POLYMER BIOCOMPOSITES IN THE FORM OF HYDROGEL USED FOR THE TREATMENT OF PRESSURE SORES

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Abstract

This paper presents research related to the development of hydrogel polymer biocomposites (chitosan and alginate) with an analgesic medication (lidocaine hydrochloride). The pharmaceutical availability of lidocaine hydrochloride from the hydrogel preparations was evaluated for transdermal systems in accordance with the Polish Pharmacopoeia, XI Edition. The rheological tests showed that these preparations had the characteristics of non-Newtonian, pseudoplastic liquids, for which the viscosity decreases as the shear rate increases. The developed hydrogel preparations showed bacteriostatic and bactericidal effects against both Escherichia coli and Staphylococcus aureus. The release of lidocaine hydrochloride from the tested hydrogel was a complex process that followed first-order kinetics. The rate of release could be influenced by appropriate selection of the composition of the biocomposite.

Keywords: *natural polymers, polymer biocomposite hydrogels, pressure sores, rheological and biological properties, transdermal systems*

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1. Introduction

Polymer hydrogels have a characteristic structure, which determines their unusual properties. These materials consist of polymer chains with numerous cross-links between them. Such a system resembles a three-dimensional network whose cross-linking density depends on the number of cross-links. There are free spaces inside the polymer network that are filled by water during the swelling process. This process is possible due to the presence of numerous hydrophilic groups in the polymer chains. In contact with water, hydrophilic groups dissociate, which induces additional electrostatic interactions. There is repulsion of the same ions, which increases the distance between the polymer chains and, consequently, the free space available for water increases [1]. Due to their high water content, appropriate density and plasticity, hydrogels have physical properties similar to those of soft tissues of living organisms. Water in hydrogels acts as a transport medium, thanks to which substances can be 'locked' in them and the rate of their release can be freely manipulated, thus creating a so-called intelligent drug-delivery system. Drugs placed in hydrogel release systems can be administered orally, nasally, rectally, vaginally and into the eyes. The use of hydrogels in the field of medical science is not limited to drug delivery. This type of biomaterial has a very wide range of applications, including the production of soft contact lenses, surgical implants, hybrid organs and biosensors. They are also used in pharmacy, cosmetology, tissue engineering and in the production of surgical tampons and even personal hygiene products [2–5]. The use of polymer biocomposite hydrogels for the treatment of difficult-to-heal wounds provides greater benefits compared with traditional gauze dressings, because they accelerate the wound-healing process, create a protective barrier for newly formed tissues and do not stick to the wound, which facilitates painless dressing replacement and allows following the healing process.

Pressure sores are difficult-to-treat chronic wounds. Their formation may be the result of long-term immobilisation caused by a chronic disease or systemic complications during the course of the underlying disease. Moreover, pressure sores may lead to serious systemic complications, such as dehydration, electrolyte disturbance, osteomyelitis or the development of sepsis [6, 7]. The pressure ulcer healing process is complex and involves regeneration of connective tissue and epidermis. Creating ideal conditions for this healing requires eliminating as many negative factors as possible that may delay or inhibit this process.

Chitosan is a natural cationic polysaccharide consisting of *N*-glucosamine and *N*-acetylglucosamine units linked by a β-1,4-glycosidic bond. Due to its unique biological properties, it has attracted great interest in the field of wound healing. It is an ideal material for the development of new dressings for wounds of various origins (pressure ulcers, burns and difficult-to-heal wounds such as diabetic foot and those infected by bacteria) [8]. Chitosan is used in wound dressings in various forms depending on the application: a hydrogel, membrane, foil, sponge, micro- and nanofibers or powders. As a polycation, chitosan combines with anionic polymers, which allows the creation of two- and three-dimensional structures, each with distinct porosity and mechanical strength. One of the forms mentioned most frequently in the literature is hydrogels. They consist of a three-dimensional network of hydrophilic polymers such as poly(ethylene glycol) (PEG), poly(acrylic acid) (carbomers) and poly(vinyl alcohol) (PVA). The polymer network allows for the absorption of significant amounts of water. Chitosan can be bound to the hydrogel through hydrogen bonds and hydrophobic interactions (physically) or incorporated into the network (chemically), which improves the mechanical strength of the gel [9]. Based on the literature, chitosan hydrogel has great potential in various stages

of the wound-healing process (at various stages): they reduce inflammation and have high sorption properties, thanks to which they retain water and provide a moist environment. Dressings based on chitosan hydrogel have also been described in the literature as a matrix to deliver pharmacological agents such as antibiotics, growth factors and stem cells, among others, which can further accelerate wound healing [10–12].

There is a focus on creating new antibacterial dressings for patients with difficult-to-heal wounds that are infected by antibiotic-resistant pathogenic bacteria. Such wounds are often a consequence of other serious diseases and irregularities in the functioning of our body, such as diabetes, autoimmune diseases, cancer and some medications taken on a permanent basis. Therefore, antibacterial agents, primarily antibiotics, are added to dressings. However, due to the increasing problems with combating antibiotic-resistant infections, there have been numerous attempts to combine polymer matrices with antimicrobial peptides, which may constitute a new direction in the treatment of resistant strains of pathogenic bacteria inhabiting the wound environment. Another innovative approach is the addition of biological factors, including bacteriophages and growth factors. When bacteriophages come into contact with the exudate of an infected wound, they actively fight the bacteria contained therein [13, 14]. Growth factors are often added to accelerate the healing and scarring processes and to improve the ability to repair damaged tissues. One of the most frequently used growth factors is basic fibroblast growth factor (bFGF), a polypeptide that regulates that cell growth, differentiation and angiogenesis [15].

Researchers have attempted to design adhesive nanocomposite hydrogels with photothermal antibacterial properties [10]. In one study, the authors generated a hydrogel dressing made of *N*-carboxyethyl chitosan (CEC) and Pluronic F127/carbon nanotubes (PF127/CNT). *In vivo* studies revealed their great potential as a photothermal therapy (PTT) dressing for infected wounds. The dressings showed adequate haemostatic activity, water absorption capacity, stable mechanical properties and good biodegradability [10]. Hydrogels loaded with the antibiotic moxifloxacin hydrochloride released the antibiotic depending on pH and showed good antibacterial activity. In another study, the researchers generated a chitosan hydrogel with silver nanoparticles that showed a good ability to combat difficult bacterial infections. They compared it with a commercially available reference dressing and found that the chitosan hydrogel had a stronger antibacterial effect, accelerated the healing process and showed very good organoleptic properties and proper adhesion to the wound [16].

This paper presents research related to the development of hydrogels constituting a matrix of dressing material, made of various biopolymers (chitosan and alginates) with an active substance with an analgesic effect (lidocaine hydrochloride [LidCl]). The pharmaceutical availability of LidCl from the hydrogel preparations and the rate of their release from the hydrogel through the membrane into the acceptor fluid were performed as for transdermal systems according to the Polish Pharmacopoeia, XI Edition [17]. Natural polymers from the polysaccharide group were used to construct dressings intended to treat pressure ulcers due to their specific biological properties, namely: biocompatibility; biodegradability; and the ability to accelerate the wound-healing process, to stimulate the body's immune mechanisms and to reduce the risk of infection by limiting bacterial growth [18–25]. The antibacterial activity of the hydrogel preparations against the gram-negative bacterium *Escherichia coli* and the gram-positive bacterium *Staphylococcus aureus* bacteria were assessed according to the JISL 1902:2002 standard).

2. Materials and Methods

2.1. Materials

This research used the following reagents:

- chitosan from Vanson Halo Source (USA) with an average molecular weight of 320.0 kDa, a degree of deacetylation of 77.5% and an ash content of 31 ppm;
- microcrystalline chitosan with a reduced pH of 6.65, a polymer content of 1.8 wt%, an average molecular weight of 284.0 kDa and a degree of deacetylation of 77.5% (created at the Łukasiewicz – Łódź Institute of Technology [ŁIT]);
- sodium alginate (Protanal LF 10/60), FMC BioPolymer (USA);
- glycerine, Fluka (p.a.) (USA), used as a plasticiser;
- lactic acid, Fluka (p.a.);
- sodium tripolyphosphate (TPP; Sigma-Aldrich, USA);
- calcium chloride anhydrous, Avantor Performance Materials Poland S.A. (p.a.) (Poland);
- lidocaine hydrochloride-2-(diethylamino)-*N*-(2,6-dimethylphenyl)acetamide hydrochloride monohydrate (Zakład Farmaceutyczny Amara®). LidCl is a local anaesthetic agent that can be used alone or in combination with sypatotonic amines. This derivative is used more often than LidCl as a pharmaceutical raw material for the production of painkillers [26].

2.2. Research Methodology

2.2.1. Production of Polymer Biocomposite Hydrogels Containing Lidocaine Hydrochloride

A polymer biocomposite in the form of a hydrogel (H/Ch/AlgNa/Ca/liof) was produced with microcrystalline chitosan (with a reduced pH = 6.65 and polymer content = 1.8 wt%) and sodium alginate lyophilisate containing 11 wt% calcium chloride. The chitosan microcrystalline hydrogel (MCCh) used in this research was manufactured by the authors from the raw material obtained from Vanson Halo Source according to the continuous method developed at Łukasiewicz – ŁIT [27, 28].

Two hydrogel mixtures were prepared with two weight contents of microcrystalline chitosan/alginate Na/Ca: 75:25 (H/Ch/AlgNa/Ca/liof/B) and 50:50 (H/Ch/AlgNa/Ca/liof/C). Additionally, the hydrogel preparations were cross-linked with TPP (2.0% by weight based on the dry weight of the composite). Cross-linking was carried out for 3 h at 5.5–6.0°C. In the final stage, a plasticiser (glycerine) was added to each preparation (0.4 parts by weight per 1 part by weight of the composite). LidCl was added separately to the mixtures – 3% by weight based on the dry weight of the polymers contained in the composite, in accordance with the dose recommended in the Polish Pharmacopoeia, XI Edition [17]. Table 1 presents the quantitative composition of the polymer biocomposite hydrogels.

Table 1. Quantitative composition of the chitosan/alginate-sodium-calcium biocomposite hydrogels (H/Ch/AlgNa/Ca/liof).

Sample	Quantitative composition of the hydrogel [wt%]					Total polymer content * [wt%]	pH
	MCCh	AlgNa/Ca	Glycerine	TPP	LidCl		
H/Ch/AlgNa/ Ca/liof/B	52.82	17.24	27.59	1.38	–	1.76	6.86
H/Ch/AlgNa/ Ca/liof/B/LidCl	51.72	17.24	27.59	1.38	2.07	1.77	6.91
H/Ch/AlgNa/ Ca/liof/C	35.21	35.21	28.17	1.41	–	1.82	6.70
H/Ch/AlgNa/ Ca/liof/C/LidCl	34.48	34.48	27.59	1.38	2.07	1.83	6.73

Note. *Polymer content (MCCh and AlgNa/Ca) in the composite hydrogel. Abbreviations: AlgNa/Ca, sodium alginate; LidCl, lidocaine hydrochloride; MCCh, chitosan microcrystalline hydrogel; TPP, sodium tripolyphosphate.

2.2.2. Rheological Evaluation of the Polymer Biocomposite Hydrogels Containing Lidocaine Hydrochloride

The dynamic viscosity, shear stress and shear rate were determined using a an RV DV-II digital viscometer with the Rheocalc V3.1-1 program (Brookfield, USA) at 25 and 37°C in accordance with the developed procedure [29]. The CPE-52 and CPE-41 cones were used to measure sample volumes of 0.5 and 2.0 ml, respectively. The thermostating time was 15 min.

2.2.3. Water Release Rate From the Polymer Composite Hydrogels

The rate of water loss from the polymer biocomposite hydrogels was determined. Approximately 1 g of the preparation was weighed with an accuracy of 0.0001 g and incubated at 37°C for the time necessary to obtain a constant mass. The sample was weighed at regular intervals to determine the loss of the initial mass of the preparation. The water loss process is described by equation (1) [30]:

$$m_t = m_\alpha(1 - e^{-kt}) \quad (1)$$

where m_α is the total amount of water in the preparation [g] and m_t is the amount of water that evaporated after time t [g].

2.2.4. Evaluation of the Availability of Lidocaine Hydrochloride From the Polymer Biocomposite Hydrogels

The availability of LidCl from the polymer biocomposite hydrogels was carried out at the Department of Pharmaceutical Pharmacy, Department of Applied Pharmacy, Medical University of Łódź. The tests were performed as for transdermal systems, using a paddle apparatus (Figure 1) and an extraction chamber made of a chemically neutral material (Figure 2), which consisted of a base, an overlay and, if necessary, a semi-permeable membrane (dialysis tubing) made of regenerated cellulose with a pore size approximately 25 Å (Servapor, Sigma-Aldrich, USA). The extraction chamber held the polymer biocomposite hydrogel flat, parallel to the bottom edge of the paddle mixer with the release surface facing upwards. The distance between the paddle mixer

and the surface of the polymer biocomposite hydrogel was constant at 25 ± 2 mm. Each sample weighed approximately 2.5 g. The acceptor fluid was 100 ml of 0.9% sodium chloride (physiological saline). The tests were carried out at 32 ± 0.5°C and a stirrer speed of 100 rpm. The LidCl concentration in the receptor fluid was determined spectroscopically at 262 nm after 5, 15, 30, 60, 120 and 180 min.

Figure 1. Paddle apparatus for testing *in vitro* the release rate of lidocaine hydrochloride from the polymer biocomposite hydrogels.

Figure 2. Extraction chamber.

2.2.5. Determining the Rate of Lidocaine Hydrochloride Release From the Polymer Composite Hydrogels Into an Acceptor Fluid

The rate of release of LidCl from the polymer biocomposite hydrogels into the acceptor fluid was carried out at the Department of Pharmacy, Department of Applied Pharmacy, Medical University of Łódź. A diffusion apparatus consisting of a donor chamber and an acceptor chamber, separated by a semi-permeable membrane (dialysis tubing) made of regenerated cellulose with a pore size of approximately 25 Å (Servapor; Figure 3), was used for the tests. A hydrogel preparation weighing approximately 1.0 g was applied to the membrane. Then, 25 ml of physiological saline or phosphate buffer (pH 7.4) was introduced into the acceptor cell. The contact area of the membrane with the acceptor fluid was 0.785 cm^2. The measurement was performed at 32 ± 0.5°C with constant stirring

of the acceptor fluid. The amount of the LidCl released was determined spectrophotometrically at 262 nm after 5, 15, 30, 60, 120 and 180 min.

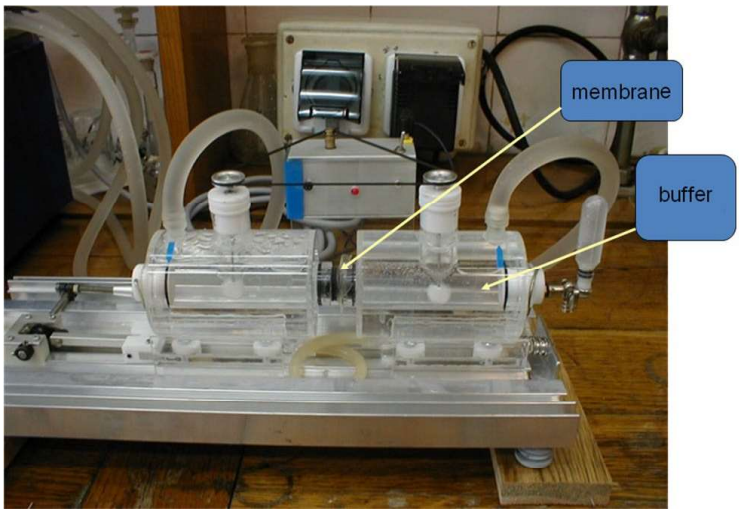

Figure 3. The photograph of a diffusion apparatus used to determine the rate of lidocaine hydrocholoride release from the polymer biocomposite hydrogels into the acceptor fluid.

2.2.6. Evaluating the Antibacterial Activity of the Polymer Biocomposite Hydrogels

The antibacterial effect of the polymer biocomposite hydrogels was determined at the Microbiological Laboratory of the Lukasiewicz – ŁIT in accordance with the institution's 'Testing of the antibacterial effect of textile products. Quantitative test' (5^{th} edition) research protocol, developed based on the JIS L 1902:2002 standard. The study consisted of assessing the inhibition of the growth of standard bacterial strains in contact with the tested sample. The number of bacteria on the sample was assessed before and after incubation for 24 h relative to the inactive sample as a control (reference material was cotton).

The number of bacteria (*E. coli* and *S. aureus*) on the control sample before incubation and the number of bacteria that survived on the control sample and test samples after incubation were determined. Based on the number of bacteria, the bacteriostatic and bactericidal activity was determined, calculated according to equations (2) and (3), respectively:

$$S = Mb - Mc, \text{ and} \quad (2)$$

$$L = Ma - Mc. \quad (3)$$

The terms in equations (2) and (3) mean the following:

S – bacteriostatic activity;
L – bactericidal activity;
Ma – common logarithm of the number of bacteria on the sample without the active agent immediately after inoculation;
Mb – common logarithm of the number of bacteria on the sample without the active agent after incubation for 24 h; and
Mc – common logarithm of the number of bacteria on the sample with the active agent after incubation for 24 h.

According to the JIS L 1902:2002 standard, L > 0 indicates bactericidal activity, while S > 2 bacteriostatic activity [31].

3. Results and Discussion

The main goal of this research was to develop a multifunctional dressing in the form of a polymer biocomposite containing the analgesic LidCl and to assess the rheological properties, water-releasing ability and biological activity. The developed dressing is intended for the treatment of difficult-to-heal wounds, in particular, pressure ulcers. Biopolymers such as chitosan and alginates are used to develop dressings due to their specific properties useful in the treatment of difficult-to-heal wounds. Chitosan is a biopolymer with biostimulatory properties that can accelerate the influx of phagocytic cells to the wound site, which cleanse the wound of necrotic tissues and stimulate the migration and proliferation of intraepithelial cells and fibroblasts. This polymer also impairs the growth of bacteria and fungi, which reduces the risk of wound infection. High biological activity predisposes this polymer to the production of modern dressings used to treat difficult-to-heal wounds (bedsores) [23, 32–37]. Alginates are one of the most frequently used natural polymers used to produce dressing materials due to their biological properties: biocompatibility, biodegradability, absorbent properties and the ability to stimulate the growth of fibroblasts. The interaction of alginates, such as sodium alginate, with divalent calcium cations (Ca^{2+}) leads to the formation of gels; this phenomenon has been used in the design and production of active dressings that have haemostatic properties and support the blood coagulation process. The ability to swell and absorb fluids becomes particularly important when using 'moist wound therapy'. A hydrophilic, moist coating is created on the wound surface, maintaining a warm, healing environment without the adverse effects of occlusion [38–41].

3.1. Assessment of the Rheological Properties of the Polymer Biocomposite Hydrogels

Polymers that have the ability to create hydrogels, retain water in their structure and have adhesive properties to the surface of damaged tissue are valuable materials for the construction of special dressings. The properties of such polymers are determined by the intermolecular forces between the chains and their spatial structure. Intermolecular interactions have a significant impact on the rheological properties of polymer solutions, as well as the rate of release of the medicinal substance from the dressings produced with their participation; moreover, the ability to create gel forms is determined by the formation of hydrogen bridges between the chains of these polymers [42–44]. Therefore, rheological tests play an important role in the development of therapeutic hydrogel dressings containing medicinal substances. The dynamic viscosity, shear stress and shear rate of the polymer biocomposite hydrogels containing LidCl were determined at 25°C (based on the measurement procedure) and at 37°C (the temperature of the human body). The results are presented in Figures 4–7.

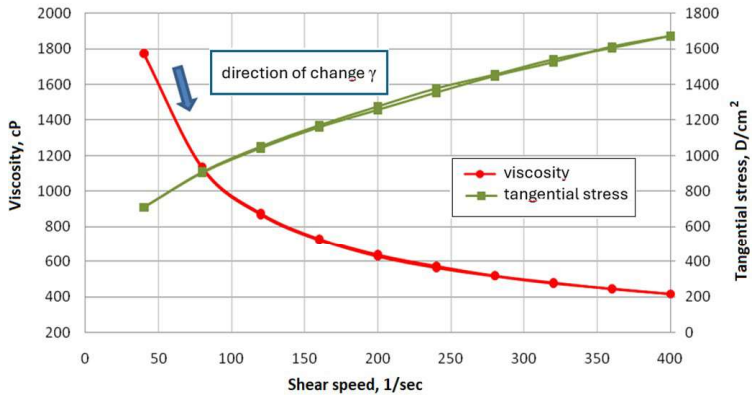

Figure 4. Dependence of viscosity and shear stress on the shear rate (γ) at 25°C for the H/Ch/AlgNa/Ca/liof/**B**/LidCl hydrogel. See Table 1 for details on the hydrogel.

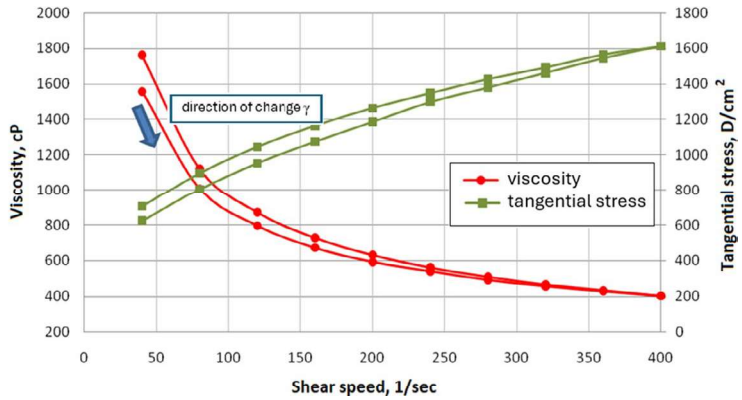

Figure 5. Dependence of viscosity and shear stress on the shear rate (γ) at 37°C for the H/Ch/AlgNa/Ca/liof/**B**/LidCl hydrogel. See Table 1 for details on the hydrogel.

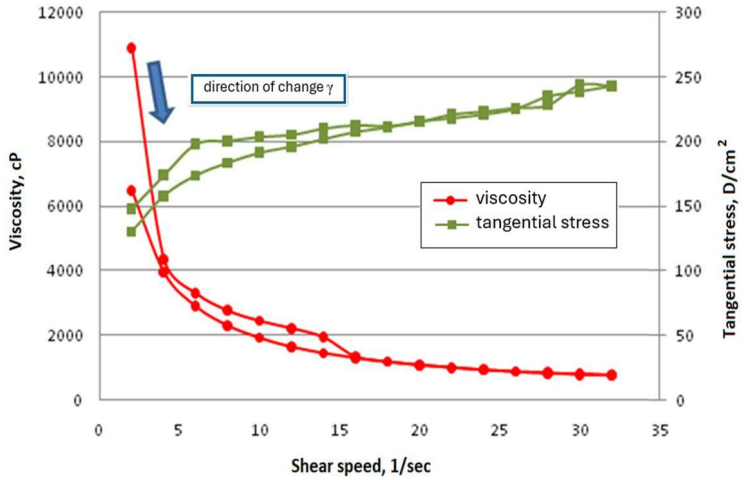

Figure 6. Dependence of viscosity and shear stress on the shear rate (γ) at 25°C for the H/Ch/AlgNa/Ca/liof/**C**/LidCl hydrogel (diluted to 1%). See Table 1 for details on the hydrogel.

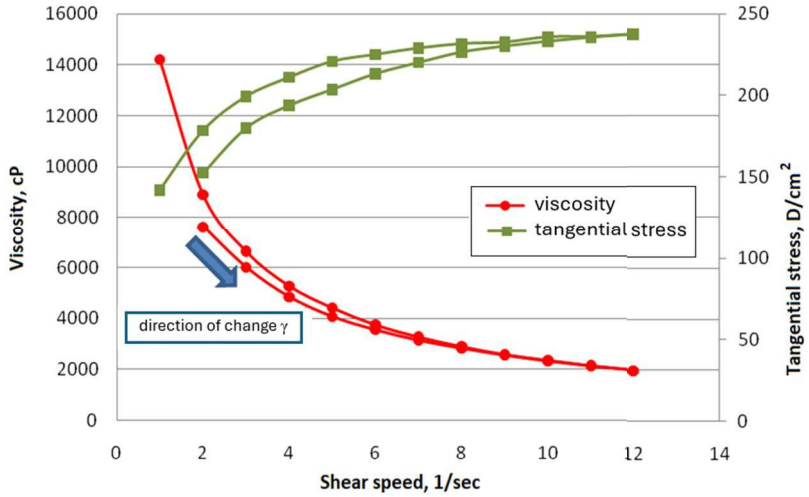

Figure 7. Dependence of viscosity and shear stress on the shear rate (γ) at 37°C for the H/Ch/AlgNa/Ca/liof/**C**/LidCl hydrogel (diluted to 1%). See Table 1 for details on the hydrogel.

Figures 4–7 show the flow curves of hydrogel preparations in the coordinate system $\eta = f(\gamma)$ and $\tau = f(\gamma)$, where η is the dynamic viscosity [cP], τ is the shear stress [D/cm^2] and γ is the shear rate [1/sec]. The tested polymer biocomposite hydrogels varied in terms of composition and showed the characteristics of non-Newtonian, pseudoplastic (shear-thinned) liquids. For most of them, there were hysteresis loops in the flow curve diagrams when the shear rate was increased or decreased. However, the following hydrogel preparations showed an antithixotropic character during shear: H/Ch/AlgNa/Ca/liof/B/LidCl and H/Ch/AlgNa/Ca/liof/C/LidCl (diluted to 1%) at 37°C. Diluting the polymer biocomposite hydrogel with water to a concentration of 1% by weight was necessary to reduce the viscosity so that it could be measured with the instrument (as describedin Section 2.2.2). At 25°C, the polymer biocomposite hydrogel (H/Ch/AlgNa/Ca/liof/B/LidCl) was rheologically stable, and its properties did not depend on shear forces. However, the H/Ch/AlgNa/Ca/liof/C/LidCl (diluted to 1%) hydrogel presented the characteristics of a thixotropic liquid thinned by shear when measured at 25°C; the 'thinning' effect persisted after the shear forces ceased. The consequence of this phenomenon was the destruction of the molecular structure of the hydrogel.

3.2. Assessment of the Water Release Rate From the Polymer Biocomposite Hydrogels

Functional dressings intended for the treatment of difficult-to-heal wounds – in particular, pressure sores – should ensure the effective action of the medicinal substance contained in them and provide appropriate comfort. An extremely important and desirable feature of dressings is maintaining appropriate moisture in the wound, ensuring greater effectiveness and faster healing. A moist environment ensures better penetration of active substances into the wound and allows easy and painless replacement of the dressing without the risk of damaging the tissue rebuilt as a result of granulation and epithelisation. Figure 8 presents the water release rate from the polymer biocomposite hydrogels.

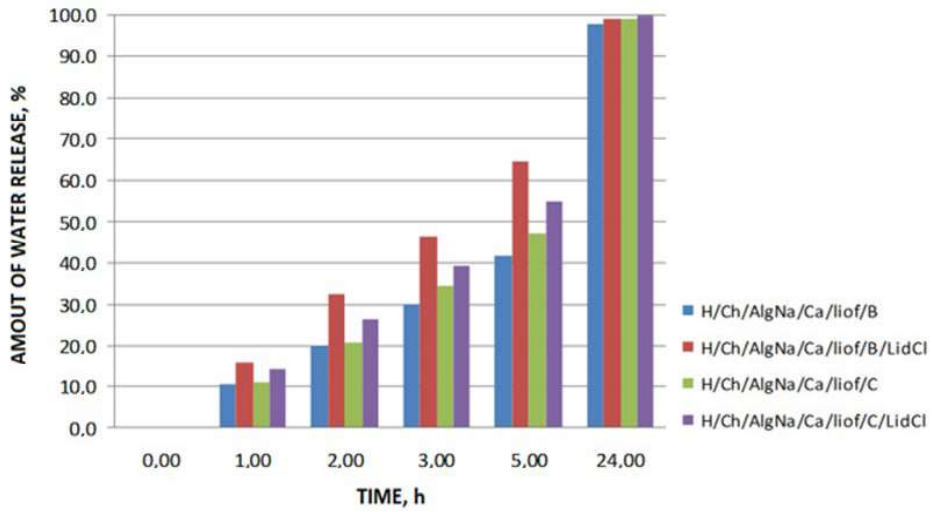

Figure 8. Changes in the amount of water released by the polymer biocomposite hydrogels H/Ch/AlgNa/Ca/liof/B, H/Ch/AlgNa/Ca/liof/B/LidCl, H/Ch/AlgNa/Ca/liof/C and H/Ch/ AlgNa/Ca/liof/C/LidCl as a function of time. See Table 1 for details on the hydrogels.

After 5 h, the polymer biocomposite hydrogels with LidCl released water faster compared with the polymer biocomposite hydrogels without LidCl. After 24 h, almost all water had been released from the polymer biocomposite hydrogels. Therefore, they can be assumed to maintain moisture at the appropriate level and allow appropriate wound healing.

3.3. Availability of Lidocaine Hydrochloride from the Polymer Biocomposite Hydrogels

As described in Section 2.2.4, the availability of LidCl from the polymer biocomposite hydrogels was determined. Table 2 lists the parameters of the kinetic equation describing the release of LidCl from the polymer biocomposite hydrogels, and Figures 9 and 10 show the amount of released LidCl as a function of time.

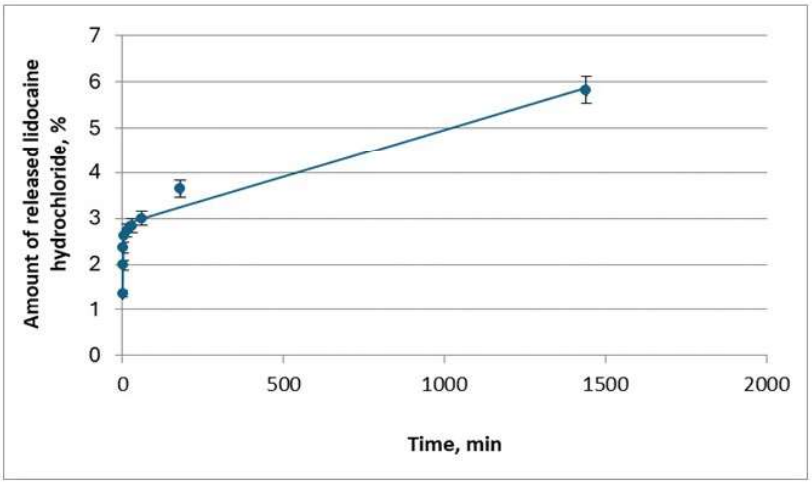

Figure 9. Release kinetics of lidocaine hydrochloride from the H/Ch/AlgNa/Ca/liof/C/LidCl hydrogel. See Table 1 for details on the hydrogel.

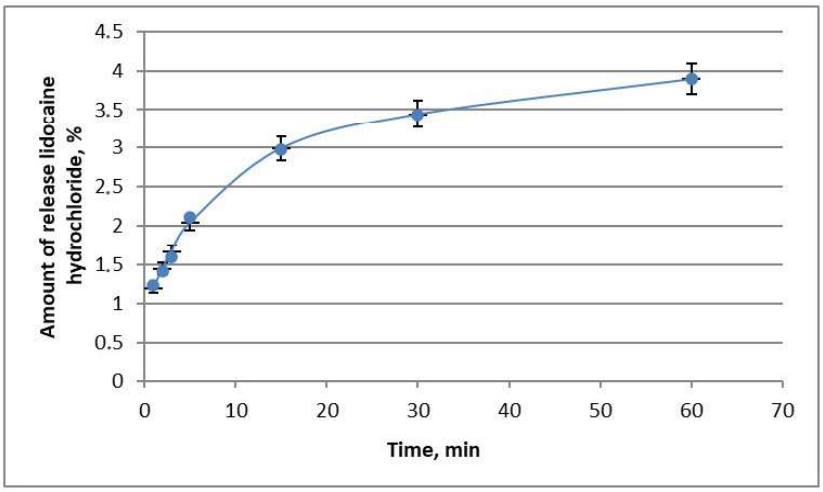

Figure 10. Release kinetics of lidocaine hydrochloride from the H/Ch/AlgNa/Ca/liof/B/LidCl hydrogel. See Table 1 for details on the hydrogel.

Table 2. Parameters of the kinetic equation describing the release rate of lidocaine hydrochloride from the polymer biocomposite hydrogels.

Sample	a_0 [%]	Stage I			Stage II		
		a_1 [%]	k_1 [min^{-1}]	$t_{0.5}$ [min]	a_2 [%]	$k_2 \times 10^5$, [min^{-1}]	$t_{0.5}$ [h]
H/Ch/AlgNa/Ca/liof/C/LidCl	0.46	2.40	0.477	1.45	97.14	2.18	530
H/Ch/AlgNa/Ca/liof/B/LidCl	0.91	2.15	0.137	5.07	96.94	14.38	80

Note. See Table 1 for details on the hydrogels. Abbreviations: a_0, $100 - a_1 - a_2$, the amount of 'unbound' lidocaine hydrochloride [%]; a_1 and k_1, constants describing the first stage; a_2 and k_2, constants describing the second stage; $t_{0.5}$, release half-life for each stage.

After 1440 min, 5.82% of LidCl had been released from the H/Ch/AlgNa/Ca/liof/C/LidCl hydrogel. After 60 min, approximately 3.89% of LidCl had been released from the H/Ch/AlgNa/Ca/liof/B/LidCl hydrogel.

The H/Ch/AlgNa/Ca/liof/C/LidCl and H/Ch/AlgNa/Ca/liof/B/LidCl hydrogels showed a similar course of LidCl release directly (without membrane usage) into physiological saline. There was a fast initial release, followed by very slow release. This pattern indicates LidCl release follows first-order kinetics and is a complex process. The determined parameters of the kinetic equation revealed a significant slowdown in the release of LidCl from the H/Ch/AlgNa/Ca/liof/C/LidCl hydrogel during the second stage of the process, with a release half-time ($t_{0.5}$) of 530 h.

3.4. Release of Lidocaine Hydrochloride From the Polymer Biocomposite Hydrogels Into an Acceptor Fluid

The developed dressing is a transdermal therapeutic system, which, in addition to the possibility of controlled drug release, allows the administration of drugs that are broken down in the gastrointestinal tract or metabolised in the liver. A diffusion apparatus consisting of donor and acceptor chambers (Figure 4), separated

by a semi-permeable membrane made of regenerated cellulose with a pore size of approximately 25 Å, was used to assess the release of LidCl from the hydrogels to the acceptor fluid, as described in Section 2.2.5. The dressings contained a medicinal substance in the form of lidocaine hydrochloride. Table 3 lists the determined values of the parameters of the kinetic equation describing the rate of release of LidCl, and Figures 11 and 12 show the amount of released LidCl as a function of time.

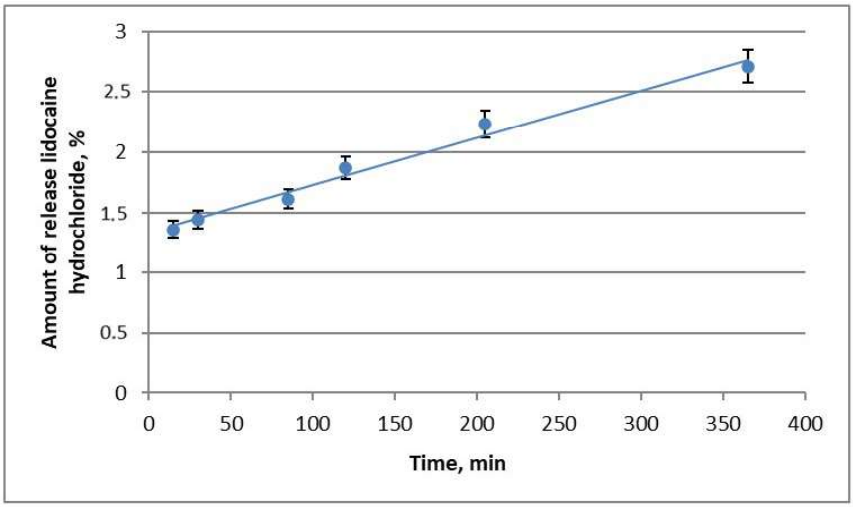

Figure 11. Release kinetics of lidocaine hydrochloride from the H/Ch/AlgNa/Ca/liof/C/LidCl hydrogel. See Table 1 for details on the hydrogel.

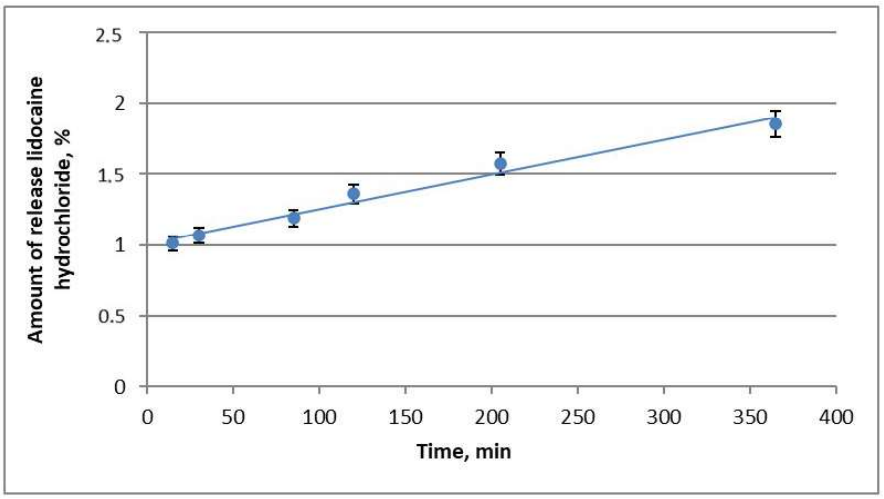

Figure 12. Release kinetics of lidocaine hydrochloride from the H/Ch/AlgNa/Ca/liof/B/LidCl hydrogel. See Table 1 for details on the hydrogel.

Table 3. Parameters of the kinetic equation describing the release rate of lidocaine hydrochloride from the polymer biocomposite hydrogels.

Hydrogel dressing symbol	a_0 [%]	Stage I			Stage II		
		a_1 [%]	k_1 [min^{-1}]	$t_{0.5}$ [min]	a_2 [%]	$k_2 \times 10^5$, [min^{-1}]	$t_{0.5}$ [h]
H/Ch/AlgNa/Ca/liof/C/LidCl	1.33	-	-	-	98.67	4.02	288
H/Ch/AlgNa/Ca/liof/B/LidCl	1.01	-	-	-	98.99	2.47	468

Note. See Table 1 for details on the hydrogels. Abbreviations: a_0, $100 - a_1 - a_2$, the amount of 'unbound' lidocaine hydrochloride [%]; a_1 and k_1, constants describing the first stage; a_2 and k_2, constants describing the second stage; $t_{0.5}$, release half-life for each stage.

After 365 min, approximately 1.85% and 2.71% LidCl had been released from the H/Ch/AlgNa/Ca/liof/C/LidCl and H/Ch/AlgNa/Ca/liof/B/LidCl hydrogels, respectively, to the acceptor fluid. These hydrogels had a release process controlled by diffusion through the membrane.

3.5. Assessment of the Antibacterial Activity of the Polymer Biocomposite Hydrogels

The gram-negative bacterium *E. coli* is a natural physiological component of the gastrointestinal flora of humans and animals and but can often cause infections, the clinical symptoms and course of which depend on the place of their occurrence [45]. The gram-positive bacterium *S. aureus* can cause local infections of virtually all tissues and organs as well as a general infection of the body, which is often life-threatening [46]. These bacteria were adopted as model pathogens, approximating the conditions occurring in the wound and causing wound infection, to assess the antibacterial activity of the polymer biocomposite hydrogels. To test the antibacterial activity, the hydrogel preparations were sterilised with ultraviolet radiation for 20 min. Based on the results in Table 4, the tested polymer biocomposite hydrogels showed bacteriostatic and bactericidal properties against *E. coli* bacteria and *S. aureus*.

Table 4. Antibacterial activity of the polymer biocomposite hydrogels containing lidocaine hydrochloride.

Biocomposite symbol	Number of bacteria [cfu/sample]	Bacterium	Bacteriostatic activity (S)	Bactericidal activity (L)
Control (cotton)	1.9×10^4	*Escherichia coli* ATCC 11229	–	–
H/Ch/AlgNa/Ca/liof/B/LidCl	< 20		7.0	3.0
H/Ch/AlgNa/Ca/liof/C/LidCl	< 20		7.0	3.0
Control (cotton)	1.3×10^4	*Staphylococcus aureus* ATCC 6538	–	–
H/Ch/AlgNa/Ca/liof/B/LidCl	7.0×10^1		4.4	2.1
H/Ch/AlgNa/Ca/liof/C/LidCl	8.8×10^4		4.9	2.3

Note. See Table 1 for details on the hydrogels. Abbreviation: cfu, colony-forming units.

4. Conclusions

Polymer biocomposite hydrogels based on microcrystalline chitosan with a reduced pH and sodium alginate and carrying the analgesic LidCl, intended for the treatment of difficult-to-heal pressure ulcers, was developed. The hydrogels exhibited the characteristics of non-Newtonian, pseudoplastic (shear-thinned) liquid, for which the dynamic viscosity decreased as the shear rate increased. The hydrogels containing LidCl released water faster than the hydrogels without LidCl. The release of LidCl from the polymer biocomposite hydrogels is a complex process that follows first-order kinetics. By changing the composition and amount of individual polymers in the biocomposite, it will probably be possible to control the release rate of LidCl, or whatever active substance is included in the hydrogel. Finally, the polymer biocomposite hydrogels showed antibacterial activity against the gram-negative bacterium *E. coli* and the gram-positive bacterium *S. aureus*.

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