Cross-linking of Gelatine Hydrogels using UV irradiation

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ZADÁNÍ BAKALÁŘSKÉ PRÁCE

(projektu, uměleckého díla, uměleckého výkonu)

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Téma práce:	**Síťování hydrogelu želatiny pomocí UV záření**

Zásady pro vypracování

Pro možné využití hydrogelu želatiny v biologických aplikacích bude tato nejprve modifikována za účelem získání methakrylamidových postranních skupin, které umožní následné zesítění pomocí radikálové polymerace účinkem UV záření s využitím fotoiniciátoru. Na takto připravených hydrogelech budou posléze zkoumány jejich viskoelastické vlastnosti.
Zásady pro vypracování:

1. Vypracování literární rešerši na zadané téma
2. Příprava modifikované želatiny
3. UV síťování želatiny
4. Charakterizace viskoelastických vlastností hydrogelů želatiny s různou hustotou polymerní sítě.
5. Vyhodnocení a diskuze získaných výsledků

Forma zpracování bakalářské práce: **tištěná/elektronická**
Jazyk zpracování: **Angličtina**

Seznam doporučené literatury:

[1] DERAKHSHANFAR, S., MBELECK, R., XU, K.G., ZHANG, X.Y., ZHONG, W., XING, M. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances. Bioactive Materials. 2018, vol. 3(2), p. 144-156.
[2] CHOI, G., CHA, H.J. Recent advances in the development of nature-derived photocrosslinkable biomaterials for 3D printing in tissue engineering. Biomaterials Research. 2019, vol. 23(1), art. no. 18.
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Termín odevzdání bakalářské práce: **17. května 2024**

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ABSTRAKT

Bakalářská práce se zabývá syntézou a charakterizací hovězí modifikované želatiny, přípravou hydrogelu pomocí radikálového síťování UV zářením a charakterizací viskoelastických vlastností takto připravených hydrogelů s různou hustotou polymerní sítě na základě reologických poznatků. V první, teoretické, části práce jsou prezentovány znalosti z oblasti hovězí želatiny a jejího hydrogelu a reologické analýzy. Druhá, praktická, část již pojednává o reálné přípravě modifikované želatiny, jejího chemického síťování za pomocí UV záření do podoby hydrogelu a jeho následné charakterizaci stran viskoelastických vlastností.

Klíčová slova: želatina, hydrogel, kolagen, fotoiniciace, viskoelastické vlastnosti

ABSTRACT

The bachelor thesis tackles the synthesis and characterization of bovine modified gelatine, the preparation of hydrogels by UV radical cross-linking and characterization of highly viscoelastic properties of gelatine hydrogels with a different density of polymer net based on rheological measurements. In the first, larger, part, theoretical knowledge of bovine gelatine and its hydrogel and the rheological analysis are presented. The second part is about the real preparation of the modified gelatine, its chemical cross-linking using the UV light into the form of a hydrogel and the subsequential characteristics of the sides of viscoelastic properties.

Keywords: gelatine, hydrogel, collagen, photoiniciation, viscoelastic properties

Acknowledgements, motto and a declaration of honour saying that the print version of the Bachelor's thesis and the electronic version of the thesis deposited in the IS/STAG system are identical, worded as follows:

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CONTENTS

INTRODUCTION

Gelatine is a clear protein gained by four types of hydrolysis; acid, alkaline, enzymatic and thermic. The result is a gel-like structure of a particular strength called pork or bovine gelatine. The properties of gelatine are determined by the properties of the collagen from which the gelatines originated.

The human body is rich in collagen and therefore in gelatine too as they fulfil numerous vital roles within the organism. These important and sophisticated bodily functions and reactions gave the humankind the desire to be able to create such materials artificially. As the humankind advances in the medical and biomedical field, several people tend to imitate and create an exact replica of the skin. That is why it is important to find such a material that has very similar mechanical properties as the skin and this is the main goal of this work, to find a hydrogel material with mechanical viscoelastic properties – flexibility and elasticity – like the skin.

While there are several lab methods that lead towards the polymer of gelatine, the cross-linking of hydrogel of bovine gelatine using UV radiation in several concentrations of methacrylic anhydride was used in this case, so that the strength and other properties of the results could be compared.

The IUPAC terminology is used throughout this Bachelor´s thesis.

I. THEORY

1 GENERAL CHARACTERISTICS AND USE OF GELATINE

In the book called „Český lékopis 2001“ [1] by the Ministry of Health in the Czech Republic, this substance is defined as a cleaned protein obtained either by alkaline hydrolysis and/or partially acid hydrolysis and/or enzymatic hydrolysis, or thermic hydrolysis of animal collagen. Hydrolysis results in gel-like or non-gel-like products.

The quality of gelatine depends on the quality of collagen. There are two types of gelatine – A and B. Both types are non-toxic.

It is created by enzymatic hydrolysis of gelatine, which means that the protein at high temperatures and the presence of enzymatic proteases decomposes on simpler compounds.

1.1 Pork gelatine – type A

It is processed by an acid hydrolysis. After washing and cutting, the skin is placed in an acid bath with a pH around 1.5 for 24 to 48 hours. Following this the skin is washed by pure water and put into stainless steel cook tubs. The material is gradually heated from 50 °C to the boiling point to achieve denaturalization and solubilization of collagen. This method is also used for fish skin or bones.

1.2 Bovine gelatine - type B

This hydrocolloid is rich in glycine, proline and hydroxyproline. It is synthesised from the alkaline digestion of collagen from bovine skin or the skin of an old cattle.

1.3 Physical and chemical properties of gelatine

The most important property, apart from hydrolysates, is the ability to create a jelly structure after the dissolving and the subsequent cooling down. After heating up the material becomes liquid. This change in the state of matter, liquid-jelly-liquid, is its specific property and is used in many food-related and unrelated technologies. According to the quality there are various degrees in the firmness of gelatine. The firmness is measured in Bloom degrees by specialists on specialised devices. Based on the data, the density is set, which is crucial for the selling price. Gelatine dissolves at 30 °C in water.

1.4 Chemical and microbial properties of gelatine

Gelatine as a foodstuff of an animal origin is under a constant very strict quality inspection done by both the producer and corresponding responsible authorities. For one thing, chemical properties are observed, which includes humidity, arsenic, copper, zinc, iron, lead, sulphur trioxide and ash content, for another thing, the level of certain microorganisms are also checked.

First of all, the total number of microorganisms is recorded. The number has to be under 1000/g. Amongst those belong coliform (none in 1 gram) and anaerobic (less than 10 in 1 gram) bacteria, clostridium perfingens (none in 1 gram), salmonella (none in 25 grams) and staphylococcus aureus (none in 0.1 gram).

During the manufacture gelatine is under a constant laboratory surveillance which watches the compliance of the indicators with the norms in effect. The whole system of checking proceeds according to the HACCP rules and the ISO 9001 regulation.

The manufacturing plants are under a constant veterinary surveillance which observes the whole manufacturing process starting with the foodstuff reception and ending with the final expedition. Every manufactured batch has a veterinary certificate.

1.5 Observable characteristics

Bloom – is an important indicator of the firmness of the gelatine gel. Measuring of it is done in a cooled solution at the concentration 6.67 wt.% (7.5 g of gelatine and 105 g of water). Gelatine gets dissolved at 60 °C and then cools down on 10 °C for 16 hours. The firmness of the jelly is measured by a *Bloom* gelometer. The princip of this device lies in the resistance of jelly against the cylinder with a diameter of 4 mm and its penetration into the depth of 12.7 mm. For instance, if the force for getting there is 200 g, the Bloom merit is 200 Bloom. The food gelatine sold on market has 80–300 Bloom. However most used type has 220–260 Bloom.

pH – this merit is set by the technological process that was used. The A type has it between 4.7–5.2 and the B type has it between 7.5–9.3.

Amino acids – the amount of protein is between 84–87 % and is composed of 18 different amino acids.

Granularity – the unit is mesh. Depending on the application, the material can be coarse-grained or powdery. Most commonly used gelatine has granularity between 20–30 mesh, which corresponds with a powder.

Viscosity – this merit is found out by a viscosimetric pipette in the solution concentrated on 6.67 wt.% at 60 °C. It ranges from 15 to 75 m^2 s^{-1}. This parameter is very important for the technological process of manufacturing certain products. For example, a low merit is required for the gumdrop production (to pour the material easily into forms) and a high merit is required for the fabrication of hard pharmaceutical capsules.

Melting Point – the temperature at which the transition from a solid state into a liquid solution begins. Numerically, is it between 28 and 30 °C.

Solidification Point – the temperature at which the transition from a liquid state into a solid state begins. Solidification happens gradually at the temperature below 25 °C.

Both points are important at some applications of gelatine for the settings of the needed temperatures during the manufacturing process or for the manipulation with some manufactured products. For increasing both points gelatine is often combined with other hydro-colloids like guar gum, xanthan and carrageenan. [8]

1.6 Industrial Use of Pork Gelatine

The pork gelatine is vastly used as the main ingredient of jelly candies, gumdrops, jellybeans and food supplements. The process of brewing bones and creating a jelly structure appeared in 1682 by Denis Papin and the word gelatine appeared around 1700. In 1754, the first patent for manufacturing gelatine was accepted in England. Since 1850 the gelatine material became known to be used in the photographic industry and 20 years later C. Voit found out that gelatine is a protein. After the World War 2, in 1950 the industrial manufacture of gelatine increased and thanks to the research development several new technologies capable of producing high-quality gelatine arose.

Following can be listed as brand-new types of gelatine:

Gelatine hydrolysates that are used as a supplement of vitally important proteins in human diet. They help renewing the joint fluid, function as a prevention of arthritis and osteoporosis. As a food supplement they are recommended for the people who put a high load on their joints and bones like sportsmen. During hydrolysis of gelatine molecule chains get shorter and that increases the digestibility and the use of amino acids inside it. Hydrolysis destroys

the gelling ability, the Bloom merit is zero, and subsequently the gelatine hydrolysate gets soluble in cold water and can be used in protein drinks.

Sliced gelatine was produced to be used in households for food preparation in a simple form.

Instant gelatine is produced by a special process enabling gelatine to dissolve in cold water. The production includes swelling instead of gelling, which is used in dessert, filling and creme preparation.

1.6.1 Food industry

On the market, gelatine is in a form either as powder or as slices. The sliced gelatine exists in Bronze, Silver, Gold, Platinum versions, which vary in the Bloom strength and weight. [10]

This industry is the biggest consumer of gelatine. The most common uses are:

- Meat products, in particular hams, aspics, decorative jelly, bonding agents for fat and water and the surface treatment of meat products,
- Fish products such as fish and sea food aspics,
- Sweets, probably the most popular products are gumdrops, soft caramel candies, marshmallows and liquorice,
- Ice cream, in *gateau* desserts and ice creams gelatine functions as a stabilizator of whipping cream, a bonding agent and a prevention of crystal formation,
- Milk products, gelatine is used as a thickener of low-fat products and as a stabilizator of whipping cream in dessert cremes. It is also often used in a decorative jelly.

1.6.2 Other industries

Given the large amount of use in several industrial areas only the most important ones are listed.

Pharmaceutical industry – gelatine is mainly used for the production of soft and hard capsules and as a bonding agent for tablets containing pharmaceutical ingredients.

Photography – modern photography cannot do without gelatine. The photographic materials that are manufactured on the basis of silver salts require gelatine as a bonding agent for individual layers of both films and photographic papers.

Metallurgy – gelatine is used in electrolytes, adding it into the mixture of zinc and cadmium leads to clearing it, which allows the production of highly cleansed metals.

Plastic production – makes of use of gelatine as a protective factor and for the ability to control the size of particles.

Paper production – gelatine as a helping bonding agent, increases the resistance against humidity and the firmness of the material.

Cosmetics – mainly gelatine hydrolysates are used in the production of cosmetic products like lotions, creams, shampoos and soaps due to the high levels of collagen nourishing the skin and hair.

1.7 Compatibility of gelatine and further research

In order to be used for specific applications gelatine is combined with other hydrocolloids or similar substances to achieve specific properties.

Thanks to a long-term use of gelatine in many industrial fields and a constant development during its manufacture new possibilities of use still emerge.

Gelatine is still going to be used a lot in the future and is going to be found useful in many other industries. [6, 8]

2 COLLAGEN

The properties of gelatine are determined by the type and properties of the collagen that the gelatine is composed of. Having this natural material allowed vertebrates to evolve into present form as it helps body cells create tissues in the presence of oxygen. Thanks to collagen vertebrates develop skeletons, spine and limbs. Therefore, it can be found in connective tissues like tendons, ligaments, cartilages, bones, vascular cells, vitreous humour, corneal, sclera, basilar membrane, skin and teeth. Connective tissue contains a lot of intracellular substance that consists of mucopolysaccharids. This substance separates fibroblasts.

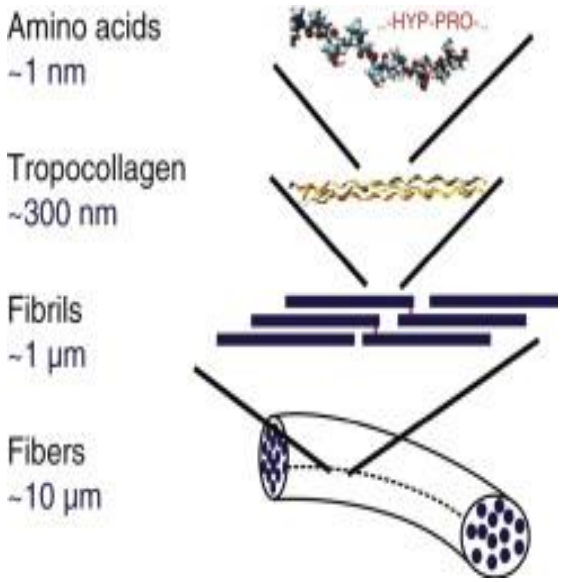

Figure 1. Illustration of collagen development. [2]

It is a complicated, fibrous, extracellular, heterogenic, water-nonsoluable protein that serves as a cornerstone of fibrous tissues. Collagen composes roughly one third of all protein in the body of mammals. Its usual forms are fibrous tissues or nets. At the moment, there are 28 different types of collagen. The main unit is called tropocollagen. The alpha 1 and alpha 2 chains create triple helix.

Its role in the process of aging is crucial due to degenerative processes and changes in collagen production. [2, 3, 13]

2.1 Hydrolysed collagen

Hydrolysed collagen (Figure 2) arises as a consequence of thermic degradation of crosslinked peptide bonds of collagen proteins. Hydrolysate is a mixture of particles of different size and weight, while their molar mass is always lower than the molar mass of native collagen. During the creation of gelatine, it is not needed to protect gentle collagen structure. Raw materials for the gelatine manufacture go through the initial process during the presence of strong acids and bases. That is why the conditions for the hydrolysate gain are more drastic and allow the use of wider range materials containing collagen (bones). Given the easy access of a large amount of the cheap material the hydrolysed collagen is usually the cheapest form of collagen protein which is also used widely in the food industry. It can be added as an ingredient ensuring better density and consistence or used as a component for the products of cheaper cosmetics and food supplements as a less valuable substitute for native collagen protein. [7]

Figure 2. Dry powder of hydrolysed collagen. [6]

2.2 Freeze-dried collagen

There is a great difference in the quality of hydrolysed collagen used for the manufacture of the freeze-dried one. The expensive one maintains intact triple helix only for living organisms. Lyophilisation is one of the methods for gaining dried products. It is based on sublimation drying which happens in the conditions under 0 ° C and lowered pressure. This is used for the substances that are sensitive for changes, especially for heating. The consequence of this process is that active substances maintain their activity without destroying their valuable components like vitamins, proteins, enzymes, minerals etc. Such products contain minimal amount of water and do not require any conservations. By diluting the products by water, the biological and organoleptic properties do not get lost. In practise, this phenomenon is used in collagen food supplements to regenerate skin, bones and

cartilages. This type of collagen is also used during the manufacture of face masks and collagen strips. Lyophilisation in general is costly and that is reflected also in the price of the products.

2.3 Animal collagen

Collagen is composed of basic protein coming from animal organisms. This group contains more than 20 different types of proteins which vary in its composition, properties, location in tissues and in the functions they fulfil. These proteins dominate in connective tissues from which skin, cartilages, bones and important body organs are derived from. Collagen proteins vary very little among each other however these differences determine their properties. The collagen of higher vertebrates is bound in more complicated way and has higher temperature of denaturation. On top of that, during the process of aging of the collagen in higher animals, outer and intracellular lateral covalent bonds are formed within their bodies and their purpose is to support the stability of collagen fibres. Along with the increasing amount of such bonds the density and durability of the collagen goes higher as well. Nonetheless the solubility decreases. Such a density of collagen grid does not give a good appeal to the human skin and it loses its firmness and agility. The net of collagen from fish skin is much sparse that the collagen from cattle and pork skin. Nevertheless, its solubility is higher. In this regard, the higher solubility allows the final product, collagen gel, to gain the native collagen form as long as a very gentle method under strict conditions is used to achieve this protein. Collagen extraction from pork and bovine skins requires aggressive processing using strong chemicals and enzymes which cause strong degradation of this protein. What is more, bovine protein can be a carrier of prions, which are potentially contagious proteins. Prions are potentially contagious proteins which usually occur in most mammals and reptiles. They are not dangerous until they change the natural confirmation after which they become prion infectious protein. Infectious prions cause deadly dangerous disease of the nervous system in animals and humans like mad cow disease or *Creutzfeldt-Jakob* disease. Fish belonging to lower organisms have never been proven to be able to create prion proteins.

2.4 Plant collagen (phytocollagen)

Into this group belong complex sugars of plant origin which on the skin create an effect of protective coat and the contraction similar to the one that happens when an animal collagen is put on. Despite that these compounds do not work in the full scale of collagen. In this case it is only a mental abbreviation or marketing term since only animals are able to produce

collagen. Collagen contains two specific amino acids – hydroxyproline and hydroxylysine – that are typical for this protein.

There is, however, a plant protein – extensine – that contains hydroxyproline, but has a very different structure. Despite the presence of the essential amino acid hydroxyproline which increases the moisture of skin its effect is only similar to the effect of collagen and cannot replace it.

2.5 Micro collagen

This term stands for smaller molecules of proteins – oligopeptides – which resemble molecules of natural collagen by its effect. These particles can affect the production of not only collagen but also other compounds of dermis. This type of collagen is manufactured in biotechnological laboratories where it is modified by adding of fat molecules which increase its stability and help micro collagen penetrate through protective layers of the skin more easily. [4]

3 CROSSLINKING

It means changing a monomer into a polymer. During this process the molecular weight changes along with the properties. A greater molecular weight provides the polymer with the greater physical and chemical durability, higher melting point and better mechanical tenacity. The necessary condition for crosslinking is the presence of reactive groups (-NH_2, -COOH, -OH) on the side of the main chain that can react with the crosslinker. There are two types of crosslinking – chemical and physical. [14]

3.1 Physical crosslinking

The physical method does not require any crosslinker. The process is initiated by applying the outer conditions like heating or cooling. The bonds that are typical for this crosslinking are Van der Waals´, hydrophobic bonds, electrostatic forces and hydrogen bonds, which are the strongest among the mentioned. Nevertheless, a great disadvantage is that such secondary bonds do not have a big bonding energy, which leads to the state of worsened mechanical durability. [15, 16]

According to the intensity and the structure of the physical net gels can be divided on weak and strong. The strong gels are firmly connected and flexible in the node points. The weak gels are flexible and elastic only when the mechanical tension is low. The irreversible damage happens when the mechanical tension reaches the merit of static flow limit. [17, 18, 23]

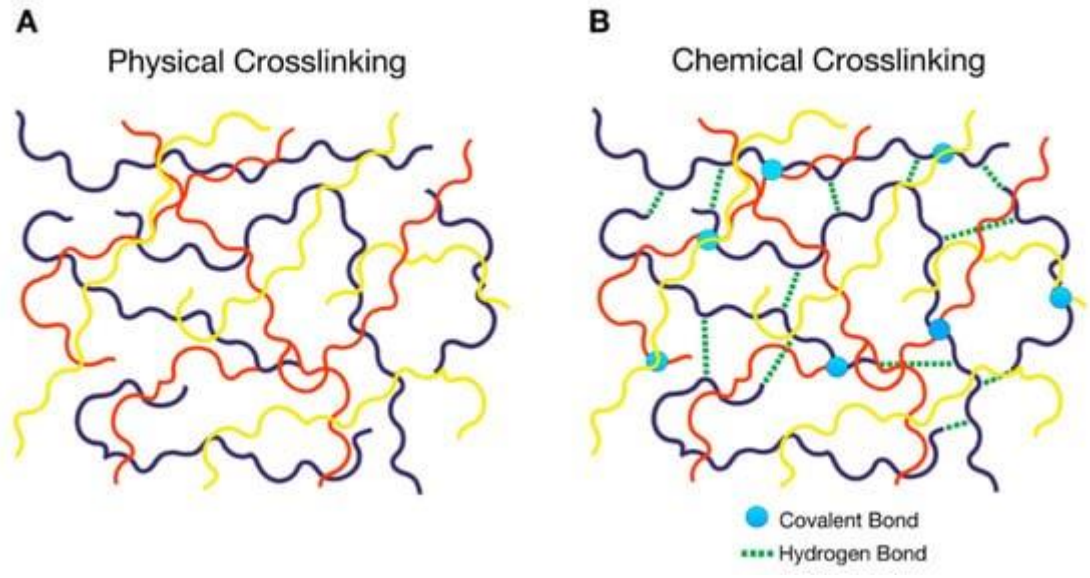

Figure 3. Physical and chemical crosslinking. [18]

3.2 Chemical crosslinking

The hydrogels crosslinked in the chemical way can contain both ionic and covalent bonds. During this process the radical polymerization of the functional groups on the main chain happens. Possibly, the crosslinking can be done also through the condensation or the usage of a crosslinker while the concentration is changing and therefore the period of crosslinking, depending on the level of crosslinking reaction, changes too. [20]

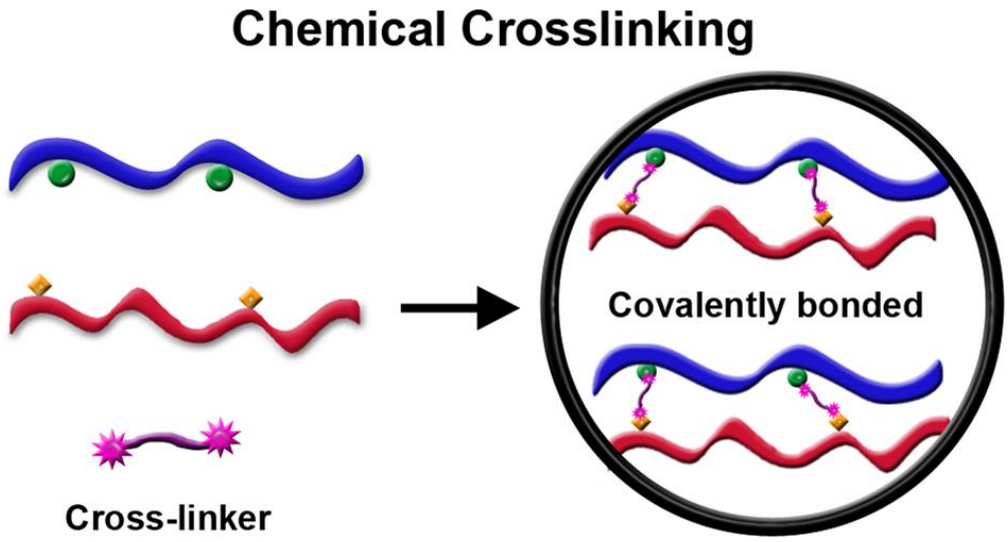

Figure 4. Chemical crosslinking. [20]

One of many methods of chemical crosslinking is done by using crosswise bonds that is characterized by the radical polymerization of a low-molecular monomer or branched homopolymers or copolymers while having the crosslinker present. This type is in practise used in the solutions made for medical or biological purposes. Another way of chemical crosslinking and creating gels is copolymerization on the polymer basis. Such reactions involve anionic and radical initiators. Some examples of these substances are benzoyl peroxide or azobisisobutyronitrile.

One of the newest methods of chemical crosslinking involves the use of enzymes. In particular, it is a synthesis of polyethylene glycol (PEG) hydrogel done by using an enzyme. The enzyme catalyses the reaction and consequently creates the amide bond among each individual polymer. The final properties of the gel can be regulated by changing the ratio between the enzyme (lysine) and the PEG.

The unique method of crosslinking makes use of the high-power radiation and lies in the use of the gamma and the electron-beam radiation for the polymerization of unsaturated compounds. The polymers, that are dissolved in water and derivatised by vinyl groups, can

be transferred to hydrogels by applying a high-energetic radiation. However, even the polymers lacking the vinyl groups can be crosslinked. In this case, applying beams on the polymer solution creates radicals. The recombination of these radicals results in the origin of covalent bonds which lead to the crosslinking of the gel. [19, 21–23]

4 RHEOLOGY

This branch of science examines general mechanical properties of substances, relations among tension, deformation and the speed of deformation. In the case of a liquid hydrodynamic relations can be examined too. As a subfield of mechanics, it is focused on the deformation and the flow of substances caused by the tension that is being put on it. The basic rheological properties are viscosity, elasticity, plasticity and weight.

Viscoelasticity modifies the pliability of biological structures (biomaterials). The variability of such properties is broad, starting with non-newtonian liquids (blood, lymph, synovial fluid) going via the diversity of soft tissues ending at the diversity of bones.

Viscosity is a physical quantity dictating the ratio between the shear stress and the change in velocity in dependence of the distance between neighbouring layers of the flowing liquid. It characterises the internal friction of the fluid and depends primarily on the attractive forces between the particles. The higher the attractive forces among particles are, the higher the viscosity of the fluid is. Generally, higher viscosity slows down the movement of the fluid or the items in the fluid.

Mathematical expressions of flow properties in liquids are rheological state equations which express the relationship between the shear stress τ and the liquid deformation. The graphic forms are flow curved lines. [5, 10]

4.1 Viscosity

Dynamic viscosity η characterises the substance and its merit depend on the pressure and the temperature. In gaseous substances its merit rises with the temperature, in liquids its merit descends with the temperature. In the SI framework its unit of measurement Pa·s = kg/m s. The reverse quantity of dynamic viscosity is fluidity ϕ.

Kinematic viscosity is a qoutient between the dynamic viscosity and the density of the liquid:

$$\nu = \eta/\rho \tag{1}$$

In practise it is used for states depending both on the dynamic viscosity and the density ρ. The unit of measurement m^2/s.

Fluids abiding by the Newton law are called the Newtonian liquids and are usually represented by low-molecular substances. The shear stress is defined as:

$$\tau = \eta \cdot \frac{du}{dx} = \eta \cdot D \quad (2)$$

τ…shear stress,

η…apparent shear viscosity which is not a constant of a substance and depends on the speed of deformation or on the shear stress,

D…speed gradient describing the changes in the shape in the flowing liquid,

du…mutual speed of the movement of shear plates by dx

4.2 Non-Newtonian liquids

The liquids that do not abide by Newton law are called non-Newtonian liquids. In practice this group is represented by solutions and polymer melts, suspensions and various pastes.

Basic types are pseudoplastic, dilatant, Bingham, thixotropic and rheopectic liquids.

Pseudoplastic liquids; the apparent viscosity descends as the speed gradient grows. According to the flow curves, there are two subgroups: real pseudoplastic and structural viscous liquids. The solutions and polymer melts, soap and detergent solutions and particular suspensions belong to this group. From the technical point of view, pseudoplasticity is useful as it lowers the amount of energy required for mixing and liquid flow in the pipage.

Dilatant liquids; the apparent viscosity ascends as the speed gradient grows. This behaviour is not very often and was observed in some highly concentrated suspensions (PVC in plastisols). Dilatancy complicates technological processes and is often supressed by changing the composition.

The speed gradient is defined as:

$$D = K \cdot \tau^n \quad (3)$$

n is index of non-Newtonian behavior, when $n >$ for dilatancy, $n <$ for pseudoplasticity,

K is a consistency coefficient.

Both pseudoplastic and dilatant liquids can exist in a form with limital shear stress, those can be toothpastes, lipsticks or runny chocolate.

Bingham liquids; those liquids have a plastic part of deformation which start flowing only after the liminal shear stress (also called yield stress), τ_0, is crossed.

$$\tau = \tau_0 \eta \cdot D \quad (4)$$

τ_0…yield stress

For example, concentrated industrial and sewage dregs.

Another group of substances has a time-dependant deformation part and the apparent viscosity changes in time along with the stress during constant shearing. The two basic types are thixotropic and rheopectic.

Thixotropic substances; the apparent viscosity descends as long as the shear stress is in action. In practice, it is observed in painting materials.

Rheopectic substances; the apparent viscosity ascends as long as the shear stress is in action. This behaviour is less common, for instance the suspension of bentonite.

The non-Newtonian liquids are very common in the industry and the viscosity defined as substance viscosity does not have physical meaning and it is needed to replace it with a curved line within the particular range of shear stress. [11]

4.3 Measuring of viscosity

The flow, fall and rotational viscosimeters can be used, however only the last type can be used to exactly characterize the flow curves of non-Newtonian liquids. The conditions of the right measuring for non-Newtonian liquids are the laminar flow in the whole extend of measuring and a well-defined geometry of the flow.

4.3.1 Fall viscosimeters

The measuring is based on the speed of the fall of a known item (usually a ball) within the liquid whose viscosity is being measured. The speed is calculated according to the Stockes´ law:

$$v = \frac{F}{6 \cdot \pi \cdot \eta \cdot r} \tag{5}$$

v…the speed of the fall of the particle,

F…resistance force,

π…a constant,

η…the dynamic apparent viscosity of the measured liquid,

r…the radius of an individual particle.

Typical examples are the Stockes´ viscosimeter (the simplest one) or Höppler´s viscosimeter.

4.3.2 Flow viscosimeters

Measuring on these devices is based on the Poisseuil´s equation for the laminar discharge of the liquid from a perpendicular pipe of a circular cut due to its own weight:

$$\eta = \frac{\pi \cdot r \cdot h \cdot \rho \cdot g \cdot tF}{8 \cdot V \cdot l} \tag{6}$$

r…the radius of the measuring tube,

h…the height of the liquid slope,

ρ…the density of the liquid,

g…gravitational acceleration,

t_F…the time of the flow,

V…the bulk of the discharged liquid,

l…the length of the measuring tube.

So that the flow is laminar, it is necessary to use a capillary tube for low viscosity samples. In practise, the merits are measured relatively, not absolutely. The unknown liquid is compared to the reference liquid (frequently the solvent) and the unknown viscosity is calculated as:

$$\eta = (\frac{tD}{t_0}) \cdot \eta_0 \tag{7}$$

η_0 … viscosity of the solvent,

t_D…the time of the discharge of the measured liquid (diluted polymer solutions),

t_0… the time of the discharge of the solvent.

The most common types are Engler´s, Ostwald´s, Kohl´s and Ubbelohdeo´s viscosimeters.

4.3.3 Rotational viscosimeter

In this case the sample is subjected to the shear between two defined surfaces while one of them is not moving and the other one is rotating/oscilating. The impact of the rotation at different velocities is measured and recorded by the related software.

Most common types are dynamic rheometers in various geometrical setting like concentric cylinders, plate-plate, cone-plate. Instead of the concentric cylinders geometry, especially for more viscous liquids, plate-plate and cone-plate geometries are used. In both cases the measured liquid is put into a narrow tube between surfaces, so during the experiment only a little of the sample is needed. In this case the whole sample subjected to the constant speed of shear deformation (shear rate) and both final and side effects are suppressed. The tempering of the sample is very efficient due to a big cooling area and a thin layer. The disadvantage of this geometry is a fact that it cannot be used for every suspension and dispersion (big particles interrupt flow relations in the wedge-shaped gap).

Models cone-plate and plate-plate, the upper cone or plate rotates while the bottom plate stays still. A constant rotating moment is usually applied on the upper part and the whole system is put through the shear stress. The result of this experiment is setting the dependence of the apparent viscosity on the shear rate.

Basic factors affecting the rheology are the volume fraction of the inner-phase concentration, inner-phase viscosity, outer-phase viscosity, the distribution of particles and the character of the stabilizing film. [12]

4.4 Approaches to measuring viscoelastic behaviour

Loss Modulus, G'', (deformation energy lost through internal friction when flowing) characterizes the viscous portion of the viscoelastic behaviour.

Internal friction between layers and by that between molecules and particles of a fluid causes viscous behaviour. The friction always goes in the direction of the development of frictional heat in the sample and of the transformation of deformation energy into heat energy. That is absorbed by the sample and fully used by internal friction processes, which means that it does not affect the further behaviour of the sample. This loss is also called energy dissipation.

Storage Modulus, G', (stored deformation energy) describes the elastic portion of the viscoelastic behaviour.

Thanks to the elastic portion of energy, changing the shape of the material using force does not lead to overstretching or destroying the material. It is stored in the deformed material and when the force is not present anymore the stored energy helps the structure to reform back into its original shape. [9]

II. ANALYSIS

5 MATERIALS

The whole practical part was inspired by the scientific article "Gelatine acrylamide with improved UV crosslinking and mechanical properties for 3D biofabrication". [14]

5.1 Bovine gelatine

The used type comes from the Sigma-Aldrich company in the form of a sterile light powder. The gel strength is roughly 225 g Bloom.

All of the listed materials were used in the form that they were delivered in and were not additionally cleansed in any way. The experiment also required ethanol and distilled water to be used to clean the tools and the workplace.

5.2 Phosphate Buffered Saline

Liquid, sterile, transparent chemical used in the creation of methacrylic gelatine was purchased from the Sigma-Aldrich company. Its pH is 7.4. The function of a buffer is muting the pH divergences of the mixture.

5.3 Methacrylic Anhydride

Supplied by the Sigma-Aldrich company, this liquid is stored in dark-tanned glassy bottle. Its presence for a certain amount of methacrylic anhydride (further MAA) in the mixture ensures that methacrylic groups will be added to the bovine gelatine.

5.4 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propan-1-one

Functioning as a photoinitiator, this substance comes from the Ciba Speciality Chemicals N. V. A, and initiates radical polymerization. It absorbs the UV light, breaks down and free radicals get created. By this, a radical chain polymerization was started. The quality of the photoinitiator is directly proportional to the quality of the product.

6 SYNTHESIS OF MODIFIED GELATINE

To prepare 10 % (bulk) solution of bovine gelatine, substances bovine gelatine, phosphate buffered saline, methacrylic anhydride and dialysis tubing cellulose membrane were needed. The right ration and weight of bovine gelatine and phosphate buffered saline was; 0.1 mL PB saline, 1 g bovine gelatine.

Figure 5. Mixture of bovine gelatine, MMA and phosphate buffered saline.

In practice, 20 g of bovine gelatine (powder) were weighted, added it into a glassy flask, 200 mL of phosphate buffered saline were poured in and a magnetic stirring pole was dropped inside. This item ensured proper mixing of the solution. It was propelled by a magnetic stirrer placed under it. Roughly half of the flask was in the glassy ball of water that was heated up to 50 °C by a magnetic stirrer. The mixture was left under such conditions for approximately one hour until the solid gelatine got completely dispersed in the liquid. After that, MAA was squirted inside through a syringe. In the first case, the bulk of MAA was 12 mL and in the second case it was 2 mL. Such compounds were then left for two hours getting properly mixed at 1000 spins per minute at the constant temperature of 50 °C.

Figure 6. Filtration of excessive MMA groups (12 mL conc.).

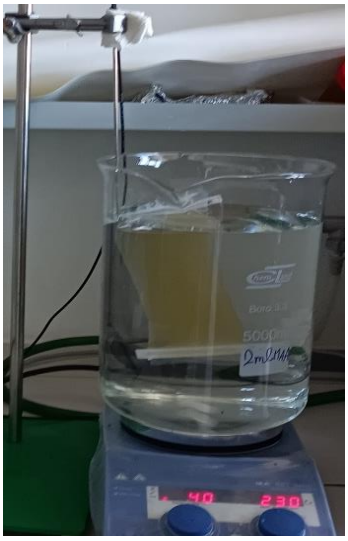

Figure 7. Filtration of excessive MMA groups (2 mL conc.).

Then the glassy flasks were put out of the water and away from heat and magnetic field on the desk. The dialysis tubing cellulose membrane was prepared by cutting away the appropriate length and closing one end with a plastic buckle. Such a material was used to hold the mixture. Dialysis belongs among methods of replacement of kidney function. Healthy kidneys filter waste matter out of blood and get the blood rid of excessive liquid.

These two functions are replaced by dialysis. In the experiment, the membrane was used as a filtering sack for the unreacted MAA that was not added to the gelatine chains. The mixture was poured into the membrane and placed into a jar with distilled water and mixed by a magnetic stirrer for a week.

Figure 9. Solid modified gelatine (12 mL).

Functioning as a membrane, it filtered out the MAA into distilled water around and left the content inside with methacrylic groups attached. Using a magnetic pole, I took the magnetic stirrer out of the balloon and poured the mixed content into the cellulose membrane and finally sealed it with another plastic buckle. The cellulose sack was floating in a big glassy jar full of distilled water at the 40 °C for the next seven days. On the bottom of the jar, there was a magnetic stirring pole making the sacks rotate. In a week under such conditions the sack was taken out, freeze-dried and then froze to the temperature of 192 °C using liquid nitrogen. This substance was transferred to the **lyophilizator and left there freezing for 28 hours.**

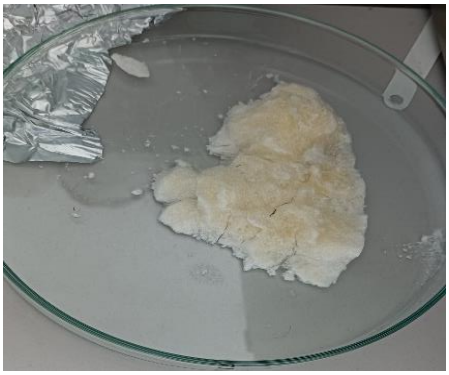

Figure 8. Solid modified gelatine (2 mL).

The results of these reactions were white, solid, dried gelatines with the concentration – 2 mL – of MAA groups. After this whole process the same staps were repeated but the MAA concentration was 12 mL.

The first bulk with 2 ml of MAA was not large enough and that was why on the same day when the results were received, another amount of mixture with 2 ml of MAA got created. The substances of both concentrations were cut, crushed by a stainless steel cutter and put into dry clean plastic containers. After the successful modification of end groups of gelatine using acrylamid function groups, the next step was to verify the influence of these groups on cross-linking of gelatine polymer by the UV light into the form of hydrogel. The photoiniiator of the reaction was 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propan-1-one known by its commercial name Igracure 2959. Firstly, this photoiniciator was in the concentration of 0,13 wt.% on the amount of gelatine dissolved in 10 mL water while being intensely mixed (1000 rpm = revolutions per minute) for 10 minutes at the 40 °C.

During the manipulation with the photoiniciator and subsequently with the reaction system it was necessary to work in a darkroom to prevent their activation by the day light. After the UV photoiniciator was completely dissolved in water the modified gelatine was added in the amount of 15 wt.% of the whole mixture while maintaining the originally used parameters of mixing and the time for dissolving the gelatine for the next 15 minutes.

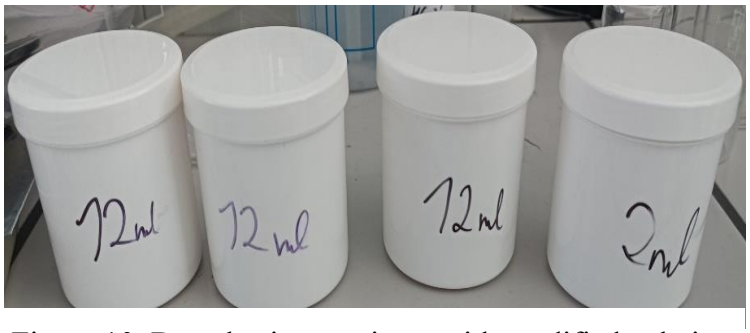

Figure 10. Dry plastic containers with modified gelatine.

After the preparation of reaction mixture for UV cross-linking several UV lamps with different UV intensities with peak around 365 nm were tested. The result of this optimization was the conclusion that UV cross-linking would be realized directly by the adjustment of the rotation rheometer with the plate-plate geometry (25 mm diameter; 0.5 mm gap) using the UV lamp OmniCure Series 1000 UV–VIS with the mercury discharge tube with relatively broadscale filter ranging between 320 and 500 nm (ensured by covering of the Ingracure 2959 reactive zone), which brought another advantage in limiting the unnecessary manipulations with the prepared hydrogel. The UV light lamp was manufactured in Canada by the Lumen Dynamics. This manipulation could lead to the infringement of its structure when the particular hydrogel was directly tested for its mechanic properties after it hardened in the geometry of rotation rheometer, which was used to suppress a possible hydrogel slide on the walls of the geometry. In order to verify the intensity of the UV light on mechanical properties of such hydrogels three merits – 410, 840 and 1850 mW cm^{-2} - were used. It was modulated by positioning of the UV lamp curtain and the period of exposure 10 minutes at 25 °C. For the future reference, the shadowing of UV curtain will be presented in the matching order 022, 045 and 090.

For the purpose of observing mechanical and viscoelastic properties the modular rheometer Physica MCR502 (Anton Paar, Austria) with the temperature check by the unit TC 30 and thermal chamber CTD600 were used for the cross-linking of studied hydrogels. This chamber prevents the access of the day light to the sample. All of the measurements were running under the same parameters used for the preparation of hydrogels at 25 °C. Following

experiments were selected to determine the quality of polymer net of the hydrogel from the modified gelatine by UV cross-linking depending on the applied intensity of UV light in the process of their cross-linking, (i) development of viscoelastic moduli depending on the applied angular frequency (100–0.1 rad s^{-1}) at the constant deformation 1 % with the frequency of recording 5 points/decade of angular frequency (further „*frequency sweep*“), (ii) verification of sufficient static yield stress range of hydrogels investigated by a gradual increase in the shear stress (10–1000 Pa with recording 10 points/decade of the shear stress), (iii) creep and creep relaxation at the working shear stress of 500 Pa and variable recording of points for a sufficient record of the beginnings of the load and unload of hydrogels.

Figure 11. Rheometer Anton Paar MCR 502.

For the purposes of a relevant study on the UV crosslinking of modified gelatine into the form of a hydrogel within the limits of the Bachelor‘s thesis it was necessary to fix several parameters like:

- the concentration of the modified gelatine in the original solution (15 wt.%), the amount of the used photoiniciator (0.13 wt.% on the whole amount of gelatine),
- the thickness of the cross-linked substance (0.5 mm set by the distance in the plate-plate geometry),
- the period of UV cross-linking (10 minutes, in this time frame it was not reached balanced merits of observed viscoelastic moduli, the reaction could have lasted longer),

- a wide filter of the UV light wavelengths during the process of cross-linking (for example focusing on the wave length of 365 nm would probably increase the activity of the photoiniciator Igracure 2959),
- temperature during the UV cross-linking (25 °C, it was possible to expect that the higher temperature gets the more significant the cross-lining was),
- etc.

The matrix of samples of variable parameters is depicted in the Table 1. It is obvious that within the Bachelor's thesis there were 6 various samples of gelatine hydrogels studies for viscoelastic properties.

Table 1 – Variable parameters influencing the degree of cross-linking of MAA modified gelatine cross-linked using the UV light

Amount of MAA	Intensity of UV light at cross-linking (going through curtain)		
	410 mW cm^{-2} (022)	840 mW cm^{-2} (045)	1.85 W cm^{-2} (090)
2 mL	✓	✓	✓
12 mL	✓	✓	✓

6.1 Process of the UV cross-linking

Cross-linking of the modified gelatine was observed in a real time of the reaction using the record of a viscoelastic moduli. The Figure 12 depicts the process of the UV cross-linking for the sample containing a higher amount of end methacrylate function groups on gelatine chains depending on the intensity of the applied UV light. The examined sample was firstly analysed at constant deformation of 1 % and the frequency of 1 Hz for 60 seconds. After that the UV lamp was turned on and cross-linking process started. From the development of the storage and loss moduli a significant development of a cross-linking structure in the arising gelatine hydrogel was observed. While the loss modulus stayed almost the same, the storage modulus reached within the time of UV irradiation cross-linking particular merits. In the Figure 12, there can be seen a noticeable influence of the applied intensity of the UV light and can be stated that with the increasing intensity more of the photoiniciator Igracure 2959 involved in the cross-linking was activated meaning a higher increase in the merits of the storage modulus was observed in the time. Having proved the reproducibility of the measurement, repetitive measuring with a new reaction system was conducted for the highest UV light intensity.

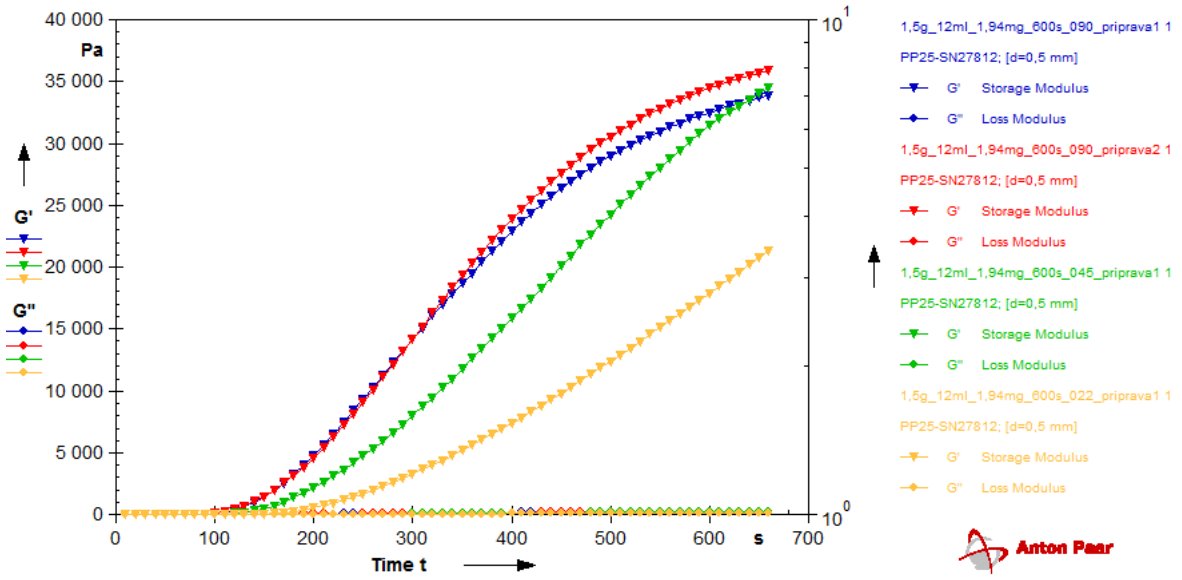

Figure 12. Development of viscoelastic moduli in time during UV cross-linking of system with 12 mL MAA used for gelatine modification.

In the Figure 13 an analogous dependence with the same course for the sample containing modified gelatine with a lesser amount of end function groups meaning for its modification 2 mL of MAA were used. From the course of cross-linking in the Figures 12 and 13 is obvious that the merit of the storage modulus was not constant for even a single examined sample, which means that with passing time the cross-linking density of the hydrogel from the modified bovine gelatine would get developed.

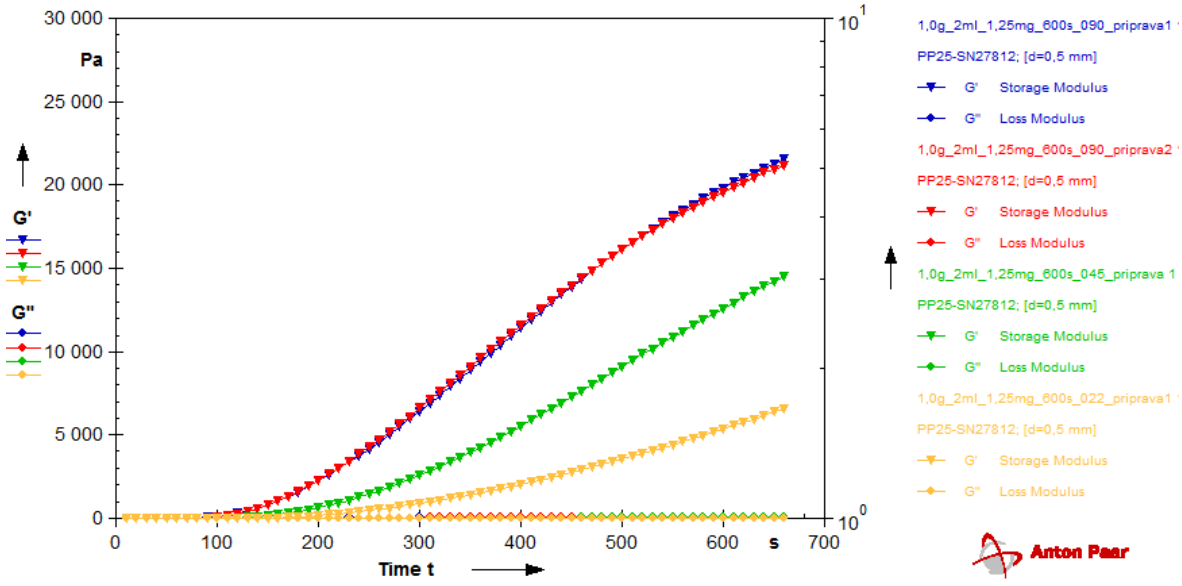

Figure 13. Development of viscoelastic moduli in time during the UV cross-linking of the system with 2 mL of MAA used for the gelatine modification.

Overall projection of the cross-linking course is shown in the Figure 14. It is obvious that the gelatine samples containing higher amount of methacrylamide functional groups (12 mL of MAA) reach under comparable circumstances distinctly higher values of the storage modulus. A comparable end value of a storage modulus after the reaction initiated by the UV light across the MAA amount used for gelatine modification is for the sample containing 12 mL of MAA and the lowest intensity of UV light used and the sample containing 2 mL of MAA and the highest intensity of the UV light used. Nonetheless, already from the course (slopes) of dependences of storage modulus can be predicted more significant final value of cross-linking density for the sample with 12 mL of MMA set by a higher amount of methacrylamide functional groups capable of engaging in the final spatial network in gelatine hydrogel.

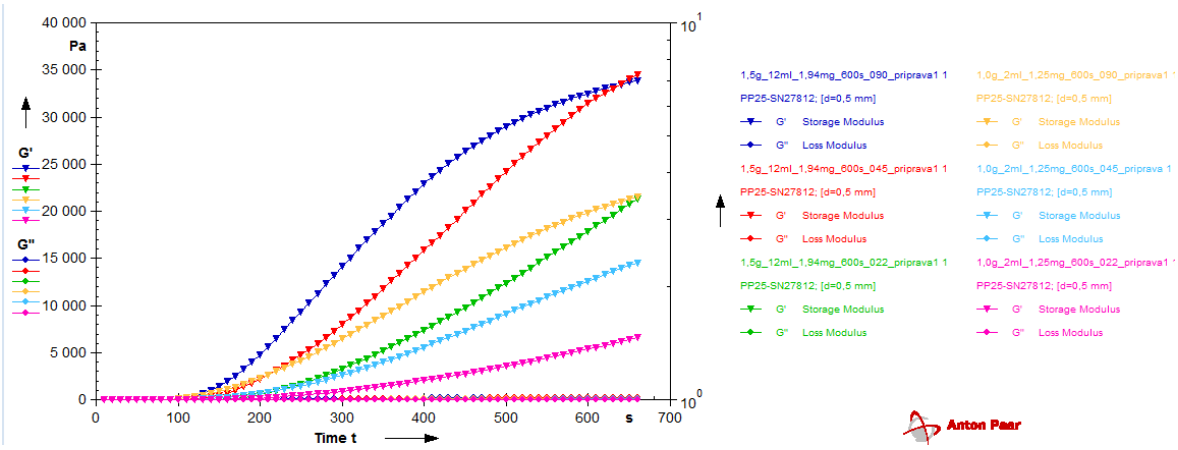

Figure 14. Development of viscoelastic moduli in time during the UV cross-linking for all studied variants of MAA-modified gelatine.

6.2 Dynamic behaviour of studied hydrogels

From the practical point of view, it is important to know the response of the created polymer network on its dynamic loading which can be taken into consideration in a broader time scale with the advantage of observing through the frequency sweep experiment. Lower frequencies mean in general long processes while high frequencies in essence mean very fast processes. In the Figure 15, the frequency sweep for the sample of modified gelatine with 2 mL of MAA is depicted where it is clear that the values of the storage and loss moduli are practically independent on the applied angular frequency, which corresponds to the systems that have already created the 3D network structure. The dependence of the complex viscosity confirms the actuality about time consideration mentioned above, at low angular frequencies the samples are showing high viscosity. As the angular frequency increases the complex viscosity decreases, which can be described as a shear thinning

behaviour. The Figure 15 shows a more developed polymer network of modified gelatine for a system cross-linked at a higher UV light intensity, which is in accordance with the results observed in the Figure 14. This case also shows the reproducibility of the formation of polymer network in the new sample, in particular at the highest UV light intensity applied.

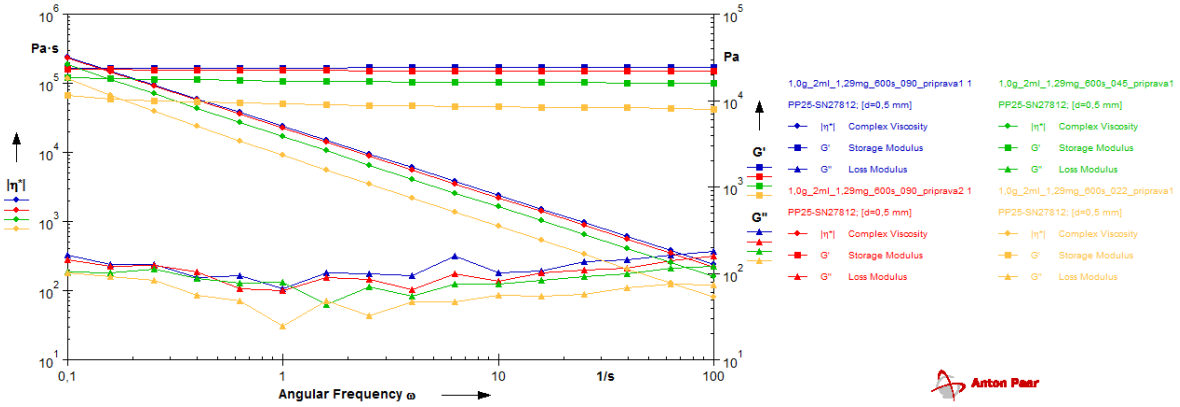

Figure 15. Frequency sweep for the systems with 2 mL of MAA used for gelatine modification.

In an analogous way that applied for the results in the Figure 15 frequency sweep results for the sample of the modified gelatine with 12 mL of MAA can be described (Figure 16).

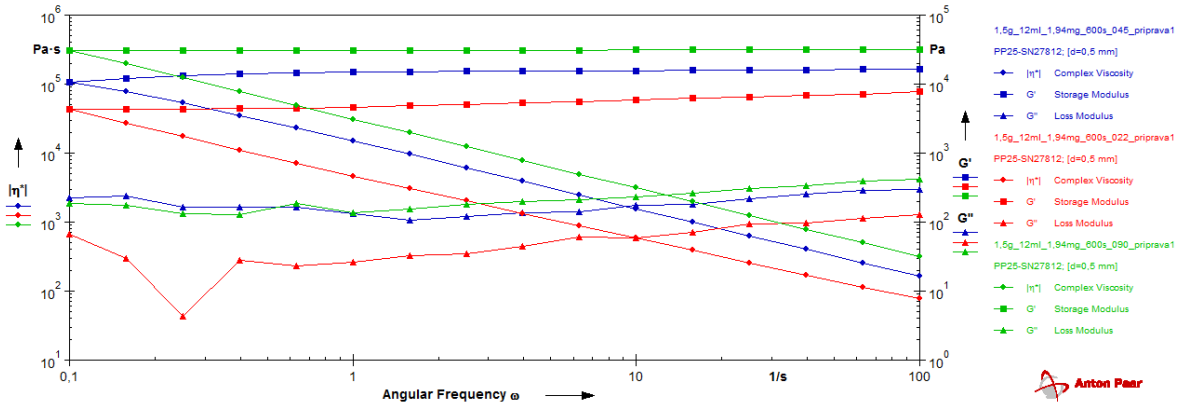

Figure 16. Frequency sweep for the systems with 12 mL of MAA used for gelatine modification.

During the observation of the specifically storage modulus values from the frequency sweep measurements (Figure 17) an interesting fact can be observed. The difference among individual types of gelatine lies in a different amount of MAA used for their modifications but is not that noticeable as it was in the Figure 14. On the contrary, at lower intensities of UV radiation and at lower applied angular frequencies (slower processes), the values of the storage moduli for gelatin with a lower amount of functional groups (2 mL MAA)

even prevail over the values of the elastic moduli for gelatin with a higher amount of functional groups, which can be caused by the lower elasticity of the polymer network, when the same amount of UV initiator used in the case of a larger amount of functional groups had more of them to choose from, which prevented the macroscopic development of a compact polymer network.

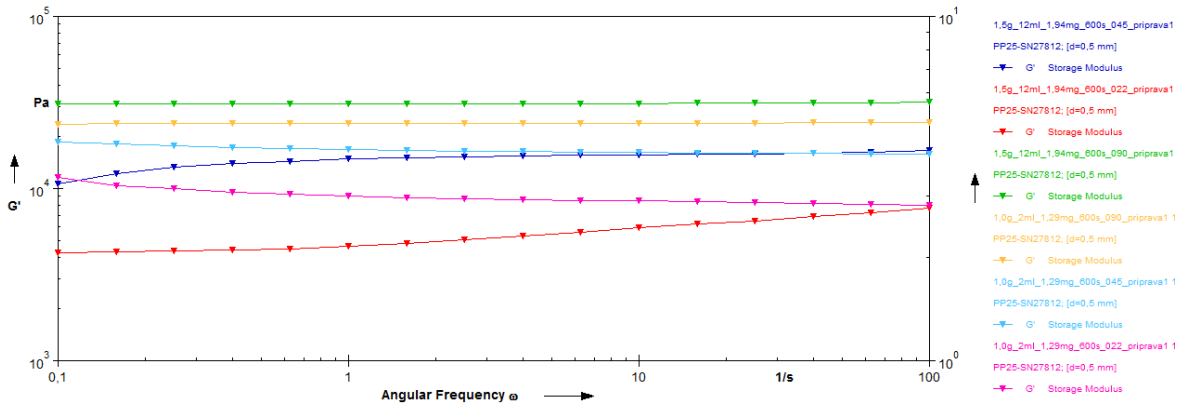

Figure 17. Dependence of the storage modulus on the angular frequency of the studied gelatine hydrogel samples.

6.3 Long-term static load of studied gelatine hydrogels

Apart from the appraisal of the dynamic behaviour of the created gelatine hydrogels it is also needed to assess their long-term behaviour at the static load, which corresponds with the creep test. For this purpose, it is important to estimate the real load of hydrogels during the applications where, as indicated in the Figure 18, the value of the applied constant shear stress significantly affects the interpretation of the creep test for the same sample.

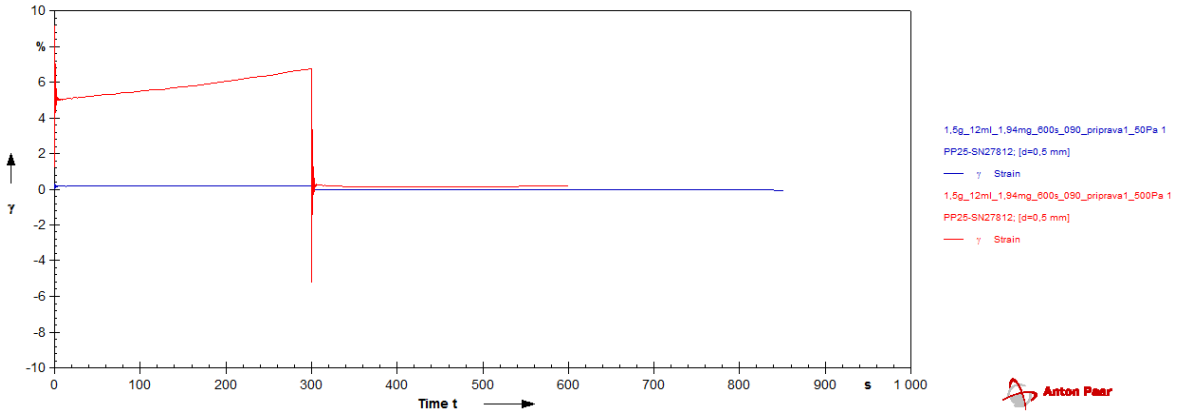

Figure 18. Comparison of the influence of shear stress applied on the creep behaviour of the gelatine hydrogel sample.

With the regard to the interesting character of the polymer network found out through the results of the frequency sweep, the creep test is going to be discussed for the gelatine

sample that originated from 2 mL of MAA. It is obvious from the Figure 19 that the elastic character of the hydrogel samples rises along with the increasing intensity of the UV light used for the cross-linking, which is demonstrated in the Figure 14. In the sample that originated at the lowest intensity of the UV light the phenomenon called creep ringing appeared, which in practice means trembling of the sample at the beginning of the action of a constant load (shear stress) or, vice versa, at its release. This phenomenon is the more noticeable the higher the elastic part in the system is. For the mentioned creep in the Figure 19, the constant shear stress 500 Pa was induced, which is still below the level of the static yield stress. This level was verified for every studied version of modified gelatine.

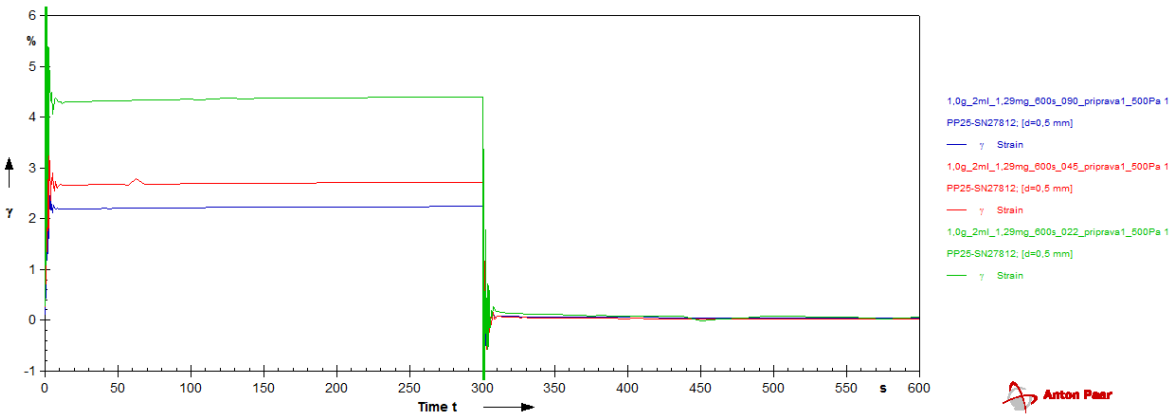

Figure 19. Creep test for samples of gelatine hydrogels with a lower content of methacrylamide functional groups.

7 USE IN PRACTICE

Cross-linking happened in all samples by using the UV light. The first half had 2 mL of MAA and the other half had 12 mL of MAA.

Findings and results of this work could be used in the healthcare industry. The cross-linked polymer could theoretically be used to support the healing of burns or it could be used for 3D-printing of skin replacements. A synthetic skin would be firstly grown in a lab and then put on the injured site.

However, putting this into practice requires a lot more research to be done. Further research could be focused on changing certain parameters that were used during the experiment, like the period of UV light exposure, the concentration of each material or the presence of an antimicrobial agent, in order to create such a material that would be almost identical with a real human skin.

CONCLUSION

The main goal of the Bachelor´s thesis was met. Based on the literature review of several scientific articles I selected a possible variant of the gelatine modification made by the MMA groups and its subsequential UV crosslinking using the photoiniciator.

During the experimental part more than one UV lamp was used. It was found out that the use of the UV lamp (OmniCure Series 1000 UV–VIS) was effective in every case and produced the best results, i.e. stable polymeric net and denser gel-like consistency when the intensity was 90 %.

Additionally, properties like elasticity (Storage Modulus) and viscosity (Loss Modulus) were measured by the rheometer Anton Paar MCR 502 in different concentrations of methacrylamide (MMA), 2 ml and 12 ml, and to conclude, the more elastic hydrogels of gelatine were obtained for samples with higher amount of MMA used for the gelatine modification enabling development of more complex crosslinked network compared to the variant with lower content of MMA end groups. Further, the higher intensity of UV light used for the crosslinking process, the higher the elastic properties of gelatine were obtained as well.

At this point of research, it cannot be stated which concentration, 2 or 12 mL of MMA, would suit an injured skin more as the experiment does not include any comparison with the real skin or its alternative. In order to make this estimation more research needs to be done.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations:

g	gram
Hz	Hertz
IUPAC	International Union of Pure and Applied Chemistry
kg/m s	kilogram/meter second
MAA	methacrylamide
mL	milliliter
mm	millimeter
mW cm^{-2}	millwatt/square centimeter
Pa s	Pascal second
PEG	polyethylene glycol
pH	per hydrogen
rad s^{-1}	radian/second
UV	ultraviolet

Symbols:

%	percentage
wt.%	weight percent
°C	degree Celsius
G'	storage modulus
G''	loss modulus
ϕ	fluidity
v	kinematic viscosity
η	apparent shear viscosity
ρ	density

τ	shear stress
τ_0	yield stress
D	speed gradient
du/dx	mutual speed of the movement of shear plates by dx
n	index of non-Newtonian behavior
v	the speed of the fall of the particle
F	resistance force
π	value of pi
r	the radius of an individual particle
η_0	viscosity of the solvent,
t_D	the time of the discharge of the measured liquid (diluted polymer solutions)
t_0	the time of the discharge of the solvent
h	the height of the liquid slope
g	gravitational acceleration,
t_F	the time of the flow
V	the bulk of the discharged liquid
l	the length of the measuring tube

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