

Use of Box–Behnken design for optimization of compounded medication: acyclovir capsules report

Suelen da Silva Reis^a, Valdemir da Silva Quintanilha Junior^a, Gabriella da Silva Boto^a, Thalita Martins da Silva^b, Elizabeth Valverde Macedo^{a,b}, Carlos Augusto de Freitas Peregrino^{a,b}, Samanta Cardozo Mourão^a, Emeli Moura de Araújo^{*a}

^aDepartamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal Fluminense, Niterói, RJ, Brazil; ^bLaboratório Universitário Rodolpho Albino, Universidade Federal Fluminense, Niterói, RJ, Brazil

*Corresponding author: emeliaraujo@id.uff.br

Campus compounding pharmacies play an important role in public health. *Herpes simplex* is one of the most common viral diseases in humans, which generates a great demand for acyclovir capsules in compounding pharmacy. It is well known that the formulation's components influence the effectiveness of the drug. The objective of this study is to show the applicability of Box-Behnken design in optimization of a compounded formulation and to evaluate the effect of excipients on dissolution and drug content in acyclovir 200 mg capsules produced at UFF's University Pharmacy (FAU). The formulations were prepared and evaluated for average weight test, uniformity of dosage units and *in vitro* dissolution, while meeting pharmacopoeial specifications. A statistical analysis showed that sodium starch glycolate, Aerosil®, influences drug content and dissolution results. Magnesium stearate shows no influence on the dissolution at different concentrations but influences the assay results. A numerical optimization was applied to adjust the formulation variables based on the foresaid responses, accomplishing the best formulation that will be prepared and dispensed at FAU upon medical prescription.

Keywords: Acyclovir. Dissolution. Excipients. Design of experiment. Capsules.

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Introduction

Campus compounding pharmacies are important sources of medicines at low cost and individualized therapy, always ensuring the quality of the products dispensed.

The current legislation for compounding pharmacies (1) requires, as far as capsules quality control test is concerned, weight determination, standard deviation, and relative standard deviation. These analyses establish whether there was a uniform distribution of the powder mixture in the analyzed batch. On the other hand, they do not provide any information on the homogeneity of the encapsulated powder mixture. Therefore, it is feasible that a particular formulation meets the pharmacopoeial acceptance criteria for the average weight and, at the same time, falls on non-conformity for the content uniformity test and *in vitro* dissolution test, which are important parameters for quality assessment of oral solid dosage form (2).

Process or formulation variables could impact the medicine quality, the optimization of these parameters could be carried out using the Design of Experiments (DoE) approach.

DoE is a part of quality by design and can be used to generate design space, which determines the multidimensional combination and interaction of independent variables that have been demonstrated to provide a product of desired quality (3). The Box-Behnken technique is capable of analyzing different variables and of helping to select the optimal composition with minimum number of experiments, saving time and resources (3, 4).

The University Pharmacy of Fluminense Federal University (FAU), founded in 1996, has a respectful track record in compounding pharmacy including the production of liquid, semi-solid dosage forms and capsules. Currently, it has been reformulating its products to optimize quality parameters, including acyclovir capsules.

Acyclovir (Figure 1) is an antiviral drug that is primarily used for the treatment of *Herpes simplex* virus (HSV) as well as infections due to *Varicella zoster* and *Herpes zoster* virus. It is a guanosine analogue with a functional aliphatic group that is present in the side chain responsible for stopping the virus replication process and competitively inhibiting the viral DNA synthesis. Due to its potent selectivity, it is currently the antiviral of choice for the treatment of the different forms of herpetic infection produced by HSV-1 and HSV-2 (5).

The available dosage forms of acyclovir are capsules, tablets, suspension, and topical ointment (6) being included on the World Health Organization's (WHO) list of essential medicines needed in basic health system (7). The demand for acyclovir capsules in the FAU has been increasing since the compounding allows the supply of medicines in individualized formulations at a lower cost (8). The present work aimed to optimize the FAU's acyclovir formulation based in DoE and considering the effect of excipients on pharmacopoeial quality. Furthermore, to show the applicability of Box-Behnken design in the development of formulations in compounding pharmacies, taking advantage of its reduced number of experiments.

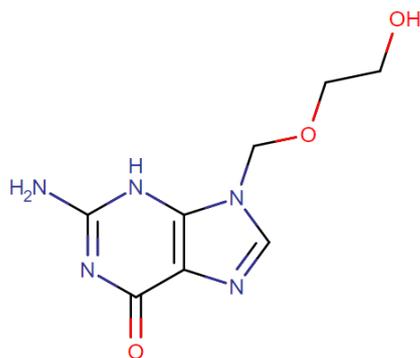


Figure 1. Molecular structure of acyclovir.

Experimental section

Chemicals and reagents

Acyclovir (Fragon, Brazil), colloidal silicon dioxide – Aerosil® (Farnos, Brazil), magnesium stearate (Farnos, Brazil), sodium starch glycolate (Blanver, Brazil), microcrystalline cellulose (Galena, Brazil), sodium hydroxide (Vetec, Brazil), hydrochloric acid (Proquimos, Brazil), acetic acid (Synth, Brazil). Deionized water was obtained from the water deionizer Gehaka model 0510LX (Gehaka, São Paulo – 96 Brazil).

Experimental design

A Box–Behnken factorial design with three variables in three levels was used to study, to optimize, and to evaluate the main effects, namely, interaction, and quadratic effects of the selected independent variables on the response variables.

The Box–Behnken was carried out in *Statistica 12.5 Software* to evaluate the effects of the independent variables on the *in vitro* dissolution test and assay of 200 mg acyclovir capsules. All the experimental tests were carried out at the University Laboratory Rodolpho Albino (LURA / UFF).

Selection of excipients and their amounts was based on FAU's original formulation of 200 mg acyclovir capsules, composed of Aerosil® (0.5%) and magnesium stearate (1%). Sodium starch glycolate was chosen as a disintegrating agent and it is commonly used in concentrations between 2% and 8%, with the optimum concentration about 4% in oral solid dosage forms (9). The independent variables studied were the excipients in different concentrations: A) Aerosil®, B) magnesium stearate, C) sodium starch glycolate. Each independent variable level was expressed by (-1) for the low level, (0) for the medium and (1) for the high level as shown in Table 1. The influence of each excipient in the formulation's performance was evaluated using the absence of each one in the low level (-1). Table 2 presents the matrix factorial design that includes 15 formulations. Formulation 7 is the original formula, and the central point was made in triplicate. The effect of the variables on the acyclovir dissolution and assay were studied (response variable).

Table 1. Levels decoded of independent variables used in DoE.

Variables	Level / % p/p		
	Low (-1)	Middle (0)	High (+1)
Sodium starch glycolate	0	4	8
Aerosil®	0	0.5	1
Magnesium stearate	0	0.5	1

Table 2. Matrix factorial design of acyclovir capsules.

Formulation	Excipients (% p/p)		
	Sodium starch glycolate	Aerosil®	Magnesium stearate
1	0	0	0.5
2	8	0	0.5
3	0	1	0.5
4	8	1	0.5
5	0	0.5	0
6	8	0.5	0
7	0	0.5	1
8	8	0.5	1
9	4	0	0
10	4	1	0
11	4	0	1
12	4	1	1
13	4	0.5	0.5
14	4	0.5	0.5
15	4	0.5	0.5

Each formulation contained acyclovir 0.1992g (equivalent to 0.2 g of acyclovir) and the excipient quantitative composition was described in Table 2. Microcrystalline cellulose was used as a diluent in *quantum satis* (q.s.) to complete each capsule volume, with values corresponding from 45,21 to 55,39% of the concentration (w/w), depending on the formulation. The powder mixtures were prepared by manually mixing in geometric dilution, then filled into size 00 capsules (10). A total of sixty (60) capsules were obtained in each experiment.

The response variables used for optimization of the formulation were average weight, acyclovir content and amount dissolved at 45 minutes from dissolution test.

The results were expressed as mean \pm standard deviation (SD) using Microsoft Excel software (Redmond, WA, USA). The statistical analysis was performed by analysis of variance (ANOVA) using *Statistica 12.5 Software*, and the results were compared graphically by Pareto chart of standardized effects and by 3D response surface plot generated by the software. Significance level was considered at $p < 0.05$.

Average weight

The weight determination was carried out by using 20 units, according to Brazilian Pharmacopoeia 6th edition (11) method. The average weight was calculated by comparing the difference between full and empty capsules. The

uniformity of weight was evaluated based on variation limits for the average weights present in the Brazilian Pharmacopeia. The standard deviation and relative standard deviation were calculated. It was considered 7.5% (more or less) as a variation limit (11).

Acyclovir content

The concentration of acyclovir was determined by UV spectrophotometry (Perkin Elmer, Model: Lambda 35 UV/VIS Spectrometer) following the method uniformity of dosage units by weight variation, as described in Brazilian Pharmacopeia 6th edition (12). Twenty individual capsules were accurately weighed (Shimadzu, Model: AUY220) before and after removing their content, then the average weight was recorded. The content of the capsules were triturated by using mortar and pestle where the equivalent of 0.1 g of acyclovir was transferred to a 100 mL volumetric flask. Then, 60 mL of a sodium hydroxide 0.1 M solution was added and put into sonication for 15 minutes without heating. Thereafter, the flask was completed with 0.1 M NaOH, homogenized and filtered. Then 15 mL of this solution was transferred to a 100 mL volumetric flask, adding 5.8 mL 2 M hydrochloric acid and 50 mL deionized water, homogenized and the flask was filled with deionized water. Subsequently, 5 mL was transferred to a 50 mL volumetric flask and filled with acetic acid 0.1 M. The absorbance at 255 nm was determined using 0.1 N hydrochloric acid as blank. A calibration curve, ranging from 5 to 18 µg/mL, was constructed by the absorbances at 255 nm using 0.1 N hydrochloric acid as solvent ($y = 56624x + 0.0176$; $R^2 = 0.9940$).

Dissolution tests

The dissolution tests were carried out in a dissolution tester (Nova Etica, Model 299) according to the acyclovir capsules monograph of U.S. Pharmacopeia (USP) (13): apparatus 1 (basket), speed of 100 rpm, 900 mL of 0.1 N hydrochloric acid solution at $37 \pm 0.5^\circ\text{C}$. The filtered aliquots of 1 mL were removed from each vessel at 45 minutes, transferred to 10 mL volumetric flask and filled with 0.1 N hydrochloric acid.

The absorbance at 254 nm was determined by UV spectrophotometry (Perkin Elmer, Model: Lambda 35 UV/VIS Spectrometer) using 0.1 N hydrochloric acid as blank. USP (13) recommends not less than 75% of acyclovir dissolved in 45 minutes of test. The same calibration curve described in acyclovir content section was used.

The dissolution results obtained were the variables of the experimental planning and were analyzed in *Statistica 12.5 Software*, performing ANOVA followed Tukey's *post-hoc* test.

Optimization of formulation using desirability approach

In *Statistica 12.5 Software*, numerical optimization was applied to optimize the formulation variables based on the aforesaid responses.

Three formulations were prepared using the predicted values and desirability for the dissolution (FA), acyclovir content (FB) and the original FAU's formulation (FC). The dissolution profile was determined for each formulation, for this test it was used the same conditions and method described above (13), but 1 mL were removed from each vessel at 5, 10, 15, 20, 30, 45 and 60 minutes, with immediate replacement of fresh medium to keep the balance of the sink condition (14). Thereafter, the samples were filtered, and the percentages of drug dissolved were determined in a UV spectrophotometer (Perkin Elmer, Model: Lambda 35 UV/VIS Spectrometer) at 254 nm using 0.1 N hydrochloric acid as blank. The dissolution profiles were obtained. The same calibration curve described in acyclovir content section was used.

The dissolution efficiencies (DE%) were determined for the ratio among the area under the dissolution curves, between the times zero and 60 minutes (AUC_{0-60}) calculated applying the trapezoidal method, and the total area of the rectangle (AUC_{TR}) defined for the ordinate (100% of dissolution) and for the abscissa ($t=60$ minutes) (15). The statistical analysis of variance (ANOVA) followed Tukey's *post-hoc* test, using *Statistica 12.5 software*, was performed considering as significant the values of $p < 0.05$.

Results and Discussion

All formulations met the requirements for average weight according to the (limit variation of 7.5%) preconized by Brazilian Pharmacopeia 6th edition (11) and showed low relative standard deviation (RSD) values, between 1.35-3.58% (Table 3).

Influence of independent variables on the acyclovir content

The 15 formulations proposed in experiment design presented results of drug content ranging between 89.94-100.5% (Table 4).

The results of acyclovir content were loaded in *Statistica 12.5 Software*. Figure 2 illustrates the interplay between different factors studied for their effect on acyclovir content. The results were present as the response at different combinations of two variables, keeping the third variable at its middle level.

The three-dimensional response surface curves demonstrate that magnesium stearate shows a positive influence in intermediate concentration, while Aerosil® and sodium starch glycolate in lower or higher concentration (Figure 2).

Table 3. Average weight of the developed formulations (1-15).

Formulation	Average weight (g) X ± S (RSD%)	Min Value (IL)	Max Value (SL)
1	0.4639 ± 0.0098 (2.10%)	0.4390 (0.4291)	0.4790 (0.4987)
2	0.4768 ± 0.0151 (3.16%)	0.4507 (0.4410)	0.4982 (0.5125)
3	0.4398 ± 0.0123 (2.79%)	0.4140 (0.4068)	0.4580 (0.4727)
4	0.4577 ± 0.0094 (2.05%)	0.4400 (0.4233)	0.4760 (0.4920)
5	0.4527 ± 0.0090 (1.99%)	0.4387 (0.4187)	0.4680 (0.4866)
6	0.4660 ± 0.0113 (2.42%)	0.4434 (0.4310)	0.4898 (0.5009)
7	0.4516 ± 0.0161 (3.58%)	0.4290 (0.4177)	0.4780 (0.4854)
8	0.4660 ± 0.0101 (2.17%)	0.4490 (0.4311)	0.4860 (0.5010)
9	0.4724 ± 0.0141 (2.98%)	0.4460 (0.4369)	0.4980 (0.5078)
10	0.4445 ± 0.0051 (1.16%)	0.4350 (0.4112)	0.4520 (0.4778)
11	0.4715 ± 0.0099 (2.11%)	0.4550 (0.4361)	0.4870 (0.5068)
12	0.4447 ± 0.0108 (2.42%)	0.4130 (0.4113)	0.4580 (0.4780)
13	0.4552 ± 0.0110 (2.41%)	0.4390 (0.4211)	0.4810 (0.4893)
14	0.4587 ± 0.0081 (1.76%)	0.4440 (0.4243)	0.4730 (0.4930)
15	0.4571 ± 0.0061 (1.35%)	0.4460 (0.4228)	0.4700 (0.4914)

X = average, S = standard deviation, RSD = relative standard deviation, Max value = maximum value of weight; Min value = minimum value of weight (n=20), SL = superior weight variation limit, IL = inferior weight variation limit.

Table 4. Acyclovir content of the developed formulations (1-15)

Formulation	Acyclovir content (%) X ± S (RSD%)
1	98.68 ± 0.62 (0.63%)
2	98.13 ± 1.27 (1.29%)
3	97.17 ± 8.00 (8.24%)
4	100.5 ± 1.81 (1.79%)
5	96.65 ± 3.10 (3.21%)
6	92.91 ± 2.23 (2.40%)
7	93.26 ± 3.44 (3.69%)
8	91.97 ± 2.36 (2.57%)
9	92.72 ± 2.76 (2.97%)
10	92.18 ± 3.46 (3.75%)
11	89.94 ± 4.44 (4.94%)
12	90.53 ± 4.03 (4.46%)
13	90.17 ± 3.79 (4.21%)
14	91.02 ± 4.85 (5.32%)
15	91.32 ± 3.22 (3.53%)

X = average, S = standard deviation, RSD = relative standard deviation.

Pareto charts were plotted to represent ANOVA tests and determine the effect of the independent variables, their interactions, and their quadratic effects on the dependent variables. Positive (+) and negative (-) coefficients show the synergistic effect and the antagonistic effect, respectively (14). Pareto chart (Figure 3) corroborate that

sodium starch glycolate and Aerosil® have a negative quadratic influence on the assay, while magnesium stearate has a quadratic and linear influence on the result, with p-values lower than 0.05 (Figure 3).

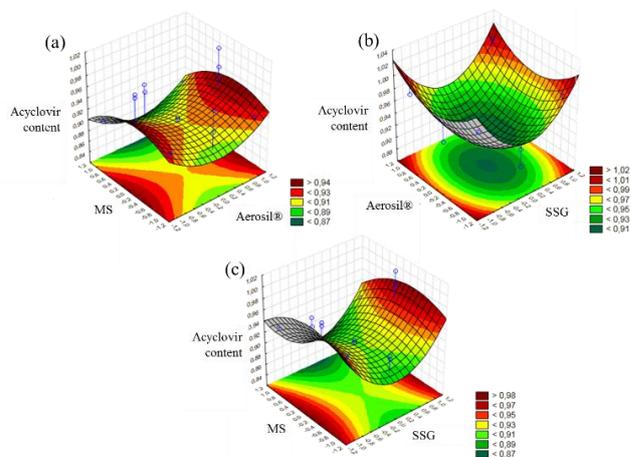


Figure 2. Response surface plots showing the effect of the three variables on the acyclovir content: (a) the effect of magnesium stearate and Aerosil®; (b) the effect of Aerosil® and sodium starch glycolate; (c) the effect of magnesium stearate and sodium starch glycolate. MS = magnesium stearate, SSG = sodium starch glycolate.

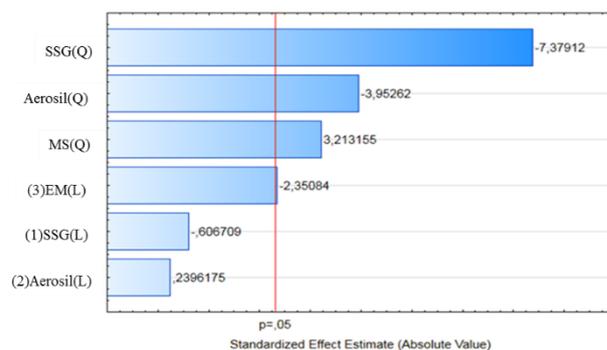


Figure 3. Pareto chart of standardized effects for acyclovir content. MS = magnesium stearate, SSG = sodium starch glycolate, Q = quadratic, L = Linear.

Influence of independent variables on the dissolution test

All formulations met the requirements for dissolution test of USP 44 (13), where not less than 75% was dissolved in 45 minutes of testing, with values between 83-91% of acyclovir dissolved in 45 min (Table 5).

The data from the 15 formulations (Table 5) were analyzed in the *Statistica 12.5 Software*. Figure 4 illustrates the interplay between the variables studied and their effect on acyclovir dissolution. The three-dimensional response surface curves demonstrate that magnesium stearate show no influence on the dissolution at different concentrations, while sodium starch glycolate at medium concentration and Aerosil® at higher concentration displayed higher dissolution results (Figure 4).

Table 5. Percentages of acyclovir dissolved for the developed formulations (1-15).

Formulation	Acyclovir dissolved (%) X ± S (RSD%)
1	90.57 ± 0.028 (3.07 %)
2	89.37 ± 0.033 (3.66%)
3	90.98 ± 0.032 (3.55 %)
4	88.51 ± 0.021 (2.39 %)
5	88.66 ± 0.020 (2.29 %)
6	89.21 ± 0.033 (3.70 %)
7	89.01 ± 0.046 (5.14 %)
8	88.58 ± 0.011 (1.26 %)
9	90.69 ± 0.040 (4.45 %)
10	90.42 ± 0.017 (1.84 %)
11	88.99 ± 0.023 (2.62 %)
12	91.50 ± 0.023 (2.56 %)
13	90.87 ± 0.025 (2.75 %)
14	91.05 ± 0.027 (2.91 %)
15	91.04 ± 0.025 (2.76 %)

X = average, S = standard deviation, RSD = relative standard deviation.

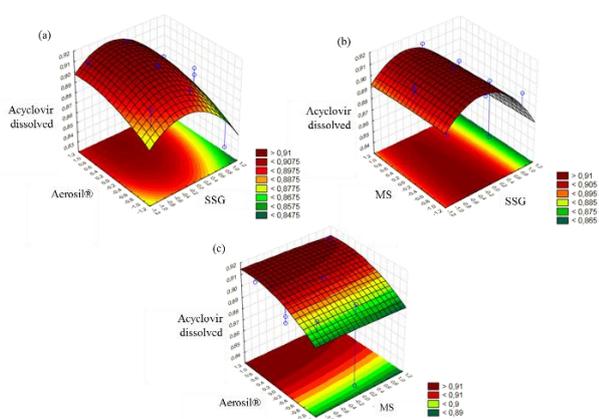


Figure 4. Response surface plots showing the effect of the three variables on the acyclovir dissolved: (a) the effect of Aerosil and sodium starch glycolate; (b) the effect of magnesium stearate and sodium starch glycolate; (c) the effect of Aerosil® and magnesium stearate. MS = magnesium stearate, SSG = sodium starch glycolate.

Figure 5 shows ANOVA tests represented by Pareto chart and corroborates that sodium starch glycolate generates a statistically significant influence on the percentage of dissolved acyclovir (p-value < 0.05), influencing the dissolution in a quadratic way through the interaction with the other excipients of the formulation.

DoE has been widely used in the pharmaceutical industry for research and development of formulations. The statistical design for empirical experiments leads to more reliable results, saving time and reduction of number repetitions of the experiments. The response surface methodology studies the relationship between the response and their effective factors where regression models are used for analyzing the response. The experiments in this methodology study the effects of various factors and evaluate obtaining the optimal conditions to achieve the desired response. The most common model of this method is Box–Behnken, that is a

spherical design and is also utilized for the optimization (14).

Previous works have demonstrated the applicability of Box–Behnken design to observe the excipients' effect on formulation. Dayal et al. (2005) (16) studied the effect of excipients on properties of nasal drug delivery system hydroxyurea- contains. Using Box–Behnken experimental design, it was possible to predict and to identify the major excipients that influences product quality.

In other study, the experimental design was used to optimize and evaluate of excipients (crosslinked caboxymethyl cellulose, sodium starch glycolate and starch) on domperidone maleate release from tablets, considering the main, interaction and quadratic effects. After statistical analyses it was observed the increase of dissolution using the combination of carboxymethyl cellulose and sodium starch glycolate (17).

In the same way, in development of fast dissolving tablets, the Box–Behnken design allow to predict an optimal formulation, considering excipients kinds and concentrations, from evaluation of variables interactions (18).

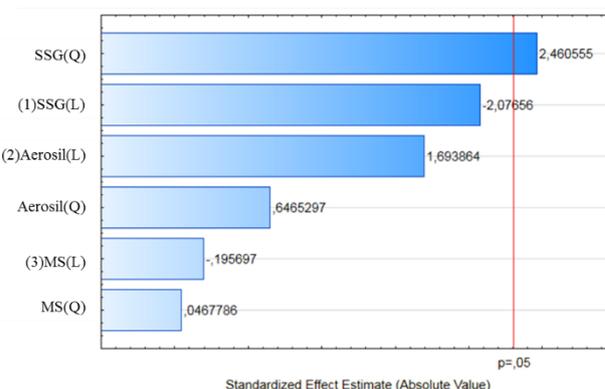


Figure 5. Pareto chart of standardized effects for acyclovir dissolved. MS = magnesium stearate, SSG = sodium starch glycolate, Q = quadratic, L = Linear.

Optimization of formulation using desirability approach

The purpose of the optimization step is to determine the ideal level of each independent variable to prepare a product with the best quality attributes (3). In the present study, the main desirable outcome is to provide an acyclovir formulation with the best dissolution profile. In *Statistica 12.5 Software*, numerical optimization was applied to improve the formulation variables based on the aforesaid responses.

Figure 6 demonstrates that the highest value of desirability for the acyclovir content (0.99145) could be achieved at low level (-1) of sodium starch glycolate (0%), higher level (+1) of Aerosil® (1%) and medium level (-0.2) of magnesium stearate (0.4%).

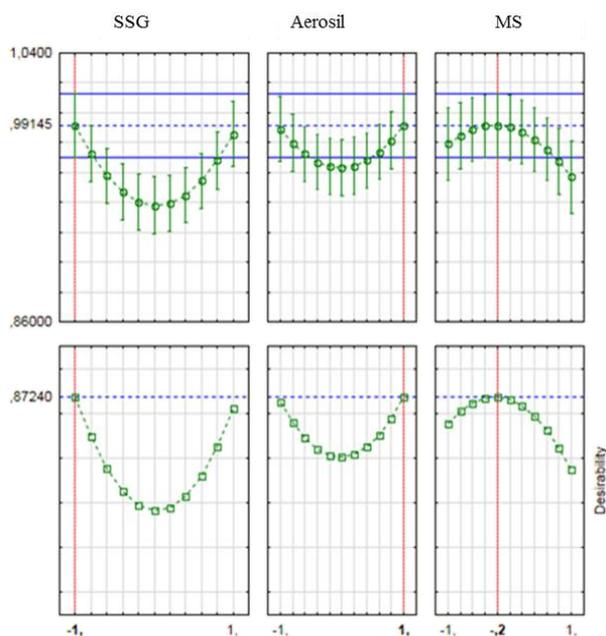


Figure 6. Graphical representation of predicted and desirability values profile for acyclovir content. MS = magnesium stearate, SSG = sodium starch glycolate.

Figure 7 demonstrates that the highest value of desirability for the acyclovir dissolution (0.95906) could be attained at medium level (-0.2) of sodium starch glycolate (3.2%), higher level (+1) of Aerosil® (1%) and medium level (+0.4) of magnesium stearate (0.7%).

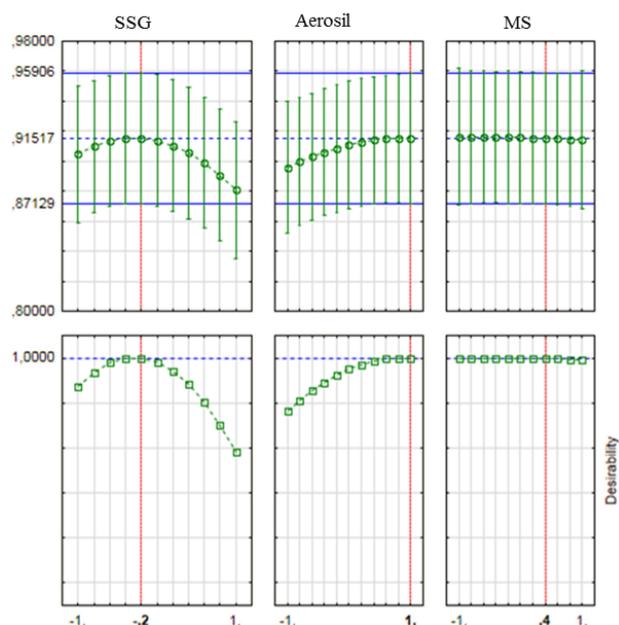


Figure 7. Graphical representation of predicted and desirability values profiles for acyclovir dissolution. MS = magnesium stearate, SSG = sodium starch glycolate.

The theoretical formulations for dissolution (FA), acyclovir content (FB) and the original formulation (FC) (Table 6) were prepared, and the dissolution profile determined. As described in a previous section, the

microcrystalline cellulose was applied as q.s. acting as a diluent in each formulation.

Table 6. Compositions of the theoretical formulations.

Component	Percentage (%)		
	FA	FB	FC
Acyclovir	48.16 (0.1992 g)	49.46 (0.1992 g)	49.98 (0.1992 g)
SSG	3.2	-	-
Aerosil	1	1	0.5
MS	0.7	0.4	1
MC	45.48	48.5	50.25

MS = magnesium stearate, SSG = sodium starch glycolate; MC = microcrystalline cellulose.

Acyclovir is one of the most important antiviral drugs but has several problems due to its physical properties (19). The oral bioavailability of acyclovir is about 20% with an elimination half-life of 1–2 h. The drug has a narrow absorption window and is primarily absorbed from the region of stomach and upper portion of the small intestine (4). Solubility, permeability, and drug product dissolution determine the rate and extent of drug absorption from immediate-release solid oral dosage forms, such as tablets and capsules (20).

The dissolution performance is one of the most relevant parameters in a formulation evaluation as it governs the release rate of the drug from the solid form and hence its bioavailability (3).

The dissolution study refers to the evaluation of the position change of the solute molecule from its original and interacting environment, to a liquid environment with different molecules, providing information on the drug's *in vivo* behavior, since the absorption speed is linked to the dissolution process, reflecting its characteristics of quality and bioavailability (21).

Several factors can influence these characteristics, including the choice of excipients such as lubricants and disintegrants that will provide the formulation with good fluidity and uniform sizing, as well as allowing adequate conditions for the drug to dissolve, respectively (22). In this sense the excipients study is particularly important, since their choice can change both positively and negatively, as an example, the high hydrophobicity of one of these excipients leads to difficulty in wetting and reducing its dissolution rate.

They also influence the handling process, considering the mixing operations, the care with previously mixed powders so that they do not suffer segregation during their handling, and the flow of the powder into the capsules and their leveling, processes that can change the uniformity of the final product (23).

The dissolution test was carried out to evaluate and compare the release profiles of acyclovir from different formulations FA, FB and FC (Table 6). The dissolution profiles of each formulation are summarized in Figure 8. Similar results were found for three formulations.

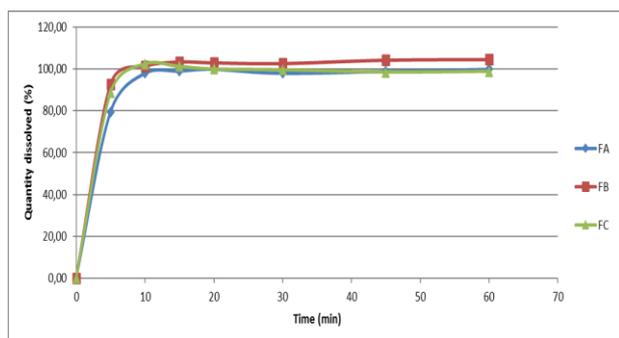


Figure 8. Acyclovir formulations dissolution profile in formulations (FA), (FB) and (FC).

The dissolution efficiency (DE) can be used to compare the dissolution profiles. The results of DE confirm the profiles graphical analysis (Figure 8) and the values (Table 7). The results were compared using ANOVA followed by Tukey's *post-hoc* test and indicates that there are no differences between the dissolution profiles of the 3 formulations.

The requirements for dissolution test is not less than 75% of acyclovir dissolved in 45 minutes of testing (13), in this study more than 75% of acyclovir was released within 5 min for formulations A, B and C (Table 8).

Table 7. DE values (%) of acyclovir formulations (FA), (FB) and (FC).

Formulation	DE (%)
	$\bar{X} \pm S$ (RSD%)
A	68.29 \pm 1.62 (2.37 %)
B	72.08 \pm 1.02 (1.41 %)
C	69.86 \pm 2.77 (3.96 %)

X = average, S = standard deviation, RSD = relative standard deviation.

Table 8. Quantity dissolved (Q) of acyclovir in formulations (FA), (FB) and (FC) at 5 and 10 minutes (n=3).

Formulation	Q 5min (%)	Q 10min (%)
	$\bar{X} \pm S$ (RSD%)	$\bar{X} \pm S$ (RSD%)
A	79.52 ^a \pm 0.60 (0.76%)	97.95 ^a \pm 3.75 (3.82%)
B	92.77 ^b \pm 2.27 (2.45%)	101.39 ^a \pm 2.26 (2.33%)
C	88.68 ^b \pm 5.54 (6.24 %)	102.29 ^a \pm 4.59 (4.49 %)

a, b = Same letters in the same column do not show statistically significant differences according to ANOVA and Tukey's *post-hoc* test.

Rapid dissolution requires >85% of active ingredients be dissolved in 30 min (20). More than 97% of acyclovir was dissolved from formulations A, B and C within 10 min, indicating rapid dissolution (Table 8). If dissolution is very rapid, the limiting step for oral absorption would be intestinal membrane permeation or gastric emptying, and not drug dissolution (20).

Vaithianathan *et al.* (2016) (20) studied the influence of 14 common excipients in cimetidine and acyclovir capsules. The quantities were higher than those used in typical immediate-release solid oral dosage forms. The dissolution profile and a human pharmacokinetic study was conducted. Through three four-way crossover BE studies, 12 common excipients were found to not impact

BCS class III drug absorption in humans: sodium lauryl sulfate, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate.

Although formulation A contained 3.2% sodium starch glycolate it showed a theoretical influence on the dissolution of acyclovir (Figure 5), but there was no statistically significant difference in the dissolution profile (Figure 8), DE (Table 7) and in the dissolution in times 5 and 10 minutes (Table 8). Therefore, the addition of this excipient is not necessary in the proposed formulation.

Over-mixing of magnesium stearate is known to slow the dissolution by forming a coating around drug and other excipients dissolution and possibly reduce drug absorption (20). Although formulation C contains 1% magnesium stearate, the dissolution profile did not show any statistically significant difference (Table 7) compared to formulation A and B which contain 0.7% and 0.4% magnesium stearate, respectively.

The three formulations also showed no statistically significant difference at times 5 and 10 of dissolution (Table 8), showing that this variation from 0.4 to 1% of magnesium stearate did not impact the dissolution of acyclovir. This corroborates what has been shown in the three-dimensional response surface curves (Figure 4) and Pareto chart (Figure 5) that demonstrate that magnesium stearate shows no influence on the dissolution at different concentrations.

The variation of 0.5 to 1% of colloidal silicon dioxide (Aerosil®) also did not seem to affect the dissolution rate as shown in tables 7, 8 and figure 8, which was also shown in figures 4 and 5.

The theoretical formulations were tested for acyclovir content. USP 44 (13) recommends for acyclovir capsules not less than 93% of the labeled amount of acyclovir. Only on FB, the theoretical formulation for acyclovir content achieved this minimum result (Table 9). The uniformity of dosage units by weight variation was calculated according to Brazilian Pharmacopoeia (11) and FB achieved the lower acceptance value (7.96) meeting the requirements ($AV \leq 15$). This formulation was predicted by the software to be the formulation that would have the best results for acyclovir content and was chosen as the optimized formulation to be produced in FAU.

Table 9. Results of acyclovir content for formulations (FA), (FB) and (FC).

Formulation	Acyclovir content (%)
	$\bar{X} \pm S$ (RSD%)
A	92.02 \pm 2.38 (2.58 %)
B	94.44 \pm 0.22 (0.24 %)
C	88.07 \pm 1.54 (1.75 %)

X = average, S = standard deviation, RSD = relative standard deviation.

Conclusions

The technique Design of Experiments (DoE) is an excellent tool for evaluating the influence of several parameters

affecting the formulation at low cost and with small number of experiments. By employing this method, it was possible to evaluate the influence of the excipients on acyclovir 200 mg formulations, and the applicability and robustness of the method was demonstrated by the *software* prediction of which formulation would generate the best acyclovir content in the capsules, allowing the University Pharmacy (FAU) to offer a pharmaceutical product with higher quality under suitable production process conditions.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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