POLYMER BIOCOMPOSITES USED IN BEDSORES TREATMENT

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Abstract
Investigations are presented in the preparation of composite dressing material based on two biopolymers -chitosan and sodium alginate with the addition of sulfanilamide as medication designed for the healing of bedsores. The dressing was prepared in the form of film. The biopolymers used in the construction of the film make the dressing biodegradable and resorbable in the wound's environment. Mechanical properties of the film were tested: thickness, extension strength, tenacity and elongation at maximum stress The ability of the material to match the wound was examined, too, as well as the transmission of water vapor. Sulfanilamide as bacteriostatic agent was added to the prepared composites. Mechanical and sorption properties of the composite dressings with addition of the active substance depend largely on their composition. The sorption properties were tested before and after addition of the medication .The release of the medication is intricate and proceeds according to kinetics of first order. Susceptibility of the composite materials to hydrolytic and enzymatic degradation was assessed.

Key words: *polymeric biocomposites, dressing, film, bacteriostatic agent, bedsore.*

1. Introduction

Bedsores are since long time a serious problem particularly in palliative care prompting an ever increasing demand for dressing materials capable of providing an efficacious protection and healing of the bedsores, and of playing the role of drug carriers. Such materials ought to be made of special polymeric materials that promote the absorption of active substances, stimulate wound healing, and reveal adequate mechanical and sorption properties.

Bedsores are defects of the skin and deeper layers of tissue caused by long lasting oppression and/or friction. The result is in tissue gangrene particularly in the parts of the body where the bones directly oppress soft tissue causing a reduced or limited blood circulation [1, 2]. Healing of bedsores is a complex process which leads to a regeneration of connecting tissue and epidermis. It was found that wounds heal faster in moist environment, the healing depending largely upon temperature and oxygen concentration. Multifunctional dressings of a new generation can provide proper microclimatic conditions in the wound's environment [3]

Thanks to specific biological properties like ability to accelerate granulation and wound epithelialization, polyaminosaccharides, chitosan and sodium alginate in particular, lend themselves exquisitely as materials for the construction of wound dressings [4 - 8]. Since several years in the Institute of Biopolymers and Chemical Fibres, research and development works have been conducted in biomaterials made of biopolymers mainly polysaccharides designed for uses in medicine and veterinary [9 - 15].

Herein presented are investigations in the preparation of a composite dressing material based on two biopolymers: chitosan and sodium alginate with an addition of sulfaniloamide. The dressings are designed for the healing of bedsores in all phases of their appearance. Sufaniloamide, p-aminobenzensulfonamide exerts bacteriostatic activity against a number of bacteria, staphylococcus and streptococcus in particular. The medication is being used in the healing of pus skin diseases and running sores [16]

2. Materials and methodology

2.1. Materials

- Chitosan, delivered by Vanson Halo Source USA characterized by: average molecular mass ($\bar{M}_v$) = 320 kDa, deacetylation degree (DD) = 77.5%, ash content = 40 ppm.
- Modified chitosan lactate with pH = 6.2 - 6.6 polymer content = 1.96%, $\bar{M}_v$ = 316.0 kDa, DD = 77.5%.
- Sodium alginate (Protanal 10/60) by FMC BioPolymer Co.
- Plasticizer – glycerol, by Fluka Co.
- Bacteriostatic agent - Suphaniloamide, p-aminobenzensulphonamide, by Sigma-Aldrich.
- Lactic acid, analytically pure – by Fluka Co.
- Phosphate buffer, pH= 7.4.
- Lysozyme – muramidase from chicken protein, EC 3.2.1.17, by Merck Co with activity of 50 000 U/mg. The enzyme was used in the biodegradation of the composite film.

2.2. Preparation of chitosan-alginate biocomposites in film form with addition of an active substance

Chitosan lactate with pH in the range of 6.2 - 6.6 and sodium alginate, blended in the percentage proportion of 85:15; 75:25; 50:50, were used in the preparation of chitosan-alginate composites which constituted the basic dressing material in the form of a film. Sulfanilamide was added to the blends in the amount of 6% on dry mass of the polymers. The amount of the component was adopted in accordance with recommendations of the Polish Pharmacopeia concerning doses for outside uses [17]. To provide good elasticity and wound fitness, glycerol was also added to the blend in the amount of 0.4 weight parts per 1 weight part of the composite (calculated on dry mass of the polymers). The film composite with thickness in the range of 0.043 – 0.1 mm was prepared by casting of the blend onto Teflon plates and consecutive drying at 20 °C for 24 - 48 hours.

2.3. Estimation of mechanical properties of the film composites

Mechanical properties were tested in the Laboratory of Metrology of IBChF under accreditation certificate AB 338. The composite materials were examined according to following standards:

- PN-EN ISO 4593:1999 *„Plastics. Film and sheets. Testing of thickness by mechanical scanning"*– thickness [mm];
- PN-EN ISO 527-3:1998 *„Plastics. Testing of mechanical properties at static extension" Testing conditions for film and sheets"*- tenacity [MPa], elongation at max. tension. [%];
- PN-EN 13726-4:2005 *„Inactive medical devices. Methods of direct testing of wound dressing. Part 4: Ability to match"* – permanent deformation [%], extensibility [N/cm];
- PN-EN-13726-2:2005*„Methods of direct testing of wound dressing . Part 2: Transmission of water vapor through dressing with semipermeable film"* - transmission of water vapor [gm^{-2} $24h^{-1}$] **Assessment of the imbibition properties of the composite film**

The imbibition was assessed by water retention value (WRV) and absorption capacity. Water retention value (WRV) was estimated according to the equation [18].

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

where:

m_1 - mass of the sample immersed in water for 20 hours and centrifuged for 10 minutes at 4000 r.p.m.

m_0 - mass of the sample after up-drying at 105 °C.

Samples sized 2 cm × 2 cm were prepared for the estimation of absorption capacity The samples were immersed in demineralized water for 0.25; 0.5 h; 3.0 h; 5.0 h; and 24.0 h, and then dried and weighted .

2.5. Estimation of the amount of aminosaccharides released in the course of degradation of the composite film

The amount of aminosaccharides was estimated by colorimetry with 3,5-dinitrosalicilic acid (DSN). Absorbance (E) of the tested sample against a zero sample is measured with spectrometer Helios at wave length $\lambda = 540$ nm [19].

2.6. Assessment of the release rate of the medication from composite film

The delivery speed of the medication was examined according to Polish Pharmacopeia VII edition with the use of the spatula apparatus (see ***Figure 1***) [20, 21]. Selected chitosan-alginate composites in film form with a mass of 0.04 g were tested. Composition of the tested composites: weight proportion of chitosan to alginate - 85:15, 75:25, 50:50% with 6% of sulfanilamide. A 0.9% aqueous NaCl served as acceptor fluid. The testing was carried out at 37 °C, and 100 r.p.m. of the agitator. Concentration of the medication in the acceptor fluid was measured by spectrophotometric analysis at wave length of 259 nm, and time intervals of: 1, 2, 5, 10, 15, 30, 60, 120, 180 min and 24 h. The testing was made at the Faculty of Applied Pharmacy of Medical University, Łódź.

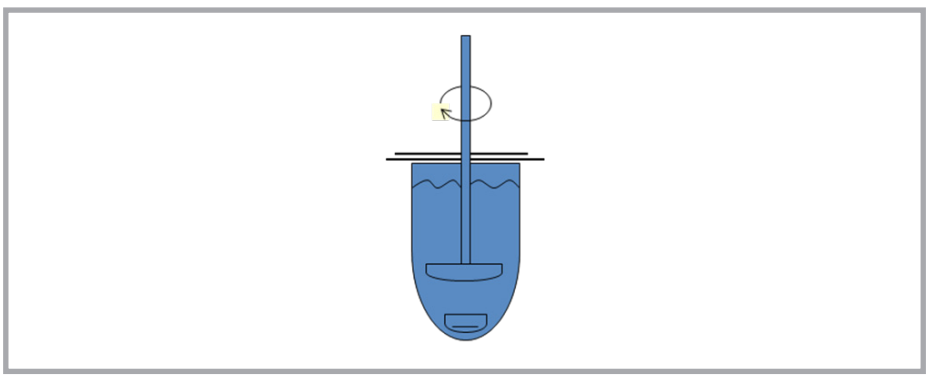

Figure 1. *Apparatus for the in vitro testing of the delivery of medication from transdermic therapeutic systems (TTS).*

2.7. Estimation of the susceptibility of the composite materials to hydrolytic and enzymatic degradation

The composite materials in film form were analyzed in respect of their susceptibility to hydrolytic and enzymatic degradation. The degradation was conducted for 7 days at 37 °C in a phosphate buffer at pH = 7.40 and bath module of 1:350 w/w. A lysozyme with concentration of 200 μg/cm^3 was applied in the enzymatic degradation. The films from the singular degradation times were taken out of the bath, filtered on a Büchner funnel, washed with distilled water at 50 °C and then with 70% ethanol, and freeze-dried eventually to constant weight. The run of the degradation was assessed by change of pH, mass loss and concentration of aminosaccharides formed in the course of the hydrolytic and enzymatic degradation. Photographic documentation was prepared for all tested preparations.

3. Results and discussion

Objective of the reported investigations was the preparation of composite film materials designed for the healing of bedsores and characterized by adequate elasticity and strength, and a sufficient transmission of water vapor. The dressings made of the material ought to fit well to the wound, provide a good contact with the afflicted tissue, and allow an

easy patient's deportment. With the dressing, the wound should be protected against further defects caused by accumulation of excessive amounts of fluids [22].

3.1. Assessment of mechanical properties of the film composite materials

Table 1 contains the results of the testing of mechanical parameters of the film composite materials.

Table 1. *Mechanical properties of film composite materials (all preparation contain 0.4 weight parts on 1 part of dry mass of both polymers).*

Symbol of sample	G69/B/1	G69/B/1/Sul	G69/B/2	G69/B/2/Sul	G69/B/3	G69/B/3/Sul
Composition of the Chit : Alg composite, wt%	85 : 15		75 : 25		50 : 50	
Amount of sulfanilamide, wt%	-	6.0	-	6.0	-	6.0
Tenacity, MPa	8.40	13.7	9.78	6.15	24.1	20.7
Elongation at max. tension, %	95.6	77.8	85.0	103.0	95.6	73.7
Permanent deformation, %	-	3.07	-	5.20	-	4.53
Extensibility, N/cm	-	1.48	-	1.48	-	2.78
Transmission of water vapor, $g \cdot m^{-2} \cdot 24h^{-1}$	-	6738	-	8588	-	10559

The film of the 50 : 50% composition showed highest tenacity (24 MPa) and elasticity on the level of 96%. Amongst the tested compositions that material has shown the highest extensibilty of about. 3 N/cm and water vapor transmission of 10559 $g \cdot m^{-2} \cdot 24h^{-1}$ The addition of 6% of sulfanilamide resulted in a slight decrease of the parameters.

3.2. Assessment of the imbition properties of the composite film

It is hydration that eases the permeation of the active substance from the carrier-dressing through epidermis. Water enhances skin permeability by swelling keratin fibres and increasing the intercellular space [23].

The ability to bind water is therefore an important feature of the dressings: the higher the absorption properties the higher is the efficacy of the preparation and the better its penetration into skin. That was the reason why were the imbibition properties of the prepared film dressings assessed. Examined were absorption ability and water retention value (WRV) of the prepared composites. Results are presented in ***Figures 2*** and ***3*** (see page 116).

From ***Figure 2*** it can be deducted that the absorption ability of the composite film increases with the dipping time in demineralized water. The addition of sulfanilamide did not limit the water absorption in the preparations with higher chitosan content (85:15, 75:25) while in the preparations with the balanced content of both polymers (50:50) it caused a distinct increase of the absorption up to about 100% both after 30 minutes 24 hours.

It was shown that the tested preparations reveal a high capacity to bind water. It results from ***Figure 3*** that WRV depends upon the composition of the preparations. WRV increases with the increase of the sodium alginate component to a maximum for the bal-

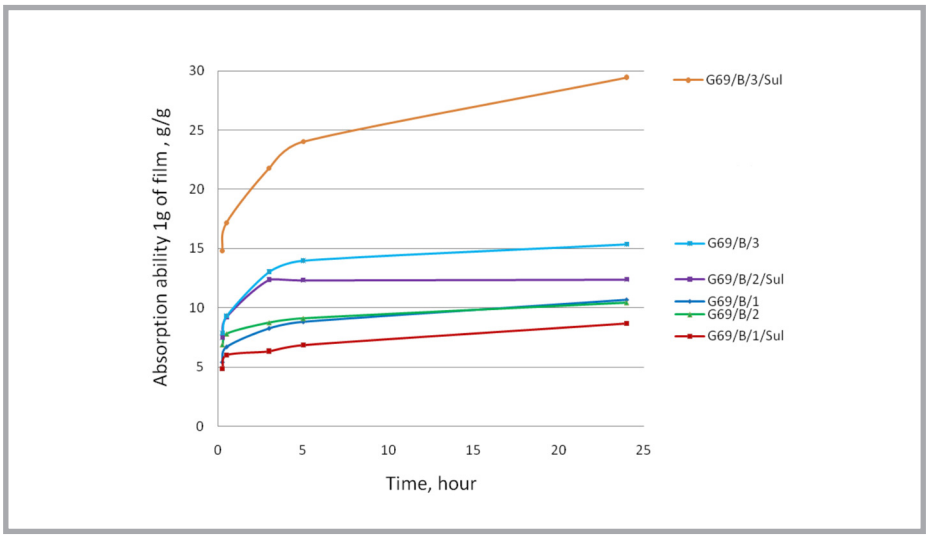

Figure 2. *Change of absorption ability of 1 g of composite film with and without sulfanilamide in dependence on time.*

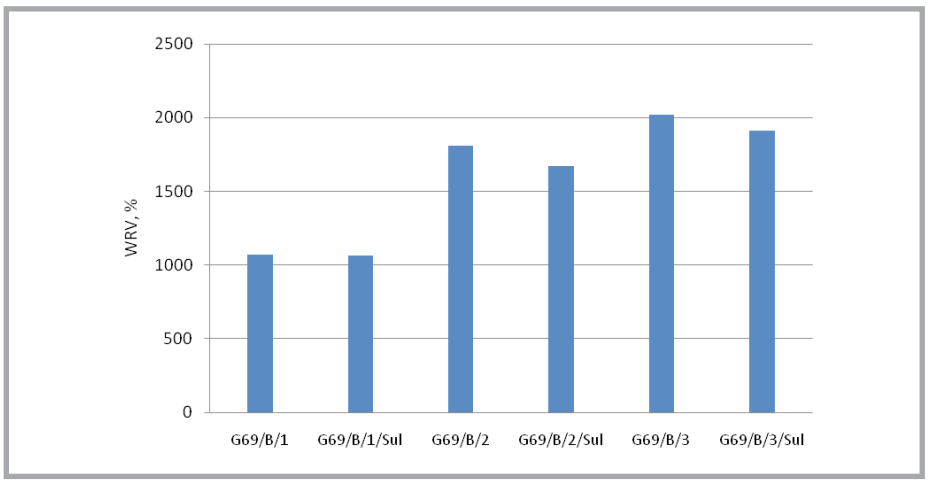

Figure 3. *Change of WRV of composite film with and without sulfanilamide.*

anced content of both polymers. Addition of the active substance has no significant influence upon WRV.

3.3. Assessment of the release of sulfanilamide from the composite films

The testing of the release of sulfanilamide from the composite films was carried out in the Faculty of Applied Pharmacy of the medical University, Łódź. The release is

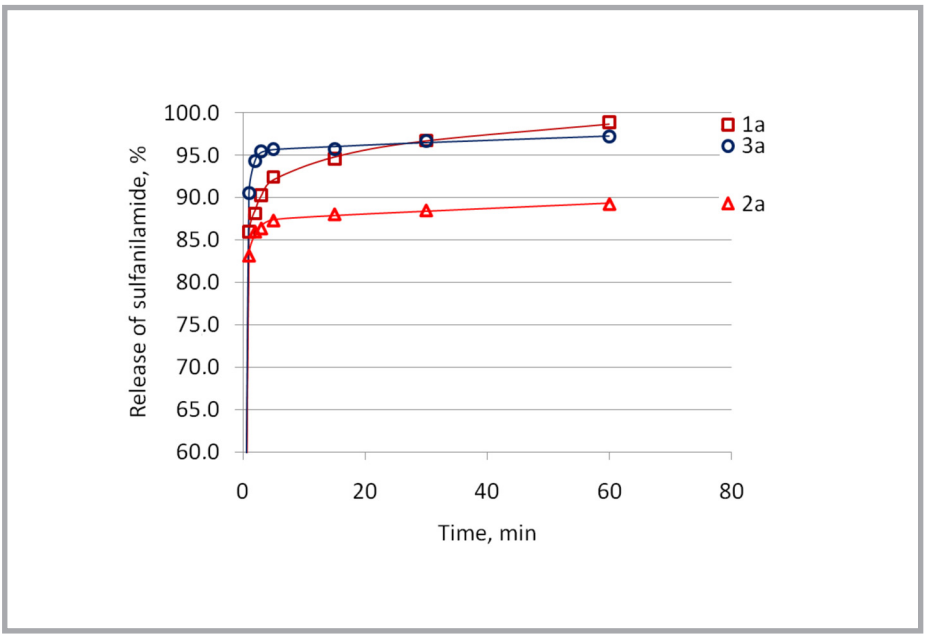

Figure 4. *Kinetics of sulfanilamide release from composite films: 1a - G69/B/1/Sul (85:15), 2a - G69/B/2/Sul (75:25), 3a - G69/B/3/Sul (50:50).*

defined as a transmission of the active substance contained in the drug to the environment from which it can be absorbed by the organism**.** The rate of the release depends on the form of the drug and on the transport speed of the drug from the point it was given to the point of absorption [20].

The testing concerned the release of sulfanilamide from the composite films with a mass of 0.04 g differentiated by their composition. Following preparations were tested chitosan lactate and sodium alginate in the proportion of 85:15, 75:25, 50:50 with addition of 6 % sulfanilamide and 0.4 weight parts of the plasticizer on 1part of dry mass of both polymers. The results are shown in ***Figure 4***.

Pondering over the results presented in ***Figure 4*** leads to the inference that the composition of the composite materials presents the crucial parameter influencing the release rate of sulfanilamide. The slowest release 89% after 60 minutes was found in the preparation marked G69/B/2/Sul (75:25). The results confirm that the release is a complex process proceeding according to kinetics of 1st order.

It may be assumed that sulfanilamide is fixed in the film on its surface or dispersed in its interior or permanently associated by physical-chemical or chemical bonds. In the first case sulfanilamide is instantly released (by dissolving speed). The dispersed portion is released by way of diffusion. The slowest release proceeds with the chemically or physical-chemically bound part.

3.4. Examination of the susceptibility of the composite film to hydrolytic and enzymatic degradation

The composite preparation G69/B/3/Sul (50:50) containing balanced amounts of the two polymers and sulfanilamide was subjected to hydrolytic and enzymatic degradation Assessed during the proceeding degradation were pH, mass loss and concentration of aminosaccharides. Considering it that the composite films are designed for uses in dressings for the healing of bedsores and that they active use is short lasting, 7 days were assumed as testing time. Photographic documentation was prepared for all preparations. ***Table 3***. presents the degradation conditions.

Table 3. *Conditions of the hydrolytic and enzymatic degradation of composite film.*

Parametr of degradation	Hydrolytic degradation	Enzymatic degradation
Time, days	0, 1, 2, 7	0, 1, 2, 7
Temperature	37 °C	37 °C
Medium	Phosphate buffer, pH = 7.40	
Enzyme	non	Lysozyme, concentr. 200 μg/cm^3
Bath module	1 : 350 w/w	

Results of the examination of the susceptibility of composite film to hydrolytic and enzymatic degradation are compiled in ***Table 4***.

Table 4. *The run of the hydrolytic and enzymatic degradation of the composite film as function of time.*

Degradation time	Hydrolytic degradation			Enzymatic degradation		
	pH	Mass loss, %	Concentration of aminosaccharides, g/g	pH	Mass loss, %	Concentration of aminosaccharides, g/g
0	7.40	0	0	7.40	0	0
1	7.35	14.73		7.36	15.25	0.035
2	7.33	16.64		7.35	18.67	0.042
7	7.32	16.26		7.33	19.58	0.046

The results presented in ***Table 4*** indicate that the composite film marked G69/B/3 /Sul (50:50) is not prone to hydrolytic degradation providing its stability when in contact with the afflicted tissue during the assumed application time, and protection against external factors. After 2 days in the phosphate buffer the mass loss amounted to 16% without formation of aminosaccharides. After the next 5 days, a further mass loss was not observed. In ***Figure 5*** shown are macroscopic images of the composite film during the proceeding hydrolytic degradation.

The proceeding enzymatic degradation of the polymeric composite was assessed similarly as in the hydrolytic degradation. Mass loss was over 19% after 7 days of the test and the concentration of the delivered aminosaccharides on 1 g of the preparation was 0.046 g/g. In ***Figure 6*** shown are macroscopic images of the composite film during the pro-

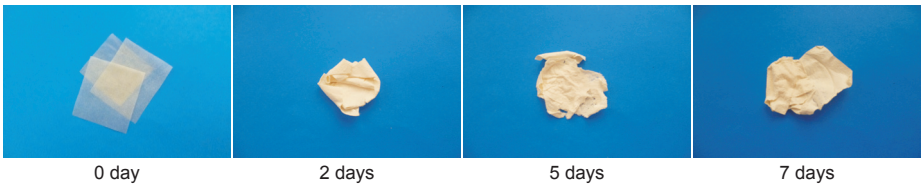

Figure 5. *Macroscopic image of the composite film G69/B/3/Sul (50:50) during hydrolytic degradation.*

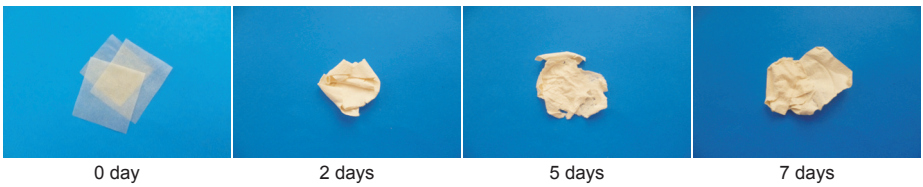

Figure 6. *Macroscopic image of the composite film G69/B/3/Sul (50:50)/ during enzymatic degradation.*

ceeding enzymatic degradation. The obtained results indicate that the prepared film is fit for the healing of bedsores

4. Summary

- In the course of the research a composite preparation in film form was prepared, based on chitosan lactate and sodium alginate.
- The chitosan-alginate composite films are characterized by high sorption. The composite containing balanced amounts of both polymers show a sorption ability of over 29.5 g/g and a high water retention value (WRV) = 2000%.
- The composition of the chitosan-alginate composite is the ruling factor in the release of the introduced medication - sulfanilamide, and of the mechanical properties as tenacity, elasticity, permanent deformation and transmission of water vapor.
- The release of the medication from the biocomposites is a complex process proceeding according to kinetics of 1st order.
- The composite show an insignificant susceptibility to enzymatic degradation in the presence of lysozyme at concentration of 200 μg/cm^3. Mass loss in the tested preparation was 19.6% after 7 days of the test, and the concentration of the delivered aminosaccharides amounted to about 0.046 g/g of the preparation.
- The prepared composites in the form of film complied with basic criteria concerning mechanical and sorption properties which recommends the material as candidate for the uses in bedsore-healing dressings.

5. Acknowledgment

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