

Properties and active substance release kinetics from gelatin-alginate matrices

Janusz Pluta, Dorota Haznar

Department of Applied Pharmacy, Wrocław Medical University

Summary

The aim of the study was to evaluate the effect of composition and technological processing on pharmaceutical availability of active substance as well as on the properties of porous gelatin-alginate matrices. The active substance carrier included glycerol or peanut oil apart from gelatin and sodium alginate, and some matrices were additionally modified with calcium lactate.

The obtained matrices were characterized by good sorption properties and high resistance to proteolytic enzymes. The release of the model antibiotic followed the pattern of first order kinetics, while half-release time in vitro (in the experimental conditions) was 1.5 to 3 hrs.

Key words: drug carrier, gelatin, sodium alginate, sponge

Tissue infection is a serious clinical problem. Conventional therapy in the form of systemic administration of an antibiotic is often little effective and not free from numerous adverse effects. In order to achieve therapeutic levels at the site of infection, it is necessary to administer very high doses of the drug, as only a small portion reaches the target tissue. Thus topical therapy is used alternatively, with relatively good result. The search for such a drug carrier that would enable a prolonged sustained release of the therapeutic levels in the infected tissue and at the same time not evoking tissue reaction and undergoing bioresorption is still going on.

Trials were undertaken to incorporate an antibiotic in tissue glues. Fibrin glues are well known biomaterials with good bioavailability, haemostatic effect and good adhesion properties. However, obtaining a sustained release of the drug is problematic as the drug release from fibrin glues is very quick [3].

Moreover, the application of chitosan or collagen sponges soaked with an antibiotic immediately before use was investigated. However the drug was released very quickly in the first phase and the action of the antibiotic was only slightly prolonged [1, 2].

Prolonged therapeutic concentration of the drug in bone tissue (i.e. for about 10 days) was obtained using constant implants formed of biodegradable lactic acid polymers and copolymers and cross-linked gelatin [4, 5]

A promising drug forms which in the future may find a wide application in therapy and prevention of infection are hydrogel films made of polymers such as hyaluronic acid, cross-linked chitosan and gelatin. In vitro studies revealed a satisfactory drug release kinetics on swelling. They could find application during surgical procedures in order to prevent infections [6-8].

The aim of our study was to obtain a porous gelatin-alginate matrix to be used as active substance carrier that might find application in the treatment of poorly supplied tissues such as bones. To produce the matrix, materials commonly used in pharmaceutical technology, well tolerated and non-toxic were used. The applied polymers: gelatin and sodium alginate are commonly used, well tolerated and biocompatible polymers. Preliminary experiments revealed that matrices containing only the above mentioned polymers were too fragile, hard and brittle, for this reason the addition of elasticizing agents proved necessary. Thus glycerol, which occurs naturally in human organism and peanut oil, which is used as oil solvent in injections were added as elasticizing agents.

1. MATERIALS

Pig skin gelatin 180 Bloom (Fluka –BioChemika), Alginic acid sodium salt (Sigma), Glycerol 86% (PPH POCH Gliwice), Peanut oil (PPH POCH Gliwice), 0.1 N Hydrochloric acid (PPH POCH Gliwice), Pepsin (BTL Gliwice), Cefradine (Polfa Tarchomin).

2. PREPARATION OF MATRICES

The initial products for the production of gelatin-alginate matrices were obtained by mixing sterile 20% solution of gelatin with 2% solution of sodium alginate in 9:1 weight ratio. The obtained mixtures were next lyophilized and the dry products were pulverized.

Prescribed amount of ground dry gelatin-alginate mixture was swelled in purified water and next dissolved heating in water bath. The amount of water was such as to obtain 20% concentration of gelatin. 1 g of cefradin was dissolved in a portion of the measured water and added to the mixture on foaming.

Then glycerol or peanut oil were added to the solution in such an amount that their content was 1%, 3%, 5% and 10% respectively in relation to gelatin. The mixture was foamed by high-speed stirrer and the foam was transferred onto Petri's pans and lyophilized for 24 hrs (Steris – Lyovag GT – 2E). C and D sponges were additionally modified with calcium lactate. Calcium lactate in the form of a solution was added at the end of foaming in an amount constituting 2% of the amount of sodium alginate. Composition of individual matrices is presented in Table 1.

3. RESEARCH METHODOLOGY

The spongy matrices were evaluated as to their colour and the degree of porosity. Moreover, the following physicochemical parameters were assessed: theoretical density, sorption capability and resistance to washing. Also pharmaceutical availability of cefradine was assessed for certain dressings. Obtained results were analysed statistically by means of Anova/Manova.

3. 1. Investigation of theoretical density

Lyophilized matrices were cut to fragments sized 1 cm x 1 cm and next measured exact to 0.001mm and weighed exact to 0.0001g using SARTORIUS MC1 scales. Density of matrices was calculated by means of the formula:

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

In which: D – density, m – mass of the segment, g,h,a – matrix fragment dimensions.

The measurements were repeated 8 times for each version of the sponge and an arithmetic mean of individual measurements was calculated. The obtained results are presented in Table 2.

3. 2. Investigation of sorption capability

Sponge samples measuring 1cm x 0.5cm were weighed exact to 0.0001g and placed in previously weighed beakers containing 5 ml of distilled water. The samples were removed from beakers after 30 minutes and the beakers were weighed again. The sorption capability of the material was calculated on the basis of water loss from the beaker according to the following formula:

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

In which: S_w – the amount of sorbed water, W_a – weight of the beaker prior to the assessment, W_b – weight of the beaker after removal of the matrix, W_s – sponge weight.

The measurements were 8 times repeated for each version of the dressing and next an arithmetic mean and standard deviation were calculated. The obtained results are presented in Figure 2.

3. 3. Investigation of resistance to washing.

Investigation of the resistance of matrices to washing was performed using an apparatus for active substance release from tablets according to Ph. Eur. 2004 [9].

Previously weighed 1 cm x 1 cm samples of matrices were placed in beakers containing 1% solution of pepsin in 0,1N HCl at 37°C and stirred at 50 revolutions/minute. After 30 minutes the residues of the matrices were removed and washed with distilled water. Next they were dried over silicone gel to obtain solid mass. The dried residue was weighed and the percentage loss of its mass was calculated according to the following formula:

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

In which: $\%P$ – percentage of the residue, W_{sb} – weight of the dry residue, W – initial weight of the matrix.

The measurements were 8 times repeated for each version of the matrix and next an arithmetic mean and standard deviation were calculated. The obtained results are shown in Figure 3.

3. 4. Pharmaceutical availability of cefradine

Pharmaceutical availability of cefradine was examined for selected versions of gelatin-alginate sponges. The investigation was performed in a diffuser consisting of a donor chamber and an acceptor chamber separated by a semi-permeable membrane (Figure 1). The investigation was carried out at 37°C with the diffuser being shaken at 50 revolutions /minute. The chambers contained 20 ml of water each. A previously weighed 1 cm x 1.5 cm sample of matrix was placed in the donor chamber. The amount of released cefradine was measured spectrophotometrically with the samples taken after 15, 30, 45, 60, 90, 120, 150 and 180 minutes.

Each version was 4 times measured and the results were used to calculate arithmetic means and standard deviations. The obtained results were used to plot the relationship between $\ln (100\% - \% \text{ of the released drug})$ and time as well as to determine the release kinetics trend line. The obtained results are presented in Fig. 2-7.

4. DISCUSSION OF RESULTS

All the prepared gelatin- alginate sponges were creamy-white in colour and had distinct porous structure. The increase of glycerol or peanut oil content did not decrease porosity of the material. A reverse effect was observed in case of calcium lactate.

4. 1. Theoretical density

Performed measurements, the results of which are presented in Table 2, revealed that the quantitative composition of sponges affects significantly their theoretical density. The obtained results ranged from 0.182 g/cm³ (C3 version) to 0.570 g/cm³ (B10 version).

A directly proportional relationship was found between the content of glycerol or peanut oil and theoretical density, which was more significant with addition of the latter substance. Addition of calcium lactate caused a significant decrease of theoretical density of the matrices, both in sponges containing peanut oil as well as in those containing glycerol.

The obtained results are consistent with the regularity of maintaining porous structure observed in the preliminary investigations.

4. 2. Sorption capability

The effect of the content of gelatin, sodium alginate and an elasticizing agent – glycerol or peanut oil as well as the effect of cross-linking factor such as calcium lactate on sorption capability of the sponges were investigated.

In case of sponges not modified with calcium lactate, the matrices containing peanut oil were able to absorb less water than sponges containing glycerol (Fig. 2). The only exception was B3 matrix, which revealed the highest sorption capability in this group (4.443 gram of water per 1 gram of the matrix). The amount of water absorbed by 1 g of the matrix containing peanut oil was from 2.186 to 3.163 gram, while for glycerol containing matrices it was from 2.984 to 4.334 g respectively.

Sponges modified with calcium lactate displayed significantly higher sorption capability, and a significant difference was observed between sponges containing glycerol and those containing peanut oil. After modification, matrices with glycerol increased the amount of absorbed fluid both in relation to respective non-modified versions as well as to the version with peanut oil.

Moreover, a direct relationship was found between increase in the content of the elasticizing agent and decrease of sorption capability. The results correlate in a way with the data presented above. Sponges containing lower levels of glycerol or peanut oil preserved their porous structure better and were characterized by lower relative density. This suggests that sorption capability is associated with binding water by polymers (sodium alginate and gelatin) and preserving it in the porous texture of the material.

4. 3. Resistance to washing

Investigations, the results of which are presented in Figure 3, revealed that composition of the matrices affects significantly their stability to washing, including the action of proteolytic enzymes.

Matrices with the addition of glycerol (version A) and of peanut oil (version B and D) were characterized by a similar stability under experimental conditions. On the other hand, sponges containing glycerol and modified with calcium lactate were least resistant to the action of proteolytic enzymes.

The results of investigations of resistance to washing correlate significantly with the results of investigations of sorption capability and theoretical density (characterizing the degree of maintenance of porous structure) of gelatin-alginate matrices. Sponges revealing weak sorption capability were least degradable. For example, B5 matrix, which absorbed the least amount of fluid, was destabilized only in about 33%, while version C5 sponges, able to absorb the highest amount of fluid in sorption capability investigation, were disintegrated in over 57%.

It seems that well preserved porous structure facilitates entrance of the fluid to the matrix, thus increases the surface exposed to the action of proteolytic enzymes.

4. 4. Pharmaceutical availability of cefradine

The investigation of pharmaceutical availability of cefradine was performed for selected gelatin-alginate matrices. The obtained results are presented in Figures 4-7. The results indicate that release of cefradine from gelatin-alginate matrices follows the pattern of first order kinetics, what was confirmed by high values of correlation coefficients of the curve illustrating logarithmic relationship of % residue by time. The release kinetics order was observed not to be associated with qualitative composition of the sponges, while the content of individual components plays a decisive role in the amount and rate of the antibiotic release from matrices.

The shortest cefradine half-release time was obtained for non-modified sponges containing glycerol as elasticizing agent. Cross-linking of the matrix with calcium lactate prolonged insignificantly the half-release time (Table 3).

Half-release time was 1.34 h for non-modified sponges with glycerol and 1.91 h for sponges with glycerol modified with calcium lactate.

In case of matrices with the addition of peanut oil, cross-linking with calcium lactate accelerated the release of the antibiotic. The half-release time shortened from 2.99 h to 1.59 h following calcium lactate modification.

It seems that the maintenance of a porous structure plays a significant role in the cefradine release time from gelatin-alginate sponges, as it facilitates penetration of the fluid into the sponge and faster dissolution of contained in it antibiotic.

Comparing our earlier investigations (Haznar et al. 2003), the initial lyophilization of gelatin–alginate mixture plays a significant role in the obtained order of release kinetics. Initial lyophilization of the gelatin-alginate mixture, which was subsequently used to produce the matrices, resulted in a model release of the drug following a pattern of first order kinetics,

while the release of drugs from matrices without initial lyophilization of the gelatin-alginate mixture approached the zero order kinetics.

Anova/Manova analysis demonstrated statistically significant differences in kinetics parameters (release rate constant, half-release time and total released %) between sponge A and sponge C modified with calcium lactate, as well as between sponges B and D and A and B. Differences between sponges C and D proved statistically insignificant.

5. DISCUSSION

Obtained results demonstrate the addition of both, elasticizing agents (glycerol or peanut oil) as well as cross-linking agent (calcium lactate) exert a significant effect on physico-chemical parameters of the sponge. The addition of glycerol and – to a larger extent – of peanut oil causes increase of its density by “overloading” the matrix. On the other hand it seems that decrease of the parameter resulting from cross-linking of the matrix with calcium ions is due to fixation of the sponge structure (“stiffening”) on foaming by altering the solubility of alginate. Moreover, a correlation between density of the matrix and its sorption capability was found. Sponges containing lower amounts of glycerol or peanut oil and sponges cross-linked with calcium ions which better maintain the porous structure and have lower density were characterized by higher sorption capability. This may suggest that the sorption capability is conditioned by binding water by biopolymers (alginate and gelatin) and its retention in the pores of the material.

Similarly, correlation was found between the resistance to washing and density and sorption capability of gelatin-alginate matrices. Sponges revealing poor sorption capability were less susceptible to washing out in water. For instance, B5 matrix, which absorbed less fluid was washed out in about 33%, while C5 sponges, which absorbed the most fluid on sorption capability testing, underwent the highest degree washing – in over 57%.

It seems that well maintained porous structure facilitates penetration of fluid to the matrix and thus increases the surface on which proteolytic enzymes may act.

It also seems that considering the pharmaceutical availability, the release rate of cefradin from gelatin-alginate sponge is associated with the degree of maintenance of the porous structure, what facilitates penetration of the fluid into the sponge and faster dissolution of contained antibiotic.

Comparing earlier studies [10], it appeared that preliminary freeze-drying of the gelatin-alginate mixture exerts a decisive role on the order of the release kinetics. Preliminary

freeze-drying of the mixture of gelatin and sodium alginate which was next used to produce the matrices resulted in the model active substance release according to first order kinetics. When the active substance was released from matrices that were obtained without preliminary lyophilization of the gelatin-alginate mixture, the release kinetics approached zero order, while the obtained half-release times were significantly prolonged (about 30 h).

6. CONCLUSIONS

1. Using biopolymers such as gelatin and sodium alginate, it is possible to obtain porous carriers for drugs which will undergo bioresorption.
2. Depending on the composition, matrices with various degree of porosity can be obtained, what has a significant effect on their physico-chemical properties and pharmaceutical availability of the active substance.

LITERATURA

- [1] Wachol-Drewiek Z., Pfeiffer M., Scholl E.: Comparative investigation of drug delivery of collagen implants saturated in antibiotic solutions and a sponge containing gentamicin. *Biomaterials* (1996), 17, 1733-1738.
- [2] Oungbho K., Müller B.W.: Chitosan sponges as sustained release drug carriers. *Int. J. Pharmaceut.* (1997), 156, 229-237.
- [3] Bong Gyu Yu et.al.: Development of a local antibiotic delivery system using fibrin glue. *J. Controll. Release* (1996), 39, 65-70.
- [4] Schmidt C., Wenz R., Nies B., Moll F.: Antibiotic in vivo/in vitro release, histocompatibility and biodegradation of gentamicin implants based on lactic acid polymers and copolymers. *J. Controll. Release* (1995), 37, 83-94.
- [5] Fan H., Dash A. K.: Effect of cross-linking on the in vitro release kinetics of doxorubicin from gelatin implants. *Int. J. Pharmaceut.* (2001), 213, 103-116.
- [6] Yi Luo, Kirker K. R., Prestwich G. D.: Cross – linked hyaluronic acid hydrogel films: new

- biomaterials for drug delivery. J. Controll. Release (2000), 69, 169-184.
- [7] Shu X. Z., Zhu K. J., Song W.: Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. Int. J. Pharmaceut. (2001), 212, 19-28.
- [8] Kuijpers A. J. et al.: Controlled delivery of antibacterial proteins from biodegradable matrices. J. Controll. Release (1998), 53, 235-247.
- [9] European Pharmacopoeia 2002 – 4th Edition – Council of Europe, Strasbourg 2001.
- [10] Haznar D., Pluta J.: The influence of composition on physicochemical properties and active substance release from gelatin-alginate sponge. Polimers in Medicine (2003), 33 (4), 17-27.

Address of the authors:

Department of Applied Pharmacy

Wrocław Medical University

ul. Szewska 38, 50-139 Wrocław, Poland

tel /fax +48 71 784 03 17

e-mail: haznar@bf.uni.wroc.pl

Fig. 1. Diffusion apparatus (1 - donor chamber, 2 - acceptor chamber, 3 - semi-permeable membrane)

Ryc. 1. Schemat aparatu dyfuzyjnego (1 - komora donorowa, 2 - komora akceptorowa, 3 - błona półprzepuszczalna)

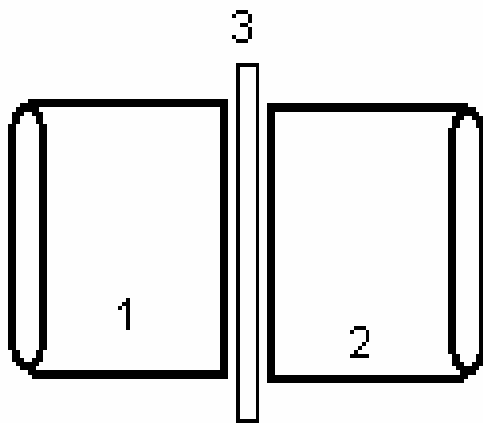


Fig. 2. Mean amount of water absorbed by 1 g of sponge
Ryc. 2. Średnia ilość wody zaadsorbowanej przez 1g gąbki

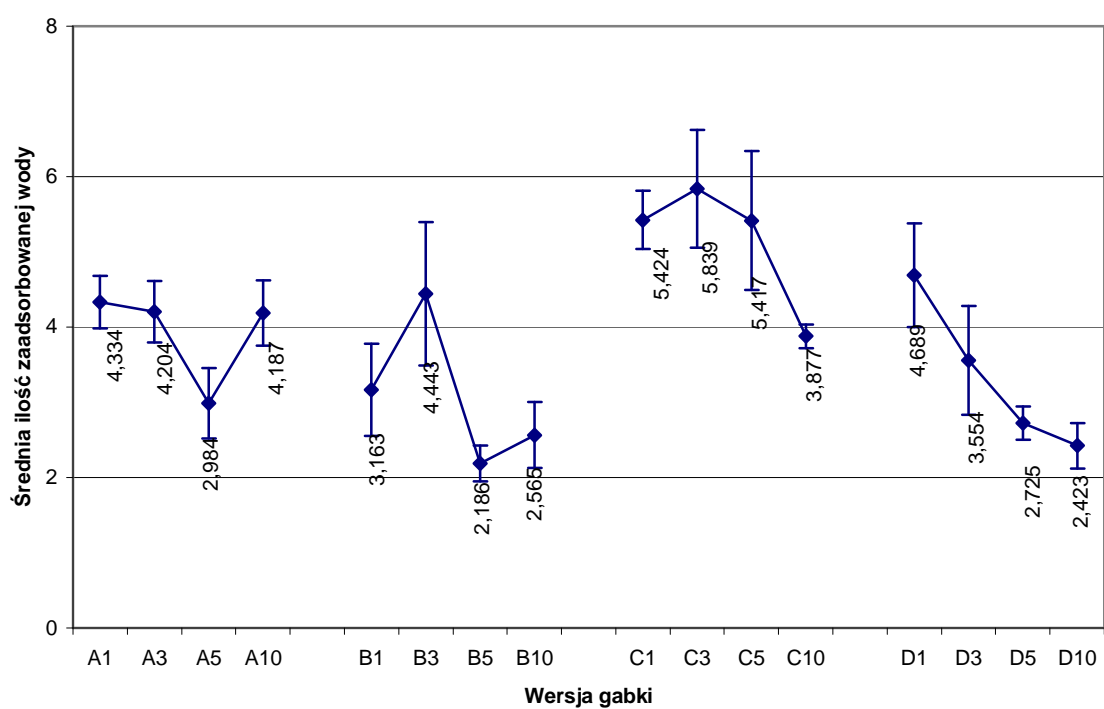


Fig. 3. Mean percentage of residue after washing in relation to composition
Ryc. 3. Średni procent pozostałości po rozmywaniu w zależności od składu

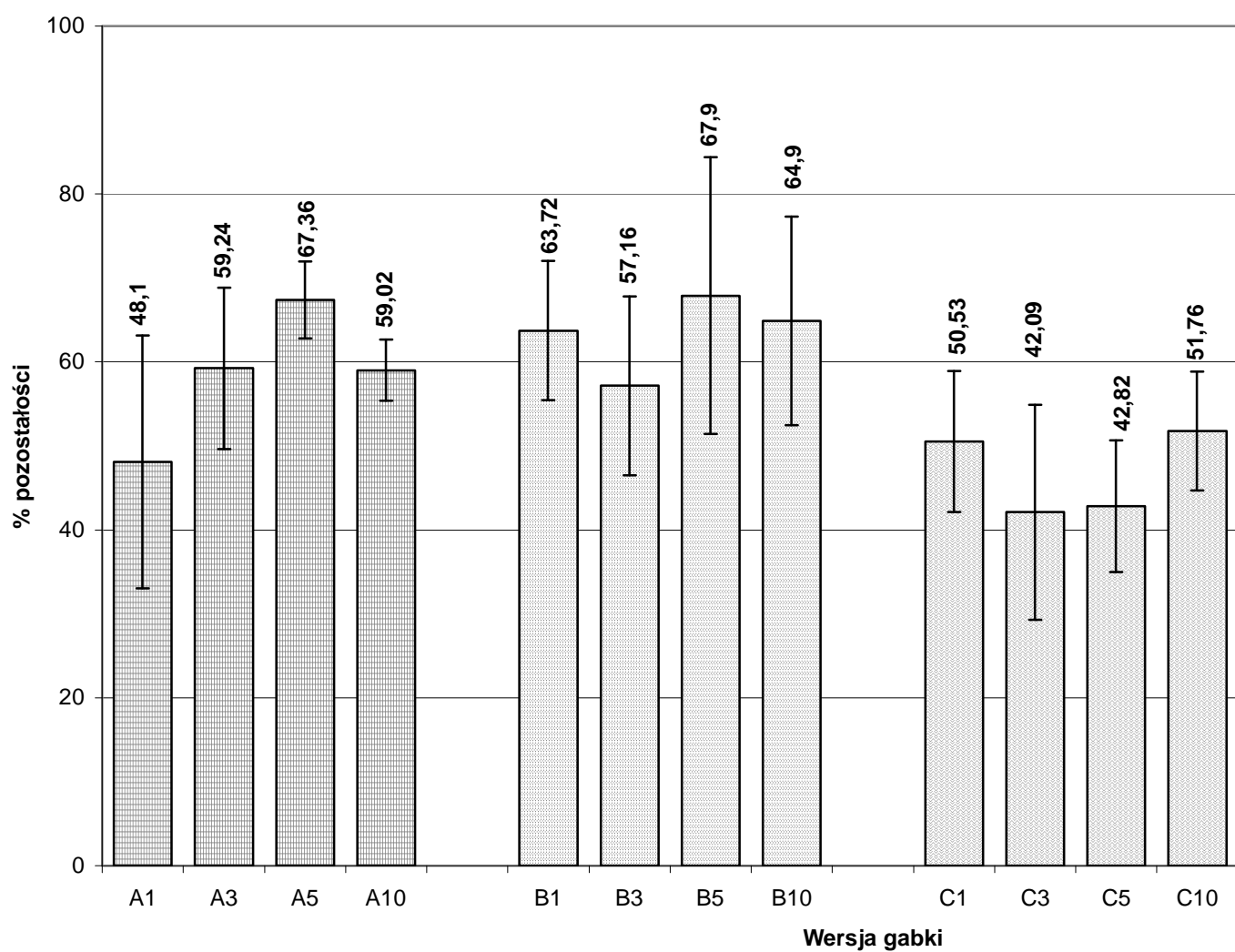


Fig. 4. Kinetics of cefradine release from A3 sponge

Ryc. 4. Kinytyka uwalniania cefradyny z gąbki wersji A3

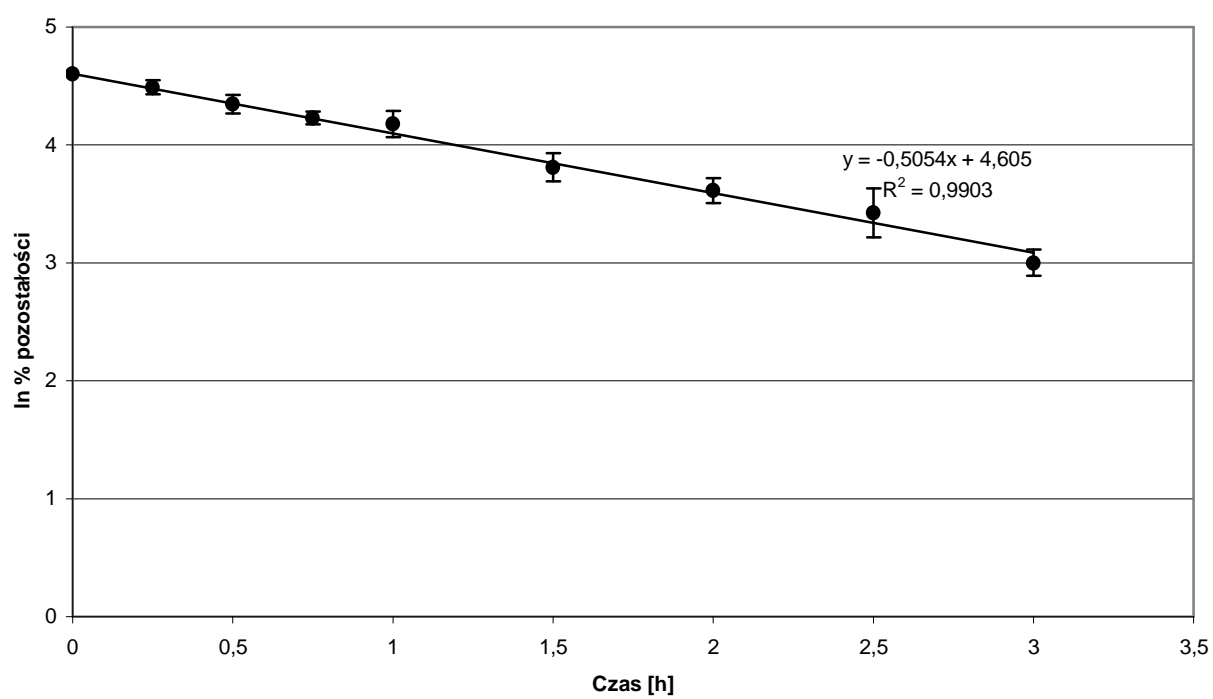


Fig. 5. Kinetics of cefradine release from B3 sponge
Ryc. 5. Kinetyka uwalniania cefradyny z gąbki wersji B3

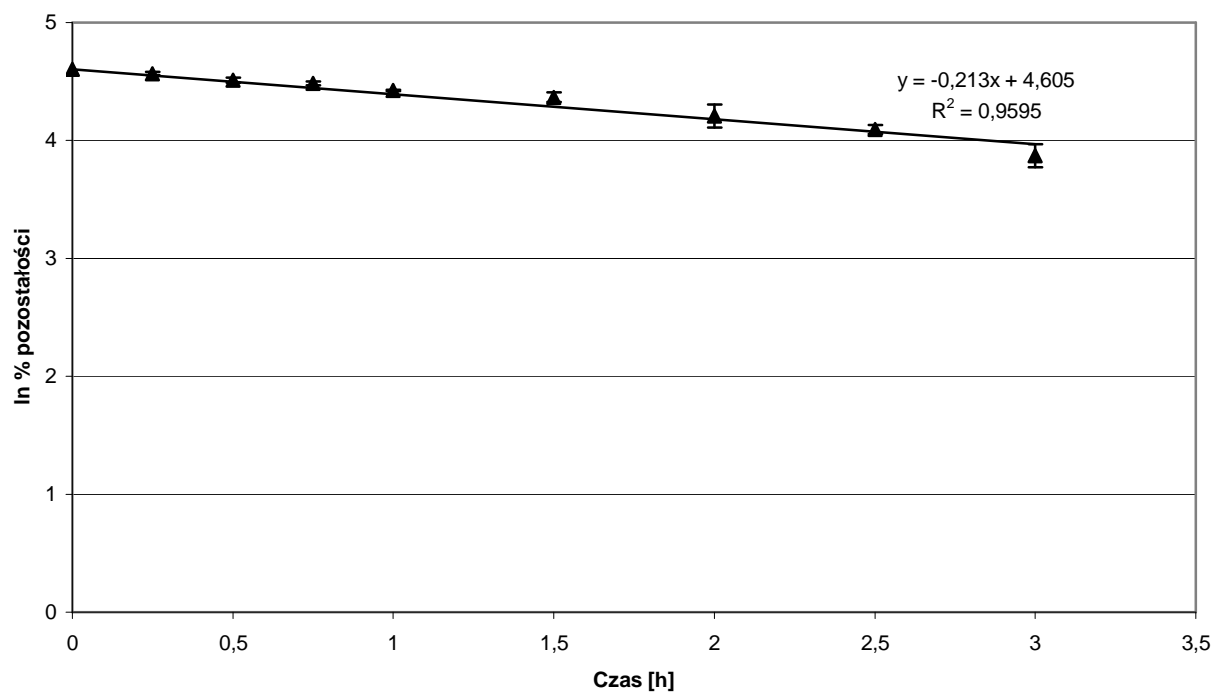


Fig. 6. Kinetics of cefradine release from C3 sponge

Ryc. 6. Kinetyka uwalniania cefradyny z gąbki wersji C3

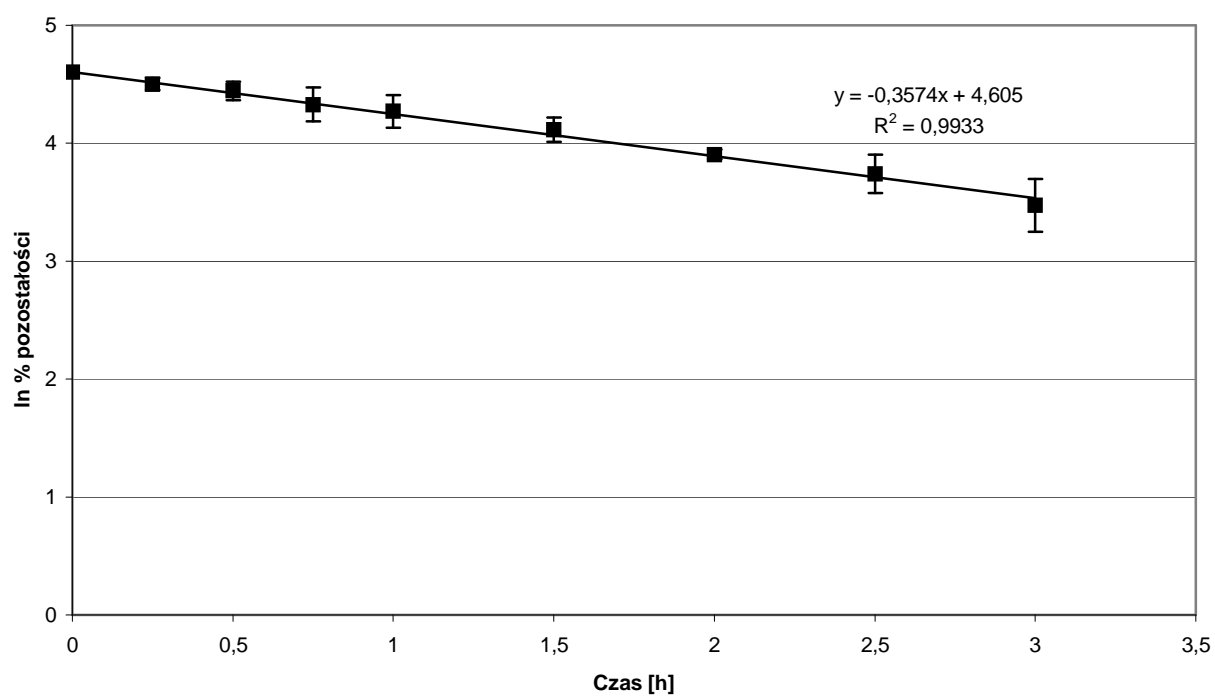


Fig. 7. Kinetics of cefradine release from D3 sponge

Ryc. 7. Kinetyka uwalniania cefradyny z gąbki wersji D3

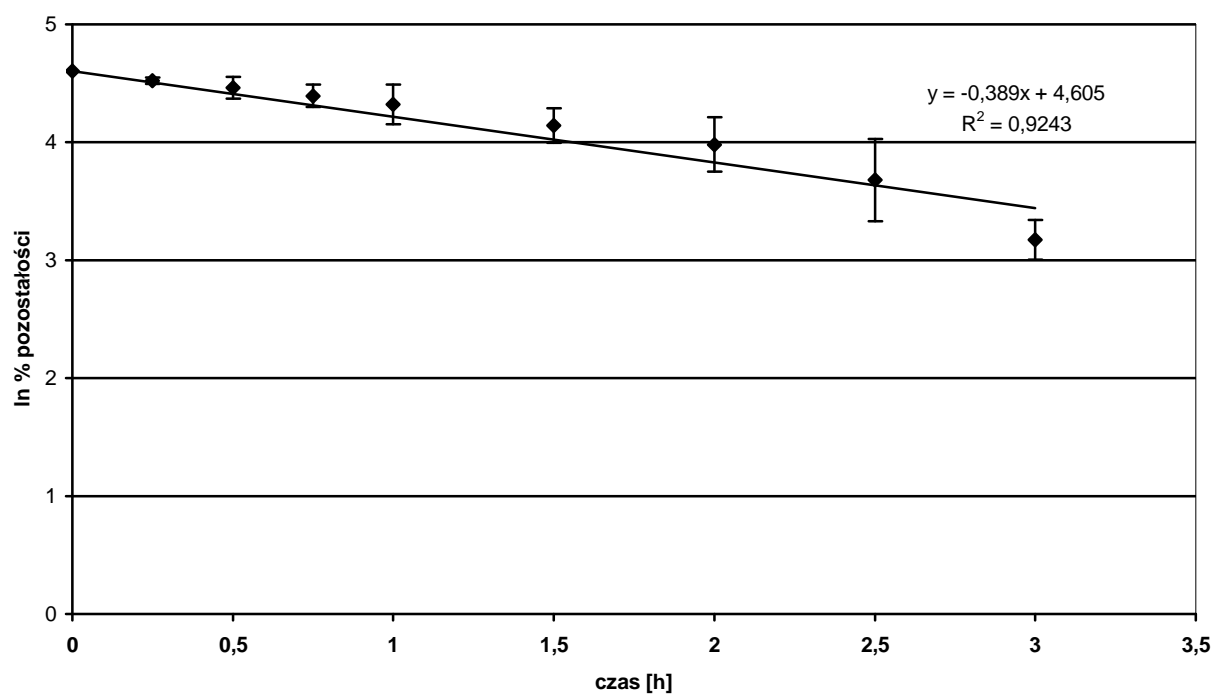


Table 1. Composition of sponges

Tabela 1. Skład gąbek

Sponge version	<i>Gelatin-sodium alginate ratio</i>	Sodium lactate content	<i>Glycerol(GL) or peanut oil (OA) content</i>	Sponge version	<i>Gelatin-sodium alginate ratio</i>	Sodium lactate content	<i>Glycerol(GL) or peanut oil (OA) content</i>
Wersja gąbki	<i>Stosunek żelatyny do alginianu sodu</i>	Zawartość mleczanu sodu	<i>Zawartość glicerolu (GL) lub oleju arachidowego (OA)</i>	Wersja gąbki	<i>Stosunek żelatyny do alginianu sodu</i>	Zawartość mleczanu sodu	<i>Zawartość glicerolu (GL) lub oleju arachidowego (OA)</i>
A1	90:1	-	1%GL	C1	90:1	+	1%GL
A3	90:1	-	3%GL	C3	90:1	+	3%GL
A5	90:1	-	5%GL	C5	90:1	+	5%GL
A10	90:1	-	10%GL	C10	90:1	+	10%GL
B1	90:1	-	1%AO	D1	90:1	+	1%AO
B3	90:1	-	3%AO	D3	90:1	+	3%AO
B5	90:1	-	5%AO	D5	90:1	+	5%AO
B10	90:1	-	10%AO	D10	90:1	+	10%AO

Table 2. Mean density of the sponges [g/cm³]

Tabela 2. Średnia gęstość gąbek [g/cm³]

Sponge version Wersja gąbki	Mean density [g/cm ³] Średnia gęstość [g/cm ³]	±S.D.	Sponge version Wersja gąbki	Mean density [g/cm ³] Średnia gęstość [g/cm ³]	±S.D.
A1	0,205	0,011	C1	0,1941	0,006
A3	0,229	0,013	C3	0,182	0,014
A5	0,0186	0,034	C5	0,193	0,013
A10	0,438	0,029	C10	0,211	0,020
B1	0,249	0,035	D1	0,287	0,019
B3	0,275	0,012	D3	0,289	0,044
B5	0,405	0,062	D5	0,267	0,013
B10	0,569	0,150	D10	0,358	0,043

Table 3. Half-release time and total amounts of the active substance in relation to the sponge composition

Tabela 3. Okresy półuwalniania oraz całkowite ilości substancji leczniczej w zależności od składu gąbek

Sponge version Wersja gąbki	Total % of released active substance after 3h Całkowity % uwolnionej substancji leczniczej po 3h	$T_{0,5}$
	[%]	[h]
A3	78,64	1,34
B3	51,80	2,99
C3	67,15	1,91
D3	75,76	1,59

