*AAPS PharmSciTech* 2001; 2 (1) article 1
(http://www.pharmscitech.com).

# Statistical Optimization of Gastric Floating System for Oral Controlled Delivery of Calcium

*Submitted: November 6, 2000; Accepted: January 11, 2001*

Shoufeng Li,[1*] Senshang Lin,[1,4] Yie W. Chien,[1,5] Bruce P. Daggy,[2] and Haresh L. Mirchandani[2]

[1]Controlled Drug-Delivery Research Center, Rutgers College of Pharmacy, 41 Gordon Road, Suite D, Piscataway, NJ 08854

[2]SmithKline Beecham Consumer Healthcare, Parsippany, NJ 07054

[3]Current address: Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St John's University, 8000 Utopia Parkway, Jamaica, NY 11439

[4]Current address: Provost and Professor, Kaohsiung Medical University, 100 Shuh Chen 1st Rd, Kaohsiung 807, Taiwan

## ABSTRACT

The development of an optimized gastric floating drug delivery system is described. Statistical experimental design and data analysis using response surface methodology is also illustrated. A central, composite Box-Wilson design for the controlled release of calcium was used with 3 formulation variables: $X_1$ (hydroxypropyl methylcellulose [HPMC] loading), $X_2$ (citric acid loading), and $X_3$ (magnesium stearate loading). Twenty formulations were prepared, and dissolution studies and floating kinetics were performed on these formulations. The dissolution data obtained were then fitted to the Power Law, and floating profiles were analyzed. Diffusion exponents obtained by Power Law were used as targeted response variables, and the constraints were placed on other response variables. All 3 formulation variables were found to be significant for the release properties ($P < .05$), while only HPMC loading was found to be significant for floating properties. Optimization of the formulations was achieved by applying the constrained optimization. The optimized formulation delivered calcium at the release rate of 40 mg/hr, with predicted n and $T_{50\%}$ values at 0.93 and 3.29 hours, respectively. Experimentally, calcium was observed to release from the optimized formulation with n and $T_{50\%}$ values of 0.89 (± 0.10) and 3.20 (± 0.21) hours, which showed an excellent agreement. The quadratic mathematical model developed could be used to further predict formulations with desirable release and floating properties.

**Key Words:** Calcium, Gastric floating system, Oral controlled delivery, Statistical optimization, Response surface methodology (RSM)

## INTRODUCTION

The development of oral controlled-release drug delivery systems has been hindered by the fluctuation in gastric emptying time, the variation in pH in different segments of the gastrointestinal (GI) tract, and the difficulty of localizing an oral delivery system in a selected region of the GI tract [1]. A gastric floating drug delivery system (GFDDS) [1-8] can overcome at least some of these problems and is particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments. The GFDDS is be able to prolong the retention time of a dosage form in the GI tract, thereby improving the oral bioavailability of the drug [2]. Some studies [9, 10] have been conducted to evaluate various pharmaceutical excipients that could be used to achieve the floating of dosage forms.

Calcium, a very important building mineral for our bones, is absorbed primarily in the duodenum as a result of the presence of active absorption sites (calcium-binding protein [CaBP]) in the upper GI tract [11, 12]. Many factors that affect calcium absorption have been discussed in the literature, and interested readers should consult pertinent articles [13-15]. In view of this unique absorption characteristic, the gastric residence time of a calcium-containing formulation should be prolonged to permit calcium to reach the site of active absorption in a controlled manner; the oral

***Corresponding Author:** Shoufeng Li; (Current address) Pharmaceutical and Analytical Development, Novartis Pharmaceuticals Corporation, 59 Route 10, East Hanover, NJ 07936; Telephone: (973)781-4331; Facsimile: (973)781-4556; E-mail: shoufeng.li@pharma.novartis.com

bioavailability of calcium could therefore be increased. A controlled delivery system for calcium would be beneficial for patients with hypohydrochloria syndrome because calcium would be released in a controlled fashion rather than as a burst phenomenon as in the conventional form.

Many sets of experiments may have to be performed to develop an optimal GFDDS formulation before the in vivo testing can be initiated. The use of response surface methodology (RSM), first developed by Box and Wilson [16], has been proven to be a useful tool in the development of solid dosage forms [17, 18]. Some attractive features of this methodology are that RSM is suitable for simulating the curvature feature of the real situation design space due to the intrinsic property of the design; a mathematical model that describes the contribution of each independent variable to the response variables can be developed; and pure error can be evaluated by replication of the center point, which is an integral part of the design, therefore minimizing the required number of experiments. The basic procedure of RSM includes experimental design, regression analysis, optimization algorithms, and validation. The specifics of the technique have been well described by Box and Wilson [16].

The objective of this study was to develop an optimized GFDDS formulation using calcium carbonate as a model compound. To achieve the objective, the contribution of several independent formulation variables of the mixed polymeric GFDDS fabricated from a combination of polymers was examined. Independent variables that were evaluated included the loading of hydroxypropyl methylcellulose Methocel® K100LV (Dow Chemical Co, Midland, MI), as well as the loadings of citric acid and magnesium stearate. The ranges of these formulation variables were chosen on the basis of the results obtained from the preliminary studies conducted in this laboratory. Dependent variables studied included release parameters such as the diffusion exponent (n), time for the release of 50% of calcium loading (T50%), and floating properties such as the area under the floating kinetics curve ($AUC_f$) and the residual floating force ($F_r$). Specifically, the in vitro release and floating studies of 20 formulations, prepared according to the Box-Wilson design, were performed using USP type II dissolution apparatus and an online continuous floating monitoring system, respectively. Dissolution data were fitted to the Power Law (a kinetic model widely used to describe the initial portion [% dissolution ≤ 60%] of the release profiles) to obtain the release parameters—n and $T_{50\%}$. The floating parameters, $AUC_f$ and Fr, were obtained directly from the floating kinetics curves. The data obtained were further analyzed. The diffusion exponent was used as the target optimization parameter with constraint being put on the other 2 release parameters: 2 hours < $T_{50\%}$ < 4 hours and Rel (percentage of calcium release at 6 hours) > 80%. All the response variables were fitted to a quadratic model; a Lagrangian function was then constructed. The optimized formulation was calculated by solving the Lagrangian function. A validation experiment was performed to validate the mathematical model and to compare the experiment results with the theoretical values of the response parameters predicted from the mathematical model.

## MATERIALS AND METHODS

### *Materials*

Calcium carbonate (Lot #: A-6-313-24) was received from SmithKline Beecham Consumer Healthcare (SBCH/Parsippany, NJ). Methocel® K100LV (Lot #: JL 08012N21), a commercially available grade of hydroxypropyl methylcellulose (HPMC) from Dow Chemical Co (Midland, MI), was also supplied by SBCH. Other materials were purchased from commercial sources: citric acid (CA) from Sigma Chemical Co (St Louis, MO), magnesium stearate (MgSt) from Fisher Scientific Co. (Fairlawn, NJ), and hard gelatin capsules (size 000, manufactured by Eli Lilly Co) from Frontier Co (Norway, IA).

### *Experimental Design*

A central, composite Box-Wilson design (**Table 1**) consists of 8 full factorial design points, 6 axial points, and 6 center points. This design generally involves dependent variable Y and several independent or controlled variables $X_1$, $X_2$...$X_k$. The response surface can be expressed as Y = f ($X_1$, $X_2$....Xk).

The 3 independent formulation variables selected for this particular study were $X_1$, Methocel® K100LV loading level; $X_2$, CA loading level; and X3, mMgSt loading level. All other formulation and processing parameters, such as the level of active ingredient and processing conditions, were kept invariant throughout the study. The dependent variables included the following: Y1, diffusion exponent (n); Y2, time for 50% of calcium to be released (T50%); Y3, percentage of calcium released at 6 hours (Rel); Y4, maximum floating force ($F_{max}$); Y5, time to reach the maximum

floating force ($T_{max}$); Y6, area under the floating kinetics curve ($AUC_f$); and Y7, residual floating force ($F_r$).

**Table 1. Central Composite Box-Wilson Design**

| Formulation Variables | | | |
|---|---|---|---|
| **Formulation No.** | **HPMC K100LV ($X_1$), mg** | **Citric Acid ($X_2$), mg** | **Magnesium Stearate (X3), mg** |
| F1 | 150 | 80 | 20 |
| F2 | 250 | 80 | 20 |
| F3 | 150 | 140 | 20 |
| F4 | 250 | 140 | 20 |
| F5 | 150 | 80 | 40 |
| F6 | 250 | 80 | 40 |
| F7 | 150 | 140 | 40 |
| F8 | 250 | 140 | 40 |
| F9 | 115.9 | 110 | 30 |
| F10 | 284.1 | 110 | 30 |
| F11 | 200 | 59.54 | 30 |
| F12 | 200 | 160.46 | 30 |
| F13 | 200 | 110 | 13.18 |
| F14 | 200 | 110 | 46.82 |
| F15 | 200 | 110 | 30 |
| F16 | 200 | 110 | 30 |
| F17 | 200 | 110 | 30 |
| F18 | 200 | 110 | 30 |
| F19 | 200 | 110 | 30 |
| F20 | 200 | 110 | 30 |

## Preparation of Calcium GFDDS

All the gastric floating formulations contained a fixed amount of active ingredient—625 mg CaCO3. Briefly, calcium carbonate, CA, and K100LV were mixed in a mortar and pestle for 5 minutes to obtain a homogeneous blend. MgSt was then added and blended for an additional 3 minutes. The resultant mixture was carefully weighed and manually filled into size 000 hard gelatin capsules for dissolution and floating studies.

## In Vitro Characterization of GFDDS Release Study

Dissolution studies were conducted using a standard USP paddle dissolution apparatus (DT6R/Erweka Instrument Co, Milford, CT). In all dissolution studies the paddles were rotated at a speed of 100 rpm in 900 mL of simulated gastric fluid (SGF) at 37 ± 1°C. A series of samples (1 mL each) were withdrawn at predetermined intervals for a period of up to 12 hours. The samples were filtered and then analyzed by a calcium analyzer (Calcette/Precision Systems, Inc, Natick, MA), which used EGTA (Glycol ether diamine tetraacetic acid (C14H24N2O10)) solution as titrant, calcium gluconate, and calcette reagent cell activator to generate a certain level of fluorescence. The reaction cell was activated just before each set of measurements, and the Calcette was calibrated by a calcium standard solution before each use.

## Floating Study

To provide quantitative measurements of the floating force, an online continuous floating monitoring system modified from Timmermans et al [19] was designed. The setup consisted of an analytical balance interfaced with a PC via RS232C interface; data were continuously collected at 60-second intervals for up to 8 hours.

Following calibration of the floating apparatus, one capsule was inserted into the sample holder basket and the holder was then immersed into the SGF (900 mL of 0.1 N HCl with 1.8 g of NaCl) maintained at 37°C by a reaction beaker. All other process variables were kept constant.

## Release Profile Analysis

Dissolution data were fitted to the Power Law. Two kinetic parameters—n and $T_{50\%}$—can be determined by nonlinear fitting of the data to the Power Law [10, 20]:

$$\frac{M_t}{M_\infty} = kt^n \tag{1}$$

where $M_t/M_\infty$ represents the fraction of calcium released at time $t$, $k$ is the kinetic constant characterizing the polymeric system, $t$ is the release time, and $n$ is the diffusion exponent. Furthermore, $T_{50\%}$ can be calculated by

$$T_{50\%} = \sqrt[n]{\frac{0.5}{k}} \tag{2}$$

All curve fitting, simulation, and plotting conducted in this work used SigmaPlot and EXCEL.

## Regression Analysis

The contribution of the different formulation variables was compared using analysis of variance (ANOVA) at $P$ = .05 significance level. An SAS program was written for all the regression analysis, quadratic model in the form of

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \quad (3)$$

where Yi is the level of response variable; βi is the regression coefficient; $X_1$, $X_2$, $X_3$ stand for the main effect; $X_1X_2$, $X_2X_3$, $X_1X_3$ are the interactions between the main effects; and $X_1^2$, $X_2^2$, $X_3^2$ are quadratic terms of the independent variables that are used to simulate the curvature of the designed sample space.

A backward elimination procedure was used to fit the data to the quadratic model. It examined successive terms from the original regression equation until the statistical criteria (Mallow $C_p$ statistic) started to rise. It then restored the last term, leaving the equation that minimized the Mallow $C_p$ value [21].

### *Response Surface Plot*

The quadratic model obtained from regression analysis allowed us to build a 3-dimensional graph in which the dependent variable Y was represented by a curvature surface as a function of $X_i$. The relationship between the response and independent variables can be directly visualized from the response surface plot. The information that the 3-dimensional graph conveyed was the same as that from the mathematical equation.

### *Statistical Optimization*

The application of mathematical optimization in the pharmaceutical field was first reported by Fonner et al [17], using the Lagrangian method as a constrained optimization technique. Later developments in computer science have enabled the incorporation of the optimization algorithm into the experimental design software. For example, X-STAT uses a simplex optimization technique [22], and the feasibility program and grid search have been reported by Schwartz et al [23]. In this research article, both the simplex technique using X-STAT software and the Lagrangian method using the EXCEL solver function were used in the optimization.

## RESULTS AND DISCUSSION

Calcium has a low bioavailability of only about 25% to 35%, which leaves a large margin for improvement [11]. This might be due to either an incomplete calcium release or a short residence time of the dosage form; both issues are drug delivery related. The absorption window of calcium is in the duodenum and upper jejunum, where calcium-binding protein (CaBP) exists [12]. If the calcium dosage form can be retained in the stomach as long as possible, therefore, to give the absorption window the maximum exposure to calcium, the bioavailability of calcium could be improved. GFDDS is one approach to prolonging the dosage form retention that could achieve this goal.

When designing the GFDDS, the two mechanisms most frequently used were low density and gas generation [26]. A low-density drug delivery system could be achieved by using HPMC in a polymeric delivery system. HPMC has been widely used as a low-density, hydrocolloidal system [4, 25]. Upon contact with water, a hydrogel layer would be formed to act as a gel boundary for the delivery system. mixing of various grades of HPMC or HPMC with other polymers yielded desirable polymer properties more often than did a specific HPMC alone. Another mechanism of floatation, gas generation, was also incorporated into the calcium GFDDS. This was achieved by incorporating CA into the delivery system. When water penetrated into the calcium GFDDS, CA would react with calcium carbonate and generate carbon dioxide, which was trapped in the polymeric system and helped the floatation of the delivery system. Besides that, calcium citrate formed from the reaction of CaCO3, and CA was more soluble and bioavailable [13].

Our preliminary observations suggested that capsules containing 5% to 10% HPMC were able to float in the dissolution medium for only a few minutes in dissolution studies. In contrast, at higher HPMC levels (15%-25%), the capsules were able to sustain their flotation in the dissolution medium throughout the entire experimental period. Therefore, in this study, a relatively wide range of HPMC loading (13%-27%) has been selected in order to obtain the optimum HPMC loading level.

### *Release Properties*

Release profiles from the 20 formulations (**Table 1**) of Box-Wilson design are shown in **Figures 1-3**. **Figure 1** illustrates the release profiles of the first 8 factorial design points. **Figure 2** shows the results of 6 axial points, and **Figure 3** shows the results of the 6 central points.

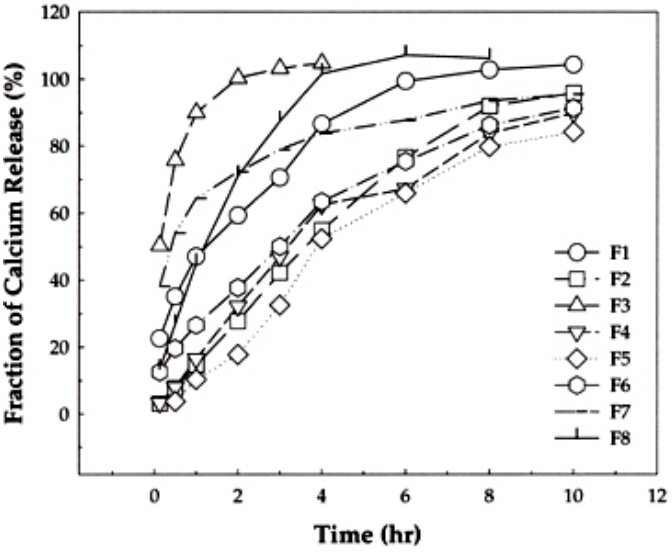


**Figure 1. Calcium release profiles for formulations prepared from 8 factorial points of Box-Wilson design.**

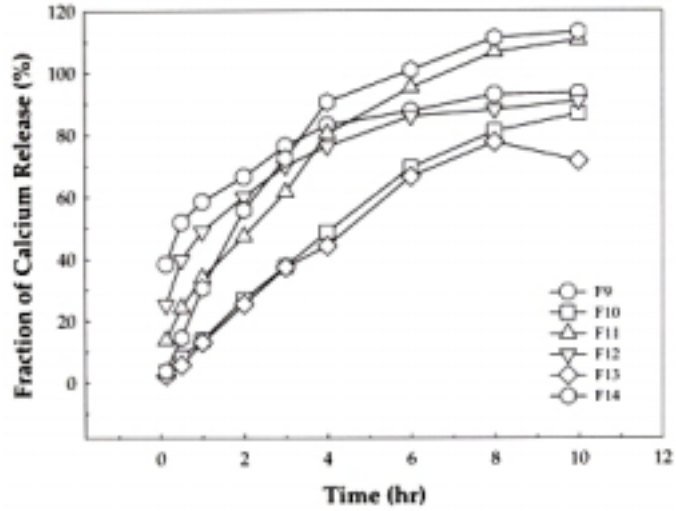


**Figure 2. Calcium release profiles for formulations prepared from 6 axial points of Box-Wilson design.**

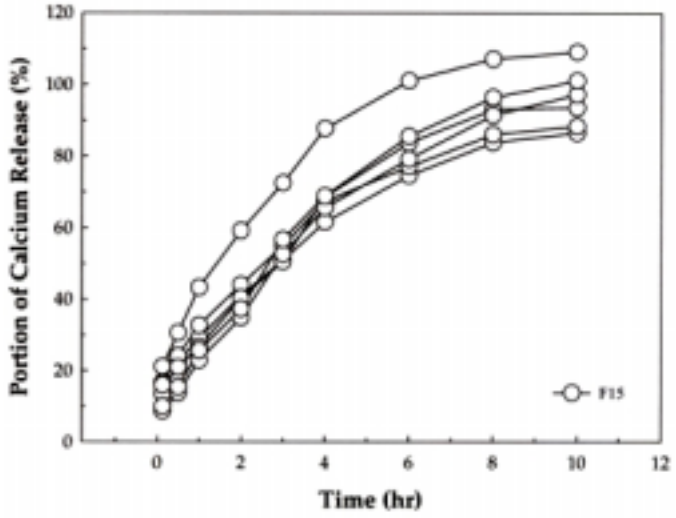


**Figure 3. Calcium release profiles for formulations prepared from 6 central points of Box-Wilson design.**

It is clear from **Figure 1** that other than formulations 3 and 7, the formulations showed a linear pattern of calcium release, at least in their initial phase, indicating the appropriate choice of the range of the formulation variables. Formulation 3 and 7, however, had low HPMC (150 mg) and high CA (140 mg) loading (**Table 1**), both of which contributed to the much faster release of calcium from the delivery system.

**Figure 2** illustrates the calcium release profile from the 6 extreme points from the Box-Wilson design. Formulations 9 and 12, which had the lowest loading of HPMC (115.9 mg) and the highest loading of CA (160.46 mg), were the worst in terms of controlling the release of calcium.

Replicates of the center point in **Figure 3** were used to calculate the pure error due to the experimental procedure, which enabled the determination of lack of fit (LOF) of the suggested regression model. Clustering and overlapping of the release results from **Figure 3** indicated that the experimental errors caused by the procedure were small enough to generate meaningful fittings for the response variables.

Following the procedure described in the Methods section (Equation 1), calcium dissolution data were fitted to the Power Law. Different n values of Equation 1 represent different drug release mechanisms [10]. When n is around 0.45, the fickian diffusion phenomenon dominates, and n between 0.45 and 0.89 is an anomalous transport, often termed as first-order release. After the n value reaches 0.89 and above, the release can be characterized by case II and super case II transport, which means the drug release rate does not change over time and the release is characterized by zero-order release. In this case, the drug release is dominated by the erosion and swelling of the polymer. Most of the fittings gave high $r^2$ values close to 1.0 (**Table 2**). Diffusion exponent values thus obtained ranged from 0.15 to 1.06. Two formulations, F3 and F7, which did not show a linear fashion in their initial release profile (**Figure 1**), had very low n values—0.24 and 0.26 respectively. Low HPMC and high CA settings for these formulations were at least partially responsible for the incapability of the system to control the release of calcium from GFDDS. Other formulations gave relatively higher n values, such as formulations F2, F5, and F6, which had values around 0.90. For the 6 axial points from the Box-Wilson design, a similar phenomenon was observed. Formulations with low HPMC and high CA settings

**Table 2. Results of Release and Floating Parameters for Formulations Prepared from Box-Wilson design**

| Formulation No. | Diffusion Exponent n | Time for 50% of Calcium Release $T_{50\%}$ (hour) | Ca Release at 6 Hour Rel (%) | Correlation Coefficient[1] | $F_{max}$[2] (gram) | $T_{max}$[3] (minute) | $AUC_f$[4] (gram·minute) | $F_r$[5] (gram) |
|---|---|---|---|---|---|---|---|---|
| F1 | 0.38 | 1.25 | 99 | 0.9979 | 7.81 | 15 | 53.04 | 6.33 |
| F2 | 0.97 | 3.46 | 77 | 0.9993 | 8.23 | 29 | 56.13 | 6.44 |
| F3 | 0.24 | 0.25 | 100 | 0.9969 | 9.17 | 10 | 48.13 | 5.20 |
| F4 | 0.54 | 2.90 | 76 | 0.9995 | 9.22 | 14 | 56.26 | 6.30 |
| F5 | 0.92 | 3.03 | 80 | 0.9999 | 8.57 | 24 | 48.74 | 5.36 |
| F6 | 0.90 | 3.33 | 67 | 0.9812 | 8.11 | 32 | 56.96 | 6.71 |
| F7 | 0.26 | 0.36 | 88 | 0.9999 | 8.99 | 12 | 52.54 | 5.42 |
| F8 | 0.62 | 1.19 | 107 | 0.9970 | 8.68 | 41 | 60.80 | 6.97 |
| F9 | 0.15 | 0.77 | 88 | 0.9965 | 10.48 | 17 | 36.98 | 3.85 |
| F10 | 0.87 | 4.02 | 69 | 0.9995 | 8.15 | 31 | 55.26 | 6.64 |
| F11 | 0.85 | 4.48 | 67 | 0.9949 | 7.79 | 33 | 56.03 | 6.80 |
| F12 | 0.32 | 1.07 | 86 | 0.9983 | 10.00 | 11 | 47.29 | 4.17 |
| F13 | 0.48 | 2.23 | 95 | 0.9939 | 9.02 | 20 | 52.34 | 6.14 |
| F14 | 1.06 | 1.57 | 101 | 0.9988 | 7.96 | 32 | 56.35 | 6.55 |
| F15 | 0.41 | 1.37 | 100 | 0.9868 | 9.11 | 16 | 54.16 | 6.38 |
| F16 | 0.49 | 2.84 | 75 | 0.9918 | 8.46 | 14 | 53.17 | 6.34 |
| F17 | 0.47 | 2.27 | 77 | 0.9841 | 8.89 | 34 | 54.17 | 5.62 |
| F18 | 0.56 | 2.65 | 84 | 0.9568 | 7.72 | 30 | 55.26 | 6.45 |
| F19 | 0.76 | 2.78 | 79 | 0.9899 | 8.65 | 31 | 62.35 | 7.06 |
| F20 | 0.72 | 2.62 | 86 | 0.9874 | 9.68 | 20 | 68.16 | 7.92 |

1. Correlation coefficient: R-square value obtained by nonlinear fitting of calcium release profile to the Power Law
2. $F_{max}$: maximum floating force
3. $T_{max}$: Time to reach maximum floating force
4. $AUC_f$: Area under the floating force curve
5. $F_r$: Residual floating force at the time of termination of experiment

**Table 3. Estimation of Regression Coefficient for Different Response Variables Obtained from SAS Backward Regression Procedure**

| Coefficient | Independent Variables | N | $T_{50\%}$ | Rel | $F_{max}$ | $T_{max}$ | AUC | $F_r$ |
|---|---|---|---|---|---|---|---|---|
| $b_0$ | | 0.5683 | 2.4327 | 83.42 | 8.76 | 24.23 | 57.76 | 6.613 |
| $b_1$ | HPMC | 0.1718 | 0.8212 | -5.27 | -0.31 | 5.75 | 4.28 | 0.6445 |
| $b_2$ | CA | -0.18 | -0.9083 | 5.85 | n/a | -4.39 | n/a | -0.3934 |
| $b_3$ | MgSt | 0.1042 | n/a | n/a | 0.52 | 4.47 | n/a | n/a |
| $b_4$ | HPMC*MgSt | n/a | -0.5038 | 6.5 | n/a | n/a | n/a | n/a |
| $b_5$ | HPMC*CA | n/a | n/a | 6.0 | n/a | n/a | n/a | n/a |
| $b_6$ | MgSt*CA | n/a | -0.4363 | n/a | n/a | n/a | n/a | n/a |
| $b_7$ | HPMC*HPMC | n/a | n/a | n/a | n/a | n/a | -3.35 | -0.3927 |
| $b_8$ | CA*CA | n/a | n/a | n/a | n/a | n/a | n/a | -0.3079 |
| $b_9$ | MgSt*MgSt | 0.0518 | -0.2569 | 5.62 | n/a | n/a | n/a | n/a |
| $R^2$ | | 0.8493 | 0.9103 | 0.781 | 0.5877 | 0.6594 | 0.6344 | 0.6818 |
| Model Probability | | 0.0042 | 0.0004 | 0.0213 | 0.2417 | 0.1243 | 0.1603 | 0.0964 |

$b_0$, $b_1$, …, $b_9$: Regression coefficient of each term from nonlinear fitting of quadratic model
HPMC, CA and MgSt: Main effect of formulation variables
HPMC*MgSt, HPMC*CA and MgSt*CA: Interaction between formulation variables
HPMC*HPMC, CA*CA and MgSt*MgSt: Quadratic term of formulation variables

had the lowest diffusion exponent values. Diffusion exponent values for the 6 center points of the design were found to be between 0.4 and 0.8. Some diffusion exponent values were less than 0.45, for example those of formulations 3, 7, 9, and 12, which have very fast calcium release from the delivery system. Theoretically, the diffusion exponent should be at least 0.45. Because Power Law could be used to simulate only the first 60% of the drug release, this left only 2 or 3 points to be used for the nonlinear fitting for the fast release formulations. The inadequate data points resulted in inadequate fitting, which explained the low diffusion exponent values for those formulations.

$T_{50\%}$ values of different formulations were obtained by applying Equation 2. The results are summarized in **Table 2**. Those formulations, which released calcium rapidly within the first couple of minutes of the experiment, corresponded to low $T_{50\%}$ values (F3, F7, F9, and F12). Several formulations had their $T_{50\%}$

values around the desired range of between 2 and 4 hours.

The values of another response variable, the release of calcium at 6 hours (Rel), of the 20 formulations were obtained directly from the calcium release profiles (**Figures 1-3**). Formulations with low $T_{50\%}$ values generally have a high Rel value (**Table 3**).

The fact that some formulations with low HPMC loading and high CA loading have a very fast release of calcium is probably because the ability to hold calcium in the system becomes weaker due to the decrease of polymer concentration. Meanwhile, the increase in CA resulted in more gas generation, which helped to facilitate the release of calcium.

MgSt could serve as a water-resistant agent, so the addition of MgSt could slow water penetration into the mixed polymeric system, thus maintaining the dryness of the interior core of the delivery system and improving the floating capacity of the delivery system.

## *Regression Analysis*

A backward stepwise regression (SAS Institute) was used to generate the quadratic equations for each response parameter. Each response parameter listed in **Table 2** was fitted to a second-order polynomial model (Equation 3), and the regression coefficients for each term in the regression model were summarized in **Table 3** together with $r^2$ of the regression model.

Parameter estimation was given in SAS output, and those parameters with a probability of above 5% were not used in the final model. The final coefficient estimates for n, $T_{50\%}$, Rel, $F_{max}$, $T_{max}$, $AUC_f$, and $F_r$, summarized in **Table 3**, were used for further optimization.

The 6 replicated center points in the experimental design provided the design with the ability to assess the pure error of the experiment and enabled the model's LOF to be checked. In this case, LOF could be evaluated using the SAS PROC RSREG command, and the result indicated that the model was sufficient and had no LOF (**Table 4**). When the F-ratio of LOF and pure error mean square error is around or less than 1.0, the total mean square error can be used in data analysis. Therefore, a pooled estimate of $s^2$ could be obtained by recombining the pure error and LOF sums of squares into the residual sum of square.

**Table 4. Summary of PROC RSREG Results in Analyzing Lack of Fit (LOF)**

| Analysis of Variance (ANOVA) for n | | | | | |
|---|---|---|---|---|---|
| Source | DF | Sum Square | Mean Square | F-Ratio | Pr > F |
| Total | 19 | 1.376 | | | |
| Model | 9 | 1.168 | 0.130 | 6.26 | 0.0042 |
| Residual | 10 | 0.207 | 0.021 | | |
| Lack of fit | 5 | 0.107 | 0.021 | 1.06 | |
| Pure error | 5 | 0.101 | 0.020 | | |
| ANOVA for $T_{50\%}$ | | | | | |
| Source | DF | Sum Square | Mean Square | F-Ratio | Pr > F |
| Total | 19 | 27.77 | | | |
| Model | 9 | 25.36 | 2.818 | 11.6 | 0.0004 |
| Residual | 10 | 2.41 | 0.241 | | |
| Lack of fit | 5 | 0.89 | 0.177 | 0.582 | |
| Pure error | 5 | 1.52 | 0.305 | | |
| ANOVA for Rel | | | | | |
| Source | DF | Sum Square | Mean Square | F-Ratio | Pr > F |
| Total | 19 | 2781 | | | |
| Model | 9 | 2173 | 241.4 | 3.969 | 0.0213 |
| Residual | 10 | 608.2 | 60.82 | | |
| Lack of fit | 5 | 194.7 | 38.94 | 0.471 | |
| Pure error | 5 | 413.5 | 82.7 | | |

**Table 5. Residuals and 95% Confidence Interval (CI) for Diffusion Exponent - n**

| Dependent Variable N | Predict Value | Lower 95% CI | Upper 95% CI | Residual |
|---|---|---|---|---|
| 0.42 | 0.506 | 0.3698 | 0.6421 | -0.086 |
| 0.9 | 0.8496 | 0.7135 | 0.9858 | 0.0504 |
| 0.94 | 0.7144 | 0.5783 | 0.8505 | 0.2256 |
| 0.96 | 1.0581 | 0.9219 | 1.1942 | -0.0981 |
| 0.24 | 0.1459 | 0.00981 | 0.2821 | 0.0941 |
| 0.56 | 0.4896 | 0.3535 | 0.6257 | 0.0704 |
| 0.26 | 0.3544 | 0.2182 | 0.4905 | -0.0944 |
| 0.66 | 0.698 | 0.5619 | 0.8342 | -0.038 |
| 0.2 | 0.313 | 0.18 | 0.4459 | -0.113 |
| 0.87 | 0.891 | 0.7581 | 1.024 | -0.021 |
| 0.5 | 0.4267 | 0.2938 | 0.5597 | 0.0733 |
| 0.93 | 0.7773 | 0.6443 | 0.9102 | 0.1527 |
| 0.88 | 0.9048 | 0.7718 | 1.0377 | -0.0248 |
| 0.31 | 0.2992 | 0.1663 | 0.4322 | 0.0108 |
| 0.41 | 0.602 | 0.5434 | 0.6606 | -0.192 |
| 0.49 | 0.602 | 0.5434 | 0.6606 | -0.112 |
| 0.47 | 0.602 | 0.5434 | 0.6606 | -0.132 |
| 0.56 | 0.602 | 0.5434 | 0.6606 | -0.042 |
| 0.76 | 0.602 | 0.5434 | 0.6606 | 0.158 |
| 0.72 | 0.602 | 0.5434 | 0.6606 | 0.118 |

A residual plot of the obtained data showed homogeneity of the data, and no data transformation seemed necessary. The residual values and plots for diffusion exponents are shown in **Table 5** and **Figure 4**. All the experimental data points are uniformly distributed around the mean of the response variable. Transformation would be necessary if the residual plot of the variable were skewed or scattered in certain patterns, such as a microphone pattern.

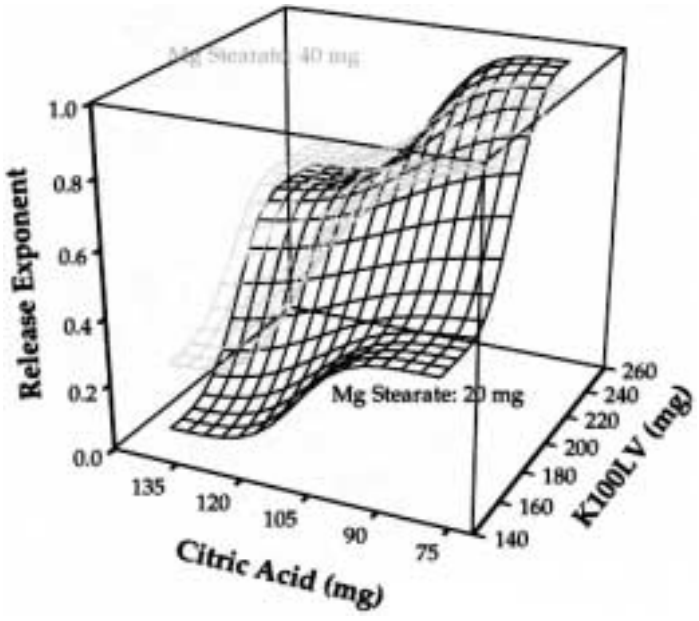


**Figure 4. Residual plot for diffusion exponent - n. The residual of n was calculated by the difference between the experimental and predicted values. The predicted values were obtained by calculation using the quadratic model listed in Table 3.**

## *Effect of Formulation Variables on Release Properties by Mathematical Modeling*

The regression equation obtained for the diffusion exponent was:

$n = 0.5684 + 0.1718(HPMC) + 0.1042(MgSt) - 0.1800(CA) + 0.0518(MgSt)^2$

($r^2 = 0.8493$)

The information the equation conveyed was:

(1) $r^2$ was high, indicating the adequate fitting of the quadratic model;

(2) As HPMC loading increased, the drug delivery system gained more control over the release of calcium, resulting in an increased diffusion exponent value;

(3) A similar kind of behavior was observed for MgSt (a positive coefficient), probably because MgSt is a water-resistant agent, so delayed water diffusion resulted in a more uniform calcium release;

(4) For CA, the opposite effect was observed. It had a negative coefficient, which meant that an increased amount of CA could cause a decrease in the diffusion exponent value; therefore, a smaller amount of CA is better for controlling calcium release from the drug delivery system.

By the same token, for $T_{50\%}$, the mathematical equation bears the form of:

$T_{50\%} = 2.43 + 0.82(HPMC) – 0.91(CA) - 0.50(HPMC)*(MgSt) - 0.44(MgSt)(CA) - 0.26(MgSt^2)$

($r^2 = 0.9103$)

As the amount of polymer increased, $T_{50\%}$ of the drug release increased too, which was quite comprehensible because as water uptake slowed, the water diffusion and calcium release also slowed. Yet as the amount of CA increased, $T_{50\%}$ decreased.

The ANOVA table (**Table 4**) for the release properties of the formulations demonstrates that the model was significant for all response variables. The 3 main effects were as follows: HPMC, CA, and MgSt were significant to the diffusion exponent ($Y_1$). Similarly, HPMC and CA were also important in terms of $T_{50\%}$ ($Y_2$) and Rel ($Y_3$). The quadratic term of MgSt was significant for all the response variables (**Table 3**).

## *Floating Dynamics*

The floating properties of the calcium GFDDS could be obtained by using a floating capable polymer and gas-generation mechanism. It was believed that a minimal level of floating strength was required to maintain the floating of GFDDS [1]. However, no information is currently available on the in vivo threshold value of floating force [24]. Further, in vivo behavior of the systems can be complicated by a meal, position of the subject, and other factors [27]. Thus, the floating of a GFDDS was a complicated behavior and its kinetics could be unpredictably difficult. This led us to believe that the floating capabilities of the oral drug delivery device could be maintained to its maximum capacity without compromising calcium release from the system.

A typical floating kinetics curve, which was obtained by filling 500 mg of HPMC K100LV into size 000 hard gelatin capsules, is shown in **Figure 5**. Several important parameters were adopted to characterize the floating kinetic profile of GFDDS—maximum floating force ($F_{max}$), time to reach maximum floating force ($T_{max}$), area under the floating force curve ($AUC_f$), and residual floating force at the time of termination of the experiment ($F_r$).

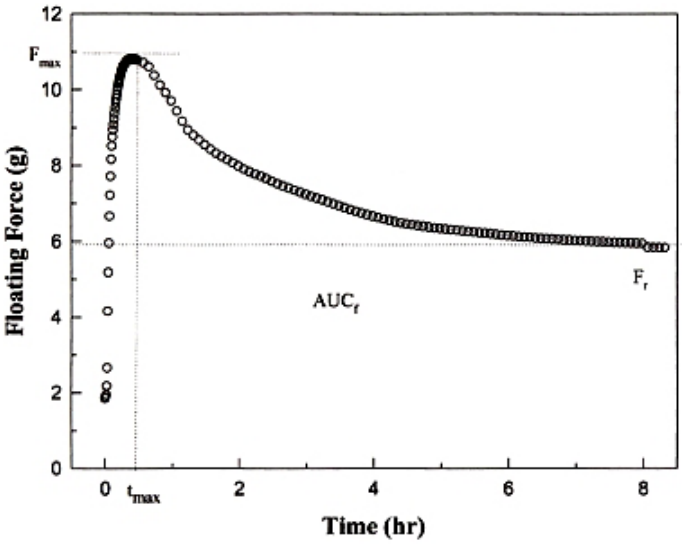


**Figure 5. Typical floating kinetics profile of GFDDS obtained by the continuous floating monitoring system. Test unit consisted of 500 mg of HPMC K100LV filled into a size 000 hard gelatin capsule. The measurement was conducted according to the procedure described in the Floating Study section.**

Floating data were obtained by the continuous floating kinetics monitoring system introduced in the Materials and Methods section. Therefore, values of 4 parameters obtained ($F_{max}$, $T_{max}$, $F_r$, and $AUC_f$) for the 20 formulations of Box-Wilson design were also included in **Table 2**.

The maximum floating force that the GFDDS could achieve ranged from 7.72 to 10.48 g. Time required to reach the peak value was found to be between 10 and 41 minutes, indicating a fast achievement of floating force. This was probably due to the incorporation of the 2 floating improvement mechanisms, MgSt and CA.

Using AUC values to represent the overall effect of drug absorption has been well accepted in the pharmacokinetic field, while floating AUC was also a good measurement of the overall floating capacity of the GFDDS. Its calculation was based on the linear trapezoidal rule, and the AUC values for this series of formulations were between 36.98 and 68.16 g/min. The lowest $AUC_f$ corresponded to the lowest HPMC settings (formulation 9). Most of the $AUC_f$ values were found to be around 50 g/min. Because the floating measurement was an online continuous measurement and our major objective was to make comparisons between formulations, the whole measurement procedure was conducted under static conditions and the gastric floating system would reach an equilibrium under such experimental conditions after a certain period of time. The magnitude of the equilibrium was expressed by Fr values. Fr values vary between 3.85 and 7.92 g. All the floating data were submitted for ANOVA test.

Quadratic model fitting for $AUC_f$ showed an $r^2$ of 0.63, and the *P* value for the model was 0.16 (**Table 3**). The fitting was less significant probably due to the lack of variance in the floating data. Data of the floating properties of the GFDDS formulations were fairly homogeneous. Only the loading of HPMCK100LV was found to be significant for AUC: Higher polymer loading corresponded to better floating properties.

The $r^2$ value for the Fr model fitting was 0.68 with a *P* value of 0.096. Again HPMC and its quadratic terms had the greatest impact on the residual floating force of the GFDDS formulations.

ANOVA test results indicated that the HPMC loading level had a significant effect on $T_{max}$, $AUC_f$, and Fr. The quadratic term of HPMC also showed a significant effect on AUC. The other 2 formulation variables, CA and MgSt loading, were not as significant in the selected experimental range. However, depending on the amount added, the presence of MgSt could contribute to the difference in the floating capacities of the dosage forms [1].

## *Response Surface Plot*

Graph presentation of the data can help show the relationship between the response and independent variables. Graphs gave information similar to that of the mathematical equation obtained from statistical analysis. The response surface graph of the diffusion exponent was presented in **Figure 6** as an example, which covered the entire variable range of the design.

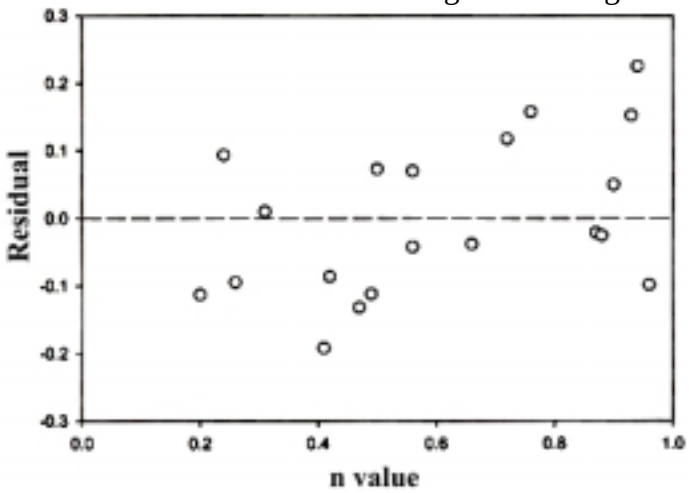


**Figure 6. Response surface plot for diffusion exponent - n.**

The response surface plot (**Figure 6**) illustrated that more MgSt achieved better control of the calcium release. Diffusion exponent values rose as HPMC loading went from lower to upper level. For CA, it seemed less was always the rule within the design space. The diffusion exponent can vary a lot over the design space, with the most preferable ranges being the lower range of CA, the higher range of MgSt, and the higher range of HPMC. This conclusion agreed with the analysis we discussed in the previous section.

## Constrained Optimization

A constrained optimization technique was used to generate the optimum setting for the formulation using maximization of the diffusion exponent as our major optimization objective. Readers are suggested to refer to Fonner et al [17] for the theory part of the constrained optimization. Among different techniques that were available for solving the constrained optimization problem, the most popular method appeared to be the Lagrangian and Simplex methods. In this study, optimization was undertaken using both simplex techniques incorporated in X-STAT package [22] and the Lagrangian method introduced by Fonner et al [17], which could be achieved by using the EXCEL SOLVER function.

Maximization of the diffusion exponent would be favorable because it indicated a more uniform release rate (ie, approach zero-order release) from the polymeric system. Meanwhile, a certain constraint was applied on $T_{50\%}$ ($2 < T_{50\%} < 4$ hours) and calcium release at 6 hours (Rel > 80%). Floating parameters were not included in the formulation optimization because floating properties for the whole batch of formulation were satisfactory, and little variation in floating data was observed.

Optimization results therefore obtained were included in **Table 6**. The optimum formulation had a setting of $CACO_3$ (625 mg), HPMC (250 mg), CA (85 mg), and MgSt (20 mg). There was excellent agreement between the measured and the predicted data for most of the characteristics in terms of the diffusion exponent, $T_{50\%}$ (Table 6). As shown in **Figure 7**, the experimental release profile (symbol and solid line) of the predicted formulation agreed quite well with the release profile predicted from the response surface model (dashed line). A more constant release rate in the early portion of the profile was achieved by maximizing the diffusion exponent (n) using response surface methodology.

**Table 6. Composition of the Optimized Formulation Obtained by Constrained Optimization Technique and Comparison of Experimental and Predicted Values of Release Parameters**

| Optimized Formulation | | |
|---|---|---|
| $CACO_3$ | | 625 mg |
| HPMC K100LV | | 250 mg |
| Citric Acid | | 85 mg |
| Magnesium Stearate | | 20 mg |
| Formulation | Diffusion exponent (± SD) | $T_{50\%}$ (hour ± SD) |
| Experiment | 0.89 (± 0.10) | 3.20 (± 0.21) |
| Predicted | 0.93 | 3.29 |

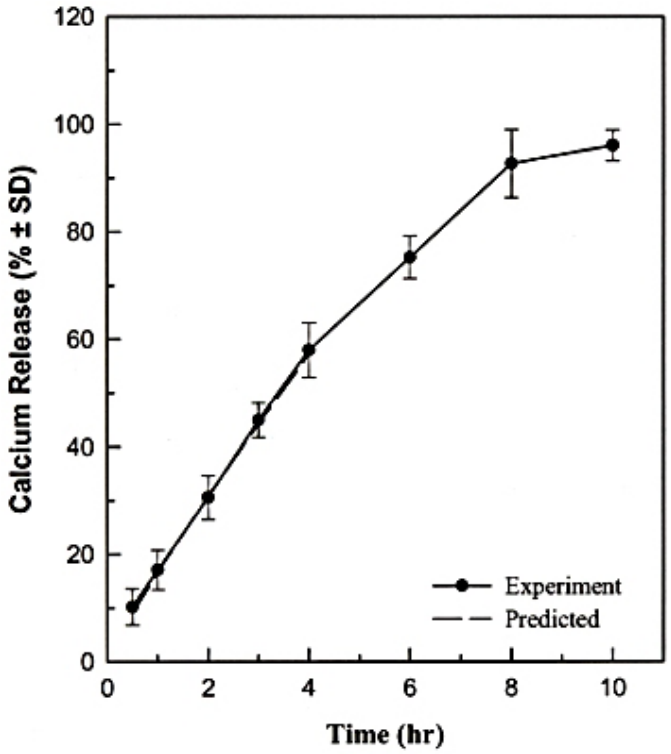


**Figure 7. Comparison of experimental and predicted calcium release profile for the optimized formulation.**

# CONCLUSIONS

A rotatable, central composite design was performed, and the desired release of calcium from GFDDS was achieved by carefully monitoring the selection of formulation variables. Further, the results from floating studies suggested that the desired floating profile of GFDDS could be achieved while maintaining the desirable release properties of the GFDDS formulation.

The statistical approach for formulation optimization is a useful tool, particularly when several variables are to be evaluated simultaneously. The mathematical model generated by regression analysis can be used to predict and optimize the formulation variables. The prediction

from the model and the experimental results in this study conform to each other quite well, indicating the validity of the method. The 3 formulation variables evaluated in this study are all very important in affecting the release profiles of the formulations; however, only the HPMC loading level is important within the selected range for floating properties of the GFDDS. Overall, a controlled-release intragastric floating system for calcium has been successfully developed using the constrained optimization method. A step-by-step procedure of the statistical method has been illustrated in this study, which can be extrapolated to the development of other drug delivery systems.

## ACKNOWLEDGEMENTS

The authors would like to thank the Parke-Davis chair of endowment for providing a predoctoral fellowship to Dr. Shoufeng Li and SmithKline Beecham Consumer Healthcare for providing funding support for this project.

## REFERENCES

1. Moes AJ Gastroretentive dosage forms. *Crit Rev Ther Drug Carrier Syst.* 1993;10(2):143-159.

2. Menon A, Ritschel WA, Sakr A. Development and evaluation of a monolithic floating dosage form for furosemide. *J Pharm Sci*. 1994;83(Feb):239-245.

3. Jimenez Castellanos MR, Zia H, Rhodes CT. Design and testing in vitro of a bioadhesive and floating drug delivery system for oral application. *Int J Pharm*. 1994;105(Apr 25):65-70.

4. Watanabe K. Preparation and evaluation of intragastric floating tablet having pH independent buoyancy and sustained release property. *Arch Pract Pharm Yakuzaigaku.* 1993;53:1-7.

5. Desai S, Bolton S. Floating controlled-release drug delivery system: in vitro-in vivo evaluation. *Pharm Res.* 1993;10(Sep):1321-1325.

6. Thanoo AC, Sunny MC, Jayakrishnan A. Oral sustained-release drug delivery systems using polycarbonate microspheres capable of floating on the gastric fluid. *J Pharm Pharmacol*. 1993;45(Jan):21-24.

7. Hilton AK, Deasy PB. In vitro and in vivo evaluation of an oral sustained-release floating dosage form of amoxicillin trihydrate. *Int J Pharm*. 1992;86(10):79-88.

8. Xu WL, Tu XD, Lu ZD. Development of gentamicin sulfate sustained-release tablets remaining-floating in stomach. *Yao Hsueh Hsueh Pao*. 1991;26(7):541-545. Chinese.

9. Gerogiannis VS, Rekkas DM, Dallas PP, Choulis NH. Floating and swelling characteristics of various excipients used in controlled release technology. *Drug Dev Ind Pharm*. 1993;19(9):1061-1081.

10. Chueh HR, Zia H, Rhodes CT. Optimization of sotalol floating and bioadhesive extended release tablet formulations. *Drug Dev Ind Pharm*. 1995;21(15):1725-1747.

11. Heaney RP. Calcium bioavailability. *Bol Asoc Med P R*. 1987;79(1):27-29.

12. Allen LH. Calcium bioavailability and absorption: a review. *Am J Clin Nutr*. 1982; 35(4):783-808.

13. Pak AY, Harvey JA, Hsu MC. Enhanced calcium bioavailability from a solubilized form of calcium citrate. *J Clin Endocrinol Metab.*1987;65(4):801-805.

14. Pointillart A, Gueguen L. Meal-feeding and phosphorus ingestion influence calcium bioavailability evaluated by calcium balance and bone breaking strength in pigs. *Bone Miner*. 1993;21(1):75-81.

15. Nickel K. P. Calcium bioavailability from bovine milk and dairy products in premenopausal women using intrinsic and extrinsic labeling techniques. *J Nutr*. 1996;126(5):1406-1411.

16. Box GEP, Wilson KB. On the experimental attainment of optimum conditions. *J Roy Statist Soc*. 1951;13(B):1.

17. Fonner AE, Buck JR, Banker GS. Mathematical optimization techniques in drug product design and process analysis. *J Pharm Sci*. 1970;59(11):1587-1596.

18. Franz RM, Sytsma JA, Smith BP, Lucisano L J. In vitro evaluation of a mixed polymeric sustained release matrix using response surface methodology. *J Control Release*. 1987;5(Sep):59-172.

19. Timmermans J, Moes AJ. Measuring the resultant-weight of an immersed test material: I. Validation of an apparatus and a method dedicated to pharmaceutical applications. *Acta Pharm Technol*. 1990;36(3):171-175.

20. Colombo P, Bettini R, Santi P, De Ascentiis A, Peppas NA. Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water transport. *J Control Release*. 1996;39(May):231-237.

21. Mallow A. L. Some comments on $C_p$. Technometrics. 1999;15:661-675.

22. Murray J. X-STAT Menu. 1992.

23. Schwartz JB, Flamholz JR, Press RH. Computer optimization of pharmaceutical formulations I: General Procedure. *J Pharm Sci*. 1973;62(7):1165-1170.

24. Timmermans J, Moes AJ. Cutoff size for gastric emptying of dosage forms. *J Pharm Sci*. 1993;82(Aug):854.

25. Oth M, Franz M, Timmermans J, Moes A. The bilayer floating capsule: a stomach-directed drug delivery system for misoprostol. *Pharm Res*. 1992;9(3):298-302.

26. Ichikawa M, Watanabe S, Miyake Y. A new multiple-unit oral floating dosage system. I: Preparation and in vitro evaluation of floating and sustained-released characteristics. *J Pharm Sci*. 1996;80(11):1062-1066.

27. Timmermans J, Moes AJ. Factors controlling the buoyancy and gastric retention capabilities of floating matrix capsules: new data for reconsidering the controversy. *J Pharm Sci*. 1994;83(Jan):18-24.