

# **Physico-chemical properties of commercial active alginate dressings**

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## **Summary**

The aim of this study was to analyse the changing physico-chemical properties of commercial active alginate wound dressings and to evaluate an attempt to improve their effect through chemical modification.

The dressings were modified by treating them with specially designed solutions of sodium lactate and arachis oil. Then selected physico-chemical properties (sorption and desorption, theoretical density, pharmaceutical availability of chemical compounds, resistance to washing) were compared for each raw and modified dressing.

**Key words:** commercial active alginate wound dressings, physico-chemical properties

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## **INTRODUCTION**

Wounds are common injuries frequently occurring in human's life. Since the dawn of mankind people have been trying to develop the most suitable method of wound dressing. Various materials were used to this end, ranging from hot olive oil and wax in antiquity to cotton fabric in the 19th century and flax, gauze, viscose and paraffin in the 20th century [1]. Treating wounds has always followed the same pattern: cleaning, covering them to prevent from additional injuries or dirtying, and changing the

dressing frequently in order to remove pathological secretions from the injured place and to keep the wound dry [2].

Over years, research led to developing a new concept of wound treatment with maintaining constant moisture within the wound as the most important factor. Clinical observations have revealed that the secretion produced by the wound surface contains oxygen and nutrients that ensure proper tissue growth and promote granulation and adequate epidermization. This applies to every type of wounds, both clean and contaminated. Thus the once well-established opinion that frequent wound drying improves healing proved false [2]. Research has allowed for developing and producing all kinds of modern dressing materials, synthetic and natural [1], known as active dressings. These dressings maintain high moisture between the wound and the dressing; remove excessive exudate and toxic components; allow for gas exchange between the wound and the environment; make an effective barrier for bacteria and other microorganisms; are free from toxic substances; protect the newly formed tissues; can be easily removed from the wound surface without causing any injury [3].

The main types of active dressings available on the market are: hydrocolloids, hydrogels, alginate dressings, polyurethane dressings, mixed/combined dressings [3]. Absorptive dressings include calcium or calcium-sodium alginates obtained from alginate acid salts. The dressings come either as fibrous sheets (for shallow wounds) or in the form of compressed, rope-like plaits (for cavity wounds). By absorbing exudate, they form gel around every fibre, which binds both the exudate and the bacteria it contains [1]. These are highly absorbent dressings. The fibres trapped in a wound are readily biodegraded without producing symptoms of intolerance or allergic reaction.

Alginate is a well-known polysaccharide obtained from seaweeds, chiefly the brown algae (*Phaeophyceae*) [4-5] or produced extracellularly by some bacteria such as *Azotobacter vinelandii*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* [6-8].

Chemically, alginate acid is a linear copolymer consisting of two monomers [9]:  $\beta$ -D-mannuronic acid radical (M) and  $\alpha$ -L-guluronic acid radical (G), joined by glycosidic links  $\beta$ -1,4 and  $\alpha$ -1,4. The D-mannuronic unit has  ${}^4C_1$  conformation, while the L-guluronic unit has  ${}^1C_4$  conformation, irrespective of which unit is their closest neighbour [10].

Because of their unique properties, such as natural origin, biocompatibility and relatively low production costs, alginate macromolecules are used in pharmaceutical applications as carriers for immobilizing cells, enzymes and proteins as well as for

controlled release of drugs [9]. Because of their specific properties such as promoting wound healing combined with high absorption, hemostatic properties and ion-exchange capacities of alginate fibres, alginate dressings are increasingly used in medical applications, mainly as dressing materials.

The first stage of producing a ready dressing material is fibre forming and then producing woven sheets. For specific medical applications, the fibrous sheets require additional finishing such as adding superabsorbents, plating or perforating to make them more penetrable for liquids [10].

The physical microstructure that the fibres eventually receive can be modified. As a rule, this modification is caused by deliberate actions in order to obtain better, enhanced properties of the fibre and consequently improved, and often new, properties of the textiles made of them. The modification of the physical microstructure may result from physical, chemical or biological treatment of the fibre [11].

Thus, the aim of this study was to analyse the changing physico-chemical properties of commercial active alginate dressings and an attempt to improve them through chemical modification using sodium lactate (cross-linking agent) and arachis oil (elasticizer).

## **MATERIALS AND METHODS**

The following materials and reagents were used in the examination:

- active alginate dressing Medisorb A (produced by TZMO SA, Torun);
- $10^{-5}$ M solutions of dyes 1F, 2F, 5F and 6F;
- 2% solution of sodium lactate;
- arachis oil;
- 0.1% solution of pronase E in 0.1M HCl;
- Ringer's solution (8.600g NaCl, 0.3000g KCl, 0.4800g CaCl<sub>2</sub>;  
all were dissolved in distilled water in a 1dm<sup>3</sup> measuring flask).

## **METHODS OF EXAMINATION**

For the purpose of the examination,  $10^{-5}$ M solutions of four dyes: 1F, 2F, 5F, 6F were prepared. All four of them are non-genotoxic analogs of carcinogenic azo-dyes

synthesized at the North Carolina State University, Raleigh (USA). The figure below shows the structural formulae of some of them (fig. 1).

### **Method of modifying alginate dressing**

Below are presented the methods of modifying the samples of the commercial Medisorb A alginate dressing. They were placed in 4 weighing bottles containing 5ml of the earlier prepared dye solutions. The weighing bottles with samples were placed in an exsiccator. After 30 minutes, dyed dressing samples were removed and dried in a laboratory drier at 35°C. Dry samples were weighed to constant weight.

Samples of raw and dyed dressing were placed in weighing bottles with 5ml of 2% solution of sodium lactate.

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In each case, the bottles with samples were placed in an exsiccator. After 15 minutes, the modified dressing samples were removed and dried in a laboratory drier at 35°C. Dry samples were weighed to constant weight.

For raw and modified dressings, the following physico-chemical parameters were determined: theoretical density, sorptive capacity for water and dye solutions, resistance to washing and pharmaceutical availability.

### **EXAMINATION OF THEORETICAL DENSITY**

The dressing was cut into 1cm×1cm fragments and weighed on an analytical balance. Density of the dressing was calculated by means of the formula:

$$D = m/g \times h \times a$$

where:  $D$  – density,  $m$  – mass of the fragment,  $g$ ,  $h$ ,  $a$  – dressing dimensions [13].

For raw and modified dressings, 10 measurements were conducted, and an arithmetic mean and standard deviation were calculated.

## EXAMINATION OF SORPTIVE CAPACITY

### Examination of sorptive capacity for water

For examining the sorptive capacity, samples of raw dressing sized 1cm × 0.5cm and weighed on an analytical balance were used. The samples were placed in previously weighed weighing bottles containing 5ml of distilled water. After 30 minutes, the samples were removed and the bottles were weighed again.

### Examination of sorptive capacity for dye solutions

For examining the sorptive capacity, samples of raw dressing and samples modified with sodium lactate and arachis oil sized 1cm × 0.5cm and weighed on an analytical balance were used. The samples were placed in previously weighed weighing bottles containing 5ml of previously prepared 10<sup>-5</sup>M solutions of the 1F, 2F, 5F and 6F dyes. After 30 minutes, dressing samples were removed and the weighing bottles were weighed again. The sorptive capacity of the dressing was calculated on the basis of water loss from the weighing bottle according to the formula [13]:

$$S_w = (W_a - W_b) / W_s$$

Where:  $S_w$  – amount of sorbed water or dye solution,  $W_a$  – weight of the weighing bottle prior to the examination,  $W_b$  – weight of the weighing bottle after removing the sample,  $W_s$  – sample weight.

## EXAMINATION OF RESISTANCE TO WASHING

Samples of raw and dyed dressing as well as of dressings modified with sodium lactate and arachis oil sized 1cm × 1cm were placed in flasks containing 20ml of 0.1% pronase solution (Pronase E from *Streptomyces griseus*) in 0.1M hydrochloric acid. The contents were stirred using a magnetic mixer at 50rpm. After 30 minutes, the residue from the dressing was removed and dried in a laboratory drier at 35°C. The dried

samples were weighed and the percentage of the residue was calculated according to the formula [13]:

$$\%P = (W_{sb} \times 100\%) / W$$

where: %P – percentage of residue,  $W_{sb}$  – weight of dry residue, W – initial weight of sample.

## PHARMACEUTICAL AVAILABILITY

A raw dressing as well as one modified with sodium lactate and arachis oil were cut into 1cm × 1.5cm fragments. The samples were placed in flasks containing 50ml of 10<sup>-5</sup>M dye solutions. Then the flasks were placed in a shaker at 50rpm. After 15 minutes, 10ml of dye solution were removed and replaced with 10ml of Ringer's solution. After another 15 minutes, 10ml of dye solution in Ringer's solution were removed. This was repeated seven times. The examination was conducted at 37°C. Spectrophotometric (UV/VIS) analysis of the amounts of solutions was carried out using a "Specol" spectrophotometer produced by Carl-Zeiss Jena.

## RESULTS

### Examination of theoretical density

The quality content of the dressing samples was found to substantially affect the values of theoretical density.

The theoretical density of a raw dressing ranges from 0.0357g/cm<sup>3</sup> to 0.0530g/cm<sup>3</sup>, which means that the dressing has a significantly heterogenous structure. Average theoretical density is 0.0443g/cm<sup>3</sup> (±S.D. 0.0064). Adding a modifier, both sodium lactate and arachis oil, resulted in increased theoretical density.

For a dressing modified with 5ml of 2% solution of sodium lactate, the theoretical density ranges from 0.0947g/cm<sup>3</sup> to 0.1010g/cm<sup>3</sup>. Average theoretical density is 0.0977g/cm<sup>3</sup> (±S.D. 0.0020). For samples modified with arachis oil, the theoretical density ranges from 0.1055g/cm<sup>3</sup> (for a sample modified with 1 drop of oil)

to  $0.1555\text{g/cm}^3$  (for a sample modified with 5 drops of oil). Average theoretical densities are  $0.1149\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0061$ ) and  $0.1400\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0072$ ) respectively.

Combined modification of samples with 1 drop of arachis oil and 5ml of 2% solution of sodium lactate results in lower theoretical density of the dressing compared with samples modified with only 1 drop of arachis oil without sodium lactate. Average theoretical density of samples modified with sodium lactate and 1 drop of arachis oil is  $0.1063\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0098$ ), whereas for samples modified with only 1 drop of oil it is  $0.1149\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0061$ ).

Adding 5ml of 2% solution of sodium lactate to samples modified with 5 drops of arachis oil results in increased theoretical density of the dressing, which averages  $0.2173\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0813$ ), while for samples modified with only 5 drops of arachis oil it is  $0.1400\text{g/cm}^3$  ( $\pm\text{S.D. } 0.0072$ ).

Figure 2 shows these average theoretical densities for all the samples examined. Each modification increases theoretical density. This is best visible for the dressing modified with 5 drops of arachis oil or with sodium lactate and 5 drops of arachis oil. Thus, the theoretical density of the samples examined is mostly affected by the amount of arachis oil added to them, as it makes the samples “heavier” and consequently increases their density.

### **Examination of sorptive capacity**

The aim of the examination was to investigate the capability of raw dressing to sorb water and dye solutions. The capability of modified samples to sorb dye solutions was also examined. Table 1 shows the calculated values of water sorption for samples of a raw dressing. This ranged from 30.21 to 40.97 with the average value of 33.70 ( $\pm\text{S.D. } 2.85$ ).

When the sorptive capacity of a raw dressing was examined for dye solutions, the results depended on the kind of the dye used. The best sorbed solution was that of 1F – 39.705 ( $\pm\text{S.D. } 3.761$ ), followed by 2F – 36.48 ( $\pm\text{S.D. } 4.853$ ) and 5F – 30.06 ( $\pm\text{S.D. } 2.202$ ). The least sorbed solution was that of dye 6F, where the value was just 24.74 ( $\pm\text{S.D. } 2.308$ ).

Cross-linking modification of samples using sodium lactate resulted in their substantially decreased sorptive capacity for dye solutions. The results were as follows: 8.73 ( $\pm\text{S.D. } 2.520$ ) for 1F; 7.33 ( $\pm\text{S.D. } 0.665$ ) for 5F; and 6.97 ( $\pm\text{S.D. } 0.645$ ) for 6F.

The least sorbed solution was that of dye 2F, where the value was just 4.707 ( $\pm$ S.D. 0.281). The amounts of dyes sorbed by the samples are shown in Figure 3.

Sorption of dyes by the samples modified with arachis oil as an elasticizing agent depends on the quantity of the oil used. The highest sorption was found for samples modified with 1 drop of oil and the lowest for samples modified with 5 drops. Also, the samples modified with 1 and 3 drops of oil had the best sorption for dye 1F – 19.59 ( $\pm$ S.D. 1.910) and 16.17 ( $\pm$ S.D. 1.270) respectively. The samples modified with 5 drops of arachis oil had the best sorption for dye 6F – 9.083 ( $\pm$ S.D. 0.525).

Modifying the samples with both sodium lactate and arachis oil results in their significantly lower sorptive capacity. The samples modified with sodium lactate and 1 drop of arachis oil had the highest sorption of 8.59 ( $\pm$ S.D. 2.019) for dye 1F, and the lowest, of 4.19 ( $\pm$ S.D. 0.323), for dye 2F. After adding five drops of arachis oil, the sorption of dye 5F decreases to 3.89 ( $\pm$ S.D. 0.159). The amounts of dyes sorbed depending on the modifier used are shown in Figure 4.

Correlation between the dressing density and its sorptive capacity was also found. Samples containing less arachis oil, and thus having lower density, proved to have better sorptive properties. It may be concluded that the sorptive capacity of the dressing is affected by the alginate binding water and retaining it in the pores of the material.

### **Examination of resistance to washing**

The best resistance to washing was found in samples dyed with dyes 1F and 2F, for which the percentage of residue was 65.2% and 65.5% respectively. The samples dyed with dyes 5F and 6F had a slightly lower resistance to washing.

Also examined was the percentage of residue for dyed dressings modified with sodium lactate and arachis oil depending on the dye used. Lower resistance to washing, compared with dyed-only samples, was found in samples dyed and modified both with sodium lactate and 5 drops of arachis oil. The highest resistance was found in the sample dyed with dye 5F, where the percentage of residue was 64.2%.

Less resistant to the enzyme were the samples dyed with dyes 1F, 2F and 6F, where the percentages of residue were 62.8%, 62.3% and 58.9% respectively. Resistance to washing for a dressing dyed and modified with sodium lactate and arachis oil depending on the dye used is shown in Figure 5.

The analyses proved that adding elasticizing or stiffening modifiers does not improve the resistance of commercial dressings to proteolytic enzymes. It was found out that modifying the samples of dressings hardly affects their resistance to proteolytic enzymes.

### **Pharmaceutical availability**

A UV/VIS analysis of the availability of dye solutions for a commercial alginate dressing revealed ion exchange occurring between the dressing and the dye solution with Ringer's solution.

In particular, the biggest changes of absorbance and molar concentration were recorded for the solutions of dyes 1F, 2F and 6F. Chart 6 shows the function of this parameter (fig. 6).

By observing the process of ion exchange between the dye solutions and the modified sample, it was found that the biggest changes of absorbance and molar concentration in time occur in the solutions of 1F and 2F (Table 2). Compared with the absorbance values for these solutions recorded in the previous examination, absorbance for the solution of 1F and 2F increased, which implies a more intensive ion exchange.

The solution of dye 5F in Ringer's solution, in which the modified samples were immersed, shows decreased absorbance, whereas that of dye 6F in Ringer's solution shows increased absorbance with decreased ion exchange (Figure 7).

In time, insignificant changes of molar concentration of the solutions of dyes 5F and 6F in Ringer's solution were recorded. It may be inferred that the ion exchange occurring between the dye solution, Ringer's solution and the dressing is strongly affected by how well the porous structure is preserved. Earlier, Figures 3 and 4 showed that, among the dyes used, 1F is best sorbed by both raw and modified active dressing. This observation is confirmed in the process of ion exchange.

### **DISCUSSION**

The results obtained show that adding both an elasticizer (arachis oil) and a cross-linking agent (sodium lactate) changes the physico-chemical parameters of the active alginate dressing Medisorb A.

By comparing the results of this examination, obtained for commercial alginate dressings, with similar examinations for lyophilized matrices [13], some interesting trends in changing physico-chemical parameters of the dressings were found. The biopolymers examined are subject to changes on molecular and supermolecular levels, not only during their production, but also during their use.

The study revealed that a commercial dressing with an undefined heterogenous structure also has good, repeatable characteristics of variable physico-chemical parameters.

## CONCLUSIONS

In particular, the following was observed:

1. The commercial dressing has a largely heterogenous structure and a well-preserved porous structure of dressing.
2. The composition of dressing samples affects the value of theoretical density. Adding a modifier, either sodium lactate or arachis oil, resulted in increased theoretical density of the dressing, which was more visible for arachis oil. The amount of arachis oil added to the samples makes them “heavier” and consequently increases their density.
3. The sorptive capacity of a raw dressing for dye solutions depends on the kind of dye. Modifying the samples with sodium lactate resulted in decreased sorptive capacity for dye solutions, which suggests rebuilding of the supermolecular structure of the matrix and decreasing the number of amorphous areas capable of sorbing the dyes. Simultaneous modification of the samples with sodium lactate and arachis oil leads to a considerable decrease of sorptive capacity.
4. Correlation between the dressing density and its sorptive capacity was found. Samples containing less arachis oil, and thus having lower density, proved to have better sorptive properties.
5. Modifying the samples hardly affects their resistance to proteolytic enzymes.
6. The trend in absorbance changes for all the dye solutions tested suggests that there is an ion exchange occurring between the dressing and the dye solution with the components of Ringer’s solution. The most intensive ion exchange occurs between the dressing and the solution of dye 1F (most sorbed by the matrix) with the components of Ringer’s solution (mainly  $\text{Na}^+$  ions). The ion exchange occurring between the dye solution, Ringer’s solution and the dressing

is strongly affected by how well the porous structure is preserved. A well-preserved porous structure facilitates the penetration of solutions into the dressing.

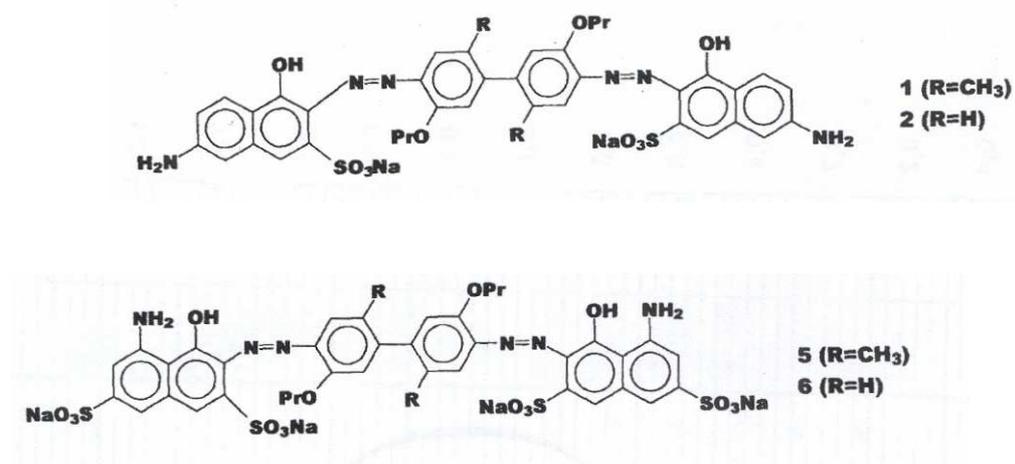
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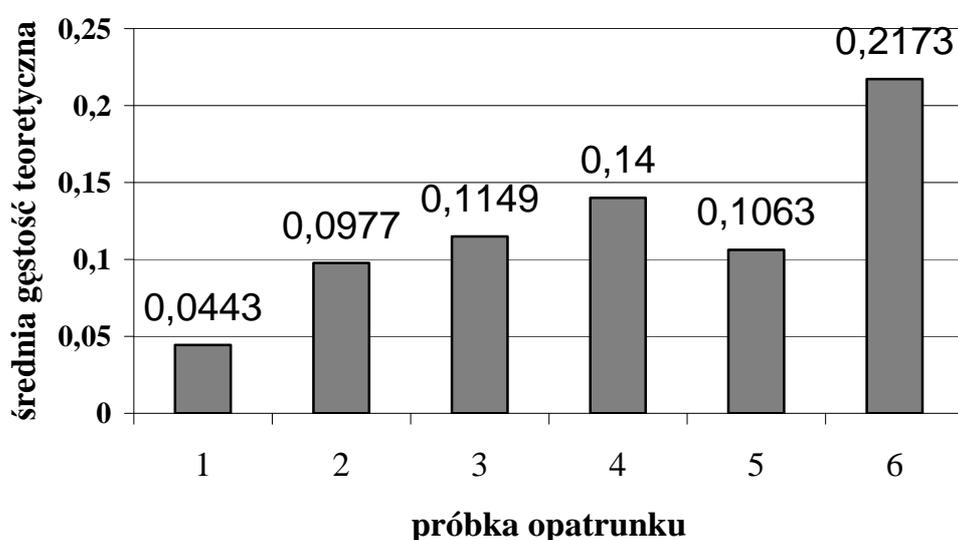
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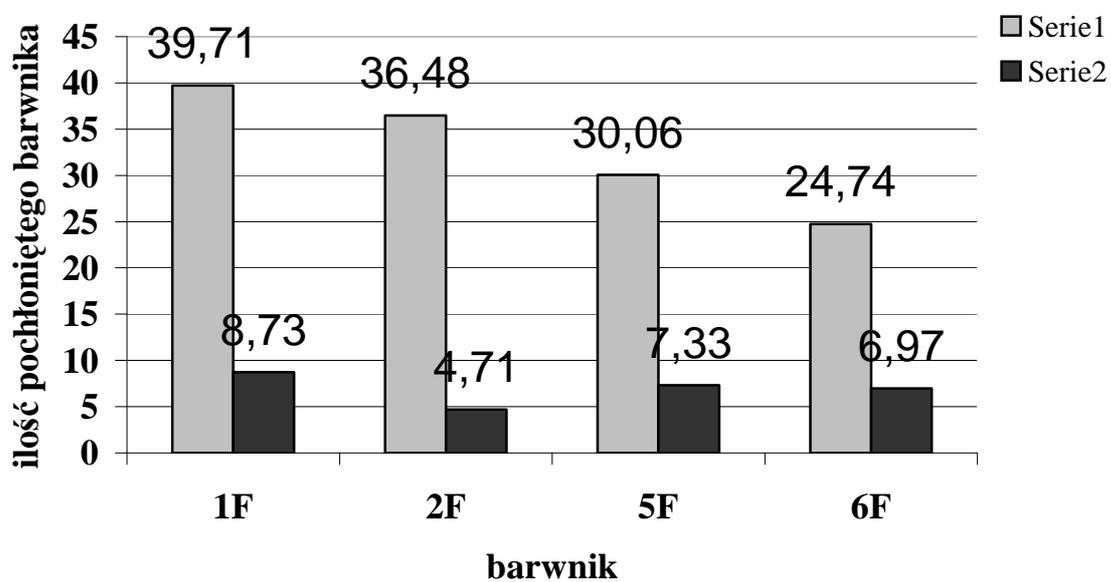
Ryc. 1. Wzory strukturalne wybranych do badań modelowych barwników azowych

Fig. 1. Structural formulae for the model azo-dyes selected for analysis



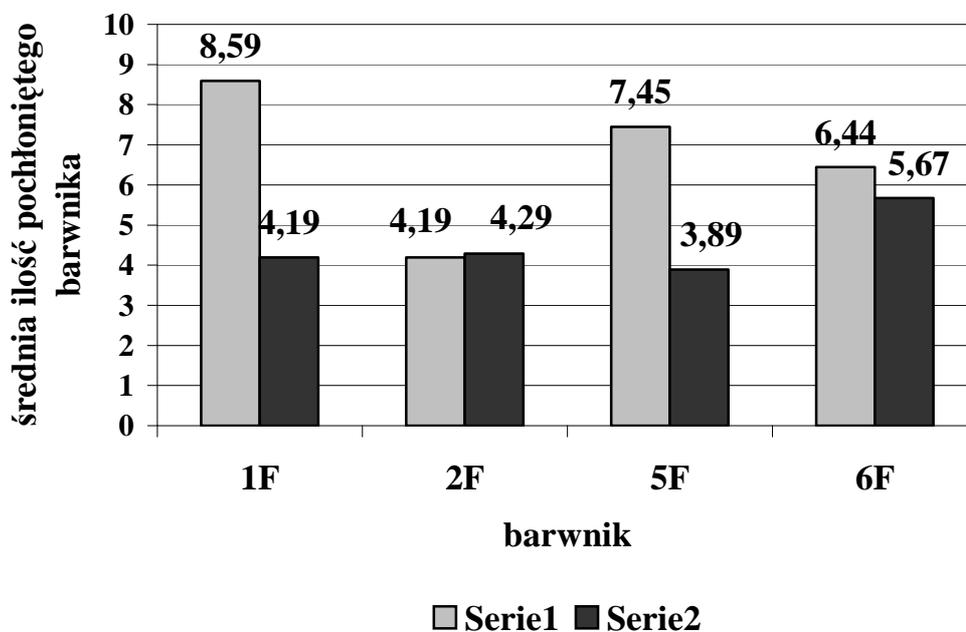
Ryc. 2. Zestawienie średniej wartości gęstości teoretycznej [g/cm<sup>3</sup>] dla badanych próbek opatrunku: 1 - próbka surowa, 2- próbka modyfikowana mleczanem sodu, 3 - próbka modyfikowana 1 kroplą oleju arachidowego, 4 - próbka modyfikowana 5 kroplami oleju arachidowego, 5 - próbka modyfikowana mleczanem sodu i 1 kroplą oleju arachidowego, 6 - próbka modyfikowana mleczanem sodu i 5 kroplami oleju arachidowego

Fig. 2. Average values of theoretical density [g/cm<sup>3</sup>] for the dressing samples examined: 1 - raw sample; 2 - sample modified with sodium lactate; 3 - sample modified with 1 drop of arachis oil; 4 - sample modified with 5 drops of arachis oil; 5 - sample modified with sodium lactate and 1 drop of arachis oil; 6 - sample modified with sodium lactate and 5 drops of arachis oil



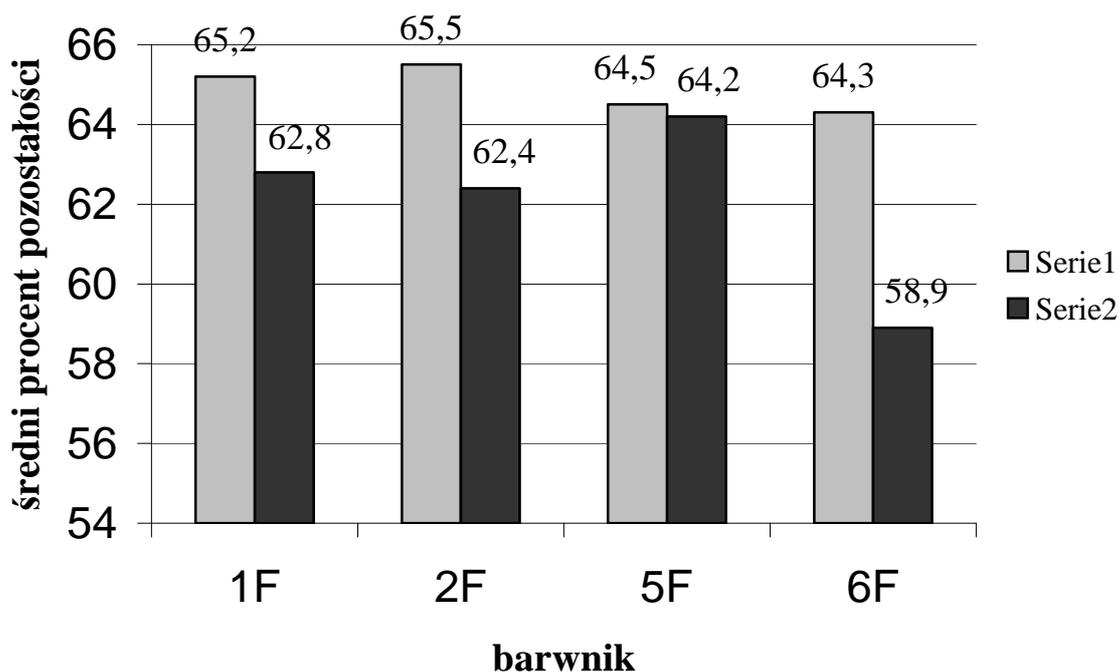
Ryc. 3. Zestawienie ilości pochłoniętego barwnika przez surowy opatrunek (seria 1) oraz przez opatrunek modyfikowany mleczanem sodu (seria 2) zależne od rodzaju zastosowanego barwnika

Fig. 3. Amount of dye sorbed by a raw dressing (series 1) and by a dressing modified with sodium lactate (series 2) depending on the kind of the dye used



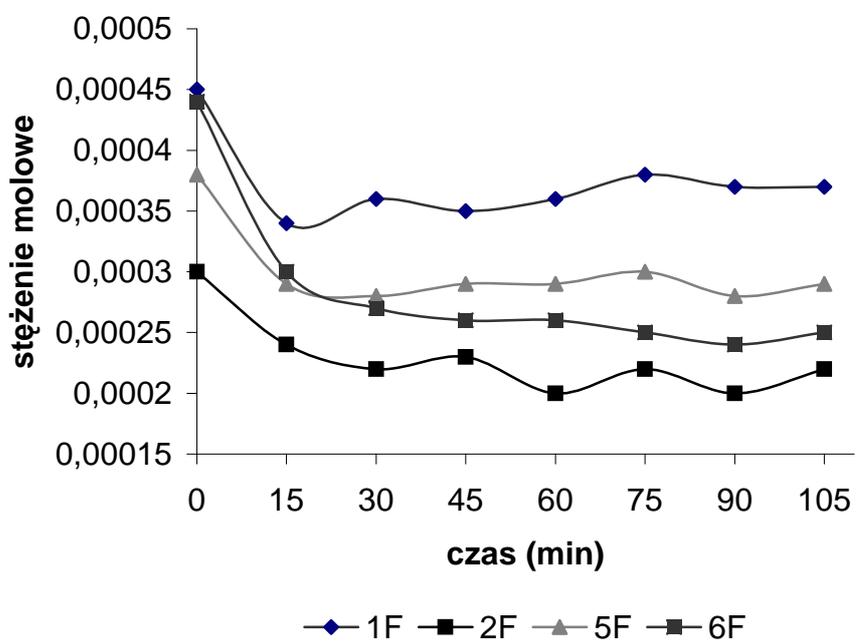
Ryc 4. Zestawienie ilości pochłoniętego barwnika przez opatrunek modyfikowany mleczanem sodu i olejem arachidowym w zależności od ilości użytego oleju (1 kropla oleju arachidowego – seria 1; 5 kropli oleju – seria 2)

Fig. 4. Amount of dye sorbed by a dressing modified with sodium lactate and arachis oil depending on the amount of the dye used (1 drop of arachis oil – series 1; 5 drops – series 2)



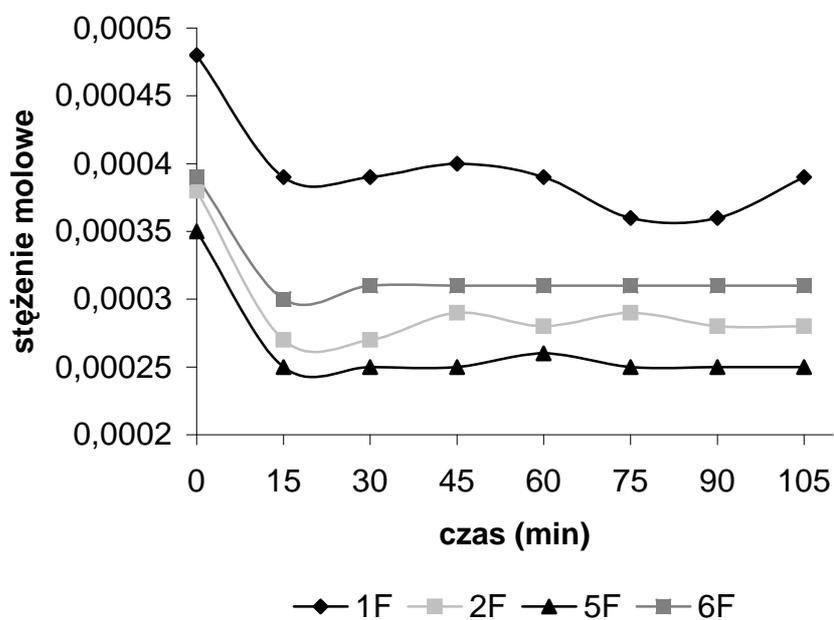
Ryc. 5. Średni procent pozostałości wybarwionego opatrunku po rozmywaniu, w zależności od zastosowanego barwnika (seria 1). Średni procent pozostałości opatrunku modyfikowanego mleczanem sodu i olejem arachidowym oraz wybarwionego po rozmywaniu, w zależności od zastosowanego barwnika (seria 2)

Fig. 5. Average percentage of residue from a dyed dressing after washing depending on the dye used (series 1). Average percentage of residue from a dressing modified with sodium lactate and arachis oil, and dyed after washing, depending on the dye used (series 2)



Ryc. 6. Zmiana stężenia molowego roztworów barwników w czasie (dla surowego opatrunku)

Fig. 6. Change of molar concentration of dye solutions in time (for a raw dressing)



Ryc. 7. Zmiana stężenia molowego roztworów barwników w czasie (dla opatrunku modyfikowanego 5ml mleczanu sodu i 3 kroplami oleju arachidowego)

Fig. 7. Change of molar concentration of dye solutions in time (for a dressing modified with 5ml of sodium lactate and 3 drops of arachis oil)

TABELA 1. Ilość wody pochłoniętej przez próbki surowego opatrunku

Table 1 Amount of water sorbed by samples of raw dressing

Lp.	Wa [g]	Wb [g]	Ws [g]	Sw
1	23,8324	23,6476	0,0030	33,00
2	23,1100	22,9233	0,0043	32,75
3	53,0792	52,8972	0,0055	33,10
4	23,0874	22,8615	0,0065	34,75
5	27,7715	27,5690	0,0060	33,75
6	27,6230	27,4755	0,0036	40,97
7	30,0250	29,8356	0,0060	31,57
8	27,8956	27,7055	0,0056	33,95
9	26,7348	26,5998	0,0050	30,21
10	28,4816	28,2771	0,0037	32,98
Średnia	-	-	-	33,70
Odchyl. Stand.	-	-	-	2,85

TABELA 2. Zestawienie średnich absorbancji i stężeń molowych dla roztworów barwników w płynie Ringera, w którym zanurzono surowe i modyfikowane mleczanem sodu i 3 kroplami oleju arachidowego opatrunki aktywne

Table 2. Average absorbances and molar concentrations for dye solutions in Ringer's solution, in which raw dressings and dressings modified with sodium lactate and 3 drops of arachis oil were immersed

	A	c [mol/dm <sup>3</sup> ]		A	c [mol/dm <sup>3</sup> ]		A	c [mol/dm <sup>3</sup> ]		A	c [mol/dm <sup>3</sup> ]
	surowy			surowy			surowy			surowy	
1F	0,497	0,00045	2F	0,180	0,00030	5F	0,375	0,00038	6F	0,269	0,00044
	0,361	0,00034		0,138	0,00024		0,275	0,00029		0,187	0,00030
	0,369	0,00036		0,130	0,00022		0,272	0,00028		0,184	0,00027
	0,366	0,00035		0,136	0,00023		0,275	0,00029		0,181	0,00026
	0,391	0,00036		0,125	0,00020		0,275	0,00029		0,181	0,00026
	0,380	0,00038		0,131	0,00022		0,276	0,00030		0,178	0,00025
	0,375	0,00037		0,124	0,00020		0,273	0,00028		0,177	0,00024
	0,375	0,00037		0,127	0,00022		0,275	0,00029		0,178	0,00025
	modyfikowany		modyfikowany		modyfikowany		modyfikowany				
	0,521	0,00048	0,224	0,00038	0,350	0,00035	0,249	0,00039			
	0,403	0,00039	0,168	0,00027	0,260	0,00025	0,189	0,00030			
	0,405	0,00039	0,170	0,00027	0,260	0,00025	0,187	0,00031			
	0,411	0,00040	0,186	0,00029	0,263	0,00025	0,194	0,00031			
	0,408	0,00039	0,182	0,00028	0,267	0,00026	0,195	0,00031			
	0,392	0,00036	0,188	0,00029	0,263	0,00025	0,194	0,00031			
	0,389	0,00036	0,184	0,00028	0,265	0,00025	0,194	0,00031			
0,405	0,00039	0,185	0,00028	0,265	0,00025	0,193	0,00031				