Synthesis and Characterization of Hydrogels Based on Tulipalin A and Graphene Oxide

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Zásady pro vypracování:

Student se bude v rešeršní části zabývat Tulipalinem A a grafen oxidem obecně. Podrobně se bude věnovat hydrogelním materiálům, a nakonec nastíní možné aplikace takovýchto systémů.V experimentální části bude optimalizovat hydrogelů Tulipalinu A s N-isopropylacrylamidem. Tyto systémy bude charakterizovat pomocí různých analytických metod, spektroskopických technik, termických metod a nakonec zhodnotí jejich chování pomocí rotačního reometru. Bude také hodnotit vhodnost materiálů pro jejich využití v medicinálních aplikacích.

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ABSTRAKT

Bakalářská práce se zabývala přípravou a charakterizací hydrogelů na bázi tulipalinu A s příměsí oxid grafenu pro použití v medicínské oblasti. Daná práce obsahuje dvě části. Teoretickou část zabývající se jednotlivými komponenty samotného hydrogelu a současně metodotogií, která objasňuje principy provedených měření. Praktická část obsahuje postup výroby hydrogelů a jejich charakterizace pomocí snímací elektronové mikroskopie, hodnocení viskoelastických vlastností a schopnosti botnání, výsledky těchto zkoušek a diskuzi k dosaženým výsledkům.

Klíčová slova: Hydrogely, tulipalin A, oxid grafenu, medicína.

ABSTRACT

This bachelor thesis was focused on the preparation and characterization of the hydrogels based on tulipaline and graphene oxide as an active filler for application in medicine. This thesis includes theoretical part aimed on the theoretical backgroun of the individual components of the hydrogel as well as on the describtion of the methodology clarifying the principle of the performed measurements. The practical part including the fabrication procedure of the hydrogels and their characterization using scanning electron microscopy, investigation of the viscoelastic properties and swelling capabilities, results of the measurements and discussion of obtained results.

Keywords: Hydrogel, tulipaline A, graphene oxide, medicine.

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I hereby declare that the print version of my Bachelor's/Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

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INTRODUCTION

Carbon is the second most abundant element within the human body and the fourth most abundant element in the universe (by mass). This position in universe makes carbon the chemical basis for all known forms of life on earth, so because of that graphene could be the best material in ecologically friendly direction [1, 2]. However, due to the 2D shape and very small thickness of the individual graphene sheets, it might be in some specialized form dangerous to cells in water [3]. Since the discovery of graphene, provide the significant advancements within different scientific disciplines, the most affected area of its implementation covers the electronics and biotechnology. Therefore, this thesis is mainly aim on the synthesis of the copolymers based on renewable monomer Tulipaline A and further to fabrication of the hydrogels based on the mentioned copolymer as well as of graphene oxide (GO) sheets as functional filler. Such copolymers were characterized from the composition point of view by FTIR technique. The basic properties of the fabricated hydrogels were investigated such as internal structure using scanning electron microscopy, swelling by gravimetric investigation and finally the mechanical properties using viscoelastic characterization. This thesis provides a brief overview on the hydrogels, GO as a functional filler and basics characterizations in its theoretical part and is further focused on the Tulipanine - N-isopropyl acrylamide copolymer hydrogels with GO as a filler and shows basic characterization of the fabricated materials.

I. THEORY

1 MATERIALS

1.1 Hydrogels

Generally, hydrogels are crosslinked polymers (Figure 1). The hydrophilic structure gives the ability to swell and hold large amounts of water(or some other liquids including polar solvents), while the ability of resistance to dissolution arises from cross-links between network chains a cross-linking density [5]. The process of crosslinking in hydrogel depends on chemical or physical character of the polymer properties and conditions during an experiment. Hydrogels can be synthesized in various “classical” chemical ways. In the most cases the process includes one-step procedures like polymerization and parallel cross-linking of multifunctional monomers [5], as well as multiple step approach, that involves synthesis of polymer molecules having reactive groups and their subsequent cross-linking, possibly also by reacting polymers with appropriate cross-linking reagents [5]. For control of the heat of polymerization and the final hydrogels properties, diluents can be used, such as water or other aqueous solutions. Then, the hydrogel mass needs to be washed to remove impurities left from the preparation process. These include non-reacted monomer, initiators, cross-linkers, and unwanted products produced via side reactions. It is able to design and synthesize polymer networks with molecular-scale control over structure such as cross-linking density and with such properties as mechanical strength and biodegradation [6,5]. Hyrogels have received respectable attention in the past 50 years, due to an enormous spectrum of differences in chemical structure and ways of crosslinking, various hydrogels could be prepared for various applications in biomedical and pharmaceutical scopes [7].

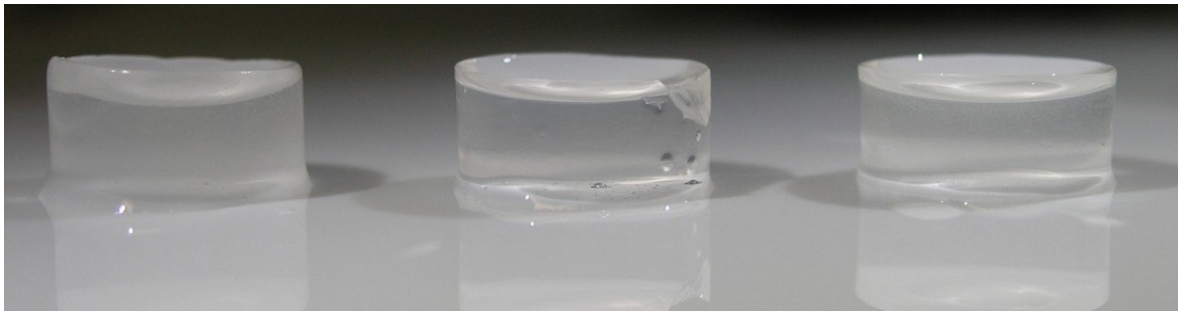

Figure 1: Image of the hydrogel in the swollen state [8].

1.1.1 Classification of hydrogel products

The hydrogels can be classified on various bases as detailed below:

1.1.1.1 Classification based on source

Hydrogels could be splitted into two groups based on their natural or synthetic origins.

The methods of preparation lead to formations of some interesting classes of hydrogels for science. These can be presented by the following groups:

a) Homopolymeric hydrogels have a polymer network that compounds single species of monomer, which is a basic structural unit comprising of any polymer network. Homopolymers may have cross-linked skeletal structure depending on the character of the monomer and type of polymerization technique.

b) Copolymeric hydrogels are made of two or more different type of monomer with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network.

c) Multipolymers is an important class of hydrogels, is made of two different independent cross-linked synthetic and/or natural polymer component, contained in a network form. In semi-IPN hydrogel, one component is a cross-linked polymer and other component is a non-cross-linked polymer.

1.1.1.2 Classification based on configuration

The classification of hydrogels depends on their physical structure and chemical composition can be classified as follows:

a) Amorphous (non-crystalline).

b) Semicrystalline: A complex mixture of amorphous and crystalline phases.

c) Crystalline.

1.1.1.3 Classification based on type of cross-linking

Hydrogels are divided into two categories based on the chemical or physical nature of the cross-link junctions. Chemically cross-linked networks have permanent connection, while

physical networks have transient junctions that arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions.

1.1.1.4 Classification based on physical appearance

Hydrogels appearance as matrix, film, or microsphere depends on the technique of polymerization involved in the process of preparation.

1.1.1.5 Classification according to network electrical charge

Hydrogels may be categorized into four groups on the basis of presence or absence of electrical charge located on the cross-linked chains:

a) Non-ionic (neutral).

b) Ionic (including anionic or cationic).

c) Amphoteric electrolyte (ampholytic) containing both acidic and basic groups.

d) Zwitterionic (polybetaines) containing both anionic and cationic groups in each structural repeating unit.

Hydrogel-forming natural polymers include proteins such as collagen and gelatine and polysaccharides such as starch, alginate, and agarose. Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization methods. [6]

1.1.2 Applications of hydrogels

As was mentioned before, hydrogels are three-dimensional cross-linked hydrophilic polymer networks, that are able to swell or de-swell reversibly in water and hold relatively large volume of liquid in swollen form. Hydrogels can be designed with controllable feedback as to expand or shrink with local changes in external environmental conditions. Hydrogels are capable to perform volume transition in response to a variety of physical and chemical initiative, where the physical impulses include temperature, electric or magnetic field, light, pressure, and sound, while the chemical stimuli include pH value, ionic strength, solvent composition and molecular types [6].

From definition follows-hydrogels are polymer networks having hydrophilic properties. But in some cases during preparation of hydrogels based on hydrophilic monomers, hydrophobic monomers can be used to regulate the properties for specific applications of final hydrogel for example as hygiene products, contact lenses, tissue engineering scaffolds, wound dressings and drug delivery systems (Figure 2) [9,10].

In general, hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers can be hydrophobic and chemically stronger compared to natural polymers. The mechanical strength of synthetic polymer has some advantages as well as disadvantages. Because of strong structure the degradation rate is slow enough, but on the other hand, mechanical strength provides decent level of durability. These two contrary properties should be balanced through optimal ratio of product [11,12].

Figure 2: Applications of hydrogels [13].

1.1.3 Hydrogel Production

In the most brief sense, a hydrogel is simply a hydrophilic polymeric network cross-linked in some fashion to produce an elastic structure. Thus, any technique which can be used to create a cross-linked polymer can be used to produce a hydrogel. Copolymerization or

cross-linking free-radical polymerization is usually used to create hydrogels by reaction of hydrophilic monomers with multifunctional cross-linkers. Natural or synthetic water-soluble linear polymers are cross-linked to form hydrogels in following ways:

a) Linking polymer chains via chemical reaction.
b) By ionizing radiation to generate main-chain free radicals which could recombine as cross-link junctions.
c) Physical interactions such as entanglements, electrostatics, and crystallite formation.

Any of the different polymerization techniques could be used to form gels [6,5].

1.2 Tulipalin A

Tulipalin A - in systematic nomenclature is also known as α-Methylene-γ-butyrolactone (MBL) (Figure 3). Synthetic α-methylene-γ-butyrolactone was studied in the 1940s. Natural α-methylene-γ-butyrolactone was first isolated in 1946 from the American Kandyk (Erythronium americanum) [14]. In standard conditions tulipalin A is liquid transparent odourless substance that is found in the form of glycoside (Tuliposide A) in relatively high concentrations (0.2–2 wt %) in different parts of tulips. In addition, MBL can be produced by other way, from biomass sugar-based itaconic anhydride or by biosynthesis from pyruvate and acetyl coenzyme A [4].

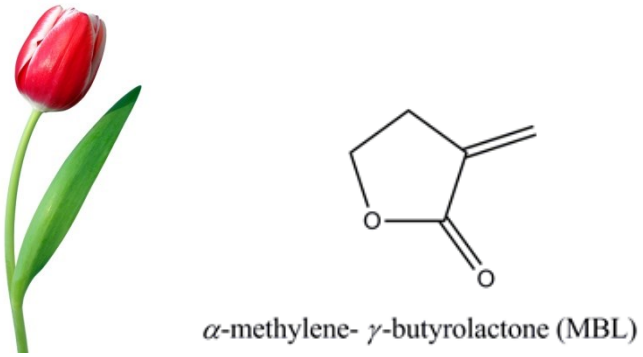

Figure 3: Illustration of the plant Tulip (left) and schematic illustration of the monomeric unit used for polymerization (right) [15].

1.2.1 Polymerization and structure

MBL consists of a five-member lactone ring and an exocyclic carbon–carbon double bond. Because of this type of structure it can serve as a dual monomer enabling both radical po-

lymerization and ring-opening copolymerization. The reactivity force of the MBL exo-cyclic double bond is high and as a result the MBL could be polymerized through the double bond by different polymerization techniques as free radical polymerization, group-transfer polymerization, coordination polymerization with metallocene. Also, tulipalin A has autopolymeric character. Poly(α-methylene-γ-butyrolactone) (PMBL) has high glass-transition temperature of 195 °C. MBL presents in various copolymers decent optical properties, also some resistance to heat, weathering, scratching, and solvents. Recently, emulsion copolymerization of MBL with acrylic acid in the presence of cross-linker was used to prepare polymer particles [4]. Subsequent saponification with sodium hydroxide leads to partial opening of the MBL ring and superabsorbent properties of resulting material (Figure 4) [4].

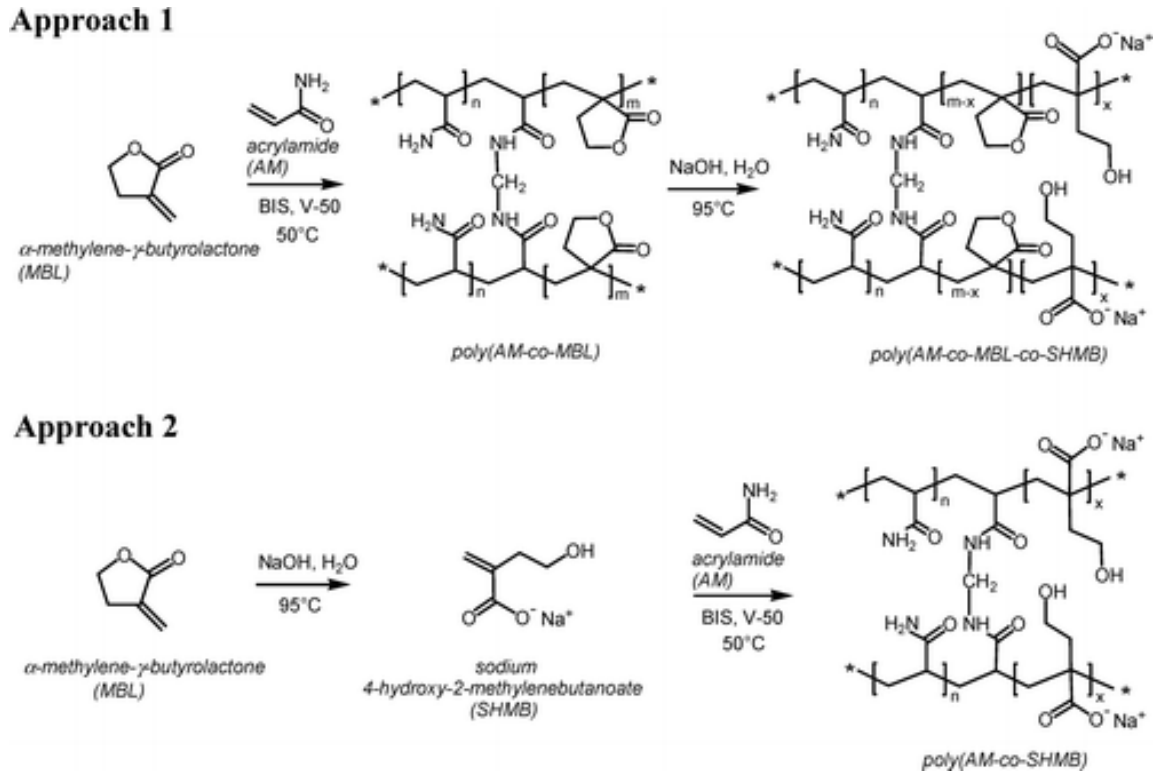

Figure 4: Structure of tulipalin A and polymerization [4].

1.2.2 Properties

In toxicology Tulipalin A is allergenic substance that causes rash. Also is known that α-Methylene-γ-butyrolactone could be potential fungicidal agent [14,16]. The fungicidal properties of the extract of tulip bulbs were first proved by the Dutch biochemists in 1966. The active ingredients of this extract, not yet identified, were called tulipalin; in 1967 two

independent groups of researchers identified hypothetical tulipalin A as a well-known α-methylene-γ-butyrolactone [17]. All the tulipales and tuliposides inhibit the development of pathogenic fungi, but tulipalin A shows the fungicidal properties most actively. The minimum concentration at which oppression of pathogenic fungi occurs Fusarium oxysporum f. tulipae, Gibberella zeae and Rhizoctonia solani, 5-10 times less than the necessary concentrations of other tulipalin and tuliposides. With respect to Pythium ultimum, all these substances are equally effective. Tulipalin A inhibits all forms of gray mold caused by fungi of the genus Botrytis; this is the only natural substance that depresses the development of a specific parasite of the tulips Botrytis tulipeae, albeit as efficiently as other gray mold. The German Federal Institute for Risk Assessment classifies tulipalls as Category B allergens ("contact allergic reactions are very likely"). Regular contact of a person with tulips and alstromerias leads to rapid sensitization of the body to tulipalin A and a characteristic professional disease of flower growers - tulip dermatitis.

Manifestations of the disease often coincide with the symptoms of fungal skin and nail lesions. Upon contact with the bulbs of tulips, the erythema of the skin of the hands first, then its keratinization and cracking. At the onset of the disease, itching is often noted, followed by tingling in the fingers. Often there are lesions of the nails: cracking of the nail, onycholysis (exfoliation of the nail), abscesses of the nail bed. In rare cases, tulip dermatitis extends beyond the shoulder girdle; There are cases when an allergy leads to speech disorders.

Tulipalin, being a natural antibiotic, has been repeatedly tested in medicine and pharmaceuticals (for example, in 2011, researchers from Oxford showed the possibility of using natural tulipalin for the synthesis of the antitumor drug methylene lactacin). As of 2014, these experiments have not given a practical result, in the pharmaceutical industry, tulipolines are not used yet [18].

1.2.3 Application

Tulipalin A is considered as one of the candidates for the role of a "green" (renewable) monomer, a raw material for the production of plastics that can be extracted from plants without the use of non-renewable reagents and energy carriers. The first polymers based on pure α-methylene-γ-butyrolactone and copolymers of α-methylene-γ-butyrolactone and acrylonitrite are transparent, very hard but fragile, were patented in the US in 1947 [19]. In

the later literature, various experimental polymerization technologies are described, usually not pure tulipalin, but its mixtures with other organic compounds. For example, polymethylene butyrolactone (PMBL, copolymer of tulipalin A and γ-methyl-α-methylene-γ-butyrolactone) is similar in properties to polymethylmethacrylate (PMMA), and advantageously differs from it by a greater glass transition temperature (195 °C versus 100 °C in PMMA) and the best mechanical and optical properties [20]. Copolymers of tulipalin A, characterized by increased resistance to abrasion and ultraviolet radiation – a possible perspective replacement of acrylic soils, paints and varnishes in the automotive industry.

1.3 Graphene oxide

Graphene oxide (GO) - is oxidized graphene. While pure graphite is originally three-dimensional (3D) system consisting of individual layers with delocalized hexagonal honeycomb structure in plane and bonded to each other by common C-C bond in out of plane direction, the graphene and GO have two-dimensional (2D) structure, mostly consisted of several layers (Figure 5)[21]. There are some special method how to prepare single-sheet graphene and GO described by.[1,21] In addition, GO is oxidized to exfoliate the carbon layers, using carboxyl, hydroxyl, and epoxide groups, that appear on the surface of graphene sheets. Nowadays graphene oxide is a very attracted material due to affordable cost, easy production, and possibility to be converted to graphene [21]. The agents of oxidation react with graphite, a lot of oxygen-containing functional groups have been introduced onto both sides of a single graphite sheet to surmount intermolecular bonds of van der Waals forces between the plates as was already proposed by Brodie and Hummers [22]. The interval of interlayer spacing of graphite is increased from 0.335 nm to more than 0.625 nm for GO [23]. As known GO is based on graphene it means that GO preserves some properties from graphene. For example: It is the thinnest discovered compound, the thickness is just about one atom. Also it is the lightest known material (1 square meter coming in at around 0.77 milligrams), the strongest compound discovered (approximately 100-300 times stronger than steel) [24]. It is so strong that a 1 m^2 hammock, that is not heavier than a cat´s whisker, could bear the weight of an cat without any breaking [24].

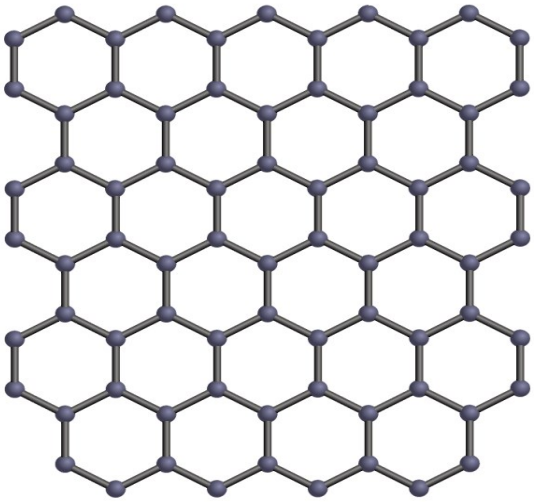

Figure 5: The structure of graphene [25].

1.3.1 Properties

Graphene also allows scientists to observe some of the ghost-like quantum effects, that before were just theoretical aspect of science. One such phenomenon is a variant of Klein tunnelling, which was firstly created by the Swedish physicist Oskar Klein in 1929. This tunnel effect in quantum physics describes how particles can time to time pass through a barrier that would in normal case block them. The main rule is - the larger the barrier the smaller the chance of quantum particles passing through. However, this hypothesis cannot be applied to electron´s trip in graphene – in some conditions they move ahead as if the barrier did not even exist [24].

Further notable properties of graphene are its amazing levels of light absorption approximately at 2.3% of white light. The unbelievable properties of graphene and GO exist because of unique molecular structure that contains small sp^2 carbon domains surrounded by sp^3 carbon domains. GO also has oxygen-containing hydrophilic functional groups, so that water molecules can easily insert into the interlayer [24, 22].

1.3.2 Application

Using electrochemical and optical properties of graphene, it is possible to develop highly effective, and simultaneously miniature biosensors for monitoring the neurological status in patients after a stroke or brain injury [26,27]. Similarly, based on graphene, it will be possible to create a framework for healing lesions of nervous tissue. In recent times, great

interest has arisen around biosensors based on graphene. In work it is told about the sensor, formed on oxide of graphene, which selectively detects DNA in solutions [27]. It has also been found that graphene can deliver oligonucleotides to living cells to detect biomolecules [28, 27]. Graphene or composites based on it were used to modify the electrodes in the electrochemical recognition of various biomolecules, including glucose, DNA and proteins, with high sensitivity. In addition, graphene can also be used in conjunction with other nanomaterials to build various biosensors [2,27]. Another kind of use of graphene in medicine can be attributed graphene paper, which has a pronounced antibacterial effect. The study of the antibacterial effect of graphene nanostructures showed how graphene oxide can be used as a coating material for the implant surface. As in many other areas, the study of biomedical applications of graphene expands, but is mainly at the initial stage. Progress in this area is still exciting and encouraging, but there are a number of challenges that researchers face and that must be overcome. One of such tasks is a thorough and in-depth understanding of the interaction of graphene - tissue, especially the mechanism of cellular uptake. Such knowledge contributes to the development of effective delivery of drugs, biosensors and other applications (Figure 6) [2]. Also, graphene and graphene oxide can accelerate the growth, differentiation and proliferation of stem cells, and therefore are very promising in tissue engineering, regenerative medicine, and other biomedical fields [29,28,27]. Systematic study is highly desirable for solving safety problems before the practical application of graphene in biomedicine.

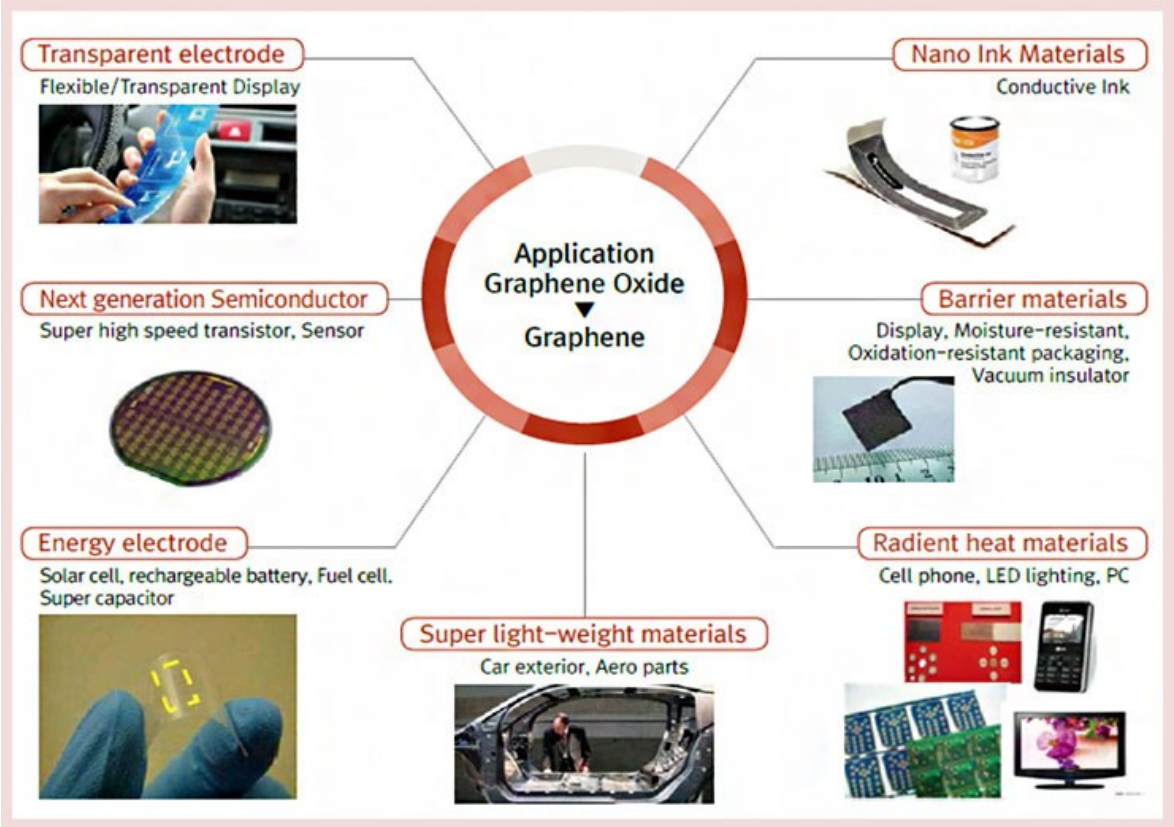

Figure 6: Applications of GO [30].

1.3.3 Cytotoxicity

The toxicity of graphene and graphene oxide is another major problem. Preliminary results show that the physico-chemical properties are closely related to cytotoxicity. Scientists from the University of California at Riverside came to the conclusion that graphene can be dangerous. When the material enters the groundwater, the hexagonal structure of graphene begins to break down, the microparticles quickly lose stability, collapse and can not bring significant harm [3]. The molecular structure of graphene in the form of nanoparticles is able to rupture the membranes of organisms living cells, which causes its significant toxicity [3].

2 METHODS AND INSTRUMENTS

In this work properties of hydrogels were measured and observed by electron microscope, rotational rheometer, swelling test and cytotoxicity test.

2.1 Microscopy techniques

Microscope is an optical device for obtaining enlarged images of objects (or components of their structure) that are invisible to the naked eye.

2.1.1 Electron microscope

The basic principles of electron microscopy are similar to the principles of light microscopy; the major difference is that electromagnetic lenses, not optical lenses, focus a high velocity electron beam instead of visible light. Because electrons are absorbed by atoms in air, the entire tube between the electron source and the viewing screen is maintained under an ultrahigh vacuum. The resolving power of an electron microscope is 1000-10000 times greater than the resolution of a traditional light microscope and for modern best instruments it can be less than one angstrom. To obtain an image in an electron microscope, special magnetic lenses are used that control the movement of electrons in the column of the device by means of a magnetic field [31].

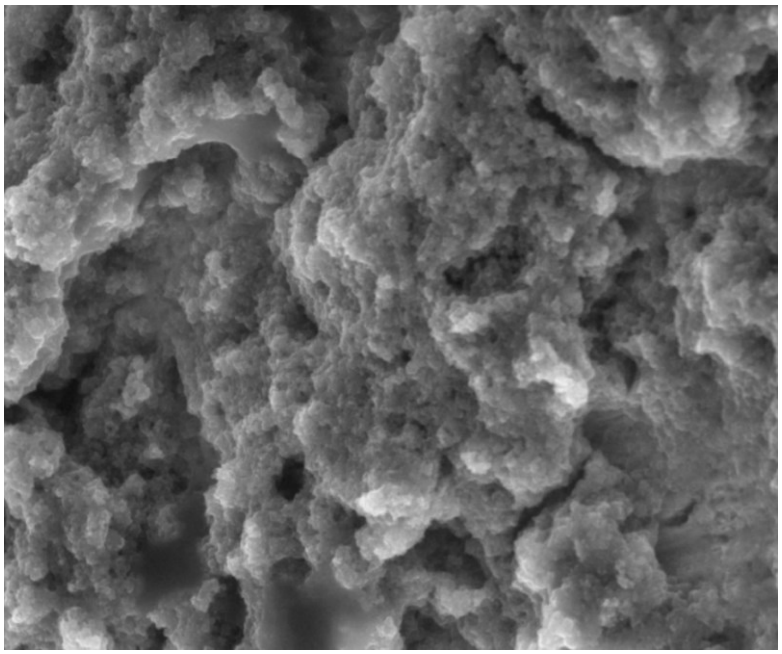

Figure 7: SEM photo of hydrogel [32].

2.2 Rheology

Rheometry is the experimental technique, that used to measure the rheological properties of materials. Rheology could be defined as the branch of science of the flow and deformation of substance which describes the interrelation between deformation, force and time.

Rheometer is applied to measure flow properties. Generally, rheometers could be splitted into two basic types.

a) rotary - for low shear speed measurements (10-2 - 102 s^{-1})
b) capillary - for medium and high shear rates (10 - 103 s^{-1}) [33]

2.2.1 Rotational rheometer

This type of rheometers creates an azimuthal flow and offers a wide dynamical range. Rotational rheometers are usually could be employed for high viscous media [34].

Types of rotational rheometers (Figure 8)

a) Roller-cylinder type. This geometric system is optimal for materials with low viscosity. The polymer melt is subjected to shear stress between two concentric cylinders, one of which rotates at a constant angular velocity and the other is stationary.
b) Cone-plate type. The advantage of this type is that conditions of the flow are strictly defined.
c) Plate-plate type. The major difference between plate-plate type and others is that the shear deformation velocity is dependent on the distance between the plates of the system [33].

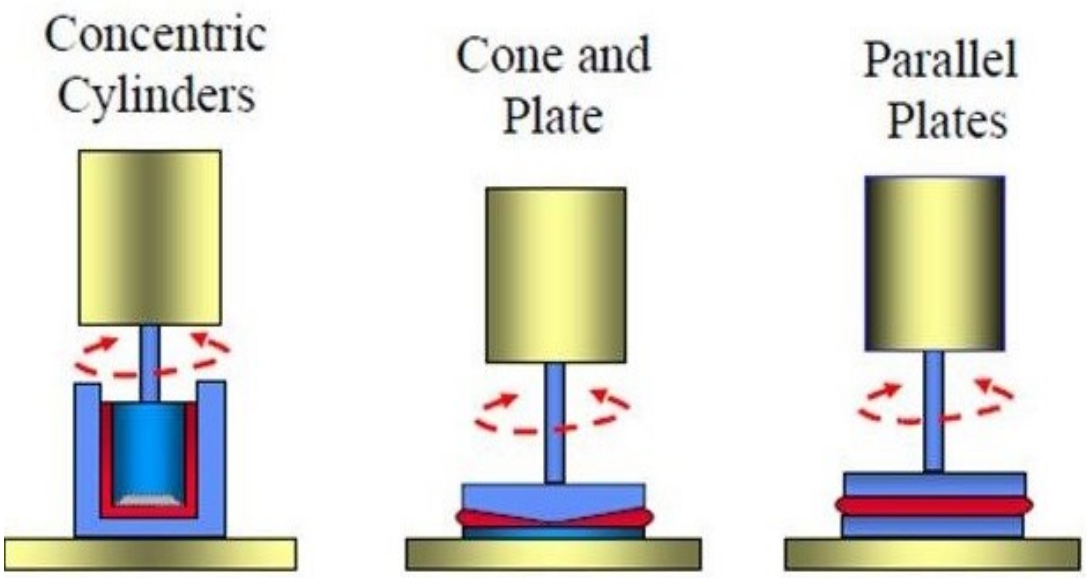

Figure 8: Types of geometries [35].

2.2.2 Viscoelastic properties

Storage modulus - G′, loss modulus - G″ and loss factor - tan δ, are one of the most important hydrogel properties monitored against strain, time and frequency. The loss factor, tan δ, is defined as G″/G′. To re-emphasize, G′ measures the deformation energy stored during shear process of a test material and G″ is representative of the energy dissipated during shearing. If G″ > G′ (tan δ > 1), the sample behaves more like a viscous liquid while, conversely, when G′ > G″, and, thus, tan δ < 1, the sample behaves more like an elastic solid [36].

2.3 Swelling properties

The sorption measurement was used to find out a mass of water that hydrogel is able to absorb. For successful result temperature, pressure and humidity have to be stable during whole period of measurement. Principle of the method is – ultra dry sample of hydrogel is needed to weigh and then put in pure water. Weight of sample is measured by stated period of time. Final step is comparison of weight between dried form of sample and swelled form. Due to known time and weights is possibility to create sorption isotherm. The variable H in Figure 9 is the same as the SD used in the practical part of this BP.

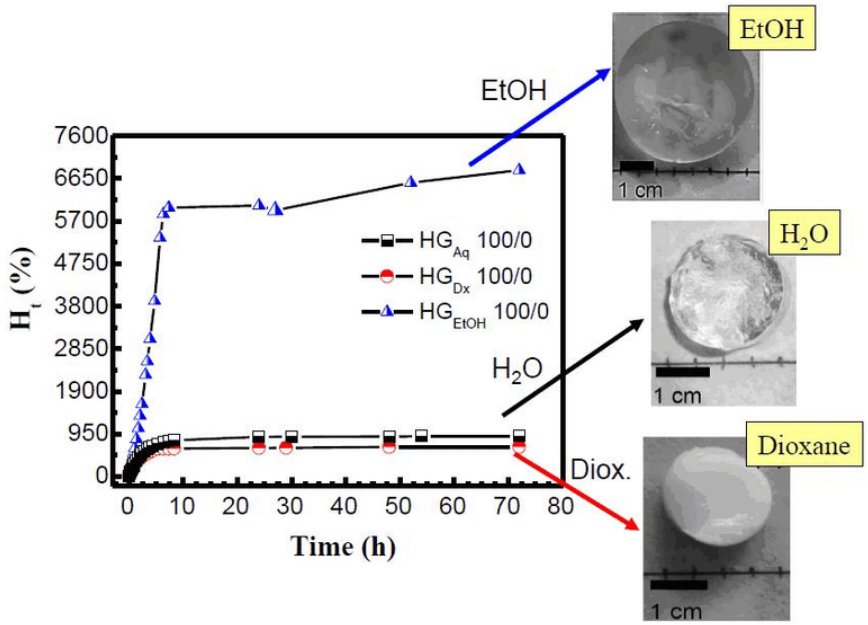

Figure 9: Swelling isotherm of the hydrogels [37].

2.3.1 Cytotoxicity and antibacterial properties

Cytotoxicity is the property of object to be toxic to cells. As example of toxic agent could be venom, alkaloid or immune cell [38, 39]. Cell killers agent could negatively influence membrane and inner structures of cell, that usually evokes necrosis (death of cell) [38]. Test of cytotoxicity is provided by monitoring life functionality of cells that are in contact with potential cytotoxic agent.

The common approach and the principle of testing the biological behavior of materials begins with simple in vitro tests mostly based on cell cultures, which generally deal with fields such as toxicology, microbiology or applied biology. These tests are able to determine the local toxicity caused by the test material. If these experiments and material efficiency tests yield promising results, longer and more demanding in vivo tests will be undertaken. Experimental animals are usually used to find out how it would work in real body system. But nowadays, alternative methods that are more gentle to animals or are omitted altogether. Clinical studies are the last level of this process. It should be borne in mind, among other things, that the number of subjects who come in contact with the material is often high. This significantly increases the likelihood that some patients may discover problems based on specific individual immunity. This problem is addressed by the testing methods used in individual patients [40].

II. ANALYSIS

3 MAIN AIMS OF THE BACHELOR THESIS

- The creation the hydrogels for medical application

The hydrogels would contain tulipalin A as one of the major components and graphene oxide as minor part. These materials were chosen because of their amazing behaviour and should possess suitable properties in medical field. The products could be applied as wound dressings, drug delivery systems, hygiene products, or tissue engineering scaffolds.

- Synthesis and fabrication of the new types of hydrogels
- Structural characterization of the hydrogels using SEM.
- Mechanical properties using viscoelastic measurements
- Swelling capabilities using gravimetric measurements
- Suitability elucidation medical application using cytotoxicity evaluation

4 EXPERIMETNAL PART

The content of the experimental part is made in such a way as to achieve the goals set work.

4.1 Materials

α-methylene-γ-butyrolactone (MBL, 97%) (Sigma-Aldrich, Germany), N′-methylenebis(acrylamide) (BIS, 99%) (Sigma-Aldrich, Germany), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (V-50, 97%) (Sigma-Aldrich, Germany), Hydrochloric acid (1M) (own production), sodium hydroxide (Sigma-Aldrich, Germany), N-Isopropylacrylamide (NIPAM, 97%) (Sigma-Aldrich, Germany), 2-Bromo-2-methylpropionyl bromide (BIBB) (Sigma-Aldrich, Germany), CuBr catalyst (Sigma-Aldrich, Germany), (graphene oxide (GO) (own production), distilled water (own production).

4.2 Instruments

Rotational Rheometer (Anton Paar, MCR 502, Austria) with parralel plate geometry,when the upper plate has 10 mm in diameter was used. Constant temperature was achieved using peltier cell (Anton Paar, MCR 502, Austria). Scanning electron microscope (Phenom pro) was used and following parameters were used for image aquisition(voltage : Default: 5 kV, 10 kV and 15 kV, resolution: < 10 nm (BSED) & < 8 nm (SED))

4.3 Methods

In the next text will be described the synthesis of hydrogels and process of measurement properties of the samples.

4.3.1 GO production

Graphene oxide (GO) was made from graphite powder using the modified Hammer method. The product is separated on a high speed centrifuge, operating at 10000 rpm for 20 minutes at 25 ° C, and the purification process is based on the dispersion of GO in 0.1 M HCl and - centrifugal separation. The procedure was repeated with distilled water several times until the pH reached 7. The particles were then lyophilized after purification to remove residual water and give a brown powder. The BiBB initiator was immobilized according to the procedure described elsewhere. Polymerization from the surface of GO par-

ticles was accompanied by the procedure described below. The presence of oxygen was minimized by degassing the system followed by several freezing cycles. Finally, the CuBr catalyst was added during argon flow and the polymerization was carried out at 70 ° C for 2 hours. The product was purified by filtration using DMF, acetone and diethyl ether and dried by lyophilization.

4.3.2 Polymerization of hydrogel

SHMB (Sodium 4-Hydroxy-2-methylenebutanoate), BIS, NIPAM and V-50 are present in all mixtures, while the GO particles were mixed only to half of reactions. Six hydrogels had to be created with GO (0.022g which is 0.73%) and six without GO. Then the reaction mixture was added to water. The solution was purged with nitrogen for 15 min to eliminate oxygen, injected into heat resistant plastic mould fixed by bulldog clips and sealed off by paraffin film. Polymerization was carried out at 70 °C for 7 h. Ratio of individual components is presented in tables 1, 2, 3, 4, 5 and 6.

Table 1: Composition of the Sample 1.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	8	0,72
			BIS	1	0,009	**SHMB**	2	0,18
water	70	2,1						

Table 2: Composition of the Sample 2.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	6	0,54
			BIS	1	0,009	**SHMB**	4	0,36
water	70	2,1						

Table 3: Composition of the Sample 3.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	4	0,36
			BIS	1	0,009	**SHMB**	6	0,54
water	70	2,1						

Table 4: Composition of the Sample 4.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	8	0,72
			BIS	1,5	0,0135	**SHMB**	2	0,18
water	70	2,1						

Table 5: Composition of the Sample 5.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	6	0,54
			BIS	1,5	0,0135	**SHMB**	4	0,36
water	70	2,1						

Table 6: Composition of the Sample 6.

COMPONENT	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION [%]	WEIGHT [g]	SUBSTANCE	PROPOTION	WEIGHT [g]
non-water complex	30	0,9	**V-50**	0,2	0,0018	**NIPAM**	4	0,36
			BIS	1,5	0,0135	**SHMB**	6	0,54
water	70	2,1						

4.3.3 Microscopy

This method allows optically explore the structure and texture of prepared samples. For such investigation samples were dried using lyophilisation for 72 hours. All hydrogels were dried prior to the insertion to microscope. The cross-section of all samples was investigated in order to see the structural properties of the prepared hydrogels and how the structure is affected by presence of the GO particles.

4.3.4 Viscoelastic properties

In order to investigate the viscoelastic properties of hydrogels rotational rheometer Anton Paar MCR 502 was used. The investigation was performed on the swollen samples. Firstly the linear viscoelastic range was established as is shown in the following part (Figs. 17-29) then it was measured dependence of storage and loss moduli on the frequency, to properly see the mechanical performance of the prepared hydrogels as well as further effect of the GO addition to the hydrogels. The investigated frequency range was (0.-10 Hz). All measurements were carried out at 25 ° C.

For every sample was determinated dependence of storage modulus and loss modulus on strain and also storage modulus and loss modulus at frequency.

4.3.5 Swelling test

Samples of hydrogel with cylindrical shape 15 mm in diameter and 1 mm thick were immersed in distilled water at room temperature, and water sorption was determined using an analytical balance. The equilibrium water content (EWC) in weight percent, expressing the maximum amount of the water swollen by the hydrogel at given condition, was calculated from eq 1

$$\mathrm{EW}\ [\%] = \frac{\mathrm{W}\infty - \mathrm{Wd}}{\mathrm{W}\infty} \times 100 \qquad (1)$$

where W_∞ is the weight of the swollen condition and W_d is weight of the lyophilized form of hydrogel. [4]

The rate of the hydrogel water absorption could be expressed by degree of swelling (SD), it was calculated from eq 2

$$\mathrm{SD}\ [\%] = \frac{\mathrm{Wt} - \mathrm{Wd}}{\mathrm{Wt}} \times 100 \qquad (2)$$

where Wt is the weight of hydrogel at different time intervals and Wd is weight of the lyophilized form of hydrogel. [4]

4.3.6 Cytotoxicity test

4.3.6.1 Cultivation and trypsinization

Cultivation - the technique for increasing cell count.

Trypsinization - the technique for separating the cells from a material.

After confluence, cells were inspected on a microscope and transferred to a laminar box. A medium was aspirated from the culture vessel (T75) so as not to damage the cells. The cells were rinsed with 0.2 ml/cm^2 of phosphate buffer (PBS), equivalent to 15 ml, from the media, and then the buffer was aspirated. Additionally, 0.1 ml/cm^2 of trypsin corresponding to 7.5 ml was added to incubate the incubator for 15-20 minutes to break the cell-to-cell binding. The release of the cells was continuously checked on a microscope. After discontinuation of the batch, 7.5 ml of medium was added to the flask and the solution was pipetted into a tube and placed in a centrifuge which had been pre-heated to 37 °C. During centrifugation, which took place for 3 minutes at 1100 rpm, the cells were sedimented, allowing the medium to be aspirated with trypsin. The number of cells thus obtained was

approximately 2 × 107, which was then diluted with the medium to a desired concentration of 1 × 105 cells/ml. The cell suspension thus obtained was diluted into a 96-well plate with 1 µl suspension in each well and 12 well plates in each well, where 1 ml of the cell suspension was in each well. Cell plate was incubated for 24 hours.

4.3.6.2 Extract fabrication and application

To the tested hydrogels, the culture medium was added so that the concentration corresponded to 0.1 g of particles/ml of medium. The slurry thus formed was placed in a shaker for 24 hours at 450 rpm and 37 °C. After 24 hours of cell culture incubation, the 96-well plate medium was aspirated. 100 µl of the medium was replaced in the reference portion with fresh media and 100 %, 75 %, 50 %, 25 %, 10 % and 1 % platelets were added to the cells and incubated in the incubator.

4.3.6.3 MTT test

After 24 hours of culturing the cells in a 96-well plate with varying concentrations of the extract, the medium was aspirated from all wells. Pure medium and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide - MTT (Molecular Probes, USA) were added to each well. The cells were stored for an additional 4 hours in the incubator. During this time, MTT was metabolized to formazan, which is star-shaped. Furthermore, the medium with MTT was aspirated and 100 µl of dimethyl sulfoxide-DMSO (Molecular Probes, USA) was added to dissolve formazane, which was allowed to work for 15 minutes. The resulting coloration was then measured by a spectrophotometer at a wavelength of 570 nm, the resulting absorbance corresponding to the number of living cells. With higher live cell counts, the concentration of metabolized formazan will increase and absorption will increase.

The test was provided by EN ISO 10993-5: cytotoxicity equal to 1 corresponds to 100 % cell survival, values of > 0.8 are assigned to no cytotoxicity, 0.6–0.8 mild cytotoxicity, 0.4–0.6 moderate toxicity, < 0.4 severe cytotoxicity.

5 RESULTS AND DISCUSSION

5.1 Fabrication of the hydrogel

According to the Figure 10, two basic components such as Tulipaline A and N-isopropylacryl amide were copolymerized, where n and m are numbers showing the amount of the individual component. As was shown in the Tables (1-6) various composition of the final hydrogels have been used, including the copolymer various ratios between (NIPAM and SHMB), various amount of cross-linker (BIS) and addition of the GO particles. Impact of the composition of the fabrication procedure, as well as on the final properties of the hydrogels is disccussed below in the thesis more in detail.

During the hydrogel fabrication, the final material does not showed the transparent samples as is usual for hydrogel material, but cloudy-like systems have been received. Such behaviour is mostly caused due to the fact that, SHMB monomer was not properly opened during ring-opening procedure and is present in the hydrogel structure in its closed form (Figure 11b).

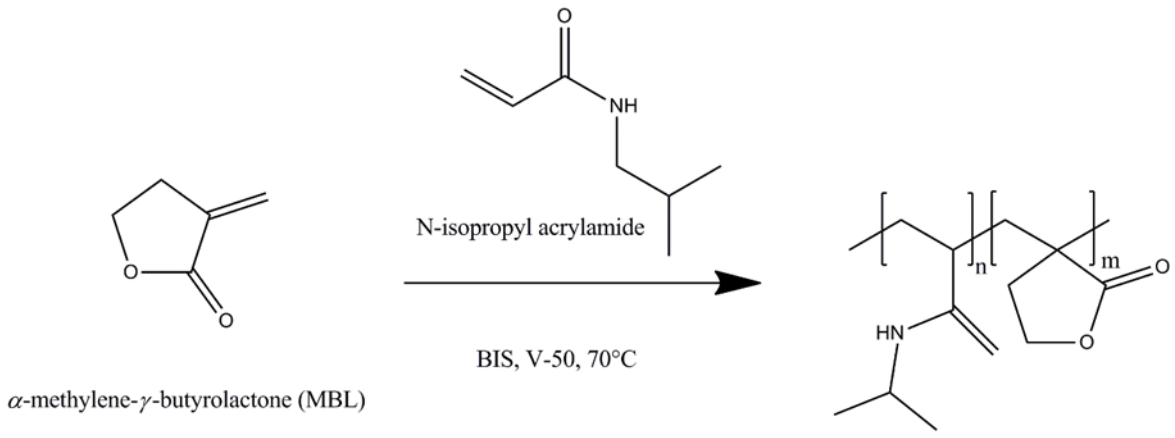

Figure 10: Polymerization

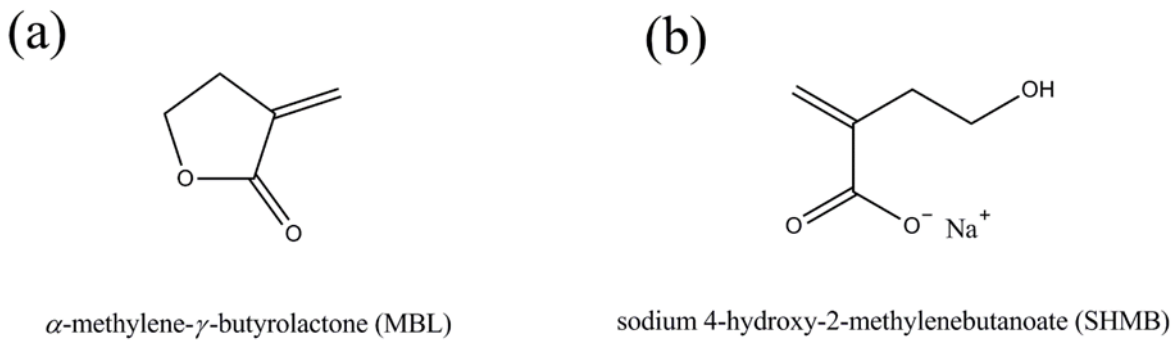

Figure 11: The chemical structures of MBL and SHMB.

The fabrication procedure was successful for samples 1, 4, 5, 6, 1 GO, 4 GO, 6 GO, and were suitable for different types of measurements, such as SEM investigations, viscoelastic evaluation, swelling tests and finaly cytotoxicity elucidation. Samples 2, 3, 2 GO, 3 GO, 5 GO were not reacting at all or react only partialy, which was confirmed by its consistency showing liquid-like behaviour. The main reason for such behaviour was higher amount of the reactive species mainly hydrogyl groups present in the GO as well as in SHMB when placed to the water and thus polymerizations as well as cross-linking reactions were unsuccessful. Finally, such samples could not be measured, because the liquid-like behaviour does not allow the aforementioned characterization usually used for properly cross-linked hydrogels.

5.2 Morphology of the hydrogels

Generally, the hydrogels haмy porous structure in order to be able to absorb as much water as possible, while the mechanical properties sustain on the same level. From this point of view of the SEM investigation provide and information if the porous structures is present in the samples. As can be seen from the Fig. 10, even the resolution of the used SEM is not high the porous structure of the sample 1 GO can be seen in this case also the manipulation with the hydrogel was easy and there was not problem with cross-setion creation for such SEM investigation. Similar properties were also obtained for the rest of the hydrogels. Please see the Figs. 10-16. Moreover, it can be seen that all sample showed porous structure, just sample 4 does not show it clearly most probably due to the low resolution of the SEM as well due to the magnification used for such investigation.

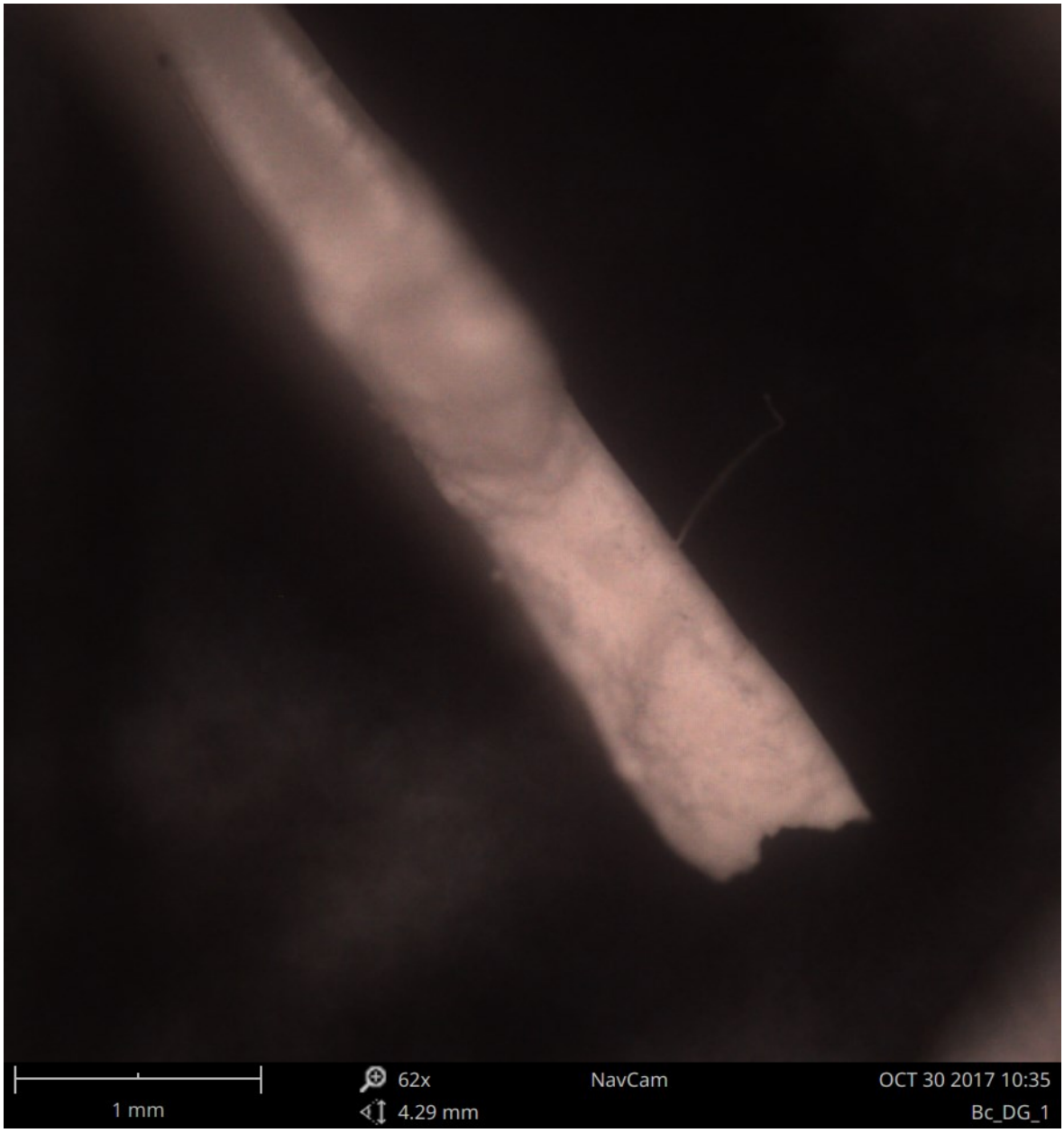

Figure 12: SEM image of the cross-section of the Sample 1.

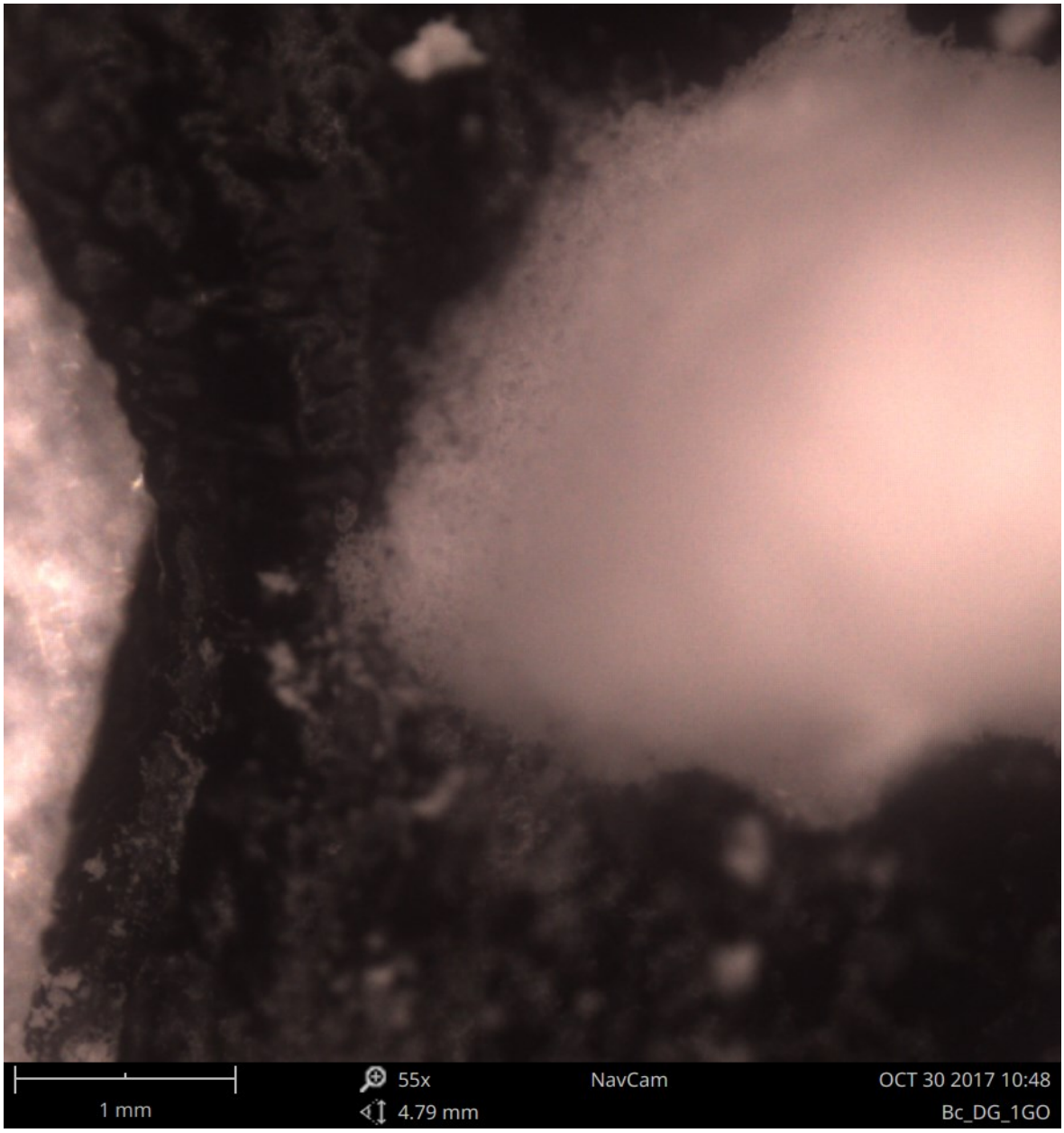

Figure 13: SEM image of the cross-section of the Sample 1 GO.

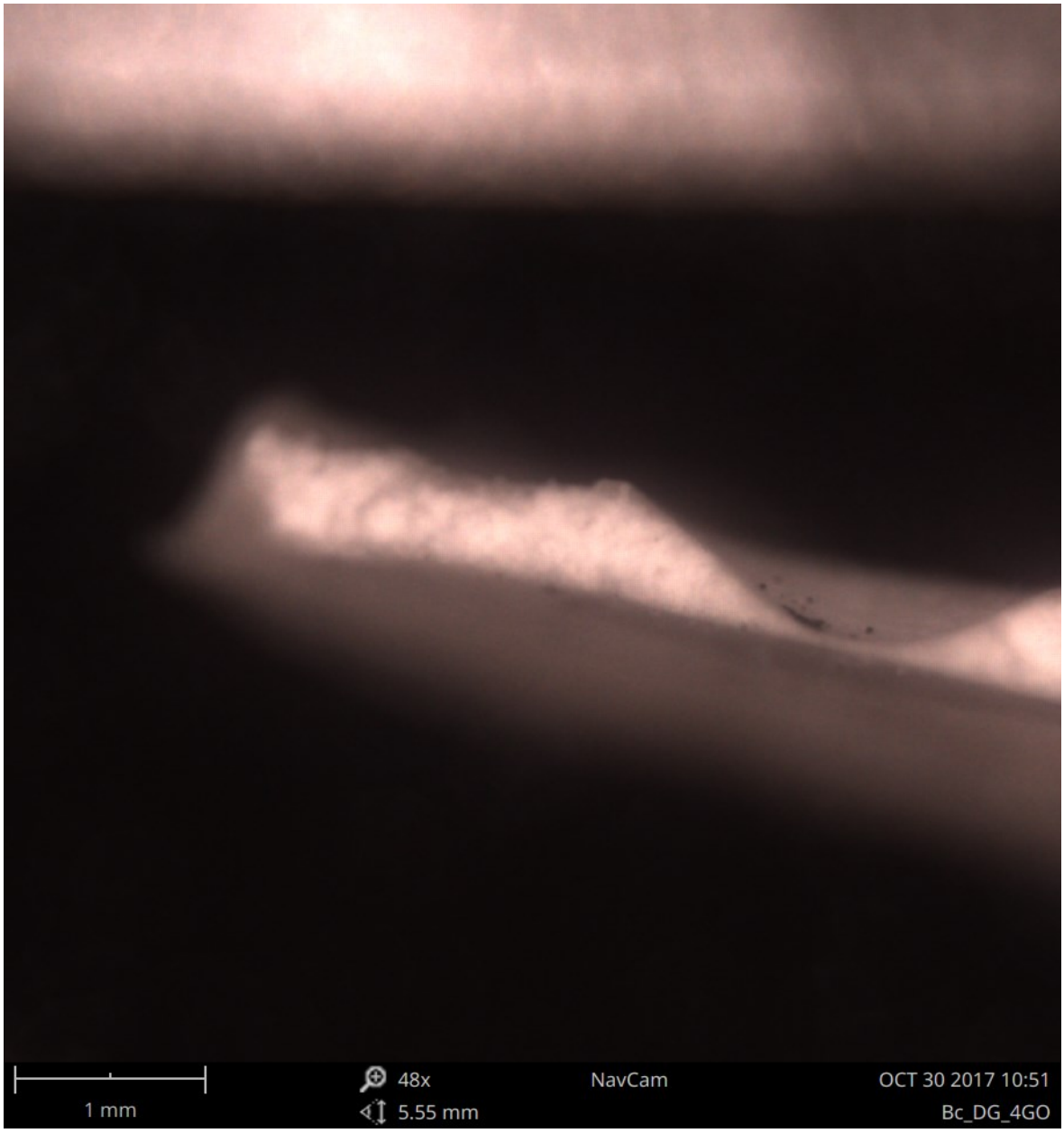

Figure 14: SEM image of the cross-section of the Sample 4.

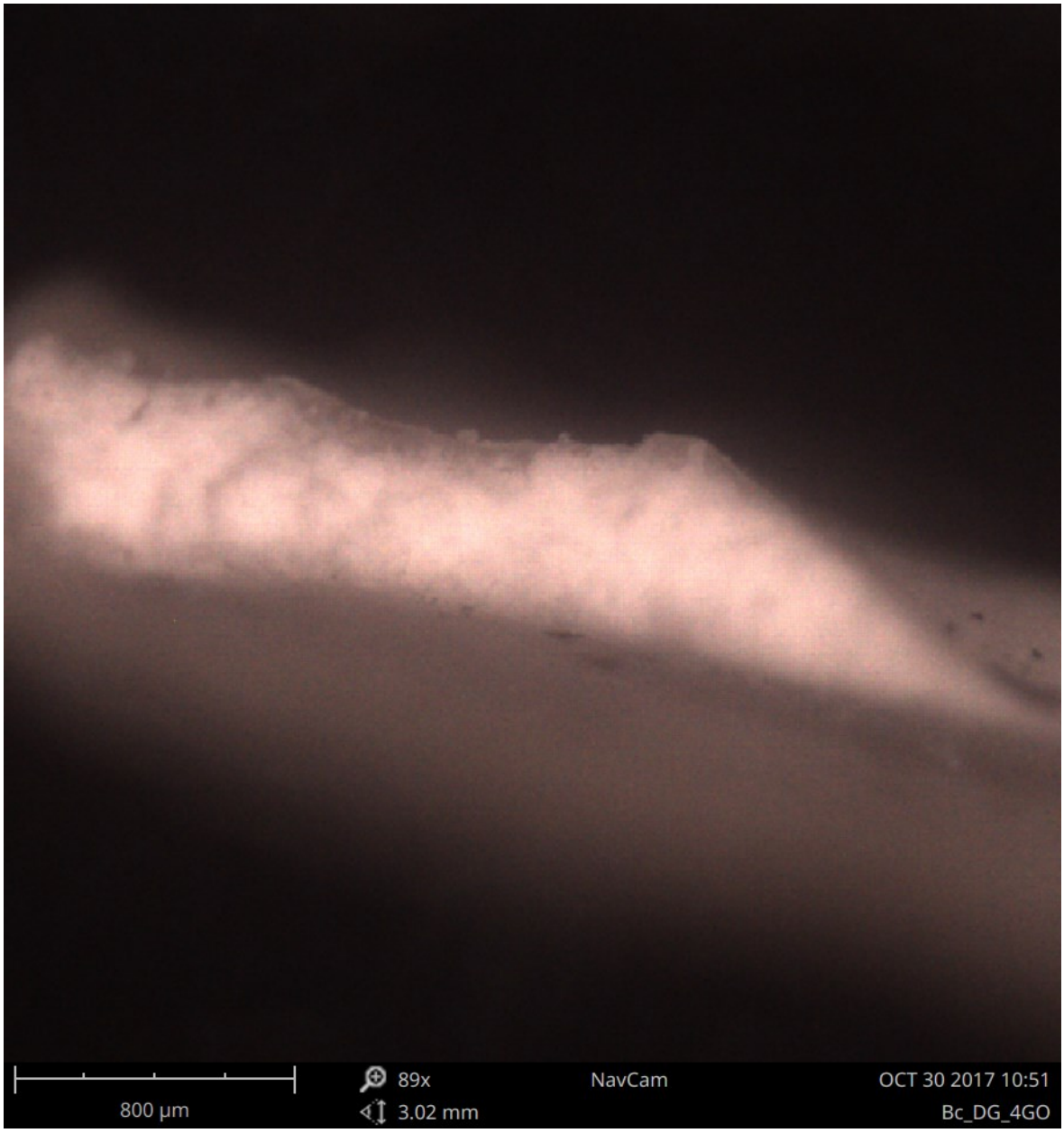

Figure 15: SEM image of the cross-section of the Sample 4 GO.

Figure 16: SEM image of the cross-section of the Sample 5.

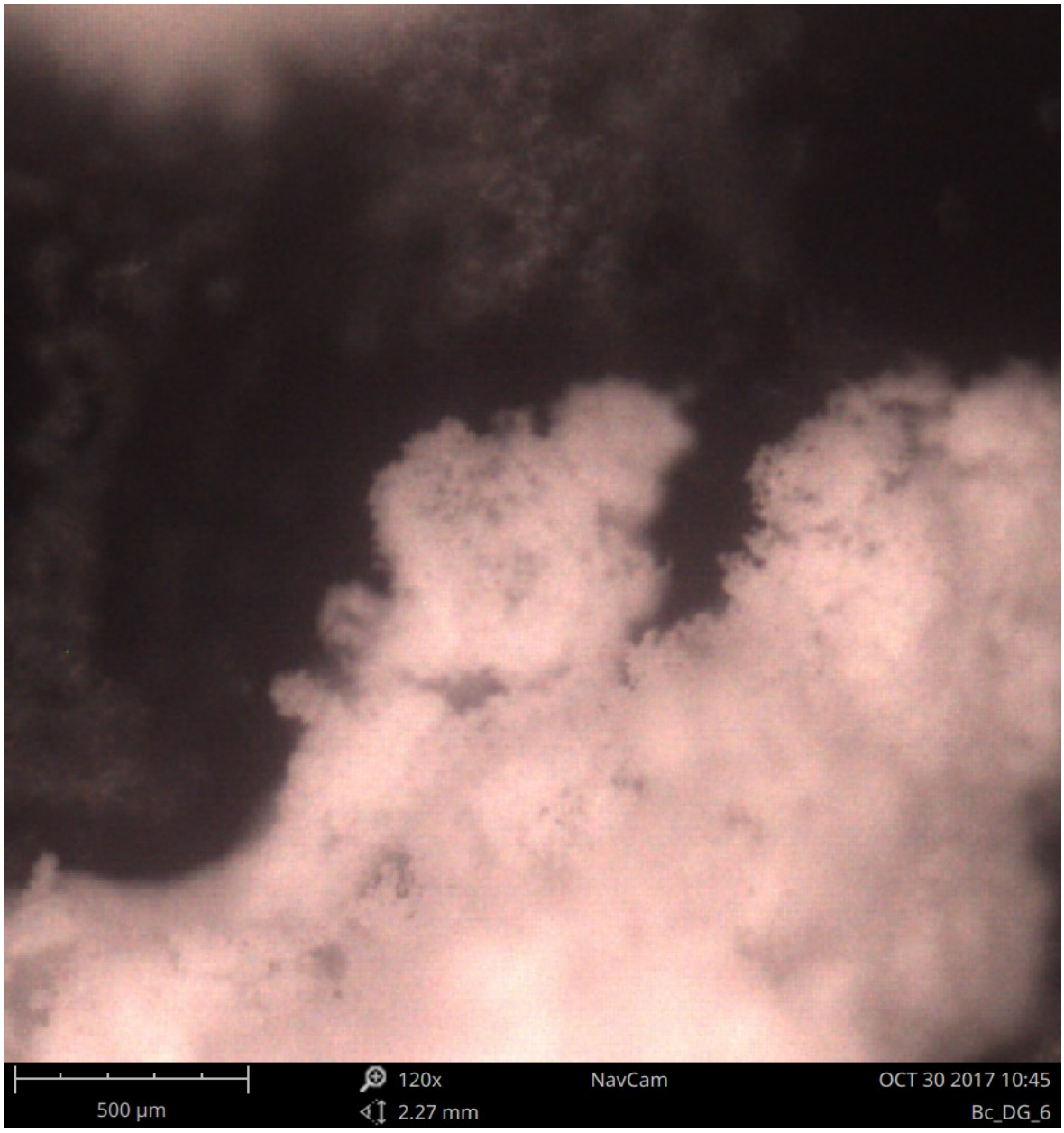

Figure 17: SEM image of the cross-section of the Sample 6.

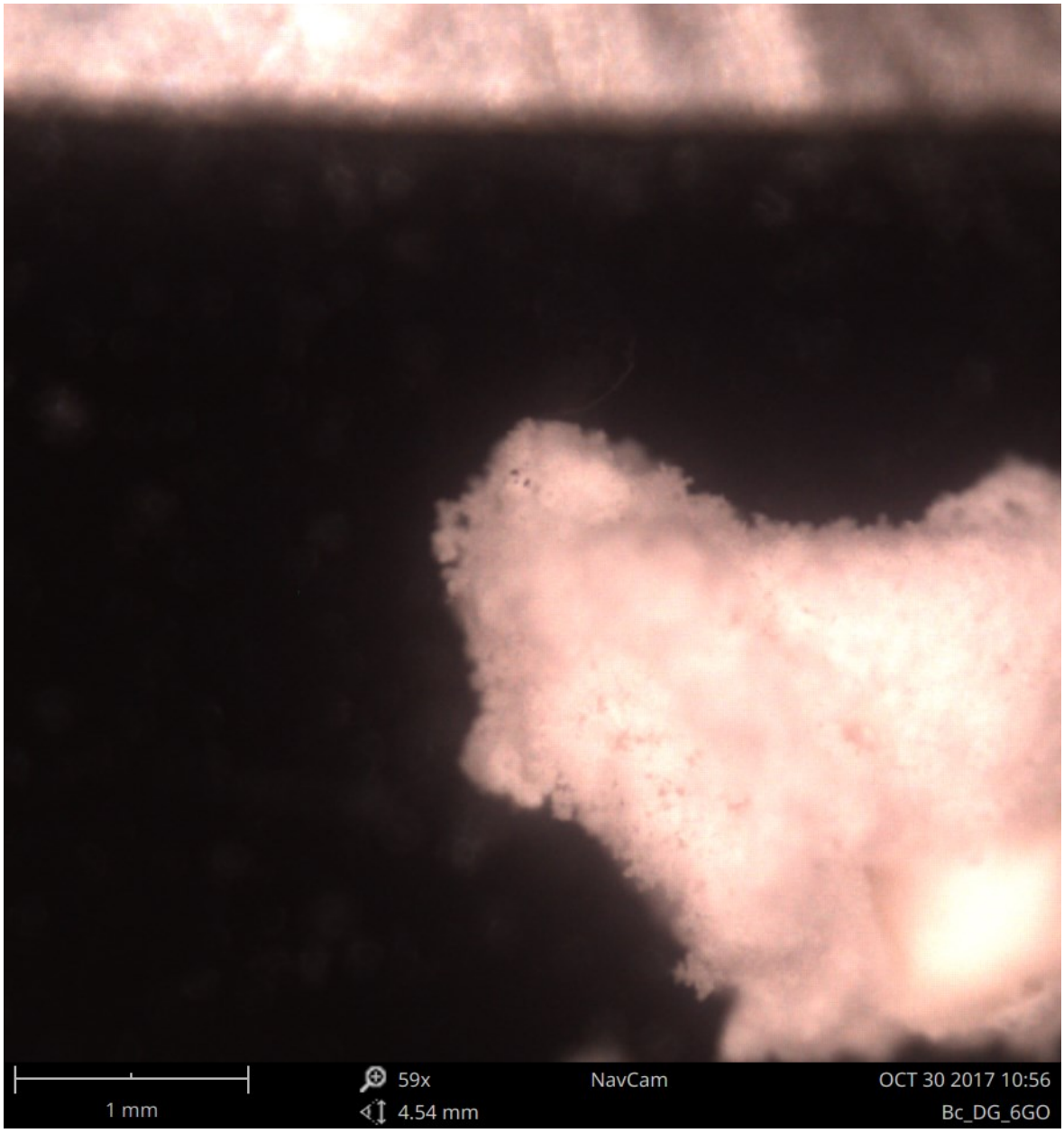

Figure 18: SEM image of the cross-section of the Sample 6 GO.

According to performed images and the hydrogels observation after polymerization, it is expected that GO could negatively influence polymerization of hydrogels. Cross-linking reaction was not effective because of GO presence which reacts with the radicals created during initiation. As a confirmation of such claim, the fabrication of the hydrogel 5GO failed, while in the sample without GO (sample 5) it was successful. The samples without graphene oxide have more solid and smooth structure than similar samples with GO, for example: 1 vs 1GO, 4 vs 4GO. Finally, in case of samples 6 and 6GO, here are negligible optical differences indicating that GO incorporation to the sample was significantly improved.

5.3 Viscoelastic properties

In order to investigate the mechanical performance of the prepared hydrogels, the evaluation of the viscoelastic properties using rotational rheometer in the oscillation regime was used. From this point of view two basic characteristic were measured. Firstly, strain dependence of the viscoelastic moduli on the strain, to obtain the linear viscoelastic region as well as critical deformation, where the sample exhibit significant rupture. Secondly, the frequency dependence on the viscoelastic moduli, providing the information, if the material is cross-linked as well as the absolute value of the Storage modulus reflecting the mechanical performance of the fabricated hydrogels.

As can be seen in the Figure 19, the value of storage modulus started to decrease at 0.3 % strain deformation slightly up to 1 %. If the deformation further increase the more pronounced damage of the Sample 1 occurs due to the fact that storage modulus significantly decreases while the loss modulus increase and thus overall elasticity of the material decreases. Very similar behavior was observed for the rest of the samples, just the values of the critical deformation are changing depending on the hydrogel composition, which will be individually discussed.

Figure 20 represents the frequency behavior of the Sample 1, it can be seen that the viscoelastic moduli are independent on the frequency indicating well-developed cross-linked network. Also the values of Storage modulus is around 2000 Pa, which is very similar to those already observed for similar type of hydrogel [4]. For the measurement the range from 0.1 Hz to 10 Hz was used, however, probably due to the wet contacts between the plates of rheometer and sample, the valid data are up to 2.5 Hz, although whole measure range have been presented.

If the GO particles are placed to this hydrogel during fabrication process, the impact on the mechanical properties can be seen. Firstly in Figure 21, the slight decrease can be seen from 0.7 % up to 2 %, which is improved in comparison to hydrogel without GO and aslo here there is no rapid decrease of Storage modulus and seems that material can be applicable to the higher shear deformations. In case of the absolute value of the Storage modulus, it was found to be lower (Figure 22) showing just 800 Pa, while without GO possess nearly 2000 Pa. Such behavior can be described as an effect of more flexible structrure due to the presence of GO and reflecting lower cross-likning density present in the hydrogel.

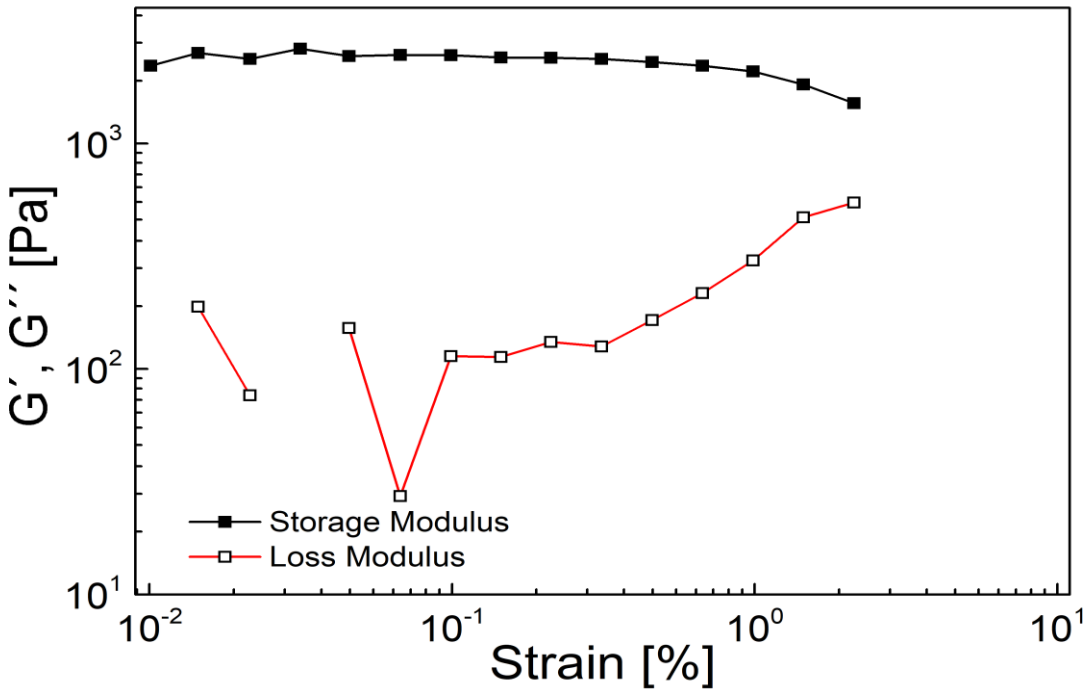

Figure 19: The dependence storage and loss modulus on strain for the Sample 1 (amount of cross-linker 1%).

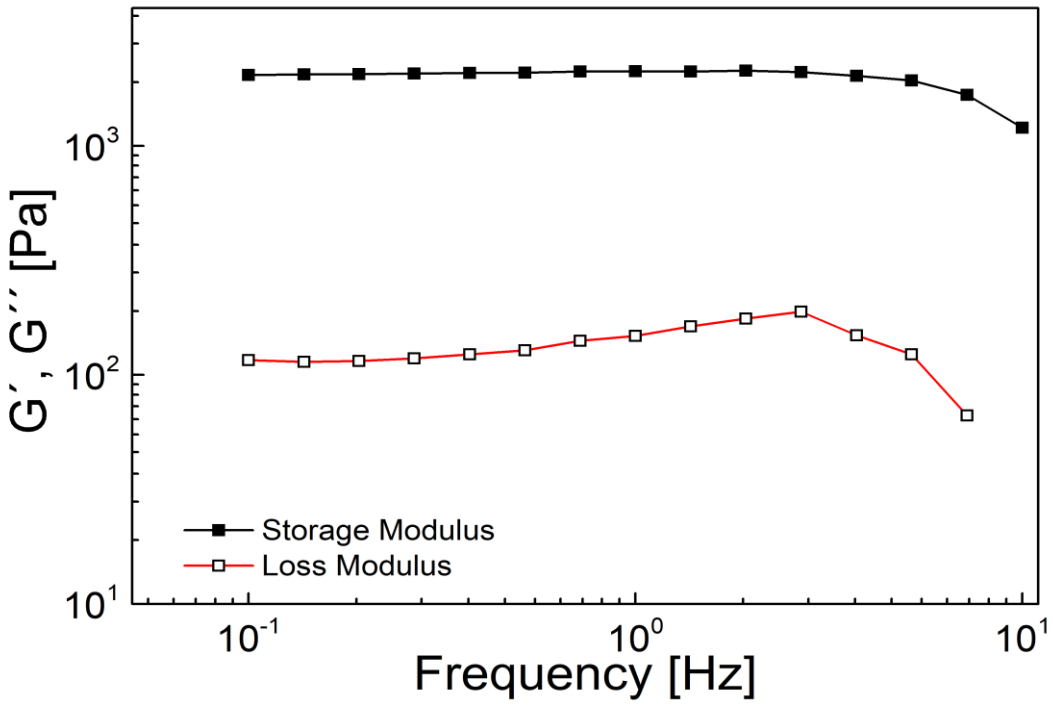

Figure 20: The dependence storage and loss modulus on frequency for the Sample 1 (amount of cross-linker 1%)

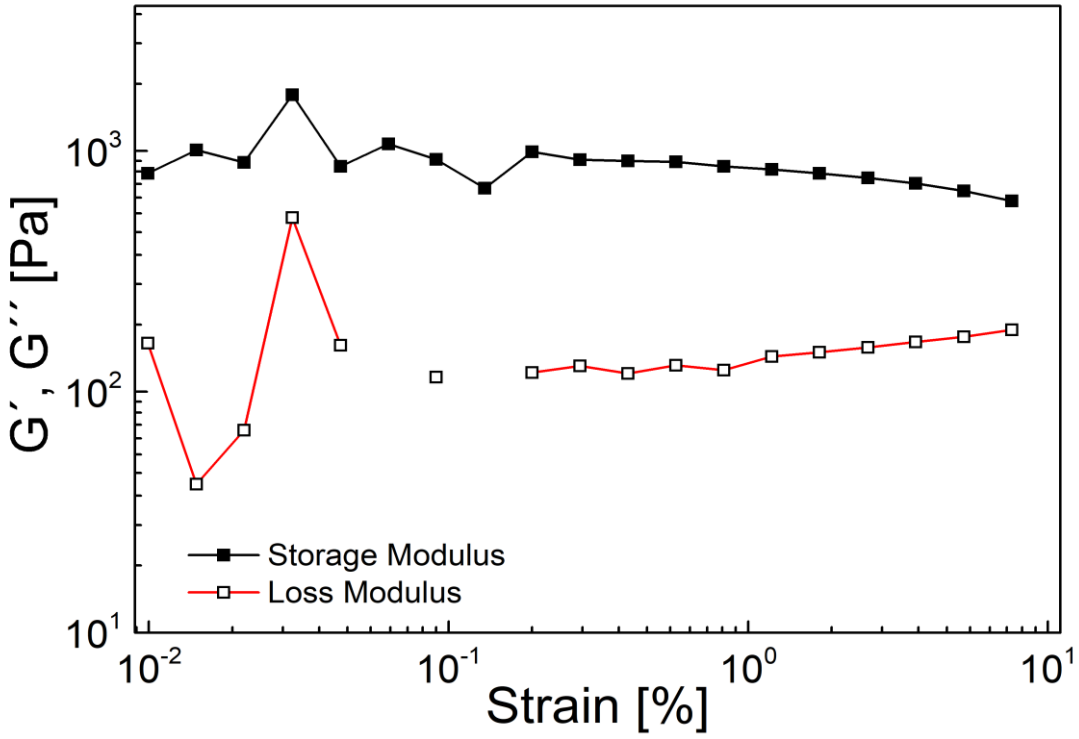

Figure 21: The dependence storage and loss modulus on strain for the Sample 1 GO (amount of cross-linker 1%, amount of GO 0.73%)

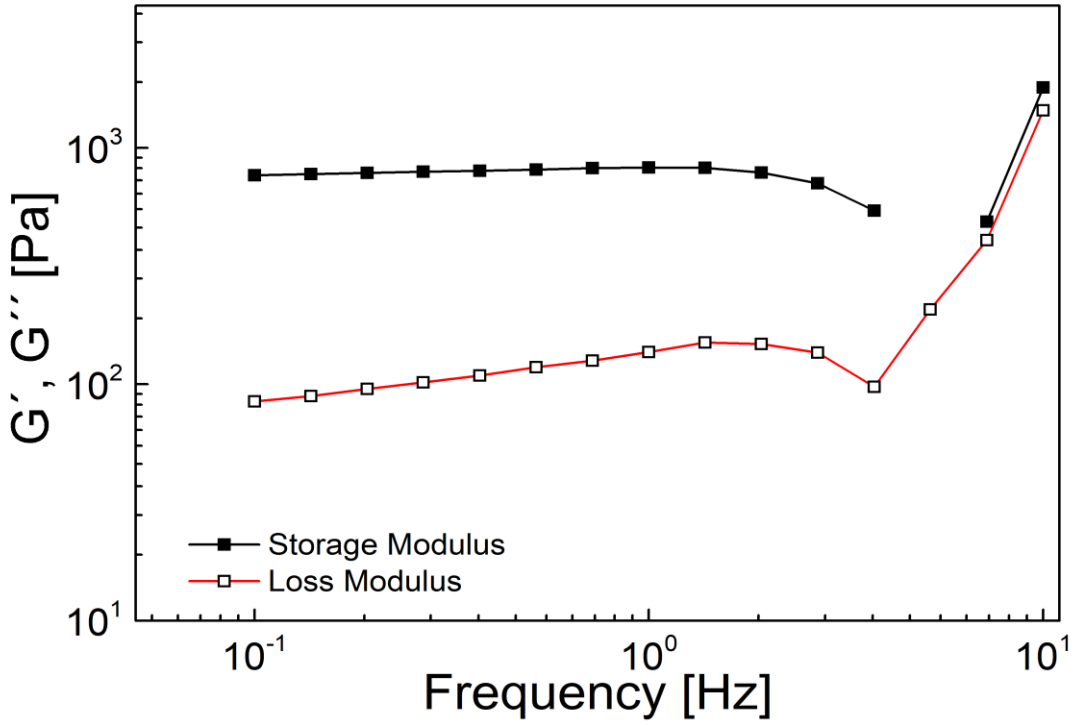

Figure 22: The dependence storage and loss modulus on frequency for the Sample 1 GO (amount of cross-linker 1%, amount of GO 0.73%).

In order to investigate the effect of the cross-linker the Sample 4 has been prepared and has the same composition but higher amount of the cross-linker. As can be seen in the Figure 23, the Storage modulus decreases slightly from 1 % up to 3 % of strain deformation and also can be measure to higher deformations up to 8 %, which was not possible for Sample 1 having lower amount of cross-linker and thus lower cross-linking density. However, the absolute value of Storage modulus from the frequency sweep reaches near 1000 Pa (Figure 24, which is significantly lower than in case of the Sample 1. So it can be concluded that, for such type of hydrogels, the presence of higher amount of cross-liker lead to the sample with enhanced deformation capability, while the Storage modulus is negavely affected.

Completely different situation can be visible in the case of Sample 4 containing the GO particles in the hydrogel composition. The deformation capabilities (Figure 25) are prolonged to the 4 % of strain deformation when the decrease of Storage modulus is very slight, while the measurable deformation was significantly enhanced to 20 % indicating the significant improvement of the deformation capabilities. Moreover, also the absolute value of the Storage modulus incrase to 2200 Pa (Figure 26), which more than 100 % enhancement in comparison to sample without GO as well as higher than the Sample 1 having only 2000 Pa. Therfore can be clearly concluded that addition of the higher amount of the cross-linker provide a significant synergism with GO particles in the form of improvement of mechanical performance of the fabricated hydrogels.

As was already mentioned, the presence of the GO suppressed the cross-linking reaction. In case of the Sample 5 where also the ratio of the monomeric units is changed, the mechanical properties were investigated. In Figure 27, it can be seen that the higher amount of the Tulipane A monomer decreases the mechanical performance and strain deformation is only 2 % and is measurable up to 6%. Also the absolute value of the Storage modulus (Figure 28) is only 750 Pa. Such behaviour can be ascribed as a low degree of copolymerization between the monomeric units, which finaly provide cross-linking sample with very low cross-linking density.

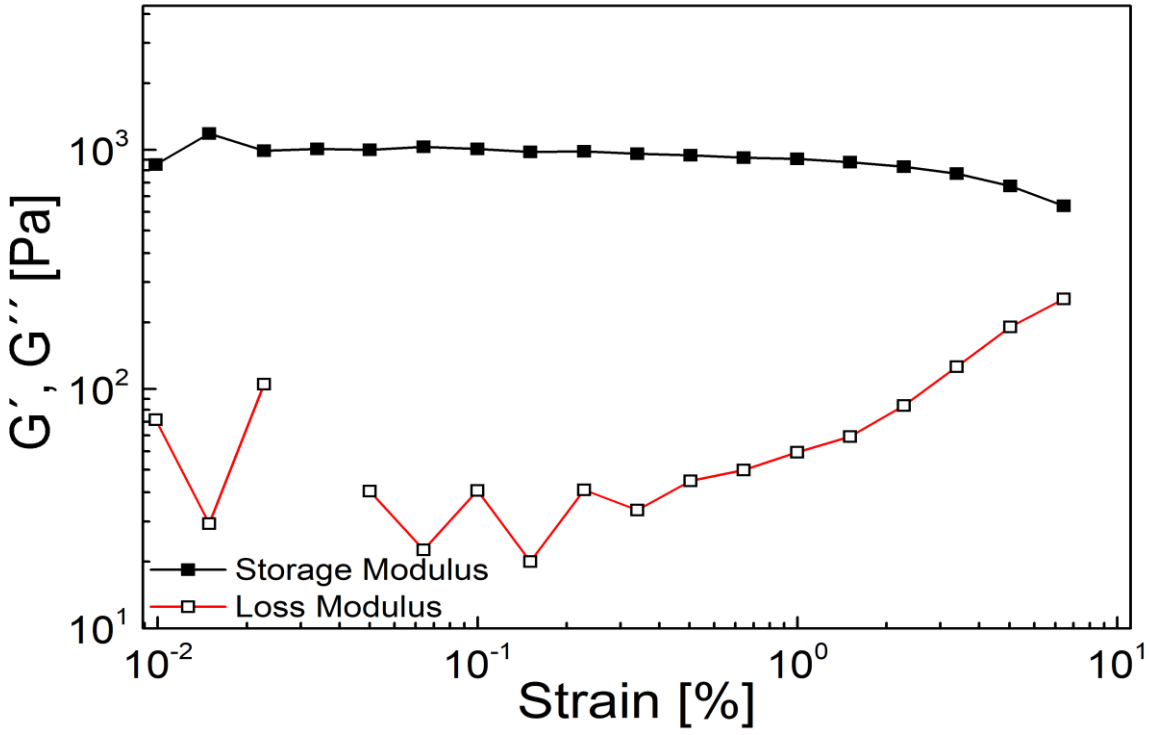

Figure 23: The dependence storage and loss modulus on strain for the Sample 4 (amount of cross-linker 1.5%).

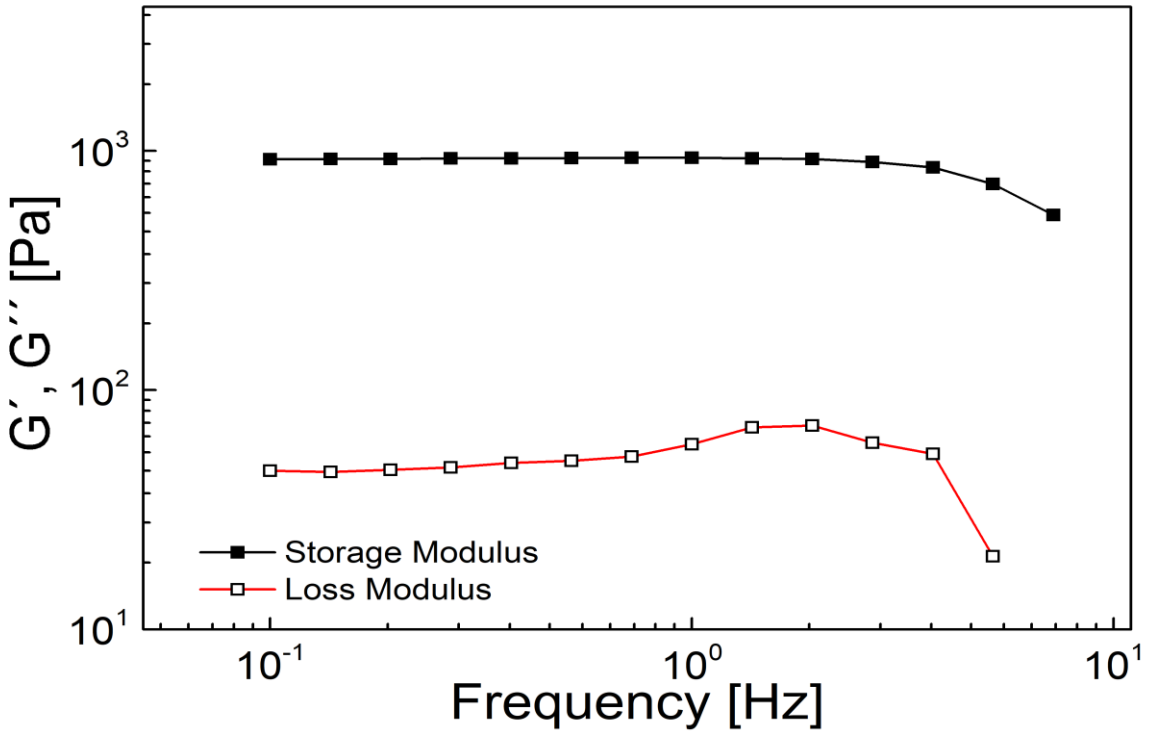

Figure 24: The dependence storage and loss modulus on frequency for the sample 4 (amount of cross-linker 1.5%)

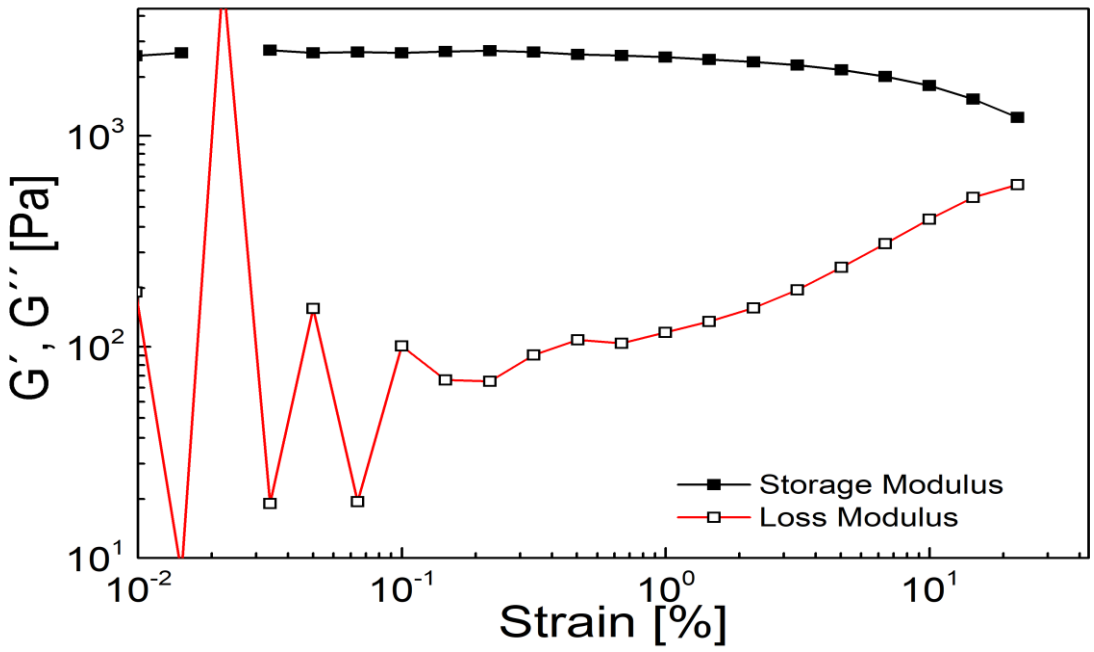

Figure 25: The dependence storage and loss modulus on strain for the Sample 4 GO (amount of cross-linker 1.5%, amount of GO 0.73%).

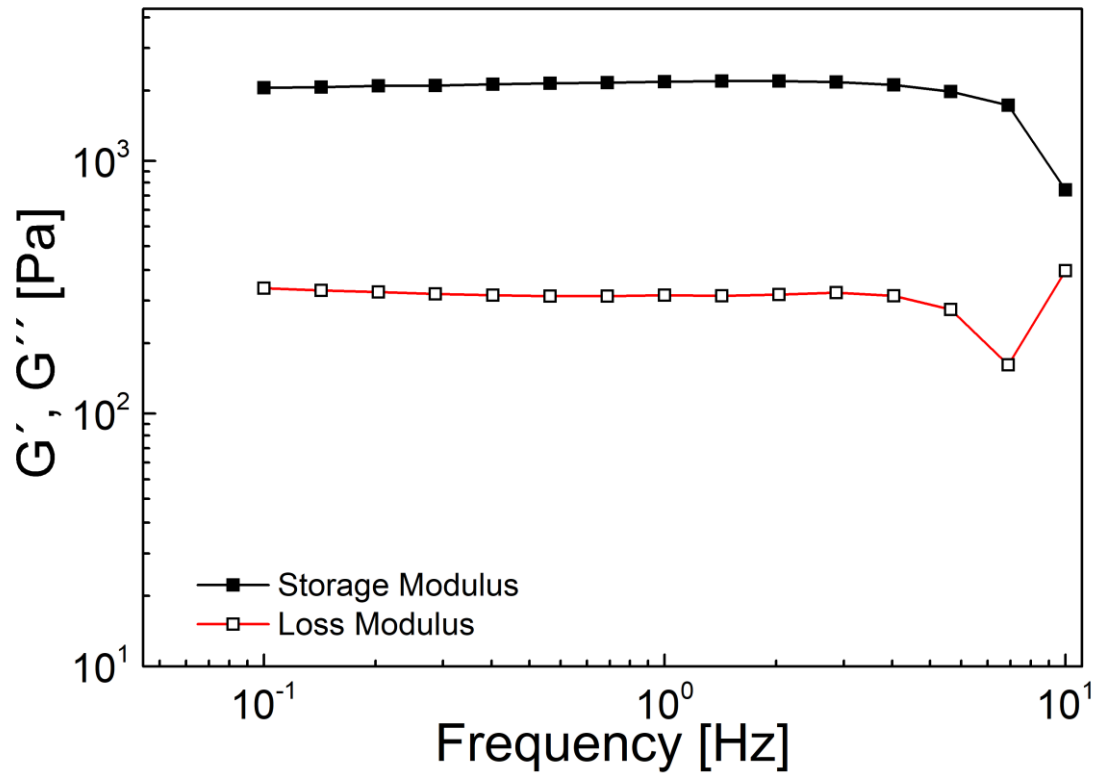

Figure 26: The dependence storage and loss modulus on frequency for the Sample 4 GO (amount of cross-linker 1.5%, amount of GO 0.73%).

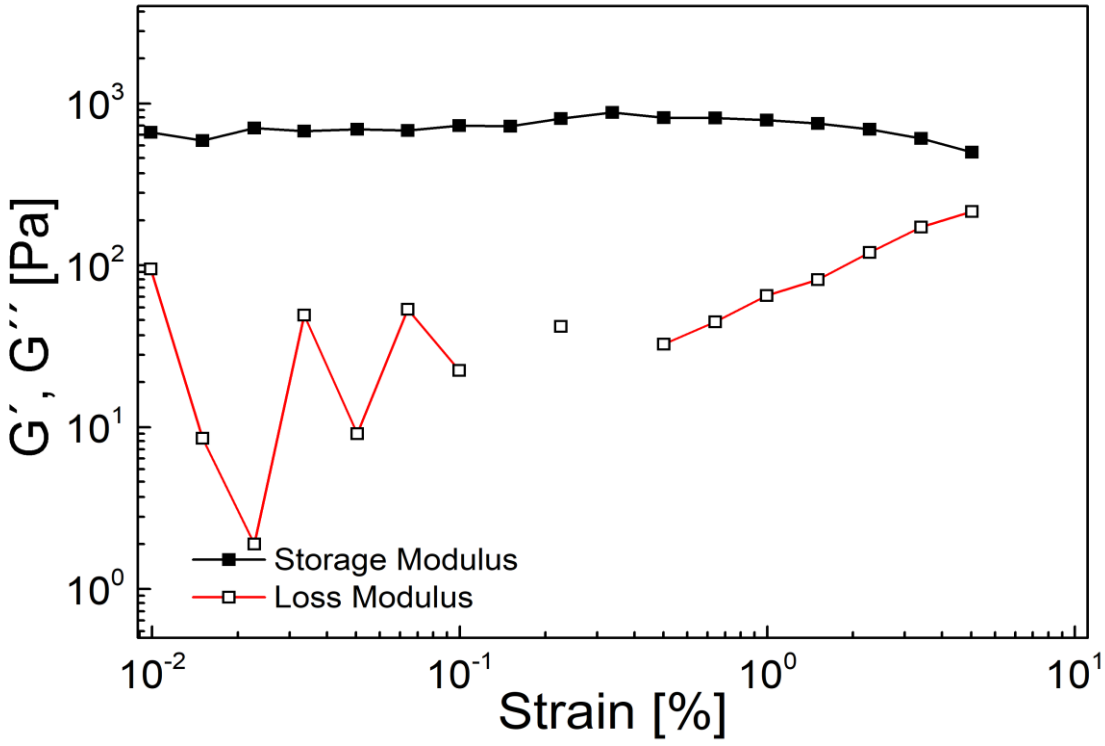

Figure 27: The dependence storage and loss modulus on strain for the Sample 5 (amount of cross-linker 1.5%).

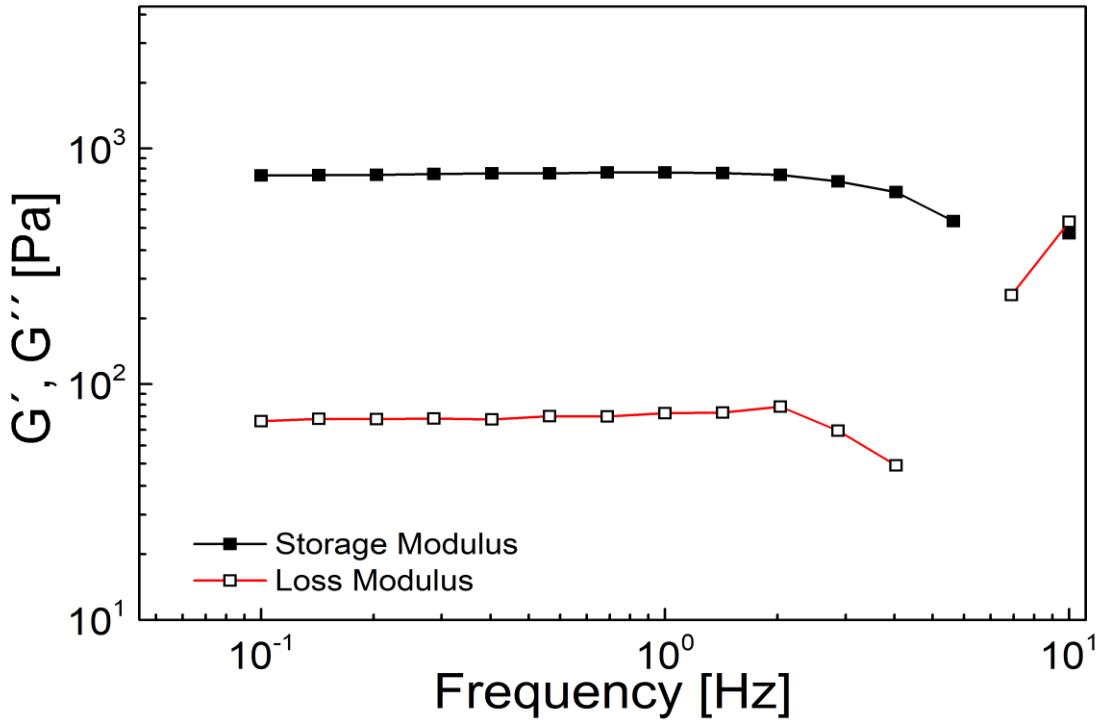

Figure 28: The dependence storage and loss modulus on frequency for the Sample 5 (amount of cross-linker 1.5%).

In order to elucidate the effect of the ratio between the monomeric units also the Sample 6 has been fabrication and containing the higher amount of the Tulipane A. Such sample according to the both investigation have moderate deformation capability (Figure 29) and very low absolute values of the Storage modulus (Figure 30) only 400 Pa. In this case, the Sample 6 containing higher amount of Tulipaline units in the copolymer chain, provide a sample with lower mechanical performance.

However, the situation is very different if the GO particles are added to the hydrogel during fabrication step. It can be seen very slight decrease in the Storage modulus with increasing strain deformation, means that hydrogel is very stable up to 3 % of strain deformation (Figure 31). On the other hand, the absolute value is the highest for such composition having nearly 3000 Pa. This behavior is mainly caused by the fact that higher amount of the cross-linker, next to the higher amount of the Tulipaline and presence of GO particle have a significant synergy in case of cross-linking density and thus provide hydrogels with excellent mechanical properties.

Therefore can be concluded, that the composition of the hydrogel has significant effect on the final mechanical performance. The amount of cross-linker mainly improving deformation capabilities, the higher amount of the Tulipaline A portion decreases the absolute values of Storage modulus, but improving the stability of the hydrogel upon deformation and finally the addition of the GO particles positively enhancing the absolute values of the Storage modulus. Thus can be stated, that by the varying of the individual components present in the hydrogel, the mechanical properties can be finely tuned based on the requirements of the potential applications.

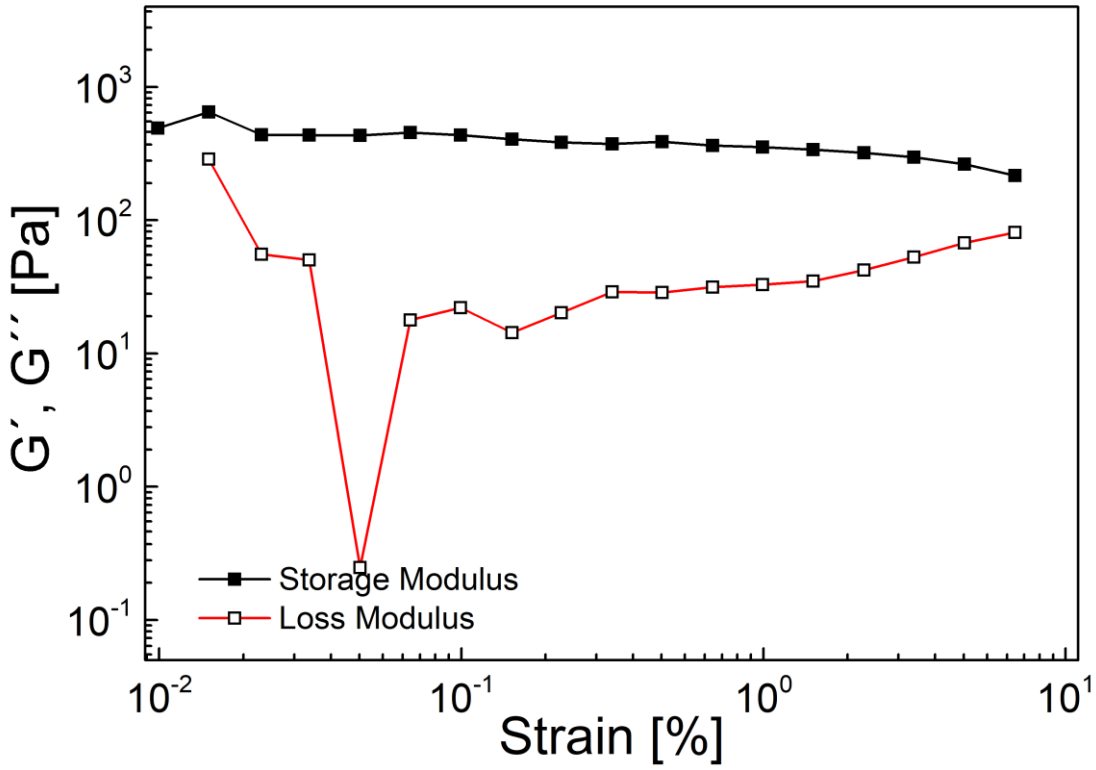

Figure 29: The dependence storage and loss modulus on strain for the Sample 6 (amount of cross-linker 1.5%)

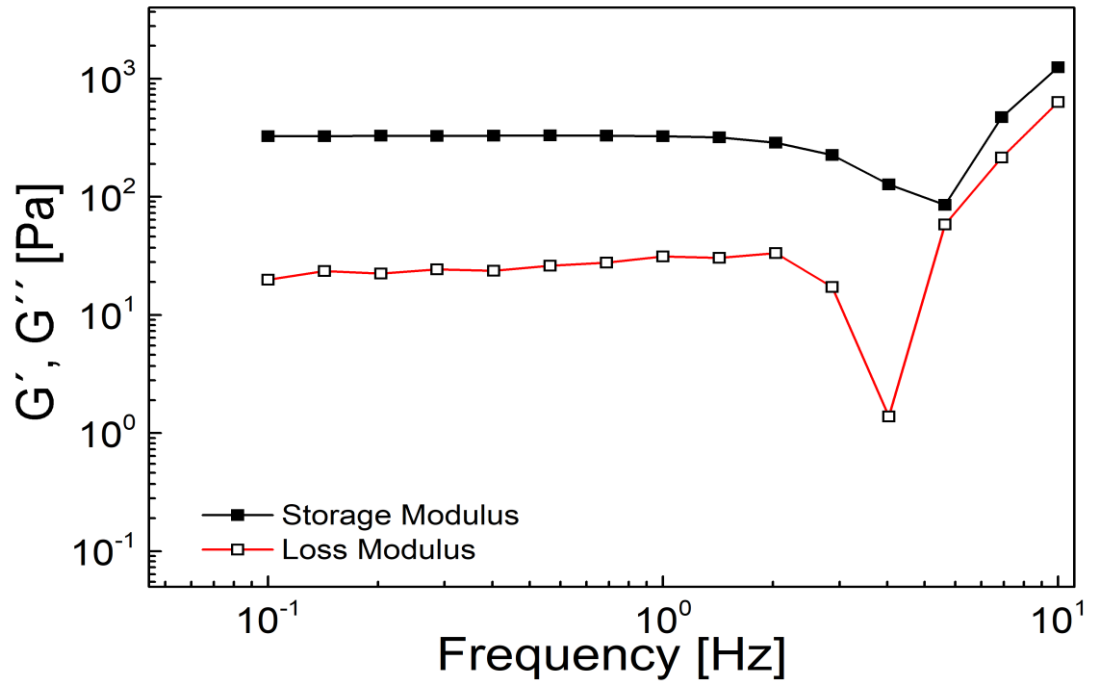

Figure 30: The dependence storage and loss modulus on frequency for the Sample 6 (amount of cross-linker 1.5%)

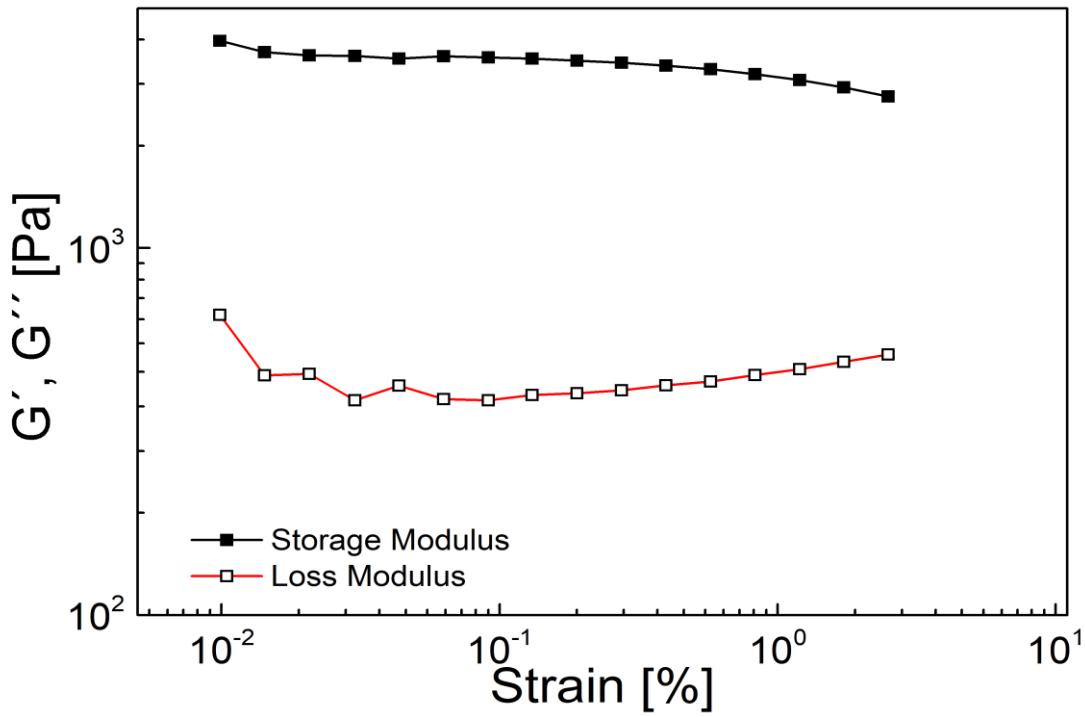

Figure 31: The dependence storage and loss modulus on strain for the Sample 6 GO (amount of cross-linker 1.5%, amount of GO 0.73%).

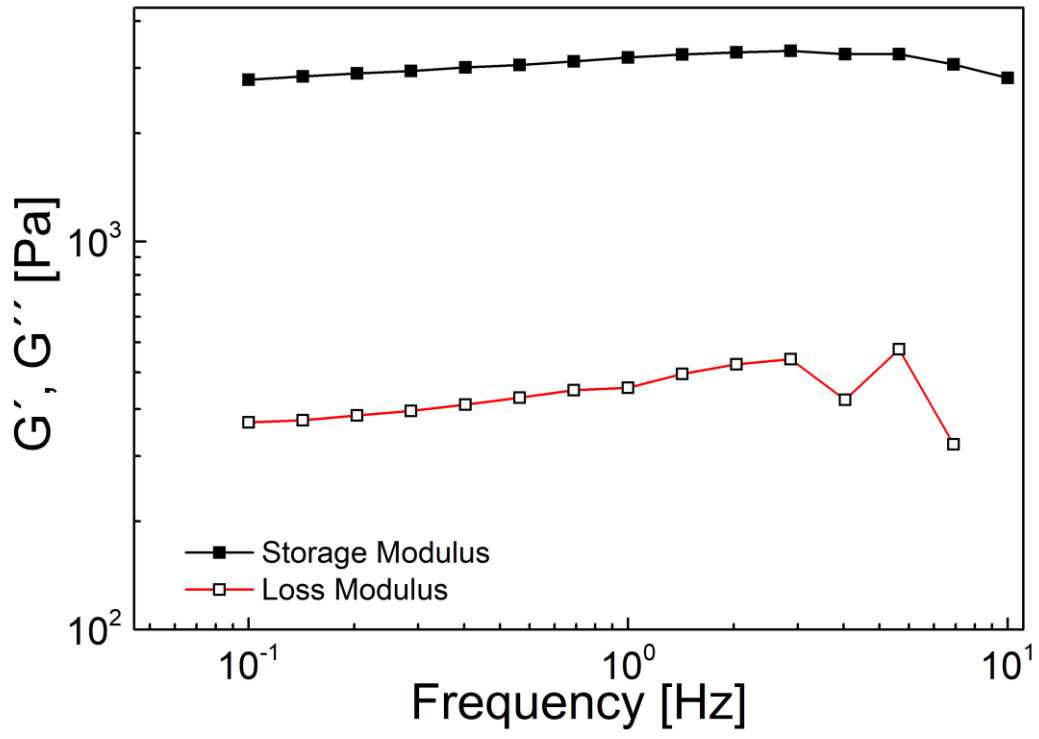

Figure 32: The dependence storage and loss modulus on frequency for the Sample 6 GO (amount of cross-linker 1.5%, amount of GO 0.73%).

5.4 Swelling capabilities

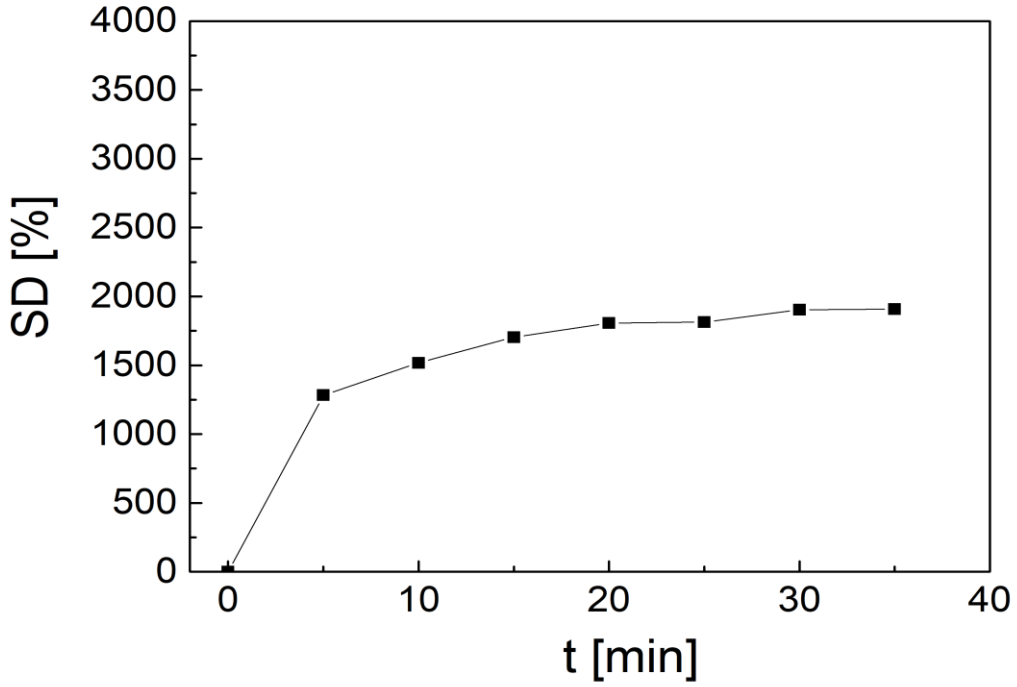

Figure 33: The dependence of SD on the time for the Sample 1.

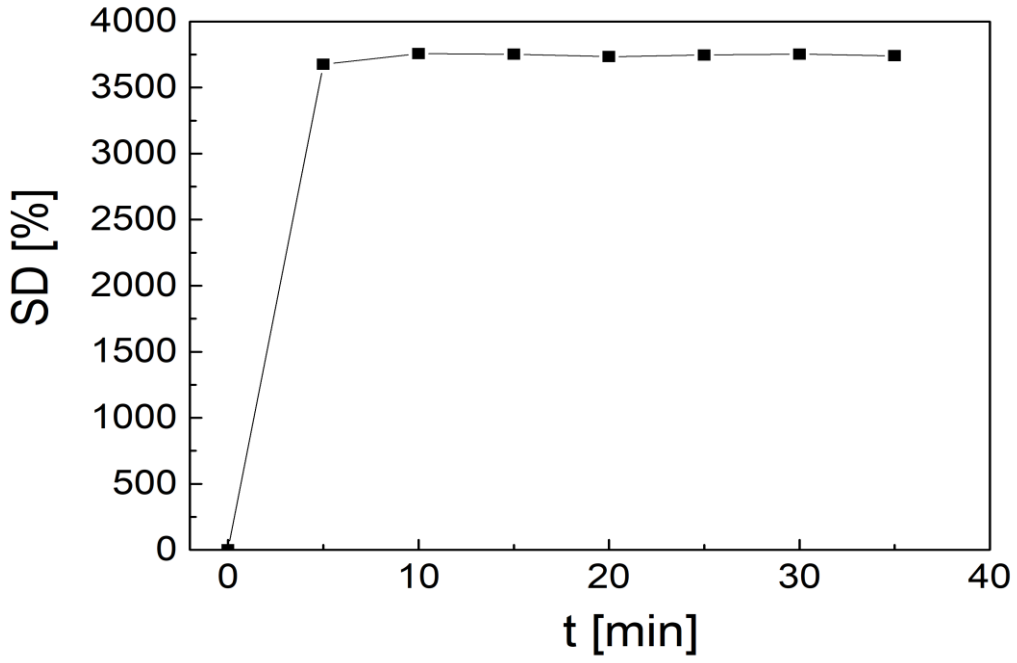

Figure 34: The dependence of SD on the time for the Sample 1 GO.

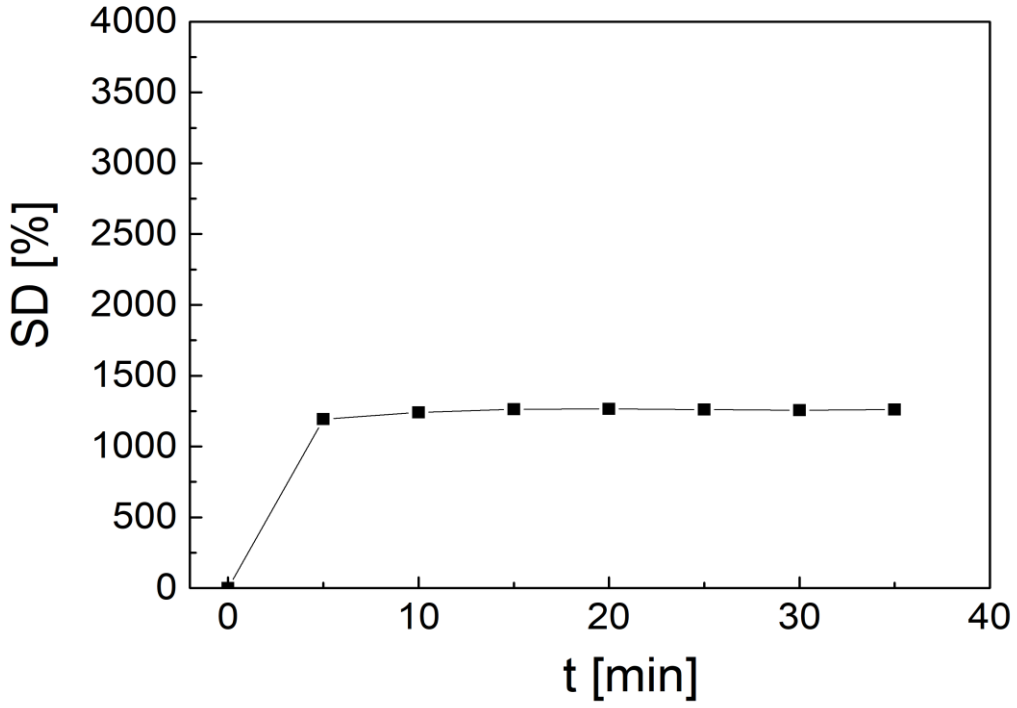

Figure 35: The dependence of SD on the time for the Sample 4.

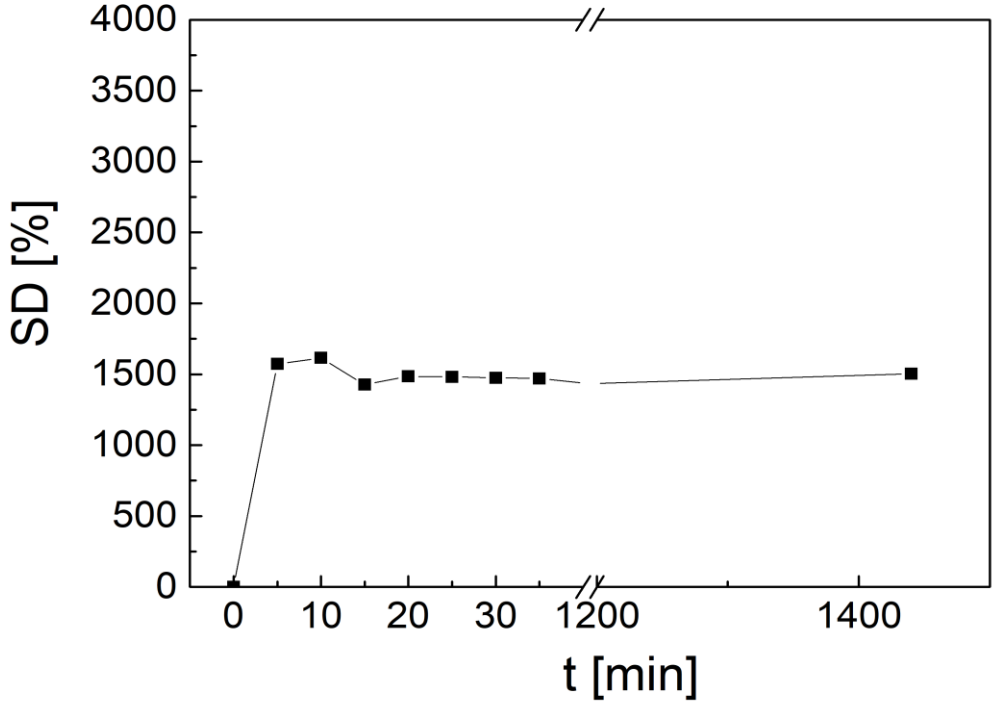

Figure 36: The dependence of SD on the time for the Sample 4 GO.

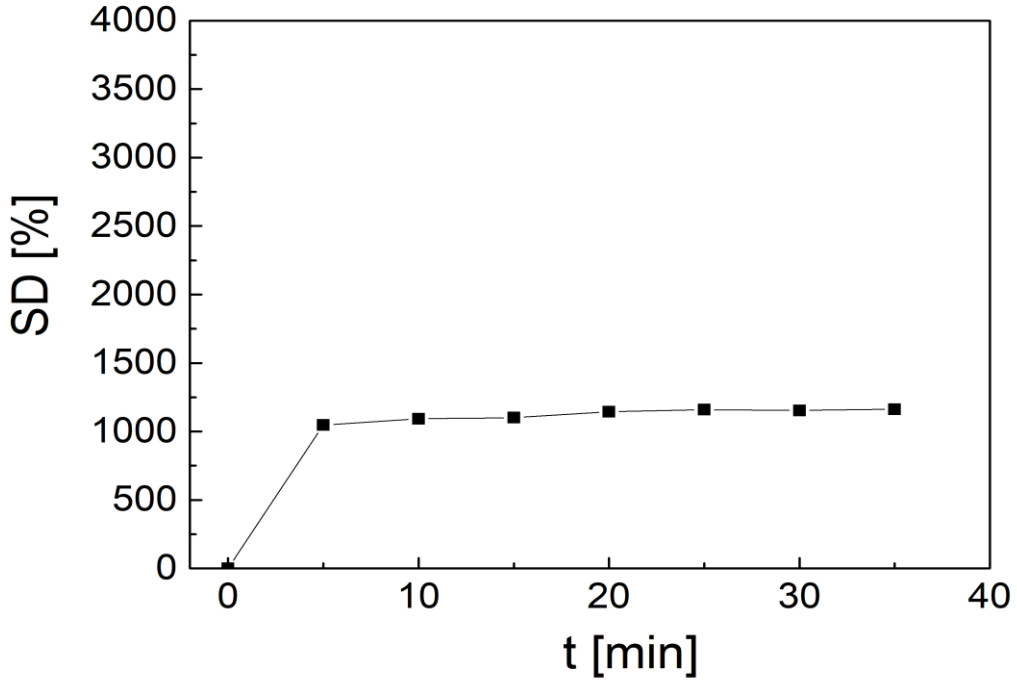

Figure 37: The dependence of SD on the time for the Sample 5.

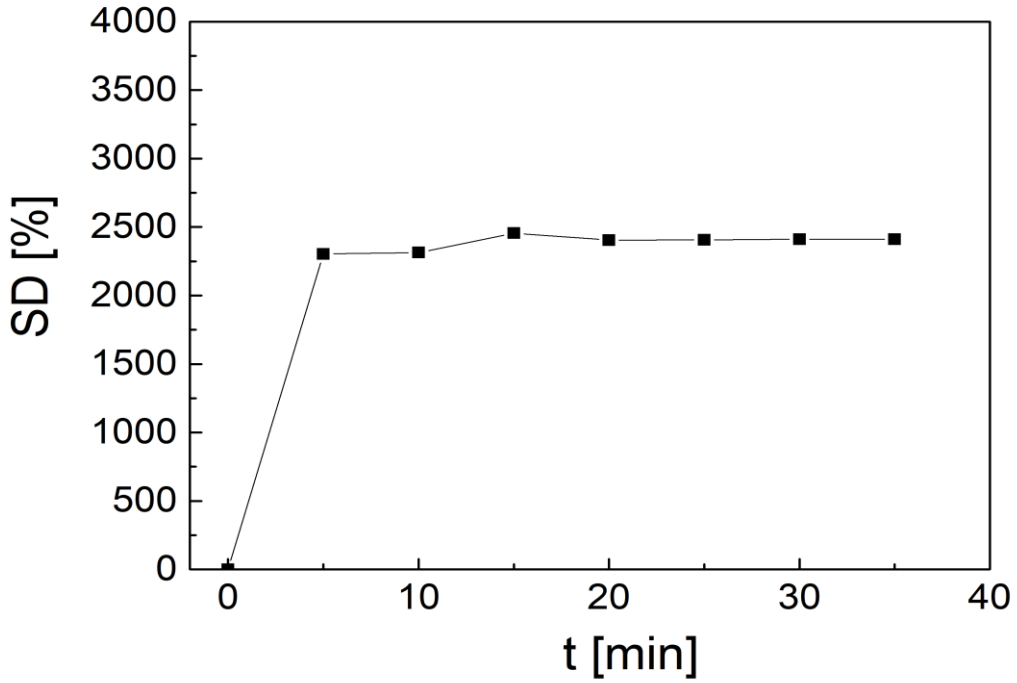

Figure 38: The dependence of SD on the time for the Sample 6.

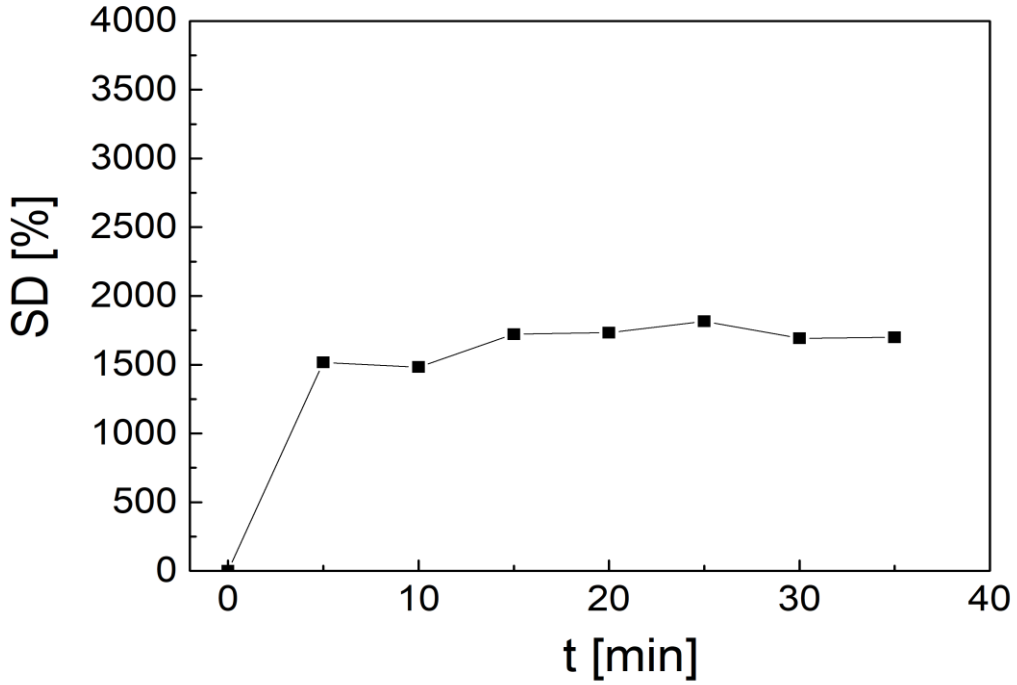

Figure 39: The dependence of SD on the time for the Sample 6 GO.

Table 7: The values of SD and EWC for various types of hydrogels.

Sample	Max.SD[%]	EWC[%]
1	1950	94,5
1GO	3740	97,4
4	1260	92,6
4GO	1470	93,6
5	1163	92,1
6	2411	96,0
6GO	1700	94,4

In the Figure 36 there is the range of the investigation up to 1440 minutes. It can be seen, that after 35 minutes the hydrogel absors the maximum amount of water, which is not changed even if the test is performed further. Therefore, the rest of the Figures (33-35 and 37-39) including results only in the period from 0 to 35 minutes. It was further found, that GO and 1% of cross-linker have tendence to improve swelling properties. This is probably caused due to the fact that, smaller amount of cross-linker provide system with lower cross-linking density which is very similar to effect of the GO in case of Sample 1 and thus shows enourmous capability to water absorbtion. Rest of the samples containing higher amount of the cross-linker and thus provide generally lower values of SD. Such results well-corelates with those obtained from viscoelastic investigations. From the rest of the results can be clearly seen that the higher amount of the Tulipaline provide system with enhanced swelling degree as well as GO due to the presence of the –OH hydroxyl group present in the structure. Futher, the cross-linker suppres the swelling degree since the higher cross-linking density does not provide free space for water absorption. All important values are summarized in Table 7.

5.5 Cytotoxicity evaluation

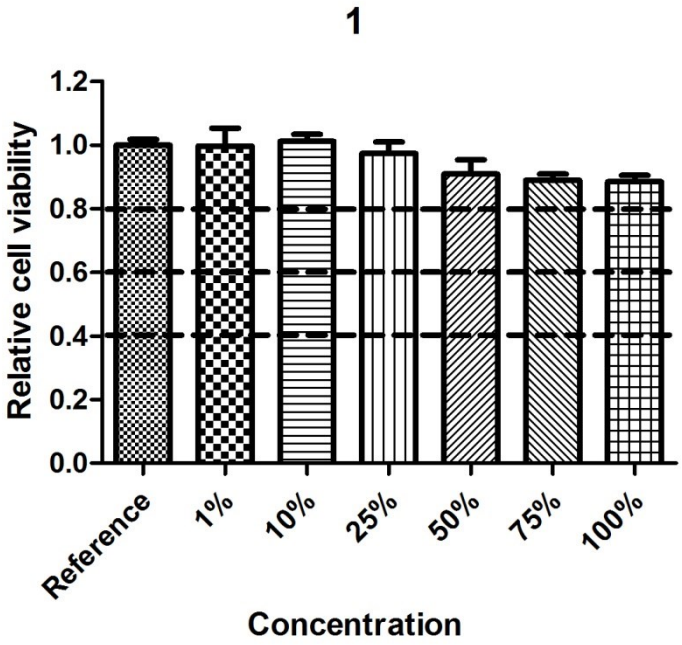

Figure 40: The dependence of relative cell viability on concentration for the Sample 1.

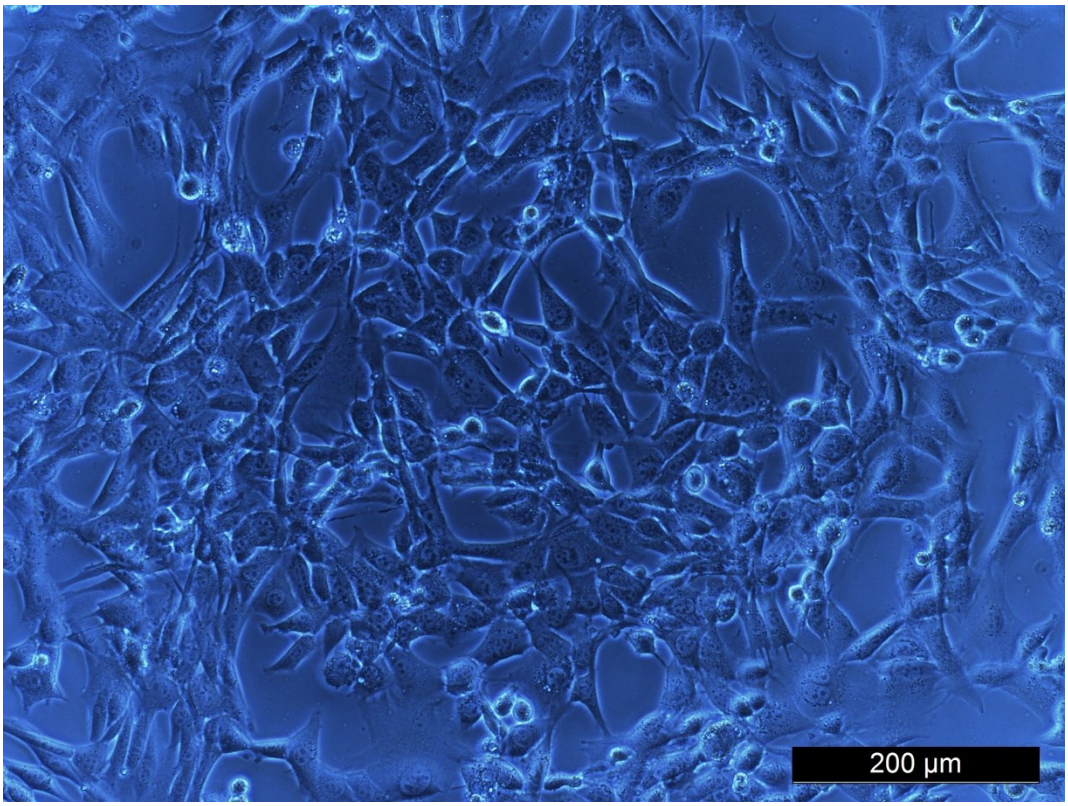

Figure 41: The cell viability with concentration 100% for the Sample 1.

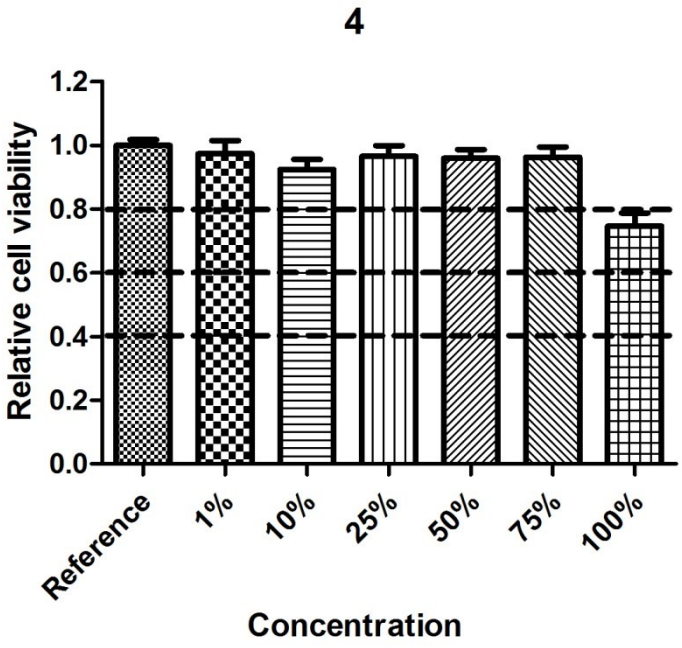

Figure 42: The dependence of relative cell viability on concentration for the Sample 4.

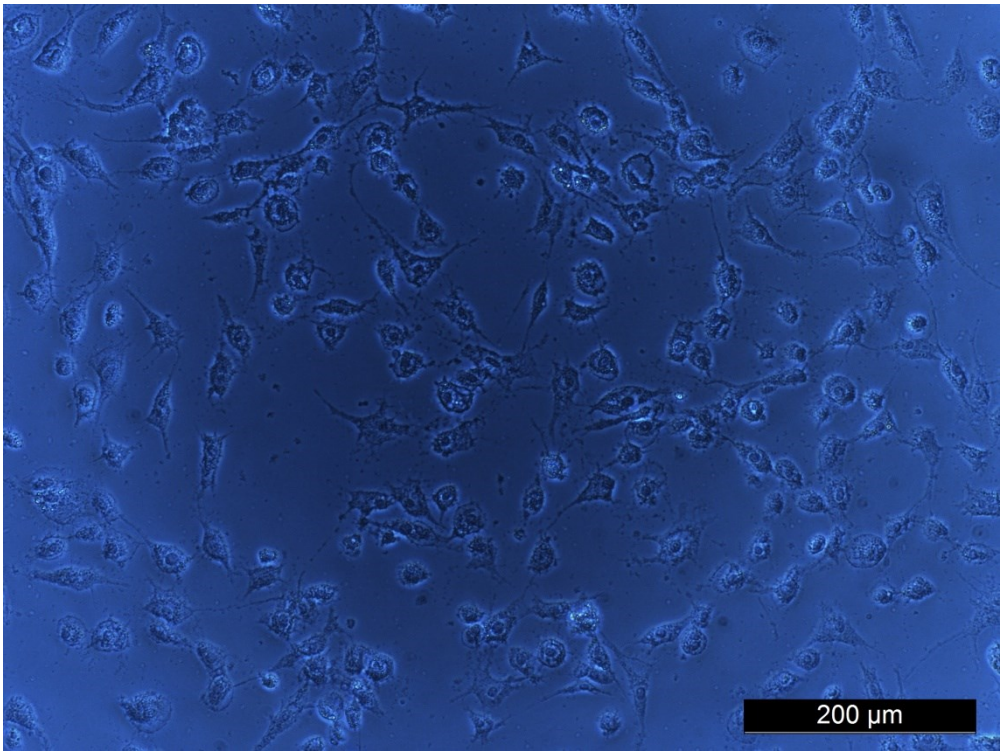

Figure 43: The cell viability with concentration 100% for the Sample 4.

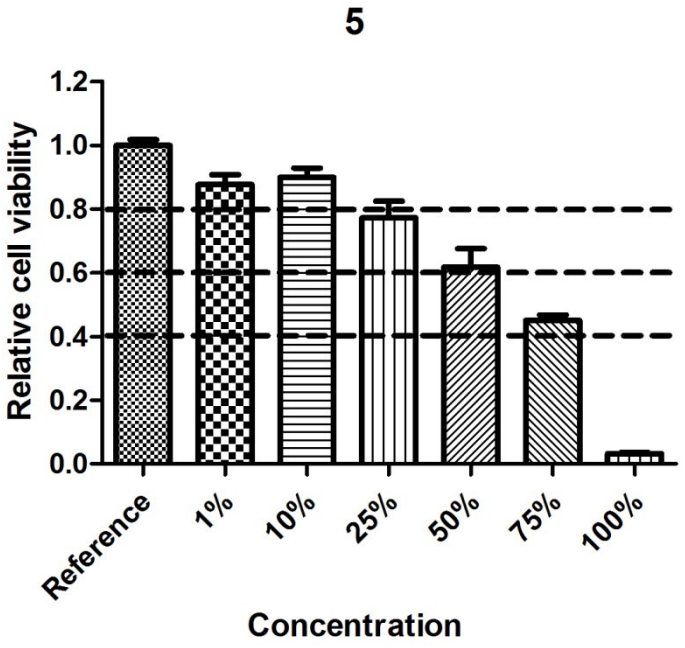

Figure 44: The dependence of relative cell viability on concentration for the Sample 5.

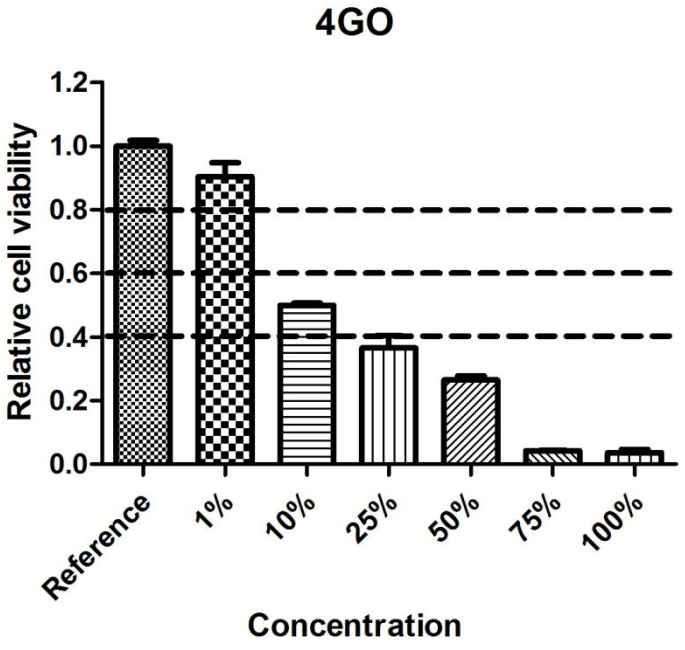

Figure 45: The dependence of relative cell viability on concentration for the Sample 4 GO.

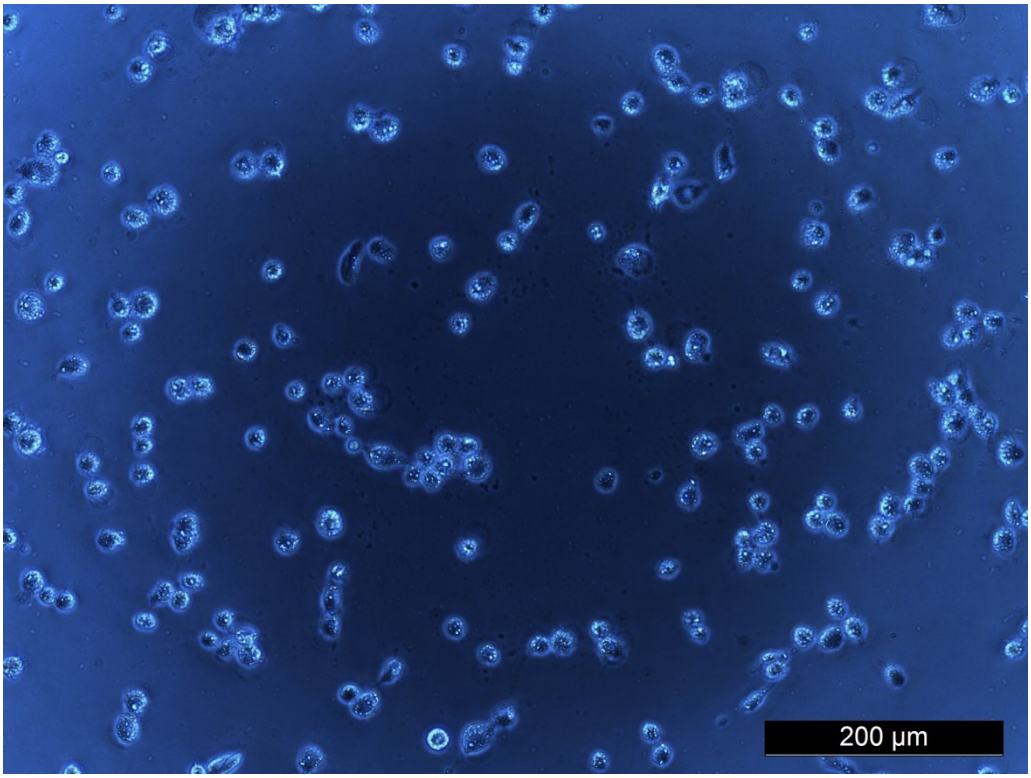

Figure 46: The cell viability with concentration 100% for the Sample 4 GO.

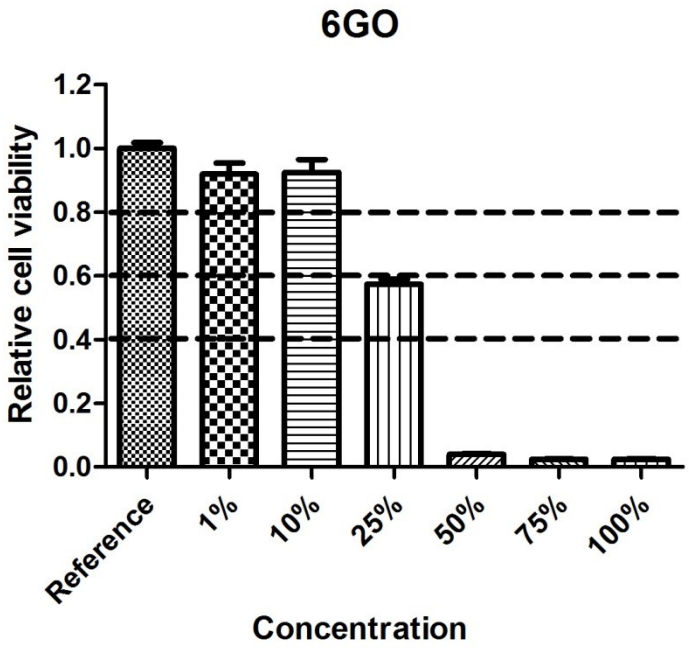

Figure 47: The dependence of relative cell viability on concentration for the Sample 6 GO.

According to the results of test graphene oxide has tendency to negatively influence hydrogels. For example according to the Figure 40, the Sample 1 in concentration 100% reduces viability appromixately about 20%. On the other hand, the sample 4 GO in concentration 100% reduces viability about 90% (Figure 43). Such samples are the same however, the amount of cross-linker is different and the presence of GO, which is finally responsible for such enormous decrease. Also in the case of the Sample 6 GO survived just 5-3% of cells (Figure 45). The less cytotoxic hydrogel is the Sample 1, the viability is about 90% (Figure 38). In the Figure 39 and 44 is difference between a form of cells. Dead cells do not have adhesion on material and have „ ball shape“. As can be seen, the samples with GO show much worse results. It could be caused by penetration cell membrane by graphene oxide unique 2D dimensional shape of particles or by lower degree of polymeration due to the presence of GO and thus free Tulipalin A monomer as an unreacted residue presenest in the sample. Here can be also stated that two samples namely Sample 1 GO and Sample 6 were not investigated due to the inapropriate size and weigth and thus the typical procedure for cytotoxicity could not be performed.

CONCLUSION

According to the performed investigations of morphology, viscoelastic properties, swelling capabilities and cytotoxicity evaluation, the GO presence is able to considerably affect the properties of hydrogels. In view of the results it is possible to suppose that GO is most probably responsible for hindering of the process of polymerization and thus the fabrication of hydrogels is not possible. The samples without graphene oxide have more solid and smooth structure than similar samples with GO. The fact was found out that GO and 1% of cross-linker could have tendency to improve swelling properties significantly, due to lower cross-linking density, influenced by GO and provide the hydrogel with bigger inner space and the Samples have better swelling capability. The amount of the Tulipaline A, cross-linker and GO could seriously change mechanical properties of the fabricated hydrogel. The amount of cross-linker mainly improving deformation capabilities, the higher amount of the Tulipaline A portion decreases the absolute values of Storage modulus, but improving the stability of the hydrogel upon deformation and finally the addition of the GO particles positively enhancing the absolute values of the Storage modulus. The results from cytotoxicity evaluation showed that the synthesized hydrogels with graphene oxide are not suitable in applicaton in the medical field because of high level of repression viability of cells. From the investigated hydrogels, just Sample 1 and Sample 4 fulfil the requirements of medical grade hydrogels, while whole investigated hydrogels containing GO particles failed. However, very promising Sample 1 GO was not investigated due to the inappropriate size and weigth of the sample, but this will be the step out future research interest.

Finally, can be concluded that this thesis clearly proved that it is possible to prepare hydrogels based on copolymers of Tulipaline A and N-isopropylacrylamide in various ratios. Also in some cases the GO addition is possible and significantly influence the mechanical, swelling and cytotoxicity properties similarly as presence of various amount of cross-linker. Therefore, the modulation of the overall properties of the fabricated hydrogels can be tailored with respect to the potential applications.

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LIST OF ABBREVIATIONS

GO	Graphene Oxide
NIPAM	N-Isopropylacrylamide
BIS	N′-methylenebis(acrylamide)
V-50	2,2′-azobis(2-methylpropionamidine) dihydrochloride
SHMB	Sodium 4- hydroxy-2-methylenebutanoate
MBL	α-methylene-γ-butyrolactone
SEM	Scanning electron microscope
SD	Degree of swelling
EWC	The equilibrium water content
G′	Storage modulus
G″	Loss modulus

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LIST OF EQUATIONS

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(2) $\mathrm{SD}\ [\%] = \frac{Wt\text{-}Wd}{Wt} \times 100$ 32