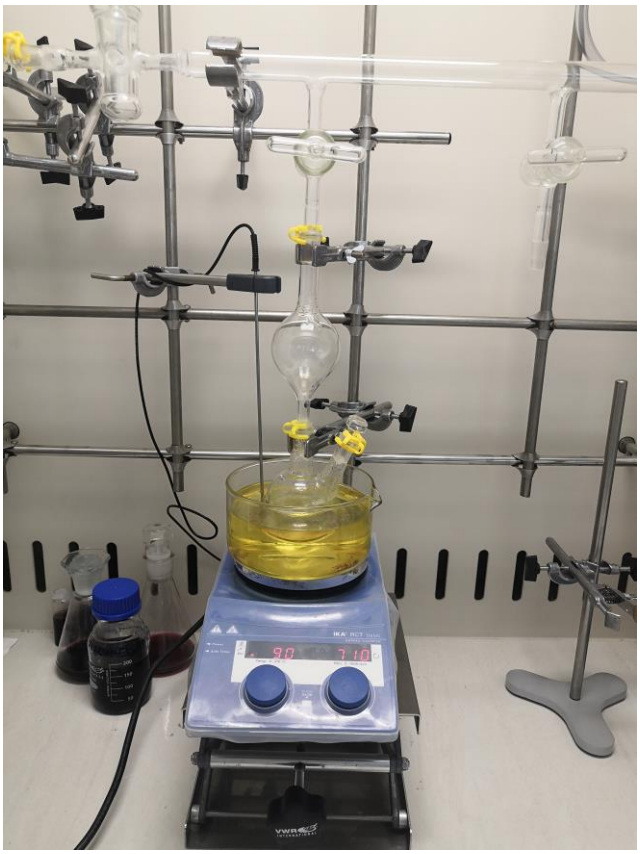

*Figure **15**. Vacuum line with apparatus for methacrylation of Gum Karaya.*

*Figure **16**. Apparatus for water stripping.*

4.4 Physicochemical and Chemical Analysis

Chemical and physicochemical analyses were carried out to elucidate the structure characterization (deacetylation, double bonds forming) and behaviour in a specific environment. The starting material (OGK) and DGK were characterized as well as MGK which was prepared from DGK. All MGK analyses were performed to determine the double bonds. Furthermore, a solubility test of the resulting MGK was accomplished to use MGK as a potentially sprayable hydrogel.

4.4.1 Solubility Test

The solubility test was performed on a final MGK owing to the future utilization in the hydrogel preparation. The test on OGK was carried out during the deacetylation – OGK swells only in water. Solubility of DGK was proved and described during the MGK preparation (see 4.3.2). For hydrogel preparation, ultrapure water was used as a solvent. MGK (0.1 g, 1 wt. %) was added into the round bottom flask with ultrapure water (9.9 g), the flask was put on the reflux condenser and the apparatus was wrapped with aluminium foil. The mixture was stirred at a speed of 400 rpm at 90 °C for 2 h until MGK was dissolved. On the contrary, chloroform was employed as a solvent for NMR analysis. 0.5 wt. % solution of MGK in deuterated chloroform was heated at 40 °C in a 2cm^3 glass vial for 3 h until the homogeneous solution was formed.

4.4.2 Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy

The energy transferred by IR radiation can excite the vibrational and rotational states of the molecule, but there is no excitation of electrons; this is the principle of IR spectroscopy. If the dipole moment changes during vibration, IR radiation is absorbed, which ultimately results in characteristic bands for the individual functional groups. Fourier transform infrared (FTIR) spectrometers provide high sensitivity, resolution, and data acquisition speed. All wavelengths are detected and measured simultaneously using a Michelson interferometer. A beam of IR radiation is passed through the ATR (attenuated total reflection) crystal in such a way that it reflects at least once off the internal surface in contact with the sample. The detector records the attenuated IR beam as an interferogram signal, which can be used to generate an IR spectrum.[57]

All ATR-FTIR spectra were recorded using Vertex 70/70v Bruker spectrometer with a diamond crystal with 64 scans from 800 to 4500 cm^{-1} at resolution 2 cm^{-1}. The measured data were processed in Opus program (version 7.5), Origin, and MS Excel. The measurements were carried out on solid samples at room temperature.

4.4.3 Raman Spectroscopy

When electromagnetic radiation interacts with matter, the scattering appears except for the absorption. Raman spectroscopy is based on inelastic scattering where energy is exchanged between radiation and matter. Raman scattering can be defined as an inelastic collision of a photon with a molecule with the result of vibrational and rotational states of a molecule

changing. The scattering is composed of two types of scattering, actually. Stokes scattering represents the situation when the sample receives part of the energy from the radiation and the photon with lower energy is emitted. On the other hand, a photon with higher energy is emitted during the anti-Stokes scattering (*Figure **17***). Unlike IR spectroscopy with changes in a dipole moment, Raman spectroscopy detects the polarizability tensor changing. Due to this fact, Raman spectra provide bands which correspond to symmetric bonds between homonuclear atoms, *e.g.* C=C. Raman spectroscopy is a kind of complementary method to IR spectroscopy.[58,59]

After the methacrylation, the presence of double bonds cannot be identified by IR spectroscopy. For this reason, Raman spectroscopy with Raman confocal microscope SOL Nanofinder II with 633 nm laser, lens magnification 50/51×, grating range 600, time of measurement 30, 60, 90 or 120 sec was employed. All samples were measured after freeze-drying in a solid state except for GMA in liquid form.

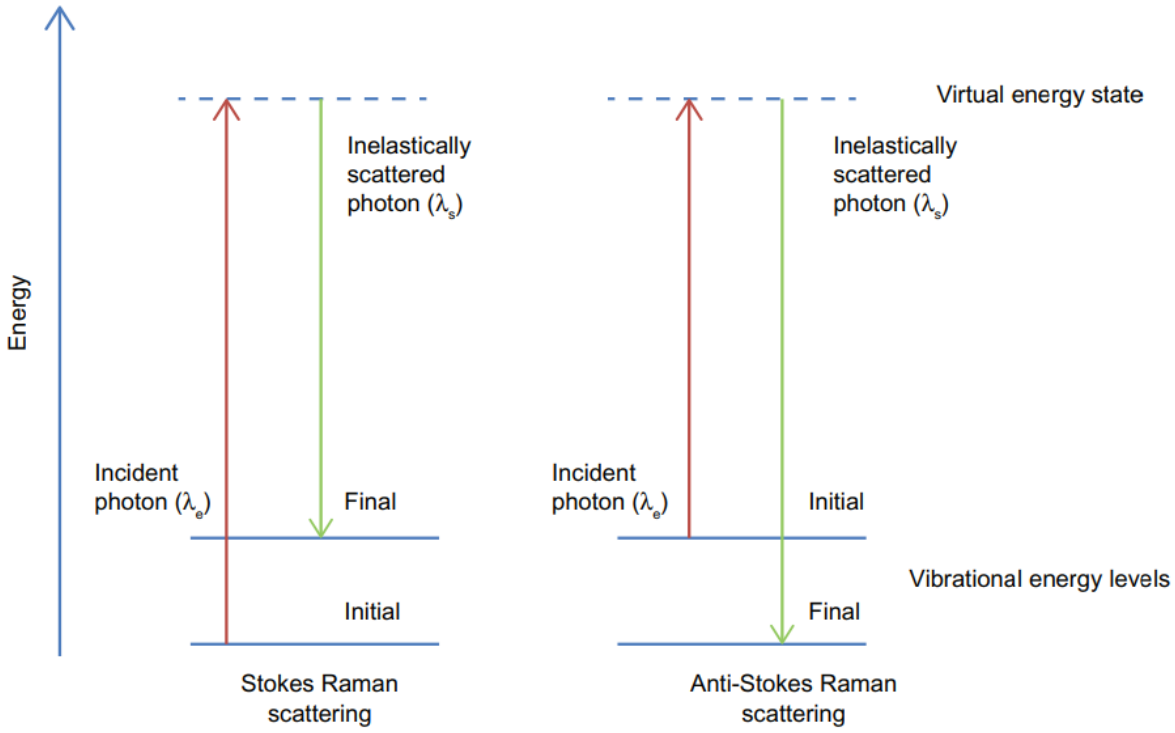

*Figure **17**. The difference between Stokes and anti-Stokes scattering.*[58]

4.4.4 ^{1}H Nuclear Magnetic Resonance Spectroscopy

The principle of nuclear magnetic resonance (NMR) is to monitor the absorption of radiofrequency radiation by a liquid sample that is fitted into the strong magnetic field. A fundamental parameter in NMR spectroscopy is the nuclear spin that is equal to the sum of all nucleons. If the nuclear spin is non-zero and non-integer (*e. g.* ^{1}H, ^{13}C, ^{19}F), the nucleus is active, and the relevant response is received in NMR spectroscopy. Nuclear spins acquire different energy due to the external magnetic field action, a system of levels is observed and the system can excite electrons after the radiofrequency radiation. Subsequently, the spin returns to the original energy level, the absorbed energy is emitted and the emission is detected.

Another parameter in NMR is chemical shift, which is given by a change in the resonant frequency of a given nucleus.[60]

^{1}H NMR spectra were measured on Bruker Avance NEO with working frequency 700 MHz spectrometer at 30 °C at Masaryk University; 5 mm triple-resonance (^{1}H-^{13}C-^{15}N) cryoprobe optimized for ^{13}C detection with cooled ^{1}H, ^{13}C and ^{15}N preamplifiers. Chemical shifts are reported in unit ppm (parts per million; δ) and they are related to residual peak non-deuterated chloroform (^{1}H NMR: δ = 7.26 ppm).

5 mg of MGK was dissolved in 1 cm^{3} of deuterated chloroform and the mixture was heated at 40 °C for 3 h. After that, 0.6 cm^{3} of homogeneous solution was placed into NMR cuvette and the sample was measured. The resulting spectrum was interpreted using MestreNova software.

5 RESULTS AND DISCUSSION

5.1 Syntheses

The scheme (*Figure **18***) and the procedure for the preparation of the modified GK are discussed below. The prepared modified resin was not fully characterized and the methacrylation itself was not optimized completely by Petra Waclawiková. The biggest problem encountered previously was NMR analysis, resp. the insolubility of the prepared MGK in any solvent.[56]
DGK was prepared without any problem according to the literature.[49] OGK was insoluble in water, but it swells only in water. pH adjustment increased the solubility and DGK was soluble, eventually the subsequent synthesis was performed.
Optimization of MGK preparation involved changing the amount of GMA, significantly, shortening the dissolution time of DGK in water, and stripping the solvent (water) before the addition to the reaction flask. Gaseous substances in the solvent could play a role in the reaction and thus the gases were eliminated by stripping.
DGK dissolution was very reluctant. Complete dissolution in a short interval (1 h) was achieved only in case of almost maximum speed of the magnetic stirrer. Furthermore, constant displacement of the magnetic stirrer in the flask using a rod with a ferromagnetic tail was essential.
All syntheses were carried out with pH changing after dissolving of DGK. The pH was adjusted to 10 – 11 with NaOH solution before the GMA addition. The pH of the solution decreased rapidly from alkali to neutral after GMA addition. After completion of the reaction, the reaction mixture was centrifuged. In the beginning, the sediment was not almost formed, therefore the centrifugation could be omitted from the preparation process possibly. The centrifuged homogeneous solution was precipitated into pre-cooled ethanol, and then a white fine polymer film was obtained.

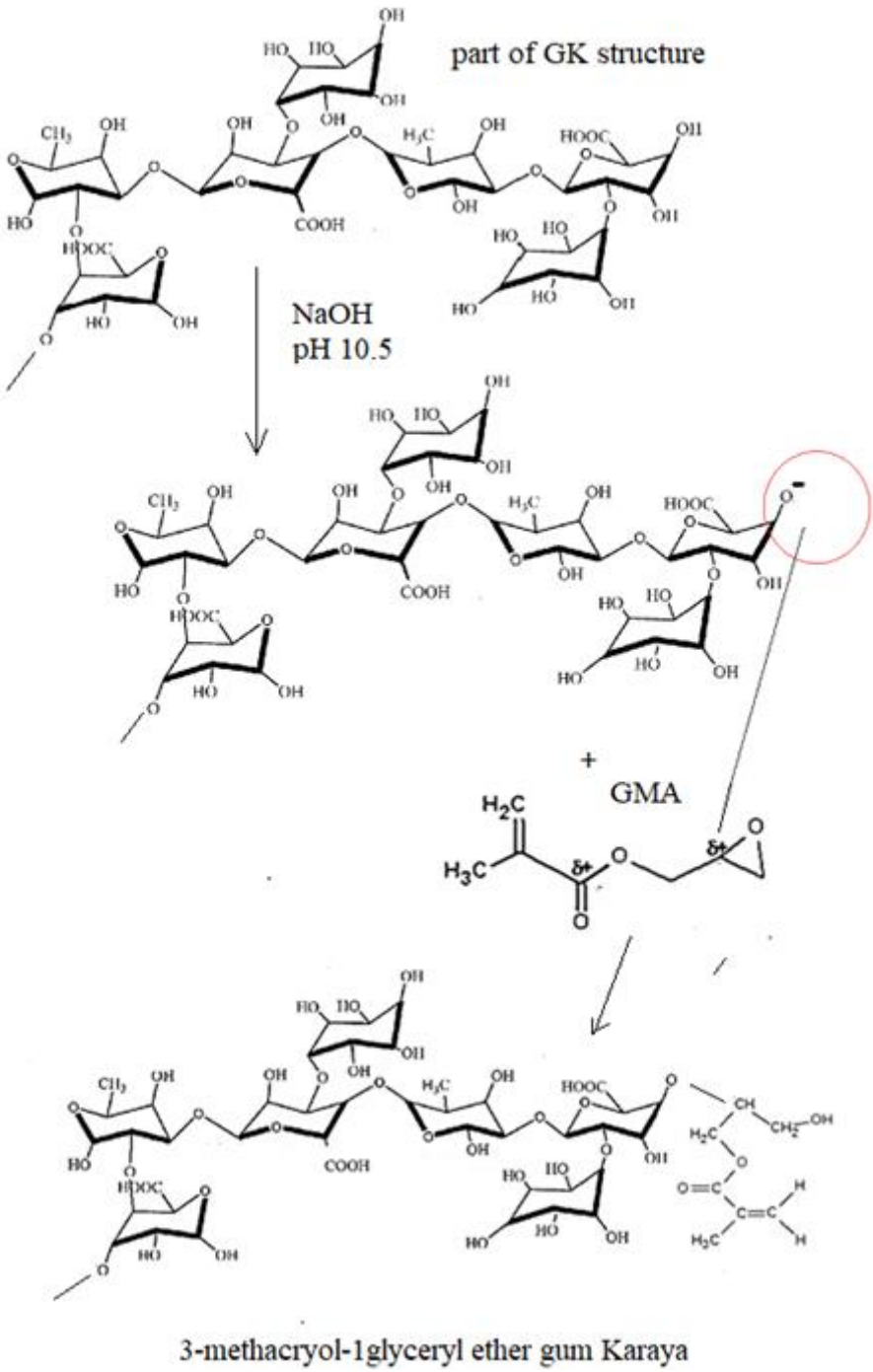

*Figure **18**. Brief scheme of DGK and MGK preparation.*[56]

5.2 Physicochemical and Chemical Analysis

5.2.1 Solubility Test

MGK was able to dissolve in ultrapure water at 90 °C after 2 h. DGK and all MGK samples was dissolved completely in deuterated chloroform at 40 °C after 3 h and thus the described NMR analysis was accomplished.

5.2.2 Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy

It was necessary to identify the characteristic vibrational bands for OGK. The specific bands in the FTIR spectrum for the OGK are indicated below (*Figure **19***), and they are correlated fully with Petra Waclawiková's spectra.[56] The most fundamental band in the spectrum corresponds to the vibration of the C=O group. This functional group should not appear in the spectrum of DGK owing to the process of deacetylation – removing acetyl groups. Subsequent further syntheses, methacrylations, were performed on DGK, so none of the MGK spectra should provide a characteristic vibration corresponding to the original C=O group (1250 and 1725 cm^{-1}, streching). Band 1045 cm^{-1} corresponds to C–O–C glycosid bond; 1380, 1425, 1600 cm^{-1} corresponds to –COO streching; 2945 cm^{-1} corresponds to $–CH_3/–CH_2$ streching; 3400 cm^{-1} corresponds to –OH intermol. H bonds.

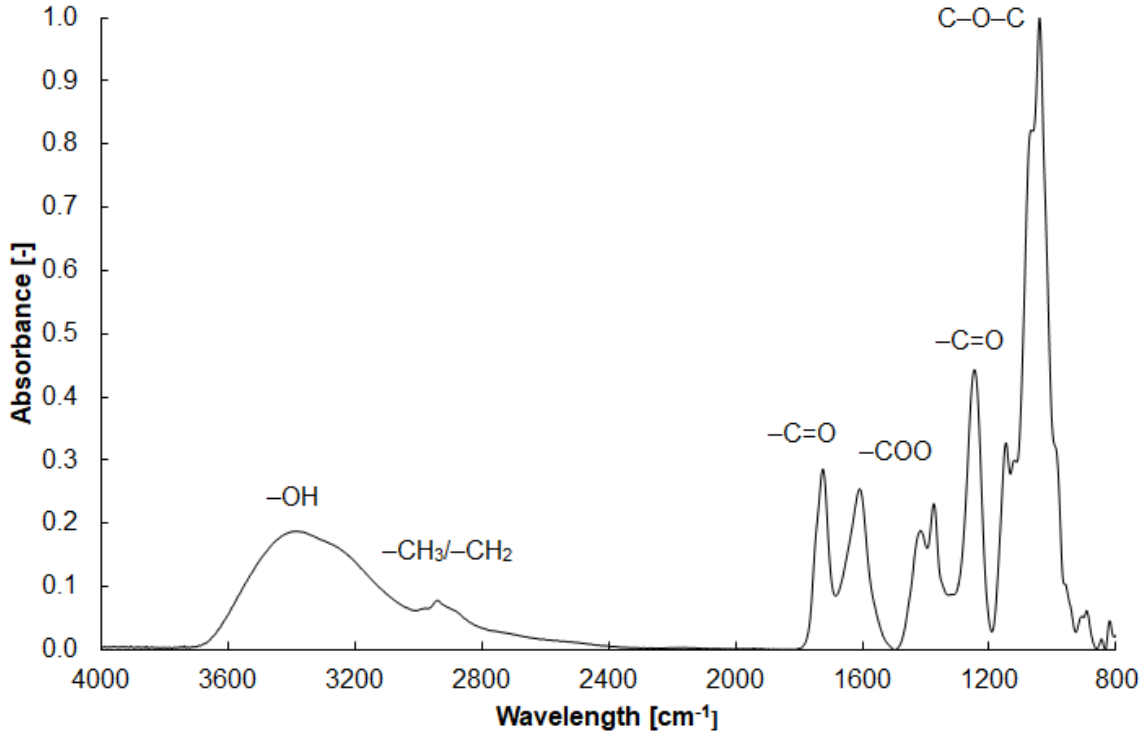

*Figure **19**. FTIR spectrum of OGK.*

The spectra of the OGK and DGK are depicted below (*Figure **20***). The deacetylation occurred totally (the vibrational band for C=O disappeared). The hypothesis was confirmed further. DGK, unlike the OGK, was able to dissolve in water. The OGK swelled in the water purely.

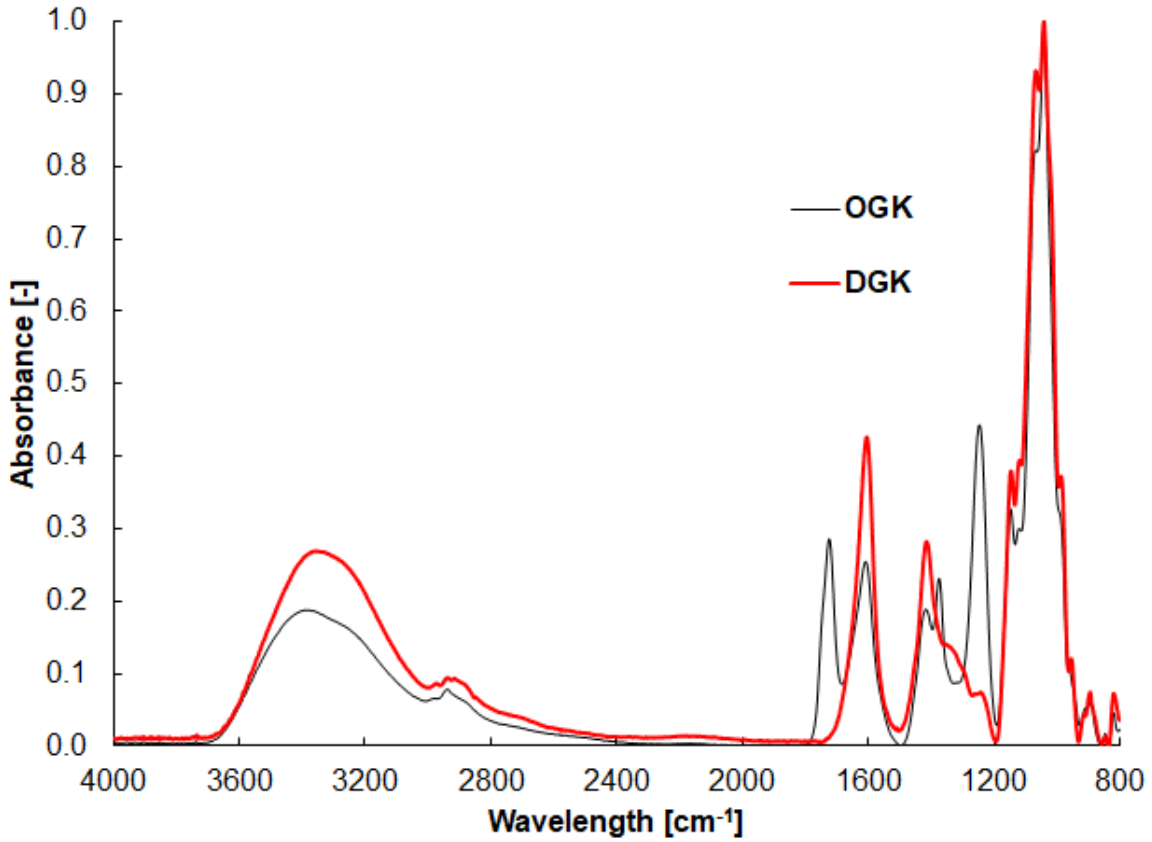

*Figure **20**. FTIR spectra of OGK and DGK.*

If the methacrylation with GMA were performed successfully, double bonds would be formed to DGK. After that, we should observe the characteristic vibration of the C=C bond in the FTIR spectrum. This vibration can be expected at a value of about 1640 cm^{-1}.[62] Although all spectra of MGK differ from the spectra of DGK (*Figure **21***), the characteristic vibration corresponding to the double bond can not be identified directly. However, Raman spectroscopy can help us to identify unambiguously the presence of double bonds. Another bands are as follows: 1045 cm^{-1} C–O–C glycosid bond; 1300, 1400, 1600 cm^{-1} –COO streching; 1700 cm^{-1} –C=O streching; 2945 cm^{-1} $–CH_3$/$–CH_2$ streching; 3400 cm^{-1} –OH intermolecular H bonds.

All MGK spectra are normalized, an approximate quantitative analysis could be determined from them. Nevertheless, NMR spectroscopy is a far better method for quantitative analysis. This analysis is described below.

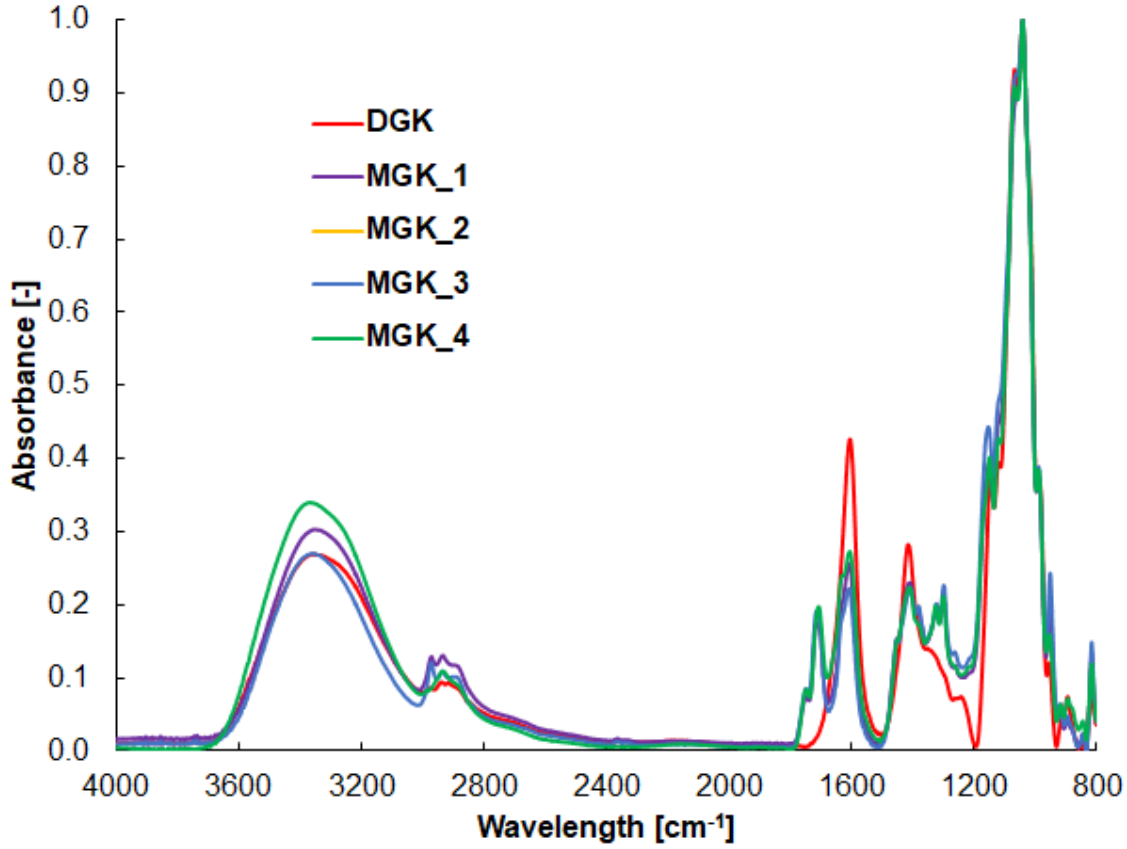

*Figure **21**. FTIR spectra of DGK and all MGK.*

5.2.3 Raman Spectroscopy

Raman spectroscopy was chosen as a method for qualitative analysis of starting materials GMA and DGK, and product MGK furthermore. The presence of double bonds in MGK can not be confirmed completely by ATR-FTIR, thus Raman spectroscopy was utilized. Above that, the method may be employed for liquid as well as solid sample measurement, therefore GMA was measured only by this spectroscopy (*Figure **22***).

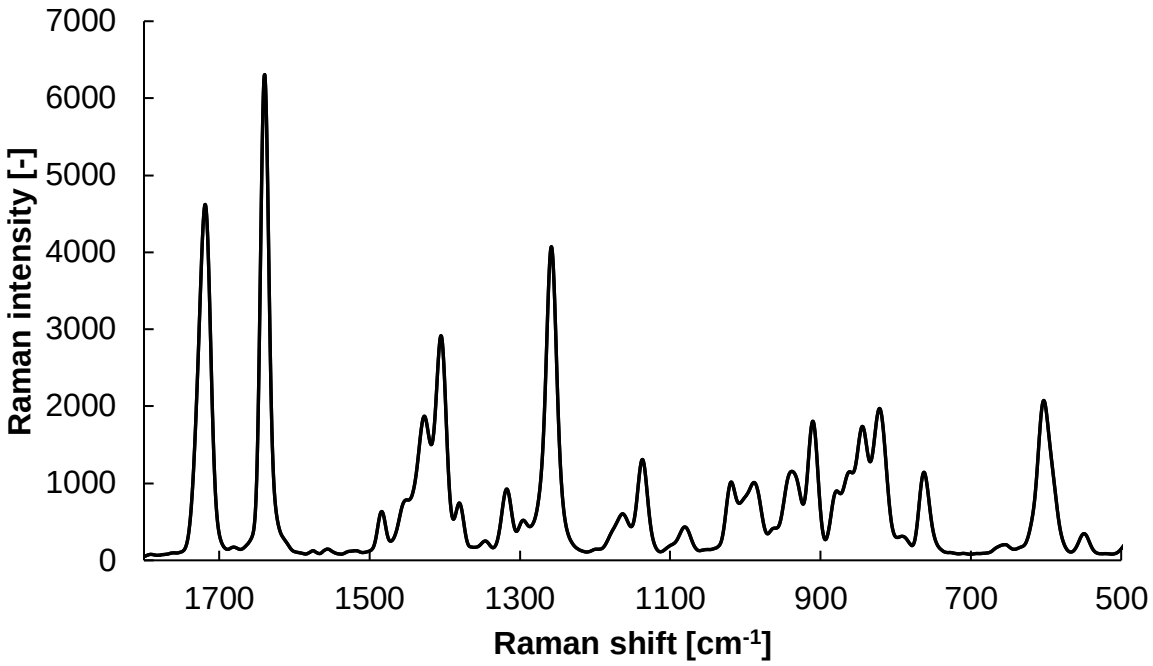

*Figure **22**. Raman spectrum of GMA.*

The vibrational valence of about 1640 cm^{-1} corresponds to a double bond in the spectrum of the methacrylate product. *Figure **23*** compares the values of DGK and MGK. The valence of about 1640 cm^{-1} does not appear in DGK, unlike the MGK spectrum. Each spectrum of MGK looks similar and thus *Figure **23*** depicts only DGK and MGK_2 measurements. Another characteristic bands are: 850 cm^{-1} C−O−C glycosid bond; 1400 cm^{-1} $–CH_3$ streching; 1680 cm^{-1} –COO streching. Although GMA and MGK show similar peaks, the peaks in the MGK spectrum do not correspond to unbound GMA (see NMR analysis below). NMR analysis was chosen as a method for quantitative information about substitution by GMA owing to the fact that Raman spectroscopy can be employed for qualitative analysis only.

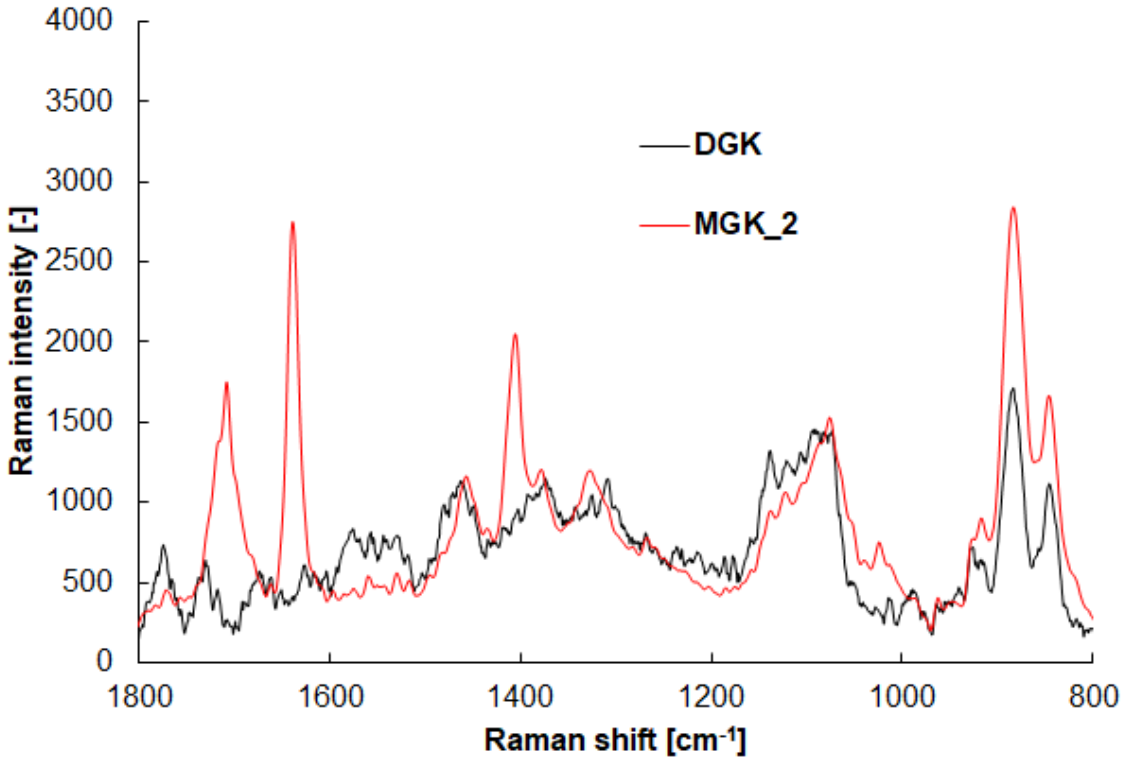

*Figure **23**. Raman spectra of DGK and MGK_2.*

5.2.4 ^{1}H Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR spectroscopy was employed as a suitable method for qualitative and quantitative analysis of MGK. DGK and MGK ^{1}H NMR spectra were compared with each other (*Figure **24***) and all MGK spectra confirmed that methacrylation of vinyl protons was confirmed at 6.15 and 5.63 ppm and methyl protons appeared at 1.96 ppm (*Figure **25***). Similar chemical shifts are described in other publications that refers to favourable methacrylation via GMA, too.[63]

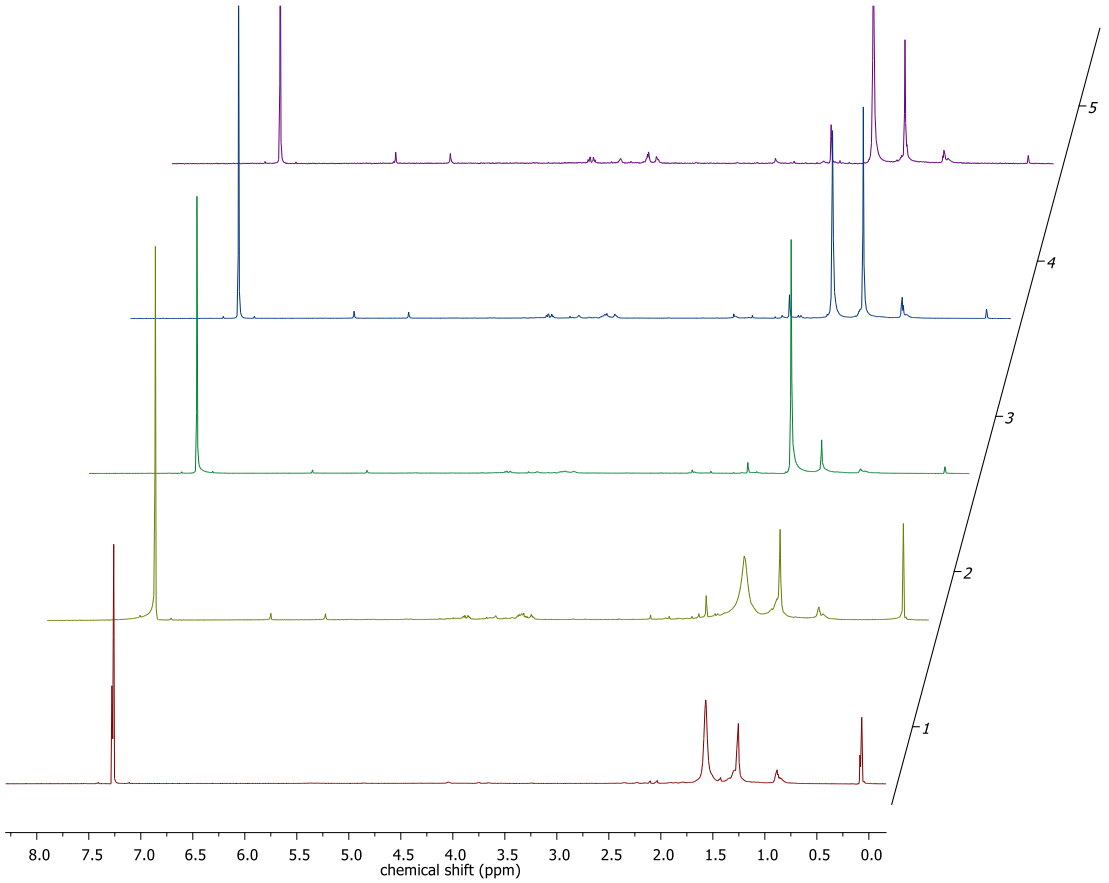

*Figure **24**. [1]H NMR spectra of DGK (1) and MGK_1 – MGK_4 (2 – 5).*

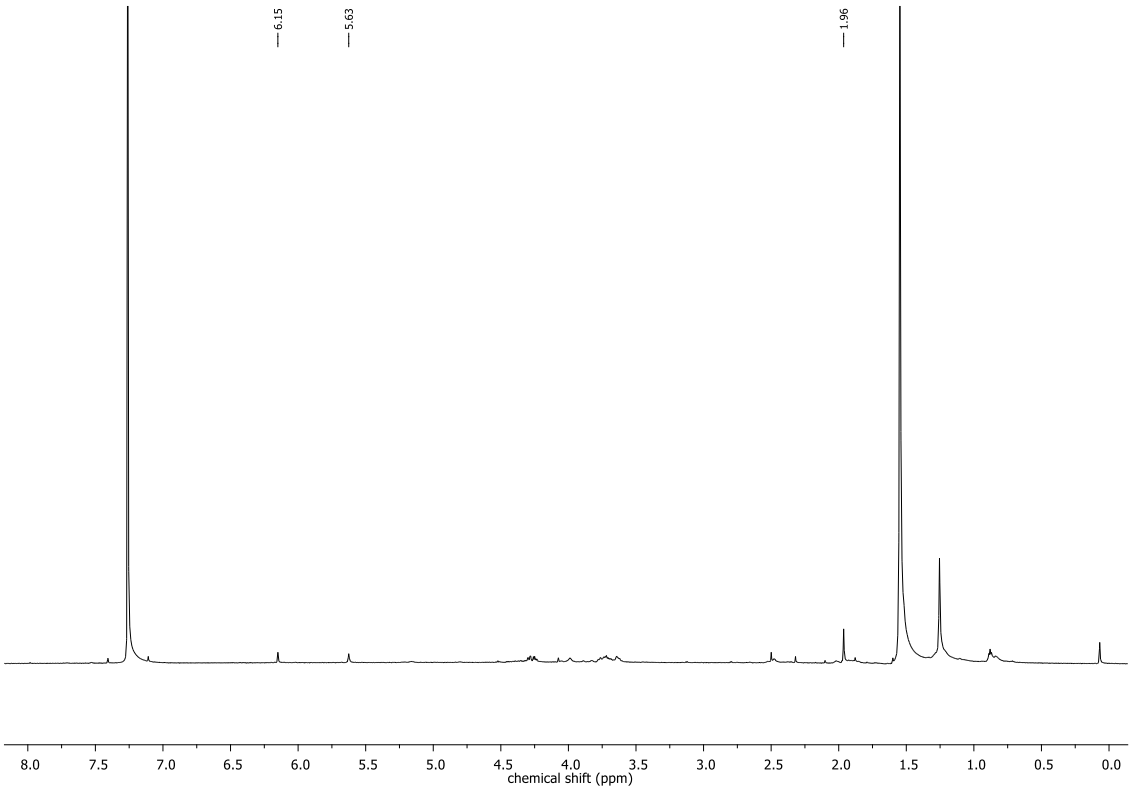

*Figure **25**. [1]H NMR spectrum of MGK_4. Peaks correspond to vinyl protons and methyl protons after methacrylation.*

Qualitative analyses cannot determine which synthesis provided the most methacrylated polymer. Thus, degree of substitution (DS) was chosen as a suitable method for the quantitative determination. The DS of MGK was determined by comparing the intensity *I* of the peak that corresponds to vinyl hydrogen with protons of the (1→4)-α-D-GalA-(1→2)- α-L-Rhap units of DGK from the ^{1}H NMR spectra (*Figure **26***) according to the literature by using the modified equation[64]

$$DS = \frac{\frac{1}{2} \cdot I_{MGK,vinyl\ hydrogen}}{I_{(1\rightarrow4)-\alpha-\mathrm{D}-\mathrm{GalA}-(1\rightarrow2)-\ \alpha-\mathrm{L}-\mathrm{Rhap}}} \cdot 100$$

The calculation of DS was carried out for each MGK (*Table **2***).

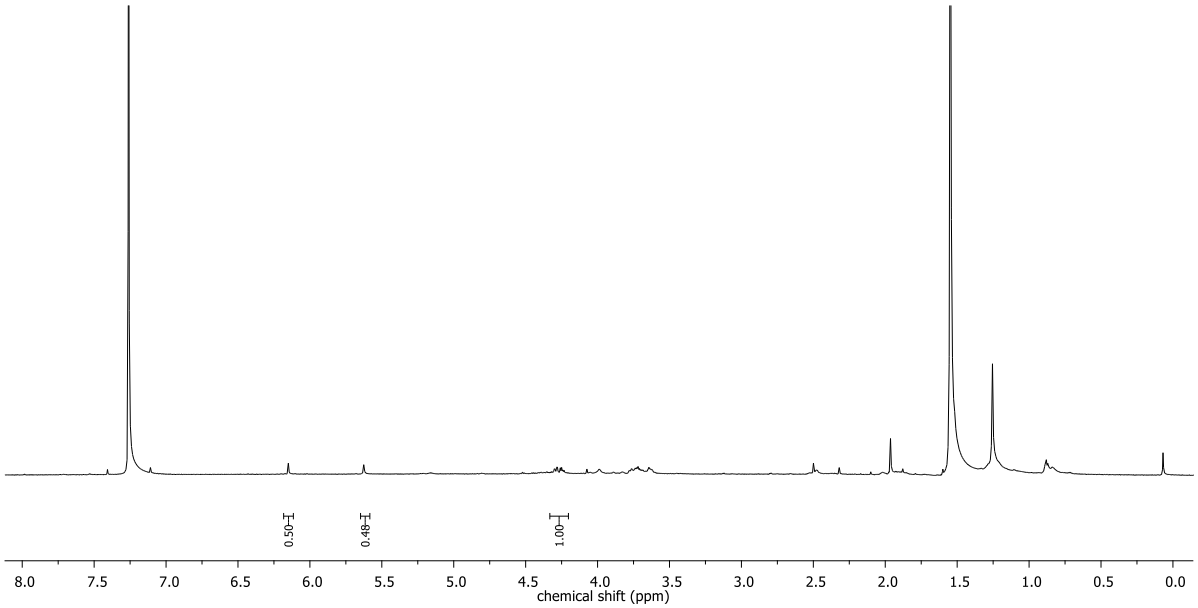

*Figure **26**. ^{1}H NMR spectrum of MGK_4 with intensity of vinyl protons and protons of sugar unit.*

*Table **2**. Screening of conditions for the methacrylation with DS.*

Synthesis[a]	GMA : GK units	GMA equiv.	GMA [cm^3]	Time [h]	DS [%]
MGK_1	1 : 2	1.0	1.71	24	23.0
MGK_2	1 : 2	1.2	2.05	24	25.5
MGK_3	1 : 2	1.2	2.05	48	22.5
MGK_4	1 : 1	1.2	4.10	24	25.0

[a] All reactions were carried out on a ca. 0.4 g GK scale.

The similar DS in each synthesis refers to the fact that the different conditions (reaction time, amount of GMA) do not lead to an appreciable change. Even though the reaction time is much longer, the DS is the lowest. Despite the almost analogous DS in each synthesis, the result itself is positive in contrast to results of the other publication (10 % approximately).[64]

6 CONCLUSIONS

The bachelor thesis describes a synthetic strategy for the preparation of a chemically modified polysaccharide gum Karaya. Gum Karaya was chosen as the natural polysaccharide with healing and antibacterial effects, which was freed firstly of acetyl groups in a strong base because the functional groups limit its solubility. Subsequently, deacetylated gum Karaya was modified by synthetic glycidyl methacrylate to form methacrylated gum Karaya.

Successful removal of acetyl groups was confirmed by the attenuated total reflectance Fourier transformed infrared method. However, this method could not be used to unambiguously prove the presence of double bonds in the polysaccharide after methacrylation. Raman spectroscopy was chosen as an alternative method to demonstrate double bonds qualitatively. The method confirmed the presence of double bonds, fortunately. From a quantitative point of view, it was desirable to use the nuclear magnetic resonance spectroscopy to find the degree of substitution under different reaction conditions. The available data show that the discussed conditions do not have a significant effect on the degree of substitution of the product, a significantly longer reaction time even slightly reduces the degree of substitution. Therefore, synthesis 1 or 2 (MGK_1 or MGK_2) should be chosen for the repeated experiments.

Due to its potential use in medicine as a sprayable hydrogel, the solubility of methacrylated gum Karaya in water, an inorganic solvent commonly used anywhere in the world, was tested. On the contrary, for nuclear magnetic resonance analysis, it was necessary to dissolve the sample in an organic deuterated solvent ($CDCl_3$). MGK was able to dissolve in both mentioned solvents.

Another assignment should be the effort to cross-link the prepared modified polysaccharide, determine its properties, and try to use it in a sprayable form for wound healing (complicated and deep skin defects), undoubtedly.

7 LIST OF ABBREVIATIONS

3D	three-dimensional
ATR	attenuated total reflection
DGK	deacetylated gum Karaya
DMA	*N*,*N*-dimethylacetamide
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DS	degree of substitution
FTIR	Fourier transform infrared (spectroscopy)
GelMA	gelatin methacryloyl
GG	gellan gum
GK	gum Karaya
GMA	glycidyl methacrylate
HEMA	hydroxyethyl methacrylate
IR	infrared (light)
Li-TPO	lithium phenyl-2,4,6-trimethylbenzoylphosphinate
MGK	methacrylated gum Karaya
MMA	methacrylic acid
OGK	original gum Karaya
PHEMA	poly(2-hydroxyethyl methacrylate)
PI	photoinitiator
PVA	polyvinyl alcohol
ROS	reactive oxygen species
TEA	triethylamine
UV	ultraviolet (light)
VIS	visible (light)

8 REFERENCES

1. Niaz, K., Khan, F. & Shah, M. A. Analysis of carbohydrates (monosaccharides, polysaccharides). *Recent Adv. Nat. Prod. Anal.* 621–633 (2020) doi:10.1016/b978-0-12-816455-6.00018-4.
2. Historically, D., Fischer, E. & Bel, L. Chapter 1. Structures of the Open-chain Forms of Reducing Sugars, and their Carbonyl Group Reactions. in *Carbohydrate Chemistry and Biochemistry* 1–40 (2008). doi:10.1039/9781847558015-00001.
3. Murray, R. K. *et al. Harperova Ilustrovaná biochemie*. (Galen, 2012).
4. Al-Assaf, S., Coqueret, X., Dahlan, K. Z. H. M., Sen, M. & Ulanski, P. *The Radiation Chemistry of Polysaccharides*. (2016).
5. Alemdar, M. & Ceylan Tuncaboylu, D. Rheological Analysis of Polysaccharide Hydrogels. *Starch/Staerke* (2020) doi:10.1002/star.202000198.
6. Li, J. & Mooney, D. J. Designing hydrogels for controlled drug delivery. *Nat. Rev. Mater.* **1**, 1–18 (2016).
7. Tavakoli, J. & Tang, Y. Hydrogel based sensors for biomedical applications: An updated review. *Polymers (Basel).* **9**, 1–25 (2017).
8. Schlegel, P. N. *et al.* Effective long-term androgen suppression in men with prostate cancer using a hydrogel implant with the GnRH agonist histrelin. *Urology* **58**, 578–582 (2001).
9. Aswathy, S. H., Narendrakumar, U. & Manjubala, I. Commercial hydrogels for biomedical applications. *Heliyon* **6**, e03719 (2020).
10. Williams, D. F. *Biofunctionality and Biocompatibility*. *Materials Science and Technology* (2006). doi:10.1002/9783527603978.mst0160.
11. Hago, E. E. & Li, X. Interpenetrating polymer network hydrogels based on gelatin and PVA by biocompatible approaches: Synthesis and characterization. *Adv. Mater. Sci. Eng.* **2013**, (2013).
12. Hoffman, A. S. Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **64**, 18–23 (2012).
13. Pellá, M. C. G. *et al.* Chitosan-based hydrogels: From preparation to biomedical applications. *Carbohydr. Polym.* **196**, 233–245 (2018).
14. Turgis, J. D., Vergé, C. & Coqueret, X. Composition effects on the EB-induced cross-linking of some acrylate and methacrylate copolymers. *Radiat. Phys. Chem.* **67**, 409–413 (2003).
15. Nguyen, K. T. & West, J. L. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials* **23**, 4307–4314 (2002).
16. Nguyen, M. K. & Alsberg, E. Bioactive factor delivery strategies from engineered polymer hydrogels for therapeutic medicine. *Prog. Polym. Sci.* **39**, 1235–1265 (2014).
17. Benedikt, S. *et al.* Highly efficient water-soluble visible light photoinitiators. *J. Polym. Sci. Part A Polym. Chem.* **54**, 473–479 (2016).
18. Meereis, C. T. W. *et al.* BAPO as an alternative photoinitiator for the radical polymerization of dental resins. *Dent. Mater.* **30**, 945–953 (2014).
19. Steyrer, B., Neubauer, P., Liska, R. & Stampfl, J. Visible light photoinitiator for 3D-printing of tough methacrylate resins. *Materials (Basel).* **10**, 1–11 (2017).
20. Alasiri, R. A., Algarni, H. A. & Alasiri, R. A. Ocular hazards of curing light units used in dental practice – A systematic review. *Saudi Dent. J.* **31**, 173–180 (2019).
21. Stark, G. Light. https://www.britannica.com/science/light. [cit. 2021-01-21]
22. Franson, J. Principal of Structural Chemistry. https://sites.google.com/a/coe.edu/principles-of-structural-chemistry/relationship-between-light-and-matter/electromagnetic-spectrum. [cit. 2021-01-21]

23. Monteiro, N. *et al.* Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry. *Dent. Mater.* **34**, 389–399 (2018).
24. Lighted, A. What is UV Light. http://www.aqualightech.com/UV.asp?id=305. [cit. 2021-01-21]
25. Griesbeck, Axel G., M. O. and F. G. *CRC handbook of organic photochemistry and photobiology*. (CRC Press, 2012).
26. Jandt, K. D., Mills, R. W., Blackwell, G. B. & Ashworth, S. H. Depth of cure and compressive strength of dental composites cured with blue light emitting diodes (LEDs). *Dent. Mater.* **16**, 41–47 (2000).
27. Oktay, E. A. *et al.* Dental resin curing blue light induces vasoconstriction through release of hydrogen peroxide. *J. Photochem. Photobiol. B Biol.* **185**, 41–45 (2018).
28. Caló, E. & Khutoryanskiy, V. V. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur. Polym. J.* **65**, 252–267 (2015).
29. Rimmer, S. *Synthesis of hydrogels for biomedical applications: control of structure and properties*. *Biomedical Hydrogels* (Woodhead Publishing Limited, 2011). doi:10.1533/9780857091383.1.51.
30. Petrochemicals Europe. https://www.petrochemistry.eu/sector-group/methacrylates/ (2020). [cit. 2021-01-21]
31. Methacrylate Producers Asociations Inc. http://www.mpausa.org/about-methacrylates/ (2020). [cit. 2021-01-21]
32. Wichterle, O. & Lím, D. Hydrophilic Gels for Biological Use. *Nature* **185**, 117–118 (1960).
33. Simonsen, L., Hovgaard, L., Mortensen, P. B. & Brøndsted, H. Dextran hydrogels for colon-specific drug delivery. V. Degradation in human intestinal incubation models. *Eur. J. Pharm. Sci.* **3**, 329–337 (1995).
34. Delattre, C., Laroche, C. & Michaud, P. Production of microbial and fungal polysaccharides. *Adv. Ferment. Technol.* 483–522 (2007).
35. Edman, P., Ekman, B. & Sjöholm, I. Immobilization of proteins in microspheres of biodegradable polyacryldextran. *J. Pharm. Sci.* **69**, 838–842 (1980).
36. Möller, S., Weisser, J., Bischoff, S. & Schnabelrauch, M. Dextran and hyaluronan methacrylate based hydrogels as matrices for soft tissue reconstruction. *Biomol. Eng.* **24**, 496–504 (2007).
37. Klemm, D., Heinze, T., Liebert, T., Heublein, B. & Hornig, S. Functional Polymers Based on Dextran BT - Polysaccharides II. *Polysaccharides II* **205**, 199–291 (2006).
38. Monteiro, N. *et al.* Photopolymerization of cell-laden gelatin methacryloyl hydrogels using a dental curing light for regenerative dentistry. *Dent. Mater.* **34**, 389–399 (2018).
39. Nichol, J. W. *et al.* Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials* **31**, 5536–5544 (2010).
40. Prajapati, V. D., Jani, G. K., Zala, B. S. & Khutliwala, T. A. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydr. Polym.* **93**, 670–678 (2013).
41. Coutinho, D. F. *et al.* Modified Gellan Gum hydrogels with tunable physical and mechanical properties. *Biomaterials* **31**, 7494–7502 (2010).
42. Pacelli, S. *et al.* Injectable and photocross-linkable gels based on gellan gum methacrylate: A new tool for biomedical application. *Int. J. Biol. Macromol.* **72**, 1335–1342 (2015).
43. Dhiman, M., Sharma, L., Singh, A. & Sharma, M. M. Exsitu Conservation Using In vitro Methods of an Endangered Plant Sterculia urens Roxb.: A High Volume Trade Plant for Gum Karaya. *Ind. Crops Prod.* **158**, 113015 (2020).
44. Dhua, M., Maiti, S. & Sen, K. K. Modified karaya gum colloidal particles for the

management of systemic hypertension. *Int. J. Biol. Macromol.* **164**, 1889–1897 (2020).
45. Raj, V., Lee, J. H., Shim, J. J. & Lee, J. Recent findings and future directions of grafted gum karaya polysaccharides and their various applications: A review. *Carbohydr. Polym.* **258**, 117687 (2021).
46. Lujan-medina, G. A. *et al.* Karaya Gum : General Topics And Applications. *Macromol. an Indian J.* **9**, 2013 (2013).
47. Silva, S. C. C. C. *et al.* Antibacterial and cytotoxic properties from esterified Sterculia gum. *Int. J. Biol. Macromol.* **164**, 606–615 (2020).
48. Bie, Y., Yang, J., Nuli, Y. & Wang, J. Natural karaya gum as an excellent binder for silicon-based anodes in high-performance lithium-ion batteries. *J. Mater. Chem. A* **5**, 1919–1924 (2017).
49. Postulkova, H. *et al.* Study of effects and conditions on the solubility of natural polysaccharide gum karaya. *Food Hydrocoll.* **67**, 148–156 (2017).
50. Verbeken, D., Dierckx, S. & Dewettinck, K. Exudate gums: Occurrence, production, and applications. *Appl. Microbiol. Biotechnol.* **63**, 10–21 (2003).
51. Singh, B., Sharma, V. & Pal, L. Formation of sterculia polysaccharide networks by gamma rays induced graft copolymerization for biomedical applications. *Carbohydr. Polym.* **86**, 1371–1380 (2011).
52. Bidarakatte Krishnappa, P. & Badalamoole, V. Karaya gum-graft-poly(2-(dimethylamino)ethyl methacrylate) gel: An efficient adsorbent for removal of ionic dyes from water. *Int. J. Biol. Macromol.* **122**, 997–1007 (2019).
53. Raizaday, A. *et al.* Development of pH sensitive microparticles of Karaya gum: By response surface methodology. *Carbohydr. Polym.* **134**, 353–363 (2015).
54. Padil, V. V. T., Nguyen, N. H. A., Ševcu˚, A. & Černík, M. Fabrication, Characterization, and Antibacterial Properties of Electrospun Membrane Composed of Gum Karaya, Polyvinyl Alcohol, and Silver Nanoparticles. *J. Nanomater.* **2015**, (2015).
55. Fosso-Kankeu, E., Mittal, H., Waanders, F., Ntwampe, I. O. & Ray, S. S. Preparation and characterization of gum karaya hydrogel nanocomposite flocculant for metal ions removal from mine effluents. *Int. J. Environ. Sci. Technol.* **13**, 711–724 (2016).
56. Waclawikova, P. Blue-light crosslinked hydrogels from modified natural gum for medical application. (Brno University of Technology, 2020).
57. Skoog, D. A., West, D. M., Holler, F. J. & Crouch, S. R. *Analytická chemie*. (VŠCHT Praha, 2019).
58. Wang, W., McGregor, H., Short, M. & Zeng, H. Clinical utility of Raman spectroscopy: current applications and ongoing developments. *Adv. Heal. Care Technol.* 13 (2016)
59. Das, R. S. & Agrawal, Y. K. Raman spectroscopy: Recent advancements, techniques and applications. *Vib. Spectrosc.* **57**, 163–176 (2011).
60. Günther, H. *NMR Spectroscopy: Basic Principles, Concepts and Applications in Chemistry*. (Wiley, 2013).
61. Reis, A. V. *et al.* Reaction of glycidyl methacrylate at the hydroxyl and carboxylic groups of poly(vinyl alcohol) and poly(acrylic acid): Is this reaction mechanism still unclear? *J. Org. Chem.* **74**, 3750–3757 (2009).
62. Oehr, C., Müller, M., Elkin, B., Hegemann, D. & Vohrer, U. Plasma grafting - A method to obtain monofunctional surfaces. *Surf. Coatings Technol.* **116**–**119**, 25–35 (1999).
63. Kollarigowda, R. H. Novel polysaccharide nanowires; Synthesized from pectin-modified methacrylate. *RSC Adv.* **5**, 102143–102146 (2015).
64. Padil, V. V. T., Senan, C. & Černík, M. Dodecenylsuccinic Anhydride Derivatives of Gum Karaya (Sterculia urens): Preparation, Characterization, and Their Antibacterial Properties. *J. Agric. Food Chem.* **63**, 3757–3765 (2015).