BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 50, n. 3, jul./sep., 2014
http://dx.doi.org/10.1590/S1984-82502014000300009

Article

The effect of dissolution medium, rotation speed and compaction pressure on the intrinsic dissolution rate of amlodipine besylate, using the rotating disk method

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The aim of this study was to evaluate the effect of dissolution medium, rotation speed and compaction pressure on the intrinsic dissolution rate (IDR) of the antihypertensive drug amlodipine besylate, using the rotating disk method. Accordingly, a fractional factorial design (3^{3-1}) was used, employing dissolution media (water, phosphate buffer pH 6.8 and HCl 0.1 M), rotation speed (50, 75 and 100 rpm), and compaction pressure (1000, 1500 and 2000 psi) as independent variables. The assays were randomized and statistically compared using the Statistica® 11 software program. Significance testing (ANOVA) indicated that the dissolution medium had a considerable impact on the IDR of amlodipine besylate. Analysis of the linear and quadratic components of the variables led to the proposition of a mathematical model that describes the IDR as a function of the parameters studied. Conversely, the levels of compaction pressure and rotation speed employed during experimental planning were less relevant, especially when the assay was conducted in the HCl 0.1 M medium.

Uniterms: Amlodipine besylate/intrinsic dissolution. Biopharmaceutical classification system. Drugs/experimental design. Antihypertensive drugs/Amlodipine besylate.

A finalidade do presente trabalho foi avaliar o efeito do meio de dissolução, velocidade de rotação e pressão de compactação na velocidade de dissolução intrínseca (VDI) do fármaco anti-hipertensivo besilato de anlodipino, usando o método do disco rotativo. Dessa forma, foi utilizado um planejamento experimental do tipo fatorial facionado (3^{3-1}) utilizando como variáveis independentes o meio de dissolução (água, HCl 0,1M e tampão fosfato pH 6,8), velocidade de rotação (50, 75 e 100 rpm) e pressão de compactação do fármaco (1000, 1500 e 2000 psi). Os ensaios foram randomizados e comparados estatisticamente pelo software Statistica® 11. A análise de variância (ANOVA) indicou que o meio de dissolução exerce considerável impacto na VDI do besilato de anlodipino. A análise das variáveis em seus componentes lineares e quadráticos permitiu a proposição de um modelo matemático que descreve a VDI em função dos parâmetros estudados. Por outro lado, os níveis de pressão de compactação e velocidade de rotação empregados exercem efeito menos relevantes, especialmente quando o ensaio é conduzido em HCl 0,1 M.

Unitermos: Besilato de anlodipino/dissolução intrínseca. Sistema de classificação biofarmacêutica. Fármacos/delineamento experimental. Fármacos/anti-hipertensivos/ Besilato de anlodipino.

INTRODUCTION

Intrinsic dissolution is the study of the dissolution and release of a drug from a surface consisting of the drug itself, under constant agitation, pH and ionic strength of the medium. This release can be expressed numerically by a value called the intrinsic dissolution rate (IDR), characteristic that allows the solubility of a given active pharmaceutical ingredient to be assessed (Healy *et al.*, 2002).

With regards to the solubility test - executed, for example, by the shake-flask method (Baka, Comer, Tacáks-Novák, 2008) - the intrinsic dissolution test has the advantages of requiring a smaller quantity of drug and a shorter assay time. Furthermore, the variability of

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results is usually less significant, thus enabling a better description of the phenomenon. Finally, the *in vivo / in vitro* correlation can be better defined, since it measures the rate at which a drug is dissolved (Issa, Ferraz, 2011; Yu *et al.*, 2004).

Accordingly, intrinsic dissolution is an important tool for the physicochemical characterization of drugs in their solid states and constitutes a test allowing intervention in the dissolution behavior of the active pharmaceutical ingredient in certain physiological environments, regardless of the action of excipients in the formulation. For this purpose, rotary disks (Wood's apparatus) or fixed disks can be used as intrinsic dissolution apparatuses (Stelle, Austin, 2009; Viegas *et al.*, 2001).

However, it is important to consider that the intrinsic dissolution rate can be affected by many factors, such as the crystalline properties of the drug (occurrence of polymorphism and solvation), the conditions of the dissolution medium (temperature, ionic strength and viscosity) and hydrodynamics, dictated largely by the stirring rate (Issa, Ferraz, 2011; Sehic *et al.*, 2010; Stelle, Austin, 2009; Zakeri-Milani *et al.*, 2009; Bartolomei *et al.*, 2006).

The influence of these factors on the IDR can be studied by means of a suitable statistical analysis. One widely used method consists of changing the levels of the variable under study, whilst keeping the others completely constant (one factor at a time). Although it is possible to evaluate the effect of a particular factor on a specific response, this procedure requires a large number of tests, which can prove inefficient and economically unfeasible (Kincl, Turk, Vrecer, 2005).

In this sense, experimental design is a promising technique, since it allows the variables that most influence the response of a phenomenon to be determined. In the specific case of fractional factorial designs, the relationship among different factors can be explained by the response surface method, which allows us to assess the factors with the greatest impact on the studied response, thus enabling the process to be optimized for improved productivity and efficiency. The advantage of applying experimental design lies in the rationalization of testing, cost reduction and more satisfactory agreement between expected and obtained values (Box, Hunter, Hunter, 2005; Montgomery, 2001).

Currently, there are few studies in the related literature that statistically evaluate the influence of parameters employed (at different levels) in dissolution testing, indicating that this important tool is not yet widely used in the pharmaceutical area (Issa *et. al.,* 2013; Polonini *et al.*, 2011; Parojčić *et al.*, 2001).

Amlodipine besylate (methyl ethyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate), empirical formula $C_{20}H_{25}ClN_2O_5$, and molecular weight 408.9 $g.mol^{-1}$, is an antihypertensive drug of the third generation of dihydropyridines, introduced into therapy in the 1980s by Pfizer Inc., under the brand name Norvasc®. Its structure (Figure 1) contains a long chain called 2-amino ethyl methyl, which protects the dihydropyridine ring from oxidation caused by the enzyme cytochrome P_{450} (Katoh *et al.,* 2000). Despite being a highly soluble drug, there is no consensus about which class it belongs to in the Biopharmaceutical Classification System, due to its intermediate bioavailability, which is 65% (Lindenberg, Kopp, Dressman, 2004).

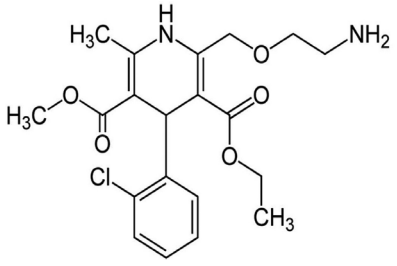

FIGURE 1 – Chemical structure of amlodipine.

Moreover, there is no record in the literature of any intrinsic dissolution tests involving this drug or what parameters may influence its IDR. Thus, the aim of this study was to evaluate the effect of dissolution medium, rotation speed and compaction pressure on the intrinsic dissolution rates of the drug amlodipine besylate, using the rotating disk method.

MATERIAL AND METHODS

Raw material

A sample of the antihypertensive drug amlodipine besylate (Cadila Healthcare, Gujarat, India) was used for testing. The API, in the form of raw material, was within its use-by date and properly protected from light in suitable packaging.

Experimental design

Intrinsic dissolution tests were planned with a fractional factorial design (3^{3-1}), in which three factors were stipulated (rotation speed, medium and compression pressure), each having three levels, stipulated as -1, 0 and +1, in accordance with Table I.

TABLE I - Independent variables (factors) and levels used in the experimental design for intrinsic dissolution tests of amlodipine besylate

Factors	Levels		
	-1	0	+1
Dissolution medium	Phosphate buffer pH 6.8	Water	HCl 0.1M
Compaction pressure (psi)	1000	1500	2000
Rotation speed (rpm)	50	75	100

Accordingly, a set of 12 experiments was generated (9 from the fractional factorial design and 3 taking into account the central levels of the variables), as shown in Table II. Tests were fully randomized in order to eliminate any bias in results. The effects of interaction among the factors were not included in the model, since such a procedure would be of more use in a full factorial study (in which all 27 possible combinations would be evaluated), which was beyond the scope of this investigation.

Finally, the influence of the variables was analyzed by means of statistical calculations, including analysis of variance (ANOVA), Pareto charts, media and surface-response graphs, all carried out with the Statistica 11.0 software program (Statsoft, Tulsa, USA).

TABLE II - Description of assays using coded levels (-1, 0, +1) for each of the factors employed in the fractional design for intrinsic dissolution tests of amlodipine besylate

Assay	Compaction Pressure	Dissolution medium	Rotation Speed
1	-1	-1	-1
2	-1	0	+1
3	-1	+1	0
4	0	-1	+1
5	0	0	0
6	0	+1	-1
7	+1	-1	0
8	+1	0	-1
9	+1	+1	+1
10	0	0	0
11	0	0	0
12	0	0	0

Intrinsic dissolution

Intrinsic Dissolution tests were carried out on a VK 7010 dissolution apparatus (Varian Inc., Palo Alto, CA, USA), to which rotating discs containing about 250 mg of the drug, previously compressed by a hydraulic press (American Lab, Charqueada, SP, Brazil), were coupled. Each assay was performed in triplicate within vessels containing 900 mL of medium. Samples were collected at 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 minutes and quantified by ultraviolet spectrophotometry in a Cary 50 UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA), at a wavelength of 237 nm.

IDR values were obtained by plotting the amount of drug dissolved (mg) against time (minutes) on graphs. The slope of the curves yielded the dissolution rate of the drug in $mg.min^{-1}$, which, when divided by the surface area of the compressed drug (0.5 cm^2), gave the IDR of amlodipine besylate, according to Equation 1 (United States Pharmacopeia, 2012).

$$IDR = \alpha/A \qquad \text{(Equation 1)}$$

- IDR = intrinsic dissolution rate ($mg.cm^{-2}.min^{-1}$)
- α = slope of the amount of drug dissolved x time ($mg.min^{-1}$)
- A = surface area of the compressed drug (0.5 cm^2)

RESULTS AND DISCUSSION

Table III shows the IDR values obtained. It can be noted that the linear regression coefficients obtained were satisfactory, with values greater than or equal to 0.99. Furthermore, the values suggest that amlodipine besylate is a highly-soluble drug, according to the Biopharmaceutical Classification System.

Figure 2 plots the linear regression of the amount of API dissolved (mg) against time (min) required in order to obtain the dissolution rate of the drug. Some assays lasted less than three hours due to detachment of the compressed drug from the apparatus matrix. Thus, in order to calculate the IDR of amlodipine besylate, the time that the compressed drug remained in the matrix was also considered. Finally, the third test was not completed because the compressed drug detached right at the beginning of the assay. To enable statistical analysis, the IDR was considered to be greater than 5 $mg.cm^{-2}.min^{-1}$ in this case.

Table IV shows the estimated regression coefficients and significance tests of the components of the factors

TABLE III - IDR values obtained for each assay, with the respective linear regression coefficients (R^2)

Assay	IDR ($mg.cm^{-2}.min^{-1}$)	R^2
1	1.143	0.994
2	0.229	0.999
3	> 5	*
4	0.223	0.995
5	0.233	0.998
6	4.591	0.998
7	0.319	0.996
8	0.192	0.997
9	1.221	0.995
10	0.187	0.999
11	0.199	0.999
12	0.264	0.998

* Regression coefficient not obtained due to detachment of the compressed drug in the beginning of the test.

studied. It appears that the linear term of the pressure and rotational speed, as well as linear and quadratic terms of the dissolution medium, are those comprising the mathematical model that explains the intrinsic dissolution rate of amlodipine besylate (Equation 2).

$$IDR_{(Amlodipine)} = 1.400 - 0.773\ (CP) + 0.074\ (CP)^2 + 1.784\ (DM) - 0.628\ (DM)^2 - 0.708\ (RS) + 0.356\ (RS)^2 \quad \text{(Equation 2)}$$

where: CP = compaction pressure; DM = dissolution medium; RS = rotation speed.

Analysis of variance of the factors (taking into account the combination of linear and quadratic effects) showed that the dissolution medium was the variable with the greatest impact on the IDR of amlodipine besylate (Table V).

The Pareto chart (Figure 3) is consistent with the result obtained in the analysis of variance. It is possible to ascertain that, although the linear components of compression pressure and rotation speed exert an impact on the IDR (which explains the significant values found for these parameters, from a statistical standpoint), the effect caused by the dissolution medium is much more pronounced when compared to the other factors.

Additionally, the media graph (Figure 4) shows that the IDR of amlodipine besylate was not subject to large variations when either water or phosphate buffer pH 6.8 is used as a dissolution medium. However, there is a significant increase in the IDR value when HCl 0.1 M is employed as a medium.

As stated earlier, amlodipine besylate is highly-soluble in aqueous media, according to the Biopharmaceutics Classification System. However, the greatest IDR values were obtained when 0.1 M HCl was used as a dissolution medium, even causing the compressed material to loosen, regardless of the compaction pressure used (as observed in assays E3, E6 and E9). This behavior is due to the basic property of amlodipine besylate (pKa=8.7), indicating that it is much

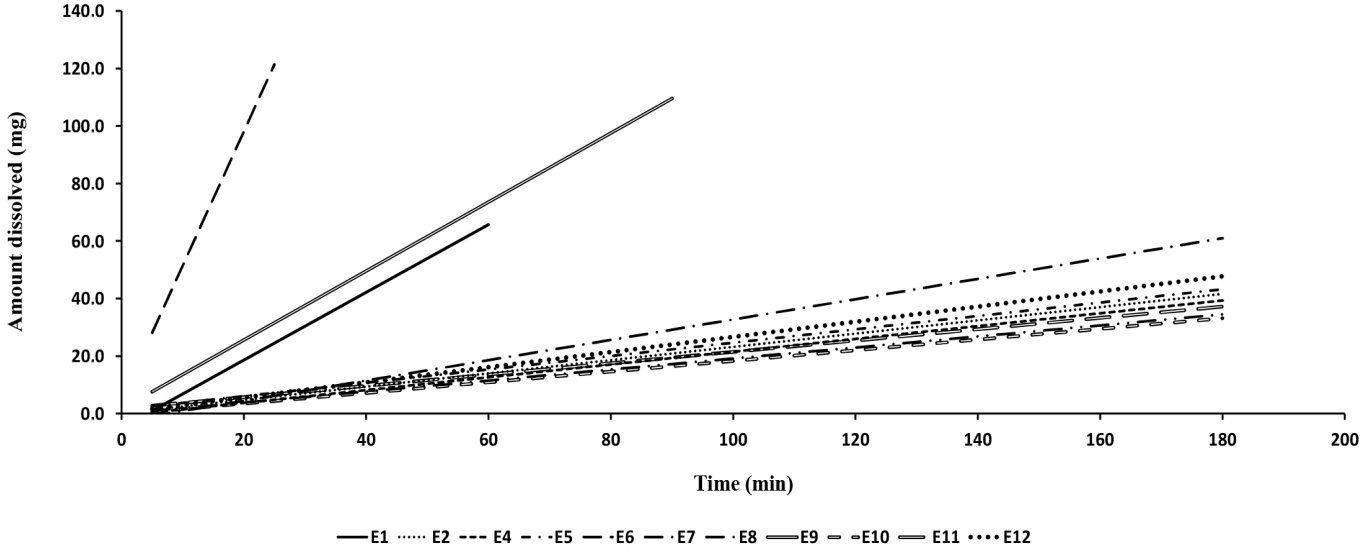

FIGURE 2 – Linear regression of the points pertaining to the amount of amlodipine besylate dissolved against time, in the intrinsic dissolution tests carried out according to Table III.

TABLE IV - Estimated regression coefficients and significance tests of linear (L) and quadratic (Q) components of the factors studied. Result is significant for p <0.05 (**bold**)

Components	Coefficient	P	Standard error	Confidence level (-95%)	Confidence level (+95%)
Intercept	**1.40**	**0.000**	**0.20**	**0.89**	**1.91**
Compaction pressure (L)	**-0.77**	**0.028**	**0.25**	**-1.43**	**-0.12**
Compaction pressure (Q)	0.07	0.723	0.20	-0.43	0.58
Dissolution medium (L)	**1.78**	**0.000**	**0.23**	**1.18**	**2.39**
Dissolution medium (Q)	**-0.62**	**0.032**	**0.21**	**-1.18**	**-0.07**
Rotation speed (L)	**-0.70**	**0.038**	**0.25**	**-1.36**	**-0.05**
Rotation speed (Q)	0.36	0.200	0.20	-0.31	0.71

TABLE V – Significance tests (ANOVA) for the factors studied. Result is significant for p < 0.05 (**bold**)

Factor	Sum of squares	Degrees of freedom	Mean of squares	F	p
Compaction pressure	3.64	2	1.82	4.67	0.071
Dissolution medium	**24.37**	**2**	**12.18**	**31.26**	**0.001**
Rotation speed	3.41	2	1.70	4.38	0.079
Error	1.95	5	0.38	**	**

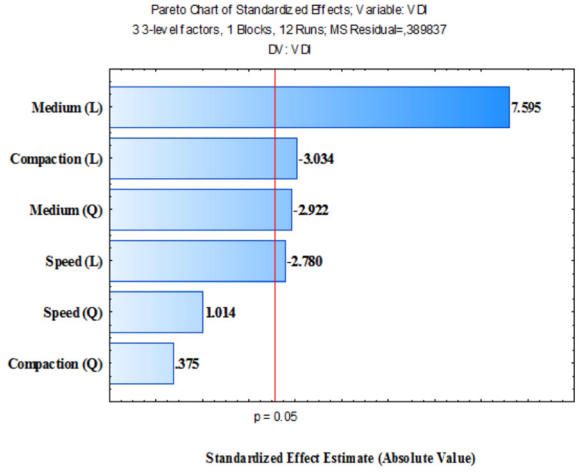

FIGURE 3 - Pareto chart representing the analysis of variance for linear (L) and quadratic (Q) components of the parameters studied. Results are significant for p > 0.05.

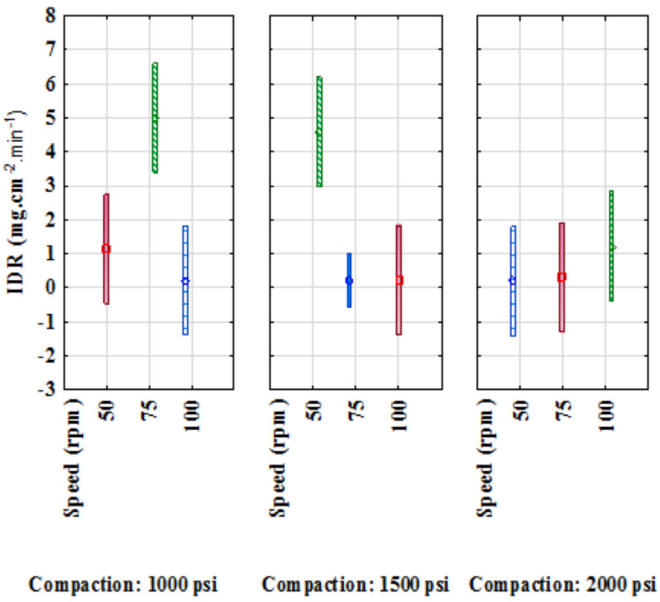

FIGURE 4 – Mean values of amlodipine besylate IDR ($mg.cm^{-2}.min^{-1}$). Error bars indicate the standard deviation of the media.

more soluble in an acidic medium (Shoin *et al.*, 2010; van Zwieten, 1994).

Analysis of the media graph enables us to infer that the compaction pressure has some influence on the dissolution of the material, but has no significant impact on the IDR of amlodipine besylate. In all the media studied, it can be noted that the 1000 psi compression pressure yielded the highest value for the intrinsic dissolution rate, which supports the theory that the dissolution medium can easily penetrate between the particles of this structure, increasing its wettability and hence facilitating dissolution of the compound. However, it is important to mention that, from a statistical standpoint, the results yielded by analysis of variance do not enable us to reject the null hypothesis;

i.e., that there is no relationship between compaction pressure and intrinsic dissolution rate.

The viability of the fractional factorial design (3^{3-1}) was ascertained through a residual analysis where a normal probability plot of the errors was designed, based on the residual values as a function of the expected IDR values. The points corresponding to the residual errors are randomly distributed near the line (Figure 5), indicating

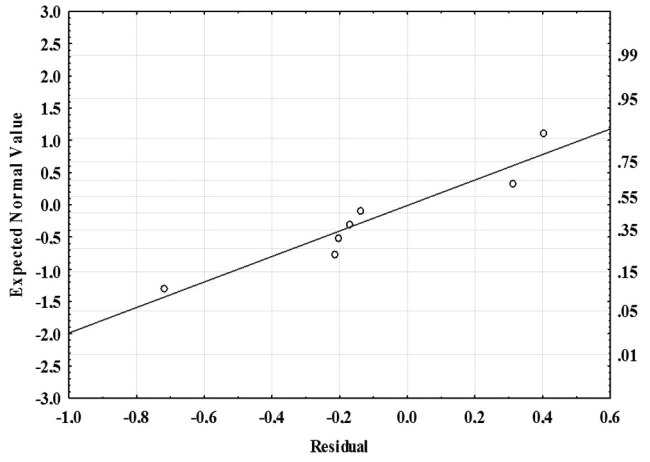

FIGURE 5 - Normal probability plot of errors, based on the residuals obtained from the ID values for amlodipine besylate.

the homogeneity of variances and validity of the fractional factorial model proposed for this study (Montgomery, 2001).

A comparison of the influence of the factors on the IDR, considered two by two, is represented in the surface-response graphs (Figure 6). It is evident that the lower the compaction pressure, the greater the tendency of the drug to loosen and solubilize, thus increasing the IDR. However, when compared to the dissolution medium, the compaction pressure itself does not seem significant, since the IDR increases significantly at all points when the drug is tested with HCl 0.1 M, which represents the highest level of the dissolution medium variable.

Finally, a comparison of the other two factors shows that the lower the compaction pressure and the higher the speed, the more the IDR of amlodipine besylate increases. However, the absence of a sharp curvature demonstrates that these factors are not significant in the response of the dependent variable, corroborating the results of the statistical analyses previously mentioned.

Based on these results, it is possible to establish the most favorable conditions for assessing the intrinsic dissolution rate of amlodipine besylate. The dissolution medium consisting of water only had the lowest IDR variability, which is especially evident in the media graph

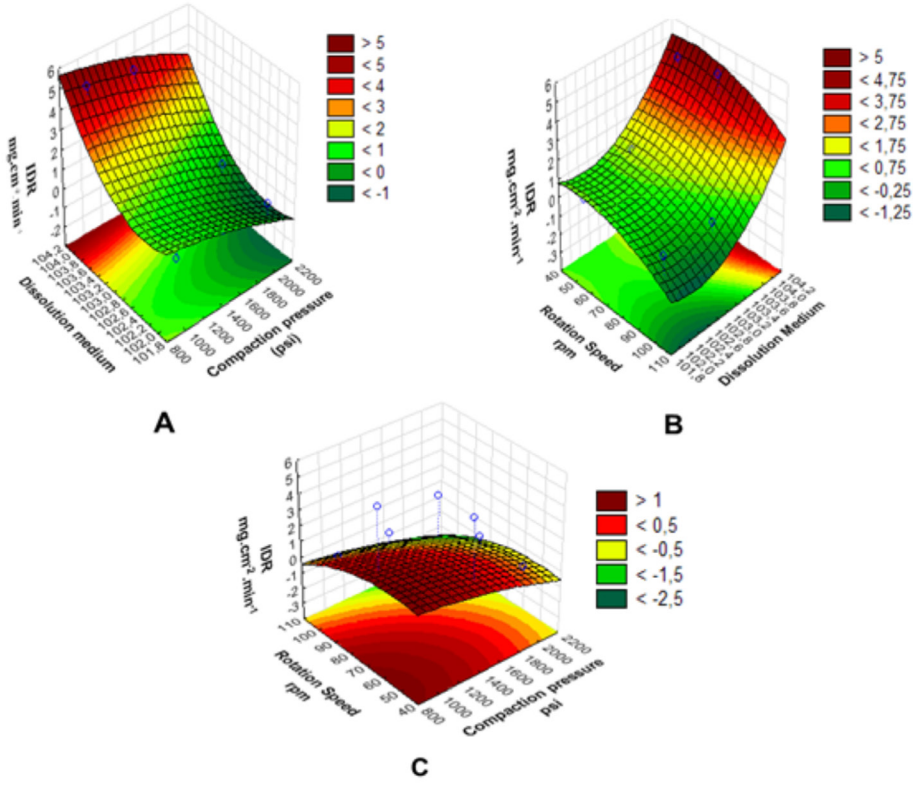

FIGURE 6 - Surface-response graphs for the IDR of amlodipine besylate compared to the studied factors (A) dissolution medium x compaction pressure, (B) dissolution medium x rotation speed, and (C) compaction pressure x rotation speed.

(Figure 3). Accordingly, using this dissolution medium at any pressure (1000 to 2000 psi) and speed (50-100 rpm) ensures the compacted drug does not disintegrate during intrinsic dissolution assay, maintaining a constant surface area and enabling a suitable investigation of the IDR of amlodipine besylate.

CONCLUSION

Based on these results, the IDR of amlodipine besylate was found to be affected mainly by the dissolution medium used for testing. The levels of compaction pressure and rotation speed employed had less impact on the dependent variable and, according to the statistical data, are not significant factors in the IDR of the drug.

ACKNOWLEDGEMENTS

The authors would like to thank the National Council of Scientific and Technological Development (CNPq) for the scholarship.

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Received for publication on 10th April 2013
Accepted for publication on 09th August 2013