

The Use of Response Surface Methodology to Evaluate the Impact of Level 2 SUPAC-IR Changes on the In Vitro Release of Metronidazole and Ranitidine from a Fixed-Dose Combination Tablet

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ABSTRACT

The purpose of this study was to evaluate the effect of different levels of disintegrant (croscarmellose sodium, CCS), binder (polyvinylpyrrolidone K30, PVP-K30), and lubricant (magnesium stearate) on the in vitro release of metronidazole (MTZ) and ranitidine (RTD) from a solid oral fixed-dose combination tablet. The excipient levels investigated were Level 2 changes in component and composition described in the Scale-Up and Post Approval Changes for Immediate Release (SUPAC-IR) guidance (1). Batches of tablets (1000 units) were manufactured by wet granulation using a Saral high-shear mixer granulator and a Manesty B3B rotary tablet press. Weight uniformity, friability, and disintegration of all tablets were assessed, and all batches complied with compendial specifications. The amount of drug released (Q) at ten minutes was dependent on the levels of CCS in the formulation, and the effect of PVP-K30 and magnesium stearate was dependent on the levels of CCS. Synergistic interactions between independent variables were observed for the Q_{10} value for RTD, whereas PVP-K30 and magnesium stearate exhibited an antagonistic effect on the Q_{10} values for MTZ and RTD. The use of response surface methodology facilitated an investigation into the effect of Level 2 component and composition changes, as described in SUPAC-IR, on the in vitro release of MTZ and RTD from a fixed-dose combination (FDC) solid oral dosage form (SODF).

INTRODUCTION

Statistical design of experiments (DOE) has been used in the pharmaceutical industry to facilitate the optimization of manufacturing processes, formulations, and analytical methods (2) for over three decades (3, 4). The use of DOE permits the formulation scientist to study simultaneously the effects of multiple factors that may impact or determine product quality during the optimization process (5, 6). Furthermore, mathematical models can be generated to produce graphical representations that describe the variability of responses from a system as a function of the predetermined input factors thought to impact the manufacturing procedure (5). The application of statistical design and mathematical equations in the development, improvement, or optimization of pharmaceutical processes is defined as response surface methodology (RSM) (6–9).

The use of RSM for the determination of significant input factors in the in vitro release of an active pharmaceutical ingredient (API) from dosage forms is a common phenomenon (10–12). However, in most cases, RSM has been used to establish the effect of input factors on the in vitro release of solid dosage forms that contain a single API (13, 14). Consequently, this study aimed to investigate the effect of Level 2 component or composition changes

described in SUPAC-IR on the in vitro release of a SODF that comprised two API in a fixed-dose combination.

MTZ and RTD are Class 1 and 3 compounds, respectively, as defined by the Biopharmaceutics Classification System (BCS) (15) and are both used for the treatment of peptic or duodenal ulcers (16). MTZ and RTD, both highly water-soluble, were selected to investigate the effect(s) of different levels of disintegrant, binder, and lubricant on the in vitro release profiles of these compounds from FDC tablets.

METHODS

Materials

RTD and MTZ were purchased from Changzhou Longcheng Medicine Raw Material Co., Ltd, (Changzhou City, Jiangsu, China) and Huanggang Hongya Pharmaceutical Co. Ltd (Huanggang, Hubei, China), respectively. Analytical standards for HPLC analyses were procured from Sigma Aldrich (St Louis, Missouri, USA). Microcrystalline cellulose PH 102 (MCC), polyvinylpyrrolidone-K30 (PVP-K30), croscarmellose sodium (CCS), colloidal silicon dioxide, and magnesium stearate were purchased from Aspen Pharmacare (Port Elizabeth, Eastern Cape, South Africa). Acetonitrile (200-nm UV cutoff) and methanol (215-nm UV cutoff) were purchased from Romil Ltd (Waterbeach, Cambridge, UK). Sodium hydroxide pellets and potassium dihydrogen orthophosphate were purchased from Merck Chemicals Ltd (Modderfontein,

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Table 1. Formulation Used for the Manufacture of MTZ and RTD Tablets

Raw material	Composition	
	(% w/w)	Actual Amount (g)
Ranitidine	16.8	84.0
Metronidazole	50.0	250.0
Microcrystalline cellulose	27.0	135.0
Polyvinyl pyrrolidone–K30	1.0	5.0
Croscarmellose sodium	4.0	20.0
Colloidal silicone dioxide	0.2	1.0
Magnesium stearate	1.0	5.0

Gauteng, South Africa), and triethylamine was procured from SaarChem Pty Ltd (Krugersdorp, Gauteng, South Africa).

Equipment

All raw materials were weighed using a top-loading analytical balance Model PM4600 (Mettler Instruments, Zurich, Switzerland) with a sensitivity of 0.01 g. Blending and granulation were undertaken using a Saral vertical axis high-shear mixer granulator fitted with a 3.5-L bowl (Saral Engineering Company, Vapi, India). A model 7521-001 Cole–Palmer peristaltic pump (Cole–Palmer Instruments Co., Barrington, Illinois, USA) consisting of a spray gun fitted with a pressure gauge was used for the addition of binder solution. The granules were dried using a Memmert dry-heat oven (Mettmer GmbH Co, Schwabach, Germany), and tablet compression was performed on a Manesty B3B rotary tablet press (Manesty, Speke, Liverpool, England) tooled with six 12-mm, flat-faced punches. All materials were sieved using wire cloth sieves conforming to DIN 4188 standards.

Method of Manufacture

RTD, MTZ, and MCC were weighed according to the formulation summarized in Table 1, passed through a screen of 850- μ m aperture size, and blended with half of the CCS at an impeller speed of 100 rpm. The resultant blend was granulated using a 12.5% m/v solution of PVP–K30 at impeller and chopper speeds of 100 and 1000 rpm, respectively. The granulation was mixed for a further minute after completion of wetting of the powder mass, and the power consumption of the equipment was used to establish the endpoint of granulation prior to tray drying in an oven set at 50 ± 0.5 °C for 24 h. The dried granules were passed through a 350- μ m screen and lubricated for an additional 3 min with previously screened colloidal silicone dioxide, magnesium stearate, and the remaining CCS (10 g). The lubricated granules

were compressed into 500-mg tablets at a press speed of 25 rpm to a target hardness of between 80 and 120 N.

Uniformity of Weight

The individual weights of ten randomly selected tablets were determined using a Model AG 135 top-loading electronic balance (Mettler Instruments, Zurich, Switzerland) with a sensitivity of 0.1 mg, and the average weight was calculated.

Friability

The friability of 20 randomly selected tablets was determined using a Model TA3R friabilator (Erweka GmbH, Heusenstamm, Germany). The compressed tablets were dedusted and weighed using a top-loading balance (Model PM 4600, Mettler Instruments, Zurich, Switzerland). The tablets were allowed to tumble at 25 rpm for 4 min (100 drops), removed, dedusted, reweighed, and the friability established.

Disintegration

Six tablets were selected randomly for the determination of disintegration time using a Model ZT 61 tablet disintegration apparatus (Erweka GmbH, Heusenstamm, Germany). Each tablet was placed into a cylinder of the basket rack and covered with a disc. The basket was set to oscillate vertically inside a beaker containing 700 mL of distilled water maintained at 37 ± 0.2 °C at a speed of 30 cycles/min. The time for disintegration of each tablet was recorded automatically on completion of the test.

Experimental Design

The evaluation of the main effects, interaction, and quadratic effects of the input and response variables was performed using a Box–Behnken statistical screening design that had three center points. The mathematical relationship between the input and output variables was generated using Design-Expert 8.0.4 software (Stat–Ease, Inc., Minneapolis, Minnesota, USA). The independent variable levels were established based on the Level 2 component and composition changes that have been described in SUPAC–IR (1), and these were studied at three levels (i.e., high, medium, and low). The levels and respective combination sequence of independent variables generated using the Box–Behnken approach is described in Table 2.

Statistical Analysis

The significance of the model and the model terms that were generated were analyzed using analysis of variance (ANOVA) type three (partial sum of squares) at a 5% level of significance using the statistical package Design Expert 8.0.4 (Stat–Ease, Inc., Minneapolis, Minnesota, USA). The predicted residual error sum of squares (PRESS) was used to assess which of the input factors had a significant impact on the measured response(s). A backward elimination procedure was used to fit data into the

Table 2. Levels and Combination Sequence of Independent Variables and Respective Codes Used for the Box–Behnken Design

Independent Variable	Levels Used, Actual (Coded)		
	Low (-1)	Medium (0)	High (+1)
x_1 = CCS (% w/w)	3	4	5
x_2 = PVP–K30 (% w/w)	0	1	2
x_3 = Magnesium stearate (% w/w)	0.5	1	1.5
Batch Number*	Independent Variables (%w/w)		
	x_1	x_2	x_3
RM 001	3.0	0.0	1.0
RM 002	5.0	0.0	1.0
RM 003	3.0	2.0	1.0
RM 004	5.0	2.0	1.0
RM 005	3.0	1.0	0.5
RM 006	5.0	1.0	0.5
RM 007	3.0	1.0	1.5
RM 008	5.0	1.0	1.5
RM 019	4.0	0.0	0.5
RM 010	4.0	2.0	0.5
RM 011	4.0	0.0	1.5
RM 012	4.0	2.0	1.5
RM 013□	4.0	1.0	1.0
RM 014□	4.0	1.0	1.0
RM 015□	4.0	1.0	1.0

*Indicates arrangement by standard order

□Indicates center-point formulations

different predictor equations, and the negligible effect of omission of nonsignificant factors was confirmed following comparison of the predicted and adjusted R^2 values of the full and reduced model. Furthermore, predicted versus actual data diagnostic plots were used to determine the goodness of fit of the proposed model to the experimental data.

In Vitro Release of Metronidazole and Ranitidine

The FDA suggests that a drug product is considered rapidly dissolving when no less than 85% of the label claim dissolves within 30 min in solutions of pH values that represent the gastrointestinal tract pH range (15). MTZ and RTD are classified as rapidly dissolving drugs according to BCS (15, 17, 18). The high solubilities of MTZ and RTD prevent the use of 30 min as a discriminatory sampling time point to determine the effect of formulation variables on drug release in any critical manner; therefore, 10 min was adopted as the discriminatory

Table 3. Weight Uniformity, Disintegration Time, and Friability Values for the Tablets

Parameter	Value
Weight uniformity (mg)	494.19 ± 3.15–509.91 ± 3.21
Disintegration time (min)	3.58 ± 0.49–11.41 ± 0.21
Friability (%)	<0.30

sampling time point. In vitro release studies of the FDC were performed using USP Apparatus 2 (Hanson Research SR 8 PLUS, Chartsworth, California, USA) fitted with an Autoplus Multifill and Maximizer Syringe Fraction Collector. Six tablets were dropped into the dissolution vessels each containing 900 mL of degassed 0.1 M HCl (pH 1.2). The paddles were set to rotate at 75 rpm since this speed represented a suitable compromise between the 50 and 100 rpm specifications described in the USP for RTD and MTZ, respectively. The temperature of the dissolution medium was maintained at 37 ± 0.5 °C, and 5-mL aliquots were collected for analysis at intervals of 10, 20, 30, and 45 min with replacement of an equal volume of fresh dissolution medium after removal of each sample. Correction of sample volume was performed prior to analysis of the dissolution data.

Analytical Method

MTZ and RTD were analyzed using a reversed-phase HPLC method that had previously been validated (19). Separation was achieved using a Nova-Pak C_{18} 3.9 × 150 mm HPLC cartridge column (Waters Corporation, Milford, Massachusetts, USA) and a mobile phase that comprised 9% (v/v) acetonitrile in phosphate buffer solution (50 mM) containing 0.1% (v/v) triethylamine at a pH of 6.7. A modular HPLC system (Thermo Separation Products, San Jose, California, USA) with a UV detector (Linear Instrument Co., California, USA) was used. The flow rate was set at 2 mL/min, and detection was achieved at 317 nm.

RESULTS AND DISCUSSION

Physicochemical Properties of Tablets

The weight uniformity, disintegration time (D_t), and friability of the compressed tablets complied with compendial specifications (Table 3).

In Vitro Release

For ease of determination of the quantitative and qualitative effects of input factors on drug release, the Q_{10} values for MTZ and RTD from formulations manufactured with different levels of input factors were compared with those from formulations manufactured with intermediate levels of excipient composition (i.e., the center formulations). The notation used to depict the formulations uses the ratios of CCS:PVP–K30:magnesium stearate, and this convention has been adopted for the following discussion. By way of example, the center-point formulations are denoted 40:10:10.

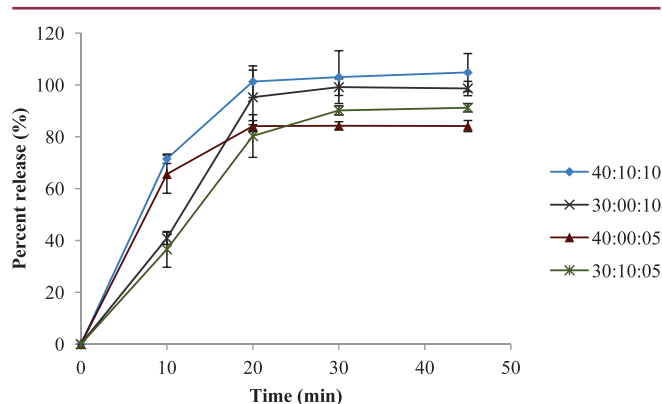


Figure 1. In vitro release profiles of MTZ for formulations containing CCS, PVP-K30, and magnesium stearate at low levels compared with the center formulation.

In Vitro Release of Metronidazole from Formulations with Low Levels of Input Factors

The effect of low levels of CCS, PVP-K30, and magnesium stearate in different ratios on the dissolution of MTZ is depicted in Figure 1. The center formulation exhibited 71% drug release within 10 min for MTZ, and 100% was released within 20 min of the commencement of dissolution testing. A reduction in the levels of CCS and the exclusion of PVP-K30 in formulation 30:00:10 resulted in a significant effect on the Q_{10} value for MTZ (i.e., 40% released); however, the total amount of MTZ released was unaffected. Formulation 40:00:05 exhibited a relatively minor decrease in Q_{10} for MTZ (i.e., 65%) when PVP-K30 was excluded and magnesium stearate was included at low levels; however, the overall amount of MTZ released was unaffected with 84% MTZ released after 45 min. A reduction in CCS and magnesium stearate to minimum levels (formulation 30:10:05) resulted in a dramatic reduction in Q_{10} for MTZ to 36%; however, approximately 91% MTZ was released within 30 min. The Q_{10} for MTZ is dependent on the content of CCS. Furthermore, the exclusion of PVP-K30 and the use of low levels of magnesium stearate have a relatively minor effect on the release of MTZ as evidenced by the changes observed for Q_{10} .

In Vitro Release of Ranitidine from Formulations with Low Levels of Input Factors

The effect of low levels of CCS, PVP-K30, and magnesium stearate in different ratios on the dissolution of RTD is depicted in Figure 2. The release of RTD from the center formulation exhibited a burst release similar to that of MTZ with a Q_{10} value of 67% and 89% RTD released within 20 min. The exclusion of PVP-K30 and the reduction of CCS to a lower level in formulation 30:00:10 resulted in a decrease in Q_{10} for RTD to 50%. However, the total drug released was unaffected since 92% RTD was released within 20 min. The reduction of magnesium stearate to 0.5% w/w and the exclusion of PVP-K30 in formulation 40:00:05 had a relatively minor effect on the Q_{10} for RTD compared with that observed for the center formulation. Formulation 40:00:05 exhibited a

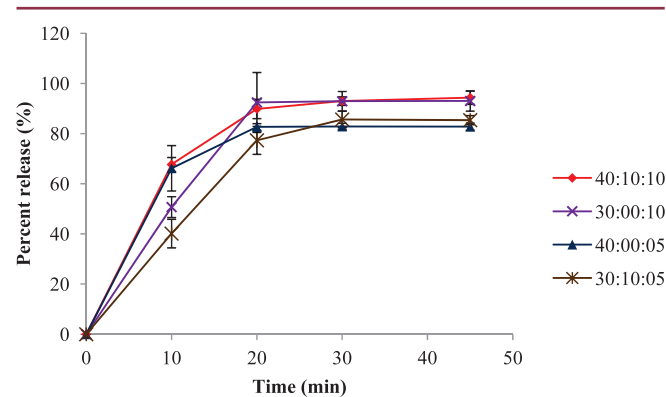


Figure 2. In vitro release profiles of RTD for formulations containing CCS, PVP-K30, and magnesium stearate at low levels compared with the center formulation.

Q_{10} of 66% for RTD, and 83% of RTD was released within 20 min. The reduction of both CCS and magnesium stearate to low levels (i.e., 3 and 0.5% w/w, respectively) in formulation 30:10:05 resulted in a Q_{10} of 40% for RTD and an almost two-fold increase in the extent of drug released to a value of 77% after 20 min compared with the Q_{10} , and 85% release was achieved within 30 min. Therefore, it is clear that the dissolution of RTD also depends on the amount of CCS in the formulation. Furthermore, magnesium stearate and PVP-K30 exhibited relatively minor effects on the dissolution of RTD compared with that observed for the center formulation. However, at low levels of CCS (i.e., 3% w/w in formulations 30:00:10 and 30:10:05), the dissolution profiles suggest that PVP-K30 has a greater retardation effect on the dissolution of RTD compared with the effect observed for magnesium stearate when using this Q_{10} approach to evaluate the data.

In Vitro Release of Metronidazole from Formulations with High Levels of Input Factors

The effects of high levels of CCS, PVP-K30, and magnesium stearate on the release of MTZ from the tablets are depicted in Figure 3. Formulation 50:20:10, in which high concentrations of CCS and PVP-K30 were used, exhibited a burst release for MTZ with a resultant Q_{10} of 82% and 97% of MTZ released after 20 min. High concentrations of CCS and magnesium stearate (50:10:15) had a relatively minor impact on the Q_{10} for MTZ compared with that observed for the center formulation. Increasing the amount of PVP-K30 and magnesium stearate to 2% and 1.5% w/w, respectively, as for formulation 40:20:15, resulted in a 46% decrease in Q_{10} for MTZ where 71% was observed for the center formulation. It is apparent that the Q_{10} for MTZ is dependent on the amount of CCS used in the formulation. Moreover, the effects of PVP-K30 and magnesium stearate on the Q_{10} of MTZ are also dependent on the CCS content. At high concentrations of CCS (i.e., 5% w/w per tablet), variations in the amounts of PVP-K30 (i.e., formulation 50:20:10) and magnesium stearate (i.e.,

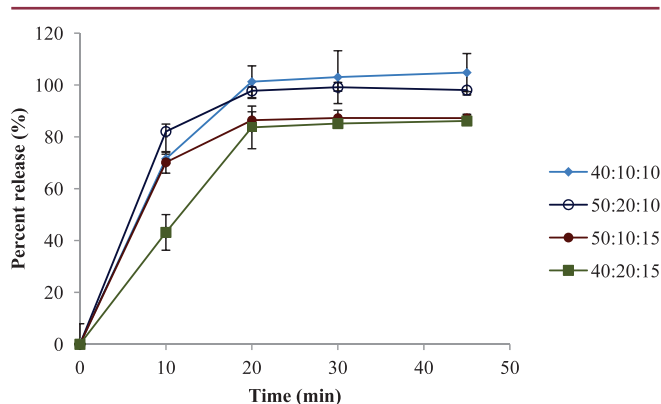


Figure 3. In vitro release profile of MTZ for formulations containing CCS, PVP-K30, and magnesium stearate at high levels compared with the center formulation.

formulation 50:10:15) exert a relatively minor effect on the Q_{10} of MTZ. However, when the content of CCS is held constant at 4% w/w as in formulation 40:20:15, an increase in PVP-K30 and magnesium stearate content results in a significant decrease in the Q_{10} value for MTZ. The effect on dissolution of varying the amount of binder at constant levels of disintegrant has been reported (20). The decrease in the value for Q_{10} observed following an increase in the content of PVP-K30 and magnesium stearate might be due to a combination of factors (e.g., formation of viscous or hydrophobic films, respectively). The inclusion of a high concentration of binder in a formulation may result in the formation of a viscous film on the surface of the tablet with a subsequent retardation of the dissolution of the API (21).

In Vitro Release of Ranitidine from Formulations with High Levels of Input Factors

The effects of high levels of CCS, PVP-K30, and magnesium stearate on the release of RTD are depicted in Figure 4. The in vitro dissolution profile of formulation 50:20:10, which was manufactured using high amounts of CCS and PVP-K30 (i.e., 5% and 2% w/w per tablet, respectively) shows that a Q_{10} value of 62% was obtained for RTD. The total RTD released (86%) was observed within 20 min. A similar profile was also observed for formulation 50:10:15 in which an increase in the content of CCS and magnesium stearate resulted in a Q_{10} of 72% and 90% RTD released within 20 min. It is apparent that intermediate levels of PVP-K30 (i.e., 1% w/w per tablet) and high levels of magnesium stearate and CCS facilitate the release of RTD. This observation indicates a possible synergistic interaction between CCS and magnesium stearate, which promotes the release of RTD.

In contrast, an increase in the content of magnesium stearate and PVP-K30 and the use of a constant level of CCS (i.e., formulation 40:20:15) results in the opposite effect. The Q_{10} for RTD was reduced to 40% compared with that of 67% observed for the center formulation. The low Q_{10} of 40% may be attributed to the high content of

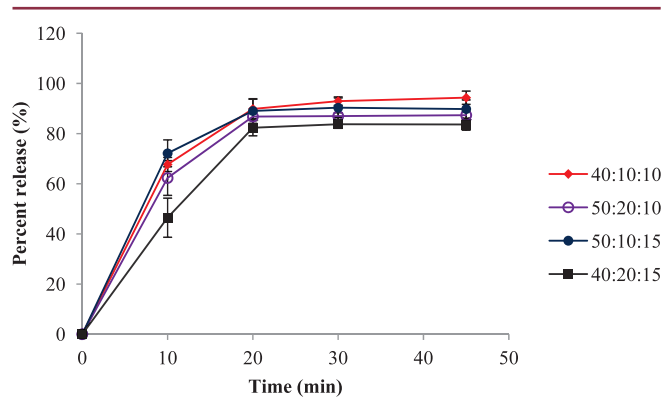


Figure 4. In vitro release profiles of RTD for formulations containing CCS, PVP-K30, and magnesium stearate at high levels compared with the center formulation.

PVP-K30 and magnesium stearate (21, 22). It is apparent that the overall Q_{10} values for formulations that contain high and low amounts of CCS are similar, which suggests that CCS has a significant impact on the rate of drug release from these dosage forms. Further, the impact of PVP-K30 and magnesium stearate on drug release is dependent on the content of CCS, which validates the fact that CCS is an important formulation factor that determines the rate of drug release. However, the relationship between the factors and their levels and impact on the rate of drug release is complex and involves some degree of interaction, as shown for formulation 50:10:15 depicted in Figure 4. Therefore, RSM was used to investigate and explore the nature of this relationship and its potential impact on the release of MTZ and RTD.

Response Surface Modeling

The significance of the model terms and a model fit comparison for percent drug release in 10 min (Q_{10}) are summarized in Table 4. The resultant polynomial equations used for the investigation of the effect of input factors on Q_{10} values for MTZ and RTD are described in eqs 1 and 2, and the predicted versus actual diagnostic plots for the response variable Q_{10} of both compounds are shown in Figure 5.

$$y_1 = 72 + 22.94x_1 - 5.98x_2 - 3.88x_3 - 9.72x_1^2 - 4.76x_2^2 - 10.83x_3^2 \quad (1)$$

$$y_2 = 65.16 + 14.78x_1 - 7.64x_2 - 1.17x_3 + 5.16x_1x_3 - 8.65x_1^2 - 6.19x_3^2 \quad (2)$$

Evaluation of eq 1 reveals that CCS has a synergistic effect on the release of MTZ, whereas PVP-K30 and magnesium stearate have the opposite effect. These results are supported by the in vitro release profiles depicted in Figures 1 and 2. Similar results were also observed following an investigation into the effect of disintegrant, filler ratio, and lubricant levels on the in vitro release of propranolol hydrochloride (13).

Table 4. Significant Model Terms and Model Fit Comparison of In Vitro Release of MTZ and RTD

Factor	RTD		MTZ	
	p value	Significance	p value	Significance
x_1 - CCS	<0.0001	Significant	<0.0001	Significant
x_2 - PVP-K30	0.0002	Significant	0.0073	Significant
x_3 - Magnesium stearate	0.3381	Not significant	0.0491	Significant
x_1x_2	0.7195	Not significant	0.1579	Not significant
x_1x_3	0.0131	Significant	0.5014	Not significant
x_2x_3	0.3020	Not significant	0.3271	Not significant
x_1^2	0.0009	Significant	0.0043	Significant
x_2^2	0.4609	Not significant	0.0894	Not significant
x_3^2	0.0063	Significant	0.0023	Significant

Model	PRESS	
	RTD	MTZ
Linear	1000.83	1463.31
Quadratic	367.24	682.17

Similarly, CCS has a synergistic effect on the release of RTD, whereas PVP-K30 and magnesium stearate have an antagonistic effect as described in Equation 2. Furthermore, CCS and magnesium stearate exhibit a synergistic interaction on the release of RTD as can be seen from the positive value of the coefficient for this factor.

The impact of independent factors (i.e., CCS, PVP-K30, and magnesium stearate) at different levels on the in vitro release rate of MTZ and RTD is shown on contour and three-dimensional response surface plots in Figures 6–8 and 9–11, respectively.

The three-dimensional response surface plot in Figure 6 depicts a curvilinear relationship between the factors and the response. The highest Q_{10} for MTZ $\geq 80\%$ is achieved when the levels of CCS are high and when those for PVP-K30 are between a low and intermediate level. In addition, $\geq 80\%$ MTZ is released when the amounts of CCS and PVP-K30 per tablet are >21 mg and <8 mg, respectively. It is evident that the antagonistic effect of PVP-K30 in the attainment of $Q_{10} \geq 80\%$ for MTZ is achieved when the content of PVP-K30 per tablet is >8 mg, at which point an increase in the content of CCS to maximum levels of 25 mg per tablet resulted in $<80\%$ drug release. A similar antagonistic effect was observed when the amount of CCS per tablet was $15 < x_1 < 21$ mg and a decrease in the Q_{10} for MTZ was exhibited when the content of PVP-K30 was >8 mg per tablet. It can be concluded that a $Q_{10} \geq 80\%$

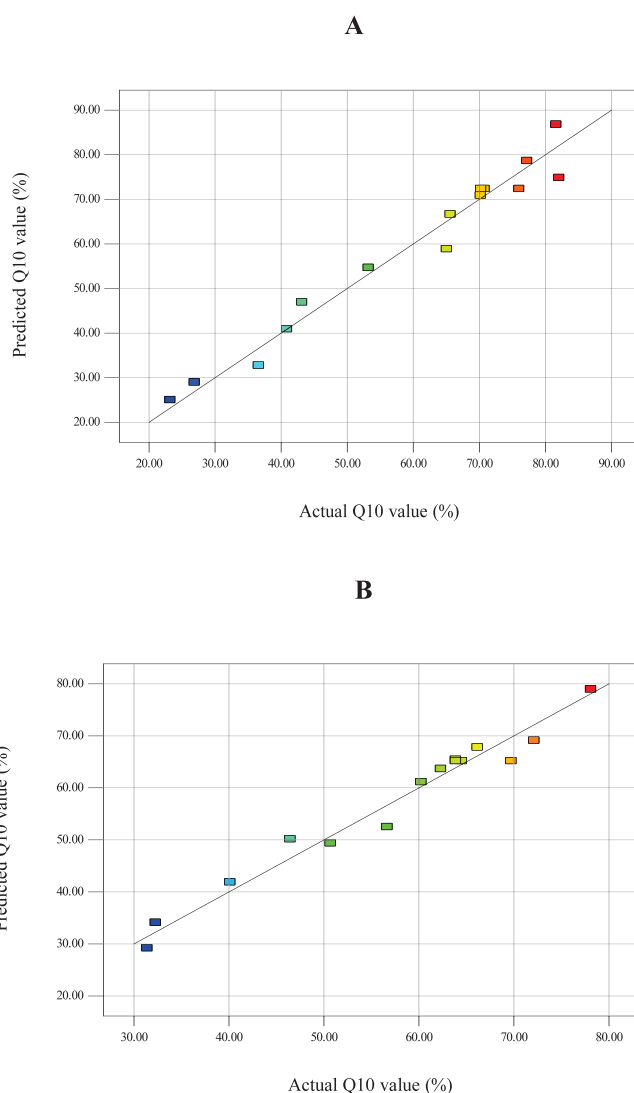


Figure 5. Predicted versus actual diagnostic plots for Q_{10} values of (A) MTZ and (B) RTD.

for MTZ may be achieved when the amount of CCS and PVP-K30 per tablet is >23 mg and <6 mg, respectively, and when an intermediate level of magnesium stearate is included in the formulation.

In general, the impact of magnesium stearate and PVP-K30 on the Q_{10} value for MTZ is minor in comparison with the effect of CCS, as shown in Figure 7. It is clear that both factors have an antagonistic effect on drug release, and an increase in either factor results in a decrease in the value of Q_{10} for MTZ. This effect is apparent when the amount of PVP-K30 is >6 mg per tablet and may in part be attributed to the highly hydrophilic nature of MTZ, which would require high concentrations of PVP-K30 to retard drug release by creating a viscous film on the tablet surface. A Q_{10} value $\geq 70\%$ for MTZ is achieved when the content of magnesium stearate per tablet (x_3) is between 2.5 and 6.5 mg and that of PVP-K30 is less than 6 mg with CCS at an intermediate level of 20 mg per tablet.

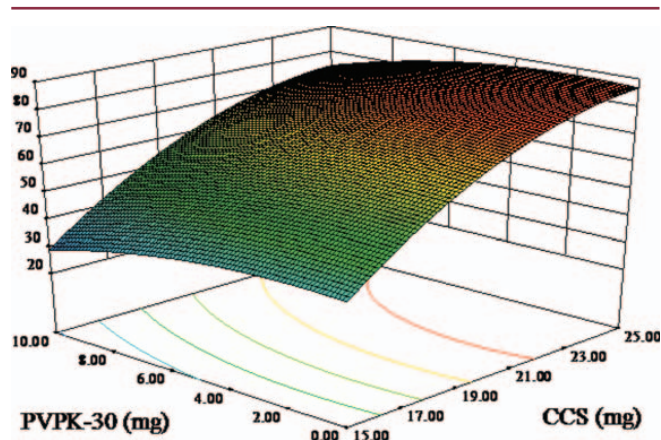


Figure 6. Three-dimensional response surface plot showing the impact of CCS and PVP-K30 on the Q_{10} value for MTZ at intermediate levels of magnesium stearate.

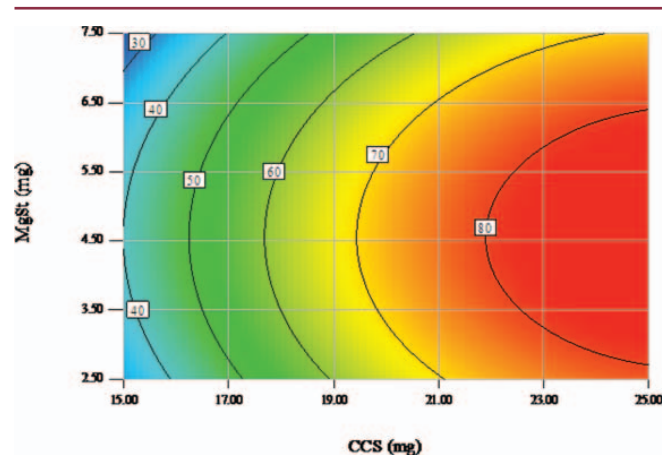


Figure 8. Contour plot showing the impact of magnesium stearate and CCS levels on the Q_{10} value for MTZ at intermediate levels of PVP-K30.

Magnesium stearate exhibited an antagonistic effect, similar to the effects observed with PVP-K30 on the dissolution of MTZ as shown in the contour plot in Figure 8. However, this effect is marked when the content of magnesium stearate per tablet is >5.5 mg. These results are supported by the in vitro release profile of formulation 40:20:15 depicted in Figure 3. The decrease in drug release observed at concentrations of magnesium stearate $>5\%$ w/w may be attributed to the hydrophobic nature of magnesium stearate that retards dissolution of the hydrophilic MTZ.

It is also evident that an increase in the levels of CCS resulted in an increase in drug release; however, a high value for $Q_{10} \geq 80\%$ for MTZ was observed when the content of CCS and magnesium stearate per tablet was >22 mg and <6.5 mg, respectively, as shown in Figure 8.

The three-dimensional response surface plot for the Q_{10} value for RTD is shown in Figure 9. It is curvilinear for the

relationship of this parameter with the amount of CCS as an input factor, whereas a linear relationship is observed for the effect of PVP-K30 levels on this measurement. As for MTZ, CCS has a profound effect on the release of RTD. An increase in the amount of CCS in the formulation results in an increase in the value of Q_{10} for RTD; for a decrease in the amount of CCS, the converse is true. In contrast, PVP-K30 appears to exert an antagonistic effect on the value for Q_{10} for RTD, and the impact is greater when the amount of CCS per tablet is <17 mg per tablet. At low levels of CCS, the D_t of the tablets is increased as a consequence of the low content of CCS (23). The increase in D_t results in a lower value of Q_{10} for RTD, since the total surface area necessary for dissolution is smaller and therefore the impact of PVP-K30 on D_t is more apparent. The response surface plot depicted in Figure 9 shows that a high Q_{10} value of ≥ 78 is achieved when the amount of CCS is >23 mg per tablet, when PVP-K30 is omitted from

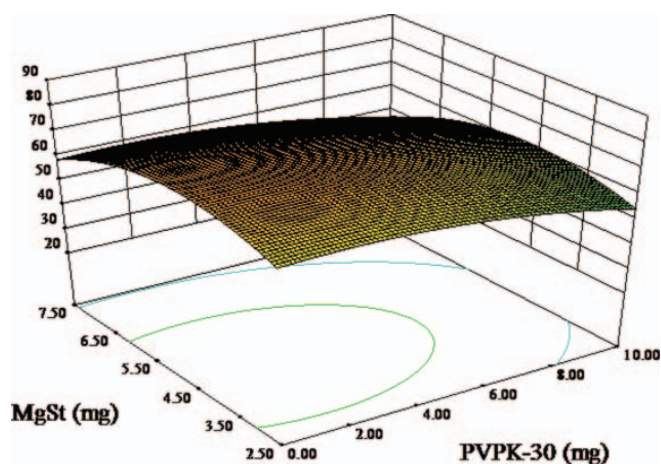


Figure 7. Three-dimensional response surface plot depicting the impact of magnesium stearate and PVP-K30 on the Q_{10} value for MTZ at intermediate levels of CCS.

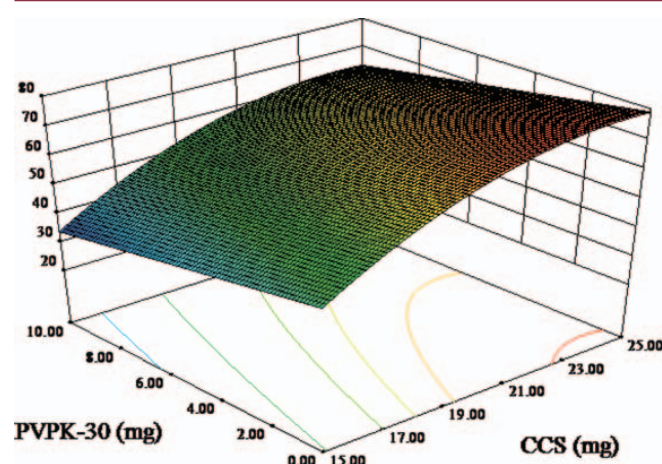


Figure 9. Three-dimensional response surface plot showing the impact of PVP-K30 and CCS on the Q_{10} value for RTD at intermediate levels of magnesium stearate.

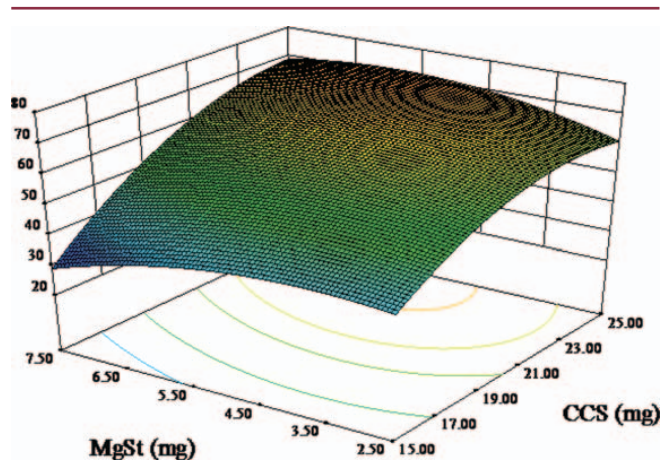


Figure 10. Three-dimensional response surface plot showing the impact of magnesium stearate and CCS on the value of Q_{10} for RTD at intermediate levels of PVP-K30.

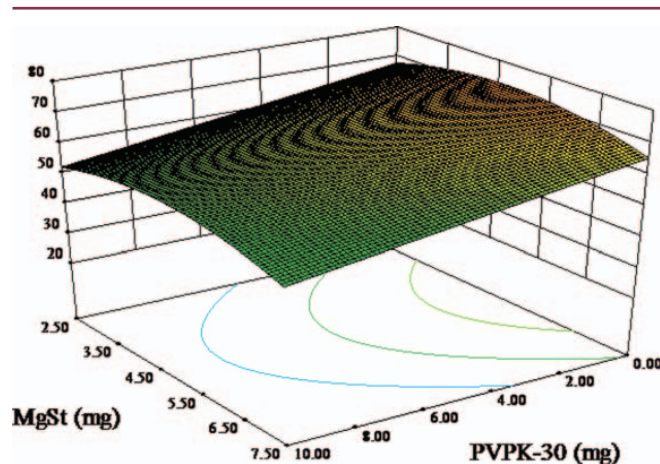


Figure 11. Three-dimensional response surface plot depicting the impact of PVP-K30 and magnesium stearate on the value of Q_{10} for RTD at intermediate levels of CCS.

the tablet formulation and intermediate levels of magnesium stearate are used.

In contrast to the effects of magnesium stearate on the value of Q_{10} for MTZ, different results are observed when evaluating the Q_{10} value for RTD. According to BCS, metronidazole is a Class 1 compound (i.e., highly soluble and highly permeable), and ranitidine is a Class 3 compound (i.e., highly soluble, poorly permeable). Consequently, the variability in physicochemical properties of these APIs is unlikely to have an impact on the Q_{10} value since we are investigating the in vitro rate of solution, which is related to solubility and not the permeability of the molecules. A curvilinear relationship is exhibited for the effect of CCS and magnesium stearate on the value for Q_{10} for RTD, and a region corresponding to maximum value for $Q_{10} \geq 70\%$ is located between intermediate levels of magnesium stearate and high levels of CCS as depicted in Figure 10. These results suggest that a synergistic interaction exists between CCS and magnesium stearate at these levels (i.e., ≥ 23 and $4.5 \leq x_3 \leq 6.5$ mg per tablet, respectively). Although this synergistic interaction has not been reported, the results are in agreement following an investigation of drug and excipient interactions with lubricants (24). Close inspection of the in vitro release profile for formulation 50:10:15 as shown in Figure 4 and evaluation of the resultant model equation for the parameter y_2 show that a synergistic interaction between CCS and magnesium stearate is evident.

The response surface plot depicted in Figure 11 reveals a linear antagonistic relationship between PVP-K30 and magnesium stearate on the value of Q_{10} for RTD. It is clear that PVP-K30 exerts a greater effect on the Q_{10} value than magnesium stearate, and consequently, the Q_{10} value is not affected with an increase in the levels of magnesium stearate in the formulation. The effect of magnesium stearate is more apparent at low levels of PVP-K30

(i.e., < 2 mg per tablet), as indicated by a Q_{10} value of $< 70\%$ for RTD when the amount of lubricant per tablet is ≥ 6.5 mg and where intermediate levels of CCS are used.

CONCLUSIONS

The use of RSM has facilitated the evaluation of the impact of SUPAC-IR Level 2 changes on the value of Q_{10} for the fixed-dose combination tablets containing MTZ and RTD manufactured in these studies. Three-dimensional and contour-response surface plots clearly demonstrate the impact of the different levels of CCS, PVP-K30, and magnesium stearate on the in vitro release of MTZ and RTD. The response surface plots show that CCS is the most significant factor that affects the measured responses, and the effects of PVP-K30 and magnesium stearate are dependent on CCS levels. Moreover, magnesium stearate has a synergistic interaction with CCS, thereby promoting the in vitro release of RTD. Further investigation is warranted to determine the nature of interaction between CCS and magnesium stearate that promotes the dissolution of RTD. The use of this approach has permitted the identification of significant factors for the formulations studied in this research and provides a framework for further investigation to solving formulation challenges.

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