ABSTRACT

Nowadays, the effective delivery of drugs to the human body is still a big problem. It is still a matter of receiving a drug with a constant concentration that is released quickly, which could be more effective. Therefore, there was a focus on the development of a new method of drug delivery using the so-called gradual release, which would prevent the rapid release (e.g., burst effect) of a large concentration of the drug into the body. This problem is solved in this work using a composite material composed of a non-woven fabric, which functions here as a polymer carrier, which in this case, carries the dye for the release kinetics stage. The polypropylene non-woven fabric is investigated here in alginate, chitosan, and gelatine polymer solutions. The materials were studied to see if and how much the polymer itself was released from the layered material into the water environment. This was measured using the viscometry method. Subsequently, the release kinetics through materials with a different number of layers was determined using the diffusion cell method. The last part of the work focuses on the study of the release of a dye (methylene blue) from a layered material that contains a gradient of this dye.

ABSTRAKT

V dnešní době je stále velký problém efektivního dodávání léčiv do lidského těla. Stále se jedná o příjem léčiva s konstantní koncentrací, která se uvolní v krátkém čase, což není velice efektivní. Proto došlo k zaměření se na vývoj nového způsobu dodávání léčiv pomocí tzv. postupného uvolňování, které by zamezilo rychlému uvolnění velké koncentrace léčiva do těla. Tento problém je zde řešen pomocí kompozitního materiálu složeného z netkané textilie, která zde funguje jako nosič hydrogelu, který v tomto případě prozatím nese barvivo pro stadium kinetiky uvolňování. Netkaná textilie z polypropylenu je zde zkoumána ve spojení s hydrogely alginátu, chitosanu a želatiny. Materiály byly studovány na to, jestli, a jak moc docházelo k uvolňování samotného hydrogelu z vrstevnatého materiálu pomocí uvolňování do prostředí vody. Toto bylo měřeno pomocí metody viskozimetrie. Následně byla zjišťována kinetika uvolňování skrz materiály s různým počtem vrstev pomocí metody difúzních cel. Poslední část práce se zaměřuje na studium uvolňování barviva (methylenové modři) z vrstevnatého materiálu, který obsahuje právě gradient tohoto barviva.

KEYWORDS

Hydrogel, layered material, gradual release, chitosan, alginate, gelatine, non-woven fabric.

KLÍČOVÁ SLOVA

Hydrogel, vrstevnatý materiál, postupné uvolňování, chitosan, alginát, želatina, netkaná textilie.

ŠUDÁKOVÁ, Anna. *Příprava, charakterizace vrstvených hydrogelových elektrospinovaných materiálů a stanovení jejich transportních charakteristik.* Brno, 2023. Dostupné také z: https://www.vut.cz/studenti/zav-prace/detail/148075. Diplomová práce. Vysoké učení technické v Brně, Fakulta chemická, Ústav fyzikální a spotřební chemie. Vedoucí práce Jiří Smilek.

DECLARATION

I declare that I prepared the diploma thesis on the topic *Preparation, characterization layered hydrogel electrospun materials and determination of transport characteristics* independently and that I cited all the used literary sources correctly and completely. In terms of content, the diploma thesis is the property of the Faculty of Chemistry Brno University of Technology and may be used for commercial purposes only with the consent of the thesis supervisor and the dean of the Faculty of Chemistry Brno University of Technology.

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Anna Šudáková

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Ing. Jiří Smilek, Ph.D., for his guidance throughout my studies, for his advice and all the time he devoted to me, especially for his encouragement during difficult times. My thanks also go to Mgr. Jan Žídek, Ph.D. for his ideas and advice during the work on this diploma thesis.

I owe my family for the greatest support they could give me. I wouldn't be where I am now without them.

Finally, I would like to thank my colleagues from the laboratory and everyone who helped me in the last month when I had a broken arm.

CONTENT

1 INTRODUCTION

Even though nowadays there are many ways to deliver drugs to the body, the problem of effective dosing, where a constant concentration would be released over a long period of time, is still not solved. During the release, a so-called burst effect occurs, which is characterized by the rapid release of a large amount of drug from the prepared material. This leads to the fact that the patient must take this drug more often and the organism can be unnecessarily burdened. This can cause mild to severe complications. For this reason, it is important to pay attention to this issue. The creation of such a material could reduce or at least minimize the need for repeated application of drugs.

Currently, research is mainly focused on the preparation of so-called smart hydrogels, which react to a certain stimulus (temperature, pH, presence of an enzyme, etc.) and, thanks to this, the incorporated drug is released. Unfortunately, it does not solve the problem of the burst effect, which can occur in these materials. Some researchers are already focusing on this issue and exploring various possibilities to prevent it. This can be, for example, influencing the method of preparation or the composition of the carrier matrix, the pH of the given environment, the number of layers in the composite material, etc. even if it was possible to extend the release time of the drug from the carrier matrix, the already mentioned burst effect still appeared. Therefore, no universal solution that would be generally valid has yet been found.

The aim of this diploma thesis is firstly the preparation of layered materials composed of non-woven fabric (polypropylene) and polymer solution (alginate, chitosan, and gelatine) of different concentrations. From these materials, it will be important to find out which polymer solutions are suitable for the preparation of these layered materials. Subsequently, materials differing in the number of layers will be prepared with the found most suitable binder, and the effect of the number of layers on the effective diffusion coefficient will be studied. Finally, layered materials will be prepared that will contain a dye gradient. Again, materials with different number of layers will be prepared. From these measurements, the effective diffusion coefficients will then be determined again, and from the graphs obtained, whether the so-called burst effect occurs or not.

2 THEORETICAL PART

The theoretical part focuses on the controlled release from layered materials and the kinetics of this release. At the same time, it deals with hydrogel's mechanical and diffusion properties and the possible use of materials for controlled and targeted release.

2.1 Hydrogels

Hydrogels are dispersion systems formed by a dispersion medium (water) and dissolved particles [1, 2]. They are polymeric materials with hydrophilic polymer chains that can bind large amounts (from 70 to 99 %) of water but do not dissolve. Due to the water content in the dispersion system, many natural materials are biocompatible because they are tissue-like due to their water content (70 %). Hydrogels can contain up to 99 % water in their structure. Since hydrogels are made up mainly of water, they show poor mechanical properties, especially for biochemical applications [3]. In addition, their physicochemical properties (e.g. viscoelastic properties, transport characteristics) are similar to native extracellular matrices [4]. From the point of view of dispersion systems, these are lyosols. The size of dissolved dispersion particles ranges from 1 to 1 000 nm. The ability to absorb water (to swell) into its structure is due to the presence of hydrophilic functional groups, which include an amino group ($-NH_2$), a carboxyl group (-COOH), or a sulfone group ($-SO_3H$) [5].

Depending on their nature, these polymer networks can be characterised, for example, by the origin of the material, method of preparation/crosslinking, composition, and electric charge [6]. Depending on their source, they can be divided into natural (e.g. collagen, hyaluronic acid, chitosan, alginate) and synthetic (e.g. poly (vinyl alcohol) (PVA), polyacrylamide (PAM)) [7].

Depending on the polymer composition, they are further divided into homopolymer, copolymer and multipolymer. Homopolymeric structures are composed of one type of monomer (e.g. chitosan, gelatine, polylactic acid, etc.). Copolymer structures are composed of at least two types of monomer units (e.g. poly (lactic acid)-poly (ethylene glycol), poly (acrylamide-co-acrylic acid) [8]), which can be differently oriented in the hydrogel structure (random, block or alternating configuration) [6].

Multipolymer hydrogels are further divided into two types. The first is an interpenetrating polymer hydrogel (IPN) formed by two independently cross-linked polymer structures forming a cross-linked structure (e.g. sodium alginate [9], collagen/chitosan, alginate/fibrin [10]). The second type is the so-called semi-interpenetrating hydrogel (semi-IPN), where one component is cross-linked, and the other is not (e.g. alginate/poly (2-hydroxyethyl methacrylate), chitosan/poly (N-isopropyl acrylamide), collagen/hyaluronic acid [10]). The way IPN hydrogels differ from semi-IPN hydrogels is based on the ability to separate them without breaking the chemical bond. This occurs in the case of semi-IPN hydrogels [10].

Division, according to configuration, divides hydrogels into amorphous, semi-crystalline or crystalline. According to the electric charge, we distinguish between neutral, ionic, amphophilic and zwitterionic hydrogels—another way hydrogels can be characterised by the type of cross-linking. In the case of physically cross-linked hydrogels (e.g. gelatine, carrageenan [11]), physical bonds are mainly created between the individual polymer chains, such as ionic interactions, hydrogen bonds or hydrophobic interactions. The transformation from a liquid to a gel structure occurs due to changes in external conditions (such as pH,

temperature, and ionic strength) [3, 5]. In the case of chemical cross-linking of hydrogels (e.g. poly (vinyl alcohol) (PVA) [12]), these are solid and permanent bonds that arise after the addition of a cross-linking agent [6]. Chemically cross-linked hydrogels use covalent bonds to form the gel [1].

These covalent bonds are much more difficult to break; therefore, changing the properties of hydrogels of chemical origin is more complex than in the case of a physical hydrogel. Chemically cross-linked hydrogels can be degraded by hydrolysis or an enzymatic reaction. In the case of ionically cross-linked alginate hydrogels, uncontrolled degradation of the material may occur depending on the ions that diffuse into the prepared hydrogel. If Na^+ ions start to penetrate this material, swelling or even loosening of the structure can occur more easily. This occurs due to the competition of Na^+ and Ca^{2+} ions. These ions cause the cross-linking to decrease over time [13].

It is possible to use them, for example, as contact lenses, catheter coating, for administering drugs, and bandages. Hydrogels can have a variety of applications. Their most significant potential is tissue engineering, as a synthetic extracellular matrix, implants, biosensors, and drug carriers [3, 14].

2.1.1 Mechanical properties of hydrogels

The mechanical properties of hydrogels are influenced by many factors; among these factors are the molecular weight of the polymer, concentration, crosslinking density, entanglement of polymer chains, crosslinking agent, and osmotic pressure [15].

The mechanical properties of hydrogels are defined by the theory of time-independent rubber elasticity and time-dependent viscoelasticity to analyse the hydrogel structure and effective crosslinking density. Hydrogels can exhibit rubber-like properties in the swollen state, where the mechanical properties rely on the time-independent renewal of polymer chains. Hydrogels in the rubbery state respond to stress immediately and return to their original state after the stress is removed. It also depends on the surrounding conditions, such as a lower temperature or long-term mechanical stress. Under such conditions, hydrogels may exhibit viscoelastic behaviour due to their intrinsic mechanical properties or the nature of forced mechanical motion. Hydrogels exhibit the highest deformation upon mechanical loading if the time scale of mechanical motion falls within the time scale of molecular motion. Elastic properties of hydrogels are achieved by controlling properties such as Young's modulus (E), tensile strength (σ), strain at failure (ε_f), and compressive strength (F) [16, 17].

Rheology is a scientific field dealing with the flow and deformation of matter under the influence of external mechanical forces. Depending on these external forces, substances can be divided into elastic, viscous and viscoelastic. Viscous substances behave in such a way that they flow under the influence of external mechanical forces. This flow will stop if the external force is removed, but the stressed material does not return to its original state. Viscoelastic substances are deformed depending on the time of application of the external force (Figure 1) [17]. An elastic material is a material that begins to deform due to the application of an external force and immediately returns to its original state after the application of this force has stopped (Figure 1). At the same time, the material will not be destroyed. The material will begin to deform if sufficient stress is applied [18].

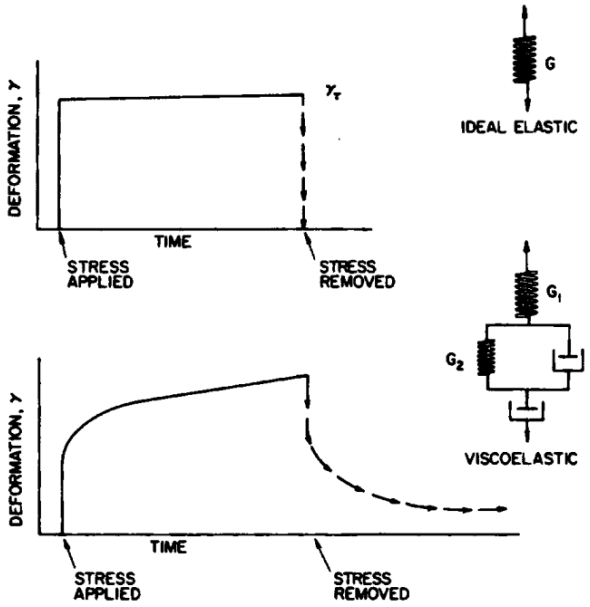

Figure 1. Graphical representation of elastic and viscoelastic behaviour [18].

Viscosity

The quantity indicating the ratio between the tangential stress and the change in velocity depending on the distance between adjacent layers during liquid flow is called viscosity. It is a quantity characterising internal friction. It depends mainly on the attractive forces between particles but also on temperature and pressure [19]. Several expressions of viscosity are used to describe colloidal systems, which differ from those used to describe pure liquids [17].

Depending on whether substances behave according to Newton's law, they are further divided into Newtonian and non-Newtonian (Figure 2).

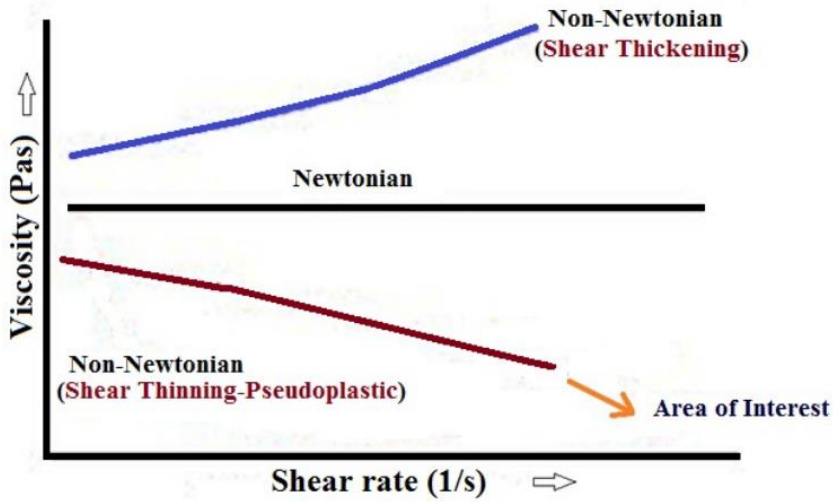

Figure 2. Typical rheogram of Newtonian and non-Newtonian fluids [20].

Newtonian fluids are those that a direct relationship between strain rate and stress can characterise (1.1). True solutions, low-molecular substances, etc., are called Newtonian liquids. If the viscosity of substances changes with the applied force, they are non-Newtonian

substances. Examples of these substances are colloidal dispersions, emulsions or even suspensions. In this case, the viscosity of substances decreases or increases under the applied force. Thixotropy is a physical phenomenon where the viscosity decreases due to the action of an external force. By removing the external stimulus, the viscosity will increase. The opposite phenomenon is rheopexy when the viscosity increases due to the action of an external force and decreases again due to relaxation [17, 21].

$$\sigma = \eta \frac{\mathrm{d}\gamma}{\mathrm{d}t} \tag{1.1}$$

Where η refers to the dynamic viscosity, γ is the shear strain, t is time [21].

The relative viscosity (1.2) indicates the ratio of the viscosity of the dispersion system (η) to the viscosity of the pure dispersion medium (η_0).

$$\eta_{rel} = \frac{\eta}{\eta_0} \tag{1.2}$$

The relative increment of viscosity (1.3) expresses the increase in the viscosity of the dispersion medium (η_0) due to the presence of the dispersion component (η).

$$\eta_i = \frac{\eta - \eta_0}{\eta_0} = \eta_{rel} - 1 \tag{1.3}$$

Swelling

Hydrogels behave viscoelastically when swollen. Therefore, they immediately react to external mechanical stress and return to their original state after removing this external force [16].

Hydrogels can absorb water from the surrounding environment into their structure. During this process, the volume of the gel increases and at the same time, its weight increases. Absorption of liquid into the xerogel environment results in lyogel. By removing the lyogel from the liquid environment, the fluid can evaporate from this structure. Swelling can be either limited or unlimited. Limited swelling is when water absorption by the xerogel stops when it reaches the elastic lyogel phase. In this case, no more liquid will be absorbed, even if it is in excess. This case mainly occurs with covalently cross-linked hydrogels when these bonds cannot be broken by the action of osmotic pressure. If it is a physically cross-linked hydrogel, there is unlimited swelling. Thus, the xerogel absorbs the liquid and does not stop even in the lyocell phase. If there is enough liquid, the bonds between individual polymer fibres can be disrupted, and these fibres begin to pass into the solvent [22].

The following definitions were introduced for the quantitative description of swelling [22]:

The degree of swelling (Q) is defined as the mass of liquid absorbed by a unit of dry xerogel (1.4).

$$Q = \frac{m_\tau - m_0}{m_0} = \frac{\rho \cdot \Delta V}{m_0} \tag{1.4}$$

Here, m_τ is the mass of the swelling gel at a particular time τ, and m_0 is the initial mass of the xerogel. The degree of swelling can therefore be determined as an increase in weight either by weighing or measuring the volume of absorbed liquid ΔV, ρ then denotes the density of the liquid. The kinetics of swelling up to a possible equilibrium state can be monitored precisely by measuring the volume [22].

The ratio of the swollen gel to the volume of the gel in the dry state gives the volume coefficient of swelling (ϕ) [22].

The degree of affinity of the hydrogel for swelling in a given solvent is then referred to as the swelling pressure. Thus, it is possible to stop the swelling of a gel if that gel is subjected to a specific amount of pressure. This prevents the solvent from entering this system [22].

Elasticity

Elasticity expresses the ability of a material to deform (react) to mechanical load immediately. Elastic properties can be achieved by controlling the properties of hydrogels. These properties include Young's modulus of elasticity (E), tensile strength (σ), strain at failure (ε_f) and compressive strength (F). Young's modulus, or tensile modulus (E), is the resistance of an elastic material to tensile stress, so it can be used to determine the internal elasticity of viscoelastic biomaterials [16].

Elasticity can be calculated using Hooke's law (1.5), which describes the relationship between stress and strain [23]:

$$\varepsilon = \frac{\sigma}{E} \tag{1.5}$$

In this equation, ε is the proportional elongation [-], σ is the tensile stress [Pa], and E is Young's modulus of elasticity in tension [Pa]. Hooke's law applies to hydrogels and biological samples when it satisfies two conditions. It must be noted that these are elastic or reversible deformations and that the relationship between elongation and stress is linear [23]. Tensile strength [16] is defined as the stress at the breaking point during the elongation of the hydrogel. Compressive strength [16] corresponds to the ability to withstand the load induced by the reduction in size. Stiffness [16] is defined as resistance to deformation caused by mechanical loading.

Porosity

Among the essential properties of hydrogels is also porosity (1.6). The latter contributes to the mechanical properties through the free volume content, size, mutual connectivity, and surface properties of the pores. Higher porosity and pore size can facilitate the delivery of nutrients and oxygen, but mechanical properties are adversely affected due to a large amount of void volume [16].

$$\phi = \frac{V_V}{V_T} \tag{1.6}$$

In this equation, the ϕ stands for porosity, V_V is the volume of void space (e.g. fluids), and V_T is the material's total (bulk) volume.

2.1.2 Transport characteristics of hydrogels

Diffusion is a process in which particles disperse spontaneously in space due to the concentration gradient. The transport of small molecules through the polymer membrane (hydrogel) occurs as a result of Brownian motion at the molecular level [24]. The force that drives this process is the concentration difference on both sides of the membrane. The process of diffusion through the polymer matrix includes sorption, diffusion, and permeation. Diffusion can be described using Fick's laws [25].

The rate of diffusion varies in each type of system. In gases, the diffusion is the fastest ($1.67 \cdot 10^{-3}$ m·s^{-1}); in liquids, it is slower ($8.33 \cdot 10^{-6}$ m·s^{-1}), and in solids, diffusion takes place the slowest ($1.67 \cdot 10^{-8}$ m·s^{-1}). This is due to the close arrangement of the molecules in the system, which prevents the passage of the molecules, and this arrangement causes resistance to the passing molecules. Diffusion in hydrogels then ranges between the rates in liquids and solids. At the same time, diffusion strongly depends on the concentration and degree of swelling [26].

Fick's first law (1.7) determines the density and direction of the diffusion flux (J). This direction then expresses the direction of the particle flow [25].

$$J = -D\left(\frac{\partial c}{\partial x}\right) \tag{1.7}$$

D in this equation corresponds to the diffusion coefficient, and ($\partial c/\partial x$) is the concentration gradient. This equation describes steady-state diffusion, i.e., the concentration does not change over time [25]. Except for the concentration gradient, no other external forces should affect diffusion [27].

Fick's second law (1.8) describes non-stationary diffusion, where the concentration gradient changes with time ($\partial c/\partial t$) and the diffusion flux changes with the position [25].

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2}\right) \tag{1.8}$$

If there is a change in the compositional profile at a specific time, a continuous diffusion process needs to be explained. This is because there is a change in concentration at a particular position in time. For this purpose, the second Fick's law was derived based on the first Fick's law and the law of conservation of matter [27].

In the case of porous materials, it is also essential to consider the shape of the pores. Since the path from one part of the material to another part of the material is not ideal and direct in everyday practice, it is necessary to introduce a new value. This so-called tortuosity (τ) (1.9) indicates the internal property of the material [28]. It is most often defined as the ratio of the actual flow (λ) path to the straight-line distance (λ_0) between the ends of the flow path [29].

$$\tau = \frac{\lambda}{\lambda_0} \tag{1.9}$$

2.1.3 Application of hydrogels

Currently, the use of hydrogels focuses mainly on the targeted distribution of drugs [5], thanks to their properties, which are similar to the extracellular matrix (ECM). The extracellular matrix is composed of soluble bioactive factors, products of homo- and hetero-typical cell-cell interactions, which serve to replicate tissue functions in vitro. This needs to be considered when creating an artificial extracellular matrix. The natural extracellular matrix consists mainly of glycosaminoglycans and fibrous proteins (collagen, elastin, laminin, and fibronectin). These structures organise themselves into nanofibrillar supramolecular networks. An adequately designed artificial matrix should support and maintain cell growth, providing appropriate mechanical, chemical and biological characteristics that match the native ECM. At the same time, it should enable the efficient transfer of nutrients and gas exchange (O_2 and CO_2). It should also remove metabolic waste and transmit signals. Most often, artificial extracellular matrices are composed of natural and synthetic hydrogels. Synthetic hydrogels (polyethylene glycol) in it ensure good mechanical properties, and natural hydrogels (collagen, agarose) are in this mixture to ensure biocompatibility [30].

Thanks to their porous structure, they are highly permeable to various types of drugs. It is possible to apply hydrophilic and hydrophobic drugs to the hydrogels, which increases their versatility. Another advantage is the modification of the surface of the hydrogel, so it is possible to deliver their content to a specific place in the human body (e.g. targeting the tumour by its acidic pH, which differs from internal surroundings where the tumour is located). At the same time, the hydrogel acts as protection against conditions in the human body, e.g., pH. Since the 1960s, hydrogels have been used to make contact lenses. The field of tissue engineering uses hydrogels as so-called scaffolds, which serve as a supporting matrix for cell growth [5].

Hydrogels can, therefore, also serve as carriers for the targeted transport of drugs throughout the body. Developing several types of hydrogels that respond to external stimuli is possible, and they are called smart hydrogels [2, 5]. One group consists of pH-sensitive hydrogels [2, 5], which contain a carboxyl group or an ammonium salt in their structure, and thus protonation or deprotonation occurs. Depending on the pH of the environment where this hydrogel is located, the shape changes and the active substance incorporated in the given hydrogel is released. This case most often occurs for the targeted treatment of cancer, as the surface of the tumour is strongly acidic [2]. Another case is temperature-sensitive hydrogels [2, 5], or thermogels, which change their structure depending on the temperature, the so-called sol-gel process. Other examples of intelligent hydrogels are hydrogels that respond to electrical impulses, light and glucose [2, 5].

2.2 Gelatine

Gelatine (Figure 3) is a protein biopolymer formed by partial hydrolysis of collagen [31]. It is mainly in pig skin, tendons, ligaments, and beef and pork bones [32]. Each gelatine may vary in molecular weight and properties, such as enzymatic denaturation and isoelectric point. This depends primarily on the source of collagen and production method; thermal or enzymatic denaturation can be used. The gelatine extraction from collagen can occur in two ways: alkaline, where gelatine type B or acidic is produced, where type A gelatine is formed. During this extraction, the triple helix structure of collagen is irreversibly disrupted, while the molecular

composition changes slightly. However, the typically recurring amino acid sequence -Gly-X-Y- and a high hydroxyproline and hydroxylysine content remain retained [31].

Depending on the method of gelatine preparation, various amino acids are degraded. For example, this is due to a reduction in isoelectric point in gelatine type B when hydrolysis of asparagine and glutamine groups occurs [31].

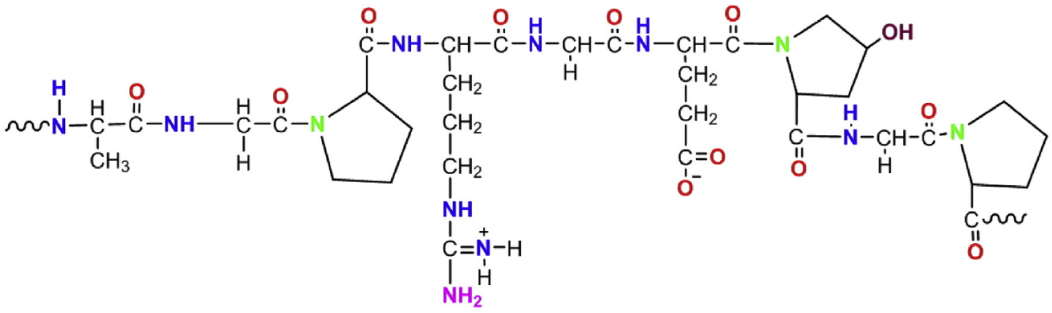

Figure 3. Representation of the chemical structure of gelatine [32].

Gelatine is a polypeptide obtained from collagen in three ways: acid, alkaline or enzymatic hydrolysis. If gelatine is obtained by acid hydrolysis, it is the so-called type A. On the contrary, gelatine obtained by alkaline hydrolysis is type B gelatine. So-called recombinant gelatine is often prepared, eliminating individual types of disadvantages. At the same time, gelatine can be produced with the desired properties advantageous for specific use [33]. In general, gelatine is very hydrophilic and very soluble. To increase mechanical properties and reduce solubility, i.e., while also reducing water degradation, it is good to crosslink gelatine before use. Physical mechanisms like microwave energy and ultraviolet radiation can cross-link gelatine. It can be activated, for example, by environmental triggers (pH, temperature, or ionic strength of the environment). Hydrogen bonds mainly form physical cross-linking, van der Waals forces, and hydrophobic interactions. Biological cross-linking or chemical cross-linking agents (formaldehyde, glutaraldehyde, glyceraldehyde, genipin, etc.) can also be used [33].

Gelatine can form both physically and chemically mesh hydrogels [34]. Depending on the preparation method, structures with a permanent network can also be formed. The physical and mechanical properties of the prepared gels then depend on the preparation process [34]. For example, lowering the temperature, high-energy radiation, plasma treatment, or dehydrothermal methods produce physically cross-linked gelatine hydrogels. The hydrogels prepared this way are thermally unstable, around 37 °C. Chemical cross-linking or modifications of side amino acid groups can prevent this. The chemical cross-linking agents provoke the formation of covalent bonds between the polymeric chains. This way, the mechanical and physical properties can be precisely defined [34]. Chemical cross-linking can occur based on a condensation reaction or using aldehydes. The most commonly used chemical cross-linkers are glutaraldehyde, formaldehyde, poly epoxy compounds and carbodiimides [35, 36].

The gelatine structure can be divided, for example, based on interactions [32]:

i. Primary, where is a one α-chain.
ii. Secondary, made up of two simple α-chains or one α-chain with a loop.
iii. Tertiary, consisting of either three simple α-chains or two simple α-chains and one loop, or one α-chain and two loops.

The incidence of the primary, secondary, and tertiary structures depends mainly on the temperature and concentration of gelatine. The secondary and tertiary structure of gelatine is stable reasons for hydrogen bonds between glycine residues and the oxygen atom of carboxylic groups contained in α-chains [32].

The gelatine can further divide into several types according to the condition in which the gelatine occurs [32]:

i. amorphous,
ii. semi-crystalline,
iii. crystalline.

Thanks to its properties, it has secured a place in many sectors, including the food and pharmaceutical industry and tissue engineering, focused on tissue such as cartilage and skin or wound bandages. It is a readily soluble biopolymer in an aqueous environment that is easily accessible and cheaper than collagen. In addition, gelatine is non-toxic, non -carcinogenic, biocompatible, and biodegradable. Its disadvantage is worse mechanical properties, such as thermal instability and a short degradation time. These disadvantages can be prevented by integrating the material [31]. The structural gel network is composed of microcrystals connected by amorphous regions twisted segments. Gelatine is characterised by its thermal reversibility [37].

2.2.1 Gelatine as a drug carrier

The use of gelatine as a carrier system for drugs has proven to be very advantageous, mainly due to its use in the form of micro and nanoparticle, fibre and even hydrogel.

Gelatine has been used as a drug delivery system in various forms, including microspheres, hydrogels, and nanoparticles. Gelatine hydrogels are excellent drug carriers because they can easily change their size, biocompatibility, absorption, and subsequent release of positively charged drugs [38]. This can be in response to an external stimulus. It can encapsulate and protect drugs, control their release, and target specific sites in the body. However, the application and suitability of gelatine as a drug delivery system depend on the properties of the drug and the intended therapeutic effect. When selecting a drug delivery system, it is crucial to consider several factors, such as biocompatibility, stability, and efficiency [33]. Depending on the selected source of collagen, its subsequent treatment, extraction, denaturation, and the degree of gelatine cross-linking, it is possible to influence the mechanical, thermal, and physicochemical properties and swelling. This allows the selection of the most suitable material for a given application, thereby obtaining the desired drug release profiles. This profile can be influenced by changing gelatine sources, molecular weight, or cross-linking degree [33].

During the preparation of the drug carrier, it is essential to monitor not only the properties of the carrier but also the drug itself. For the most effective therapeutic effect, it is necessary to keep its concentration in the blood at the minimum adequate level and, at the same time, below the level of toxicity. Medicines have their typical biological half-lives, which tell how long they can be maintained in the body in an effective concentration. The possibility of achieving prolonged or controlled release in the body is by incorporating the drug into the polymer matrix. Drug carriers with controlled release prepared this way has several advantages over classical methods. One such advantage may be better efficacy, maintaining the desired drug

concentration for a more extended period, lower toxicity and especially it offers more convenience to the patient [33].

Drug molecules tend to be trapped in pores in the hydrogel. As a result of the absorption of water from the environment, the structure swells, thereby increasing the pores between the individual fibres. This will cause the drug to diffuse through the pores into the external environment [33]. Due to the high number of functional groups, gelatine can be used for bioconjugation with agents that target a specific cell [38].

The application of layered materials for the controlled release of drugs is currently more and more investigated because it offers several advantages, such as versatility, incorporation of different molecules into the system, and control over the structure. Layered materials are mainly formed by electrostatic interactions, then by van der Waals interactions, hydrogen bonds and hydrophobic short-range bonds. The release from layered materials takes place primarily based on some external stimuli, which can be a response to a change in the pH of the environment, ionic strength, reaction to light and temperature, or enzymatic stimulation [39].

2.3 Chitosan

One naturally occurring substance is chitosan, a linear polysaccharide composed of the monosaccharide N-acetyl-D-glucosamine, and β-1,4-glycosidic bonds connect these units (Figure 4). It occurs mainly in the exoskeleton of crustaceans, insects, and the cell walls of fungi [40]. Chitosan can be obtained by partial deacetylation of naturally occurring chitin. Due to a large number of acetyl groups, rigid structure and poor solubility, makes its application worse. By increasing the degree of deacetylation, biocompatibility and degradability will increase. The degree of acetylation refers to the amount of ionised amino groups, which affects chitosan's charge density. It is a weak base that can be dissolved in diluted acid (acetic, citric). Depending on the method of preparing chitosan from chitin, it is possible to obtain chitosan with different molecular weights and degrees of deacetylation. The most common molecular weight of chitosan is around 50-2 000 kDa [41].

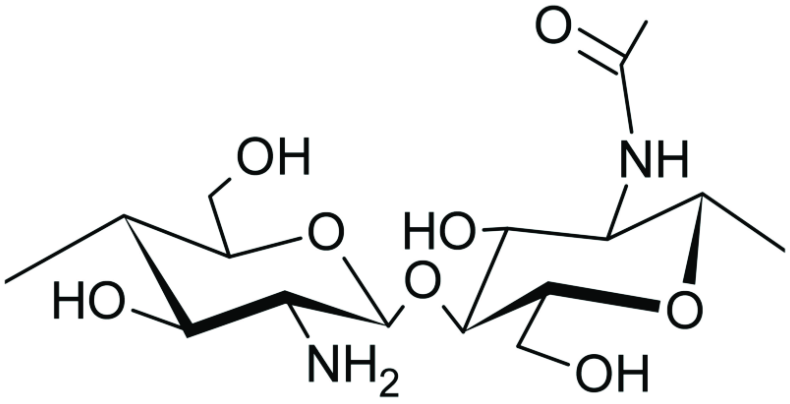

Figure 4. Chemical structure of the chitosan [42].

Due to the limited solubility of chitin in aqueous solutions, chitosan is more commonly used in industry (agriculture, industry, medicine etc.). At the same time, however, chitosan is more challenging to characterise. It is primarily a group of polymers that can be described by the number of monomer units per polymer molecule (n). This also defines the molecular weight.

The degree of deacetylation then has the most significant influence on the solubility of chitosan in an aqueous solution, which is why it is especially soluble in weak acids [43]. A hydrogel is formed by intramolecular forces, including Coulomb repulsion, hydrogen bonds, and polar forces [44].

Since it does not show irritating or allergic effects and is biocompatible with human skin, it can be used in several applications. It can be used in cosmetics, as a dye binder, as a strengthening additive in the paper industry, and in the food industry. Chitosan is a biomaterial with immunostimulating, anticoagulant, antibacterial and antifungal properties. Therefore, it can be used for wound healing in surgery or as a carrier material for drug delivery [43]. The hydrogel created from chitosan is widely applied in tissue engineering because its components are similar to the extracellular matrix [44].

2.4 Alginate

Alginate (Figure 5) is a linear polysaccharide obtained from brown algae and bacteria (*Laminaria hyperborean* and *lessonia*) [45]. This polysaccharide comprises two units, D-mannuronic acid (M) and L-guluronic acid (G), linked by a β-1,4-glycosidic bond [46, 47].

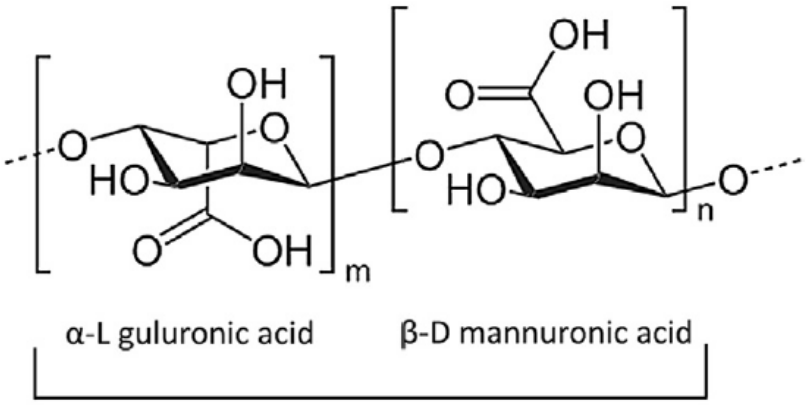

Figure 5. Chemical structure of alginate [48].

The ratio of these units can vary depending on the source of alginate and, at the same time, affects its properties, such as molecular weight, elasticity, and other mechanical properties. If alginate contains a large number of G blocks, rigid hydrogels are formed in the presence of divalent cations, e.g., Ca^{2+}, Mg^{2+}, and the so-called egg-box conformation (Figure 6) is formed. M blocks, on the other hand, show less adhesive force but have better immunostimulating activity [46]. Hydrogel formation occurs through an ionic interaction between the carboxyl group of the polymer backbone and the chelating cation [49].

Physically cross-linked alginate hydrogels lose their mechanical properties over time *in vitro* due to the flow of cross-linking agents into the surrounding medium. Divalent cations (Ca^{2+}) connect two G-blocks of alginate chains by means of ionic interchain bridges. At the same time, these bridges are responsible for the gelation of alginate solutions. For example, the loss of divalent ions can be caused by the exchange of ions with monovalent ones in the surrounding environment [13, 50]. It is also possible to introduce stable covalent bonds into alginate hydrogels, allowing greater control over mechanical and swelling properties [50]. Although alginate hydrogels are the most widely used, they have significant drawbacks. This is because

the gel dissolves due to the dissociation of the ionic bond between Ca^{2+} ions and the carboxyl groups ($-COO^-$) of the main alginate chains. For example, this makes it difficult to use as a drug carrier or scaffold for tissue engineering. One of the developed approaches to prevent this dissociation of the ionic bond is cationic polymer coatings with, for example, poly-L-lysine, chitosan, or poly(ethyleneimine). Alginate surfaces modified in this way show increased physical stability, which is only short-term. Another possibility to increase physical stability is through the formation of covalent bonds. This can occur using carbodiimide conjugation or reductive amination. Another hypothesis for how to stabilise alginate hydrogels is using a combination of ionic and covalent bonding. First, a covalent bond is formed, which is gradually replaced by ionic bonds, thereby increasing the physical stability of the alginate hydrogel [51].

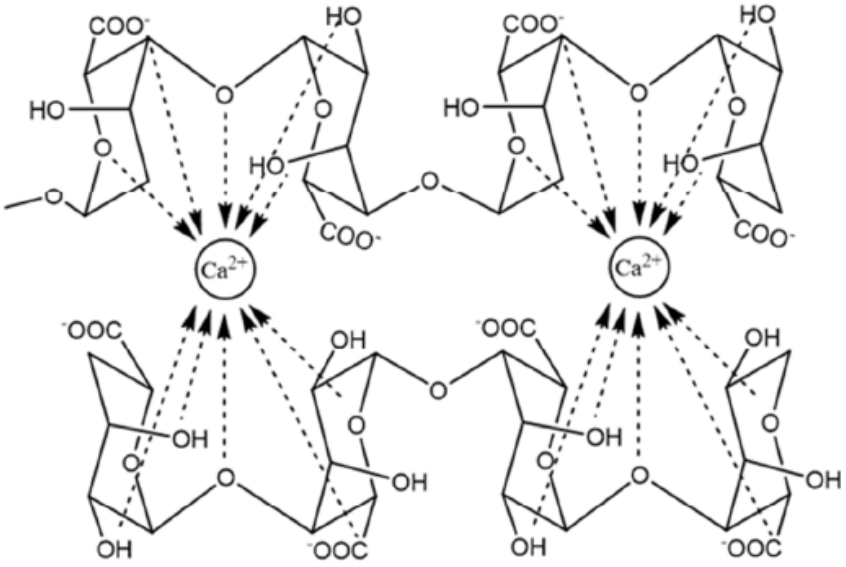

Figure 6. Connection type in the egg-box model of calcium alginate [52].

Alginate is used not only for its hydrophilicity but also because it is easily biodegradable, permeable to moisture and can absorb inflammatory exudate. At the same time, they are non-toxic, able to absorb water and create a hydrogel on the surface of wounds, which maintains a moist environment for better healing. In addition, it does not adhere to wounds, and their removal does not cause further tissue damage [46]. Physical factors control mechanical properties such as hydrogel stiffness and swelling. These factors may include cross-linking density, type of crosslinking agent, molecular weight distribution or chemical modification of the polymer [45].

The most common application of alginate is in tissue engineering, mainly used as a scaffold or bioprinting. Thanks to its properties, especially biocompatibility and viscosity, it is a relatively cheap material with fast gelation. Alginate is able to form a gel immediately in the presence of sodium or calcium ions [44]. When applied in tissue engineering, one of the most fundamental functions of alginate is that it provides mechanical integrity and can transmit mechanical signals to the cell, thereby developing tissue. It is a supporting material for constructing cartilage, bones, and sometimes skeletal muscles [47]. In clinical practice, alginate is often used, e.g., in treating heartburn and acid reflux, as a dressing material or as an appetite suppressant [53].

2.5 Transport characteristics

The apparatus (Figure 7) for determining the diffusion coefficient is composed of two cells, one of which functions as a source and the other as a receptor. Between these cells is inserted a porous membrane. Both compartments are filled with solutions of different concentrations, which are constantly mixed. After starting this experiment, two processes occur [54].

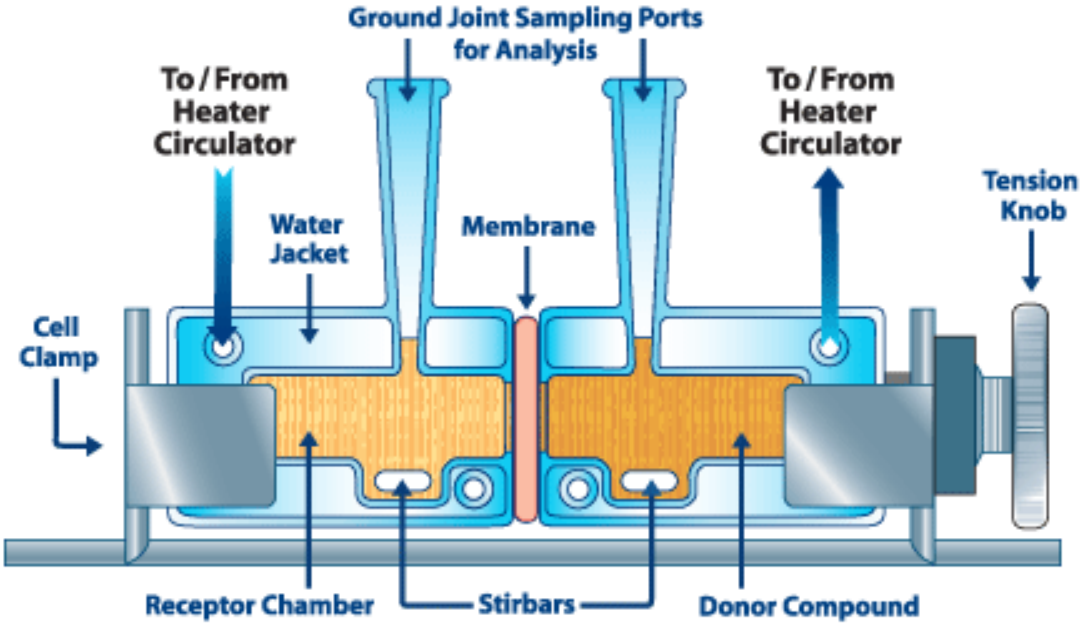

Figure 7. Apparatus of diffusion cells [55].

Diffusion itself can be divided into two steps, the first of which is sorption, and the second step is steady-state flow. During the sorption process, the dye penetrates and interacts with the prepared gel, which serves as a membrane. In this state, no dye is present in the receiving cell, which figures here as a diffusion probe. Once the concentration of the diffusion probe in the receiving cell changes, a steady state phase is reached over time. This steady state flow can be determined by fitting the linear part of the curve. It is then possible to calculate the effective diffusion coefficient from this part of the curve [54].

By measuring the concentration of solutions in both cells at different time intervals, it can then provide a relationship for calculating the diffusion coefficient (1.10),

$$\varepsilon D_{eff} = \left(\frac{\mathrm{d}n}{\mathrm{d}t}\right) \cdot \left(\frac{l}{\Delta c_{10}}\right) \tag{1.10}$$

where D_{eff} is the effective diffusion coefficient of the solute in the solvent, ε is the partition coefficient, ($\mathrm{d}n/\mathrm{d}t$) is the concentration gradient, l is the thickness of the membrane and Δc_{10} is difference of concentration in source cell and in receiving cell.

From the calculated value of the diffusion coefficient, it is possible to calculate the Stokes-Einstein radius (r) of the diffusing substance using the equation (1.11) [56, 57],

$$D = \frac{k_B T}{6\pi\eta r} \tag{1.11}$$

in which k_B is the Boltzmann constant, T is the temperature, and η is the dynamic viscosity of the solvent. In addition, it is possible to calculate the diffusion process's activation energy (E_A) since the diffusion coefficient is dependent on temperature using the Arrhenius equation (1.12) [56, 57].

$$D = A \cdot \exp\left(-\frac{E_A}{RT}\right) \tag{1.12}$$

In this equation the A is frequency factor [$l \cdot mol^{-1} \cdot s^{-1}$], and R is the gas constant [$J \cdot K^{-1} \cdot mol^{-1}$].

The diffusion process can be monitored by taking samples from both cells and measuring them on a device such as a UV-VIS spectrophotometer or conductometer. These changes can also be monitored by immersing the probes in both cells; thus, the time dependence can be observed more precisely. Spectroscopic techniques are often used because they are relatively cheap, undemanding, and accurate determination methods. As part of the diploma thesis, the diffusion and the release of substances will be measured using UV-VIS spectrometry using organic dye: positively charged methylene blue.

2.7 Methylene blue

The dye methylene blue (Figure 8) is chosen to monitor the diffusion through the formed membrane, which is generally one of the most widely used dyes. It is a heterocyclic, aromatic compound that is planar, and its molecular weight is 319.85 $g \cdot mol^{-1}$ [58]. This compound is well soluble in water (35.5 $g \cdot l^{-1}$), alcohol and acetic acid [59]. Methylene blue, or methylthioninium chloride, is a positively charged molecule with a wide range of uses from the textile industry, through medicine as a therapeutic substance, in microbiology for staining, to the field of environmental protection [58]. In its solid state, it is a green powder that changes its colour to blue when dissolved [60].

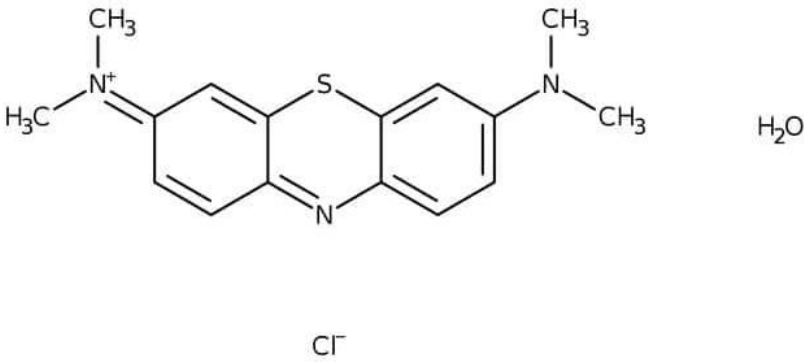

Figure 8. Molecular structure of methylene blue [61].

2.8 Mechanism of drug release

To start solving the issue of controlled drug release, it is necessary to know the release mechanism from the given structures and the factors that influence this release. In the beginning, the release mechanism is mainly controlled by diffusion. Towards the end, this mechanism is replaced and controlled more by the degradation/erosion of the material if the material is biodegradable. Several factors influence the rate of diffusion and degradation of the material. It can be water absorption, closing and opening of pores, or polymer-drug or drug-

drug interactions [62]. The closing of the pores in the hydrogel can occur at any stage, i.e. during the initial incubation of the drug as well as in the later stage of bioerosion under physiological conditions (37 °C). This closure occurs more at elevated temperature. Closed or isolated pores can reopen due to osmotic pressure. Thus, this closing and opening of pores is one of the possibilities that determines the kinetics of the release of encapsulated proteins into hydrogels. It is based on the fact that at the beginning of the incubation of proteins into the hydrogel structure, only a small part of the pores is open. The pores on the surface begin to open and connect with the pores inside the hydrogel structure once the hydrogel is placed in an aqueous environment when water enters the hydrogel. During the degradation of the polymer network, the mechanical strength of these membranes, which form the walls of the pores, is affected. At the same time, the osmotic pressure in the pores increases. This can lead to the rupture of the polymer, i.e. the previously isolated pore opens and the drug enclosed in it can be released [[63]. Investigation of the controlled release [64] of drugs from carrier structures provides information needed to further develop materials intended for the selective delivery of drug molecules and therapeutical substances to specific cells. Drug release can occur in three main ways (Figure 9):

(i) Transport through water-containing pores (diffusion).
(ii) By osmotic pumping.
(iii) Due to the dissolution of the polymer matrix (erosion).

The most common mode of release is transport through water-containing pores. The incorporated drug is usually a biopharmaceutical, such as a protein or a peptide, which are large and hydrophilic molecules that cannot be transported through the polymer network. Therefore, these substances are transported by diffusion through pores containing water. It is, therefore, a random movement of molecules controlled by a gradient of chemical potential. Diffusion is a type of conductive motion. The second type of transport is by osmotic pressure. A force, therefore, drives drug transport we can call osmotic pumping. At the same time, as the volume of the polymer increases, swelling can occur, which will cause the rearrangement of the polymer chains. The drug that is contained in the polymer can be released from it even without any transport. This happens due to the polymer's dissolution, the so-called material erosion. At the same time, pores are formed during decay, increasing the drug diffusion rate from the material [62].

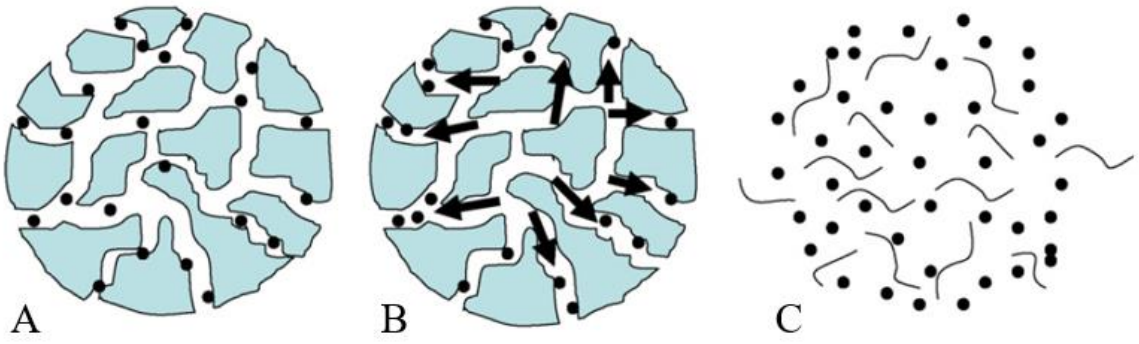

Figure 9. Types of release mechanisms: (A) diffusion through water-filled pores, (B) osmotic pumping, (C) erosion of thy hydrogel [62].

For drug transport, it is crucial to consider the properties of the polymer system, e.g., the size, porosity and density of the polymer chain [62] and the zeta potential [33]. At the same time, it is necessary to consider the surrounding conditions that affect drug release processes from the structure. It can be, for example, an increased temperature, in which chemical reactions lead to an increase in the mobility of the polymer and probably also to the closing of the pores. Salts and surfactants in the surrounding medium can induce a change in osmolality and reduce the water absorption rate. Another possible way that affects the rate of pore formation and its closing and the rate of degradation is changing the pH of the environment [62].

The equation that describes the empirical or semi-empirical function is the Peppas equation (1.13) in the form:

$$\frac{M_t}{M_\infty} = \boldsymbol{k}\, t^n \tag{1.13}$$

In this equation, M_t denotes the amount of drug released from the system at a particular time, M_∞ is the total amount of incorporated drug in the hydrogel, $\boldsymbol{k}$ is the characteristic incorporation constant of the system, and n denotes the release exponent. At the same time, this value indicates the mechanism by which the release takes place. To use this equation, it should be a diffusion-controlled mechanism without material degradation and with a constant diffusion rate. The value of n = 0.5 is when using materials in thin films, the value of n = 0.45 for cylindrical materials and 0.43 for spherical materials. If the value of the exponent is different from these, it indicates a swelling release mechanism. The most optimal release would be to achieve zero-order kinetics, reflected in the exponent equal to 1 [65].

2.8.1 Physico-chemical processes influencing the drug release

There are many ways to control the drug release rate from the polymer structure. A single mechanism is never used in a release, but individual mechanisms alternate and overlap during the process. The controlled release can then be achieved by changing the physicochemical properties of the environment. These changes mean, for example, charge, hydrophobicity, or hydrophilicity. The most common ways the release of substances from the carrier can be influenced are pH, ion gradient, ultrasound, temperature, or light.

Absorption of the water

Water absorption by the polymeric material occurs immediately after immersion or administration *in vivo*. The rate of absorption by drug carriers is swift compared to drug release. The process of water absorption is the process that creates pores in the structure. At the beginning of the release process, the pore size is too small for drug transport. As soon as the water is absorbed, the number of pores and especially their size increases, creating a porous network that enables the transport and release of the drug from the polymer structure [62].

Small hydrophobic drugs can be transported through the polymer matrix. Unlike diffusion through water-filled pores, it is not dependent on the porous structure. An important step that could reduce the release rate is dissolving the drug in water. During this mechanism, there is considerable influence by temperature [62].

Osmotic pumping

Osmotic pumping occurs when the osmotic pressure caused by water absorption causes the drug to be released from the polymer structure. This is one of the most common mechanisms for drug delivery. Water is absorbed into the channels, and osmotic pressure is created in the reservoir, which pumps the drug through the channels into the environment. It was found that osmotic transport depends only on the length of the channels, unlike diffusion transport, which depends on size and area. Transport employing osmotic pressure can be expected if the channels are longer than 60 μm. At the same time, for transport to be ensured through osmotic pressure, it is necessary to balance the inflow and outflow of water from the system. Water absorption into the system can lead to polymer cracking [62].

Hydrolysis

This is a process of cleavage of ester bonds, resulting in a decrease in the molar mass of the polymer. This phenomenon begins to occur immediately after contact with water. Hydrolysis produces acids catalysing hydrolysis, a so-called autocatalytic phenomenon that causes heterogeneous degradation inside the matrix. The effect of heterogeneous degradation is manifested in microparticles and polymer films, which have dimensions of around 10 μm. This process makes the polymer less hydrophobic as its molecular weight decreases. When we reach the limit of about 1100 Da, the resulting oligomeric structures become soluble in water [62].

Erosion

Erosion, or the loss of polymer mass, begins to occur when dissolved degradation products of the polymer diffuse into the media environment. As the polymer matrix dissolves and erodes, the pores also enlarge. Due to water absorption into the polymer matrix, drug diffusion and hydrolysis occur. Hydrolysis produces acids, which are further used for local catalytic degradation, thus dissolving the polymer inside the pores. This leads to more pores growing and joining together, creating a smaller number of larger-diameter pores [62].

Degradation products of the polymer matrix can affect the entire system in several possible ways [62]:

(i) Degradation products are acids that catalyse hydrolysis.
(ii) They can be substances that plasticise the polymer, i.e., increase the rate of water absorption and reduce the transport resistance of the polymer.
(iii) Products that can increase osmolality and water absorption.
(iv) Products can crystallise, inhibiting water absorption, degradation, and transport of substances.

Assuming that the drug is homogeneously distributed in the polymer system, it is possible to obtain identical drug release and polymer erosion profiles. This is the only release mechanism in which there is no drug transport. It is often cited as a step-controlling mechanism, i.e., it controls the diffusion rate, especially during the last drug release phase. This mechanism could be applied mainly when the drug occurs near the surface and is not transported through the system [62].

2.8.2 Drug release profile from the polymer structure

The most preferred drug release profile is zero-order release. Drug release can be monophasic and biphasic, but the most common is a triphasic profile [62].

In the case of a monophasic release profile, the active substance is uniformly dispersed in the polymer network of the hydrogel and this hydrogel influences the release rate. A two-phase release profile is shown by so-called reservoir systems, in which the active substance is enclosed in an inert membrane [66].

This release profile occurs due to heterogeneous degradation and is particularly applicable for large particles or drug delivery systems. It is advantageous to combine different sizes because it leads to a change in the drug release profile from a Fickian diffusion profile through a sigmoid profile to a zero-order profile (Figure 10) [62].

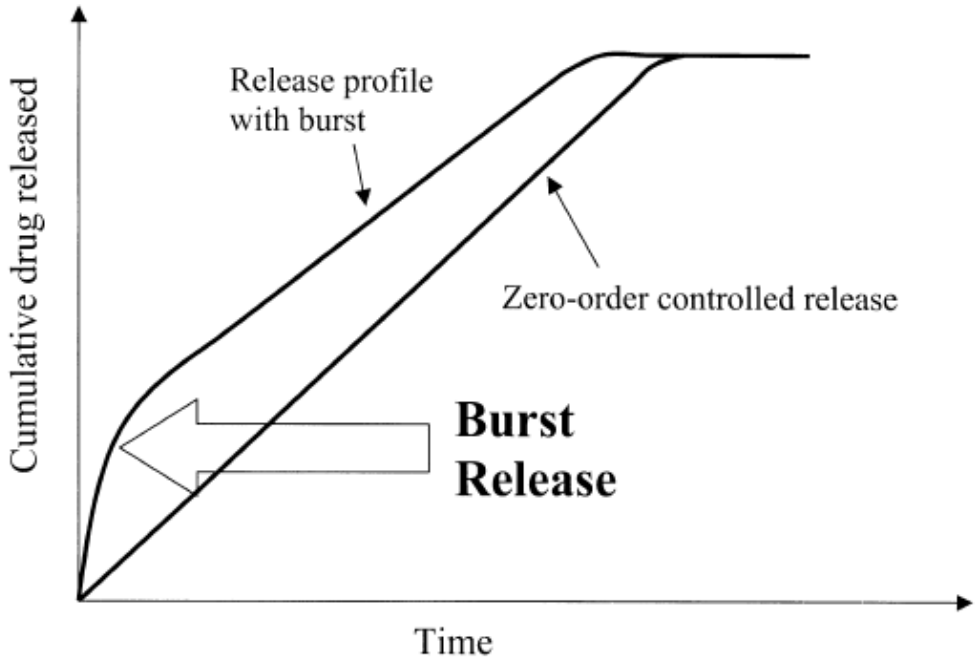

Figure 10. Schematic representation of the burst effect in a zero-order drug delivery system [69].

In the three-phase release profile, we divide them into three phases.

The first is phase I (burst effect), which is characterised by the sudden release of a large amount of drug. It is often attributed to unincorporated drug particles on the surface and is readily accessible by hydration. Rapid release of the drug from the structure can also occur through the formation of cracks and the disintegration of particles [62]. This burst effect is attributed to several physical, chemical and process parameters. However, no universal explanation for this problem has been found [69].

Phase II (lag phase) is characterised by slow release, during which the drug slowly diffuses through the pores of the polymer. At the same time, degradation and hydration of the polymer occur [62].

Phase III (secondary burst effect) is accompanied by faster drug release; this is attributed to the onset of erosion. The slow phase may not be due to a dense polymer containing a small number of pores. Inhibition of the release rate also occurs by polymer-drug, drug-drug interactions, or pore closing [62].

The most frequently mentioned effect in the release of drugs from the structure is diffusion through the pores of the polymer. This mechanism can be used primarily during the first phase of release when the polymer has yet to be eroded. Diffusion mainly describes how the drug is released. Erosion is the decisive process that affects the rate of diffusion. In general, phase I is diffusion dependent. During the study of the release of hydrophilic drugs from microspheres, some results corresponded to the fact that drug release is proportional to the square of time during phase III, indicating diffusive transport. The diffusion of pores depends mainly on the polymer's structure, i.e., how pores are formed and, conversely, how they are closed. It is essential to introduce the term effective diffusion coefficient there, which depends not only on the diffusion coefficient and porosity but also on tortuosity. Various factors, such as particle size, temperature, or environmental properties, can influence the diffusion rate. However, if there is no diffusion through the polymer during the release process or the transport is not controlled by osmotic pressure. The transport occurs mainly by diffusion through the pores [62].

3 THE CURRENT STATE OF THE SOLVED PROBLEM

This chapter deals with research that deals with the issue of hydrogels as carriers for drug release. However, the problem of the burst effect is not solved in any of them, or at least it was not solved completely.

3.1 Hydrogels for controlled release and preparation of pH sensitive hydrogels

Gupta et al. focused on the study and preparation of controlled release hydrogels and the preparation of pH sensitive hydrogels [70]. Classic hydrogel matrices that serve for the controlled release of active substances can be of two types. The first kind is the drug loosely dispersed in a polymer matrix, and these materials are easy to prepare, inexpensive, and have good performance. Drug release from this system is proportional to the square root of time, thus following the Higuchi model. The disadvantage of this system is that non-uniform release rates occur [70].

The procedure for the preparation of hydrogel (Figure 11) for controlled release is based either on the crosslinking of linear polymers or the simultaneous polymerization of monofunctional monomers and crosslinking with polyfunctional monomers. The formed hydrogels can show poor mechanical strength, which can be improved by various methods (chemical cross-linking). There is no one-size-fits-all shape for all uses of these hydrogels, and the shape needs to be adapted for each application [70].

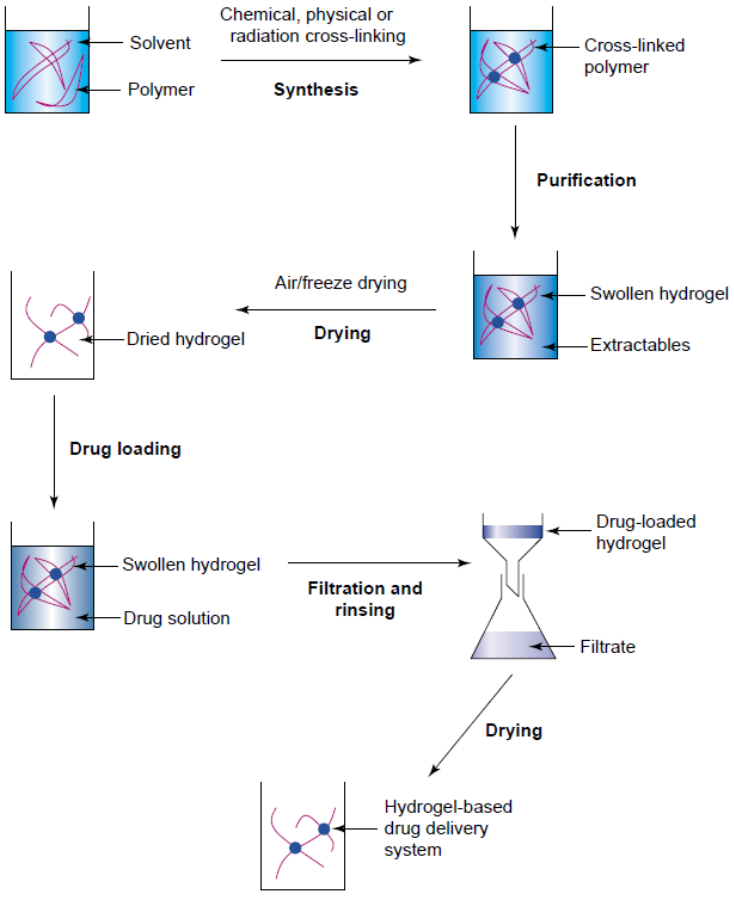

Figure 11. Schematic representation of preparation of hydrogels with incorporated drug [70].

The prepared hydrogels are glassy in the dehydrated state, the release of the drug then involves the absorption and desorption of water with the drug. This desorption is a controlled swelling mechanism of the used hydrogel. The factor that controls the rate of drug release from the polymer matrix is the resistance of the used hydrogel to swelling and shape change. They found that once the matrix absorbed enough water, the glass transition temperature of the polymer used dropped to the experimental temperature. The presence of water in the hydrogel causes swelling. The rate of absorption into the dry hydrogel (xerogel) occurs at a well-defined rate when the thickness of the hydrogel simultaneously increases. This process generally does not follow the Fickian diffusion mechanism, and this behaviour corresponds to a slow macromolecular relaxation process in the swollen state [70].

To synchronize the drug release profile with physiological conditions, they also focused on studying mechanisms that respond to physiological changes. Ideally, the system created in this way should respond to an external stimulus that starts or stops the release of the drug from the hydrogel matrix. They observed that these sensitive hydrogels to external stimuli can be completely reversible [70].

In their research, they prepared an interpenetrating network (IPN) from gelatine and dextran, this material was created as a biodegradable hydrogel that responds to external stimuli. Lipid microspheres were incorporated into this material, which served as micro reservoirs of the drug. They found that when the hydrogels were prepared below the sol-gel transition temperature, lipid microspheres were released in the presence of α-chymotrypsin and dextranase. Release was inhibited in the presence of either enzyme alone [70].

3.2 Dependence of molecular weight and structure of proteins for controlled release

Lin and Metters focused their attention on the efficient delivery of protein and peptide drugs [71], which are difficult to deliver due to their large molecular weight and specific three-dimensional structures. So far, their most common way of administration is intravenous or subcutaneous injection. The biggest problem with these substances is their susceptibility to proteolytic cleavage, which is why they have very short circulation times. For patients who take these drugs, this means repeated injections several times a day. This can cause high local toxicity and an immune response. Therefore, they focused on the preparation of a carrier for these drugs with controlled release. This carrier also includes PLGA, where gradual release was controlled by changing the molecular weight and composition of the polymer [71].

Kinetic controlled release can take place in two ways, e.g. by a suspension chain (prodrug) and/or a system that erodes the surface. In the case of pendant chains, the drug is covalently linked to the hydrogel network through cleavable spacers, this release being then controlled by the rate of spacer-bond cleavage. In the latter case, the release is controlled by the rate of surface erosion [71].

The release of covalently bound prodrugs is determined by the rate of degradation of the polymer-drug bond. These linkages tend to be designed to undergo hydrolytic degradation, allowing the rate of degradation and release to be described by simple first-order kinetic

relationships. In some cases, this is not desirable, e.g. where a more targeted release profile is needed. Therefore, they designed enzymatically cleavable spacer bonds that provide more complex release kinetics [71].

Ehrbar et al. developed a fibrin matrix that was cross-linked with pendant variants of vascular endothelial growth factor (VEGF) that were linked by plasmin-sensitive peptidyl substrates (Figure 12) [72]. Covalently bound variants of VEGF could only be released from the matrix by cleavage of modified peptide substrates. First-order kinetics were used to model the time-dependent release of VEGF, accurate prediction of release profiles needed to include matrix degradation. They considered two adjustable parameters for this prediction. The rate constant (k) was the first parameter. They hypothesized that the degradation of fibrin network bonds and plasmin-sensitive substrates used to bind VEGF to fibrin exhibits first-order kinetics. The second parameter was the number of fibrin repeating units between two crosslinks. The model they developed accurately predicted the release of these cleavable and non-cleavable VEGFs from fibrin matrices of varying density [72].

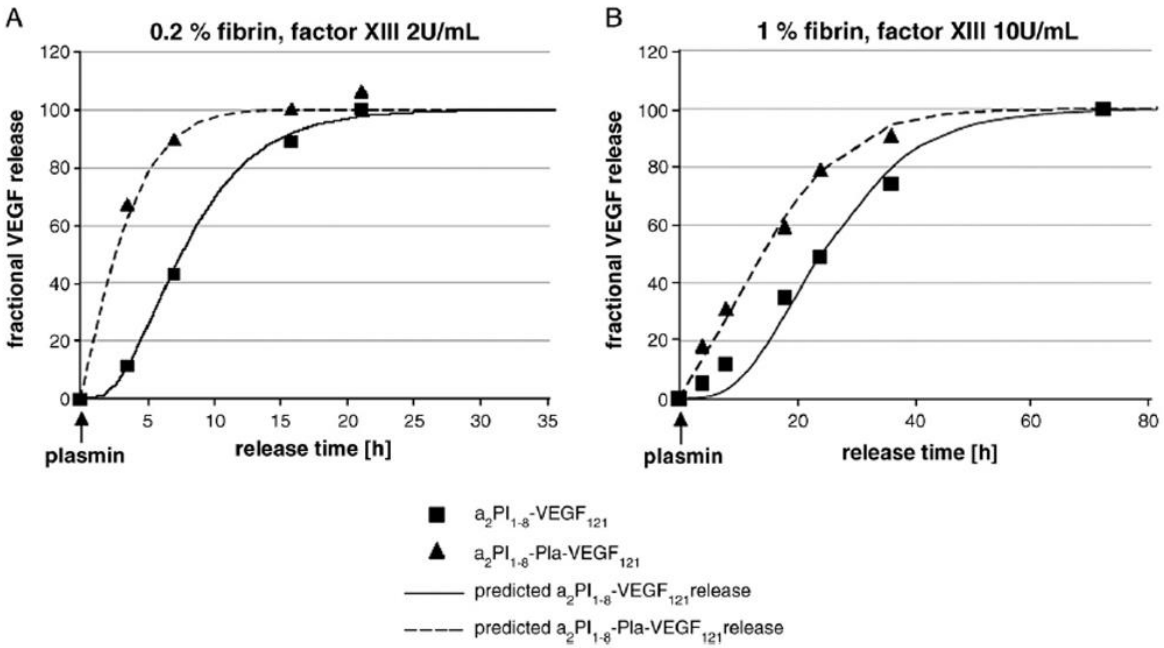

Figure 12. A mathematical model that predicts experimental observations of the release of fibrin-bound VEGF variants from low- and high-density hydrogel structures [72].

3.3 Dependence of PLGA hydrogel composition on drug release

Osswald and Kang-Mieler focused on the development of delivery systems for ophthalmic use that consisted of polylactic-co-glycolic acid (PLGA) microspheres embedded in a thermo-sensitive hydrogel [73]. To achieve prolonged drug release, they encapsulated the drug in these microspheres. At the same time, they focused on changing the ratio of lactic acid and glycolic acid, which led to an influence on the release of the drug, because the degradation time of PLGA is prolonged. At the same time, they found that by accelerating solidification, for example by increasing the speed of solvent removal, the encapsulation efficiency can then increase. The most interesting finding was that this accelerated solidification prevented drug separation from

the hydrogel, and this prevented initial cracking. Not only does it provide advantages, one of the disadvantages is that it creates highly porous microspheres [73].

In this research, they prepared PLGA microspheres filled with ovalbumin, which were further suspended in a PNI-PAAm-PEG-DA hydrogel. As soon as the prepared material was exposed to a temperature of 37 °C, the hydrogel collapsed and ovalbumin was released from the matrix, but the position of the microspheres remained (Figure 13) [73].

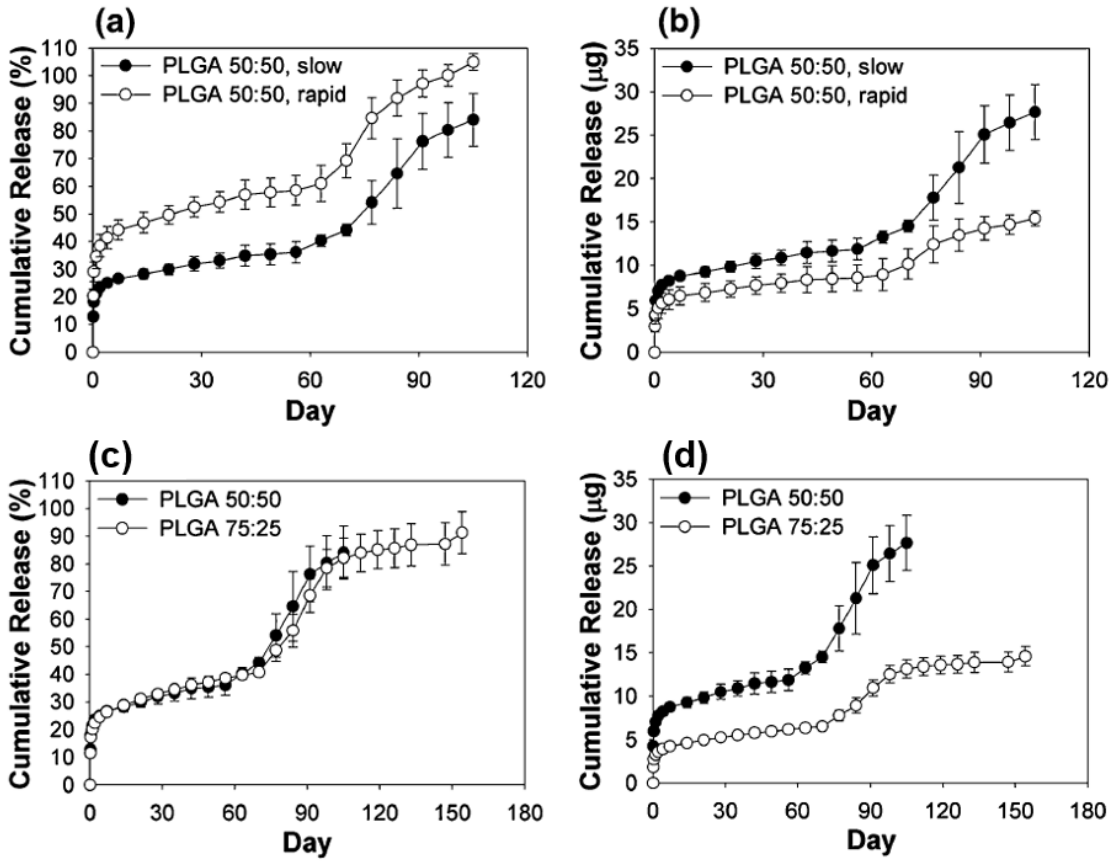

Figure 13. The amount of ovalbumin released from PLGA with different solvent evaporation rates (slow, rapid) (a, b) and with different ratios of poly(lactic) and poly(glycolic) acids (c, d) [73].

3.4 How gelatine affects drug release properties of alginate film

Thu and his team [74] appeared to study alginate films in which a drug (ibuprofen) was embedded and studied the delivery mechanism. During the observation, they came to the fact that the alginate itself had bad properties, was soft and had a rough surface. The addition of gelatine to the structure improved the physical properties and dispersion of the drug.

Their research focused on the preparation of hydrogel carriers of the drug (in this case, ibuprofen), when in a previous study they had already prepared sodium alginate hydrogels from either one layer or two layers. In the case of a two-layer hydrogel, the drug was placed in only one of the layers. However, they tried to improve this procedure, because they did not create smooth hydrogels with a homogeneously distributed drug. They chose gelatine, which should solve this problem and thus create a smooth and flexible hydrogel with a homogeneous distribution of the drug [74].

When measuring the uniformity of the drug in the hydrogel, they found that in the absence of gelatine, clusters were formed during recrystallization during the drying process, while the drug was not distributed homogeneously (Figure 14). This was subsequently confirmed by images from a scanning electron microscope (SEM) [74].

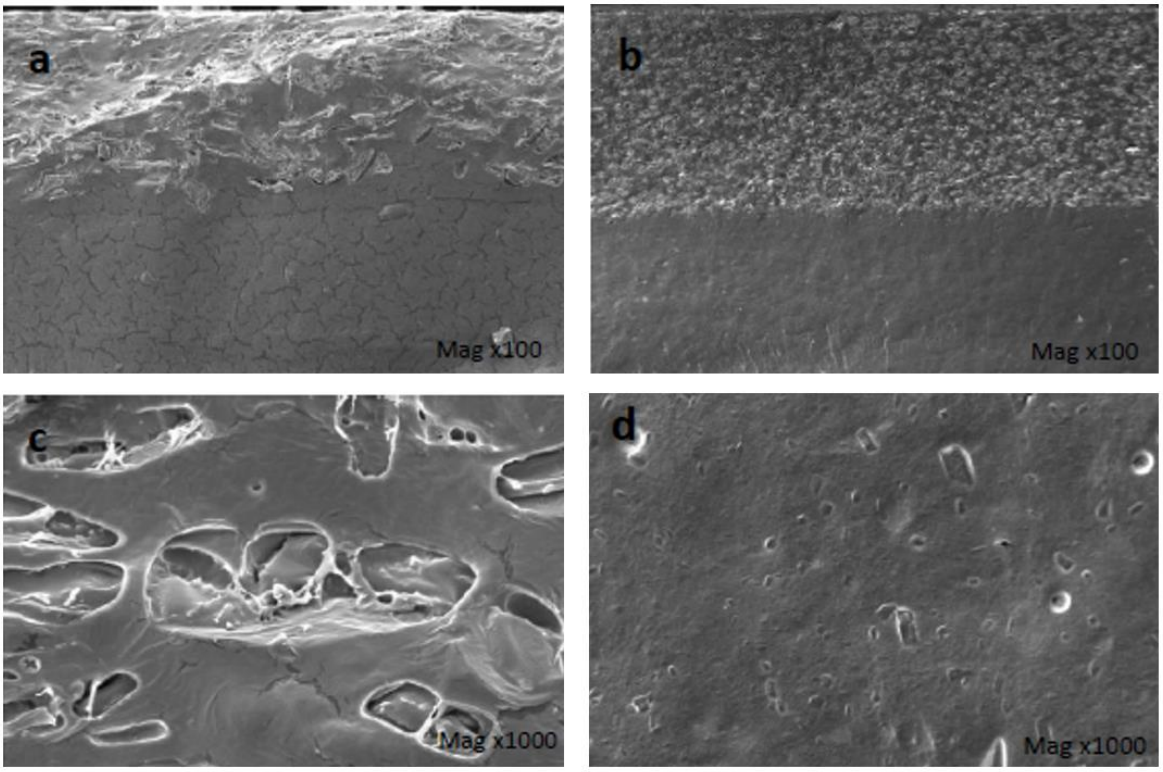

Figure 14. Cross-section of bilayer materials, (a) sodium alginate hydrogel bilayer with drug-loaded upper layer, (b) sodium alginate hydrogel bilayer with gelatine, where the upper layer contains drug, (c) sodium alginate hydrogel upper layer with ibuprofen clusters, (d) upper layer of sodium alginate hydrogel with gelatine with a homogeneous distribution of the drug [74].

They further measured on the FTIR spectrum whether the gelatine somehow interacted with the ibuprofen. Therefore, they first measured the ibuprofen itself and then all the created materials. The ibuprofen peak in this spectrum was preserved and this proves that the gelatine did not change the chemical properties of the drug in any way. This was subsequently confirmed by thermal analysis when gelatine did not affect the endothermic process of ibuprofen microaggregates [74].

Therefore, they concluded that gelatine improves the dispersion of hydrophobic drugs in the sodium alginate hydrogel and leads to the formation of only small microaggregates in the hydrocolloid film matrix, which are uniformly distributed in the matrix space [74].

3.5 Production of laminates for controlled release using electrospinning

Scafaro et al. focused their research on the preparation of layered laminates [75], which they prepared on a substrate with poly (lactic acid) (PLA) and an active substance (carvacrol, CRV), to which layers are gradually added by electrospinning (Figure 15). In this way, laminates were prepared that differed in the number of electrospun layers. The relationships between processing, structure and properties of these materials were investigated.

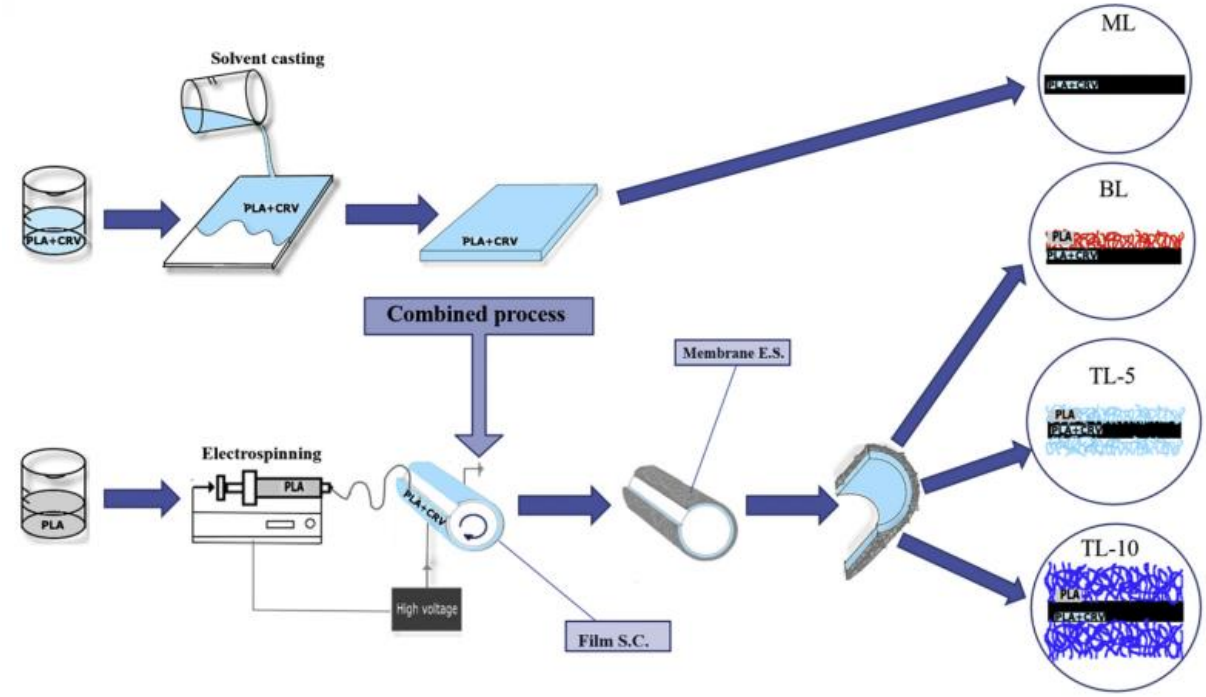

Figure 15. Schematic of the preparation of layered laminates using the electrospinning method [75].

The mono-layer (ML) exhibits typical release behaviour in thin films. There is a characteristic of rapid release of the drug in the early part of the curve (Figure 16), when the driving force is the highest, when a plateau effect is subsequently reached, this is associated with the progressive depletion of CRV after a certain time interval. After 300 hours, no drug release activity was observed from the monolayer. The maximum amount of CRV drug released was only approximately 50 % of the theoretical loaded amount, i.e. CRV originally added to the polymer solution used for solvent casting. This apparent mismatch between loaded and released content is generally observed in polymer systems containing essential oils due to the volatilization of the CRV drug during processing and due to the fact that a proportion of the CRV drug molecules remain entrapped in the structure [75].

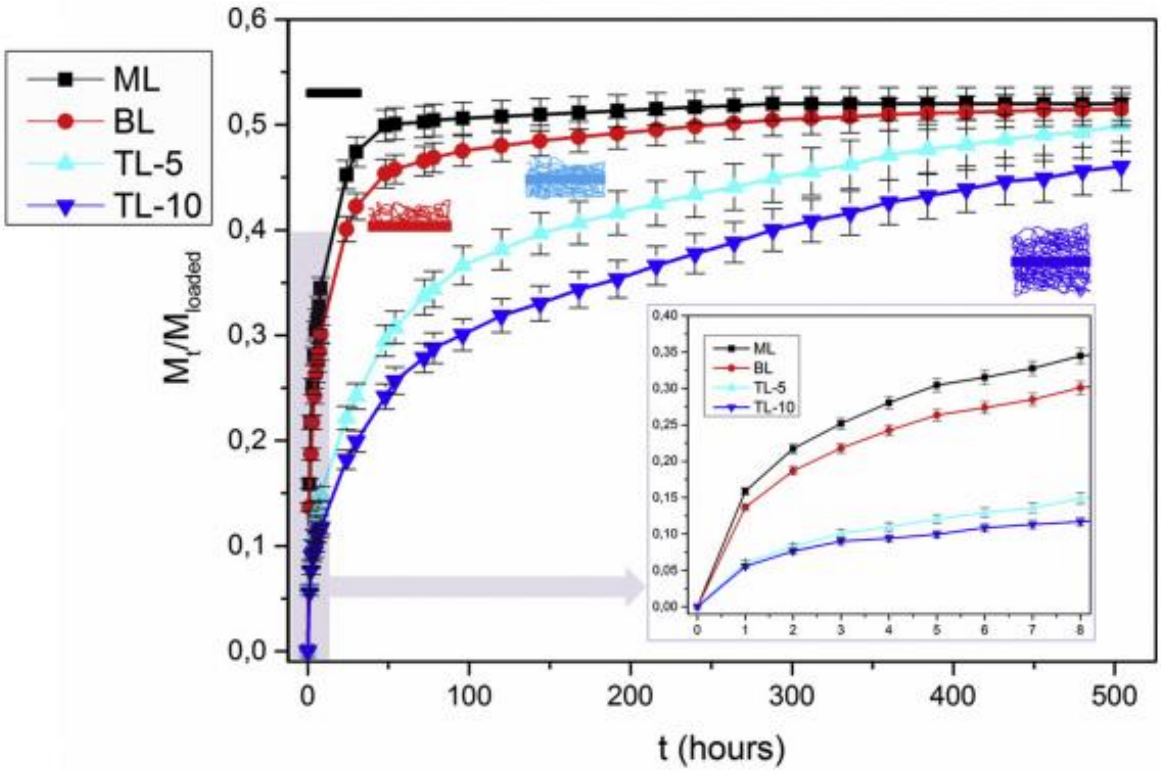

Figure 16. Release curves of active substance (CRV) from electrospun materials with different number of layers [75].

Compared to the monolayer, the bi-layer (BL**)** shows a similar release behaviour, but is characterized by a slightly lower burst release and levelling off at higher time intervals, as the M_t/M_{loaded} ratio is shown to increase monotonically, albeit slowly, but throughout the observation period, i.e. asymptotically inclined to the saturation value achieved by the monolayer [75].

The tri-layers (TL-5 and TL-10) show a progressive reduction in burst release with a subsequent increased ability to sustain gradual drug release throughout the observation period. Remarkably, none of them reached a plateau effect by 550 hours [75].

3.6 Preparation of gelatine/PVA nanofibers and their in controlled drug delivery

Yang and his group dealt with the preparation of electrospun porous gelatine networks [76]. They inserted raspberry ketone (RK) into this spun system, which allowed them to study the material's potential for controlled release. In this work, electrospinning was used to prepare RK-filled nanofibers for potential use in drug delivery. Mechanical and drug release properties were also investigated.

To study the release of RK from nanofibrous membranes formed by electrospun gelatine combined with PVA. Materials with different ratios of gelatine and PVA were prepared. The released RK was studied with a UV-VIS spectrophotometer at a wavelength of 275 nm [76].

Molecules of gelatine, PVA and raspberry ketone are soluble in water. Therefore, during their measurements, they discovered that the carrier matrices were dissolved in the aqueous solutions

and the RK was released in the so-called burst effect [76]. First, they investigated the effect of the concentration of RK incorporated into the carrier matrix (Figure 17).

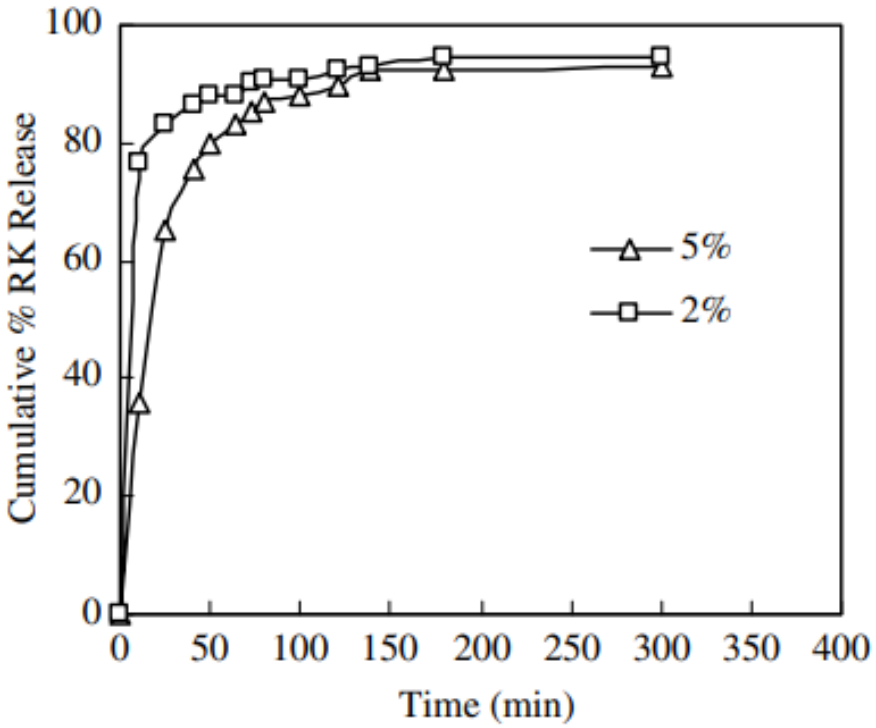

Figure 17. Released amount of RK (2 and 5 (w/w) %) from electrospun matrix of gelatine and PVA [76].

Considering that gelatine contains carboxyl and amino groups in its structure, they assumed that the pH would influence the release of RK (Figure 18). The pH values were set at 1, 4.8, and 7. In the buffer solution of pH 1, the release rate of RK and the final percentage of RK release were obviously faster and higher than those in the buffer solution of pH 4.8 and pH 7. The possible reason was, when the pH value of the buffer solution was lower than the isoelectric point of GEL (pI 7-9), the amino groups of gelatine were protonated to carry positive charges. The entire polymer network thus carried positive charges that caused the molecular chains to repel each other. In this case, the network is relaxed, and it was easy for the drug molecules to diffuse into the buffer solution. The release properties of RK at pH 4.8 (close to the pH value of human skin) and pH 7 (close to the isoelectric point of gelatine) were similar because their swelling properties were very similar [76].

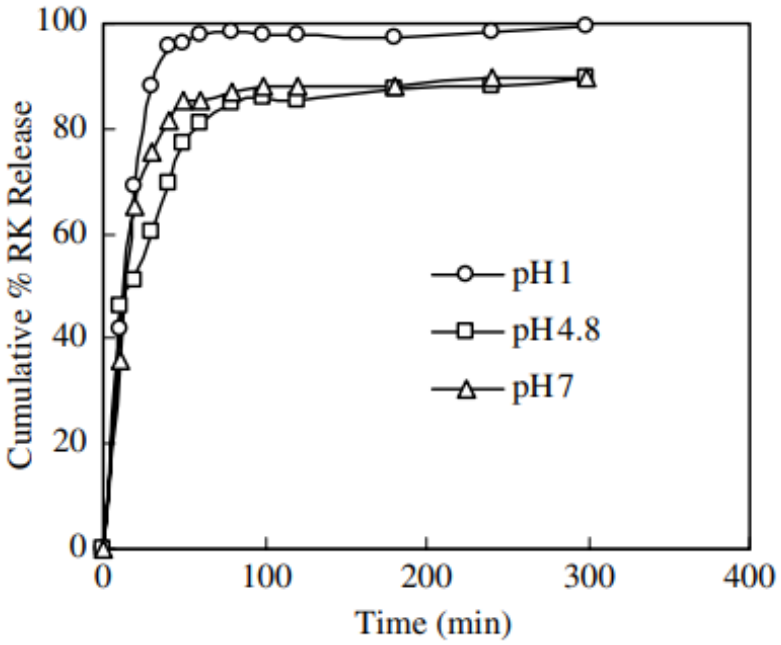

Figure 18. Released amount of RK from electrospun gelatine material and PVA in the environment of buffers of different pH [76].

The addition of PVA to electrospun gelatine was considered because gelatine showed poor water resistance and low mechanical strength. It was the addition of PVA or chemical cross-linking that increased the mechanical properties. Cross-linking was performed with glutaraldehyde for different times (Figure 19). They found that as the cross-linking time increased, the degree of cross-linking and intermolecular forces increased, resulting in slower swelling, and thus limiting the release rate [76].

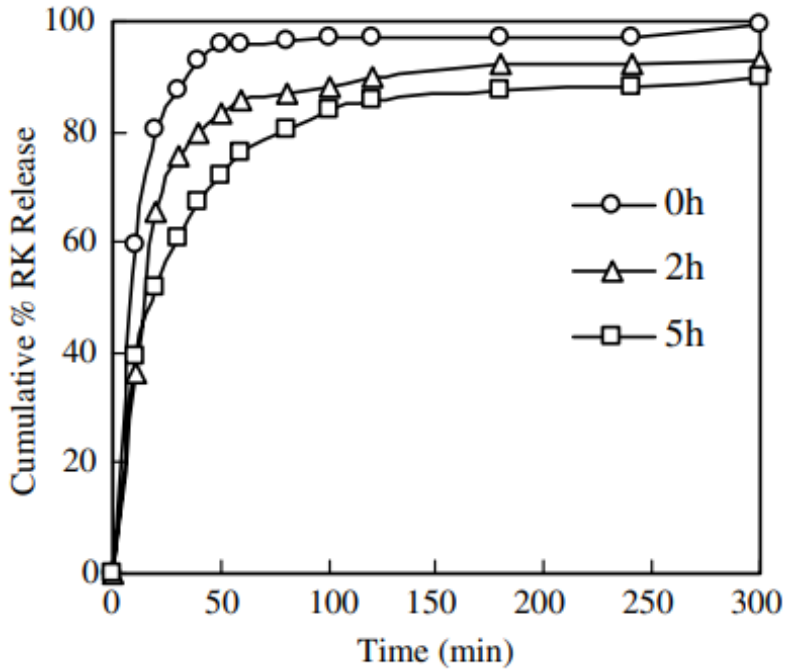

Figure 19. Released amount of RK from electrospun gelatine material and PVA with different time of cross-linking [76].

At the end, they also looked at how the ratio of gelatine and PVA affects the release of RK (Figure 20). The 7:3 material had a slightly higher release rate than the 9:1 material. They attribute this to the fact that the first mentioned material swelled more [76].

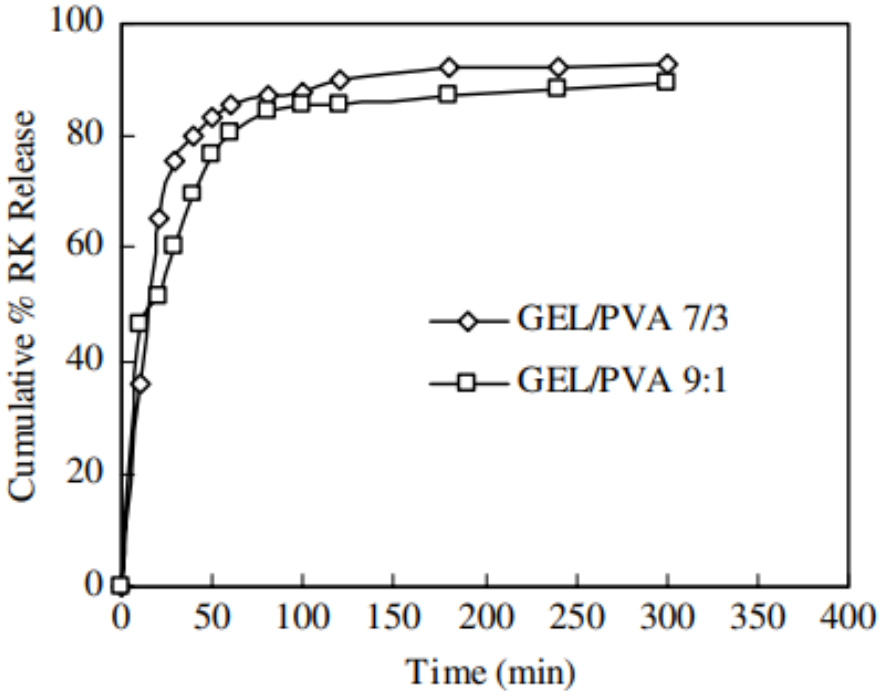

Figure 20. Released amount of RK from electrospun gelatine material and PVA with ratios of gelatine and PVA [76].

4 EXPERIMENTAL PART

The experimental part consists of a list of chemicals and materials used. Materials and chemicals. Polymer solutions of alginate, chitosan, and gelatine will be used to connect the individual layers of the non-woven fabric. All these types are chosen because they are some of the most commonly used substances in the pharmaceutical industry and medicine. Their good properties include biocompatibility, biodegradability, and the fact that they are non-toxic. Their disadvantages are poor mechanical properties, such as thermal stability, swelling or stiffness. First, the layered materials will be prepared with the mentioned polymer solutions. These materials will be connected by polymer solutions with different concentrations, when not only the effective concentration for connecting these materials, but also the stability of these materials will be determined. This entire measurement will be carried out after 72 hours of swelling of the materials in distilled water, when these materials will then be weighed and the collected water samples with the released polymers will be measured on a viscometer (Anton Paar Automated Micro Viscosimeter). From this measurement, it will be determined with which polymer solutions and with what concentration the layered materials will be further prepared. After finding out which polymer solutions is the most suitable for further measurements, layered materials with different number of layers will be prepared with this hydrogel. The effective diffusion coefficient and how it changes with increasing number of layers will be investigated on these materials. This will be investigated using the diffusion cell method (side-bi-side diffusion cells, PermeGear), where the source cell will be filled with methylene blue solution and the receiving cell with distilled water. It will also be measured how long it took for the dye to penetrate into the receiving cell. At the end, the materials will be prepared with a dye concentration gradient and the release of this dye into the aqueous environment will be monitored using a spectrophotometric probe (Optical fibres, Ocean Insight). This measurement will monitor whether the burst effect occurred or not.

4.1 Materials and chemicals

Chitosan (168 kDa)	Sigma-Aldrich Co.,
	CAS: 9012-76-4
Sodium alginate (100 kDa)	Sigma-Aldrich Co.
	CAS: 9005-38-3
Gelatine (50-100 kDa)	Sigma-Aldrich Co.,
	CAS: 9000-70-8
Acetic acid 99% g.r.	Lach-Ner, s.r.o.,
	64-19-7
Methylene blue hydrate g.r. (319.86 Da)	Penta s.r.o.,
	CAS: 122965-43-9
Non-woven fabric (polypropylene)	Beltzmann

4.2 Devices

Analytical scales	Kern ABS-N
High precision scales	Kern
Density Meter (DMA 4500)	Anton Paar
Automated Micro Viscometer (AMVn)	Anton Paar
U-3900H Spectrophotometer	Hitachi
Optical fibres (VIS)	Ocean Insight
Magnetic stirrer	Cimarec i Poly 15 and Multipoint Stirrer
Magnetic stirrer with heating	Heidolph^{TM} Hei-Tec Magnetic stirrer hotplate

4.3 Preparation of gelatine, chitosan, and alginate polymer solutions

Polymer solutions of gelatine, alginate, and chitosan were used to connect the layers of non-woven fabric (made up from electrospun polypropylene), and at the same time, they also served as dye carriers, which could be replaced by drug or active substance in the future research. The layered materials were connected by polymer solutions of gelatine, chitosan, and alginate. Subsequently, these materials were examined for the release of this polymer into water environment.

Gelatine (Gelatine from bovine skin Type B, M_w 50-100 kDa, Sigma-Aldrich Co.), alginate (Sodium alginate, M_w 100 kDa, Sigma-Aldrich Co.), and chitosan (Chitosan, M_w 168 kDa, Sigma-Aldrich Co.) polymer solutions were prepared with a concentration of 1, 2, 3, 4 and 5% (w/w). For the preparation of the 5% (w/w) alginate polymer solution, 4.00 g of sodium alginate were weighed on the high precision scales (Kern). The final volume after dissolving the weighted alginate was supplemented with distilled water to a volume of 80 cm^3. A magnetic stirrer was placed in this solution and this beaker was placed on a magnetic stirrer with 250 rpm for 24 hours.

In the case of gelatine polymer solution, 4.00 g of gelatine was weighed. The final volume after dissolving the weighted gelatine was supplemented with distilled water to a volume of 80 cm^3. A magnetic stirrer was placed in this beaker. This was all placed in a larger beaker of water that was placed on a magnetic heater, this larger beaker of water served as a water bath. The heated magnetic stirrer was set to a heating temperature of 65 °C and a rotation speed of 250 rpm. Gelatine hydrogel was formed after about 10 minutes.

To prepare the chitosan polymer solutions, it was first necessary to prepare a suitable solvent, in this case acetic acid. A stock solution of 5% (v/v) acetic acid was prepared by diluting concentrated acetic acid (99% g.r., Lach-Ner, s.r.o.). This stock solution was prepared in a 1 dm^3 volumetric flask by diluting 50 cm^3 of concentrated acetic acid with distilled water and adding distilled water to the required volume. The final volume after dissolving the weighted chitosan was supplemented with the prepared acetic acid solution to a volume of 80 cm^3. A magnetic stirrer was inserted into this mixture and the mixture was placed on a magnetic stirrer with 250 rpm where it was stirred for 24 hours.

The prepared 5% (w/w) alginate was subsequently diluted with distilled water to the remaining concentrations (4, 3, 2 and 1% (w/w)). These solutions were prepared to a volume of 40 cm^3. To create perfectly homogeneous samples, they were mixed for 1 hour on a magnetic stirrer (rpm 250). The gelatine solution (5% (w/w)) was also diluted with distilled water to concentrations of 4, 3, 2 and 1% (w/w). Again, 40 cm^3 solutions were prepared. All gelatine solutions formed were thoroughly mixed to make them homogeneous. The created solution of 5% (w/w) chitosan was also diluted after 24 hours of mixing to create other solutions with concentrations of 4, 3, 2 and 1% (w/w). These solutions were created by diluting the original solution with acetic acid to 40 cm^3, which were again allowed to stir for 1 hour on a magnetic stirrer with 250 rpm under laboratory conditions.

4.4 Preparation of layered materials and measurement of released polymer

For the preparation of layered materials, which were joined by polymer solutions, the non-woven fabric was cut to a size of 3×5 cm. From these materials, the release of these polymer solutions was subsequently determined using viscometry. The following table (Table 1) shows how the individual layered materials were prepared. To create the layered material, 16 layers of non-woven fabric were needed, these layers were interpenetrated with the prepared polymer solutions. Thus, 5% (w/w) alginate polymer solution bonded these 16 layers of polypropylene non-woven fabric. Another 16 layers of non-woven fabric were bonded with 4% (w/w) alginate polymer solution. The same procedure was followed with other concentrations of alginate solutions. Layered materials combined with chitosan and gelatine polymer solutions were prepared in the same way. The materials thus prepared were left to dry for 24 hours. The following day, the materials were cut to a size of 2×3 cm. These materials were then weighed and placed in separate containers with 20 cm^3 of distilled water. Thus, the layered materials were left for 72 hours to swell and release. After this time the materials were removed from these containers and reweighed, the water was allowed to evaporate from the materials and after another 24 hours the materials were reweighed. Distilled water in which the layered samples were left to release unreacted particles was collected (10 cm^3) for viscosity measurement. Using this measurement, the amount of released polymer and the stability of the layered materials were determined.

Table 1. Overview of the preparation of layered materials on a specific number of layers of non-woven fabric (n) connected by polymer solutions of different concentrations (w_p), the volume of polymer solutions used for each layer (V_p).

n [-]	w_p [% (w/w)]	V_p [cm^3]	description
16	5	2	The individual layers of the non-woven fabric were connected by 2 cm^3 of polymer solution (alginate, chitosan, or gelatine). After this the layered material was let to dry out at laboratory temperature for 24 hours and then cut to size 2×3 cm.
16	4	2	
16	3	2	
16	2	2	
16	1	2	

The released amount of polymer solutions (chitosan, alginate, and gelatine) from the layered structures was investigated by measuring the dynamic viscosity of the samples. This measurement was performed on an Anton Paar Automated Micro Viscosimeter using a 16 mm diameter capillary (13190613). Viscosity was measured by passing a ball with diameter 15 mm through a capillary that was tilted at an angle of 60°. To measure the viscosities of the samples,

the densities of the individual samples and water were first measured. All measurements took place at a temperature of 25 °C. First, the calibration series of alginate (Figure 21), chitosan (Figure 22), and gelatine (Figure 23) solutions were measured. The obtained data were used to provide a regression equation, which was further used to calculate the released hydrogel concentration from the layered materials. Subsequently, the samples taken from the release of hydrogels were measured.

These materials were placed in a container with 20 cm^3 of distilled water left for three days. After this time, 10 cm^3 was taken from each sample and measured on a viscometer. On the first day, calibration series of chitosan, alginate and gelatine were created. These solutions were also allowed to stand for three days and then measured on a viscometer. After three days, the weight of the swollen layered material was also measured. These materials were then allowed to dry for 24 hours before being weighed again.

4.5 Preparation of calibration solutions of alginate, chitosan, and gelatine

A series of calibration series of alginate (Figure 21) (Sodium alginate, M_w 100 kDa, Sigma-Aldrich Co.), chitosan (Figure 22) (Chitosan, M_w 168 kDa, Sigma-Aldrich Co.), and gelatine (Figure 23) (Gelatine from bovine skin Type B, M_w 50-100 kDa, Sigma-Aldrich Co.) with a concentration of 0.01, 0.015; 0.02; 0.025; 0.03; 0.05; 0.1; 0.15; 0.2 and 0.5% (w/w) were prepared. First, a stock solution of alginate, chitosan, and gelatine with a concentration of 0.5% (w/w) was prepared, created by dissolving 0.1 g of the given substance in a solvent (water or 5% (w/w) acetic acid). The stock solution was subsequently diluted to other concentrations.

The calibration curve of the alginate solutions (Figure 21) was measured on a viscometer. It provided a regression equation for calculating the mass concentrations released from the layered material into the water environment.

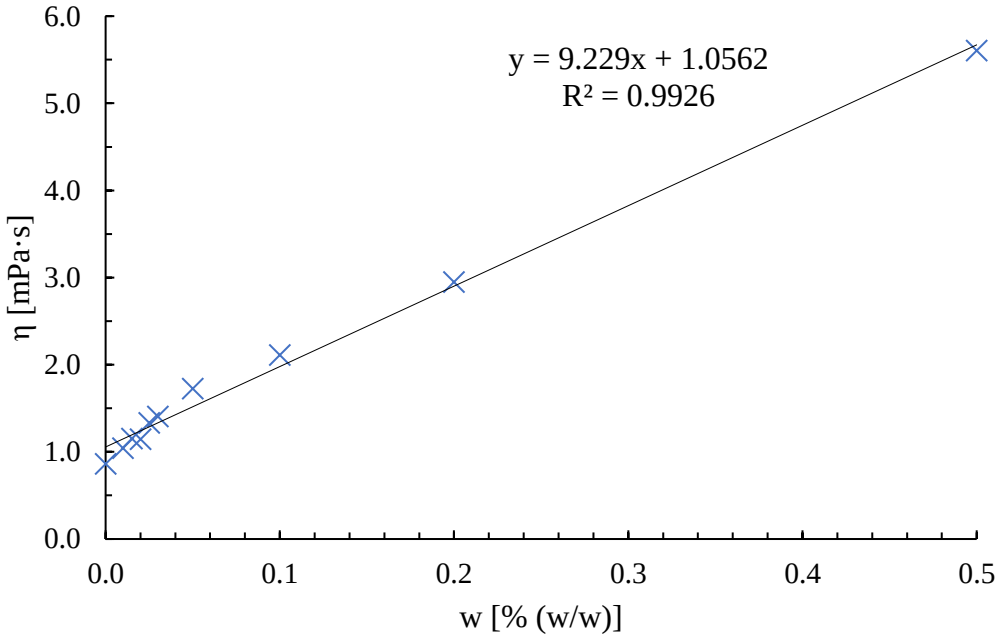

Figure 21. Calibration curve of alginate solutions with regression equation to calculate released alginate concentration.

Likewise, a calibration curve for chitosan solutions (Figure 22) was drawn up, and the regression equation was determined.

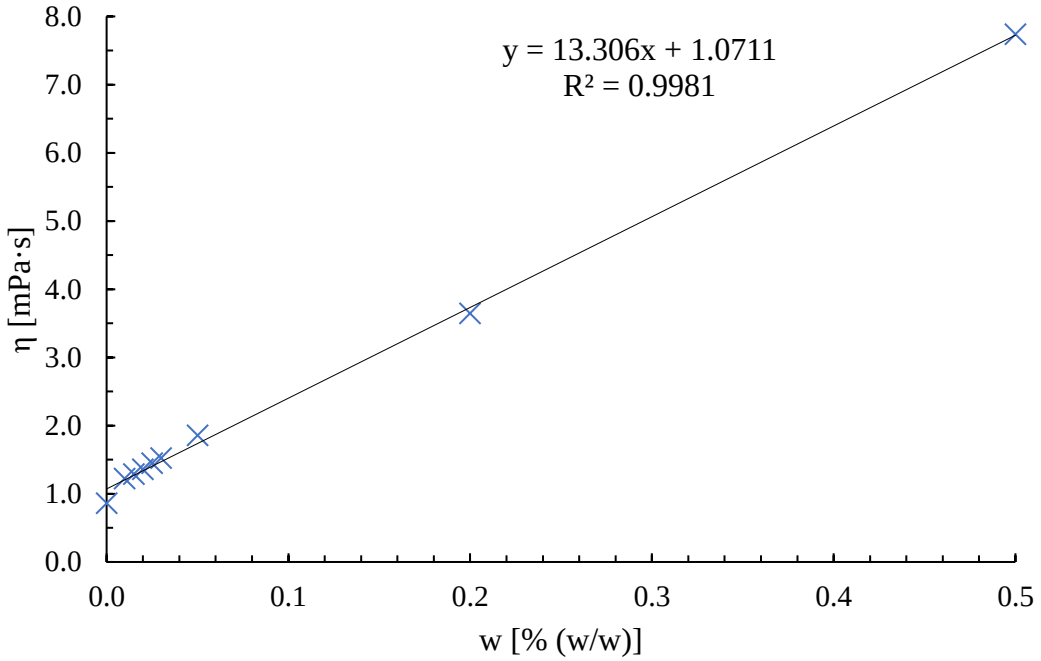

Figure 22. Calibration curve of chitosan solutions with regression equation to calculate released chitosan concentration.

A calibration curve was also created for gelatine solutions to calculate the released amount of gelatine hydrogel. This concentration was calculated using the obtained regression equation (Figure 23).

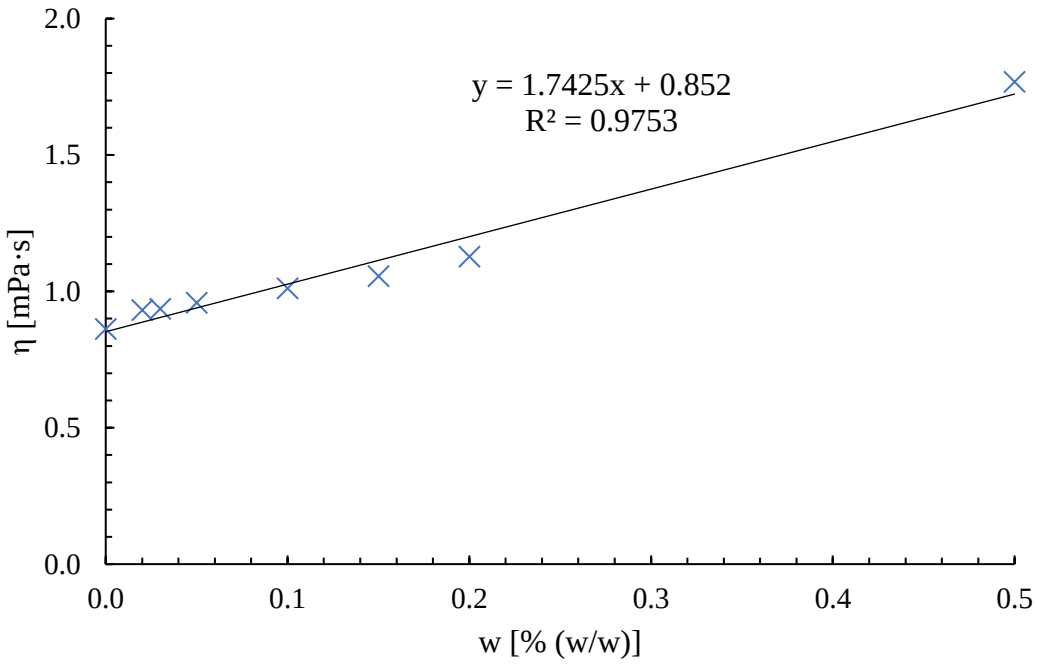

Figure 23. Calibration curve of gelatine solutions with regression equation to calculate released gelatine concentration.

Prepared stock solutions of alginate, chitosan and gelatine were prepared by weighing 0.1 g into a volume of 20 cm^3. In the case of alginate and gelatine solutions, the solutions were diluted with distilled water. For the chitosan solutions, a 5% (v/v) acetic acid solution was used as a solvent. From the stock solutions, calibration series of alginate, chitosan, and gelatine solutions were prepared in a volume of 10 cm^3.

4.6 Preparation of methylene blue solutions

The methylene blue solutions in the diploma thesis are used to determine the diffusion coefficient through the prepared layered membranes, as well as to prepare layered materials with a dye gradient for the study of gradual release.

The methylene blue (Methylene blue hydrate g.r., M_w 319.86 Da, Penta s.r.o.) stock solution was created by weighing 0.02 g of methylene blue, which was dissolved in 500 cm^3 of distilled water. This resulting stock solution had a concentration of 0.04 $g \cdot dm^{-3}$. A calibration series with concentrations of 0.001; 0.003; 0.005; 0.007; 0.01; 0.02 and 0.03 $g \cdot dm^{-3}$ were prepared from this stock solution. These calibration solutions were prepared in 50 cm^3 volumetric flasks.

This calibration series was then averaged on a UV-VIS spectrometer (Hitachi U-3900H Spectrophotometer) to obtain the regression equation. Methylene blue calibration curve samples were measured in the wavelength range 900-300 nm against distilled water (blank). By measuring the methylene blue absorption spectrum (Figure 24), it was found that the maximum absorbance was at 665 nm.

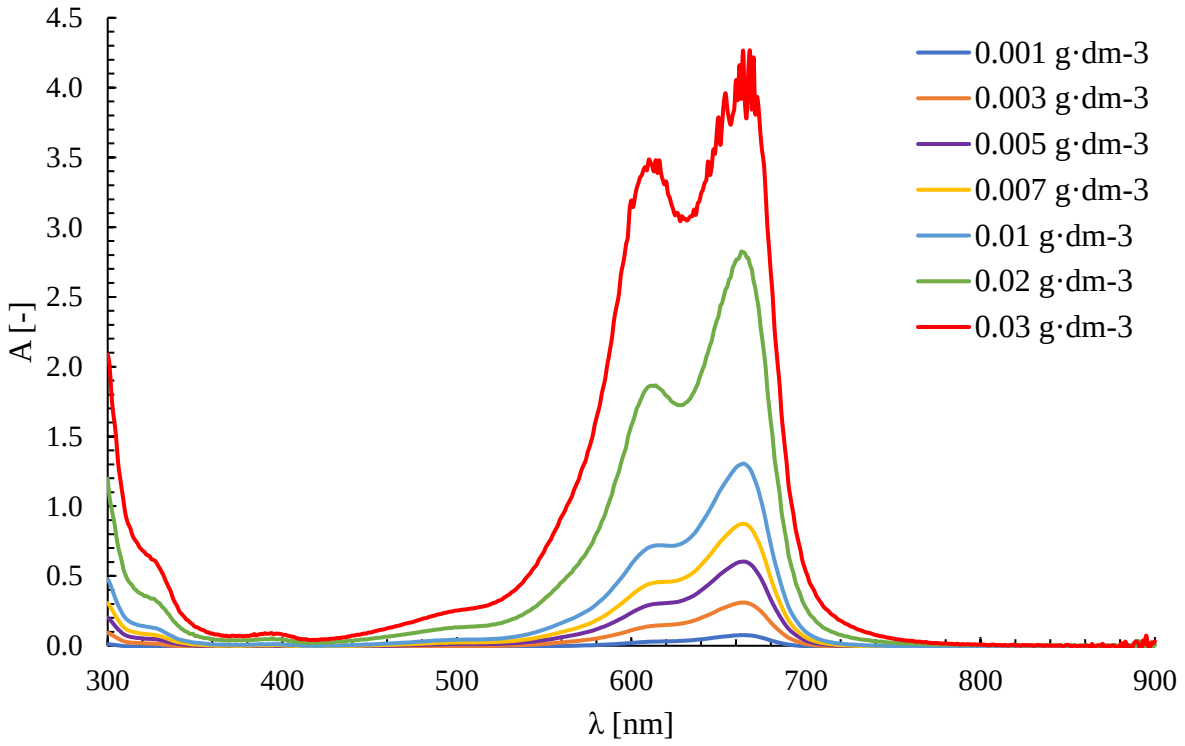

Figure 24. Absorption spectra of the methylene blue calibration series in the range of 900 to 300 nm.

The absorbance values at 665 nm were plotted against the concentration values and thus a calibration curve was created (Figure 25), from which the regression equation was determined, which was further used to calculate the concentration. Additional methylene blue solutions were prepared to form the gelatine polymer solutions containing the dye. The prepared layered material with this coloured polymer was then used to investigate whether there is a gradual and constant release of the dye from the material. Therefore, a concentration series of methylene blue (0.001; 0.003; 0.005; 0.007 and 0.01 $g \cdot dm^{-3}$) was first prepared for these polymer solutions. Therefore, a stock solution with a concentration of 0.01 $g \cdot dm^{-3}$ was prepared by dissolving 0.0025 g of methylene blue in a volumetric flask with a volume of 250 cm^3. From this stock solution, the remaining methylene blue solutions were subsequently created, which were prepared in a volume of 50 cm^3. 1.5 g of gelatine was subsequently weighed into this methylene blue concentration series to form a 3% (w/w) polymer solution, which was dissolved in 50 cm^3 of methylene blue solution. This dissolution again took place in a water bath on a magnetic stirrer with heating (250 rpm, temperature 65 °C) for 10 minutes. In this way, gelatine polymer solutions (3% (w/w)) with different concentration of dye were created for measuring the gradual release.

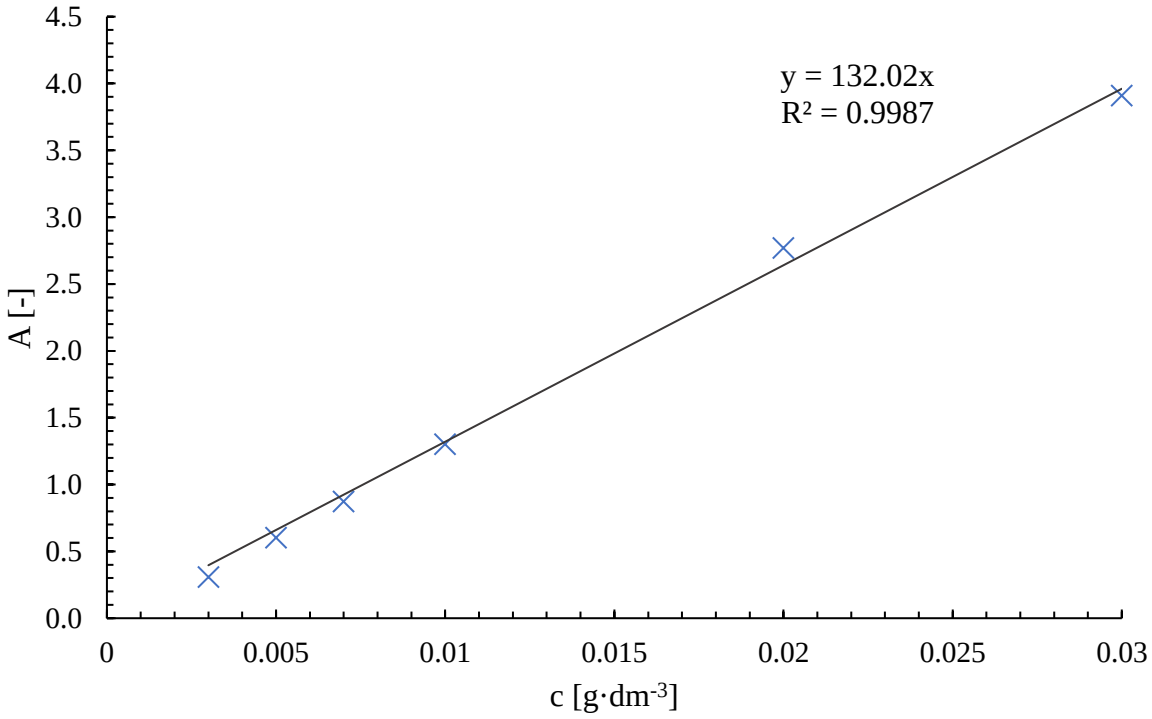

Figure 25. Dependence of absorbance on concentration of methylene blue dye and obtained regression equation.

4.7 Measurement of the diffusion coefficient through a layered membrane

The diffusion coefficient was investigated using diffusion cells. The membrane was prepared by layering the non-woven fabric that was interpenetrated with 3% (w/w) gelatine solution. These membranes differed in the number of layers, namely 1, 2, 4, 8, 16 and 32 layers.

The layered materials were interpenetrated with 3% (w/w) gelatine and allowed to dry for 24 hours. Then they were cut according to the diameter of the diffusion cell into circles (5 cm).

The materials prepared in this way were inserted between two diffusion cells which were inserted into the clamps (Figure 26). Magnetic stirrers were inserted into these diffusion cells. One of these diffusion cells (source cell) was filled with 60 cm^3 of methylene blue solution with a concentration of 0.04 $g \cdot dm^{-3}$. The second cell (receiving cell) was filled with 60 cm^3 of distilled water. These cells were filled with solutions at the same time. Then the diffusion cells were transferred to a magnetic stirrer with 250 rpm. The measurement took place at laboratory temperature. Subsequently, it was waited for the penetration of the dye into the receiving cell. Samples were taken and measured on a UV-VIS spectrometer (Hitachi U-3900H Spectrophotometer).

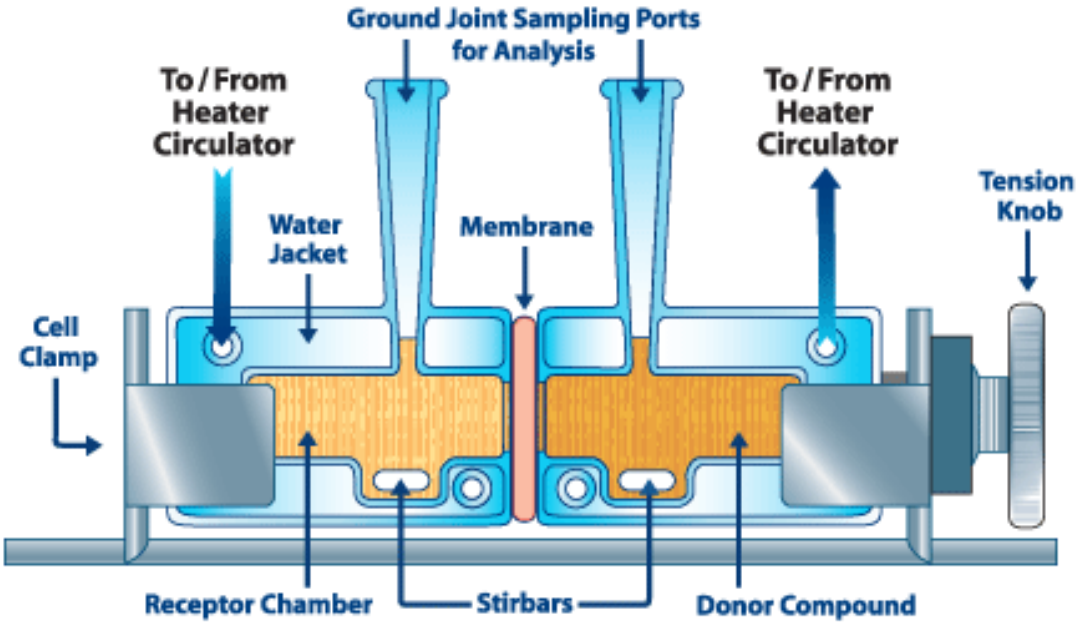

Figure 26. Apparatus of diffusion cells [55].

Methylene blue samples taken from both the receiving and source cells were measured in the wavelength range 900-300 nm using Hitachi U-3900H Spectrophotometer. These samples were measured against distilled water (blank). Absorbance values at a wavelength of 665 nm were determined from the measured methylene blue absorption spectra. These values were then entered into the regression equation and methylene blue concentrations were calculated. The values of the diffusion coefficient were subsequently obtained from these values.

4.8 Measurement of gradient release of methylene blue from layered material

For this measurement, materials with the number of layers of 8, 16 and 32 were prepared (Table **2**). These layers were not connected by pure gelatine polymer solution (3% (w/w)), the gelatine polymer solutions that contained methylene blue dye. The material consisting of 8 layers of non-woven fabric were bonded so that the first layer was coated with gelatine with a dye concentration of 0.001 $g \cdot dm^{-3}$, the second layer was coated with gelatine with a methylene blue concentration of 0.003 $g \cdot dm^{-3}$. A dye concentration of 0.005 $g \cdot dm^{-3}$ bonded two layers of nonwoven fabric, as did a concentration of 0.01 $g \cdot dm^{-3}$. The 16-layer materials were bonded in such a way that the lowest dye concentration (0.001 $g \cdot dm^{-3}$) with gelatine bonded four layers, as well as dye and gelatine concentrations of 0.003 $g \cdot dm^{-3}$ and 0.005 $g \cdot dm^{-3}$. The remaining two concentrations of methylene blue in gelatine (0.007 $g \cdot dm^{-3}$ and 0.01 $g \cdot dm^{-3}$) bonded

three layers of nonwoven fabric. The material, which had 32 layers, was divided as follows: seven layers were bonded with gelatine with a concentration of 0.001 g·dm^{-3}, and the other seven layers were bonded with gelatine with a dye concentration of 0.003 g·dm^{-3}. The remaining gelatine solutions with dye concentrations (0.005, 0.007 and 0.01 g·dm^{-3}) were connected six layers of non-woven fabric.

Table 2. A summary of the preparation of dye gradient layered materials.

n [-]	w_p [% (w/w)]	n_L [-]	c_{MB} [g·dm^{-3}]	V_p [cm^3]	description
8	3	1	0.001	2	The layered materials were prepared from a non-woven fabric (n) that was bonded with a gelatine polymer solution (w_p). This polymer solution had a dye (methylene blue) in it, so polymer solutions with different dye concentrations (c_{MB}) were prepared. Each polymer solution with a certain concentration of methylene blue bonded a certain number of nonwoven fabric layers (n_L).
		1	0.003	2	
		2	0.005	2	
		2	0.007	2	
		2	0.01	2	
16	3	4	0.001	2	
		4	0.003	2	
		4	0.005	2	
		3	0.007	2	
		3	0.01	2	
32	3	7	0.001	2	
		7	0.003	2	
		6	0.005	2	
		6	0.007	2	
		6	0.01	2	

The materials prepared in this way were allowed to dry for 24 hours. These materials were prepared in this way to achieve at least somewhat uniform distribution of the layers. Once the materials were dry, they were cut to a size of 2×3 cm. Subsequently, five of the six sides were sealed with industrial adhesive to prevent diffusion from multiple sides (Figure 27). Again, these materials were allowed to dry for 24 hours to allow the adhesive to dry sufficiently and not loosen.

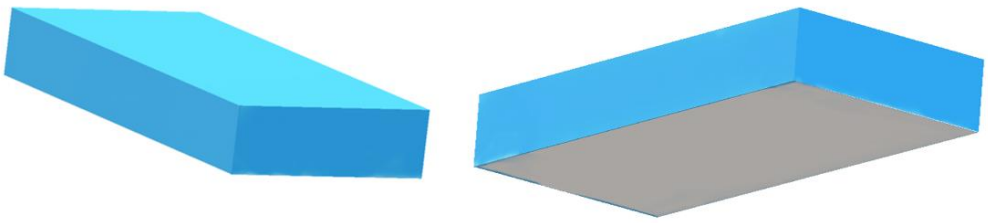

Figure 27. Schematic representation of the prepared layered materials that were glued with technical adhesive (represented by blue colour) to prevent dye diffusion from five sides.

Once these materials were prepared, they were placed in beakers with distilled water (75 cm^3) and magnetic stirrers. The beakers thus prepared were placed on a magnetic stirrer with 250 rpm. Spectrophotometric probes (Optical fibres, Ocean Insight), which read absorbance

values in the 1050-300 nm range every 10 minutes, were inserted into these beakers. The release measurement was carried out for 24 hours, after which it was switched off. To prevent water evaporation, the beakers were secured with parafilm. From these obtained data, absorbance values at 665 nm were read and concentrations were calculated using the regression equation of the calibration curve. The diffusion coefficient was also calculated from these obtained values.

5 RESULTS AND DISCUSSION

In this section, measured results from viscometry and diffusion cells are discussed. In the first part of the work, it was determined how long the materials remained stable, or disintegrated in the water environment. This means that the individual layers of the non-woven fabric joined by the polymer solution did not disconnect from each other. This was investigated because it is desirable that the nonwoven fabric and binder (polymer solution) last as long as possible. In particular, the used polymer should not dissolve or degrade in any way, as an organic dye (methylene blue) was placed in it, which should later be replaced by a drug or other active substance. When it was determined which polymer is most suitable for connecting the layers of nonwoven fabric, layered materials with different numbers of nonwoven fabric layers were prepared to study the diffusion coefficient. At the same time, the release of the dye from the layered material, which contained a gradient of this dye in its structure, and whether a controlled release occurred were also investigated.

5.1 Release of the biopolymers from layered materials

This measurement was carried out to find out whether the given polymer is suitable to be used for connecting non-woven fabric. Due to the potential application in medicine in the future, it is advisable that the polymer be as resistant as possible to release from the layered material.

For this experiment, layered materials were first prepared, where the number of non-woven fabric layers was 16. The selected polymers that interpenetrated the nonwoven fabric layers were chitosan, alginate, and gelatine. A total of five materials were prepared from each polymer solution. They differed in the concentration of the polymer that connected the non-woven fabric. Concentrations of 1, 2, 3, 4 and 5% (w/w) were selected.

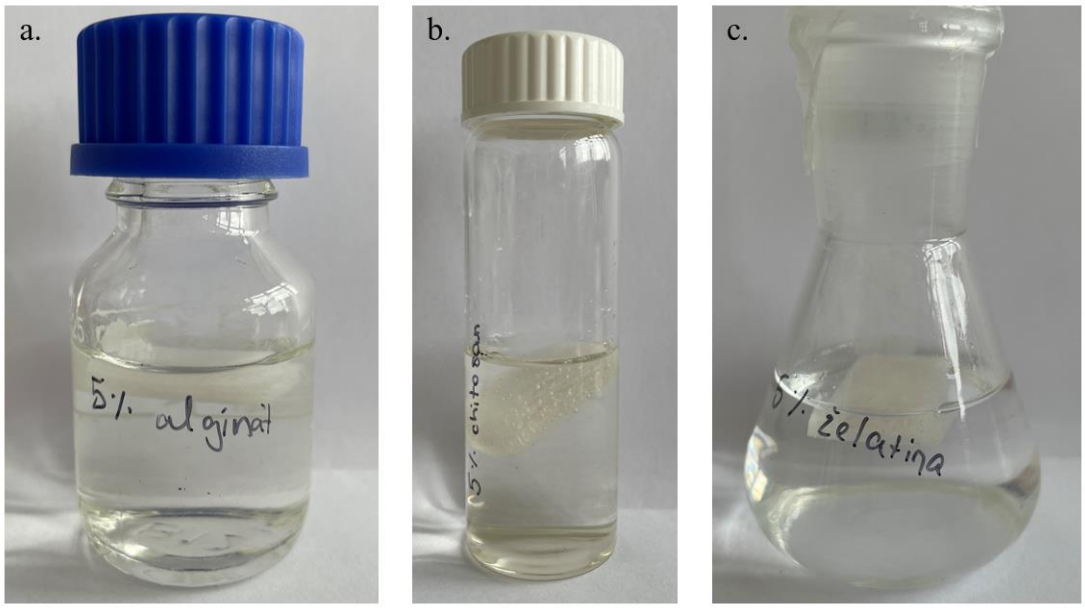

Figure 28. Figures of layered materials after 72 hours of swelling, where a. is layered material connected with 5% (w/w) alginate, b. layered material connected with 5% (w/w) chitosan, and c. layered material connected with 5% (w/w) gelatine.

It was possible to observe that even with different concentrations of the polymer that connected the non-woven fabric, different swelling occurred. This is most likely to be observed with the material with chitosan polymer solution. The alginate swelled approximately two times bigger from the original state in the case of the material with 5% (w/w) polymer solution (Figure 28). In this case, the swelling was not so significant, approximately one time bigger than the original state in the case of the layered material interpenetrated with 3% (w/w) polymer solution (Figure 29) and the material prepared with 1% (w/w) polymer solution (Figure 30) showed swelling again two times bigger from the original state. The gelatine kept the same volume, and there was no visible swelling. This swelling was characterized not only visually, but these materials were weighed before placing in water (m), after swelling (m_s) and repeated drying (m_d).

In the case of the layered materials interpenetrated with alginate (Table 3), the initial weight of the layered materials ranged from 0.09 g to 0.16 g. Their weight in the swelled state ranged from 0.46 g to 0.81 g. The average value by which the layered material swelled was 578 %. Once the materials were dried and weighed, it could be seen that that, compared to the original state, there was a loss of approximately 0.02 g to 0.03 g in each material. The amount of polymer solution released from the material was on average 27 %.

The layered material, which was connected by the biopolymer chitosan of different concentrations (Table 4), showed much greater swelling, which was also evident in the weight. Initially, the weight of these materials ranged from 0.11 g to 0.20 g. In the swollen state, the materials mostly weighed over 1.00 g, only the material bonded with 3% (w/w) chitosan weighed 0.93 g. The percentage by which the layered materials with chitosan polymer solutions swelled on average was 729 %. After drying it was possible to see from the values that an average of 0.02 g was released from each material. The released amount of chitosan polymer solution was also expressed as a percentage, in this case it was 30 %.

The weight of the layered materials connected by biopolymer gelatine (Table 5) in the original state was from 0.10 g to 0.17 g. After the materials were removed from the water after 72 hours, their weight increased to 0.56 g to 0.90 g. Thus, the gelatine-bound layered materials swelled by an average of 532 %. After drying, this the material was reweighed, and it could be seen that again about 0.01 g on average had been released from each material. The average amount of gelatine released from these materials was determined to be 11 %.

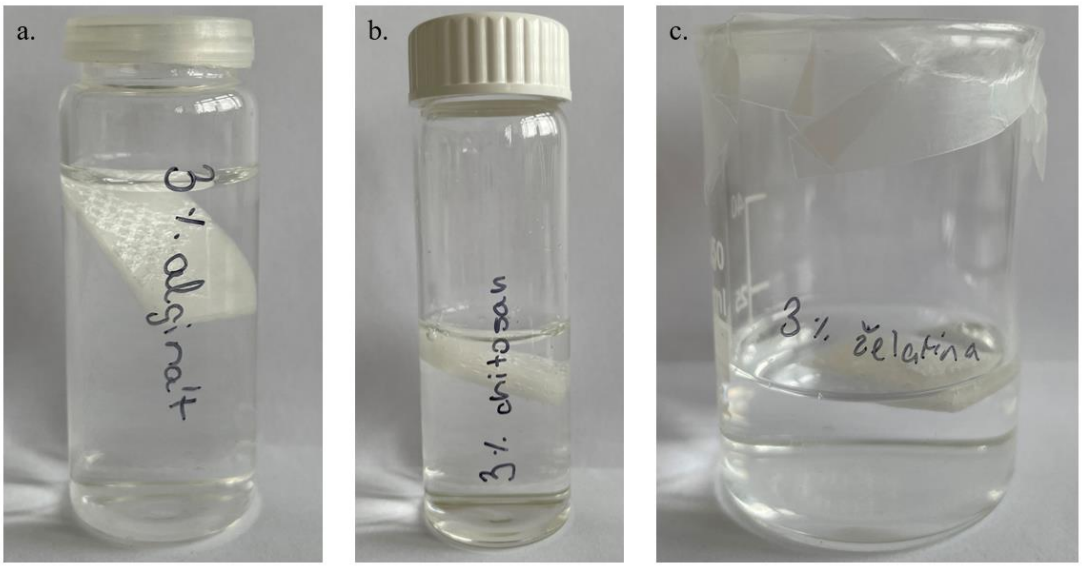

Figure 29. Figures of layered materials after 72 hours of swelling, where a. is layered material connected with 3% (w/w) alginate, b. layered material connected with 3% (w/w) chitosan, and c. layered material connected with 3% (w/w) gelatine.

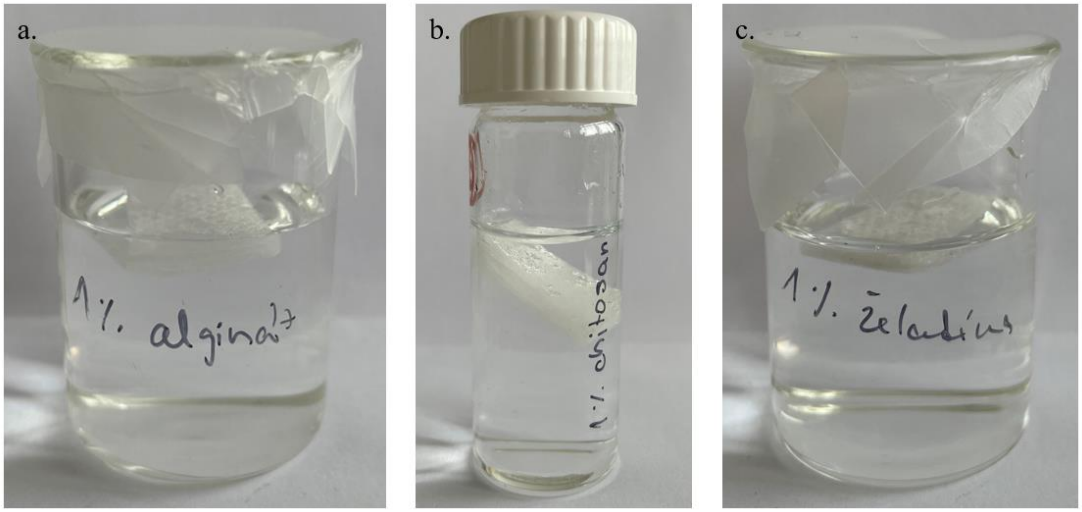

Figure 30. Figures of layered materials after 72 hours of swelling, where a. is layered material connected with 3% (w/w) alginate, b. layered material connected with 3% (w/w) chitosan, and c. layered material connected with 3% (w/w) gelatine.

The degree of swelling (Q) was calculated from the equation (1.14), where m_τ is the mass of the swelling gel at a particular time τ, and m_0 is the initial mass of the xerogel.

$$Q = \frac{m_\tau - m_0}{m_0} \tag{1.14}$$

Alginate swelled slightly after three days of measurement; its swelling coefficient showed no dependence on the concentration used (Table 3). After reweighing the layered material in a dry state, it was found that a relatively large amount of polymer had been released from the material, indicating its instability. The formed layered materials connected by alginate polymer solutions proved to be very unstable. After three days of swelling, the structure disintegrated.

Table 3. Measured weights of layered material with alginate polymer solution with different weight concentrations (w_p). The weight of the layered material was measured in a dry state (m), in a swollen state (m_s) and then again in a dry state after 24 hours of drying (m_d). From these values, the degree of swelling (Q) was calculated.

w_p [% (w/w)]	m [g]	m_s [g]	m_d [g]	Q [-]
1	0.10	0.63	0.08	5.30
2	0.09	0.46	0.06	4.11
3	0.11	0.78	0.10	6.09
4	0.14	0.81	0.11	4.79
5	0.16	0.79	0.09	3.94

The materials interpenetrated with chitosan polymer solutions absorbed a large amount of water within three days, and a significant swelling occurred (Table 4). This limits their potential use in future applications. In the table it is possible to see the individual values of the degree of swelling. Compared to the polymer solution of alginate, it can be seen that the degree of swelling is up to 1× to 2× higher in the case of chitosan. After the measurement, it turned out that, except for the material that was interpenetrated with a 2% (w/w) polymer solution, the individual layers were separated and the material with this polymer does not stick together. This was the first significant limitation for further use.

Table 4. Measured weights of layered material with chitosan polymer solution with different weight concentrations (w_p). The weight of the layered material was measured in a dry state (m), in a swollen state (m_s) and then again in a dry state after 24 hours of drying (m_d). From these values, the degree of swelling (Q) was calculated.

w_p [% (w/w)]	m [g]	m_s [g]	m_d [g]	Q [-]
1	0.11	1.03	0.09	8.36
2	0.12	1.11	0.11	8.25
3	0.15	0.93	0.10	5.20
4	0.18	1.25	0.11	5.94
5	0.20	1.22	0.12	5.10

Layered materials bonded with gelatine polymer solutions showed the similar swelling as layered materials interpenetrated with alginate polymer solutions (Table 5). They did not disintegrate as in the case of materials with alginate and chitosan. Which makes them a very promising material for further investigation of their properties such as diffusion coefficient. Only in the case of layered material bonded with gelatine with a concentration of 5% (w/w) did the top layer of polypropylene peel off. This could have been caused either by insufficient adhesion of the layer during preparation or by handling during the extraction of the material from the water. From the calculated values of the degree of swelling, it is possible to see that the values do not change much with increasing concentration of the polymer solution. Since the materials did not connect after these three days, they were still considered for further measurements.

Table 5. Measured weights of layered material with gelatine polymer solution with different weight concentrations (w_h). The weight of the layered material was measured in a dry state (m), in a swollen state (m_s) and then again in a dry state after 24 hours of drying (m_d). From these values, the degree of swelling (Q) was calculated.

w_h [% (w/w)]	m [g]	m_s [g]	m_d [g]	Q [-]
1	0.10	0.56	0.10	4.60
2	0.12	0.67	0.11	4.58
3	0.13	0.71	0.11	4.46
4	0.13	0.62	0.12	3.77
5	0.17	0.90	0.14	4.29

The measurement was started by determining the density of distilled water on an Anton Paar DMA 4500 device. The device calibrated the samples to a temperature of 25 °C. The calibration series of chitosan, alginate, and gelatine polymer solutions, as well as samples of the released polymer, were subsequently measured on this device. The obtained density values were then used to adjust the drop viscometer, specifically for calculating dynamic viscosity.

Alginate (Table 6) released ranged from 0.04 to 0.025 % (w/w). The percentage of this released amount of polymer from the layered material is about 3.89 %. This makes this material quite unstable compared to other materials. At the same time, a trend was observed where, with the increasing concentration of the polymer that connected the non-woven fabric, a more significant amount of polymer was released into the water environment. During swelling, the layered material disintegrated, which can also be seen in the larger amount of released polymer into the water.

Table 6. Measured values of density (ρ), dynamic viscosity (η) and calculated concentration of the released alginate polymer (w) from the layered material (w_p). Calculated percentage amount of released polymer from the 16-layered material (x).

w_p [% (w/w)]	ρ [g·cm^{-3}]	η [mPa·s]	w [% (w/w)]	x [%]
1	0.99737	1.4450	0.042	4.21
2	0.99741	1.4367	0.041	2.06
3	0.99779	2.1543	0.119	3.97
4	0.99811	2.7754	0.186	4.66
5	0.99859	3.1545	0.227	4.55

The amount of released chitosan from the layered material depended on the increasing concentration of the polymer solution used (Table 7), which bonded the layers of the non-woven fabric. The more significant chitosan release was due to the layered material's swelling, causing it to break up and the polymer was released from more surfaces than if it had remained intact.

Table 7. Measured values of density (ρ), dynamic viscosity (η) and calculated concentration of the released chitosan polymer (w) from the layered material (w_p). Calculated percentage amount of released polymer from the 16-layered material (x).

w_p [% (w/w)]	ρ [g·cm^{-3}]	η [mPa·s]	w [% (w/w)]	x [%]
1	0.99760	1.4486	0.028	2.84
2	0.99766	1.6609	0.066	3.28
3	0.99777	2.0694	0.110	3.66
4	0.99820	3.0376	0.215	5.37
5	0.99865	3.7538	0.292	5.85

The layered materials bonded with gelatine did not swell even after three days and kept their structure (Table 8). Only in the case of the material connected with 5% polymer solution did the last layer of the non-woven fabric peel off, which was probably caused by insufficient bonding. Thus, the polymer was not released into the water from a larger area, as with layered materials with alginate and chitosan. This made the layered materials a very suitable material for future use.

Table 8. Measured values of density (ρ), dynamic viscosity (η) and calculated concentration of the released gelatine polymer (w) from the layered material (w_p). Calculated percentage amount of released polymer from the 16-layered material (x).

w_p [% (w/w)]	ρ [g·cm^{-3}]	η [mPa·s]	w [% (w/w)]	x [%]
1	0.99729	0.9403	0.051	5.07
2	0.99731	0.9318	0.046	2.29
3	0.99734	0.9320	0.046	1.53
4	0.99729	0.9273	0.043	1.08
5	0.99740	0.9406	0.051	1.02

By interpolating the released concentration in the water environment to the used concentration with which the layered material was connected (Figure 31), it was possible to find out that there was a slightly bigger release in the case of alginate and chitosan of the polymer from the material. The average excluded amounts of polymers were 3.89 % for alginate, 3.06 % for chitosan and 2.20 % for gelatine. Especially in the case of alginate and chitosan, the dependence on molecular weight can be seen. The alginate used had a molecular weight of 100 kDa and chitosan 168 kDa. Non-woven polypropylene fabric in its structure contains pores of approximately 20 μm [77]. Therefore, as the size of the molecules increases, their release from this material decreases. In the case of gelatine, which had a molecular weight of 50 to 100 kDa (this range was given by the manufacturer), the release was still constant regardless of the polymer concentration. This polymer was, therefore, still used in the subsequent measurement. In the case of polymer solutions of alginate, and gelatine that bonded the layered materials, the percentage released was found to be similar. Therefore, the use of both biopolymers in further experiments could be considered. However, the alginate-bonded materials were disconnected after this measurement, and it was important for further consideration and use that the layered material held together. The chitosan-bonded layered materials also disconnected, making them unsuitable for further measurements.

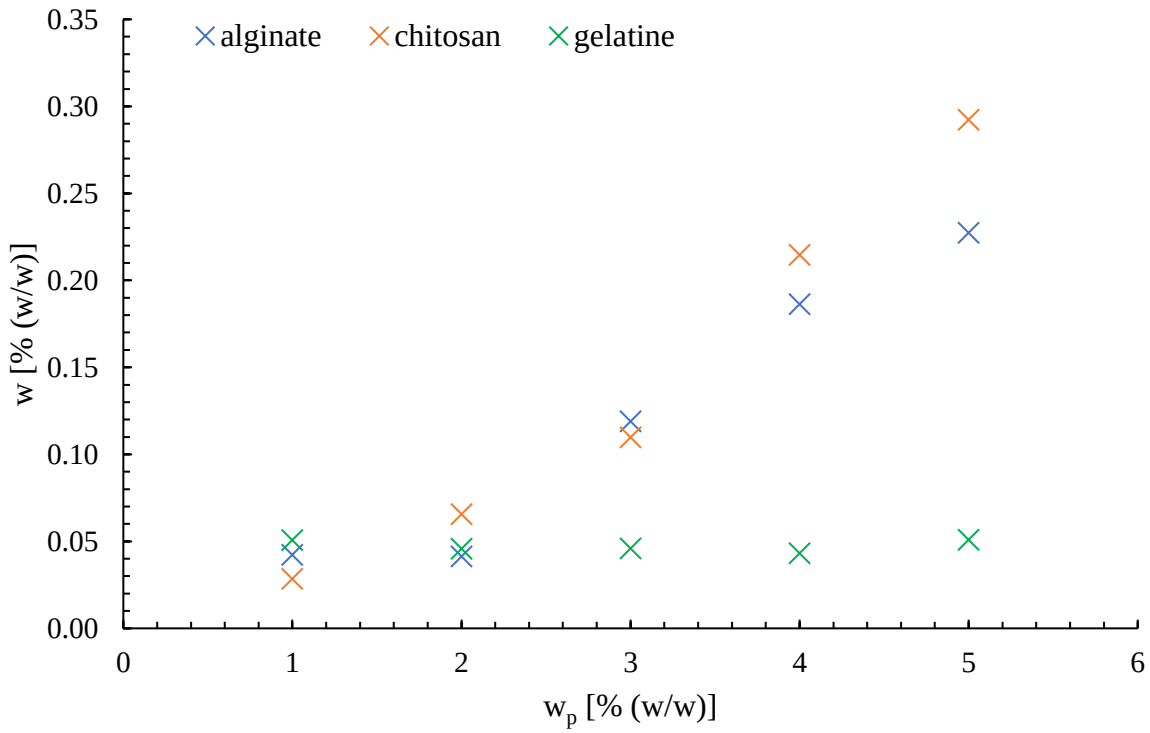

Figure 31. Dependence of the concentration of the polymer released (w) from the original amount (alginate, chitosan, and gelatine) into the water environment on the concentration of the polymer (w_p) used to connect the non-woven fabric (original amount).

5.2 Measurement of the diffusion coefficient through a layered membrane

The effective diffusion coefficient was measured by measuring the UV-VIS spectra of an organic dye (methylene blue), which passed into the receiving cell through the prepared membrane. From the previous experiment, it was concluded that the most stable non-woven fabric binder was gelatine polymer. The mass concentration of gelatine was chosen to be 3% (w/w), because it was a layered material, where the polymer was still quite strong and there was no separation of the individual layers. Membranes made of non-woven fabric (polypropylene) interpenetrated by 3% (w/w) gelatine polymer solution were prepared with the following number of layers: 2, 4, 8, 16 and 32.

The layered materials were prepared by joining the layers of non-woven fabric with a size of 8×8 cm. The membranes prepared in this way (8, 16 and 32 layers) were allowed to dry for 24 hours, then they were cut according to the shape of diffusion cells (PermeGear, Inc.) with a diameter of 5 cm. The area over which methylene blue transport took place was 12.55 cm^2. The prepared materials were inserted between the diffusion cells, the receiving cells were filled with 60 cm^3 of distilled water and the source 60 ml of methylene blue with a concentration of 0.04 $g \cdot dm^{-3}$. These prepared diffusion cells were placed on a magnetic stirrer with 250 rpm at a room temperature and individual samples were taken according to how fast the permeation of the dye occurred.

First, it was necessary to measure the absorbance of the methylene blue calibration series. The calibration series was therefore prepared with concentrations of 0.001; 0.003; 0.005; 0.007; 0.01; 0.02; 0.03 and 0.04 $g \cdot dm^{-3}$. This calibration series was measured on a UV-VIS

spectrometer (Hitachi U-3900H Spectrophotometer) in the wavelength range of 900 to 300 nm. Thanks to this measurement, absorption spectra (Figure 24) were obtained, on which it was found that the methylene blue absorption maximum was at 665 nm.

By finding this absorption maximum, the absorbance values (665 nm) were plotted as a function of the concentration of methylene blue (Figure 25). By interpolating this dependence, a regression equation was obtained, which was further used to calculate the concentration to determine the diffusion coefficient.

The formed membrane consisting of two layers (thickness was 0,0125 mm) was inserted between the diffusion cells. Dye penetration (methylene blue) was observed in the receiving cell already after the first minute of measurement. These samples were measured on a UV-VIS spectrometer (Hitachi U-3900H Spectrophotometer). Additional samples (3 cm^3 each) were taken after 1, 2 and 3 hours and were also measured on a UV-VIS spectrophotometer. The obtained values were important for determining the effective diffusion coefficient. In the case of this two-layer membrane, the effective diffusion coefficient was $1.2 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$.

The second material was a four-layer membrane with thickness 0,025 mm, which was again inserted between the diffusion cells and allowed to permeate the selected dye. The first penetration of the dye occurred after 2 hours from the beginning of the measurement. Again, the first samples, again 3 cm^3, were taken from both cells. These were again measured on a UV-VIS spectrometer. The samples taken after 3 hours and 24 hours from the beginning of the measurement were measured in the same way. From these values, the effective diffusion coefficient was determined to be $25.8 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$.

After the measurement of the first two membranes with two and four layers of non-woven fabric was completed, the membranes were allowed to dry for 24 hours, after which small residues of methylene blue could be observed on them (Figure 32). Therefore, it was possible to conclude that there is a reaction between the negatively charged dye (methylene blue) and the positively charged amino groups that occur in gelatine. At the same time, the pore size in the gelatine hydrogel ranges from 5 nm to 20 nm [78].

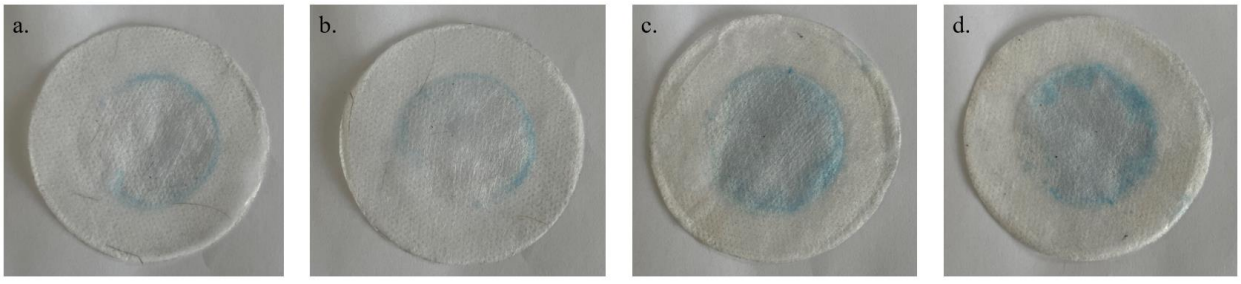

Figure 32. Membrane taken after measuring the diffusion coefficient of the number of 2 layers of non-woven fabric (a.) from the side of the source cell, (b.) receiving cell. Likewise, a membrane composed of 4 layers of non-woven fabric (c.) from the side of the source cell and (d.) the receiving cell.

The eight-layer material (thickness 0,05 mm) was placed like the previous materials between the diffusion cells, which were then filled with a solution of methylene blue (0.04 $g \cdot dm^{-3}$) in the source cell and distilled water in the receiving cell (Figure 33). Penetration of the methylene blue dye through this membrane occurred after 3 hours and at the same time the first sampling

(3 cm^3) took place, which were again measured on a UV-VIS spectrometer. The next sample collection took place 8 hours after the beginning of the measurement. The last sampling was after 24 hours. From the measured values, the effective diffusion coefficient was found to be $28.0 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$.

Figure 33. Measurement of the diffusion coefficient on side-bi-side cells with a membrane consisting of 8 layers of non-woven fabric connected by 3% (w/w) gelatine hydrogel after 8 hours.

The measurement was terminated after the system reached equilibrium. The removed membrane was allowed to dry again for 24 hours. It was then possible to observe that the membrane with this number of layers was able to retain a relatively large amount of methylene blue (Figure 34). Specifically, the retained concentration of methylene blue was 0.03 $g \cdot dm^{-3}$. The methylene blue concentration that was in the membrane after diffusion was calculated by subtracting the methylene blue concentration in the receiving cell from the methylene blue concentration in the source cell.

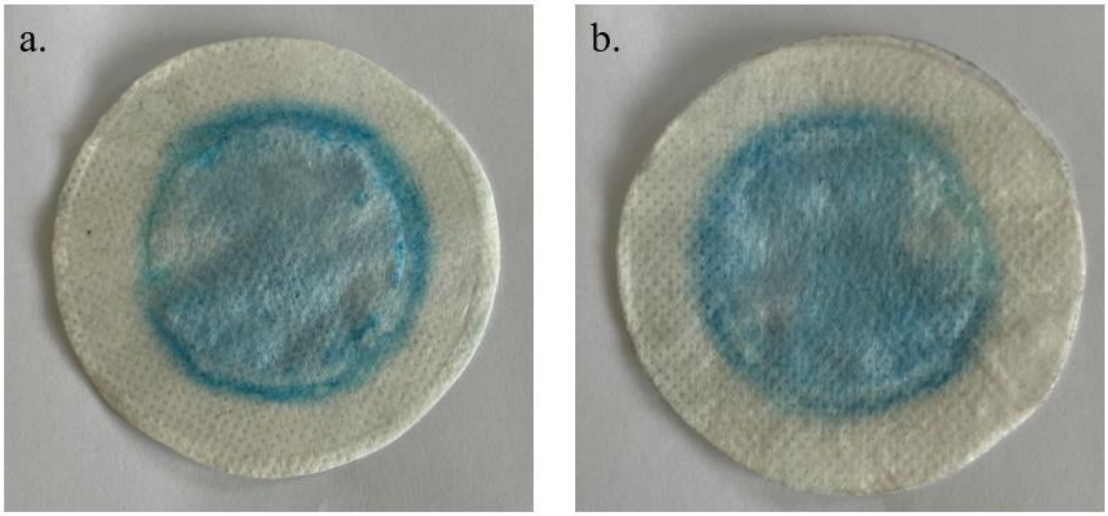

Figure 34. Membrane taken after measuring the diffusion coefficient of the number of 8 layers of non-woven fabric (a.) from the side of the source cell, (b.) receiving cell.

The material composed of sixteen layers was set in the diffusion cells in the same way with the same solution (methylene blue with a concentration of 0.04 $g \cdot dm^{-3}$) in the source cell and distilled water in the receiving cell. The penetration of methylene blue through the formed membrane with a thickness of 0.1 cm occurred after about six hours from the beginning of the measurement. After this time, the first 3 cm^3 from the receiver cell and 3 cm^3 from the source cell were also withdrawn. Another sampling of the same amount occurred after 36 hours. This was followed by measurement of the samples taken on a UV-VIS spectrometer. The obtained values were used to calculate the effective diffusion coefficient, which in the case of a 16-layered membrane was set at $27.2 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$.

The penetration of methylene blue through the 16-layer membrane made of non-woven fabric and hydrogel did not reach an equilibrium state even after 36 hours. From the samples taken during this time, it was possible to determine the effective diffusion coefficient. The membrane was left to dry for 24 hours, and it is possible to observe that the methylene blue has created various paths through the pores of the hydrogel and thus does not pass directly through the membrane (Figure 35). The retained concentration of methylene blue in this material was 0.012 $g \cdot dm^{-3}$. This leads to a significant slowing down of the passage of methylene blue, which is highly desirable in the case of controlled dye release.

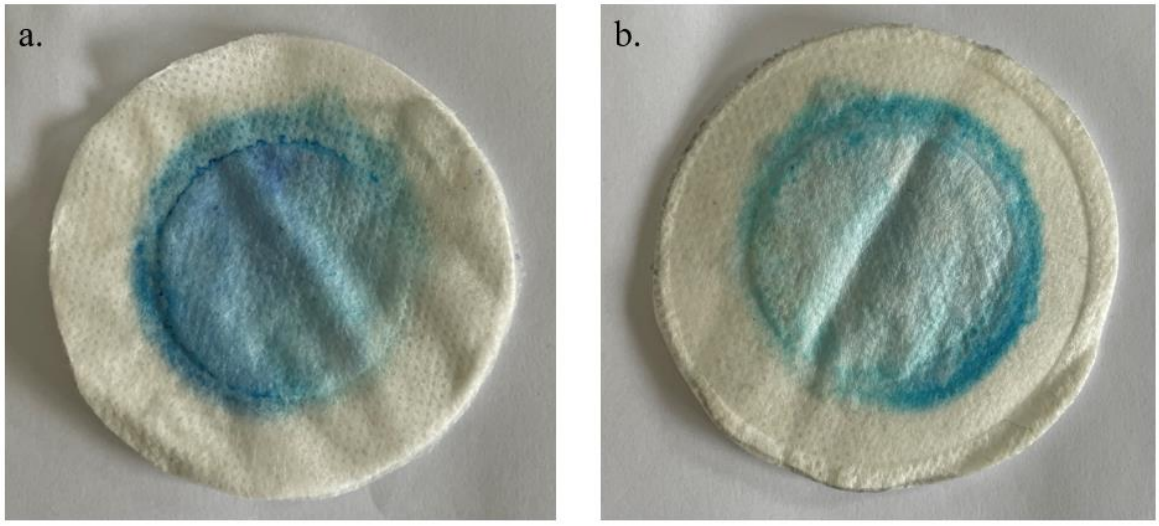

Figure 35. Membrane taken after measuring the diffusion coefficient of the number of 16 layers of non-woven fabric (a.) from the side of the source cell, (b.) receiving cell.

The last membrane used was a material composed of 32 layers of non-woven fabric (polypropylene) connected by 3% (w/w) gelatine polymer solution, this material was 0.2 cm thick. This material was placed between diffusion cells, which were then filled with methylene blue solution (0.04 $g \cdot dm^{-3}$) in the source cell and distilled water in the receiving cell. The first penetration of the dye into the receiving cell occurred after 8 hours. In this case, the dye concentration in the receiving cell was very small and the UV-VIS spectrometer would probably not have recorded this value. After this time, the first samples were also taken from the receiving and source cells. Additional samples (3 ml from source and 3 ml from receiving cell) were taken 24 hours after the beginning of the measurement. The effective diffusion coefficient was calculated to be $25.5 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$.

On the material composed of 32 layers, after drying, it was again possible to see that methylene blue diffused through the gelatine hydrogel in random paths (Figure 36). At the same time, it is again possible to see that a certain amount of methylene blue always remained in the polymer. This could again be due to opposite charges attracting each other, the negative charge belonging to the dye and the positive charge to the gelatine. In this case, the amount of methylene blue retained was 0.0077 $g \cdot dm^{-3}$.

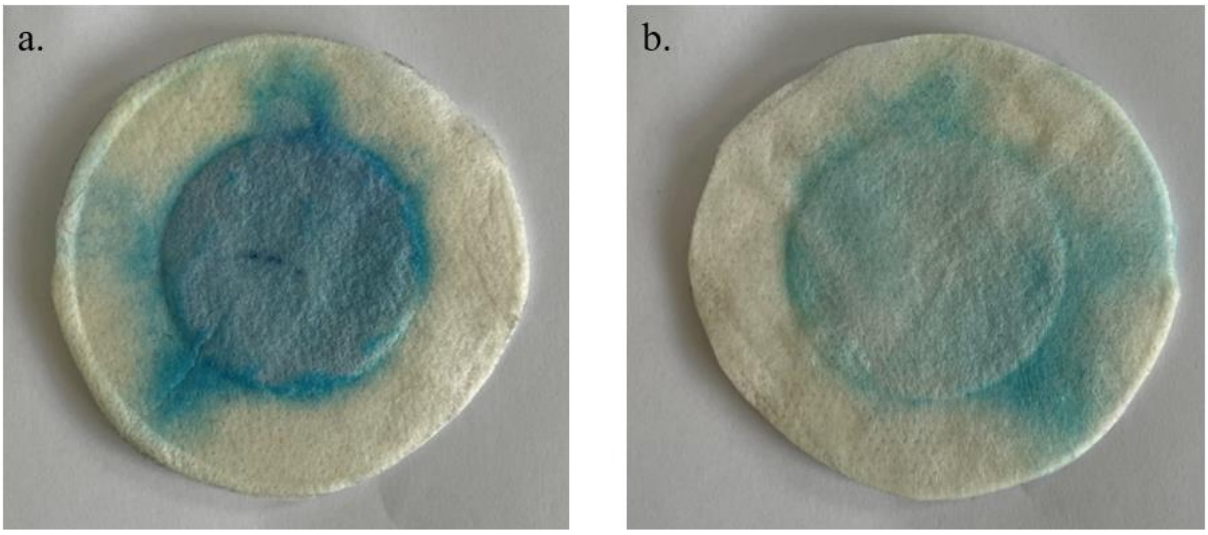

Figure 36. Membrane taken after measuring the diffusion coefficient of the number of 32 layers of non-woven fabric (a.) from the side of the source cell, (b.) receiving cell.

From the measurement of the effective diffusion coefficient (Table 9), it was found that in the case of the two-layered membrane, the diffusion was very fast. The material only had a thin layer of gelatine, so the dye didn't have to find a very long path, it also gave the impression that the dye went through a direct path. Therefore, it was possible to observe the passage of methylene blue within the first minute. The difference already occurred in the membrane composed of four layers, where a larger amount of polymer was used to connect the non-woven fabric. At the same time, the hydrogel most likely penetrated the pores of the non-woven fabric (approximately 20 µm [77]) and created more intricate paths through which the dye must diffuse. In the case of these two materials, there was still no retention of a large amount of methylene blue, this could be due to the membrane being still thin enough, so that the solvent (water) passed through it more easily. The obtained values of the effective diffusion coefficient for membranes with the number of layers 8, 16, and 32 showed that diffusion took place at approximately the same rate in these materials.

In all three materials, a certain amount of dye remained incorporated after diffusion, which was caused by the interaction of oppositely charged substances, negative methylene blue and positively charged amino groups of gelatine. Especially on materials from 16 and 32 layers, it was then possible to see that the passage of the dye did not take place in a direct way and tortuosity must be considered. It was possible to observe that the dye (methylene blue) was retained in the formed membranes. In the two-layered material, after establishing equilibrium, it was 0.0006 $g \cdot dm^{-3}$, in the four-layered material 0.0007 $g \cdot dm^{-3}$. Another measurement that reached equilibrium was in the case of a measurement with an eight-layer membrane. After this measurement, there was 0.03 $g \cdot dm^{-3}$ methylene blue remaining in the membrane. In the case of

materials from 16 and 32 layers, this measurement did not reach full equilibrium. Therefore, in the case of the 16-layered membrane, the amount of methylene blue in the structure was 0.012 $g \cdot dm^{-3}$. The 32-layered membrane had a methylene blue concentration equal to 0.0077 $g \cdot dm^{-3}$. If these systems were allowed to reach a state of equilibrium, the concentration retained in the membranes would certainly increase linearly.

Table 9. Values of the effective diffusion coefficient ($\varepsilon \cdot D_{eff}$) for individual membranes and the concentration of methylene blue inside the membrane (c_m).

number of layers	$\varepsilon \cdot D_{eff}$ [10^{-9} $m^2 \cdot s^{-1}$]	c_m [$g \cdot dm^{-3}$]
2	1.2	0.0006
4	25.8	0.0007
8	28.0	0.0300
16	27.2	0.0120
32	25.5	0.0077

5.3 Measurement of gradient release of methylene blue from layered material

Eight-layered, sixteen-layered, and thirty-two-layered materials were prepared by combining nonwoven fabric and 3% (w/w) gelatine polymer solution. This yarn was not a pure polymer solution, but a polymer that contained an organic dye (methylene blue). Specifically, polymer solutions were prepared with a methylene blue concentration of 0.001; 0.003; 0.005; 0.007 and 0.01 $g \cdot dm^{-3}$. Thus, these prepared polymer solutions combined these materials. The prepared materials were then placed in 75 cm^3 of distilled water, into which the dye was released from these layered materials. This was done because, for the time being, materials were created only with a constant source of dye, drug, or other active substance.

As it was found out from the research, during the release of substances from such materials, a so-called burst effect occurred, which is undesirable in the delivery of substances to the desired place. The solution to this problem could be the preparation of a layered material with a dye gradient in this case. The materials were prepared in such a way that the release occurred from only one side. This would first absorb the water that first passed through the material. Subsequently, there was an equalization of the concentration because the free side of the material was the one with the lowest concentration, and the higher concentration of methylene blue gradually diffused into the layers with a lower concentration.

From the absorbance values obtained, the concentration values at the specified times were calculated by recalculation using the methylene blue regression equation. These concentration values were then plotted against time (Figure 37) and it was possible to see how the release of methylene blue proceeded. From this obtained dependence, it took approximately 180 minutes for the actual release of the dye to occur. This is attributed to the penetration of water into the layered material. This was followed by the release of methylene blue at a linear rate, which indicates a gradual equalization of the dye concentrations in the material. Around the time of 310 minutes, it is possible to observe the beginning of the bending of this dependence, this was because almost all the dye from the material was already in the water environment.

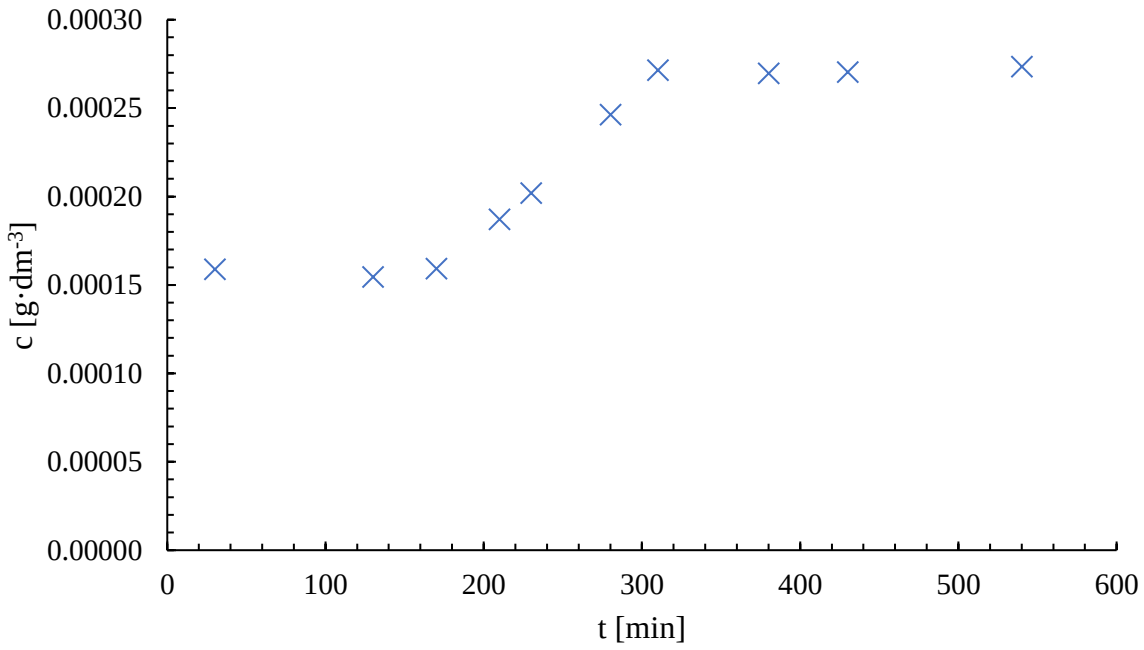

Figure 37. Dependence of the concentration of methylene blue released from the 8-layered material on time.

The dependence of the released concentration of methylene blue on time was then created from these data (Figure 38). On the graph it is possible to see that the release of methylene blue started approximately after 700 minutes of measurement, then the concentration was released at a linear rate. After about 1700 minutes, it is possible to observe a slight bending of the dependence, which was probably almost all the concentration of the dye from the material in the environment of distilled water.

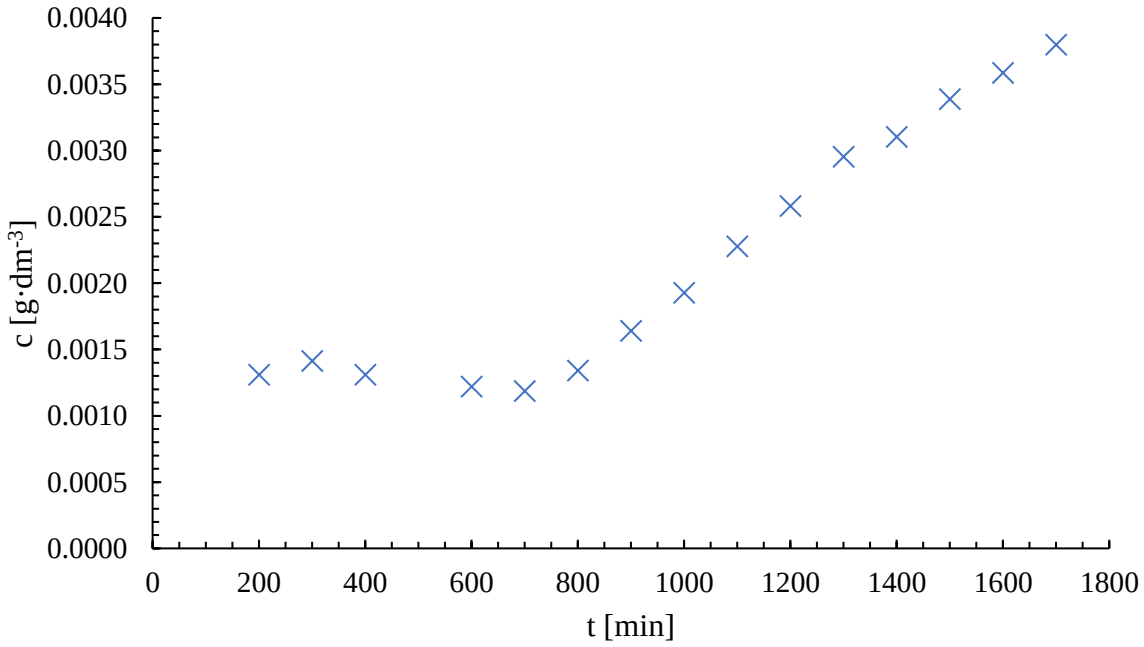

Figure 38. Dependence of the concentration of methylene blue released from the 16-layered material on time.

The last material measured in this way was a 32-layered material with a dye gradient. The dependence of methylene blue concentration on time was then created from these data (Figure **39**). This material released the first amount of dye after about 1600 minutes, after which the release rate was linear until about 2500 minutes. At this point, the bending of this dependence began to occur again, since almost all the dye had already been excluded from the material.

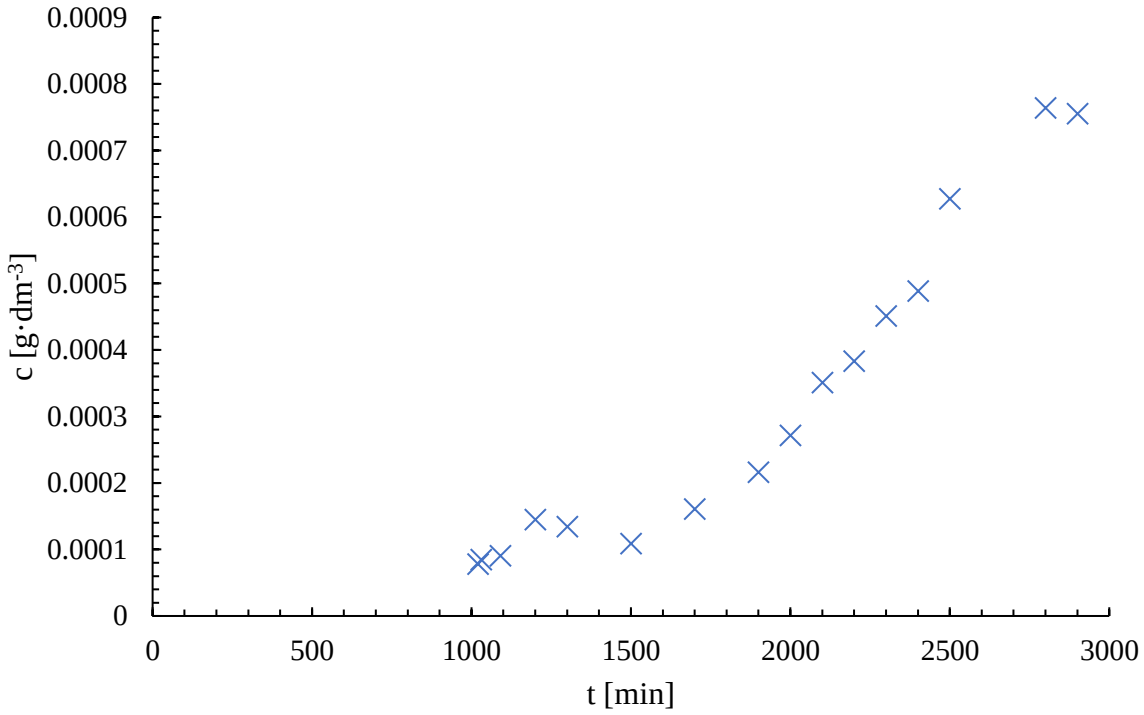

Figure 39. Dependence of the concentration of methylene blue released from the 32-layered material on time.

The effective diffusion coefficient (Table 10) was subsequently calculated from the linear regions of these dependencies of methylene blue concentration on time. For the 8 layered material connected by 3% (w/w) gelatine polymer solution, the effective diffusion coefficient was determined to be $15.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. The layered material consisted of 16 layers of non-woven fabric connected by gelatine polymer solution (3% (w/w)) had an effective diffusion coefficient of $3.5 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. The last material of 32 layers of non-woven fabric connected by 3% (w/w) gelatine polymer solution had an effective diffusion coefficient of $1.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. From the measurements of layered materials with a gradient, the effective diffusion coefficient was again obtained for each material. Again, using Excel and the correlation function, in this case showed a value of 0.13, which indicates that in this case, as the number of layers increases, the diffusion rate of methylene blue through the layered materials increases, but this the number is very close to 0 and that means there is almost no dependence.

Table 10. Values of the effective diffusion coefficient ($\varepsilon \cdot D_{eff}$) for individual membranes.

number of layers	**$\varepsilon \cdot De_{ff}$ [10^{-9} $m^2 \cdot s^{-1}$]**
8	15.3
16	3.5
32	1.3

In each of these measurements, it was possible to observe that the release values did not start from zero value. This may have been due to insufficient insulation of the vessels to prevent the measurement from being influenced by light, which varied during the day.

6 CONCLUSION

The diploma thesis focused on the issue of effective delivery, transport, and release of active substances from layered material interpenetrated with polymer solutions. The release of drugs from the layered material interpenetrated polymer solutions has the disadvantage that a so-called burst effect occurs, which appears after only a short time and a large amount of drug is expelled from the polymer. During the thesis, layered materials from non-woven fabric were prepared and this non-woven fabric was interpenetrated with polymer solutions. The release was investigated by adding an organic dye (methylene blue) to the materials and monitoring the release of this dye using UV-VIS spectrometry. Currently, there is a problem with the effective delivery of drugs to the human body. Main problem is called the burst effect, which causes a large amount of medication to be released in a short time. This problem was solved in this diploma thesis using layered materials, which had a dye gradient in its structure (polymer solution). It was considered here that the preparation of such materials would lead to a gradual replenishment of the dye. In this way, the burst effect could at least be minimized.

There are many articles in the current literature that deal with the issue of the burst effect [70]-[76]. These are mostly materials prepared from polymers and hydrogels, which are often combined in order to join the good properties (biocompatibility, biodegradability) of both materials and reduce the bad ones (mechanical strength, toxic products of degradation). This mainly means ensuring stability, better mechanical properties or improving biocompatibility and biodegradability. Although these prepared materials try to solve the already mentioned problem, a constant concentration of either a drug or another substance is always used in these materials, which enables easy detection (organic dye, etc.). That is why the mentioned burst effect still appears in these materials. As materials with a constant concentration have been investigated so far, it was assumed that the use of a concentration gradient in the prepared material could solve this problem.

First, it was investigated which of the selected biopolymers (alginate, chitosan, and gelatine) is the most suitable for connecting these non-woven fabrics. From the measurements, it was found that gelatine polymer solutions best meet the requirements for further use. Among these requirements was that the materials should last in the water environment and that the polymer itself would not diffuse out of the layered material, as this polymer further served as a carrier for the dye. At the same time, it was important for the material to remain intact and for individual layers of the non-woven fabric to not peel off, as these are materials being prepared for potential transdermal application of the drug and should be some form of patch. Especially for longer-term application, it is advisable for the material to be as stable as possible. If the problem of the burst effect is solved, there would be a significant reduction in the repeated application of the drug, or in this case the repeated exchange of the entire material with the drug. In the case of materials with a burst effect, this means for patients that they have to apply the medicine more often and this can also lead to a negative impact on the human body, especially on the liver.

After finding out what polymer will be used next (gelatine was chosen for further analysis), layered materials with different number of layers were made (from two to thirty-two layers). This was important in order to determine the effective diffusion coefficient of the prepared membrane, i.e. how diffusion takes place through this material. The material composed of two

layers had an effective diffusion coefficient set at $1.2 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$, while the four-layer material had an effective diffusion coefficient of $25.8 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. The prepared layered material consisting of 8 layers of non-woven fabric connected by a gelatine polymer solution had an effective diffusion coefficient of $28.0 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$, the sixteen-layered material had this effective diffusion coefficient determined at $27.2 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ and the material from 32 layers of non-woven fabric had it calculated to $25.5 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. And then the data was correlated. This led to the finding that there is only a small dependence, such that the rate of diffusion decreases as the number of layers increases. But it can be seen from the obtained values of the effective diffusion coefficient that the value is rather constant for materials with a number of layers greater than 4. There is therefore an assumption that other materials prepared from more than 32 layers will have an effective diffusion coefficient that will always be approximately the same as in the case of these materials. But this will have to be investigated further, whether there will be any changes in the diffusion through the membrane.

Methylene blue uptake was observed in all layered materials. This could be due to the methylene blue reacting with the gelatine as they are substances with opposite charges. Therefore, it is important to always consider that the dye will not be completely released from the structure in the next part of the measurement. This is an important finding for the future application of the drug in these materials because the dosage of the drug in these materials will have to be adapted to this.

In the case of measuring the diffusion of methylene blue from layered materials, in which methylene blue was incorporated in a gelatine polymer solution, it was possible to observe that the effective diffusion coefficient decreased with the increasing number of layers. The effective diffusion coefficient of methylene blue from the eight-layer material was determined to be $15.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$, for 16 layered material it was $3.5 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$, and the last material had an effective diffusion coefficient calculated to $1.3 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$. This may be because the dye was already contained in the materials and did not have to wait for the permeation of this dye through the entire membrane. That is why the first dye concentrates were captured even earlier. At the same time, in theory, it is possible to see a graph that shows what the release curve looks like when an unwanted burst effect occurs. From the graphs obtained during this measurement, it is possible to see that this bending of the linear part of the curve did not occur. It is therefore the first step to solve the problem of the burst effect and create a material that will be able to deliver the drug with greater efficiency.

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8 LIST OF ABBREVIATIONS

NH_2	amino group
COOH	carboxy group
SO_3H	sulfone group
PVA	poly (vinyl alcohol)
PAM	polyacrylamide
IPN	interpenetrating polymer hydrogel
semi-IPN	semi-interpenetrating hydrogel
Na^+	sodium cation
Ca^{2+}	calcium cation
E	Young's modulus
σ	tensile strength
ε_f	strain at failure
F	compressive strength
η	dynamic viscosity
γ	shear strain
η_0	viscosity of the pure dispersion medium
η_{rel}	relative viscosity
η_i	relative increment of viscosity
Q	degree of swelling
m_τ	mass of the swelling gel at a particular time
τ	time
m_0	initial mass of the xerogel
ΔV	volume of absorbed liquid
φ	density
ϕ	coefficient of swelling
ε	proportional elongation
ϕ	porosity

V_v	volume of void space
V_T	material's total volume
J	diffusion flux
D	diffusion coefficient
$(\partial c/\partial x)$	concentration gradient
$(\partial c/\partial t)$	change of the concentration over time
τ	tortuosity
λ	actual flow path
λ_0	straight-line distance
ECM	extracellular matrix
O_2	oxygen
CO_2	carbon dioxide
Gly	glycine
n	number of monomer units per polymer molecule
M	D-mannuronic acid
G	L-guluronic acid
Mg^{2+}	magnesium cation
D_{eff}	effective diffusion coefficient
ε	partition coefficient
(dn/dt)	concentration gradient
l	thickness of the membrane
Δc_{10}	difference of concentration in source cell and in receiving cell
r	Stokes-Einstein radius
k_B	Boltzmann constant
T	temperature
E_A	activation energy
A	frequency factor
R	gas constant

UV	ultra-violet light
VIS	visible light
M_t	amount of drug released from the system at a particular time
M_∞	total amount of incorporated drug in the hydrogel
k	characteristic incorporation constant of the system
n	release exponent
PLGA	poly (lactic-co-glycolic acid)
VEGF	vascular endothelial growth factor
k	rate constant
PNI-PAAm	poly(N-isopropylacrylamide)
PEG-DA	polyethylene glycol diacrylate
SEM	scanning electron microscope
FTIR	Fourier-transform infrared microscopy
PLA	poly (lactic acid)
CRV	carvacrol
ML	mono-layer
BL	bi-layer
TL	tri-layer
RK	raspberry ketone
pI	isoelectric point
M_w	molecular weight
(w/w)	mass percentage
rpm	rotation per minute
(v/v)	volume percentage
w_p	weight concentrations of prepared layered material with polymer
m	weight of the layered material was measured in a dry state
m_s	weight of the layered material was measured in a swollen state
m_d	weight of the layered material was measured in a dried state

w	concentration of the released gelatine hydrogel
x	percentage amount of released hydrogel from the material
c_m	concentration of methylene blue inside the membrane