



# The development of biodegradable hemostatic and absorbable sponges containing chlorhexidine digluconate and their *in vitro* characterization—A QbD approach

Bohdana Pavliuk<sup>1\*</sup> , Mariana Chubka<sup>2</sup> , Taras Hroshovyi<sup>1</sup> , Mariana Demchuk<sup>1</sup> , Iryna Stechyshyn<sup>1</sup>

<sup>1</sup>Horbachevsky Ternopil National Medical University, Department of Pharmacy Management, Economics and Technology, Ternopil, Ukraine.

<sup>2</sup>Horbachevsky Ternopil National Medical University, Department of Pharmacy, Ternopil, Ukraine.

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## ABSTRACT

Medical sponges are one of the most common tools used in various fields of medicine and are used to close wound surfaces in burns and trophic ulcers, showing absorbent and antiseptic properties, and stimulate tissue regeneration. The aim of this study was to optimize the composition of medical sponges with chlorhexidine digluconate based on a material of natural origin - xenoderma. The central composite design was used to establish the relationship between independent variables such as quantities of medical gelatin, propyleneglycol, and formaldehyde and dependent variables such as pH, the percentage of water absorption, the degree of *in vitro* degradation, thickness, and the time of complete dissolution, in order to obtain the optimal formulation using Response Surface Methodology. Using the concept of Quality by Design, the desired quality target product profile, critical quality attributes, risk assessment by Failure Mode Effects Analysis was determined that the number of excipients affects the pharmaco-technological parameters. It was found that sponges that contain 2% medical gelatin, 1% propyleneglycol, and 0.75% formaldehyde, are the best in composition, in terms of pH, the percentage of water absorption, the degree of *in vitro* degradation, thickness, the time of complete dissolution, in comparison with all other compositions.

## INTRODUCTION

The pharmaceutical industry is constantly looking for ways to ensure and improve the safety, quality, and efficiency of products. For this purpose, the principles of the scientific and technical approach to Quality by Design (QbD) are used, which by reducing its possible variations improves the understanding of the technological process and provides the ability to manage it, while requiring in-depth knowledge of production technologies of a particular dosage form (Ohage *et al.*, 2016; Mogal, 2016). QbD involves the analysis and control of all possible risks, assuming that each technological step of the process is a source of possible risk or changes. QbD describes an approach to pharmaceutical development that involves the development of composition and

technology and production to control the established product quality (Beg *et al.*, 2019; Suci *et al.*, 2018). Recently, the concept of QbD has become increasingly important in the development and production of medicines. QbD is recommended as one of the methods to improve the quality of pharmaceutical products by the Food and Drug Administration and the European Medicines Agency (Patwardhan *et al.*, 2017; Tefas *et al.*, 2018).

One of the essential tools of QbD in the development of optimized products and processes is “Design of Experiment (DoE),” which is based on the use of experimental designs (Budniak *et al.*, 2020; Demchuk *et al.*, 2018). Recommendations related to mathematical models are used to ensure the establishment and use of knowledge of drug technology independently and in an integrated manner (Politis *et al.*, 2017; Sylvester *et al.*, 2018).

There are currently several documents (of a recommendatory manner) that help to ensure the proper quality of QbD: ICH Q8 “Pharmaceutical Development,” ICH Q9 “Quality Risk Management,” and ICH Q10 “Pharmaceutical Quality

### \*Corresponding Author

Bohdana Pavliuk, Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. E-mail: bohdana.vons@gmail.com

*System*”, as well as the use of Process Analytical Technology or Quality by Testing (ICH 2005; ICH 2008; ICH 2009).

Today, the creation of drugs or medical devices using QbD improves quality, controls risks, and is an integral part of the development of drugs in nonpharmacopoeias dosage forms (Porfire *et al.*, 2017). Examples of such forms are medical sponges, which have wide practical application in various fields of medicine: in surgery, they are used to close wound surfaces in burns and trophic ulcers, showing absorbent and antiseptic properties, and stimulate tissue regeneration (Pavliuk *et al.*, 2020). For example, the use of a dry sponge during surgery provides the absorption of large amounts of fluid, and after the operation, such a sponge dissolves (Liu *et al.*, 2018; Marushchak *et al.*, 2017; Pavliuk *et al.*, 2020). It is currently believed that hemostatic and absorption effects are associated with the porosity of the sponges and their ability to absorb blood or exudate from the wound surface; they restore the integrity of damaged blood vessels and accelerate the recovery of damaged epidermal tissues (Azuma *et al.*, 2015). Sponges exhibit bactericidal, antiseptic, antimicrobial, regenerating, tonic, and absorbent properties and purposefully act on the focus of pathology (Pavliuk *et al.*, 2020). By method of application, sponges are divided into antiseptic, absorbent, hemostatic, vaginal, and rectal.

Since 2000, many studies have been conducted on the development of new hemostatic and absorbent agents, and there is more and more evidence of their effectiveness in comparison with conventional gauze bandages (Konstantelias *et al.*, 2016).

Therefore, the aim of this work was to develop and optimize the composition of a structured medical sponge based on a material of biological origin, namely, xenoderm, which is enriched with macro- and micronutrients, amino acids, and chlorhexidine digluconate (1%) as the active pharmaceutical ingredient (API), using QbD at each stage of technological formation (Leonard *et al.*, 2016; Lytvynuk *et al.*, 2016; Pavliuk *et al.*, 2020; Vons *et al.*, 2018).

## MATERIALS AND METHODS

### Materials

Crushed substrate of cryolyophilized xenoderm (LLC “Institute of Biomedical Technologies,” Ternopil, Ukraine), chlorhexidine digluconate solution 20% (“Sigma-Aldrich,” USA), and excipients, including medical gelatin, formaldehyde (“Sigma-Aldrich,” USA), and propylene glycol (“BASF Global,” Germany), were used.

### Methods

#### *Preparation of medical sponges*

In the xenoderm water extract, the medical gelatin [with different concentrations according to the central composite circumscribed (CCC) plan] was left at 37°C for swelling and dissolution. Then, the remainder of the xenoderm water extract was added to the gelatin solution by parts, with constant stirring until foam formation. To prepared solutions of xenoderm water extract with medical gelatin, the formaldehyde in the different concentrations was added (according to the CCC plan) to provide cross-linking. To the resulting mixtures, it was added a 1% chlorhexidine digluconate solution. The mixtures were foamed to form a stable foam that increased 2–3 times in volume. The resulting

solution was poured out into the cooled aluminum molds (size 15 \* 7 cm/100 ml) and left for 2–3 hours at temperature 20°C–22°C to provide cross-linking of the gelatin with formaldehyde and to form a framework. Thereafter, the solutions were frozen in liquid nitrogen and lyophilized during 18–20 hours (7–8 hours at temperature –30°C ± 0.5°C, 4–5 hours at temperature +30°C ± 0.5°C, and 6–7 hours at temperature +35°C ± 0.5°C). The finished dry product (medical sponge) was cut into plates of various sizes and packed in parchment bags.

Using the assessment of the quality target product profile (QTPP), the determination of the quality and efficiency of the developed medical tool was obtained, conducting a preliminary set of quality indicators that must be achieved to guarantee the desired quality, taking into account safety and efficiency. Table 1 presents the QTPP for medical sponges with chlorhexidine digluconate. In addition to defining product design requirements, QTPP will help determine quality indicators, organoleptic properties, scope, and shelf life.

#### *The critical quality attribute (CQA)*

CQA identifies features/properties that should be within the appropriate requirements, distribution, or range to ensure the required quality of medical sponges with chlorhexidine digluconate and that are typically associated with APIs, excipients, intermediates (materials during the process), and the finished product. Table 2 shows the CQAs for medical sponges with chlorhexidine digluconate.

#### *Risk assessment by failure mode effects analysis (FMEA)*

The overall risk assessment (ORA) is shown in the Ishikawa diagram (Fig. 1), which contains all process parameters that may affect or generate errors regarding the quality of the final product. The priority of critical quality indicators of sponges with chlorhexidine digluconate (1%) meets three criteria, which were evaluated on a scale (points) from 1 to 5: O: frequency of occurrence (5: for common cases, 4: for probable, 3: there is a possibility of occurrence, 2: for remote, and 1: for impossible cases); S: severity (5: catastrophic, 4: critical, 3: serious, 2: minor, and 1: insignificant); D: the ability to detect (5: impossible or difficult to detect, 4: detected with low probability, 3: moderately detected, 2: easily detected, and 1: very easily detected). Each of the critical process parameters (CPP) and each critical material characteristic (CMC) was evaluated according to these three criteria. The risk/failure priority number (RPN) was calculated by the following formula:  $RPN = O \times S \times D$  (Table 3).

#### *DoE*

Software for optimizing the composition and technology of Design-Expert trial version 12.0.0 (Ease Inc., Minneapolis, MN) was used to build the experimental design. In order to study the influence of quantitative factors on the pharmaco-technological parameters of medical sponges, three excipients were selected, namely medical gelatin, propylene glycol, and formaldehyde. The list of quantitative factors and their levels, which were studied in the development of the optimal composition and technology of medical sponges, are presented in Table 4.

The study of selected factors was carried out using a symmetric rotatable composite plan of the second order (CCC).

**Table 1.** QTPP of medical sponges.

Quality attribute	Characteristics	Justification
Dosage form	Sponge (structured) medical	Intended for external and internal use, closing wounds and ulcers of various etiologies, and stopping surgical bleeding
Application method	Local	
API	Chlorhexidine digluconate 1%	
Indication	As an antiseptic tool for wounds and bleeding of various etiologies	One of the most common over-the-counter drugs, with a wide range of antimicrobial action
Stability	At least 24-month shelf life	Store at room temperature in a tightly packed primary container
Packaging	Primary packaging: sterile cellophane-parchment packaging/blister Secondary packaging: cardboard packaging	
Appearance	White, loose, structured sponges, soft, and without foreign inclusions	
Identification of API		In accordance with the requirements of the USA Pharmacopoeia, patents
Chlorhexidine digluconate	+	
Amino acids	+	
	Analysis	
Chlorhexidine digluconate, %	0.9–1.1	
<i>In vitro</i> degradation of sponges, hours	No more than 50 hours	
Determination of water absorption, %	No less 2,800	
pH	6.6–6.9	
Sterility	Sterility of forms	

**Table 2.** CQAs.

Quality indicators	Requirements	Justification
Time of complete dissolution/degree of degradation	According to the requirements QTPP	These two indicators are interdependent and most characterize the quality of the developed tool, as they allow us to predict the time of complete dissolution of medical sponges and therefore are critical indicators
Percentage of water absorption	Must meet the established requirements	The percentage of water absorption affects the effectiveness of the developed medical tool. The higher the percentage of water absorption is, the better and to a greater extent the sponges will absorb the exudate
Quantitative content of API	Must meet the requirements ( $\pm 10\%$ )	The concentration of API is a critical indicator of quality, as violation of the tolerances of the quantitative content of API affects the pharmacological action and quality

Table 4 presents the independent variables used as formulation factors. The experimental design matrix is shown in Table 5.

During designing the optimal composition of medical sponges, they were controlled by the following six indicators:

**Study of pH change.** The pH change in solution during degradation of the sponge was determined after 10, 30, and 60 minutes and 12, 24, 36, 48, and 72 hours using a brand pH meter “MI-150.” The average pH of each series was subjected to statistical processing.

**Determination of water absorption.** To the flat-bottomed cup (preheated to  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) was added a simulation solution containing 8.398 g/l sodium chloride and 0.278 g/l anhydrous calcium chloride (the given proportion of ion content equal to the amount in human serum).

A piece of dry sponge  $1 \times 1 \text{ cm}^2$  ( $X_0$ ) was preweighed and immersed in a simulation solution for 10 minutes. Then the sponge was removed and, after removing the surface moisture with filter paper, weighed ( $X_1$ ).

Water absorption (%) is calculated as follows:

$$\text{Water absorption (\%)} = (X_1 - X_0) / X_0 \times 100\%,$$

where  $X_0$  is the mass of the sponge before absorption and  $X_1$  is the mass of the sponge after absorption.

This method was used to model the water absorption curve and determine the time of complete dissolution (degradation). For this purpose, the sponge is weighed at certain intervals until complete dissolution.

***In vitro* degradation of sponges.** Degradation can be determined by immersing a piece of sponge ( $1 \times 1 \text{ cm}^2$ ) in

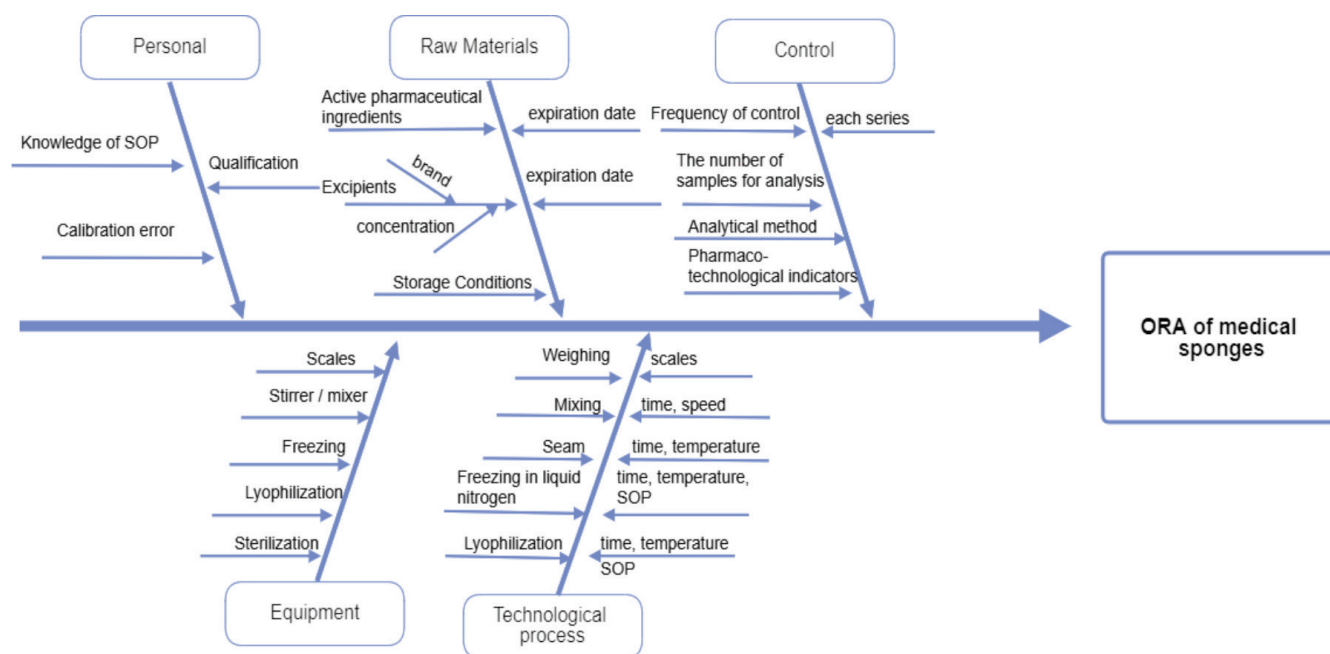


Figure 1. Ishikawa diagram.

Table 3. FMEA of risk assessment of medical sponges with chlorhexidine digluconate (1%).

CPP/CMC	Potential failure mode	Potential failure effect	Potential cause of failure	Control method	O	S	D	RPN
Drying time and temperature	Incorrect temperature or change in drying time	Humidity after lyophilization/hardness	Device error and human factor	Appearance, percentage of water absorption, and pore size	1	5	2	10
Homogeneity of the composite mass	Not matching concentration, weight, and thickness	Hardness, fragility/humidity, and mismatch of APIs concentration	Noncompliance with technology	<i>In vitro</i> degradation and percentage of water absorption	2	4	1	8
Integrity of primary packaging	Lack of sterility	Microbiological contamination	Device error and human factor	Microbiological control of the sterility of the form	3	5	3	45

Table 4. Factors studied in the development of the optimal composition of medical sponge.

Factors	Variation interval	Level of factor				
		“-α”	“-”	“0”	“+”	“+α”
$x_1$ : amount of medical gelatin, %	1.0	0.32	1.0	2.0	3.0	3.68
$x_2$ : amount of propylene glycol, %	0.5	0.16	0.5	1.0	1.5	1.84
$x_3$ : amount of formaldehyde, %	0.04	0.008	0.035	0.075	0.115	0.142

phosphate buffer solution with pH 7.4 for 24 hours. The tests were carried out in an incubation chamber (37°C). After 24 hours, the sponge is redried and the change in sponge mass is calculated. *In vitro* degradation of sponges calculated by the following formula:

$$\text{In vitro degradation (\%)} = (X_f - X_s) / X_s \times 100\%$$

where  $X_f$  is the final mass of the sponge after drying and  $X_s$  is the initial mass of the sponge before immersion.

**Appearance.** Medical sponges were monitored for appearance and characteristic organoleptic properties (color, odor, and consistency), as well as for signs of physical instability (delamination, fragility, density, and surface homogeneity).

**Table 5.** Experimental planning matrix and results of studying the characteristics of medical sponges.

№	Factors			Reviews (indicators)					
	$x_1$	$x_2$	$x_3$	$y_1$	$y_2$	$y_3$	$y_4$	$y_5$	$y_6$
1	+	+	+	6.8	2,300	26	5	0.9	89
2	-	+	+	6.6	2,400	62	4	0.9	31
3	+	-	+	6.5	2,200	26	5	0.8	92
4	-	-	+	6.8	2,400	63	5	0.9	38
5	+	+	-	6.6	2,700	26	4	0.7	92
6	-	+	-	6.5	2,750	68	5	0.6	45
7	+	-	-	6.6	2,400	31	4	0.9	77
8	-	-	-	6.7	2,300	69	5	0.9	35
9	+L	0	0	6.7	2,600	26	4	0.6	109
10	-L	0	0	6.6	2,000	96	4	0.6	25
11	0	+L	0	6.8	2,850	52	5	0.9	49
12	0	-L	0	6.6	2,850	48	5	0.9	50
13	0	0	+L	6.9	1,980	41	4	1.3	59
14	0	0	-L	6.4	2,800	49	4	0.8	54
15	0	0	0	6.6	3,000	51	5	0.9	47
16	0	0	0	6.6	2,800	47	5	0.8	51
17	0	0	0	6.7	2,900	48	4	0.8	50
18	0	0	0	6.6	3,100	49	5	0.9	49
19	0	0	0	6.7	3,200	48	5	0.7	50
20	0	0	0	6.8	3,150	47	5	0.8	51

$y_1$ : pH of sponges during degradation.

$y_2$ : Water absorption, %.

$y_3$ : *In vitro* degradation of sponges, %.

$y_4$ : Appearance, point.

$y_5$ : Thickness, cm.

$y_6$ : Time of complete dissolution, hours.

**Thickness.** The thickness of the sponges was measured using a thickness gauge KI (GOST 6507-78) with an accuracy of 10  $\mu\text{m}$ . The average thickness of the sponges of each series was subjected to statistical processing.

## RESULTS AND DISCUSSION

### *Risk assessment analysis*

According to ICH Q8, QTPP contains functions that have a direct relationship with the quality, safety, and efficacy attributes of the pharmaceutical. At the first stage of research, QTPP was developed taking into account the type of dosage form and technological features. CQA, which is determined by preliminary analysis, is the time of complete dissolution of the sponges and the percentage of water absorption.

The technological process of obtaining medical sponges consists of four main stages: preparation of the intermediate product of water extract from the xenoderm, mixing of components, freezing in a solution of liquid nitrogen, and lyophilization. Medical sponges were obtained using the process of lyophilization as one of the possible methods of drying, so an important point is

to control the drying conditions (drying time and temperature), as risk components that generally affects the mechanical properties of sponges and made a negative impact on the time of complete dissolution and *in vitro* degradation of sponges. The ORA (all factors that may affect the quality of the final product, process, instruments, personnel, and analytical techniques) was analyzed using the Ishikawa diagram (Fig. 1).

### *DoE*

Design-Expert 12.0 is an optimization software used for experimental design and statistical analysis. The planning matrix of this experiment and the results of studying the characteristics of medical sponges based on water extract from xenoderm and with 1% chlorhexidine digluconate are shown in Table 5. The reliability of the experimental plan and the reliability of the obtained results were confirmed by the values of the statistical parameters of the ANOVA test.

When studying the three factors, the second-order model has the following form:

$$y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2.$$

**Table 6.** ANOVA indicating statistical parameters for the CQAs of medical sponges.

Reviews (indicators)	ANOVA parameters					
	Source	Sum of squares	Df	Mean square	F-value	p-value
$y_1$ , pH	Model	0.1735	6	0.0289	3.24	0.0358
	Residual	0.1160	13	0.0089	-	-
	Lack of fit	0.0827	8	0.0103	1.55 <sup>a</sup>	0.3266
	Pure error	0.0333	5	0.0067	-	-
	Cor total	0.2895	19	-	-	-
$y_2$ , water absorption, %	Model	2.201E + 06	9	2.445E + 05	6.42	0.0038
	Residual	3.806E + 05	10	38,059.84	-	-
	Lack of fit	2.618E + 05	5	52,369.68	2.21 <sup>a</sup>	0.2029
	Pure error	1.188E + 05	5	23,750.00	-	-
	Cor total	2.581E + 06	19	-	-	-
$y_3$ , <i>in vitro</i> degradation of sponges, %	Model	5,713.14	9	634.79	43.06	<0.0001
	Residual	147.41	10	14.74	-	-
	Lack of fit	136.08	5	27.22	12.01 <sup>a</sup>	0.0082
	Pure error	11.33	5	2.27	-	-
	Cor total	5,860.55	19	-	-	-
$y_3$ , thickness, cm	Model	0.3908	9	5.551E-17	7.797E-15	1.0000
	Residual	0.0712	10	0.0071	-	-
	Lack of fit	0.0429	5	0.0086	1.51 <sup>a</sup>	0.3303
	Pure error	0.0283	5	0.0057	-	-
	Cor total	0.4620	19	-	-	-
$y_6$ , time of complete dissolution, h	Model	9,636.28	9	1,070.70	118.61	<0.0001
	Residual	90.27	10	9.03	-	-
	Lack of fit	78.94	5	15.79	6.97 <sup>a</sup>	0.0264
	Pure error	11.33	5	2.27	-	-
	Cor total	9,726.55	19	-	-	-

SS: Sum of squares, Df: Degree of freedom, and MS: Mean sum of squares.

<sup>a</sup>Not significant.

The statistical significance of the coefficients and the adequacy of the models were checked using the F-criterion. The nature of the influence of the studied factors was determined by the values and signs of the regression coefficients. The results of the experiment are shown in Table 6.

The relationship between the studied factors on the pH of medical sponges, after ignoring the insignificant terms, is described by the following regression equation:

$$y_1 = 6.65 + 0.083x_3 + 0.09x_1x_2.$$

Only statistically significant coefficients are included in this and other regression equations below. The regression equation shows the statistical significance of  $x_3$  (amount of formaldehyde), as well as the interaction of factors  $x_1$  and  $x_2$  (gelatin and propylene glycol content). The coefficients  $b_1$ ,  $b_2$ ,  $b_{13}$ ,  $b_{23}$ ,  $b_{11}$ ,  $b_{22}$ , and  $b_{33}$  were statistically insignificant in the regression equation.

For significant coefficients of pair interactions, the interpretation of the regression equation is carried out using a graph. The effect of formaldehyde on the pH of medical sponges, which was studied during the degradation of sponges, is shown in Figure 2. With an increasing amount of formaldehyde, more than 0.075% in the composition of the sponges increases the pH value.

As seen in Table 6, the Model *F*-value of 3.24 implies that it is significant. There is only a 3.58% chance that an *F*-value this large could occur due to the noise. *p*-values less than 0.0500 indicate model terms are significant. In this case, C and AB are significant model terms.

The most significant factor influencing percentages of water absorption is factor  $x_3$ , the amount of formaldehyde. The coefficients  $b_1$ ,  $b_2$ ,  $b_{12}$ ,  $b_{13}$ ,  $b_{23}$ ,  $b_{34}$ , and  $b_{22}$  were statistically insignificant in the regression equation.

The equation of polynomial regression for the percentage of water absorption after ignoring the insignificant terms is presented as follows:

$$y_2 = 3,026.65 - 163.22x_3 - 267.09x_1^2 - 235.28x_3^2.$$

The effect of formaldehyde on the percentage of water absorption in the composition of medical sponges is shown in Figure 3. The analysis revealed one type of dependence of the percentage of water absorption on the amount of formaldehyde because when the amount of formaldehyde increases to 0.075%, the percentage of water absorption increases and a further increase in its amount to 0.115% leads to a negative result.

The adequacy of the model describing the influence of the studied factors on the percentage of water absorption was

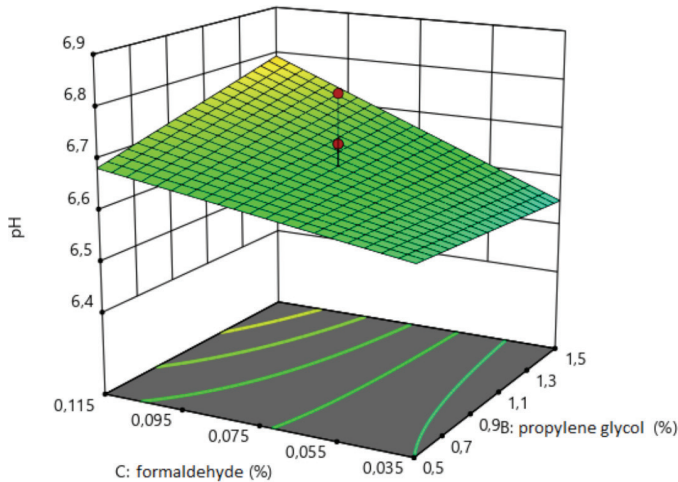


Figure 2. Surface response plot for the pH of sponges during degradation.

tested using the *F*-test. As seen in Table 6, the model *F*-value of 6.42 implies that it is significant. There is only a 0.38% chance that an *F*-value this large could occur due to the noise. *p*-values less than 0.0500 indicate that model terms are significant.

The relationship between the studied factors and the degree of degradation of medical sponges, after excluding insignificant coefficients, is described by the following regression equation:

$$y_3 = 48.52 - 19.82x_1 - 2.23x_3 + 3.24x_1^2 - 2.41x_3^2.$$

As the values of the coefficients show, the most significant factors influencing the percentage of degradation were factors  $x_1$ , the amount of medical gelatin, and  $x_3$ , the amount of formaldehyde. The coefficients  $b_2$ ,  $b_{12}$ ,  $b_{13}$ ,  $b_{14}$ ,  $b_{23}$ , and  $b_{22}$  were statistically insignificant in the regression equation. According to the data in Table 6, the Model *F*-value of 43.06 implies that it is significant. There is only a 0.01% chance that an *F*-value this large could occur due to the noise. *p*-values less than 0.0500 indicate that model terms are significant.

The influence of the amount of medical gelatin and formaldehyde on the degree of *in vitro* degradation of medical sponges is shown in Figure 4. The analysis revealed the types of the dependence of the degree of degradation of sponges on the studied factors ( $x_1$ ;  $x_3$ ), which indicate that, with increasing gelatin to 3%, the degree of degradation decreases, and therefore, the sponges will not dissolve during a long time. The concentration of medical gelatin directly affects the degree of degradation, namely from 26% to 96%. As for the effect of formaldehyde, it to a lesser extent than medical gelatin affects the degree of degradation, but with an increase in the amount of formaldehyde to 0.115%, the degree of degradation decreases.

The influence of factors on the appearance ( $y_4$ ) of developed sponges was evaluated on a 5-point scale, as in the analysis of variance. The regression equation has the following form:

$$y_4 = 4.8.$$

Thus, we can characterize that  $F_{exp}$  adequately reproduces the model; none of the studied factors within the studied intervals does affect the appearance of the developed medical sponges.

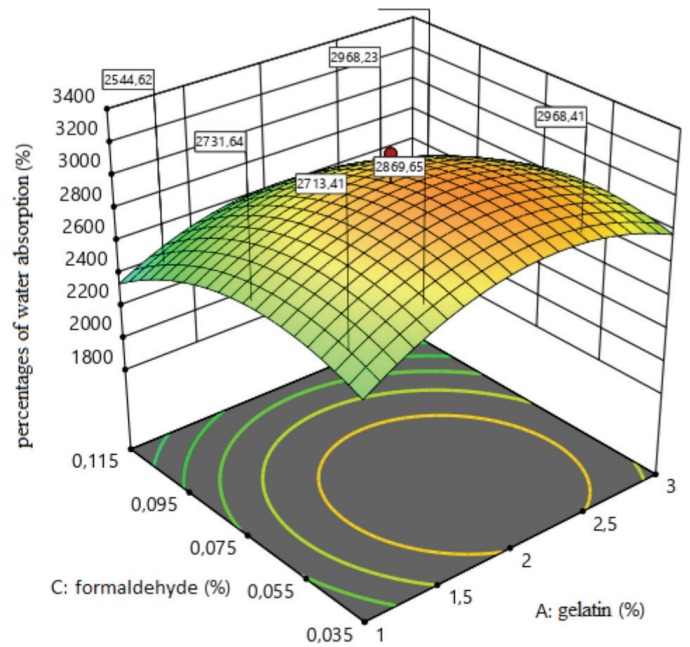


Figure 3. Surface response plot for the percentages of water absorption (%).

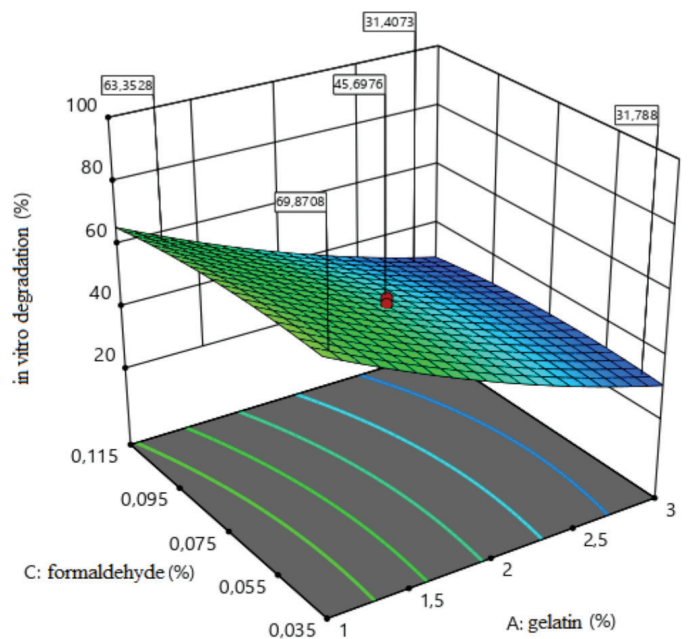


Figure 4. Surface response plot for the degree of *in vitro* degradation (%).

The relationship between the studied factors and the thickness of medical sponges, after excluding insignificant coefficients, is described by the following regression equation:

$$y_5 = 0.82 + 0.09x_3 + 0.07x_2x_3 - 0.08x_1^2 + 0.07x_3^2 \quad (F_{exp} = 0.90).$$

As the values of the coefficients show, the most significant factor influencing the thickness of the sponges is factor  $x_3$ , the amount of formaldehyde. The following coefficients were statistically insignificant in the regression equation:  $b_1$ ,  $b_2$ ,  $b_{12}$ ,

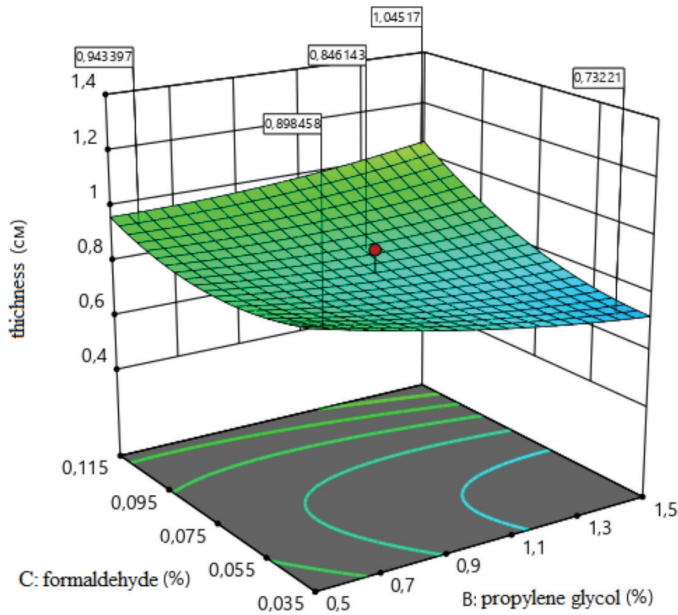


Figure 5. Surface response plot for the thickness of the sponges (cm).

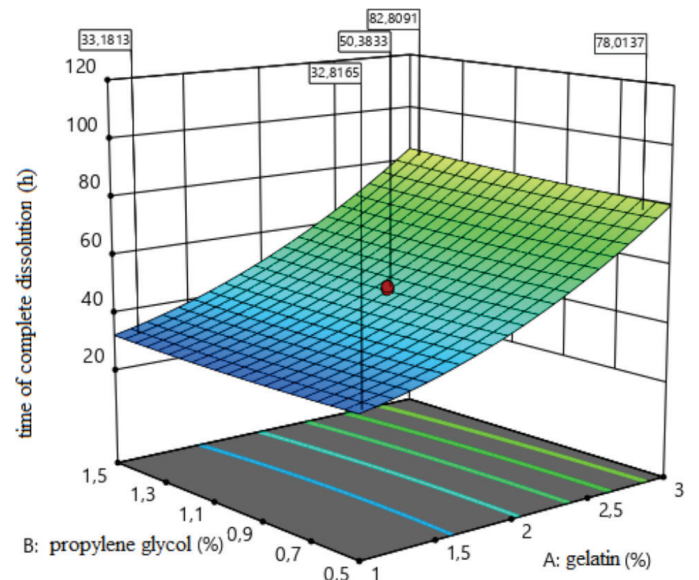


Figure 6. Surface response plot for the time of complete dissolution (hours).

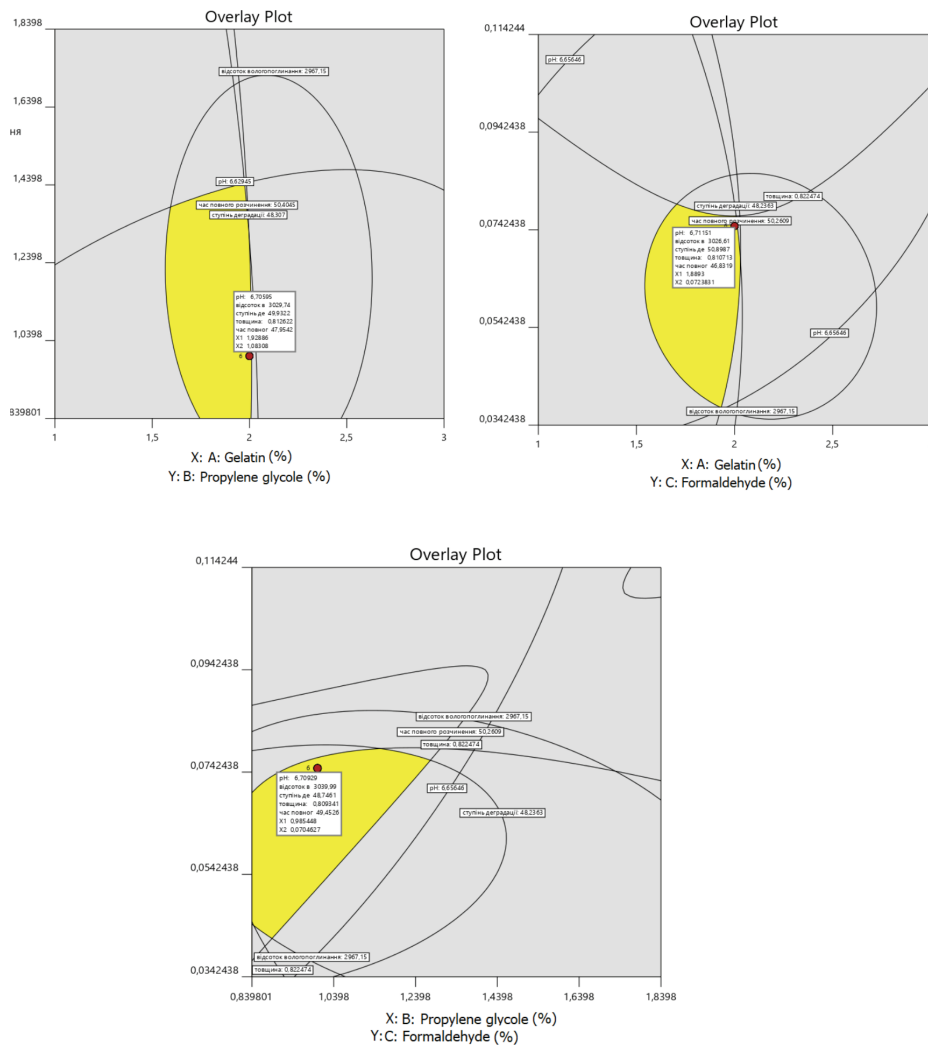


Figure 7. Optimum formulation of medical sponges.



$b_{13}$ ,  $b_{14}$ ,  $b_{23}$ , and  $b_{22}$ . The  $F$ -value model of 6.10 implies that it is significant (Table 6). There is only a 0.46% chance that an  $F$ -value this large could occur due to the noise.

The effect of formaldehyde on the thickness of medical sponges is shown in Figure 5. As a result of the analysis, the type of dependence of sponge thickness on the amount of formaldehyde was established, which indicates that, with an increasing amount of formaldehyde ( $x_3$ ) to the maximum value, the thickness of sponges increases. It leads to increased porosity and to the formation of sponges with larger sizes pores. Thus, the concentration of formaldehyde affects the thickness of medical sponges; namely with increasing concentration from 0.075% to 0.142%, the thickness of the sponges increases.

The relationship between the studied factors and the time of complete dissolution of medical sponges, after excluding insignificant coefficients, is described by the following regression equation:

$$y_6 = 49.53 + 25.06x_1 + 2.87x_1x_3 - 4.37x_2x_3 + 7.01x_1^2 + 3.30x_3^2.$$

The factor that most affects the time of complete dissolution is the amount of medical gelatin (factor  $x_1$ ). The coefficients  $b_2$ ,  $b_3$ ,  $b_{12}$ , and  $b_{22}$  were statistically insignificant in this regression equation. The model  $F$ -value of 118.61 implies that it is significant (Table 6). There is only a 0.01% chance that an  $F$ -value this large could occur due to the noise.

The effect of the amount of medical gelatin in the composition of medical sponges at the time of complete dissolution is shown in Figure 6. The analysis revealed the type of dependence of the time of complete dissolution of sponges on the content of factor  $x_1$ , which indicates that reducing the amount of medical gelatin to 0.32% accelerates the dissolution of sponges, and a further increase in the concentration causes slow, gradual dissolution.

Based on the obtained results of statistical processing of experimental data and the obtained regression equations to study the influence of factor levels on the characteristics of medical sponges, as well as based on pharmaco-technological quality indicators, the optimal composition was determined using Design-Expert 12.0.0 for the three factors studied.

The optimal composition of medical sponges was selected on the basis of the limitations of pharmaco-technological parameters established for dependent variables:  $y_1$  (6.6–6.9),  $y_2$  (2800%–3100%),  $y_3$  (45%–55%),  $y_5$  (0.8–0.9 cm), and  $y_6$  (46–50 hours) (Fig. 7).

Based on the obtained results, the optimal composition of medical sponges is proposed: the concentration of gelatin, 2.0%; propylene glycol, 1.0%; and formaldehyde, 0.075%.

## CONCLUSION

Using the concept of quality by design, the desired quality target product profile, critical quality attributes, risk assessment by failure mode effects analysis were analyzed. In order to determine the optimal composition of sponges and identify factors that may affect their quality characteristics, 20 experimental samples of medical sponges were developed and their pharmaco-technological parameters were studied. The experiment identified two main risks that affect quality, namely the duration of lyophilization and drying temperature. It was determined that the number of excipients affects the pharmaco-

technological parameters, so the most optimal is the concentration of gelatin at the “0” point, propylene glycol at the upper “+” point, and formaldehyde at the “0” point of the plan. The developed medical sponges are stable when stored at a temperature not exceeding 25°C in sterile packaging for 2 years.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

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