# FUNCTIONAL THREE-COMPONENT POLYMERIC BIOCOMPOSITES FOR THE TREATMENT OF BEDSORES

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**Abstract**

*Presented here are the results of investigations into the preparation of three-component dressing materials from various biopolymers in the form of a single-layer film which is suitable as a carrier for pain-relieving (lidocaine) and bacteriostatic (sulphanilamid) therapeutic agents. Physical-chemical, biological and usable properties of the prepared materials were tested and assessed. The amount of added active substance was adopted based on the dose recommended by the Polish Pharmacopeia for external medicinal preparations.*

*Antibacterial activity against gram (-) Escherichia coli and gram (+) Staphylococcus aureus was assessed in some of the biocomposites by quantitative methods. The cytotoxic action in direct contact with the mouse fibroblast NCTC clone 929 was also estimated.*

*Thermal analysis (DSC), infrared spectrophotometry (FTIR) and nuclear magnetic resonance spectroscopy were employed to investigate the impact of the variable contents of chitosan, alginate, carboxymethyl cellulose (CMC), and the active substance upon the chemical- and phase-structure of the prepared three-component polymeric biocomposites.*

*It was found that the quantitative composition of the biocomposites and the additive of active substances lidocaine and sulphanilamide exert a vital impact upon their physical-mechanical and usable properties (imbibition, absorption). Investigations into the release of the medicinal substance from the investigated biocomposites to an acceptor fluid led to the conclusion that the kinetics of the process may be described by a complex first order rate equation with two exponential functions.*

***Keywords:*** *Chitosan, alginate, carboxymethyl cellulose, dressing DSC, FTIR, NMR*

**Received:** 15.06.2018
**Accepted:** 18.07.2018

# 1. Introduction

Bedsores are injuries of the tissue (skin and underlying tissue) caused by a disruption of blood flow in the capillary vessels resulting from prolonged pressure on the skin and underlying tissue. The pressure lowers cell metabolism, and causes oxygen deficiency and malnutrition which, as a consequence, leads to local necrosis.

Bedsores are not only difficult wounds, they must be seen as a process that leads to very serious systemic complications like dehydration, electrolytic disorder, bone inflammation, septic infection, and may, in extreme cases, be life-threatening [1].

The healing of bedsores is a complex process aimed at the regeneration of connecting tissue and the epidermis. As many of the negative factors that could retard healing as possible should be eliminated.

The healing of difficult wounds including bedsores primarily involves special dressings along with the use of pharmaceuticals applied systemically or locally [2].

The pharmaceutical market currently offers a number of dressing materials designed for the treatment of bedsores depending upon the clinical stage of the injury. For stage one, the polyurethane film OpSite is applied, the inside of which shows adhesive properties, thus providing adherence to the skin, while the film's structure permits evaporation from the wound surface, and prevents the permeation of water and impurities from outside. Hydro-colloidal dressings, for instance the material Granuflex, have been designed for stages two and three of the sore. Hydro-colloids provide thermal isolation of the wound, thus maintaining its temperature at the level of the body.

The moist exudate beneath the dressing allows the migration of cells and prevents damage to the tissue when dressings are changed. Moreover, the dressing soothes pain by protecting against friction and other factors that could affect the wound; changing dressings is therefore painless [3]. Another group is hydro-gel dressings, destined for use in wounds with the occurrence of necrosis. Due to the high degree of hydration, they soften the necrosis and allow natural processes of autolysis and purification of the sore to occur. Aquagel, IntraSite Gel, and Purilon [4] are examples of such dressings.

Natural polymers such as chitosan, alginates and carboxymethyl cellulose show specific biological properties like biocompatibility, biodegradability, an ability to accelerate the healing of wounds, the stimulation of immunity and decreasing the risk of infection by limiting the growth of bacteria. For such features, they excellently lend themselves to the construction of dressing devices for use in the treatment of bedsores and other difficult wounds. The design of such bioactive materials involves not only the selection of a polymer with suitable physical-chemical and biological properties, but also the elaboration of a usable form of the dressing [5-13].

Presented in this work are the results of investigations into the preparation of three-component dressing materials in the form of a single-layer film with the content of a selected chitosan lactate (Ch) sodium alginate (Alg) and sodium salt of carboxylmethyl cellulose (CMC); these play the role of a carrier for the medicinal substances: analgesic – lidocaine and bacteriostatic agent – sulphanilamide. The assessment of the physicochemical, useful and biological properties of the prepared dressings is shown. The amount of the active substance was adopted in accordance with the dose recommended in Polish Pharmacopeia (FP XI, 2017) for external preparations [14].

Thermal analysis (DSC), infrared spectrophotometry (FTIR) and nuclear magnetic resonance spectroscopy (NMR) were employed to investigate the impact of the variable contents of chitosan, alginate carboxymethyl cellulose (CMC), and the active substance upon the chemical- and phase structure of the prepared three-component polymeric biocomposites. Lidocaine (Lid) is a an amide derivative used on the skin as a local anaesthetic agent, usually in the form of an aerosol or gel; it also serves as analgesic and

anti-arrhythmic drug [15]. Sulphanilamide (Sf) (*amide of para-aminobenzenesulphonic acid*) exerts bacteriostatic activity against various strains of staphylococcus and streptococcus; it is being used in various pus-forming skin diseases and abscesses [15].

# 2. Materials and Methods

## 2.1. Materials used in the research

Chitosan was delivered by Primex ehf. Iceland Company, and is characterised by the following physicochemical properties: average molecular weight ( $\bar{M}_v$)=338.0 kDa; deacetylation degree (DD)=83.2%; and ash content=40 ppm

Modified sodium lactate with pH 6.20; ash content=1.96 wt%; ( $\bar{M}_v$)=330.0 kDa; and SD=83.2%

Sodium alginate (Protanal LF 10/60), delivered by FMC BioPolymer

Sodium salt of carboxymethyl cellulose (CMC), by Aldrich Co

Plasticiser – glycerol, by Fluka Co

Lactic acid, analytically pure by Fluka Co

Calcium chloride, analytically pure by POCh Co

*Medicinal substances:*

Lidocaine – *2-(diethyloamin)-N-(2,6-dimethylofenylo)acetamid),* an analgesic agent by Sigma Co.

Sulphanilamide (*amide of para-aminobenzenesulphonic acid*) – a bacteriostatic agent by Sigma-Aldrich Co.

## 2.2. Research methods

*2.2.1. Method applied to prepare the three-component biocomposites in the form of a film with the addition of an active substance*

In order to obtain lactate, chitosan at a concentration of 2.0 wt.% was dissolved in 0.9 wt.% lactic acid; the salt obtained was then buffered with a 5.0 wt.% by NaOH of solution to obtain a pH of 6.2. Chitosan lactate with a pH of 6.2 and polymer content of 1.96 wt.% was used in the preparation of the three-component biocomposites: chitosan-alginate-carboxymethyl cellulose (Chit/Alg/KMC), which constitutes the basic materials of the dressing. The solution of chitosan salt was diluted with aqueous calcium chloride to a concentration of 1.90 wt%. For the preparation of solutions of sodium alginate and carboxymethylcellulose at a concentration of 1.7wt.%, the polymers were dissolved in water.

The sodium alginate solution with a concentration of 1.7 wt.% was next mixed with the CMC solution at 1.7wt.%. Chitosan lactate with calcium chloride was then added to the solution of sodium alginate with CMC. Three blends of chitosan/alginate/CMC with the following weight contents were prepared: 60:20:20 (Chit/Alg/CMC/1), 60:35:5(Chit/Alg/CMC/2) and 20:75:5 (Chit/Alg/CMC/3) in wt%. Medicinal substances were added to the prepared blends: lidocaine in the amount of 3 wt.% or sulphanilamide in an amount of 6 wt.% on dry mass of the polymers in accordance with the recommendations of the Polish Pharmacopeia edit. XI, 2017 [14]. Glycerol in the amount of 0.4 weight parts on 1 weight part of the composite (calculated on dry polymer mass) served to give elasticity and drapability to the prepared films. The biocomposite in the form of a film was obtained by casting the blend onto Teflon plates and drying at 20 ± 2°C 24 for 48 hours. The quantitative composition of the prepared biocomposites is presented below in Table 1.

**Table 1.** Quantitative composition of the three-component biocomposites with contents of lidocaine and sulphanilamide

| Symbol of sample | Proportion of polymers and agents contents in the biocomposite | | | | | Content of polymers (Chit, Alg and CMC) in the biocomp. wt.% | pH |
|---|---|---|---|---|---|---|---|
| | Chitosan lactate wt.% | Na-Ca alginate wt.% | CMC wt.% | Lidocaine wt.% | Glycerol wt.% | | |
| Chit/Alg/CMC/Lid | | | | | | | |
| Chit/Alg/CMC/1/ Lid | 41.90 | 14.00 | 14.00 | 2.10 | 28.00 | 1.69 | 6.45 |
| Chit/Alg/CMC/2/ Lid | 41.90 | 24.50 | 3.50 | 2.10 | 28.00 | 1.69 | 6.75 |
| Chit/Alg/CMC/3/ Lid | 14.00 | 52.40 | 3.50 | 2.10 | 28.00 | 1.69 | 6.60 |
| Chit/Alg/CMC/Sf | | | | | | | |
| Chit/Alg/CMC/1/ Sf | 41.1 | 13.70 | 13.70 | 4.10 | 27.40 | 1.69 | 6.64 |
| Chit/Alg/CMC/2/ Sf | 41.1 | 24.00 | 3.40 | 4.10 | 27.40 | 1.69 | 6.60 |
| Chit/Alg/CMC/3/ Sf | 13.70 | 51.40 | 3.40 | 4.10 | 27.40 | 1.69 | 6.70 |

*2.2.2. Examination of the three-component polymeric biocomposites by infrared spectrophotometry (FTIR)*

The Genesis Series FTIR™ device, made by Unicam (UK) and equipped with the analytical software ATI Mattson and Peak Solve, was used in the spectrophotometric examination of the polymeric biocomposites. The spectra were prepared by translucent technique in the range of 500–4000 $cm^{-1}$ of the wave number, at a resolution of 4 $cm^{-1}$ and scan number of 32. The samples were tested in the form of KBr tablets (constant ratio: 1 mg of sample in 200 mg of KBr). Liquid preparations were analysed with the use of AgCl trays [16].

*2.2.3. Structure examination of selected polymeric biocomposites by NMR spectroscopy for solid state*

The investigation of the structure of selected biocomposite films with and without the active substance lidocaine was made at the Laboratory of Structure Research of the Centre of Molecular and Macromolecular Research of the Polish Academy of Sciences, Łódź, Poland. The method of nuclear magnetic resonance spectroscopy was applied. The NMR measurements in solid state were accomplished by the use of the BRUKER AVANCE III spectrometer at a frequency of 75.47 MHz for $^{13}C$ and 121.49 MHz for nucleus $^{31}P$. The Hartmann–Hahna condition in the sequence $^{13}C$ CP/MAS was established on glycine samples while adamantan was applied as a secondary standard of the chemical shift, δ=38.48 ppm and 29.46 ppm towards tetramethylosilane (TMS) [17].


DOI: 10.15259/PCACD.23.19

The broadband probe MAS was used in the measurements. Chitosan samples were placed in the 4 mm zircon rotor ($ZrO_2$) and centrifuged at a magic angle with a speed of 8 kHz. The typical CP/MAS spectrum was recorded with a 90º proton pulse equal to 3.5 μs, contact time of 1ms, interval between the pulses of -10 s and a spectral range of 25 kHz. FIDs were collected in the time domain of 2 K pkt. The sequence of "ramp" [18] with variable pulse geometry during contact between the spins was applied in the through polarisation. Protons were decoupled by the use of the sequence *Two Pulse Phase Modulation* (TPPM) [19] with the parameter $\tau_{p=}$6.8 μs and phase angle of 20° during acquisition.

*2.2.4. Differential scanning calorimetry (DSC) of selected three-component polymeric biocomposites*

The Q100 (TA Instruments) apparatus was used for this purpose; thermal examination of the prepared materials was accomplished in a non-isothermal cycle heating-cooling-heating in the temperature range of -100 to 150°C with a heating speed of 10°C/min. The temperature of glass transition and melting were determined from the thermographs of the second heating. The obtained data were analysed by means of the Universal Analysis software (TA Instruments).

*2.2.5. Determination of the physical-mechanical properties of the three-component biocomposites*

Mechanical properties were tested in the accredited Laboratory of Metrology of the Institute of Biopolymers and Chemical Fibres (accreditation certificate No. AB 338). Basic mechanical properties of the composite materials were examined such as: tenacity at break and elongation at maximal stress according to standards PN-EN ISO 4593:1999 and, PN-EN ISO 527-3:1998. Permanent deformation and extensibility, also defined as the ability to fit, were measured according to PN-EN 13726-4:2005. Standard PN-EN-13726-2:2005 was applied to estimate vapour transmission. Results from the measurements of five samples were averaged; the tested film was 0.04 to 0.1 mm thick.

*2.2.6. Examination of the absorption properties of the three-component polymeric biocomposites*

The absorption properties of the three-component biocomposite materials were assessed by the measurement of water retention value (WRV), which was calculated according to a standard relationship [20]:

$$WRV=[(m_1 - m_0)/m_0] \cdot 100\% \qquad (1)$$

where:

$m_1$ – mass of sample kept for 20 hours in water and centrifuged afterwards at 4000 rpm for 10 minutes.

$m_0$ – mass of sample after drying at 105°C.

*2.2.7. Biological examination of selected three-component polymeric biocomposites*

2.2.7.1. Examination of anti-bacterial activity of the biocomposite materials against gram (+) Staphylococcus aureus and gram (-) Escherichia coli bacteria

The antibacterial activity of the three-component biocomposite materials in film form was tested in the Laboratory of Microbiology of the Institute of Biopolymers and Chemical Fibres according to a proprietary procedure named "Estimation of anti-bacterial activity of textiles. V edition. Quantitative test." The procedure was prepared

on the basis of the standard JIS L 1902:2002. Measured in the test is the growth inhibition of standard bacterial strains in contact with the tested sample. The number of bacteria in the sample before and after a 24 hour incubation period in comparison to an inactive sample was estimated. The bacteriostatic effect (inhibition of bacteria growth) and bactericidal effect (destruction of the bacteria by the agents contained in the sample) were calculated.

2.2.7.2. Examination of the cytotoxic action of selected three-component biocomposite materials by the method of direct contact

The assessment of the direct contact cytotoxic action was performed at the National Institute of Drugs, Dept. of Biochemistry and Biopharmacy, Warsaw, Poland on a reference cell line with mouse fibroblasts NCTC clone 929; ATCC in accordance with standard PN-EN ISO 10993-5:2009 ”Biological estimation of medical devices. The cytotoxicity was tested by *in vitro* methods. Quantitative and morphological changes in the cell cultures were assessed after 24 hours by means of a contrast-phase inverted microscope. Photographic documentation of the examination was also prepared.

*2.2.8. Assessment of the rate at which the medicinal substance is released from the three-component biocomposite preparation to an acceptor fluid*

The examination was accomplished in the Drug Store Pharmacy Department of the Faculty of Applied Pharmacy at the Medical University, Łódź, Poland. The release rate of the following medicinal substances was studied: lidocaine (Lid) and sulphanilamide from the biocomposite preparation in the form of a single-layer film with defined mass. The amounts of the used preparation samples were in the range from 0.040–0.050 g and 0.200 g for measurements with sulphanilamide and lidocaine (Lid), respectively. Measurements of the release rate of the active substance were made according to the requirements laid down in the Polish Pharmacopeia (FP VI, FP VII, FP XI) for solid drugs and trans-dermal systems [14, 21, 22] by the use of a paddle apparatus (Figure 1). Standard curves were prepared for a spectrum method (UV-Vis) at a wavelength of 259 nm for sulphanilamide and 262 nm for lidocaine, to determine the concentration of active substances in the acceptor fluid. Normal saline with a concentration of 0.9wt.% of NaCl in the amount of 100 ml was used as an acceptor fluid. Measurements were made at 35°C and at an agitator speed of 100 rpm. Lighter films were loaded with a wire spiral (few turns) to prevent floating. To determine the active substance concentration, samples of the solution (2.5 ml each) were taken in the following time sequence: 1, 2, 5, 10, 15, 30, 60, 120, 180 min and 24 h. The acceptor fluid in the measurement vessel was replenished with the same amount of standard saline as the sample taken each time.

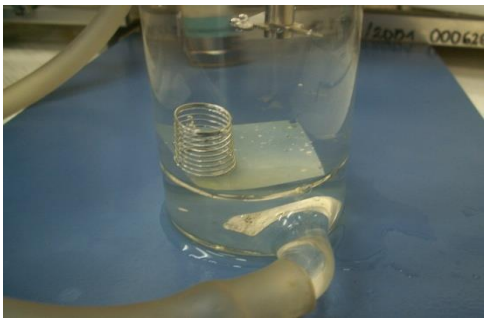

**Figure 1.** Paddle apparatus for *in vitro* measurements of the release rate of a medicinal substance from the three-component biocomposite materials in the form of a single-layer film

## 3. Results and Discussion

The main goal of the presented research was the preparation of a three-component biocomposite material in the form of a single-layer film with the addition of a medicinal substance – the analgesic lidocaine and bacteriostatic sulphanilamide. The material is intended as the main component of a dressing for the treatment of difficult-to-heal bedsores. How the contents of chitosan, alginate and carboxymethyl cellulose and the kind of bioactive compound influence the chemical and phase structure, and the properties of the prepared materials were studied in the work. The prepared dressing is a trans-dermal therapeutic system (TTS) which should have proper physicochemical, useable and biological properties. With such parameters, the dressing should allow sufficient mobility if used in movable body parts and maintain moisture in the wound environment, thus providing better conditions for the penetration of the active substance to the wound interior, and protecting the wound against external factors and secondary infections.

### 3.1 Spectrophotometric infrared analysis (FTIR) of selected three-component biocomposite materials

Infrared spectrophotometry (FTIR) is widely applied to identify intermolecular actions in blends of biopolymers. Properties of polymer blends are a result of the interactions caused by hydrogen bonds and/or electrostatic actions between functional groups of the various polymers. Figure 2 presents the chemical structure of sodium salts of carboxymethyl cellulose, sodium alginate and the biocomposite Alg/CMC.

In Figure 3, the FTIR spectra of chitosan lactate, the active substance (lidocaine) and a selected biocomposite material with addition of the active substance Chit/Alg/CMC/Lid and without the additive Chit/Alg/CMC are shown.

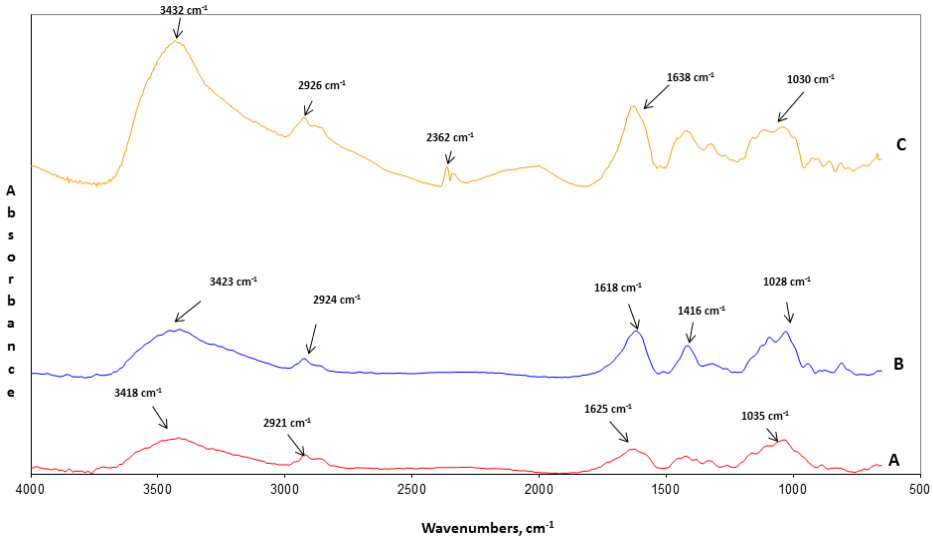


**Figure 2.** FTIR Spectra of (A) CMC, (B) sodium alginate, and (C) composite Alg/CMC

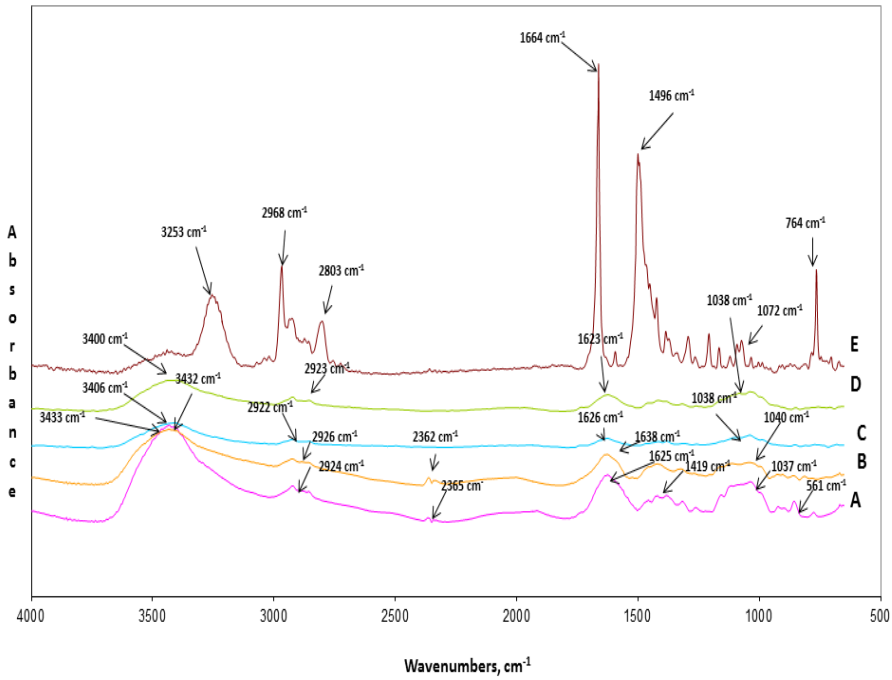


**Figure 3.** FTIR Spectra (A) chitosan lactate + $CaCl_2$, (B) composite Alg/CMC, (C) composite Chit/Alg/CMC/Lid, (D) composite Chit/Alg/CMC, and (E) lidocaine

In the FTIR spectrum of sodium alginate (Figure 2 B), two intensive absorption bands can be seen responding to symmetric (1618 $cm^{-1}$) and asymmetric (1416 $cm^{-1}$) covalent oscillations of C-O in the carboxylic ion; also present is the band (1028 $cm^{-1}$) related to the stretching oscillation of C-O-C . The FTIR spectrum of sodium alginate also reveals a strong and broad band at 3423 $cm^{-1}$ related to the stretching oscillation of O-H and the weak aliphatic band of stretching oscillation C-H at 2924 $cm^{-1}$ [23].

In the FTIR spectrum of CMC (Figure 2 A), a strong, characteristic shift band appears at 3418 $cm^{-1}$ linked with stretching oscillation of the O-H group relating to the presence of hydrogen atoms (presence of inter- and intra-molecular hydrogen bonds), and a less intensive band at 2921 $cm^{-1}$ responding to stretching oscillation of C-H related to hydrogen atoms in the methylene group [24]. The absorption band at 1625 $cm^{-1}$ confirms the presence of the COO- carboxylic group and responds to symmetric stretching oscillation. The FTIR spectrum of the Alg/CMC composite indicates that the addition of CMC to sodium alginate does not promote vital changes in interactions between the functional groups of both polymers.

On the other hand, in the spectrum of chitosan lactate with the addition of calcium chloride (Figure 3 A), the characteristic band 1625 $cm^{-1}$ can be seen, related to stretching oscillations of the carbonyl group C=O in the amide group (band of Amide I). The Amide II band, corresponding to covalent oscillation of N-H in the $-NH_2$ group, does not appear in the range of bands $\nu$=1500–1600 $cm^{-1}$. In the FTIR spectrum of lidocaine (Figure 3 E), the band at 3253 $cm^{-1}$ appears, confirming the presence of the benzene ring.

In the range of bands ν=1490–1700 $cm^{-1}$, a strong signal emerges originating from the C=C oscillation, while the signal appearing in the range of bands ν=3000–3500 $cm^{-1}$ corresponds to the amide group H-N-C=O. The absorbance in the region of 1630–1690 $cm^{-1}$ may be linked to the carbonyl groups of lidocaine [25, 26].

The addition of the analgesic lidocaine to the three-component biocomposite Chit/Alg/CMC did not cause any discernible changes in the FTIR spectrum of the composite preparation (Figure 3 C). This may indicate that the medicinal substance is only physically compounded by the biocomposite material. Confirmation of the investigations will be found in the assessment of the release rate of the substance from the prepared dressing materials formed in a single-layer film.

### 3.2. Estimation of the chemical structure of a selected three-component polymeric biocomposite by solid-state NMR spectroscopy

Structural investigation of the three-component polymeric biocomposite formed as single-layer film containing the analgesic lidocaine was accomplished by applying solid-state NMR spectroscopy (CP/MAS NMR). A formulation without the medicinal substance was also prepared for comparison. It was found that the concentration of the medicinal component is too low (at the level of 3%) to allow its unequivocal identification by the NMR technique. In Figure 4, examples of $^{13}C$ spectra of the used medicinal substance and the polymeric three-component biocomposites in the form of a film are presented.

### 3.3. Differential scanning calorimetry (DSC) of selected biocomposite materials in the formation of a single-layer film

Thermal properties of the prepared biocomposite materials in the form of a single-layer film were estimated by differential scanning calorimetry. Endo- and exothermic processes were studied, such as melting crystallisation, and phase transition from a glassy to elastic state. The thermal analysis of selected biocomposite materials with and without the active substance is shown in Table 2.

The strong affinity of polysaccharides to water is well known from the literature; their hydration depends on their molecular and super-molecular structure. Water is released at varying temperatures depending upon various interactions between water molecules and the polysaccharide chain. Endothermic peaks are correlated with the loss of water bonded with hydrophilic groups of the polymer while exothermic peaks stem from the degradation of the polyelectrolyte caused by dehydration and polymer degradation [27, 28].

In the presented thermographs of the heating of chitosan (Chit), sodium alginate (Alg) and sodium salt of carboxylmethyl cellulose (CMC), biocomposite chitosan/alginate/CMC (Chit/Alg/CMC/2) and biocomposite with active substance (Chit/Alg/CMC/2/Lid ), peaks can be observed which may relate to water which remained after drying being strongly linked with functional groups of the polymer. As a result of the first heating, the presence of only one broad endothermic peak was observed in the DSC curves responding to the melting temperature of: Chit ($T_m$=149°C), Alg ($T_m$=107°C) and CMC ($T_m$=128°C). The biocomposite chit/alg/CMC in the weight proportion of 60:35:5 reveals a decrease in the water loss temperature to 98°C. The addition of lidocaine results in a shift in the maximum of the endothermic change ($T_{m=}$116°C) and a distinct decrease of the enthalpy of the endothermic transformation ($\Delta H_{m=}$39 J/g).

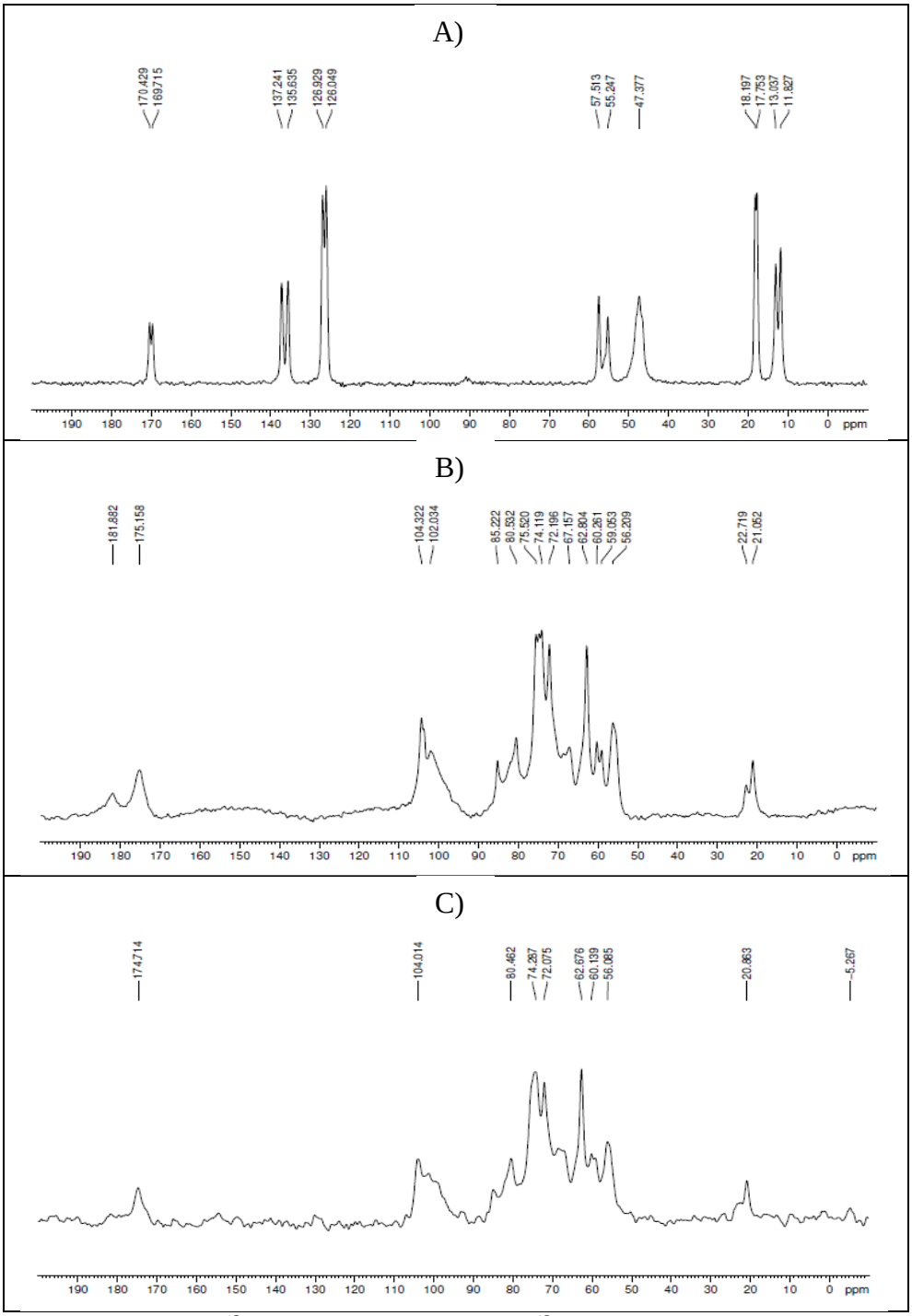


**Figure 4.** Spectrum $^{13}C$ CP/MAS of lidocaine A) ($^{13}C$=Lidocaine=RO=8 kHz) and the polymeric biocomposite chitosan-alginate-carboxymethyl cellulose: B) – with the addition of lidocaine Chit/Alg/CMC/2/Lid ($^{13}C$=Chit/Alg/CMC/2/Lid (3%)=RO=8kHz), C) – without lidocaine Chit/Alg/CMC/2 ($^{13}C$=Chit/Alg/CMC/2=RO=8 kHz)

**Table 2.** Thermal analysis of the prepared three-component polymeric biocomposite with and without the addition of the active substance.

| Symbol of the preparation | I heating | |
|---|---|---|
| | $T_m$ [°C] | $\Delta H_m$ [J/g] |
| **Chit/$CaCl_2$** | 149.3 | 97.66 |
| **Alg** | 107.9 | 68.57 |
| **CMC** | 128.6 | 167.20 |
| **Chit/Alg/CMC/2** | 98.6 | 158.50 |
| **Chit/Alg/CMC/2/Lid** | 116.8 | 39.88 |

Tm, – melting temperature (loss of water), ΔHm – enthalpy of endothermic transformation relating to melting (loss of water)
Additional information: Glycerol - Tm=18°C, Lidocaine - Tm=67–69°C

### 3.4. Estimation of the physical-mechanical parameters of the three-component biocomposite materials

Main physical-mechanical parameters were examined of the three-component biocomposite materials with a content of the analgesic lidocaine or anti-bacterial agent sulphanilamide. The impact of the substances on the mechanical parameters of the prepared materials in the form of a single-layer film was examined. The test results are compiled in Table 3.

**Table. 3.** Physical-mechanical parameters of the three-component biocomposite materials in the form of a single-layer film with and without lidocaine and with and without sulphanilamide

| Symbol of the biocomposite | Quantitative composition of composite Chit:Alg: CMC % wt | Amount of lidocaine % wt. | Tenacity MPa | Elongation at max. stress % | Permanent deformation % | Extensibility* N/cm | Transmission of moisture $g \cdot m^{-2} \cdot 24h^{-1}$ |
|---|---|---|---|---|---|---|---|
| **Lidocaine** | | | | | | | |
| Chit/Alg/CMC/1 | 60:20:20 | - | 19.2 | 70.0 | 4.76 | 2.70 | 4680 |
| Chit/Alg/CMC/1/Lid | | 3.0 | 12.7 | 88.1 | 5.02 | 1.90 | 6216 |
| Chit/Alg/CMC/2 | 60:35:5 | - | 10.1 | 69.2 | 7.74 | 2.65 | 5687 |
| Chit/Alg/CMC/2/Lid | | 3.0 | 13.5 | 91.1 | 4.76 | 1.48 | 8539 |
| Chit/Alg/CMC/3 | 20:75:5 | - | 9.0 | 23.7 | 8.05 | 3.04 | 5780 |
| Chit/Alg/CMC/3/Lid | | 3.0 | 8.81 | 52.7 | 7.20 | 1.68 | 7450 |
| **sulphanilamide** | | | | | | | |
| Chit/Alg/CMC/1 | 60:20:20 | - | 19.2 | 70.0 | 4.76 | 2.70 | 4680 |
| Chit/Alg/CMC/1/Sf | | 6.0 | 12.7 | 78.3 | 4.02 | 1.99 | 6116 |
| Chit/Alg/CMC/2 | 60:35:5 | - | 10.1 | 69.2 | 7.74 | 2.65 | 5687 |
| Chit/Alg/CMC/2/Sf | | 6.0 | 9.84 | 74.7 | 7.03 | 1.68 | 6234 |
| Chit/Alg/CMC/3 | 20:75:5 | - | 9.0 | 23.7 | 8.05 | 3.04 | 5780 |
| Chit/Alg/CMC/3/Sf | | 6.0 | 8.89 | 42.9 | 6.20 | 1.78 | 6450 |

*- force needed to extend the dressing to a defined elongation

From the mechanical parameters and moisture transmission compiled in Table 3, it may be concluded that the three-component dressings in the form of a film permit the

uninhibited evaporation of surplus moisture from the wound surface. On the other hand they prevent water and bacteria from outside to enter the wound which is thus protected from the effect of external factors. The addition of the analgesic lidocaine or antibacterial sulphanilamide causes a slight drop in the dressing's tenacity and an improvement in elasticity. The last effect is not expected to play a significant role in the practical use of materials. The best mechanical properties amongst the tested materials were recorded for the preparation Chit/Alg/CMC/2/Lid with the weight proportion of 60:35:5 for the components Chit/Alg/ CMC with the addition of lidocaine. Its tenacity amounts to 13.5 MPa, elongation at maximum stress to 91% and moisture transmission to 8500 $g \cdot m^{-2} \cdot 24h^{-1}$. Transparency of the material makes watching the wound and following-up its healing easier. The testing of extensibility and permanent deformation allows the drapability of the material to be anticipated. A dressing that loosely moves with the skin and returns to its original length after extension provides a more comfortable use.

### 3.5. Estimation of the absorption properties of the three-component biocomposite materials

The water absorption properties of the biocomposites were tested, which make the basic components of a dressing material in the form of a film destined for the treatment of bedsores. Examination was based on measurements of the water retention value (WRV) of the biocomposites. Modern dressing devices designed to heal difficult wounds, particularly bedsores, are expected to provide not only an effective action of the medicinal substance contained but also proper comfort when applied. A crucial, greatly desired feature of the dressing materials is the ability to maintain proper moisture, a prerequisite of good and fast healing. A moist environment provides better penetration of the active substances to the wound interior, and conditions for the easy and painless changing of dressings, by avoiding any damage to fresh tissue regenerated by granulation and epithelialisation. The results of the investigation are presented in Figures 5 and 6.

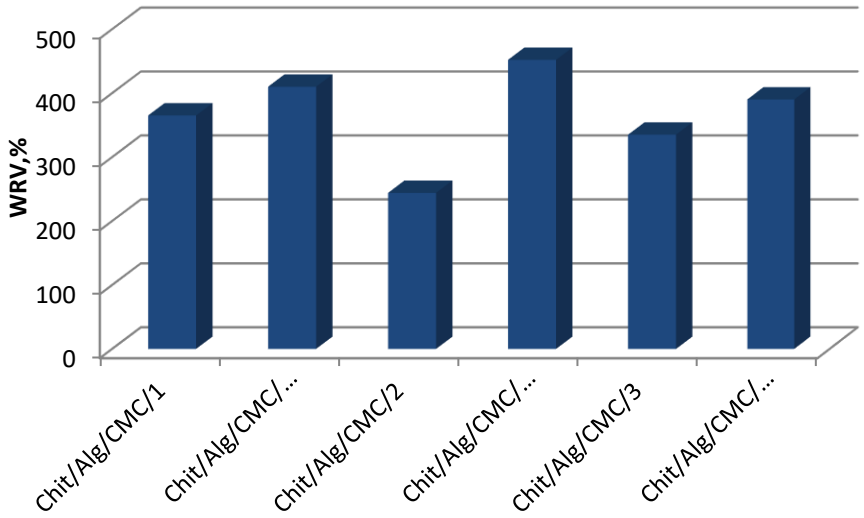


**Figure 5.** WRV of biocomposite materials Chit/Alg/CMC in the form of a single-layer film with and without the addition of lidocaine

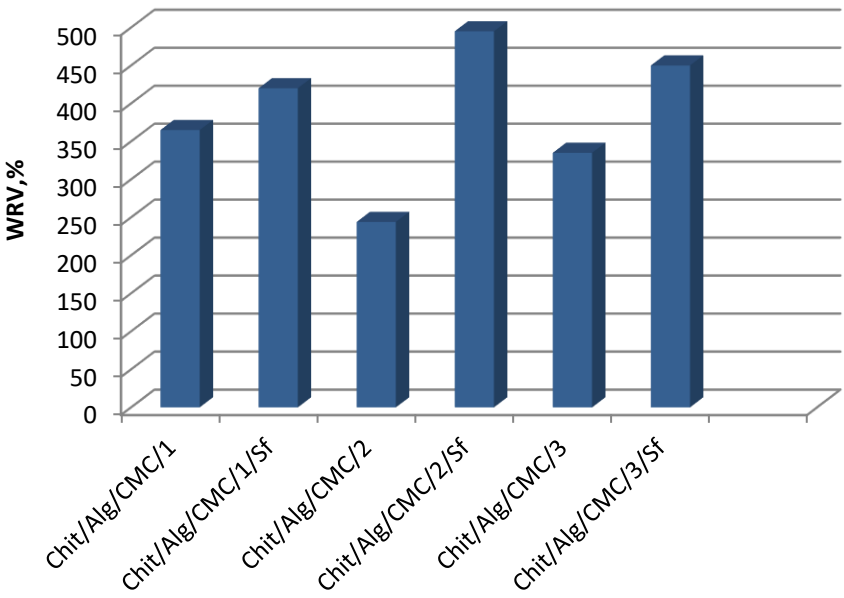


**Figure 6**. WRV of biocomposite materials Chit/Alg/CMC in the form of a single-layer film with and without the addition of sulphanilamide

The results of the WRV measurements presented in Tables 5 and 6 suggest that the parameter depends upon the quantitative composition of the prepared biocomposite materials. The addition of the medicinal agents lidocaine and sulphanilamide to the basic material results in an increase in the WRV values. Preparations denoted Chit/Alg/CMC/2/Lid and Chit/Alg/CMC/2/Sf with the weight proportion of their components Chit/Alg/CMC amounting to 60:35:5 show the highest ability to retain water.

### 3.6. Examination of the rate at which the medicinal agents are released from the three-component biocomposites

The speed at which the active agents sulphanilamide and lidocaine migrate from selected polymeric films with varied composition and mass in the range from 0.04–0.05g was studied.

The prepared dressing material constitutes a transdermal therapeutic system (TTS) which, along with its ability to slowly release drugs, also offers the administering of medications that are broken down in the alimentary tract or are metabolised in the liver [29]. A matrix TTS was applied in the tests, where the medicinal agent is evenly dispersed or suspended in a solid matrix (the single–layer film with the agent). Diffusion controls the release of the medicinal agent from the matrix system while the lipophilicity and structure of the matrix govern the rate of release.

Profiles of the release of the active substances from selected three-component biocomposites are demonstrated in Figure 7.

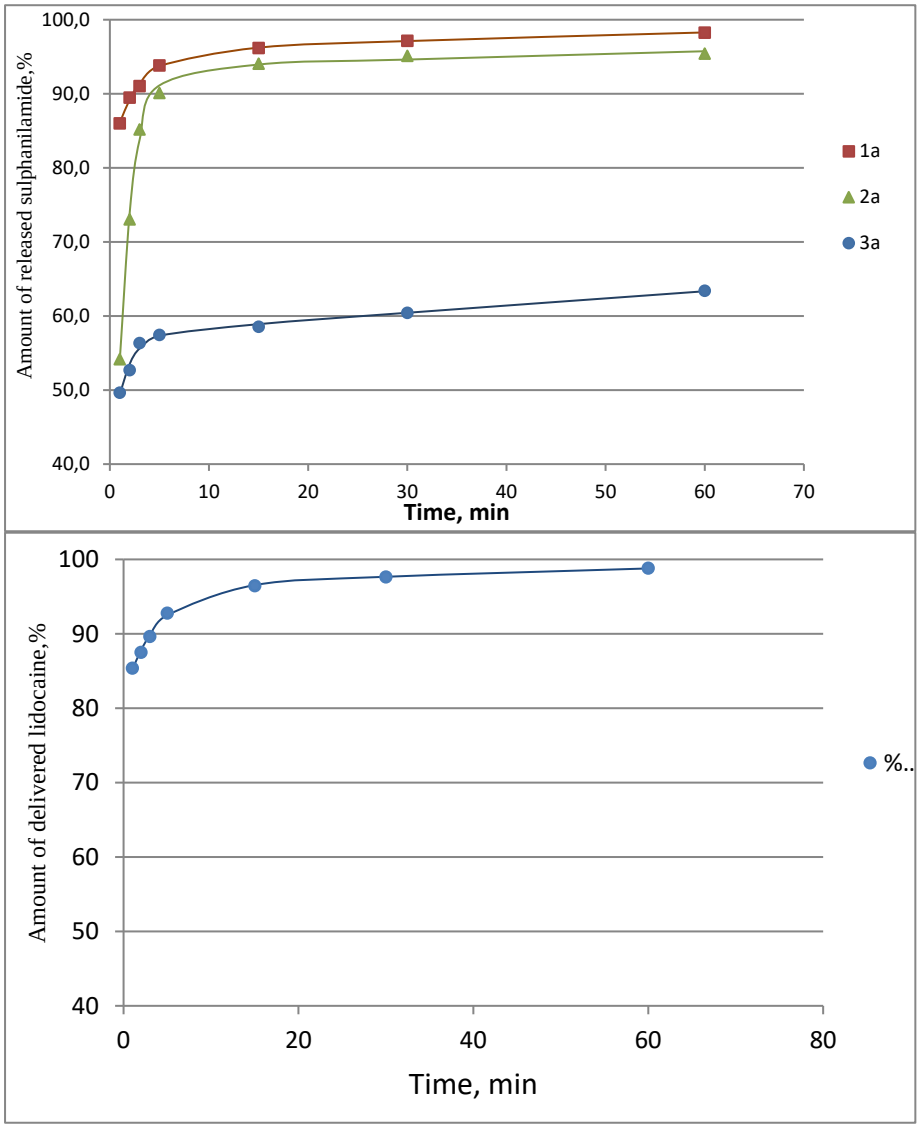


**Figure 7.** Kinetics of the release of sulphanilamide from the three-component biocomposite materials: 1a-Chit/Alg/CMC/2/Sf, 2a- Chit/Alg/CMC/3/Sf (20:75:5), 3a - Chit/Alg/CMC/1/Sf (60:20:20), 4 - Chit/Alg/CMC/2/Lid (60:35:5) (Measuring points - theoretical lines)

To describe the release profile of the drug from the biocomposite, it seems appropriate to use the scheme of the 1st order reaction process with the free expression in the following form:

$$Y=a_0 + a_1 \times (1 - \exp(-k_1 \times t)$$


DOI: 10.15259/PCACD.23.19

However, on the basis of the conducted studies, it seems that a better equation with two reactions of the 1st order should be applied, in the following form:

$$Y=a_0 + a_1 \times (1 - \exp(-k_1 \times t) + a_2 \times (1 - \exp(-k_2 \times t)$$

It may be concluded from the results shown in Table 4 that the quantitative composition of the materials significantly influences the release rate of sulphanilamide from the three-component biocomposite materials. The lowest release rate was found in the preparation Chit/Alg/CMC/1/Sf, with the component weight proportion of chitosan/alginate/CMC 60:20:20, from which 64% of the agent was released after 60 minutes.

**Table 4.** Parameters of the kinetics equation relating to the rate of release of the medicinal agent (sulphanilamide and lidocaine) from the three-component biocomposite materials in the form of a single-layer film

| Symbol of the biocomposite | $a_0$, % | Stage I | | | Stage II | | | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | | $a_1$, % | $k_1$, $min^{-1}$ | $t_{0.5}$, min | $a_2$, % | $k_2$, $min^{-1}$ | $t_{0.5}$, min | - |
| Chit/Alg/CMC/1/Sf (3a) | 41.45 | 15.85 | 0.689 | 1.01 | 42.70 | 0.00254 | 273.0 | 0.989 |
| Chit/Alg/CMC/2/Sf (1a) | 81.43 | 13.72 | 0.406 | 1.71 | 4.85 | 0.01730 | 40.1 | 0.999 |
| Chit/Alg/CMC/3/Sf (2a) | 13.67 | 79.53 | 0.707 | 0.98 | 6.80 | 0.00782 | 88.6 | 0.997 |
| Chit/Alg/CMC/2/Lid (4) | 81.81 | 13.71 | 0.274 | 2.53 | 4.48 | 0.02180 | 31.8 | 0.993 |

where:

$a_0$=– amount of free „unattached" medicinal agent in% (released less than 1 min - immediately)
$a_1$ and $k_1$ – constants for stage I - from 1 min to 5 min
$a_2$ and $k_2$ - constants for stage II –above 5 min
$t_{0.5}$ – half – period of release for both stages
$R^2$ – correlation coefficient

The results compiled in Table 4 indicate that the release of sulphanilamide from the three-component biocomposite material is a complex process proceeding according to kinetics of the 1st order. Sulphanilamide can be loosely attached onto the film surface or permanently by physicochemical or chemical bonds; it may also be dispersed in the film interior. One part of the medicinal substance is very quickly delivered (below 1 minute) as the initial dose; the delivery rate is probably related to the dissolution rate. The amount of released active substance in the stage is in the range of 13.0% to ca. 80.0%. The dispersed portion of the medicinal substance is delivered from the film by diffusion, forming the maintenance dose. The amount of sulphanilamide bound in the film interior is released with the lowest rate. In the "fast" process, the substance is released in the amount of about 13.0% to about 79.0%; the half period of the release ($t_{0.5}$) was about 1 minute. In the "slow" process, from 4.0% to 42.0% of the agent is delivered; its half-life of release is much longer: from 40 to 273 minutes. The results obtained in this part of the research indicate the possible control of the release process of sulphanilamide by changing the amount of polymers in the biocomposite film.

Kinetics for lidocaine release were tested only with the preparation denoted Chit/Alg/CMC/2/Lid, with the weight proportion of components Chit/Alg/CMC amounting to 60:35:5 (Figure 7). The release of lidocaine with that biocomposite was very fast: after 60 minutes, 99% of lidocaine was delivered. Parameters of the kinetics equation for the release of lidocaine from the used biocomposite film are shown in Table 4.

Bonds between lidocaine and the preparation Chit/Alg/KMC/2/Lid were much weaker, hence the amount of loose substance was 81.81%. About 13.7% of the total amount of lidocaine contained in the preparation was delivered during the fast stage, which lasted for about 2.5 minutes, while about 4% was released in the slow stage with the half period of release ($t_{0.5}$) of about 31 minutes.

### 3.7. Assessment of some biological properties inclusive antibacterial activity and cytotoxicity

Biocomposite preparations showing best physical-mechanical and useful properties were studied *in vitro*, examined with respect to their antibacterial activity and cytotoxicity.

#### *3.7.1. Estimation of antibacterial activity of selected three-component biocomposite materials*

The effective healing of wounds, bedsores in particular, may be impeded by the occurrence of infections caused by microorganisms originating from the natural bacterial flora of the human body or the patient's environment. This can be prevented by applying dressing materials carrying medicinal substances like antibacterial agents and analgesics, thus providing a good barrier to the pathogens.

The antibacterial action of the selected biocomposite materials was examined, on the basis of the applied research, with the aim of finding amongst the applied analgesics and antibacterial substances those which were most effective against the model bacteria Gram (-) *Escherichia coli* and Gram (+) *Staphylococcus aureus*.

*E. coli* is a type of rod-shaped bacterium that normally lives in the intestines of humans and animals. They often cause infections, whose clinical symptoms and course of the ailment depend on the place of occurrence [30]. The bacterium *S. aureus* may cause infections in any tissue and organ, or general life-threatening infections [31]. These two strains were recognised as model pathogens causing infections and reflecting the conditions in the wound.

The results presented in Table 5 are proof that all of the tested biocompsite materials reveal bacteriostatic and bactericidal activity against Gram (-) *Escherichia coli* and bacteriostatic action against Gran (+) *Staphylococcus aureus*. The activity rises when the active substances sulphanilamide and lidocaine are admixed with the tested materials. It is worth noting that the biocomposite materials themselves, without the addition of the medicinal substances, exert bactericidal action against *Escherichia coli*, and to a lesser degree against *Staphylococcus aureus.* It may be inferred from the literature that a relationship exists between the antimicrobial activity of chitosan and the characteristics of the bacterial cell wall. The polysaccharide reveals a better bactericidal and bacteriostatic action against Gram (-) bacteria than Gram (+) species due to the phospholipids and carboxylic acids contained in the bacteria cell wall [32-33].

**Table 5.** Antibacterial activity of three-component biocomposite materials with the medicinal agent as an additive

| Symbol of the biocomposite | Number of bacteria jtk/sample | Kind of bacteria | Bacteriostatic activity | Bactericidal activity |
|---|---|---|---|---|
| lidocaine | | | | |
| Reference (cotton) | $1.9 \times 10^4$/0h $1.8 \times 10^8$/24h | *Escherichia coli ATCC 11229* Bacteria gram (-) | - | - |
| Chit/Alg/CMC/2 (60:35:5) | < 20 | | 7.0 | 3.0 |
| Chit/Alg/CMC/2/Lid (60:35:5) | < 20 | | 7.0 | 3.0 |
| Reference (cotton) | $1.3 \times 10^4$/0h $5.1 \times 10^6$/24h | *Staphylococcus aureus ATCC 6538* Bacteria gram (+) | - | - |
| Chit/Alg/CMC/2 (60:35:5) | $4.7 \times 10^6$ | | 0.0 | 0.0 |
| sulphanilamide | | | | |
| Reference (cotton) | $1.9 \times 10^4$/0h $1.8 \times 10^8$/24h | *Escherichia coli ATCC 11229* Bacteria gram (-) | - | - |
| Chit/Alg/CMC/2 (60:35:5) | < 20 | | 7.0 | 3.0 |
| Chit/Alg/CMC/2/Sf (60:35:5) | < 20 | | 7.0 | 4.0 |
| Reference (cotton) | $1.3 \times 10^4$/0h $5.1 \times 10^6$/24h | *Staphylococcus aureus ATCC 6538* Bacteria gram (+) | - | - |
| Chit/Alg/CMC/2 (60:35:5) | $4.7 \times 10^6$ | | 0.0 | 0.0 |
| Chit/Alg/CMC/2/Sf (60:35:5) | $3.9 \times 10^3$ | | 3.1 | 0.5 |

### 3.8. *In vitro* assessment of the cytotoxicity of selected three-component biocomposite materials

Functional dressing materials for the healing of bedsores serve to maintain proper conditions in the wound's environment, thus assisting in the reconstruction of tissue, and protecting sores against external infections. Since the dressing comes into contact with the skin and damaged tissue, it must be made of biocompatible polymeric materials.

The cytotoxicity of the selected materials Chit/Alg/CMC/2 and Chit/Alg/CMC/2/Lid was examined to this end. The latter offers adequate useful and physical-mechanical properties, promising its possible use in the construction of dressings for bedsores. Prior

to the testing the preparations were irradiated with fast electrons at a dose of 25 kGy. The results of the examination are presented in Table 6.

**Table 6.** Assessment of cytotoxicity of biocomposite materials in the form of a single-layer film

| Symbol of the biocomposite | Medicinal substance | Cytotoxicity degree |
|---|---|---|
| Chit/Alg/CMC/2 reference | - | 0 |
| Chit/Alg/CMC/2/Lid | Lidocaine | 0 |

On the basis of the results shown in Table 6, it may be asserted that the tested biocomposite materials in the form of a film containing lidocaine, as well as the reference material, do not reveal cytotoxicity.

In Figure 8, prints from a 24-hour culture of the mouse fibroblast NCTC clone 929 presented after contact with the biocomposite samples, negative control (PVC/PV film) and positive control (latex).

The negative control (PVA/PV film) used did not affect the viability or number of mouse fibroblast cells, whereas the positive control (latex) caused a significant decrease in their number and influenced their viability. The biocomposite materials selected for testing, containing lidocaine, and a control preparation did not affect the number or viability of mouse fibroblast cells.

## 4. Conclusions

The purpose of the study presented here was to develop a material suitable for the construction of dressings designed for the healing of bedsores. Three natural polymers: chitosan, alginates and carboxymethyl cellulose (their sodium salts) (Chit/Alg/CMC) were selected to this end. These polymeric substances are already well-established in medical applications for their valuable properties like biocompatibility, antimicrobial activity, absorbency and good processability.

The polymers mixed into uniform blends were cast to prepare a single-layer film. Transparency of the film is beneficial since it enables the appearance of the wound to be watched, along with the course of healing. The blends were prepared from the singular polymers in varied weight proportions; however, the proportion of 60:35:5 of the Chit/Alg/CMC components augurs the best results due to good mechanical properties, promising sufficient strength, good vapour transportation and drapability. The weight proportion of the components can be varied to control the rate of delivery of medical substances incorporated in the three-component composite.

The study also comprised the examination of properties and actions of medical substances added to the composites: analgesic lidocaine and bacteriostatic sulphanilamide. Careful examinations were made to study possible interactions between the polymeric basic components and any medicinal substances added. NMR and FTIR spectroscopic analysis showed that the biocomposite material is only physically bonded with the medicinal substance. DSC measurements confirmed the absence of permanent interactions between the polymeric biocomposite and the medicinal substance.

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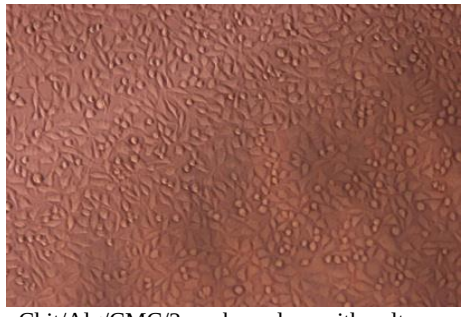
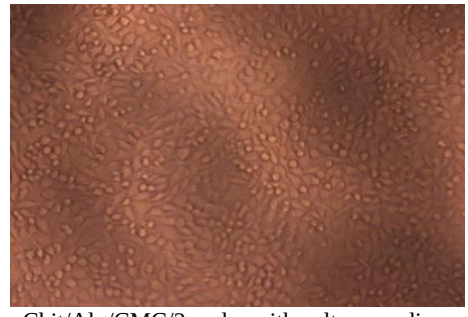
Negative control on boundary with culture medium with fibroblasts mouse NCTC, 100 x

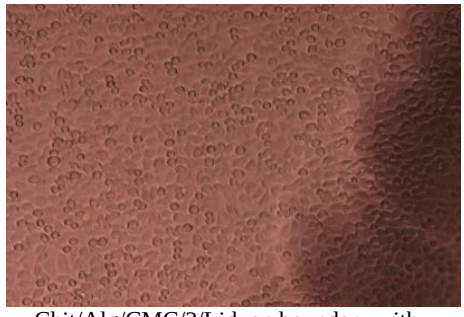
Positive control on boundary with culture medium with mouse fibroblasts NCTC,100 x



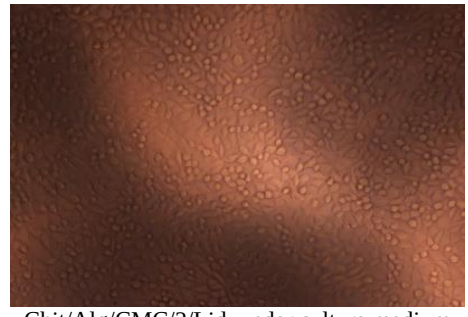
Chit/Alg/CMC/2, on boundary with culture medium with mouse fibroblasts NCTC,100 x



Chit/Alg/CMC/2,under with culture medium with mouse fibroblasts NCTC,100 x



Chit/Alg/CMC/2/Lid, on boundary with culture medium with mouse fibroblasts NCTC,100 x



Chit/Alg/CMC/2/Lid, under culture medium with mouse fibroblasts NCTC, 100 x

**Figure 8.** 24 h culture of mouse fibroblasts NCTC clone 929 after contact with samples of biocomposite with and without lidocaine, negative control and positive control

The kinetics of the release of medicinal substances from the biocomposite was studied. The release proceeds in two stages: the first major portion of the agent (lidocaine or sulphanilamide) can be quickly delivered to the wound providing the therapeutic dose; this is followed by phase II, during which smaller amounts of the agent are slowly delivered as the maintenance dose. Such a delivery scheme can be arranged by modifying the composition of the basic material.

One positive feature of a good dressing is the ability to absorb exudates from the wound. The property was assessed indirectly by the measurements of water retention

value (WRV), which indicates the potential to absorb aqueous fluids. WRV of the three polymers used is high and it goes up with the addition of both medicinal substances.

Chitosan makes up the major portion of the three-component biocomposite film; its antimicrobial property is well known, hence the composite film exerts the property as well. Experiments in this study showed that the addition of the active agents (lidocaine and sufaniamide) to the biocomposite enhances the antibacterial activity against the Gram (-) *Escherichia coli* and Gram (+) *Staphylococcus aureus*.

Biocompatibility was also assessed by examining the cytotoxicity of the prepared materials. Both the biocomposite itself selected for the testing and the test with the addition of lidocaine did not reveal cytotoxicity against the mouse fibroblast NCTC clone 929 ATCC.

Considering all of the results obtained, it may be inferred that the biocomposite Chit/Alg/CMC, particularly in the weight proportion 60:35:5, is an excellent basic material for the construction of dressings in the form of a film designed for bedsores.

## 5. Acknowledgements

*These studies were performed at the Laboratory of Structure Research of the Centre of Molecular and Macromolecular Research of the Polish Academy of Sciences in Łódź, in the Drug Store Pharmacy Department of the Faculty of Applied Pharmacy at the Medical University, Łódź,* and *at the National Institute of Drugs, Dept. of Biochemistry and Biopharmacy, Warsaw*

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