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The optimizing process of the blood coagulation powder composition with response surface methodology

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ABSTRACT

This study focuses on optimizing process of the blood coagulation powder composition with response surface methodology. For this purpose, optimizing the synthesis conditions of powder composition, the response surface methodology (RSM) was used by Box-Behnken design (BBD). The variables of this method were sodium alginate (A) and zinc oxide nanoparticles (B), prepared in combination with a fixed percentage of shell powder and measured for different reactions. results showed that according to the response surface methodology, which is the most optimal case, to introduce this method as a suitable suggestion for this kind of problem in the applications of vascular surgeries, burns, etc., and direct to future pre-clinic and clinical studies.

1. Introduction

Hemorrhagic shock continues to be the top cause of mortality on the battlefield and the second most frequent cause of death from trauma in the modern world, despite many medical advancements [1]. Uncontrolled bleeding is still one of the leading causes of preventable death after trauma and especially causes of burn trauma [2]. The issue is even more critical when complex injury patterns occur in connective anatomic regions (such as the neck, axilla, groin, and perineum) that contain many vascular structures. Also, blood loss following severe burns and autologous skin grafting in the donor area is a big challenge in burns and wound healing [3]. In all these conditions, local hemostatic agents can play a vital and efficient role in the control of threatening bleeding, which involves complex processes and limited capacity, so that in emergencies such as battlefields, hospitals, and surgery, when the hemostatic mechanism fails. The human body cannot effectively stop bleeding.

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Hemostatic materials are needed to save people's lives. Therefore, it is essential to use a substance that accelerates the hemostatic process [4]. Generally, an ideal hemostatic agent should have an immediate and consistent effect, be biocompatible, biodegradable, non-cytotoxic, and firmly adhere in a moist environment. When designing and engineering hemostatic materials, the three key considerations that should be considered are usability, durability, and cost [5,6]. Collagen [7], zeolite [8], gelatin [9], alginate [10], chitosan [11], cellulose [12], and cyanoacrylate are some of the hemostatic materials that have recently been developed for use at the bedside[13]. However, these materials' homeostatic effectiveness falls short of therapeutic demands [5,14,15]. Therefore, in the present research, significant efforts have been made to improve hemostatic materials with high efficiency. In addition, ease of use, durability, and cost are the main factors that should be considered in the design and engineering of hemostatic materials. Therefore, for blood coagulation products designing, used the natural product of sea shells with ease of access in the country. Shells play an essential role in blood coagulation due to containing 96% calcium carbonate, as well as adding biocompatible and non-toxic substances such as Niacinamide, gelatin, and zinc oxide; the role of these factors in accelerating the healing process of wounds and scars and the proliferation and migration of fibroblastic cells have been proven. Also, the way this powder works is that during the absorption of moisture by the blood-binding and wound-healing powder, a hydrogel-like environment is created, the calcium in the powder activates the coagulation cascade, and due to the vital and antibacterial elements; bacterial growth may be significantly reduced.

Optimizing a system, process, or production method's performance in order to get the most out of it is called optimization. The earlier techniques involved analyzing the impact of changes in only one element while holding the other variables constant. The "single-factor" method is the name given to this optimization technique [16]. This method's most significant flaw is the need for more testing and the failure to examine the interactions between variables [17]. Today, Bahina Sound is employed as an optimization strategy to eliminate these issues. The use of multivariate statistical techniques is done. The response surface methodology, or RMS for short, is the most well-known of the various techniques. Bax and colleagues first introduced it in 1957 [18]. The response surface method comprises statistical and mathematical modeling techniques. Moreover, when the solution is affected by several different variables, problem analysis is used. These techniques allow for estimating interactive effects and factor interactions in addition to the significant effects between components (common effects). The Box-Behnken style One of the most popular designs in the response surface method is the central composite design (CCD), often known as the BBD.

2. Experimental and theoretical methods

2.1. Materials and synthesis

In this work, seashells were gathered from the Caspian coast. In contrast, other materials, such as Gelatin, Sodium Alginate, EtOH, Niacinamide, and ZnO nanoparticles, were purchased from Sigma Aldrich.

Optimization was used to find the appropriate percentage of the desired composition of the powder. Optimization is the improvement of the performance of the system, process, or production method,

which is used to achieve maximum efficiency. Response surface method (RSM)[†] is a set of mathematical and statistical methods used for modeling, analyzing, and analyzing problems in which response is affected. In these methods, in addition to the main effects between factors, it is possible to estimate specific effects and interactions between factors (common effects). The central compound design (CCD)[‡] is the most common in the response surface method, which includes a rotating design (rotatable) in which the points are taken from the central point with a distance of 1 and 1 surface. The complete description of the process with three effective factors, which has a quadratic behavior, is expressed by the following equation:

Eq. (1).

$$Yj = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ü} X_{ü}^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j$$

Where X represents the variables, Y represents the desired answer, and b is the coefficients of the equation.

According to the response level methodology, the identified factors were selected as variables: sodium alginate (A) and zinc oxide nanoparticles (B), prepared in combination with a fixed percentage of shell powder and measured for different reactions. (Table 1)

Table 1. Symbols, codes, and actual values of independent variables

Factor	*Name*	*Units*	*Type*	*SubType*	*Min*	*Max*	*Coded Low*	*Coded High*	*Mean*	*SD.*
A	Gel	%	Numeric	Continuous	0.00	20.00	-1 ↔ 5.00	+1 ↔ 15.00	10.00	5.00
B	ZnO	%	Numeric	Continuous	0.00	4.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	1.00

Answered based on empirical observations and effectiveness missions of the developer; Coagulation time, and coagulated blood weight, symbolized as R1, R2, and R3, respectively (Table 2).

Table 2. Specified reactions in CCD methodology

Response	**Name**	**Units**	**Observation**	**Min**	**Max**	**Mean**	**SD.**	**Ratio**
R1	Time of coagulation	s	13.00	0	380	230.31	131.26	N/A
R2	Weight of coagulation	g	13.00	0	1.8211	1.11	0.40	N/A
R3	Inhibation zone (AB)	mm	13.00	0	8	5.69	1.83	N/A

[†] Response surface method
[‡] central compound design

For each of the formulations presented in the table, CCD first cleaned a specified amount of shell powder, and then zinc oxide and gel were added to it. The samples were named Std-X, where X is the row number of the sample in Table 3.

Table 3. values of independent variables and experimental and predicted values of responses entered in CCD

Std	Run	Space Type	Gel%	ZnO%	Answer 1 coagulation time	Answer 2 Coagulated blood weight	Answer 3 Inhibition zone diameter
1	11	Factorial	5	1	310	1.0552	4.5
2	10	Factorial	15	1	200	1.2058	4
3	6	Factorial	5	3	355	1.0797	6.5
4	4	Factorial	15	3	300	1.3292	6.55
5	3	Axial	0	2	400	0.9453	6
6	9	Axial	20	2	190	1.2587	6
7	5	Axial	10	0	230	1.0798	0
8	12	Axial	10	4	330	1.1898	7.9
9	13	Center	10	2	0	0	7
10	2	Center	10	2	259	1.2959	6
11	7	Center	10	2	300	1.0128	5.7
12	1	Center	10	2	255	1.2211	6
13	8	Center	10	2	295	1.0447	5.7

To evaluate the factors based on blood coagulation time response and coagulated blood weight, the powders were determined in the numbered falcons according to the CCD formula, and then the percentage of the composition of the powder was poured into these tubes, then into each of the tubes. 1.5 cc of a single human blood sample was poured into the tubes simultaneously, and the coagulation time was recorded.

After coagulation, liquids with lower rheology were removed from each tube by an angle of 60 degrees, and the remaining materials were weighed and reported to the CCD. The results of the ANOVA section reported in Table 4 show that the p-value of most coefficients has good accuracy.

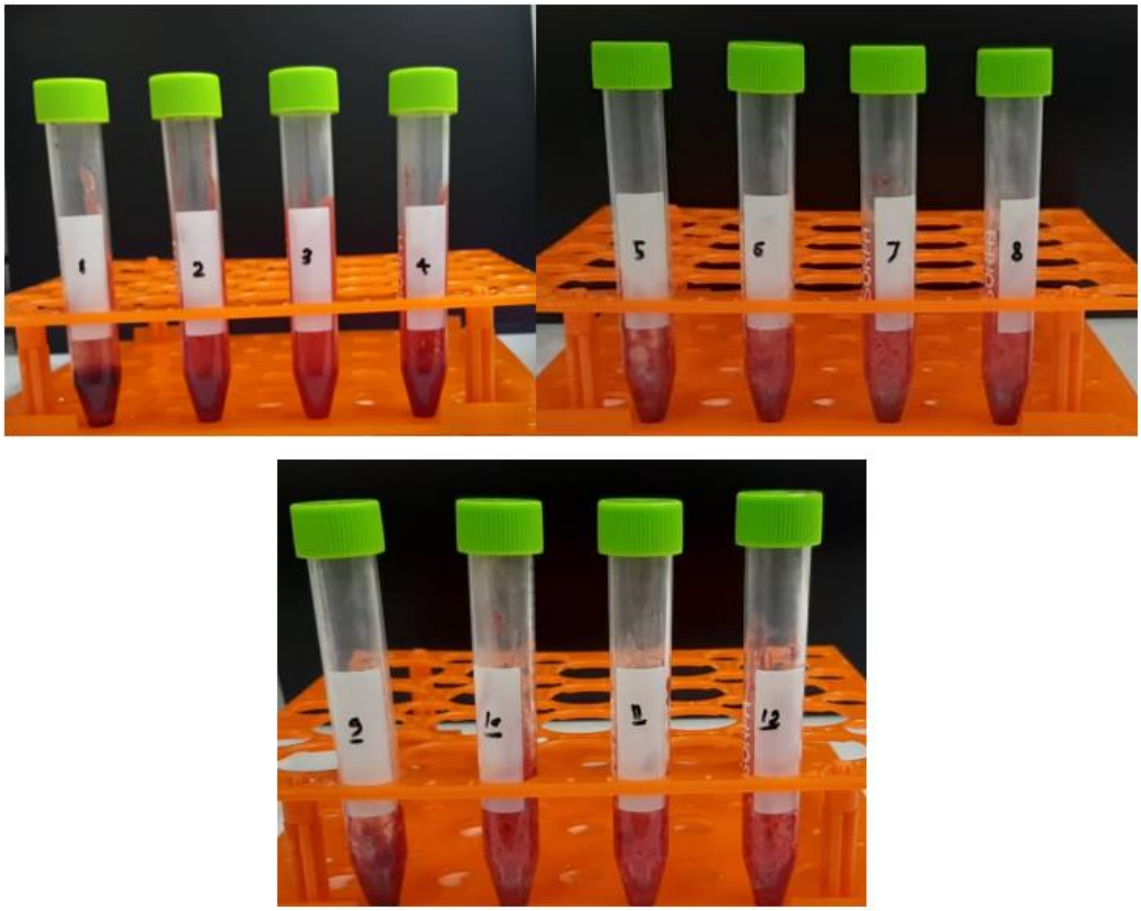

Figure 1. Powder made according to the CCD formulation in human blood samples to optimize for the best response of the hematopoiesis

3. Results and discussion

The results of the ANOVA analysis showed that the data error of this research was small and the coefficient of determination (R2) of the proposed models for the responses was higher than 0.9, and they have a normal distribution (Chart 2) because they are around the specified line, the frequency, and the variance are different. Change is not a software issue.

Table 4. p-value evaluation and accuracy of the proposed model for the responses

Response 1: Time of coagulation				***Response 2: Weight of coagulation***				***Response 3: Disk diffusion***			
Source	F-value	p-value		Source	F-value	p-value		Source	F-value	p-value	
Model	35.06	< 0.0001	significant	Model	6.77	0.0161	significant	Model	24.25	0.0007	significant
A-Gel	76.54	< 0.0001		A-Gel	12	0.0071		A-Gel	0.0507	0.8293	
B-ZnO	26.62	0.0009		B-ZnO	1.54	0.246		B-ZnO	103.75	< 0.0001	
AB	2.03	0.1921						AB	0.2273	0.6504	
								A^2	0.0902	0.7741	
								B^2	14.47	0.0089	

To set up the predictive equations, the mentioned answers were expressed based on ANOVA logic in equations 2, 3, and 4, which are based on actual values:

Eq. (2).

$$R_1 = 380.333 + 15.25 \times A + 1.25 \times B + 2.75 \times AB \quad \textbf{(2)}$$

Eq. (3).

$$R_2 = 0.9107 + 0.017115 \times A + 0.0306583 \times B \quad \textbf{(3)}$$

Eq. (4).

$$R_3 = 1.325 + (-0.0925) \times A + 3.32083 \times B + 0.0275 \times AB + 0.0015 \times A^2 + (-0.475) \times B^2 \quad \textbf{(4)}$$

This topic of two-dimensional and three-dimensional graphs of the responses to the variables is shown in figures 2 to 4. The more these images move toward yellow and even red colors, the more the answer value increases, and the closer it gets to the blue color, the decreasing effect of the variables on the answer.

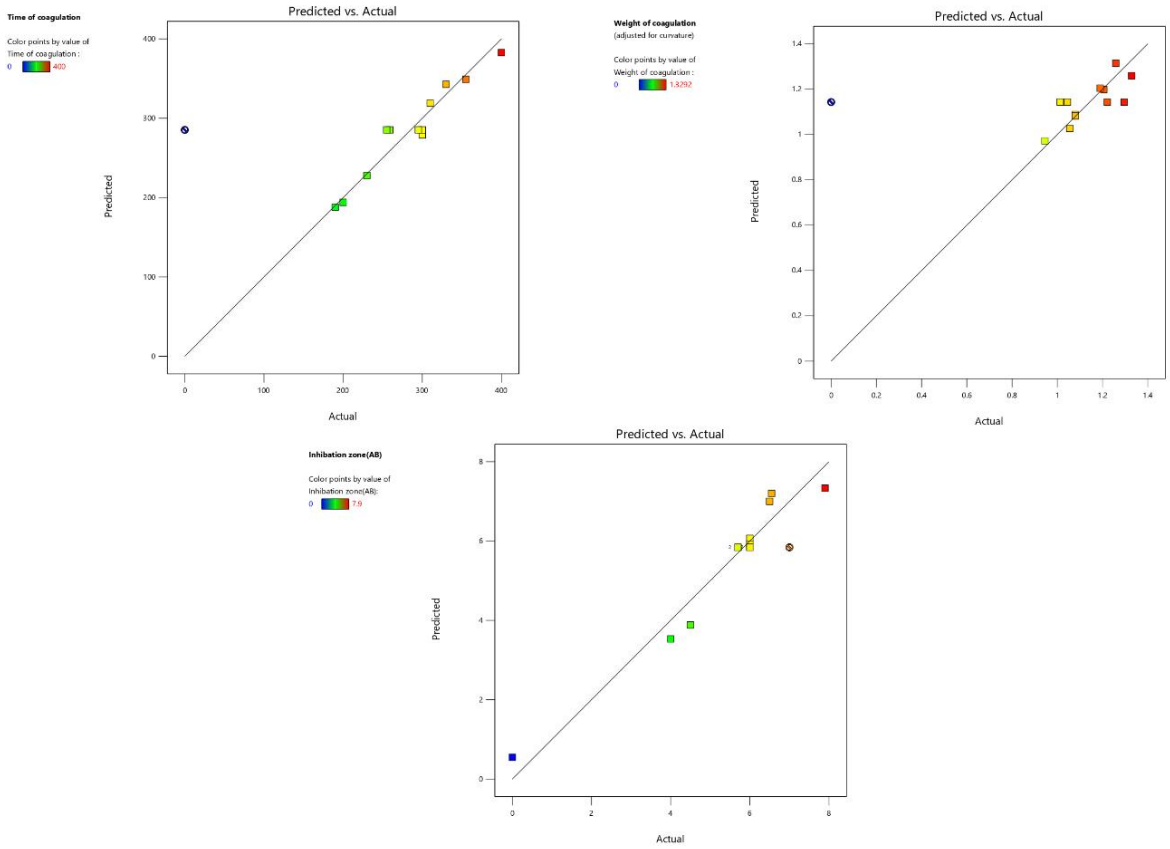

Figure 2. Normal distribution diagram of responses

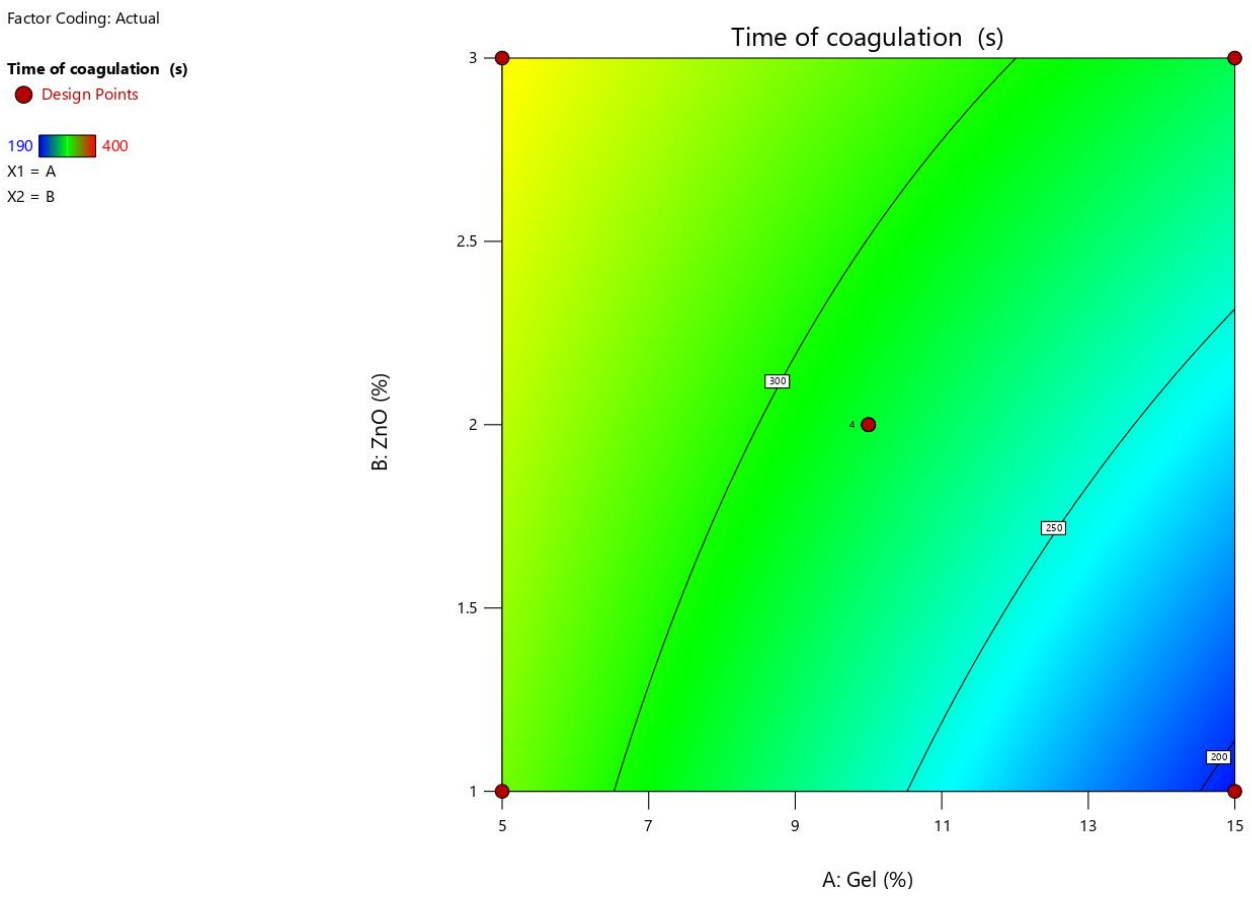

Figure 3. Two-dimensional diagram of the first response (blood coagulation time) based on changes in gel and zinc oxide factors

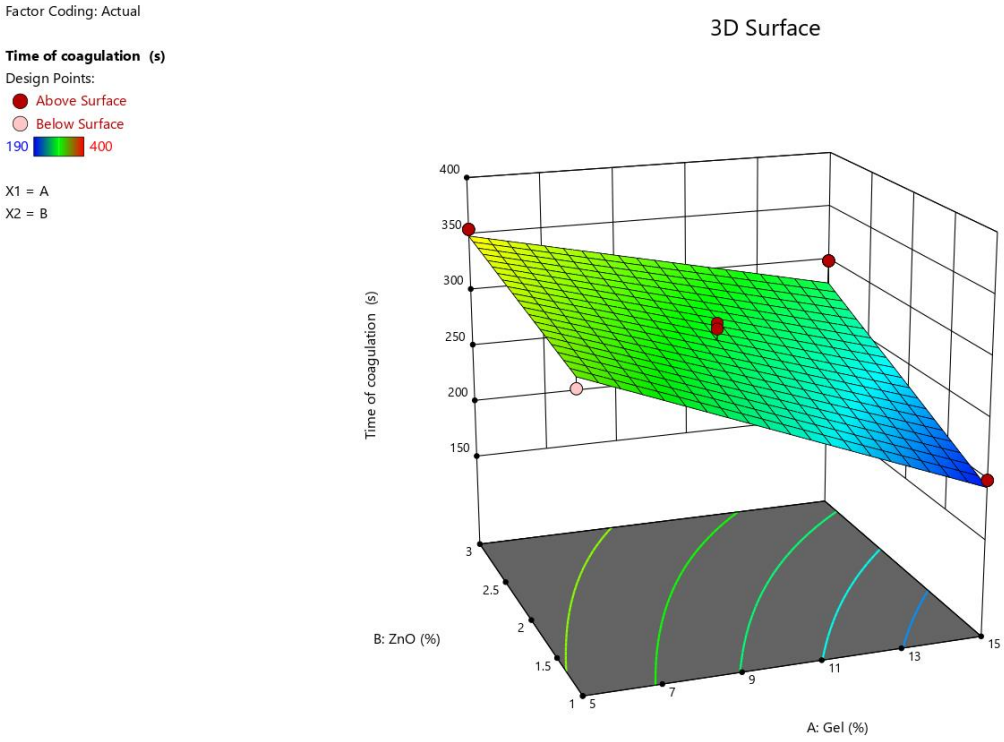

Figure 4. 3D surface plot of the first response (blood coagulation time) based on changes in gel and zinc oxide factors

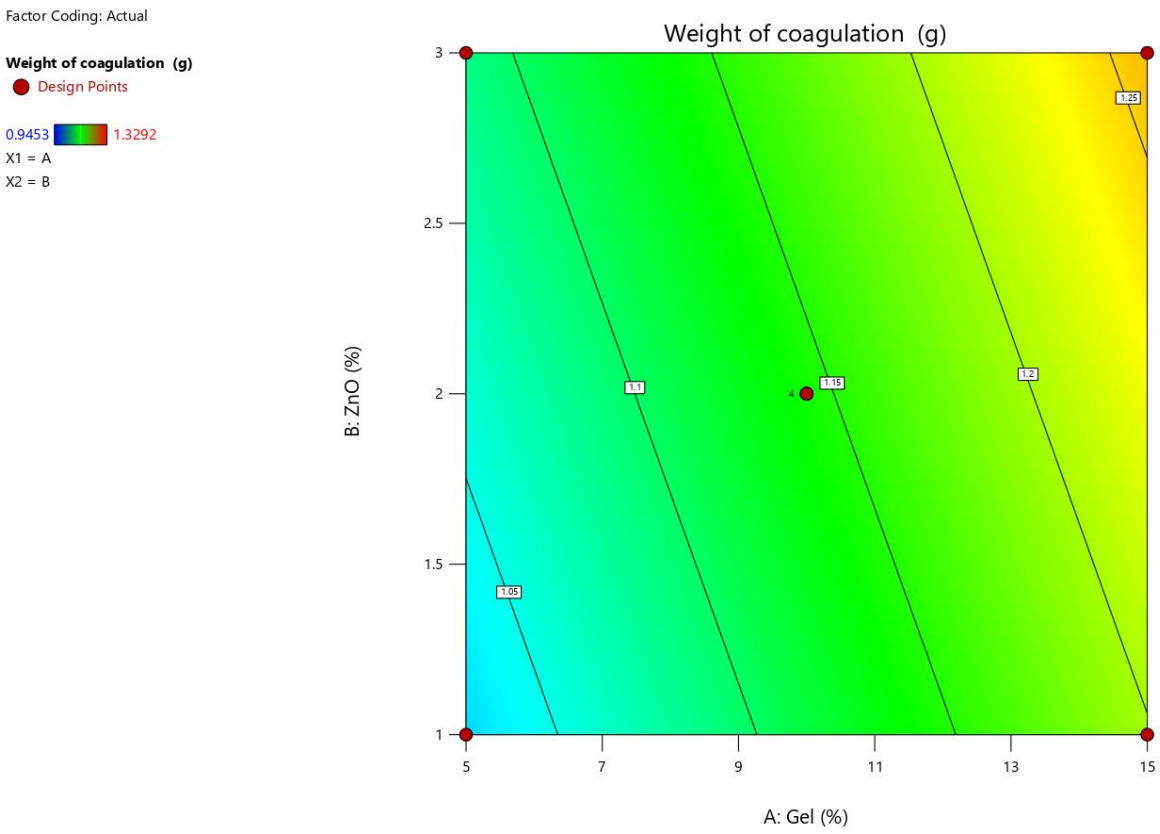

Figure 5. Two-dimensional diagram of the second response (weight of coagulated blood) based on changes in gel and zinc oxide factors

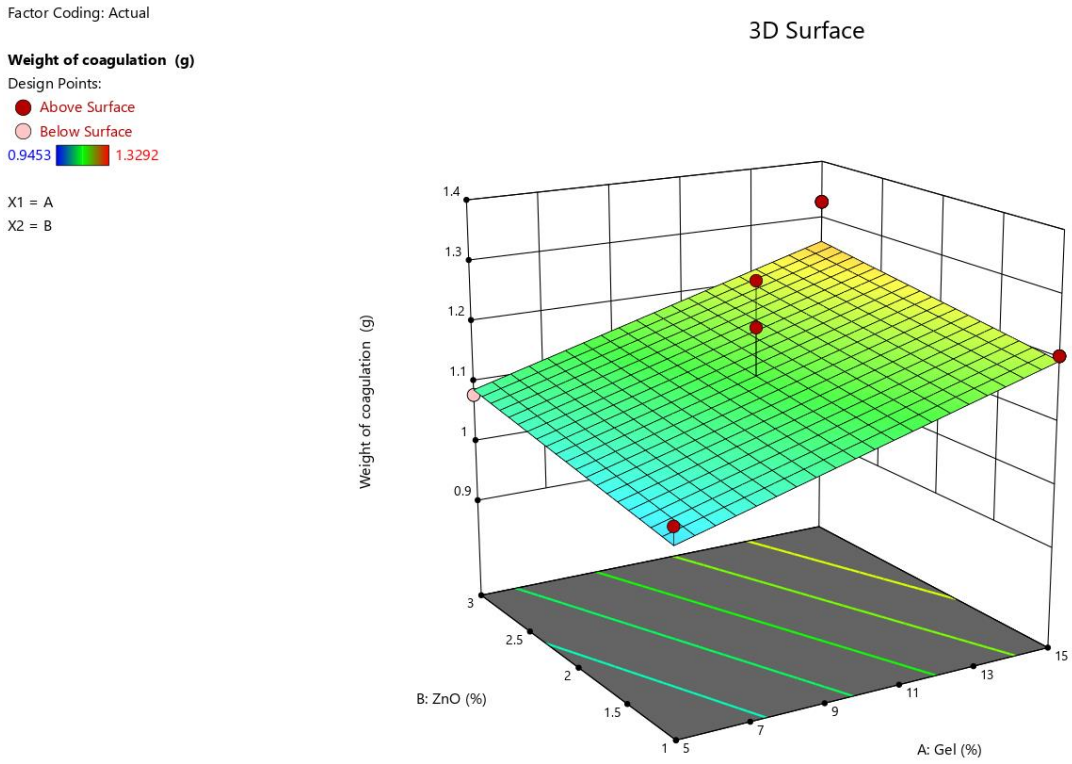

Figure 6. 3D surface plot of the second response (coagulation weight) based on gel and zinc oxide factors changes.

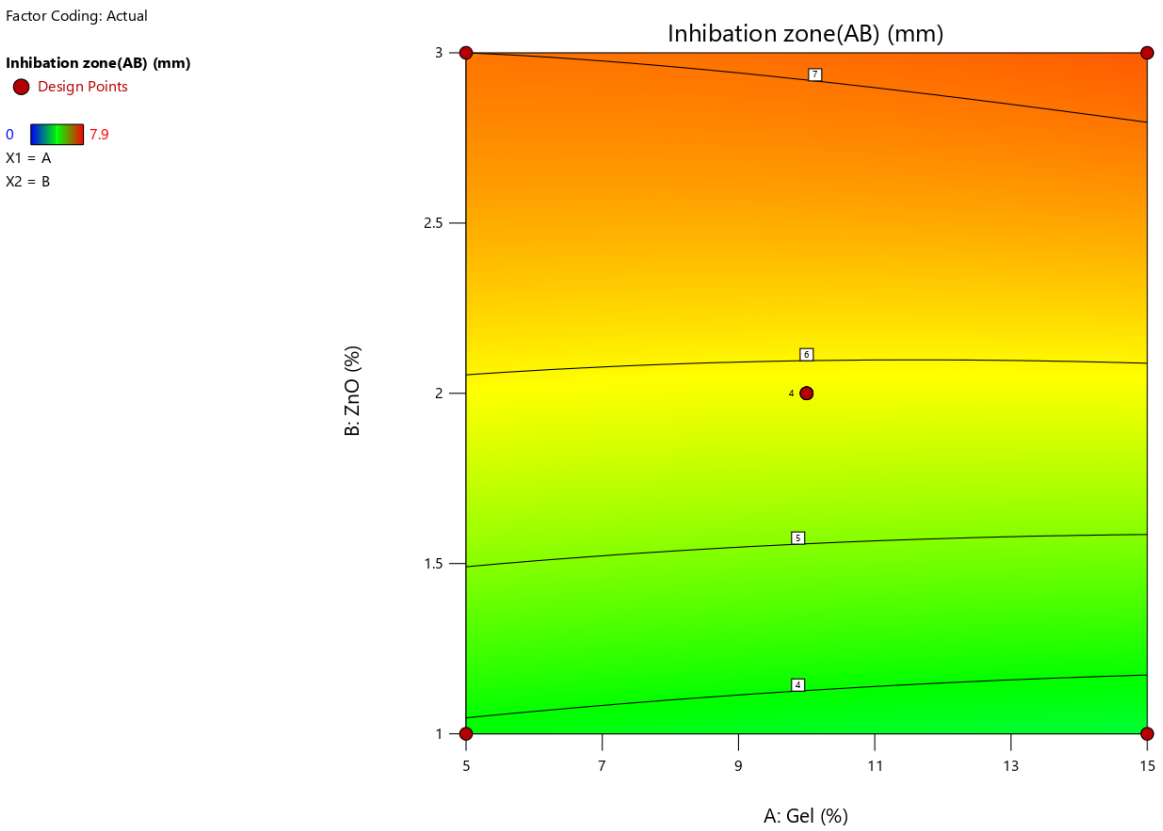

Figure 7. Two-dimensional diagram of the first response (diameter of inhibition formation) based on gel and zinc oxide factors changes.

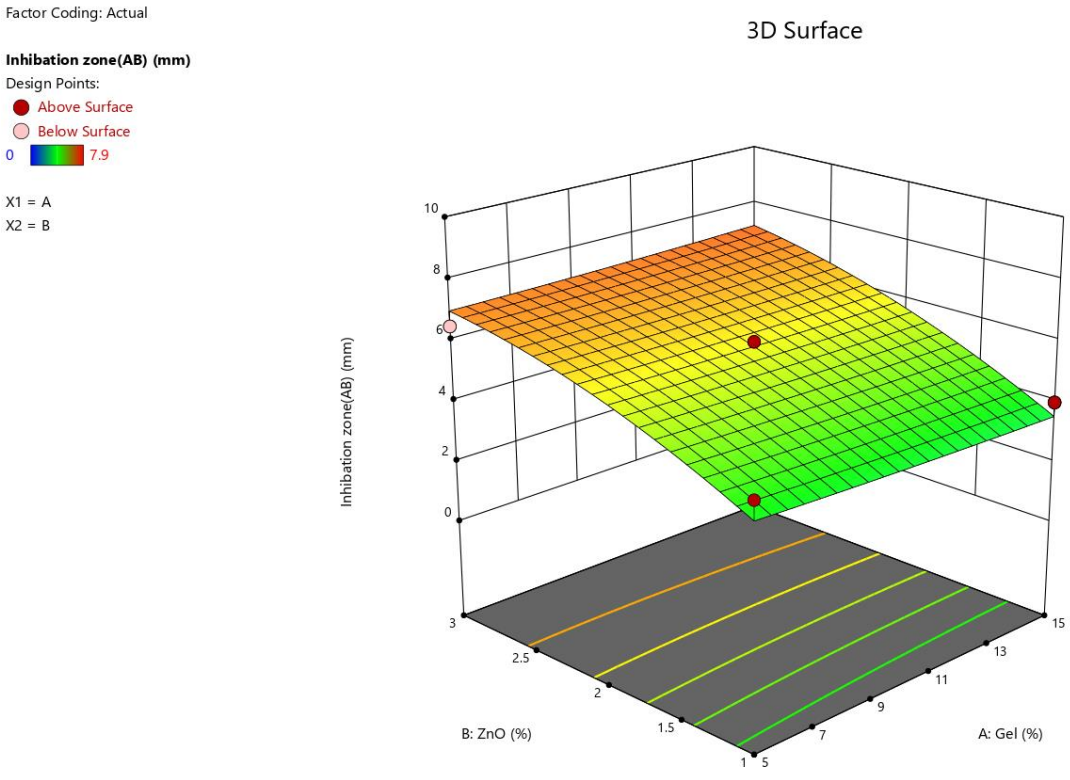

Figure 8. Two-dimensional diagram of the first response (diameter of inhibition formation) based on gel and zinc oxide factors changes.

In order to post the analysis for the final optimization of the powder in the setup using the minimization of the zinc oxide factors as well as the blood coagulation time and the maximization of the diameter of the inhibition Zone (antibacterial) specified in table 5.

Table 5. optimization setup values

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Gel	is in range	5	10	1	1	3
B:ZnO	is in range	1	3	1	1	3
Time of coagulation	minimize	190	400	1	1	3
Weight of coagulation	none	0.9453	1.3292	1	1	3
Inhibition zone(AB)	maximize	0	7.9	1	1	3

With the number of 8 outputs of goals or proposed simulation solutions specified in Table 6, it was found that the sample with the highest matching level is specified in row one.

Table 6. Suggested simulation optimization values

Number	Gel	ZnO	Time of coagulation	Weight of coagulation	Inhibition zone (AB)	Desirability	
1	10.000	2.132	289.122	1.147	6.057	0.636	Selected
2	10.000	2.138	289.310	1.147	6.067	0.636	
3	10.000	2.147	289.572	1.148	6.081	0.636	
4	10.000	2.114	288.625	1.147	6.030	0.636	
5	10.000	2.152	289.699	1.148	6.088	0.636	
6	10.000	2.167	290.139	1.148	6.112	0.636	
7	10.000	2.247	292.428	1.151	6.231	0.636	
8	10.000	2.000	285.334	1.143	5.842	0.635	

By repeating the experiment with the values suggested by the model, we reached the predicted answers, and these values are the most optimal percentage of the composition for the values of this product.

Table 7. Repetition of tests according to the suggested values of the model

Time of coagulation	*Weight of coagulation*	*Inhibition zone (AB)*
293	1.12	6.1
300	1.16	6.14
320	1.15	6

In Table 8, the statistical quality control results specify the optimal value with the final test, which confirms this optimization.

Table 8. Statistical results confirming the optimal value with the final test Confidence = 95%

Solution 1 of 8 Response	Predicted Mean	Predicted Median	Observed	SD.	n	SE Pred	95% PI low	Mean	95% PI high
Time of coagulation	289.114	289.114		19.3032	3	12.4817	260.331	304.333	317.897
Weight of coagulation	1.1472	1.1472		0.0855847	3	0.0553401	1.02201	1.14333	1.27239
Inhibation zone(AB)	6.05644	6.05644		0.576749	3	0.424199	5.01846	6.08	7.09441

This study aimed to manufacture and optimize the blood coagulation powder based on biological waste to coagulate the desired blood with the least amount of powder in the shortest possible time, which can be used in the widely used fields of biomedical sciences. In this study, we showed that according to the Response surface methodology, which is the most optimal case, to introduce this method as a suitable suggestion for this kind of problem in the applications of vascular surgeries, burns, etc., and direct to future pre-clinic and clinical studies.

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