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EVALUATING THE IMPACT OF DIFFERENT VARIABLES IN THE INTRINSIC DISSOLUTION OF METRONIDAZOLE

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Abstract

Intrinsic dissolution is a technique that enables the dissolution of a pure drug to be obtained, regardless of any effects inherent to the other components of a formulation or dosage form. The objective of this study was to evaluate the impact of different variables on the intrinsic dissolution of metronidazole, an antimicrobial drug with elevated solubility and permeability. The tests, as well as the selection of the independent variables, were executed in accordance with a fractional factorial experimental design. According to the statistical analysis carried out, the variables that have an impact on the intrinsic dissolution rate (IDR) of the metronidazole are the rotation speed and the dissolution medium, while particle size and the pressure used in the formation of the compacted drug proved not to influence the results.

Keywords: intrinsic dissolution, metronidazole, factorial design, particle size

1. Introduction

The intrinsic dissolution test is a dissolution evaluation of a pure drug, under constant conditions of factors such as surface area, temperature, rotation speed, pH and the ionic strength of the medium. This is a technique that enables a drug to be evaluated, regardless of the effects inherent to the formulation of a medication or its dosage form [1-3].

Among the applications that can be mentioned are the characterization of drugs in their solid state and the evaluation of the dissolution rate of a drug in different media [4-6].

Although many studies on the subject have been published in peer-reviewed literature, few of them go into the influence of the conditions employed in this type of test and the impact of the variables used on the intrinsic dissolution rate (IDR).

For this experiment, the drug is compressed in a matrix mold, such that the surface area that is exposed to the medium is always constant and, thus, the effect pertaining to particle size may be minimized. However, studies suggest that, according to the crystal habit of the drug, the compacted formed may be more or less organized (texturization in accordance with the preferred orientation of the crystal), which may create differences in the intrinsic dissolution rate (IDR). The micronization

process may result in a reduction of the texturization of the drug, thus creating a random crystal orientation in the compressed product, that may result in faster dissolution of the drug [2,3].

Recently, the advantages of using the intrinsic dissolution test to determine the solubility of drugs has been discussed, especially because much less material is necessary to determine the IDR, compared to the conventional solubility test, and possible alterations in the crystalline structure of the material can also be detected [6-12].

Metronidazole, whose chemical structure is presented in Figure 1, an antimicrobial drug, categorized as a class-I in the Biopharmaceutics Classification System (BCS), meaning it has high solubility and permeability, although despite this categorization, it is a pH dependent drug, which means its solubility increases at pH levels of below 2 [13-15].

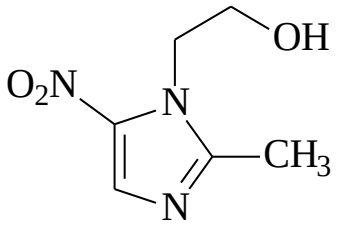

Figure 1 - Chemical structure of metronidazole

Accordingly, metronidazole proves to be an interesting model, considering that the objective of this study is to evaluate different variables in the intrinsic dissolution test for a highly-soluble drug.

2. Material and methods

2.1. Raw material

The raw material employed was donated by Micro Service Ind. Química Ltda., and was provided in three fractions with different particle sizes: A gross sample of metronidazole base (non-micronized); micro metronidazole base 01 (micronized sample) and micro metronidazole base 02 (sample subjected to double micronization).

2.2. Experimental planning

In order to develop the method and evaluate the variables in the intrinsic dissolution test of metronidazole, a fractional factorial design was developed to cover the four factors in three levels (Table 1), while the medium volume was kept constant (900 mL).

Table 1 - Variables employed in the factorial experimental planning and the different levels studied

Factors	Levels		
Compaction pressure (psi)	1000	2000	3000
Rotation speed (rpm)	50	75	100
Dissolution medium	HCl 0.1 M (101)	Water (102)	Phosphate Buffer pH 7.2 (103)
Micronization degree	0	1	2

Factorial planning of the 3^4 type indicated the necessity to execute 81 experiments. Accordingly, we attempted to optimize the study by means of a fractioned experimental design, with a reduction in the number of experiments. As it is the fractional of the 3^k series, by the evaluation of the variables on three levels, its fractioning may be reduced to 1/3, 1/9, 1/27 [16]. In

this case, a 1/3 reduction was opted for, totaling 27 experiments, which was possible with the use of the Statistica 8.0 software program.

2.3. Intrinsic dissolution

For these tests, Varian® (Varian Inc. Palo Alto, CA, United States) rotating disk apparatuses were used, coupled to VK 7010 (Varian Inc. Palo Alto, CA, United States) dissolution equipment, where about 200 mg of each sample was weighed and compacted with the aid of a hydraulic press (American Lab., Charqueada, SP, Brazil). The volume of the dissolution medium was established at 900 mL, and the tests were carried out in triplicate, with collection intervals every 5 minutes, and a total experiment time of 40 minutes. The amount of drug released was ascertained by analyzing the aliquots in a UV-VIS Cary 50 (Varian Inc. Palo Alto, CA, United States) spectrophotometer, in the application "Simple Reads". In order to carry out the calculations, straight-line equations generated by previously-constructed analytical curves in 275 nm (for HCl) and 320 nm (for the other dissolution media used) were employed.

For the calculations, carried out according to the United States Pharmacopeia (USP 2009), a graph of the amount of dissolved drug (mg) was constructed over time (seconds), and through linear regression, the value of the dissolution rate of the drug was obtained in mg/s, by the inclination of the straight line. By dividing these results by the value of the exposed surface area of the drug in the matrix (0.5 cm^2), the intrinsic dissolution rate (IDR) values for metronidazole in $mg/cm^2/s$ were obtained. The evaluation of the impact of the variables studied was carried out by statistical analysis of the data using the Statistica 8.0 software program.

3. Results

In Table 2 and Figure 2, the results obtained from the 27 experiments carried out are presented.

Table 2 - Values for dissolution rate (DR), determination coefficient (R^2) and intrinsic dissolution rate (IDR) obtained for metronidazole and the conditions employed in each experiment

Order of execution	Experiment (E)	DR (mg/s)	R^2	IDR (mg/cm^2/s)	Sample	Compaction pressure (psi)	Rotation speed (rpm)	Dissolution medium
1	27	0.0127	0.9998	0.0254	Gross	3000	100	pH 7.2(*)
2	21	0.0095	0.9994	0.0190	Micro02	3000	50	pH 7.2(*)
3	22	0.0437	0.9993	0.0874	Gross	3000	75	HCl 0.1M
4	10	0.0372	0.9987	0.0744	Micro02	2000	50	HCl 0.1M
5	23	0.0127	0.9996	0.0254	Micro02	3000	75	Water
6	3	0.0096	0.9980	0.0192	Micro01	1000	50	pH 7.2(*)
7	25	0.0525	0.9995	0.1050	Micro02	3000	100	HCl 0.1M
8	7	0.0500	0.9991	0.1000	Micro01	1000	100	HCl 0.1M
9	18	0.0136	0.9996	0.0272	Micro01	2000	100	pH 7.2(*)
10	17	0.0146	0.9997	0.0292	Micro02	2000	100	Water
11	16	0.0536	0.9996	0.1072	Gross	2000	100	HCl 0.1M
12	12	0.0097	0.9997	0.0194	Gross	2000	50	pH 7.2(*)
13	14	0.0128	0.9992	0.0256	Gross	2000	75	Water
14	13	0.0448	0.9998	0.0896	Micro01	2000	75	HCl 0.1M
15	5	0.0126	0.9994	0.0252	Micro01	1000	75	Water
16	11	0.0105	0.9996	0.0210	Micro01	2000	50	Water
17	9	0.0134	0.9996	0.0268	Micro02	1000	100	pH 7.2(*)
18	24	0.0119	0.9996	0.0238	Micro01	3000	75	pH 7.2(*)
19	1	0.0376	0.9994	0.0752	Gross	1000	50	HCl 0.1M
20	8	0.0147	0.9997	0.0294	Gross	1000	100	Water
21	15	0.0119	0.9997	0.0238	Micro02	2000	75	pH 7.2(*)
22	2	0.0104	0.9993	0.0208	Micro02	1000	50	Water
23	20	0.0102	0.9970	0.0204	Gross	3000	50	Water
24	4	0.0437	0.9989	0.0874	Micro02	1000	75	HCl 0.1M
25	6	0.0116	0.9996	0.0232	Gross	1000	75	pH 7.2(*)
26	19	0.0397	0.9991	0.0794	Micro01	3000	50	HCl 0.1M
27	26	0.0146	0.9993	0.0292	Micro01	3000	100	Water

(*) Phosphate buffer pH 7.2

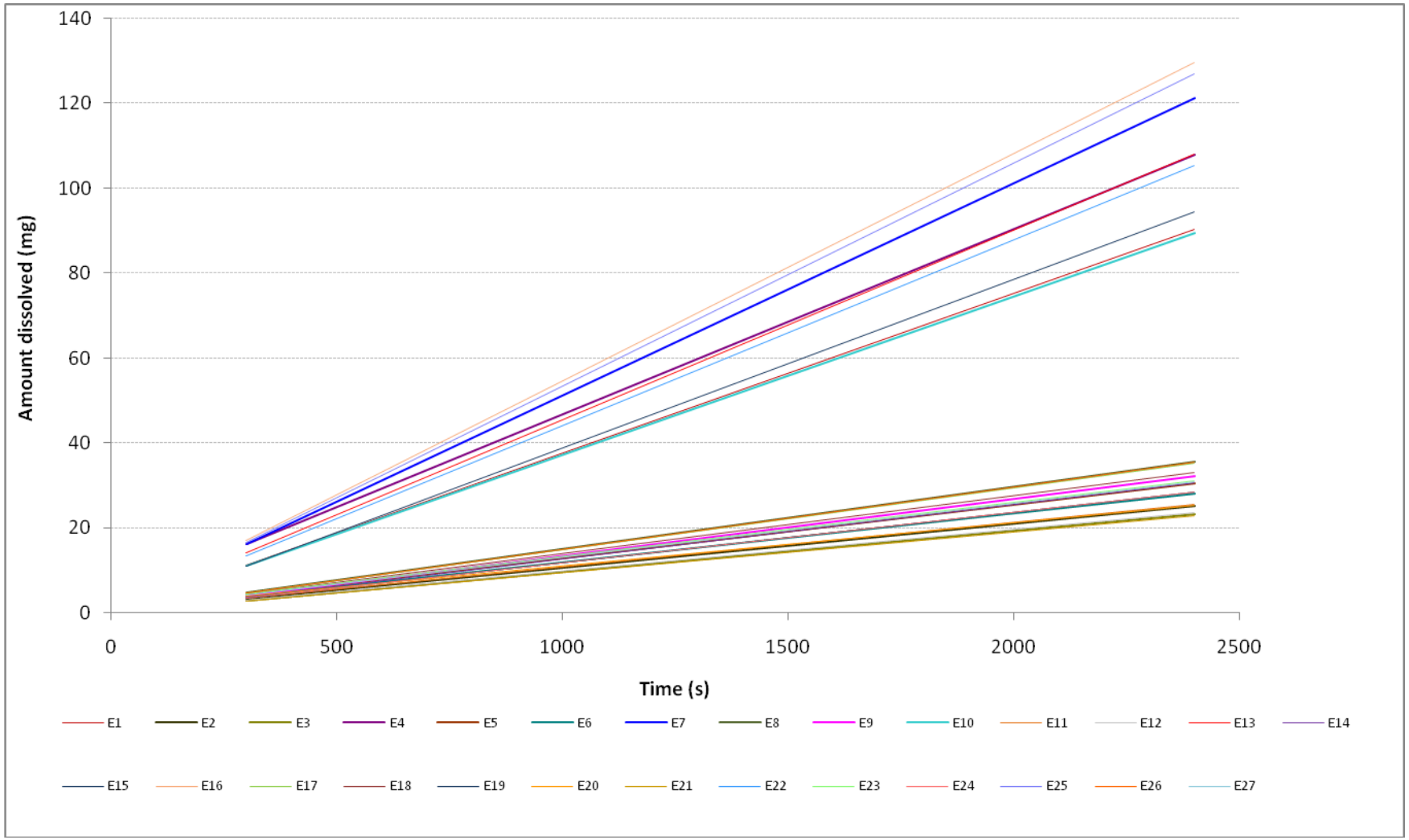

Figure 2 - Linear regression of the points for the dissolved quantity of metronidazole (mg) plotted against the collection times (s), in order to obtain the dissolution rate (DR)

The analysis of variance of the main interactions and a Pareto chart are presented in Table 3 and Figure 3, respectively.

Table 3 - Analysis of variance (ANOVA) of the IDR results obtained - the results in bold are considered significant ($p<0.05$)

Effect	Significance test for IDR ($mg/cm^2/s$)				
	Sum of squares	Degrees of freedom	Average of squares	F	p
Intercept	0.056911	1	0.056911	2304.175	0.000000
Rotation speed	0.000948	2	0.000474	19.193	**0.000034**
Compaction pressure	0.000006	2	0.000003	0.128	0.880700
Dissolution medium	0.025682	2	0.012841	519.889	**0.000000**
Micronization degree	0.000000	2	0.000000	0.009	0.991226
Error	0.000445	18	0.000025		

Percentage of explained variation ($R^2_{adjusted}$) = 97.63

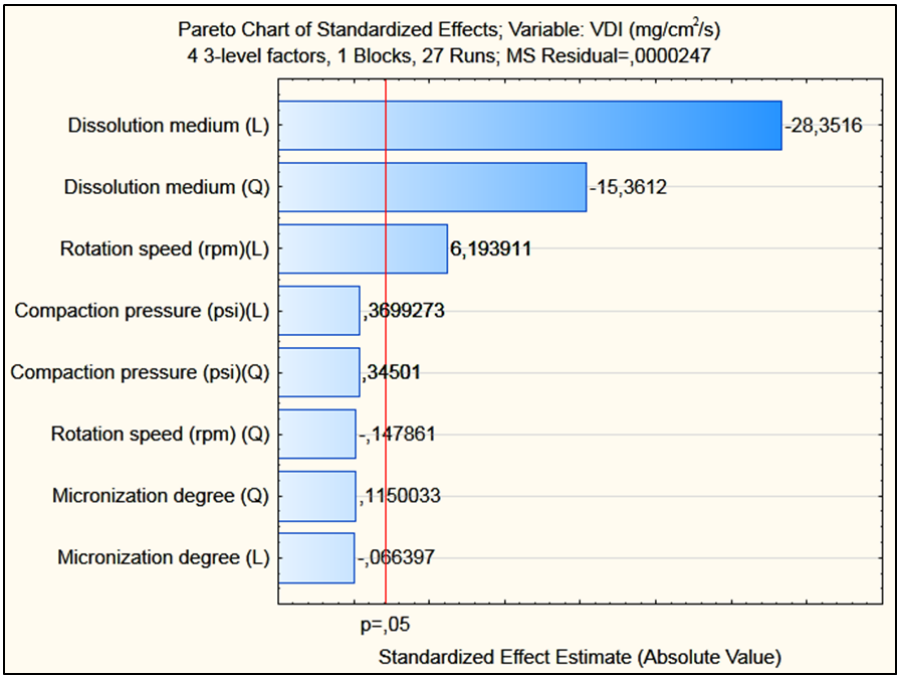

Figure 3 - Pareto chart for the effects of the variables on the IDR of metronidazole (ANOVA) - the results to the right of the red line are considered significant

An analysis of residues is presented in Figure 4.

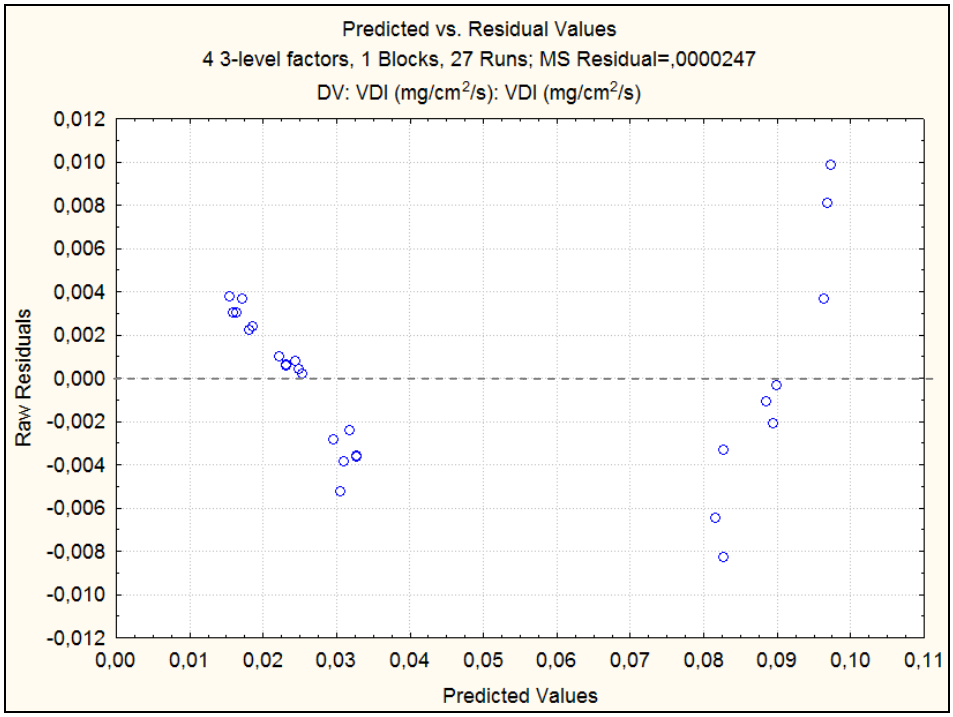

Figure 4 - Residual analysis for dependent IDR variable

In Table 4, an evaluation of the significance between the effects of the levels studied and an estimate of the regression coefficients of the staggered variables is presented.

Table 4 - Estimates per point, per interval and significance test for the regression coefficients of the evaluated factors - the results in bold are considered significant ($p<0.05$)

Effects	p	Coefficients	Standard Errors	Confidence Limit - 95%	Confidence Limit 95%
Intercept	0.000000	0.045911	0.000956	0.043902	0.047921
Rotation speed L*	**0.000008**	0.007256	0.001171	0.004795	0.009717
Rotation speed Q**	0.884096	-0.000150	0.001014	-0.002281	0.001981
Compaction pressure L*	0.715753	0.000433	0.001171	-0.002028	0.002894
Compaction pressure Q**	0.734086	0.000350	0.001014	-0.001781	0.002481
Dissolution medium L*	**0.000000**	-0.033211	0.001171	-0.035672	-0.030750
Dissolution medium Q**	**0.000000**	-0.015583	0.001014	-0.017715	-0.013452
Micronization degree L*	0.947794	-0.000078	0.001171	-0.002539	0.002383
Micronization degree Q**	0.909716	0.000117	0.001014	-0.002015	0.002248

*L = Linear effect; **Q = Quadratic effect

In the response surface graphs (Figure 5), a comparison is made of the influence of two factors on the dependent variable (IDR). It can be ascertained that the graphs that feature the dissolution medium variable are curved.

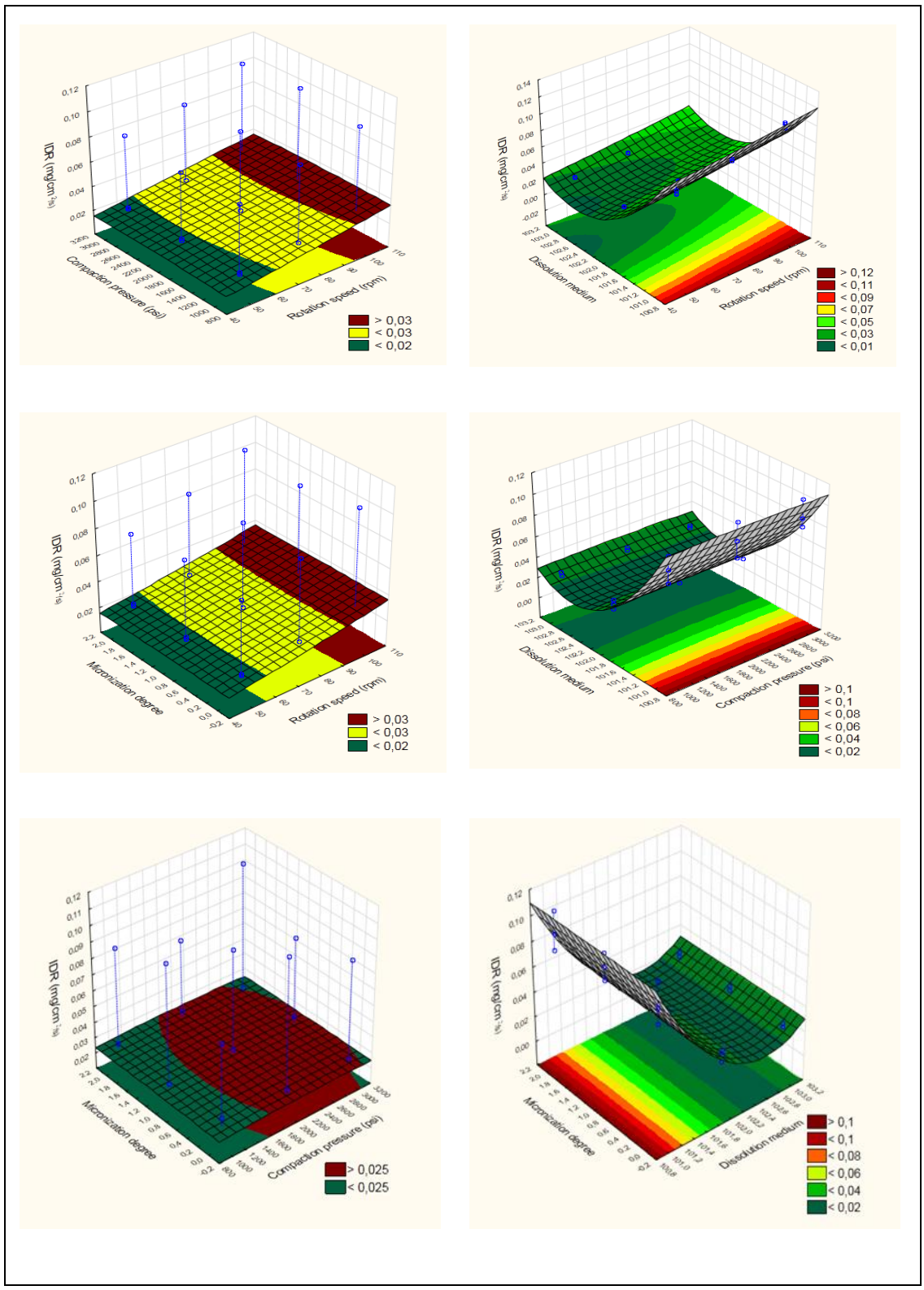

Figure 5 - Response surface for IDR (mg/cm^2/s) as a function of the factors studied

In Figure 6, graphs of the average IDR values obtained as a function of the levels employed in each one of the variables studied are presented.

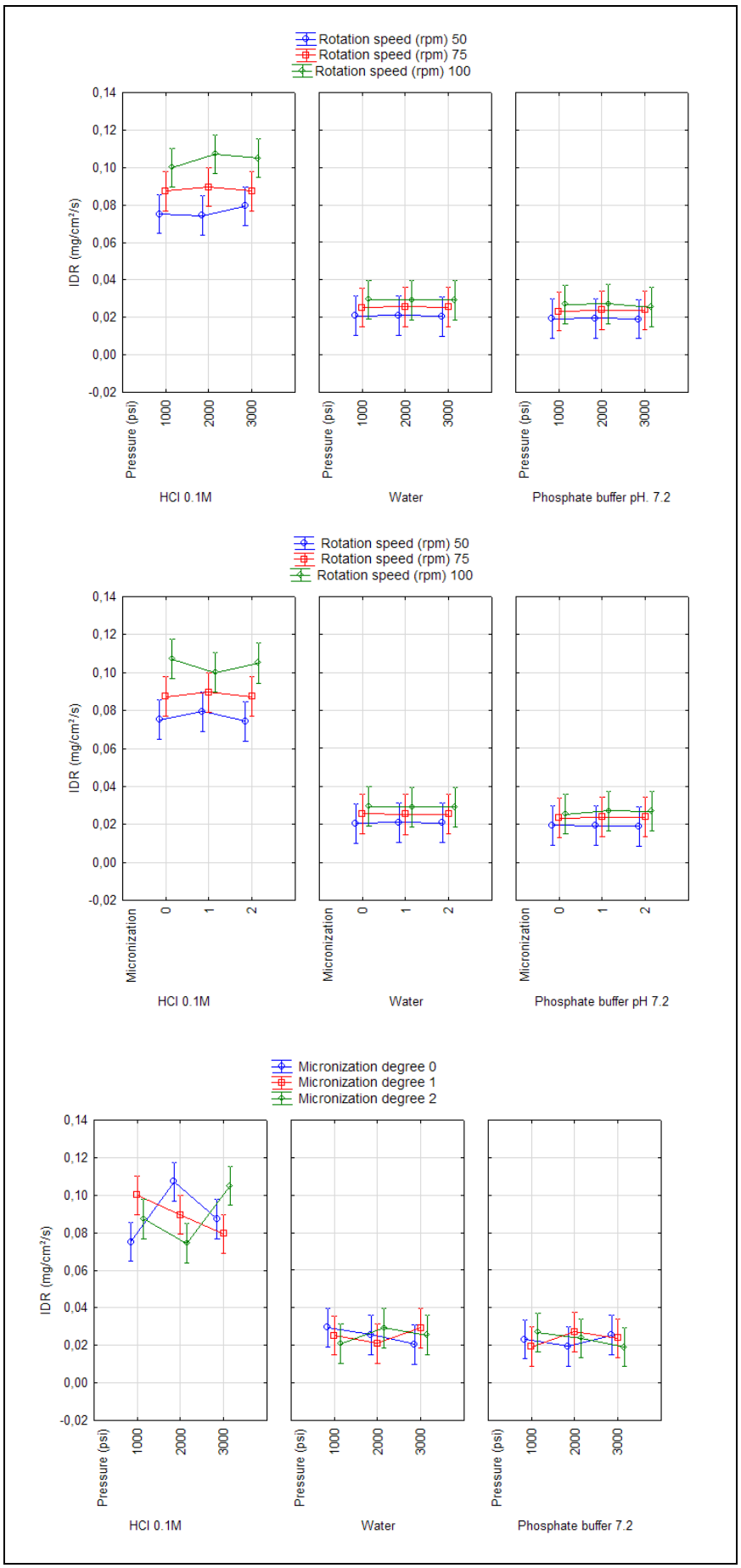

Figure 6 - Graph of IDR averages obtained between the levels of the factors evaluated

4. Discussion

According to the coefficient determination values (R^2) obtained (Table 2 and Figure 2), it was ascertained that there is linearity between the results for the dissolution rate of metronidazole, and no alterations in this profile were observed throughout the experiments.

The results presented in Table 3 and Figure 3 indicate that, among the factors studied, the rotation speed and the dissolution medium had a significant impact on the IDR of metronidazole. This observation was also evident in a study carried out by Yu et al. (2004), in an evaluation of the intrinsic dissolution rates of different drugs.

In the analysis of the residues (Figure 4) the errors were presented in a random and independent manner, thus we assume a normal distribution, which indicates the feasibility of the analysis model employed.

From the results of the significance tests of the effects (Table 4), it is possible to obtain the general equation of the model that explains the impact of the variables studied in the intrinsic dissolution rate of metronidazole. According to Eq. (1) and the Pareto chart (Figure 3), we ascertain that the contribution of rotation speed is related only to linear effect, while the contribution of the dissolution medium variable is related to linear and quadratic effects, thus having a greater influence on the intrinsic dissolution rate of the drug.

$$VDI_{metronidazol} = 0.045911 + 0.007256\ \text{rotation speed} - 0.033211\ \text{dissolution medium} - 0.015583\ (\text{dissolution medium})^2$$

(Eq. 1)

The interpretations of the surface graphs (Figure 5) are presented in Table 5.

Table 5 - Interpretation of the response surface graphs obtained for the IDR as a function of the independent variables

Variables	Interpretation
Compaction pressure X Rotation speed	The IDR is influenced only by the rotation speed, since different compaction pressures lead to similar results.
Dissolution medium X Rotation speed	An increase in the rotation induces higher IDR values in all of the media employed, especially at the lowest level of the dissolution medium, HCl 0.1 M.
Micronization degree X Rotation speed	The variable degree of micronization does not have an impact on the IDR of the drug, which is only altered with a change in the rotation employed.
Dissolution medium X Compaction pressure	As the level of the dissolution medium increases, a reductive effect in the IDR of the drug is produced, which is not altered by variations in the compaction pressure.
Micronization degree X Compaction pressure	Although the compaction pressure variable does not have a significant influence on the response, an alteration in the IDR is observed in accordance with the level used.
Micronization degree X Dissolution medium	As in the previous graphs, the degree of micronization has no effect on the IDR of the drug, which is heavily influenced by the dissolution medium.

Accordingly to Figure 6, dissolution medium is the factor with the greatest impact on the intrinsic dissolution of metronidazole, as the difference in IDR values between the lowest level used in the experimental design (HCl 0.1M) and the other levels employed (water and phosphate buffer

pH 7.2) is quite significant, a fact that can be explained by the greater solubility of metronidazole at pH levels below 2 [15].

The other variable that has a significant impact on the intrinsic dissolution of the drug is the rotation speed, albeit at a lower intensity. It can be noted that, regardless of the medium employed, the average increase of the IDR is accompanied by an increase in rotation. On the other hand, the lower intensity of the effect is related to the proximity of the results obtained, as can be observed in the dispersion bars in the graphs of the averages, and it is possible to obtain similar IDR values with different rotation speeds (Figure 6).

With regards to the compaction pressure and the degree of micronization, very close results were ascertained between the levels studied, as well as an absence of any tendency, which demonstrates the lack of significant influence in the intrinsic dissolution of metronidazole.

Although the pressure used for the formation of the compact product does not have any impact on the IDR, it is important to highlight its contribution in making the test feasible, since without it, it would be impossible to maintain the surface area of the material constant. Accordingly, any of the pressures evaluated (1000, 2000 and 3000 psi) could be employed in the analysis of metronidazole.

5. References

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