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Using an Experimental Design to Identify and Quantify the Effects of Environment Related Test Parameters on the In Vitro Mucoadhesivity Testing of a Propanolol Buccal Tablet

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ABSTRACT This study sought to identify and quantify the effects of environmental test parameters on the mucoadhesivity of a propranolol tablet. Their effects on Maximum Detachment Force (MDF) measurements were evaluated using a Box-Behnken design matrix. Prehydration time (PT) had a statistically significant negative main effect while contact force (CF) had no significant effect on in vitro MDF measurements. While contact time (CT) had no significant main or quadratic effects, it had a positive interaction effect with PT. The mathematical model was statistically validated and a PT of 3.5 min and a CT of 5 min was proposed for mucoadhesion testing by the tensile method during formulation optimization.

KEYWORDS Mucoadhesivity test methods, Propranolol, Polymeric tablet, Experimental design

INTRODUCTION

Mucoadhesion is a phenomenon where two surfaces, one of which is a mucous membrane, adhere to each other. Over the last two decades, this concept has received increasing interest for its potential to enhance localized drug delivery by retaining a dosage form at the site of action (i.e., within the gastrointestinal tract) or systemic delivery by retaining a formulation in intimate contact with the absorption site (e.g., the buccal mucosa) (Smart, 2005). Delivery systems that have been designed for mucoadhesive drug delivery include, inter alia, tablets, patches, films, disks and microspheres, ointments and gels (Ahuja et al., 1997; Salamat-Miller et al., 2005). These systems are characterized by being formulated with macromolecules/polymers that are mucoadhesive. Since retention on the mucosa and hence mucoadhesivity is an important characteristic for a drug delivery system, its assessment during formulation optimization is essential. These tests not only enable the screening of a large

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number of candidate mucoadhesives, but also the study of their mechanisms (Park & Park, 1990). Furthermore, these tests are also important during the design and development of a mucoadhesive controlled release system, as they supply invaluable data regarding compatibility, physical and mechanical stability, surface analysis and mucoadhesive bond strength (Peppas & Buri, 1985). Despite its importance, there are currently no official methods or equipment for assessing the mucoadhesivity of drug delivery systems. There are therefore several different methods of mucoadhesivity testing currently being used by various research groups working in this area. Most in vitro methods are based on the measurement of either tensile or shear stress (Takeuchi et al., 2005). Smart et al. (1984) proposed the use of the Wilhelmy plate method which is usually applied to determine surface tension. There are also reports on using a rheological approach (Hassan & Gallo, 1990) and more recently, textural analysis (Martin et al., 2003). Methods using tensile strength usually measure the force required to break the adhesive bond between a model membrane and the system under investigation. Further, widely differing test parameters at varying levels, even for the same test methods, have been reported in the literature (Caramella et al., 1994; Jones et al., 1997; Tamburic & Craig, 1997). There has been some recognition that certain dosage forms may also produce method-dependent results (Robert et al., 1988). Unfortunately, the lack of standardized techniques often leads to discordant and unclear results. Hence, an increasing number of papers are recently emphasizing the need for further studies on mucoadhesivity test methods for evaluating the performance of materials and dosage forms (Accili et al., 2004; Rossi et al., 2005; Takeuchi et al., 2005).

Mucoadhesivity measurements performed by recording the maximum detachment force of a dosage form from the mucin/mucosa can be influenced by numerous parameters (Ahuja et al., 1997). These include its intrinsic polymeric properties, the environment under which the test is undertaken and physiological variables. The effects of polymer-related factors warrant assessment during formulation optimization while physiological variables are relevant during in vivo studies. Environment-related test parameters have been shown to significantly influence in vitro mucoadhesivity testing. Some of the commonly identified environmental parameters known to affect in vitro testing between the dosage form and the substrate include: contact force, type of model substrate, contact time, pH, swelling and prehydration time (Ahuja et al., 1997; Hagerstrom & Edsman, 2001; Hagerstrom et al., 2000; Tobyn et al., 1995).

While some studies have reported the effects of some of the environmental conditions on mucoadhesivity, these have been done separately and using different methods (Chary et al., 1999; Tobyn et al., 1995). Hence, there remains a need to define suitable environmental parameters in combination for specific in vitro test conditions. While statistical experimental design is an attractive tool for product and method development, at present, there is however a lack of studies which have focused on a statistical approach to identifying and quantifying the effect of environmental parameters on in vitro mucoadhesion testing of dosage forms such as tablets. Such studies will be useful for contributing to a mechanistic understanding of the mucoadhesion phenomenon, will contribute to identifying specific parameters for a dosage form under investigation; and importantly contribute to future method identification and development of standardized official methods. In addition, it as also essential to identify suitable mucoadhesivity test parameters for a specific drug delivery system such as a buccal tablet and to validate the method.

The aim of this experimental procedure was to identify and quantify the effect of primary environment-related variables, namely prehydration time (PT), contact time (CT) and contact force (CF) on the mucoadhesive potential of propranolol HCl tablets, using the Box-Behnken response surface experimental design and tensile method of testing. Propranolol HCl a β-blocker used in the treatment of various cardiovascular disorders (Corbo et al., 1990), was chosen as a model drug since it is being widely investigated in the literature for buccal delivery (Akbari et al., 2004; Chen & Hwang, 1992; Guyot & Fawas, 2000; Remunan-Lopez et al., 1998). It is an ideal model drug for incorporation into a controlled release buccal formulation due to its short half-life (3–6 hr), low molecular weight and its extensive and highly variable first pass metabolism following oral administration (Gomeni et al., 1997). A formulation containing propranolol HCl and poly (acrylic acid) PAA was selected and subjected to analysis, following the design matrix. The mathematical model generated was employed to quantify and identify significant effects and to also propose test

variables for future mucoadhesion measurements for a propranolol mucoadhesive buccal tablet using the current method.

MATERIALS AND METHODS

Materials

Propranolol HCl was purchased from Frankel Chemicals [SA]. Poly (acrylic acid) 2100 and mucin were obtained from Sigma-Aldrich [UK]. Other excipients used to prepare the mucoadhesive tablets were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Methods

Preparation of Mucoadhesive Tablets

Flat-faced tablets (300 mg, 10 mm in diameter, 2.8–3 mm in thickness) were prepared using a Carver Press (Beckman, Scotland). Compaction pressures ranging from 2 to 5 MPa were used in order to produce a suitable tablet hardness within a range of 70–90 N. These parameters hence ensured that a friability of $<$1% was maintained (BP, 2002). The formulation comprised of propranolol HCl (80 mg), Poly (acrylic acid) (30 mg), magnesium stearate (3 mg) and dicalcium orthophosphate (187 mg).

Experimental Design

A Box-Behnken experimental design was employed in this study to statistically identify and quantify the effects of environment related parameters on mucoadhesivity testing. Response surface methodologies, such as the Box Behnken and Central Composite designs, model possible curvature in the response function (Dayal et al., 2005). The Box Behnken design was specifically selected since, in cases involving 3 or 4 factors, it requires fewer treatment combinations than a Central Composite Design. The Box Behnken design is also rotable and contains statistical "missing corners" which may be useful when the experimenter is trying to avoid combined factor extremes. This property prevents a potential loss of data in such cases.

Generation and evaluation of the statistical experimental design were performed with the Microsoft Excel 2002 Add-In, Essential Regression and Experimental Design software Version 2.2 (USA). The factors studied were contact force (CF), prehydration time (PT) and contact time (CT). These parameters were chosen as they were considered to have both from preliminary studies and the literature a significant effect on in vitro mucoadhesion testing. The levels were selected based on values previously investigated during experimental trials in our laboratories. The inclusion of center points provided a more precise estimate of experimental error and provided a measure for the adequacy of the model (lack of fit error significance). It also enabled the determination of the significance of the main, interaction and quadratic effects, i.e., which coefficients in the second order model were significantly non-zero. The response variable was the maximum detachment force (MDF). A design matrix comprising of 16 experimental runs was constructed. An interactive second order polynomial model was utilized to evaluate both the response variables:

$$Y = b_0 + b_1 * X_1 + b_2 * X_2 + b_3 * X_3 + b_4 * X_1 * X_1 + b_5 * X_2 * X_2 + b_6 * X_3 * X_3 + b_7 * X_1 * X_2 + b_8 * X_1 * X_3 + b_9 * X_2 * X_3 \quad (1)$$

Where b_0–b_9 are the regression coefficients, X_1, X_2, X_3 are the factors studied and Y is the measured response associated with each factor level combination. Stepwise forward and backward regression produced a model containing only the significant terms. The resulting equation was also subjected to a statistical evaluation and model simplification at a 95% significance level. Table 1 summarizes the factors and their levels and Table 2 summarizes the design matrix with the experimental runs, factor levels and combinations and the measured response i.e., MDF.

TABLE 1 Variables and Levels of Box-Behnken Design

Independent variables			
Factor coding	High level	Intermediate level	Low level
$X_1 \rightarrow$ PT	0.5	3.5	6.5
$X_2 \rightarrow$ CT	1.0	5.0	9.0
$X_3 \rightarrow$ CF	0.0	5.0	10.0
DEPENDENT VARIABLE			
Y1 $\rightarrow$ Maximum Detachment Force (MDF)			

TABLE 2 Experimental Matrix Illustrating Levels of Environment-Related Parameters and Results Obtained for MDF (*denotes center point measurement)

Experiment number	PT (min)	CT (min)	CF (g)	MDF (mN)
1*	6.5	9	5	462
2*	3.5	5	5	182
3	3.5	9	10	340
4	3.5	5	5	177
5	3.5	1	0	83
6	0.5	5	0	257
7*	6.5	5	10	181
8	3.5	5	5	222
9	3.5	1	10	112
10	3.5	9	0	260
11*	3.5	5	5	200
12	6.5	5	0	162
13	6.5	1	5	81
14	0.5	1	5	101
15	0.5	9	5	177
16	0.5	5	10	359

For all optimization procedures the Solver function in the Essential Regression and Experimental Design software, which incorporated the generalized linear gradient algorithm (GRG-2 algorithm), was used.

Measurement of the Maximum Detachment Force as the Response Variable

Mucoadhesivity was measured using a Lutron Digital force gauge (FG5000A, Korea). A petri dish containing mucin (30% w/w), was placed in a thermostatically controlled water bath (37.0 ± 0.5°C). The mucoadhesive tablet was attached to one side of a double-sided metal disk using cyanoacrylate adhesive. The tablet surface was hydrated with 15 µL phosphate buffered saline (PBS) pH 6.8 as per the design matrix. Experimental parameters, namely, prehydration time (PT), contact force (CF) and contact time (CT) were investigated as outlined in the design matrix (Table 2). The double-sided disk containing the tablet was then attached to the Lutron force gauge via a non-elastic connector and brought into contact with the mucin. The tablet surface was separated from the mucin (15 mm/min), using a cross head pulley until a peak detachment force was obtained. The mean ± SD of 10 individual replicates were expressed as the force required to separate the tablet from the mucin [maximum detachment force (MDF)]. As a control, the mucoadhesion experiments were conducted on tablet matrices containing PHCl, magnesium stearate and dicalcium orthophosphate only (i.e., matrices containing no mucoadhesive polymers) and the blank values were subtracted from the test values. The dependent response variable selected was maximum detachment force (MDF), which in this study has been described as the force required to separate a dosage form from the substrate.

RESULTS AND DISCUSSION

Fitting of Mucoadhesion Data to the Model

Based on the experimental design, the factor combinations yielded different mean maximum detachment forces. Table 2 summarizes the experimental runs, their factor combinations and the levels of experimental units used in the study as well as the bioadhesive forces obtained for each factor combination. In order to determine the levels of factors which yielded optimal mucoadhesivity, mathematical relationships were generated between the dependent and independent variables. Using the software described earlier, the model was fitted to the data. Repeated backward stepwise regression was used to eliminate the insignificant effects and to generate the equation for the response parameter (MDF). The regression equation together with the statistically significant coefficients and the regression significance generated for the response variable from the above procedure is presented in Table 3. The initial model was refined to include in the model only those terms for which the level of significance was below or equal to $p \leq 0.05$. Statistical testing (ANOVA) indicated that the regression model obtained was statistically significant ($p = 0.000524$).

TABLE 3 Summary of Model Coefficients and Statistical Significance

MODEL GENERATED:
Mucoadhesion = $b_0 + b_1$ * PT + b_2 * PT * CT

Coefficient	Numerical value	*p*-Value
b_0	210.92	1.120e-05
b_1	–37.01	0.00298
b_2	7.336	0.000134
Regression significance		0.000524

Examination of the Model Coefficients and Analysis of the Response Surface and Contour Plots

Coefficients with one factor represented the main effect of that particular factor, while coefficients with more than one factor corresponded to the interaction effect of those two factors on the response. A positive sign in front of the term indicated a synergistic effect, while a negative sign represented an antagonistic effect by these factors (Gupta et al., 2001) on the response variable.

Using backward elimination, the statistically significant mathematical model in Table 3 was generated. The resultant equation which represents the quantitative effect of the formulation parameters on the maximum detachment force is given below:

$$\mathrm{MDF} = 210.92 - 37.01 * \mathrm{PT} + 7.3 * \mathrm{PT} * \mathrm{CT} \qquad (2)$$

From the model generated (Table 3), it was evident that there were both positive and negative statistically significant effects on in vitro mucoadhesion testing. While PT had an antagonistic main effect on MDF, a positive interaction effect between CT and PT was identified (Table 3).

The response surface diagram and contour plot illustrated the effect of both PT and CT on in vitro MDF measurements (Fig. 1a,b). A linear relationship between PT and MDF was evident. The effect of PT produced a descending negative effect on MDF, which indicated that an increase in PT would produce a corresponding decrease in MDF. Furthermore, a statistically significant positive interaction effect between PT and CT was identified. This effect indicated that at corresponding levels of PT and CT, an increase in MDF was discernible. While it can be observed (Fig. 1a,b) that MDF increased with an increase in CT, the model generated indicated that CT does not have a statistically significant main effect on MDF.

Influence of Environmental Parameters on In Vitro Mucoadhesion Testing

The effect of the selected environmental parameters on in vitro mucoadhesion testing following model fitting, was observed to exhibit the following relationship on MDF measurements, as elaborated in the discussion that follows.

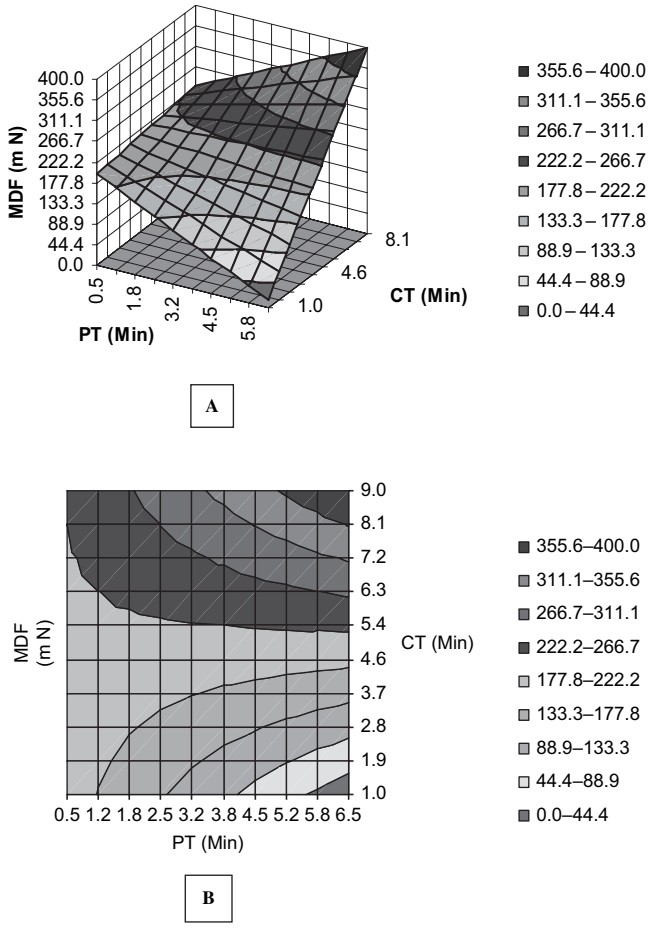

FIGURE 1 **[A] 3 Dimensional Response Surface Representation and [B] Contour Plot of PT and CT on Mucoadhesivity.**

Effect of Prehydration Time

PT on its own was found to have a statistically significant negative main effect on mucoadhesion ($p < 0.05$) as observed from the model (Table 3). This effect is best illustrated in Fig. 1, where an increase in the prehydration time is shown to be progressive from 0.5 min to 6.5 min. This considerably reduced the mucoadhesivity. The results obtained for this system clearly suggested that a minimal prehydration time demonstrated the most favourable mucoadhesion. The possible shortcomings of an increase in PT, on its own, could partially be related to the promotion of polymer disentanglement occurring too early. These results therefore implied that it may be necessary to allow this phenomenon to occur at a more gradual pace, and preferably when in contact with the mucin.

While certain experimental findings have shown a similar negative impact of PT on MDF, others have disputed this observation, and have shown that this parameter was positively correlated with in vitro

mucoadhesivity measurements (Ponchel et al., 1987; Henriksen et al., 1997). Furthermore, some studies have even shown that PT failed to demonstrate any measurable effect (Smart et al., 1984).

Based on these considerable differences among the various experimental findings, it can therefore be inferred that each system responds differently to certain parameters, and these differences may therefore necessitate identification of environment-related parameters for each specific drug delivery system being investigated and test method being employed.

Influence of Contact Force (CF)

Based on the model generated, it was evident that contact force displayed no observable significant main effect on mucoadhesivity during the in vitro testing procedure.

These findings were contrary to what was expected, i.e., an increase in the CF did not produce a corresponding increase in MDF. This observed phenomenon may be related to the fact that once the tablet surface came into contact with the mucin, all external factors (in this case CF) had no bearing on enhancing the intimacy of contact. These results also suggested that the interaction between these two surfaces at a molecular level played a more pivotal role in contributing to the MDF measurements, than any external factors. From an interfacial point of view, however, a certain contact force is required to develop a satisfactory intimate molecular contact between the mucoadhesive system and mucin/tissue, so that an interaction may be achieved in order to allow strong adhesion (Wong et al., 1999). It can therefore be assumed that in this study, CF did not play an instrumental role in contributing to MDF, and that perhaps a small initial force exerted by simply bringing the two surfaces in contact was sufficient to establish this interfacial bond.

Influence of Contact Time

From the model generated (Table 3), the effect of contact time as a main effect on mucoadhesion was not statistically significant. While it can be observed from Fig. 1 that an increase in contact time produced a corresponding increase in mucoadhesion, this effect was not statistically significant.

This observation during the increase in contact time can be related to the hydration of the polymeric tablets, which resulted in a hydrated gel layer that allowed the relaxation of the molecules. This may lead to exposure of their adhesive sites, thereby facilitating interpenetration among the molecules of the substrate to a sufficient depth in order to promote the creation of adhesive bonds (Tamburic & Craig, 1997).

The results of this finding are in agreement with a previous study, which also showed that with differing contact times (2, 8, 20 min) mucoadhesive gels did not exhibit any significant differences ($p > 0.01$) in the measured values of the tensile work and in the fracture strength when using a texture analyzer (Hagerstrom & Edsman, 2001).

Influence of Interaction Effect

While CT did not have a significant main effect, a statistically significant positive interaction effect between PT and CT on MDF was evident ($p < 0.05$). This observed relationship, however, only holds true at corresponding factor levels. For example, the interaction effect was not favored at high levels of PT and low levels of CT, but was positively correlated with MDF at intermediate and high levels of PT as CT levels increased (Fig. 1b). These results suggested that a compromise between these two parameters, by using an appropriate PT followed by a suitable contact time, was essential in order to improve in vitro mucoadhesion testing. Furthermore, the use of an apt PT and CT time corresponded to a period when the tablet interfacial layers were in the quasi-equilibrium-swollen state. This period represented a pre-swelling time necessary for the disentanglement of the mucoadhesive polymer chain, and the establishment of an intimate contact between PAA and mucin. This relationship between PT and CT therefore signified the importance of preconditioning and pre-swelling of the surface of the tablet when in contact with the mucin.

Figure 1 shows that a prehydration time (preswelling time) in combination with increased levels of CT was capable of stabilizing mucoadhesion in this system. This observation was considered as the consequence of water migration from the tablet periphery to the centre with the drying out of a consecutive mucoadhesive interface (Duchene & Ponchel, 1997).

Based on the results obtained from the model generated, a statistically significant negative main effect was exerted by PT ($p < 0.05$), and a positive interaction effect between PT and CT was also identified ($p < 0.05$). Comparisons with other experimental studies reported in the literature, have shown differing

responses with regards to some of the parameters investigated in this study. These differences therefore suggest that identification of relevant test parameters for each test method and drug delivery system being investigated is required.

Validation of the Model

In order to validate the model generated and therefore the discussion in the preceding section. Optimization (Essential Regression, 1997) was used to identify environment test parameters required in order to obtain a specified average MDF response of 209 mN. The optimization tool generated a prehydration time of 3.5 min and contact time of 5 min, as the parameters required for obtaining an average MDF value of 209 mN. A batch of tablets was subsequently prepared and subjected to mucoadhesivity testing. Using these parameters, an actual experimental MDF value of 218 ± 22.9 mN ($n = 10$) was measured for the experimental batch. Statistical analysis using a *t*-test between the predicted values and the experimental data obtained indicated no statistically significant differences ($p = 0.247$), thereby validating the model generated in the study.

Based on these observations it can be postulated that a PT of 3.5 min and a CT of 5 min were able to promote adequate preconditioning of the tablet surface, which was beneficial to mucoadhesivity testing. Furthermore, these factors would also promote interpenetration between the sialic acid residues of the mucin and the functional groups of the polymer chain. A prehydration time of 3.5 min and a contact time of 5 min may be proposed for the mucoadhesivity testing of a propranolol HCL polymeric tablet using the tensile method investigated in this study.

CONCLUSION

The aim of this study was to identify and quantify the effects of environmental test parameters on the mucoadhesion of a propranolol HCl tablet for a specified type of mucoadhesivity test method used. The factors used in this study were PT, CT and CF at varying levels. The effects of these parameters on MDF measurements were evaluated using a Box-Behnken design matrix as generated by a statistical software program. In addition to producing a statistically significant model, analysis of the results also enabled the effect of each factor to be quantified. The results showed that PT had a statistically significant negative main effect on mucoadhesion ($p < 0.05$). CF on the other hand, demonstrated no significant measurable impact on in vitro MDF measurements and this may have been the reason for it not being generated by the optimization tool as a test parameter during the validation phase. While CT had no significant main or quadratic effects, it was found to exert a statistically significant interaction effect with PT, which positively correlated with MDF ($p < 0.05$). While some effects correlated with published data, others did not, thereby confirming the dosage form and method dependent effects on MDF.

The model generated clearly demonstrated the importance of environment-related parameters on in vitro mucoadhesion testing. A CT of 5 min and a PT of 3.5 min as test parameters were identified by the optimization tool, for a predicted average response of 209 mN. The validity of the model was confirmed by the similarity in MDF for the predicted and actual experimental measurements ($p > 0.05$). It can therefore be concluded that a PT of 3.5 min and a CT of 5 min were shown to promote adequate preconditioning of tablet surface and facilitated interpenetration between the sialic acid residues of the mucin and the functional groups of the polymer chain. A prehydration time of 3.5 min and a contact time of 5 min may be proposed for future mucoadhesion testing during formulation optimization of a propranolol HCl polymeric tablet.

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