

# Development of foaming vaginal tablets with clotrimazole. Part I. Optimization of the formulation using design of experiments approach

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## ABSTRACT

**Objectives.** The aim of this study was to optimize the formulation of certain immediate release foaming vaginal tablets, with fast vaginal disintegration (part I), following their time stability (part II).

**Material and methods.** Modde optimizing software and a Box-Bhenken experimental design was used to establish an optimal formula that would provide maximum "in vitro" behaviour advantages, including stability during preservation; and the improvement of the foaming effect. There were prepared 15 formulations of foaming vaginal tablets in order to choose the optimal formula.

**Outcomes.** The main factor, with influence over all the studied parameters, is the percentage of the effervescent mixture in the formulation. The excess of citric acid has a main role in the assurance of local pH; in the improvement of the flowing properties of the powder mixtures before compression; and in the improvement of the effervescent process, having, on the other hand, an effect of decreasing the stability of tablets and increasing their humidity adsorption. Sodium lauryl sulphate, at the chosen concentration levels (0.5-1.5%), did not influence the dependent variables, it only produced a stronger resistance of the foam. The optimal formula of a foaming vaginal tablet with an optimal stability during preservation must contain 0.5% sodium lauryl sulphate, while the effervescent mixture must contain a percentage of 24% excess of citric acid, and it must represent 44% of the total amount of excipients.

**Conclusions.** The experimental determinations of the optimal formula were close to the theoretical values predicted by the program, this certifying the validity of the optimization and the conclusion draw after analysis the experimental design.

**Keywords:** foaming tablets, vaginal tablets, clotrimazole, experimental design, Box-Bhenken

## INTRODUCTION

Foaming vaginal pharmaceutical dosage forms allow the uniform spread of the doses on the whole mucosal surface of the vagina, and assures a rapid disintegration of the

pharmaceutical form, associated by a lack of flowing from the site of application (1,2). Being conceived for a mucosal route, these tablets must carry out some standards (2,3) concerning especially their

form which has to be conformed to an easily applicable device without producing vaginal lesions; and concerning their dissolution capacity, which has to assure the dissolution in a very low liquid

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volume (~2 ml), associated with a diffusion and a spread between all the vaginal mucosal velocities. Another problem is how to assure their stability in time.

Concerning the medicinal substance, we used clotrimazole as model drug, a well-tolerated (1,6,7) imidazole derivate with clinically proved local antimycotic, antibacterial, and antitrychomoniazic effect (4,5) The class of antimycotics was chosen regarding the very prevalent mycotic vaginal pathology. A number of 75% of women suffer during their life one acute vaginal candidose, 40-50% of women suffer 2-3 episodes; a number of 10% of sexually mature women have suffered from vaginal candidose during their premenstrual period. 10-20% of contaminated women suffer from a chronic form of candidose with 4 acute episodes during a year, and a percent of 20% of women are asymptomatic carriers of vaginal *Candida albicans* (6-15).

The aim of this study was to optimize the formulation of immediate release foaming vaginal tablet formulation with fast vaginal disintegration using a Box-Bhenken experimental design and Modde optimizing software. There were analysed 15 responses, concerning the properties of the powder mixtures, the properties of the tablets, considering that the studied responses are the most important characteristics of a foaming vaginal tablet.

## MATERIALS AND METHODS

### Materials

Clotrimazole (Beijing Double Crane Pharmaceutical Co. Ltd-China); citric acid (Chimopar); sodium

hydrogen carbonate (Chimopar); anhydrous sodium carbonate (Chimopar); sodium laurylsulphate (Loba Feinchemie-Austria); Ludipress Direct Compression Excipient (BASF-Germany); Methocel E15-LV-EP, Low Viscosity (Colorcon LTD U.K); Aerosil (S&D Chemicals UK); magnesium stearate (Union Derivan-Spain); anhydrous sodium sulphate (Chimopar)

### Apparatus

Analytical balance Analytical Plus (Ohaus SUA); Sieve analyzer set DIN (VEB MLW, Germany); MTM Owen (Hungary); Erweka powder mixer with infinite movement RM (Germany); Erweka SVM devices for powder density studies (Germany); Erweka GTD devices (Germany) for powder flowing time study; Exocentric tablet press Korsch EK-0 Germany; Monsanto device (Italy), for tablet's mechanical resistance study; Erweka apparatus for friability study; a special device to study the volume and the resistance of the generated foam; Humidity Balance Ohaus MB 45 (SUA); Mettler-Toledo Quatro Type MP 225 pH-meter, Switzerland; Ultra sounds bath Ultrasonics-Transsonic 700 Elma; HPLC Agilent 1100 with UV detection; column HPLC Zorbax – SB-C8.

### Software and experimental design

To perform the study, a Box-Bhenken experimental design  $3^3$  with three factors and three levels was used. Experimental design, coefficient calculation, statistic parameters calculation and

evaluation of the quality of fitting were performed with Modde 12.0 (Sartorius Stedim Data Analytics AB, Sweden). The independent variables (formulation factors considered the most influents in the case of foaming vaginal tablets) and the levels of variation are presented in table 1.

The dependent variables are presented in table 2.

### Methods

#### Formulation and preparation of the foaming vaginal tablets

After consulting the literature (16-20) we used the following ingredients to prepare foaming vaginal tablets: effervescent mixture (15-65%) with alkaline component sodium hydrogen carbonate associated with anhydrous sodium carbonate (10% from the alkaline amount for a better stability) and as acid component citric acid (in excess to obtain an optimum pH – between 4.2 and 6 after foaming), clotrimazole 10% for a 100 mg dose in an 1g weight tablet; foaming agent sodium laurylsulphate (0.5-1.5%); Ludipress as diluent; Methocel E15 2% as binder, magnesium stearate 1% as lubricant and Aerosil 1% as foam resistance enhancer.

The foaming vaginal tablets were obtained by direct compression in normal thermo-hygro-metrical conditions, using a Korsch EK 0 excentric press, equipped with droplet shaped die set punch. The powders were individually dried until a 0.5% humidity before

**TABLE 1. Independent variables (formulation factors)**

Formulation variables	Symbol	Levels		
		-1	0	+1
Sodium laurylsulphate percent	$X_1$	0.5	1	1.5
Effervescent mixture percent	$X_2$	25	45	65
Citric acid excess (%)	$X_3$	5	15	25

**TABLE 2. The matrix of the experimental design**

Exp. Name	Run Order	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>
N1	7	0.5	25	15
N2	1	1.5	25	15
N3	10	0.5	65	15
N4	13	1.5	65	15
N5	11	0.5	45	5
N6	12	1.5	45	5
N7	3	0.5	45	25
N8	8	1.5	45	25
N9	5	1	25	5
N10	2	1	65	5
N11	4	1	25	25
N12	6	1	65	25
N13	15	1	45	15
N14	14	1	45	15
N15	9	1	45	15

X<sub>1</sub> – sodium laurylsulphate percent, X<sub>2</sub> – effervescent mixture percent, X<sub>3</sub> – percent of citric acid excess in the effervescent mixture

blending. There were obtained 15 formulations of droplet shaped tablets with 2.5 cm length; 1.2 cm maximum diameter and weights between 0.897 and 1.064 grams. The resulted tablets followed the pharmacopeial requirements concerning the weight and content uniformity. The preparation and stability studies in normal thermohygommetrical conditions can assure us that the optimal formulation obtained will show maximal resistance to the environmental conditions during production and preservation.

#### Determination of the dependent variables (responses)

In order to obtain the optimal formula, we studied a number of 29 responses, these being considered by us as the most important characteristics of a foaming vaginal tablet. There were determined: flowability indexes of powder mixtures (flowing time, Carr index and Hausner ratio) using Erweka apparatus; mechanical resistance of the tablets using Monsanto apparatus; friability of the tablets using Erweka friability apparatus; the volume and resistance of the foam resulted after disintegration

in 2 ml simulated vaginal fluid (SVF) at 37°C, using a device described by the literature (2) composed by a calibrated glass test tube suspended from a ring stand in a constant temperature bath 37°C and a 1g rubber cylinder suspended by a thread, that could be gently lowered onto the surface of the foam, when the foam attained the maximal possible volume and we can determine the time until the foam will be collapsed by the 1 g weight ; foaming time of the tablets in 5 ml SVF at 37° C; the mass of carbon dioxide loosed during 20 minute foaming in 30 ml SVF at 37°C, using a gravimetric method described by the literature (21), the pH generated in the following mediums (30 ml at 37°C): distilled water, SVF pH 4.2; acetate buffer pH 5.5, to determine the pH generated by the tablet itself, but also to observe if the foaming vaginal tablet formulations have a capacity to change an eventually local pH buffer effect. An important indicator of the stability was the humidity of the powder mixtures and of the tablets, determined by using a humidity Ohaus MB 45 (USA) balance.

**TABLE 3. Dependent variables (responses) determined after preparation**

Number	Responses	Symbols
1	Carr Index for powder mixtures (%)	$Y_1$
2	Hausner Ratio for powder mixtures	$Y_2$
3	Flowing time of the powder mixtures (min)	$Y_3$
4	Mechanical resistance of the tablets (kg)	$Y_4$
5	Friability of the tablets (%)	$Y_5$
6	Foaming time (min)	$Y_6$
7	Maximal volume of the foam (ml)	$Y_7$
8	Resistance of the foam (min)	$Y_8$
9	Weight of CO <sub>2</sub> loosed by 20 minute foaming (g)	$Y_9$
10	pH generated in 30 ml distilled water	$Y_{10}$
11	pH generated in 30 ml (FVS) pH 4.2	$Y_{11}$
12	pH generated in 30 ml acetate buffer, pH 5.5	$Y_{12}$

Most of the determinations were repeated after 1 and 3 months on the tablets kept in normal thermo-hygrometrical conditions in the presence of anhydrous sodium sulphate, to conclude the stability of the formulations. The described responses and the symbols used for interpretation of the data are represented in table 3.

## RESULTS AND DISCUSSIONS

Results for the dependent variables (responses) determination  
The results obtained for the dependent variables

determinations are represented in table 4.

The results showed that there are great differences between the formulations, concerning the friability, a parameter for which we can discover excessively great values for some formulations (ex. N2 6.3%). Concerning the mechanical resistance of the tablets, the values obtained are distributed on a large scale (from 5.5 kg to 14 kg). There were obtained very good values for flowing time of powder mixtures and for pH responses for almost all the formulations. The time of

foaming showed good values, the greatest part of the results being below 10 minutes (the time established for the disintegration of a foaming vaginal tablet) (2). The volume and resistance of the foam are responses which shoes a great dependence on the formulation factors (independent variables).

As we can see, there are little differences between the humidity of powder mixtures before compression and the humidity of the tablets resulted, but the initial humidity of the powders was in all cases below 0,5% after drying. The conclusion is that during the compression process a humidity absorption phenomenon takes place. The results showed that the amount of humidity absorbed is independent on the formulation and depends on the environmental thermo-hygrometrical conditions (22).

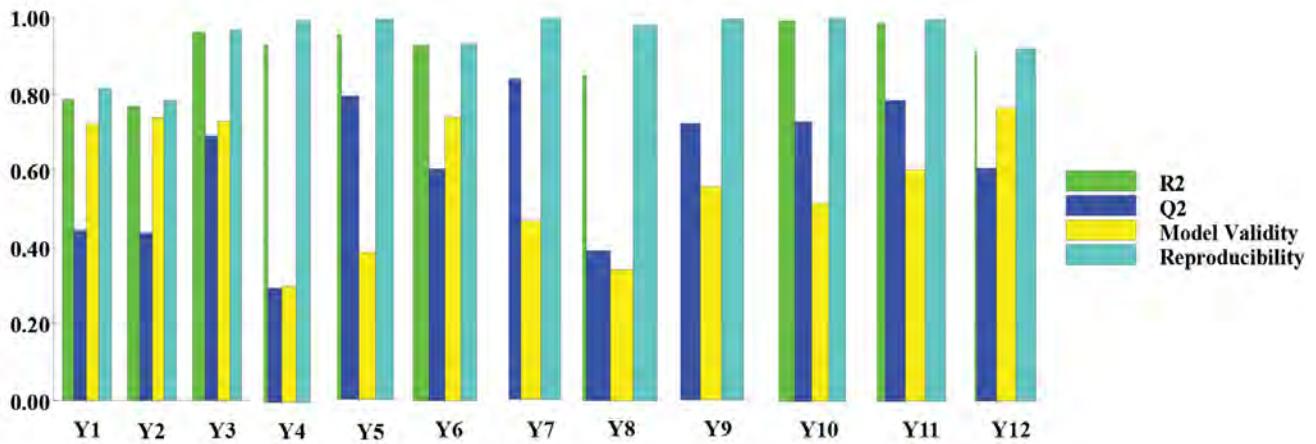
### *Fitting of the experimental data with the model*

Fitting of experimental data was realized with the Modde software, using the Partial Least Squares (PLS)

**TABLE 4. Matrix of the responses obtained for the determinations immediate after preparation**

Exp Name	$Y_1$	$Y_2$	$Y_3$	$Y_4$	$Y_5$	$Y_6$	$Y_7$	$Y_8$	$Y_9$	$Y_{10}$	$Y_{11}$	$Y_{12}$
N1	17.65	1.21	40	14	3.5	4.5	8	1.5	0.23	5.76	5.10	5.49
N2	11.11	1.13	44	12	6.3	13	5	0.083	0.221	5.85	5.00	5.5
N3	16.13	1.19	103	9	0.82	4	25	5	0.417	4.99	4.59	5.37
N4	20.63	1.26	92	7.5	0.91	10	25	6.5	0.4	5.02	4.52	5.39
N5	18.18	1.22	78	7.5	0.95	2.5	21	7.5	0.26	5.91	5.2	5.56
N6	21.21	1.27	69	8	0.83	7.5	16.5	25	0.257	6.00	5.23	5.77
N7	12.5	1.14	83	9	0.77	3.5	12	7	0.3	4.8	4.45	5.23
N8	12.5	1.14	65	5.5	0.98	7.5	12	20	0.28	4.85	4.49	5.13
N9	12.12	1.14	48	14	5.5	7	8.5	0.33	0.2	6.46	5.26	5.45
N10	20	1.25	110	9	0.47	6.7	28	5.5	0.355	5.8	4.97	5.45
N11	9.68	1.11	52	9.5	6.8	12	7	0.33	0.241	5.35	4.82	5.09
N12	20	1.25	97	5.5	0.45	7	23	6	0.425	4.24	4.22	5.2
N13	16.67	1.2	75	10	0.52	5.7	15	13.3	0.279	5.24	4.69	5.2
N14	20	1.25	79	9.5	0.83	6.5	15.5	15.5	0.27	5.31	4.75	5.3
N15	18.18	1.22	71	10	0.92	4.9	14.5	15	0.275	5.28	4.72	5.28

$Y_1$  – Carr Index (%),  $Y_2$  – Hausner Ratio,  $Y_3$  – flowing time (min),  $Y_4$  – mechanical resistance (kg),  $Y_5$  – friability (%),  $Y_6$  – foaming time (min),  $Y_7$  – foam maximal volume (ml),  $Y_8$  – resistance of the foam (min before collapse),  $Y_9$  – weight of loosed CO<sub>2</sub> (g),  $Y_{10}$  – pH water,  $Y_{11}$  – pH in SVF,  $Y_{12}$  – pH in buffer



**FIGURE 1. Histogram representing the fitting of the data after preparation, with the model**

:Y<sub>1</sub> – Carr Index (%), Y<sub>2</sub> – Hausner Ratio, Y<sub>3</sub> – flowing time (min), Y<sub>4</sub> – mechanical resistance (kg), Y<sub>5</sub> – friability (%), Y<sub>6</sub> – foaming time (min), Y<sub>7</sub> – foam maximal volume (ml), Y<sub>8</sub> – resistance of the foam (min before collapse), Y<sub>9</sub> – weight of loosed CO<sub>2</sub> (g), Y<sub>10</sub> – pH water, Y<sub>11</sub> – pH in SVF, Y<sub>12</sub> – pH in buffer

method. The results obtained are represented by histograms in fig. 1.

The ANOVA variance analysis test showed good results for most of the studied responses ( $p < 0.05$  for the model and  $p > 0.05$  for the error). We obtained anyway some deviations for the following responses: Y<sub>13</sub> (mechanical resistance after 1 month) with values of  $p$  being 0.067 for the model and 0.108 for the error; Y<sub>26</sub> (humidity of powder mixtures before compression) with values of  $p$  being 0.03 for the model and 0.022 for error; and Y<sub>27</sub> (humidity of the tablets after compression) with values of  $p$  being 0.124 for the model and 0.025 for error.

Considering that there were 29 responses studied, we can say that the fitting of the data with the model was good. Another method for the evaluation of the fitting is by drawing the dependence curves between the obtained values and the values predicted by the model. Using this method, the best fittings obtained for Y<sub>3</sub> – flowing time (min), Y<sub>6</sub> – foaming time (min), Y<sub>7</sub> – foam maximal volume (ml), Y<sub>9</sub> – weight of loosed CO<sub>2</sub> by foaming (g), Y<sub>10</sub> – pH generated in distilled

water, and Y<sub>11</sub> – pH generated in SVF (after preparation) (data not show).

#### *The influence of formulation factors on powder flow properties*

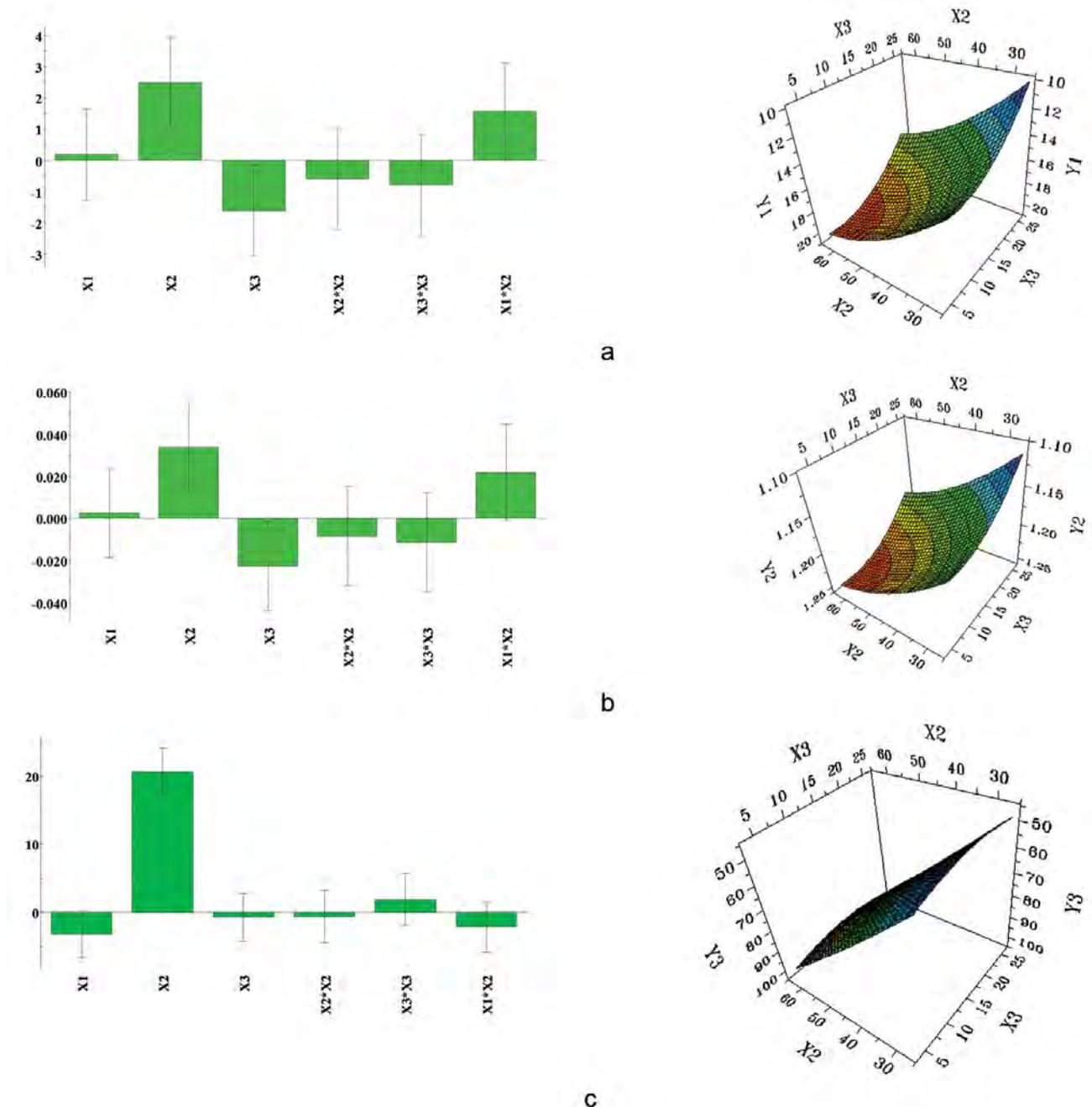
Graphs representing the influence of formulation factors on Carr's (Y<sub>1</sub>) are illustrated in Fig. 2.a. Carr's index increases with increasing the percentage of effervescent mixture (X<sub>2</sub>) and decreases with the increase of the excess citric acid (X<sub>3</sub>), being independent of the percentage of sodium lauryl sulphate. Graphs representing the influence of formulation factors on Hausner ratio (Y<sub>2</sub>) are illustrated in Fig. 2.b. Hausner ratio increases with increasing the percentage of effervescent mixture (X<sub>2</sub>) and decreases with the increase of the excess citric acid (X<sub>3</sub>), being independent of the percentage of sodium lauryl sulphate, the results being consistent with those obtained for Carr's index. Graphs representing the influence of formulation factors on flowing time of powders are illustrated in Figure 2.c. Flowing time increases with increasing the percentage of effervescent mixture (X<sub>2</sub>), so with

the decrease in the percentage of diluent Ludipress, and is independent of the other formulation variables chosen.

The results obtained for the study of the flowing time show very good flow for all formulations studied (Hausner ratio between 11.11 and 21.21; Carr's index between 1.11

and 1.26), and in terms of its variation depending on formulation factors, the results show that the effervescent mixture makes the flow difficult, probably due to interactions between mixture components, which can lead to an enhancement of powder humidity during processing. Citric acid has a

very good flow, so the excess of citric acid facilitates the flow. So, a good flow is achieved with a small amount of effervescent mixture (which corresponds to a higher ratio of diluent Ludipress), and a higher percentage of excess citric acid.



**FIGURE 2. The influence of formulation factors on powder flow**

a – Carr Index, Y1; b – Hausner Ratio, Y2 ; c – flowing time, Y3

X1 – percentage of sodium lauryl sulphate, X2 – percentage of effervescent mixture, X3 – percentage of excess of citric acid in the effervescent mixture

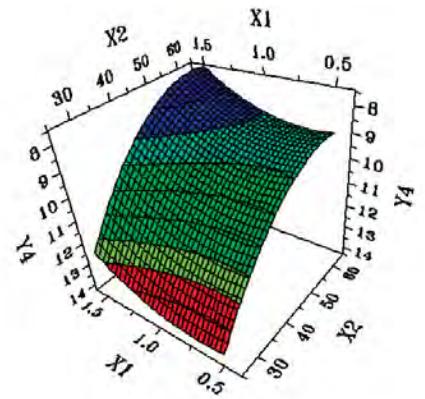
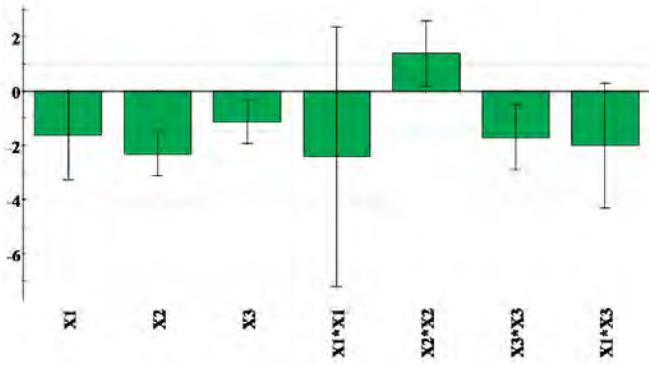
*The influence of formulation factors on tablets properties immediate after preparation*

Graphs representing the influence of formulation factors on mechanical resistance of the tablets are illustrated in Fig. 3a. The results show that the mechanical resistance of effervescent tablets does not depend on the formulation variables chosen. This is understandable because the mechanical resistance depends mainly on the compression force, which has changed from one formulation to another. However, it was not introduced in the experimental design, because it was not monitored. So, in this study the mechanical resistance and the compression force were variables that the experimental design was unable to control. However, there is a tendency of mechanical resistance decrease with increasing the percentage of all the variables chosen, so with the decrease of Ludipress percentage, and the graph has a minimum at 65% effervescent mixture and 1.5% sodium lauryl sulphate. We can thus draw the conclusion that an increase of Ludipress percentage may increase mechanical resistance. This result is understandable, given the good compressibility and compactibility properties of this excipient (16).

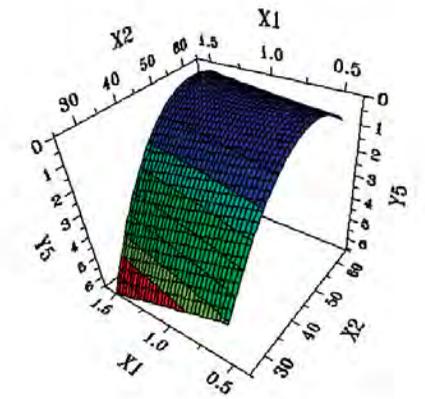
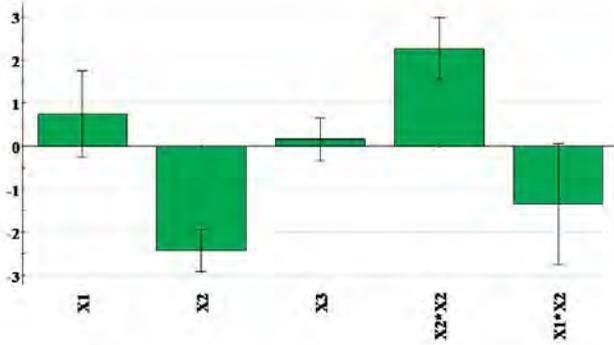
Graphs representing the influence of formulation factors on the tablets friability ( $Y_5$ ) are illustrated in Fig. 3.b. Although for friability the results are poorly fit with the model and there is a great variability of data (from improper values 5-6% to rather small values 0.45%), the friability depending generally on other factors than those chosen for the study, yet it may be observed that friability decreases with increasing the

percentage of effervescent mixture ( $X_2$ ), the chart registering a minimum at 65% effervescent mixture. Also, at 25% effervescent mixture, most formulations were inadequate in terms of friability, although they had a high mechanical resistance (from 9.5 to 14). These results suggest that the effervescent mixture reduces the friability, probably by the interactions of its components, and that there is probably an optimal ratio between the diluent and the effervescent mixture that allows these interactions and thereby obtaining tablets with appropriate friability, but this phenomenon may have negative effects on foaming and stability in time.

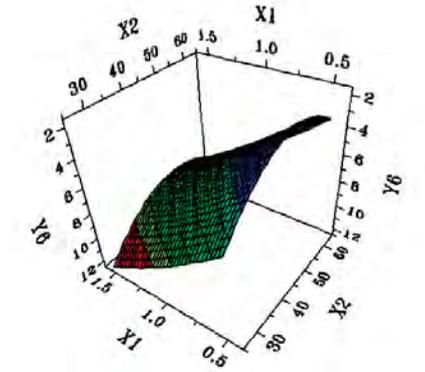
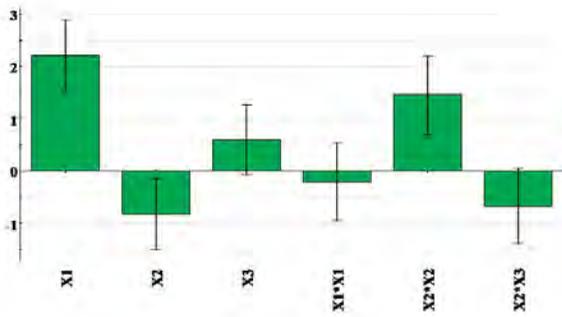
Graphs representing the influence of formulation factors on foaming time ( $Y_6$ ) are illustrated in Fig. 3.c. Foaming time increases with increasing the percentage of sodium lauryl sulphate ( $X_1$ ) and decreases with increasing the percentage of effervescent mixture ( $X_2$ ), but for this formulation variable ( $X_2$ ), a nonlinear effect is observed. Regarding the concentration of sodium lauryl sulphate, some data in the literature state that the foaming slows down the effervescence speed, due to changes in fluid properties in which the disintegration takes place (16,17,18), so the higher the concentration of sodium lauryl sulphate, the higher the duration of disaggregation time will be, but this effect may be due to the fact that with increasing the percentage of sodium lauryl sulphate a higher compression force is needed, leading to tablets that have a slow disaggregation through foaming. Regarding  $X_2$  variable (percentage of effervescent mixture), its growth decreases foaming time, because if



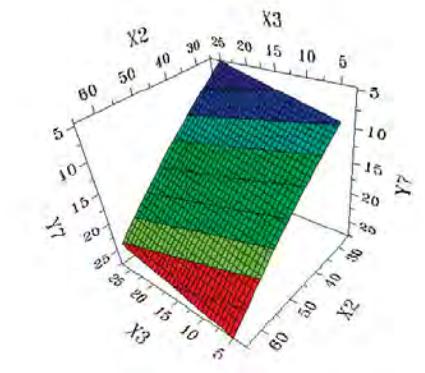
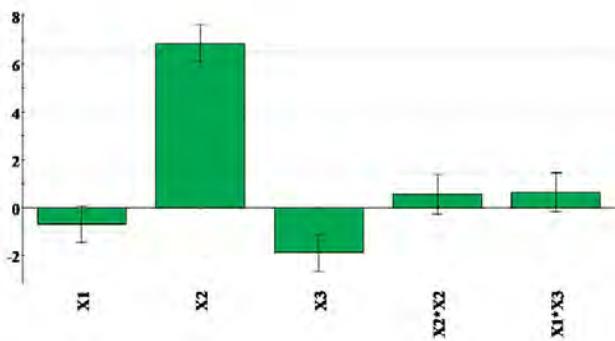
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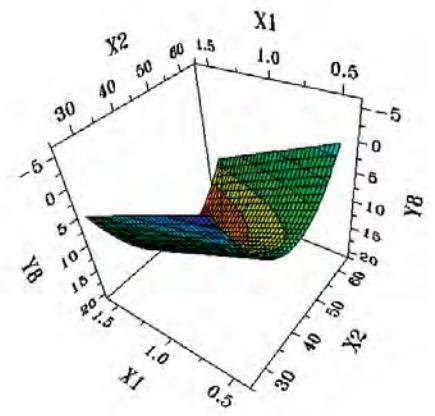
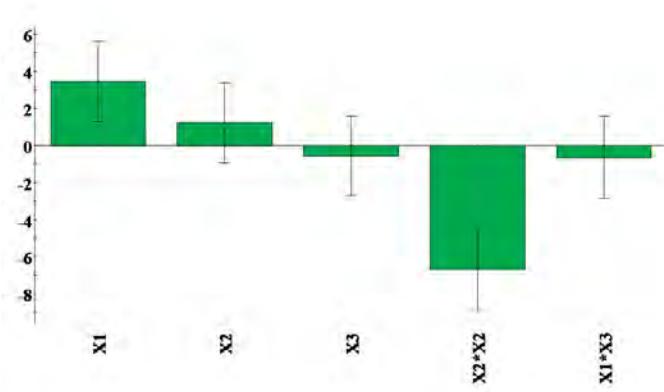
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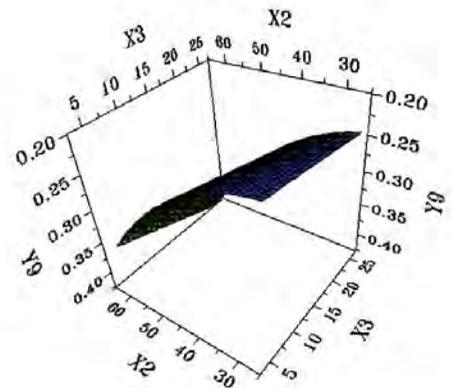
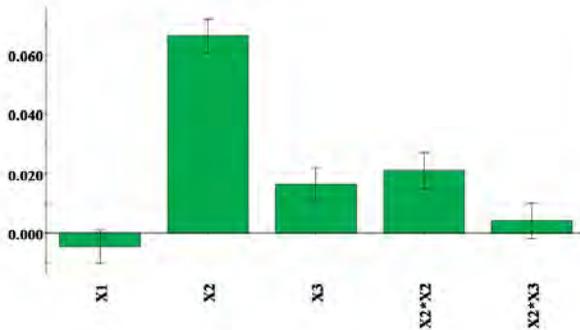
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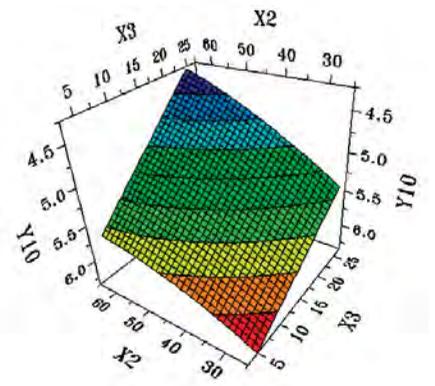
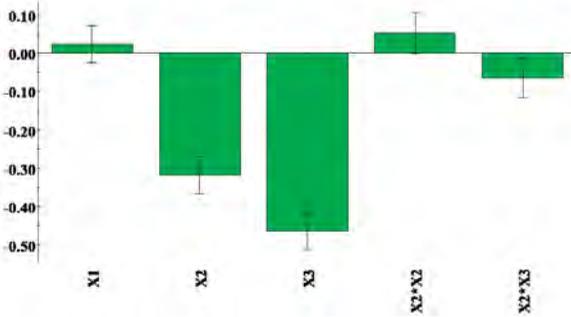
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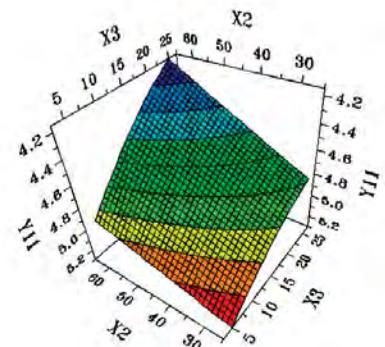
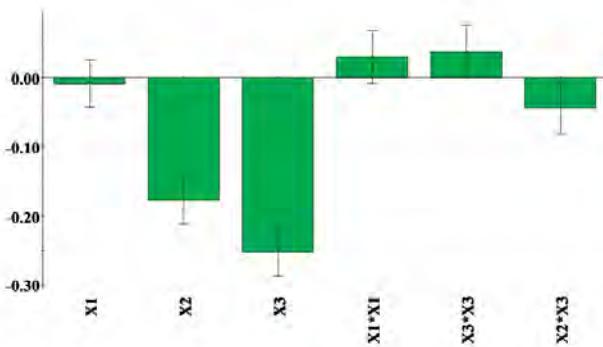
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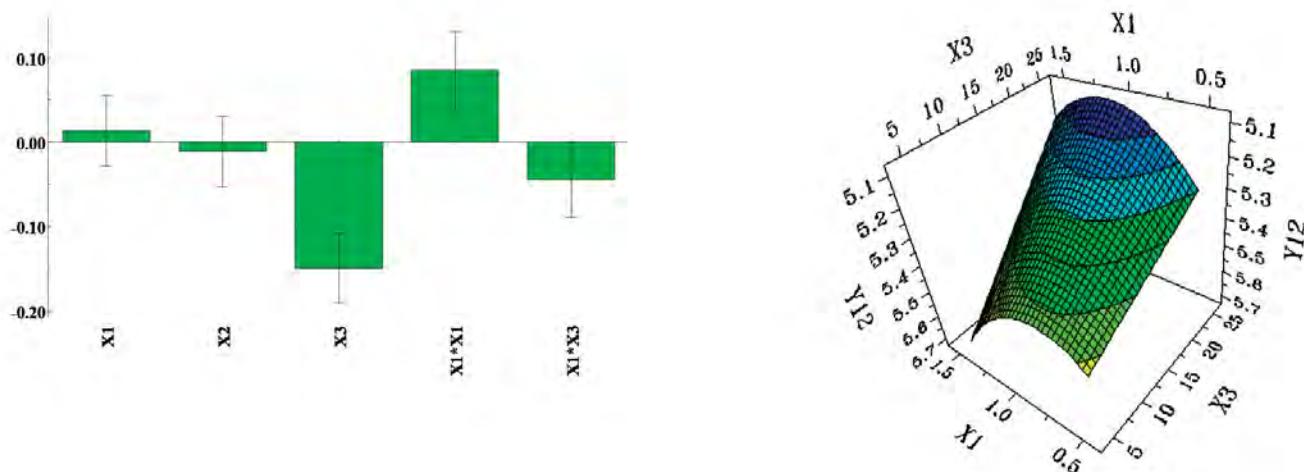
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i

**FIGURE 3. The influence of formulation factors on tablets properties**

a – mechanical resistance, Y4; b – tablets friability, Y5; c – flowing time, Y6; d – foam volume, Y7 e – foam resistance, Y8; f – amount of CO<sub>2</sub> released, Y9; g – pH generated in water, Y10; h – pH generated in SVF, Y11; i – pH generated in acetate buffer, Y12

X1 – percentage of sodium lauryl sulphate, X2 – percentage of effervescent mixture, X3 – percentage of excess of citric acid in the effervescent mixture

the amount of effervescent mixture is greater, the decomposition will be faster, with a broader interaction between the effervescent mixture components, a more abundant effervescence and a faster consumption of these components. For values greater than 45% effervescent mixture (value for which the chart has a minimum), the foaming time does not decrease with increasing the percentage of effervescent mixture, probably because over this threshold value the amount of effervescent mixture is so great that time between decomposition and consumption of the effervescent reaction is longer than for the concentration of 45%, and in this case we must also take into account the dissolution time of the effervescent mixture components in the small volume of vaginal fluid. Another explanation is that a high percentage of effervescent mixture needs a too high compression force, leading to an interaction between components during compression and a strengthening of

the tablet core, slowing down the final stage of disaggregation (1,17,19).

Graphs representing the influence of formulation factors on maximal volume of foam ( $Y_7$ ) are illustrated in Fig. 3.d. From the graphical representations it may be concluded that the volume of the foam formed in the disaggregation process depends mostly on the percentage of effervescent mixture ( $X_2$ ) and increases with its amount increase. The volume of foam depends very little on the excess citric acid ( $X_3$ ), decreasing with its increase and does not depend on the percentage of sodium lauryl sulphate ( $X_1$ ). This behaviour may be explained by the fact that to achieve the maximal volume of foam, several competing processes take place simultaneously: foam formation, foam maintenance and foam breaking. Depending on the speed of these processes, the final volume of foam will result. If the foam formation speed and its maintenance is greater than its

breaking, the volume increases; if the foam formation speed is smaller than the foam breaking speed, the foam does not increase in time, but remains at a minimum volume, or does not form at all. The results suggest that when the foam formation process is predominant, it depends only on the effervescent mixture. Perhaps increasing the percentage of effervescent mixture increases formation speed and maintenance of foam; foam breaking has a constant speed that depends on the studied environment (SVF) and is independent of the percentage of effervescent mixture and the percentage of sodium lauryl sulphate. We can say that there is no need to increase the percentage of sodium lauryl sulphate to increase the foam volume; there is probably a threshold concentration of sodium lauryl sulphate above which the foam breaking has a constant speed, and this concentration is below the minimum concentration used in the experience design, taking into

consideration the small volume of the environment used for the experiment.

Graphs representing the influence of formulation factors on foam resistance ( $Y_8$ ) are illustrated in Fig. 3.e. Although for foam volume we obtained an independent behaviour of the sodium lauryl sulphate percentage, foam resistance increases with the increase of sodium lauryl sulphate percentage ( $X_1$ ), but also with the increase of effervescent mixture percentage ( $X_2$ ); for the latter, the dependence is nonlinear. These results may be explained by the fact that a higher percentage of sodium lauryl sulphate produces a higher density foam which, because of the low surface tension of the foam spheres (16,17), withstands the mechanical action of external forces, this effect being greater with increasing the percentage of effervescent mixture that generates the foam spheres. The three-dimensional graph shows a maximal concentration at 45% effervescent mixture, suggesting that an increase of the percentage of effervescent mixture over 45% will not increase the foam resistance, but will decrease it, probably due to the fact that above this threshold value the release of carbon dioxide resulted in the effervescence is high, leading to larger foam spheres that break faster (20,21).

Graphs representing the influence of formulation factors on the amount of  $CO_2$  released after foaming ( $Y_9$ ) are illustrated in Fig. 3.f. The amount of carbon dioxide released after effervescence increases with the increase of effervescence mixture percentage ( $X_2$ ) and citric acid excess ( $X_3$ ). This behaviour is understandable (19,22), because if effervescent mixture percentage is higher, the

stoichiometric quantity of carbon dioxide resulting from the reaction of effervescence will be higher. Citric acid excess leads to the acidification of the environment and to the decrease of carbon dioxide solubility, which promotes its release.

Graphs representing the influence of formulation factors on pH values generated in water ( $Y_{10}$ ) after tablet disintegration in water are shown in Fig. 3.f. Regarding the pH produced by effervescent tablets disintegration, it may be observed that it decreases with the increase of the percentage of effervescent mixture ( $X_2$ ) and with the increase of citric acid excess ( $X_3$ ). Since all formulations contain a certain excess of citric acid, the higher the effervescent mixture percentage, the higher the amount of citric acid excess and the amount of citric acid in each tablet is, leading to a lower pH after effervescence. Another factor involved is carbon dioxide released after the effervescence reaction, which may also acidify the environment studied (19,21).

Graphs representing the influence of formulation factors on pH values recorded after tablet disintegration in simulated vaginal fluid (SVF) ( $Y_{11}$ ) are illustrated in Fig. 3.g. The results for pH values in simulated vaginal fluid show a similar dependence with the one obtained for water, but the values of final pH are lower because the pH of the environment studied is lower (SVF pH = 4.2). It can be stated that all prepared formulations develop, in simulated vaginal fluid, a suitable pH for this route of administration of the tablets (pH between 4.22 and 5.26). One can observe that in simulated vaginal fluid, effervescent tablets cause a slight increase of pH, but the values are in the physiological range (20,21).

Graphs representing the influence of formulation factors on pH values recorded after tablet disintegration in acetate buffer (pH 5.5) ( $Y_{12}$ ) are illustrated in Fig. 3.h. The graphs representing the variation of pH in acetate buffer pH 5.5 show that its change, depending on the formulation, is due only to citric acid excess ( $X_3$ ) and is independent of the percentage of effervescent mixture ( $X_2$ ). We can draw the conclusion that an excess of citric acid in the effervescent couple lowers the possible buffering capacity, while the effervescent mixture itself cannot cause such an effect, which can be demonstrated by the fact that in most cases the pH decreases and only in one case it increases slightly (pH = 5.77 for N6 formulation).

#### Optimal formula determination

The domains and theoretical levels chosen for the dependent variables and the experimental responses obtained for the optimal formula are represented in table 5.

The optimal domains for the levels of dependent variables were

chosen considering that we need an equilibrium between an easy preparation; quality assurance; time stability; easy disintegration; energetic foaming with keeping of the local mucosal environmental conditions. Based on the chosen levels for the dependent variables, the optimizing program recommended the following levels for the formulation factors, of an intravaginal foaming tablet: 0.5% sodium laurylsulphate ( $X_1$ ); 44% total amount of effervescent mixture in the formulation ( $X_2$ ); with 24% excess of citric acid in the effervescent mixture ( $X_3$ ). The optimal formula presented the following responses (properties): Carr Index for powder blend: 12.9%; Hausner Ratio for powder blend: 1.17; flowing time of powder blend: 80 minutes; mechanical resistance of the tablet: 8.5 kg; tablet friability: 0.8%; foaming time: 3.5 minutes; foam maximal volume: 18 ml; resistance of the foam: 6.5 minutes before collapse;  $CO_2$  weight loss: 0.29 g; pH generated in distilled water: 4.95; pH generated in SVF: 4.5; pH

**TABLE 5. The optimal formula, based on chosen domains; the experimental responses obtained for the prepared optimal formula**

Dependent variables	Chosen domain	Theoretical values predicted by software	Practical values obtained after preparation
Y1	<20	14.4	12.9
Y2	<1.2	1.17	1.17
Y3	50-80	77	80
Y4	5.5-9 ↓	8.64	8.5
Y5	0.5-0.99 ↓	0.69	0.8
Y6	5-10 ↓	3.17	3.5
Y7	15-20	12.43	18
Y8	5	10.3	6.5
Y9	0.25-0.35 ↑	0.29	0.29
Y10	4.5-5	4.78	4.95
Y11	4.2-4.7	4.57	4.5
Y12	5.1-5.3	5.33	5.28

$Y_1$  – Carr Index (%),  $Y_2$  – Hausner Ratio,  $Y_3$  – flowing time (min),  $Y_4$  – mechanical resistance (kg),  $Y_5$  – friability (%),  $Y_6$  – foaming time (min),  $Y_7$  – foam maximal volume (ml),  $Y_8$  – resistance of the foam (min before collapse),  $Y_9$  – weight of loosed  $CO_2$  (g),  $Y_{10}$  – pH water,  $Y_{11}$  – pH in SVF,  $Y_{12}$  – pH in buffer.

generated in pH = 5.5 acetate buffer

The theoretical values predicted by the optimizing program for the optimal formula, were very close to the practical values obtained by preparation and analysis of the recommended formula; these attesting the validity of optimization based on Modde optimizing software.

## CONCLUSIONS

Fifteen formulations of foaming vaginal fast release tablets for local antimicrobial action were prepared,

in order to choose the optimal formula with the Box-Behnken optimization design based on the Modde optimization software. The main factor involved in the behaviour of tablets immediately after preparation is the percentage of effervescent mixture. There is a very small influence of sodium lauryl sulphate on the dependent variables. The concentration of sodium lauryl sulphate was considered to be decisive only for two properties: foaming time and foam resistance. An interesting result is that the volume of foam does not depend on the percentage

of sodium lauryl sulphate (for the chosen concentration levels). The optimal formula of a foaming vaginal tablet, obtained with the Box-Behnken design, must contain 0.5% sodium lauryl sulphate, while the effervescent mixture must contain a percentage of 24% citric acid excess, and it must represent 44% of the total amount of excipients. The experimental determinations on the optimal formula were close to the theoretical values predicted by the program, this attesting the validity of the optimization, based on the Box-Behnken experimental design.

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