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Characterization of the Absorption of Theophylline From Immediate- and Controlled-Release Dosage Forms With a Numerical Approach Using the In Vitro Dissolution-Permeation Process Using Caco-2 Cells

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ABSTRACT After oral administration, drug absorption rate is recognized to be dependent on two major factors: dissolution and intestinal cells permeability. Caco-2 monolayer cells have been largely used as a permeation study model. In this study, a numerical approach funded on an exponential first-order time relationship was tested to compare immediate- and controlled-release tablets of theophylline using a dissolution-permeation system. The dissolution performance using USP II paddle apparatus was coupled to the permeability studies investigated in Caco-2 cell monolayers. The dissolved samples were taken at different times; their pH and osmolarity were adjusted to render them suitable to Caco-2 permeability studies (osmolarity = 300 mosm, pH = 7.4). The experimental data show that the dissolution fits the exponential first-order relationship rate. The permeability values were in a range of $4.45\ 10^{-6}$–$5.28\ 10^{-6}$ cm/s, and percentages of absorbed drug dose were dependent on the fraction initially present in the donor compartment, indicating that absorption of theophylline was dissolution rate limited. Plotting experimental absorbed fractions (F_a) against experimental dissolved fractions (F_d) show that permeation is the rate-limiting step in drug absorption process in the extended release form of theophylline. Our results demonstrate a general agreement between observed F_a/F_d relationships and theoretical F_a/F_d relationships obtained with our approach funded on dissolution and permeation behavior. We concluded that the couple dissolution-caco-2 system could be a useful tool to characterize intestinal permeation for a new formulation of a drug compared with the conventional one.

KEYWORDS Theophylline, Dissolution, Absorption, Caco-2, Predicting system

INTRODUCTION

The systemic availability of orally administered drugs is generally limited by two major phenomena linked to dissolution and intestinal absorption. Thus, a number of experimental approaches have been used to assess dissolution process and intestinal drug absorption. Dissolution characteristics are determined by widespread conventional methods (Ogata et al., 2001; Siewert, 1993). In addition, intestinal drug absorption may be predicted from the whole animal studies and a variety of in vitro models Bohets et al., 2001. The most commonly used models include isolated tissular segments (e.g., perfused intestinal segments, everted gut) and cultured cells (e.g., Caco-2). In this regard, Caco-2 cell studies are characterized as an intestinal permeability model (Hilgers et al., 1990).

To optimize drug formulation and its biopharmaceutical insights correlation analysis (Zerrouk et al., 2001) or modeling (Dressman & Reppas, 2000; Dunne et al., 1999) funded on dissolution and absorption data reflected by pharmacokinetic measurements have been developed with varying degrees of success. Because permeability to Caco-2 monolayer system may be directly correlated to the absorption of the drug (Artursson & Karlsson, 1991), the dissolution-absorption relationship from dissolution profile vs. permeation rate is an additional contributor to evaluate the bioavailability performance of a pharmaceutical formulation (Ginski & Polli, 1999).

According to the biopharmaceutical drug classification (Amidon et al., 1995) theophylline is a high solubility–high permeability drug. In this case, the drug absorption rate is limited by only the dissolution rate, except for immediate-release forms, which dissolve rapidly and for which gastric emptying becomes the limiting factor to absorption (Dressman et al., 1998). For this class of drugs in vitro–in vivo correlations are expected to be found if dissolution rate is slower than gastric emptying rate.

On the basis of this knowledge, we used a dissolution-absorption predicting system, taking into account the results obtained from experimental studies fitted in terms of a first-order drug dissolution and permeation. Thus, the reliability of this numerical prediction approach was evaluated in the present work by comparing the dissolution-absorption profiles of immediate- and controlled-release formulations of theophylline.

MATERIALS AND METHODS

Materials

Anhydrous theophylline was purchased from Helm laboratories; the sieved powder (250 μm) was used for all subsequent preparations of tablets. All excipients were vegetable origin. Ethylcellulose N-100 was donated by Hercules. Lactose, starch, and talc were purchased from Siphat (Tunisia), Aerosil® from Roussel, and magnesium stearate from Merck; all other chemicals were analytical grade.

Preparation of Immediate- and Controlled-Release Tablets

For each batch of tablets, the appropriate quantity of powder was mixed for 10 min in a Turbula mixer. In a second step, immediate- and controlled-release tablets were prepared by direct compression using a single-punch tabletting machine (Korsh EKO). The following control tests were performed: the mass uniformity (Mettler AT 200 balance), the breaking strength and friability [determined with a hardness tester and a friabilitator (Erweka)], and the disintegration (Prolabo apparatus).

Dissolution Rate Studies

In vitro dissolution studies were performed with six replicates using the rotating paddle method (Dissolutest Prolabo) with 1000 mL of dissolution medium, at a speed of 100 rpm and a temperature of 37°C. The Britton medium at pH 2.5 was used for immediate-release tablets. The samples were then removed at the following intervals: 10, 20, 30, 45, and 60 min. For controlled-release formulations, the pH of the dissolution medium was 2.5 at the beginning of the assay; it was changed to 4.5 at 120 min by addition of 7 mL NaOH 1N and then to 7.4 at 300 min by addition of 4 mL NaOH 1N. Assays were carried out for 480 min with sampling every 60 min. All samples were filtered through 0.2-μm syringe filters and analyzed by UV-absorption measurements (Perkin-Elmer 40 spectrophotometer) after an appropriate dilution.

Treatment of Dissolution Samples for Caco-2 Permeation Studies

Permeation studies through Caco-2 cells were conducted with isotonic and neutral pH transport medium to guarantee the viability of the cells (Ingels et al., 2002). Regarding this condition, the pH of each dissolution sample (volume=3 mL) was adjusted to 7.4 using NaOH 10N and then 2 mL of HBSS solution with addition of 0.3% NaCl and 0.2% glucose. Isotonicity was checked by an osmometer MK3. The samples tested were the ones taken at 10, 30, and 60 min for immediate-release tablets and at 120, 240, 360, and 480 min for controlled-release tablets.

Preparation of Caco-2 Monolayers

Caco-2 cells were donated by Dr. Rousset (Unity INSERM, U178, Villejuif). The cells were grown at 37°C in 25-cm^2 flasks in an atmosphere of 5% CO_2 and 95% RH using Dulbecco's modified Eagle's Media (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids (NEAA), and an antibiotic mixture of 1% penicillin/streptomycin.

Cells were passaged at confluency (every week) by using a trypsin/EDTA solution. Between passage numbers 21 and 43, cells were seeded onto Snapwell® polycarbonate filters (Corning Costar®, Brumath, France) at a density of 0.5×10^{-6} cells/cm^2. These cells were cultured for 21–28 days and then used for drug permeability studies. Cells on Snapwell® filters were placed on Grass-Sweetana diffusion chambers immediately, and 5 mL of isotonic HBSS solution (Life Technologies, Eragny, France) were placed to the basolateral side. Chambers were maintained at 37°C by using a thermostatic circulator 2219 Multipump III (UKB, Bromma Sweden) with carbogen mixing.

Drug Permeability Study Through Caco-2 Cells

Apical to basolateral permeability studies were carried out for 120 min; cells integrity was detected by measuring the TEER (ohmmeter Millicell®-ERS) and by evaluating ^{14}C mannitol permeability. Samples were removed at t=5 min and t=120 min from the donor compartment and at t=5 min, t=30 min, t=60 min, t=90 min, and t=120 min from the receiver compartment (n=3). The volume in each compartment was maintained by adding the equivalent volume of fresh HBSS solution. A 10^{-3} M, theophylline solution was also tested. At the end of the assay procedure, the removal of the filters washed with HBSS solution demonstrated that no drug was adsorbed on the polycarbonate surfaces. The concentration of ^{14}C mannitol was determined by liquid scintillation counting using a Beckman LS-3801 apparatus.

Assay Method

The concentrations of theophylline in the donor and acceptor compartments were determined by the HPLC method. The applied HPLC conditions were as follows: pump LC-10 (Shimadzu, Japan); UV detection SPD-10A (Shimadzu, Japan); column Hypersil 5-ODS (4.6 mm × 250 mm, GL Science, Japan). The mobile phase acetonitrile/methanol was pumped at 0.8 mL/min and monitored at 254 nm.

Data Analysis

Results obtained from drug dissolution studies could be written in terms of the Higuchi square root law or Hixson-Crowell cubic law but according to the approximation of Polli et al. (1996). They were successfully fitted in terms of a first-order drug release:

$$F_d = 1 - e^{-k_d t} \quad (1)$$

where k_d is the first-order dissolution rate constant for the dosage form and F_d is the fraction dissolved.

Results obtained from drug permeation studies were also fitted in terms of a first-order drug permeation:

$$F_a = 1 - e^{-k_a t} \quad (2)$$

where k_a is the first-order permeation rate constant and F_a is the fraction absorbed.

Dissolution and absorption data were fitted against Eqs. 1 and 2, respectively, to determine k_d and k_a, first-order dissolution, and permeation rate constants. All fittings were performed by using the nonlinear least-squares module of the R statistics package (R Development Core Team, 2004).

Permeability coefficients through the Caco-2 monolayers were calculated from the following equation (Camenish et al., 1998):

$$P = \frac{V}{C_0 A}\frac{dC}{dt}$$

where dC/dt (mol/mL/s) is the increase of drug concentration in the acceptor chamber during the time considered, A (1.13 cm^2) the surface exposed to the compound, V (mL) and C_0 (mol/mL), respectively, the solvent volume and the initial drug concentration in the donor chamber.

In Vitro–In Vitro Correlations Between Dissolution Studies and Caco-2 Experiments

Combining Eqs. 1 and 2 above and assuming in vivo dissolution being identical to in vitro dissolution, Polli et al. 1996 obtain the following absorption/dissolution correlation:

$$F_a = \frac{1}{f_a}\left(1 - \frac{\alpha}{\alpha - 1}(1 - F_d) + \frac{1}{\alpha - 1}(1 - F_d)^{\alpha}\right) \quad (3)$$

where f_a is the fraction of the dose absorbed at $t = \infty$ and $\alpha = k_a/k_d$ is the ratio of the first-order permeation rate constant to the first-order dissolution rate constant.

Eq. 3 was used to assess in vitro–in vivo correlations. In this context, the fraction of drug absorbed F_a has to be plotted against the fraction dissolved F_d. F_a and F_d must be paired according to identical times, whereas F_d is taken directly from the dissolution profile $F_d = f(t)$; time paired for F_a has to take into account the mean residence of the drug in the small intestine. In this way, a mean dissolution time (MDT) was used by Ginski and Polli (1999); the time T_a assigned for F_a was:

$$T_a = T_d + T_p$$

where T_p was the permeation time and T_d was calculated from the formula

$$\mathrm{MDT} = \frac{\sum_{i=1}^{n} T_{\mathrm{mid}}\Delta M}{\sum_{i=1}^{n} \Delta M}$$

where i is the dissolution sample number (e.g., for fast formulations i=1 for 10-min data, i=2 for 30-min data, etc.), n is the number of dissolution sample times, T_{mid} is the time at the midpoint between i and i-1, and ΔM is the additional drug dissolved between i and i-1.

RESULTS AND DISCUSSION

Tablet Production and Control Tests

To achieve controlled-release tablets of Theophylline, an ethylcellulose ratio of 41% was selected; this ratio was replaced by 20% starch and 80% lactose for immediate-release tablets (Table 1). The use of three lubricants (talc, magnesium stearate, and Aerosil®) led to a satisfactory flowability of the mixed powders. The tablets obtained were of good quality; they satisfied the assays of mass uniformity and breaking strength, which was higher for controlled-release tablets to ensure the sustained release of the drug (Table 2). In fact, controlled-release tablets' structure must be tough enough to guarantee the sustained release of the drug (Picker & Bikane, 1998). In our experimental conditions, the disintegration was complete in 25 sec for immediate-release tablets, whereas controlled-release tablets disintegrate and erode slowly within 4 h (Table 2). Erosion is in fact a natural tendency of ethylcellulose due to the separation of the surface particles from the matrix (Pather et al., 1998).

Dissolution Studies

Figure 1 illustrates the theophylline release rates. From immediate-release tablets, more than 80% of the drug is released after 1 h of dissolution testing as the USP requirements. The dissolution rate fits the exponential first-order relationship ($k_d = 0.033\ min^{-1}$, $p = 1.9\ 10^{-4}$).

Dissolution data obtained with the controlled-release tablets indicate that the ratio ethylcellulose/theophylline selected leads to the rate desired of about 80% of the drug released in 8 h. As expected for an inert ethylcellulose matrix, the amount of drug released was important at the beginning of the assay. Indeed, an average of 27.5% of the drug was released

TABLE 1 Formulas of Immediate- and Controlled-Release Tablets of Theophylline Used in This Study

Composition	Immediate-release tablets	Controlled-release tablets
Theophylline (%)	55.8	55.8
Ethylcellulose (%)	—	41
Starch (%)	32.8	—
Lactose (%)	8.2	—
Mg stearate (%)	2	2
Talc (%)	0.8	0.8
Aerosil® (%)	0.4	0.4

after 60 min. This amount corresponds to the drug spread on the surface of the matrix dissolving as soon as the tablet got into the contact with the dissolution medium. Then, the drug release rate decreases to suit the profile of a controlled-release dosage form. The dissolution rate fits also the exponential first-order relationship (k_d=0.0034 min^{-1}, p=7.1 10^{-8}).

Permeability Studies

The tightness of the cell-junction structure, and the paracellular permeability lighted by TEER increase with decreasing TEER (Yamashita et al., 2000). In our experimental conditions, TEER is nearly constant in a range of 365Ω. The low permeability values of a passively transported drug such as mannitol (in a range of 10^{-6}) (R Development Core Team, 2004) is also an indicator of cell integrity. It is interesting to note that the mass balance ranges from 90 to 110%. The Caco-2 permeability data (P_{app}) for each dissolution sample investigated are shown in Table 3. No significant differences were observed between the P_{app} values ranging from 4.45±0.53 10^{-5} cm/s for $CpI_{30\ min}$ to 5.28±0.71 10^{-5} cm/s for $CpCR_{480\ min}$; these results demonstrate that the compounds included in either immediate- or controlled-release tablets did not affect the absorption of theophylline.

Dissolution/Absorption Relationships From Dissolution Data and Caco-2 Permeability Studies

The absorbed fractions estimated from permeability studies are plotted against time for each dissolution sample in Figs. 2 and 3 for immediate- and controlled-release tablets, respectively. The depicted curves show that the fractions absorbed increase with the amount initially present in the donor compartment, indicating that the dissolution rate is the factor limiting the permeation rate. When the initial amounts are comparable, permeation rates are absolutely identical as observed for $CpI_{10\ min}$ and $CpCR_{120\ min}$ or for $CpI_{60\ min}$ and theophylline solution. The cumulative Caco-2 permeation and the coefficient of variation (CV) are presented in Table 3. The CV values were less than 16%, with greater amounts of drug in the donor compartment for $CpI_{60\ min}$, $CpCR_{480\ min}$ and theophylline solution. A first-order exponential time relationship could be used to express the fractions absorbed during the permeation study. Fitting the exponential law to the permeation data yields the absorption rate constant k_a and allows us to compute the α, permeation to dissolution rates ratio of Eq. 3. As expected, immediate-release form yields a much lower α (α=0.012) than the controlled-release form (α=0.17).

TABLE 2 Pharmaceutical Characteristics of the Investigated Tablets

Parameters	Immediate-release tablets	Controlled-release tablets
Weight (mg)	342.75±1.99	360.90±3.40
Breaking strength (Newton)	43.50±3.34	109.50±10.33
Friability (%)	3.56 (n=20)	0.24 (n=20)
Desegregation (min)	0.25±0.2	240±6

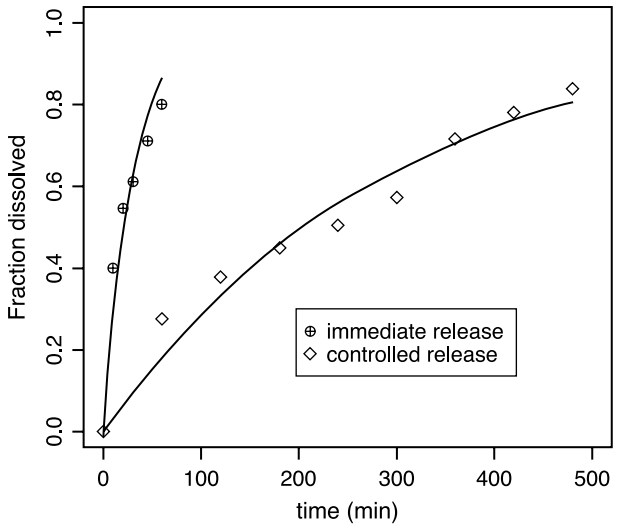

FIGURE 1 **Comparison Between Measured and Predicted Dissolution of Immediate and Controlled Release Tablets of Theophylline. The Data are Fitted on Eq. 1.**

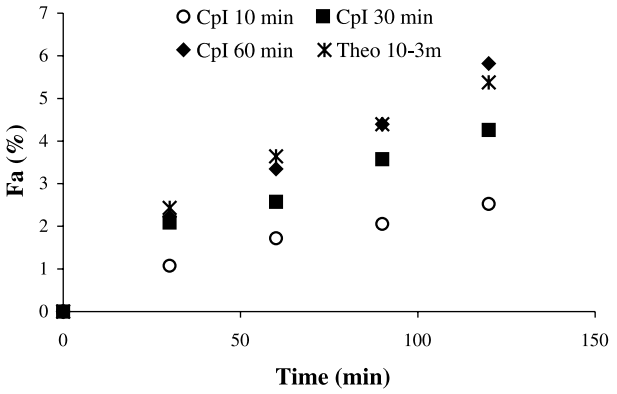

FIGURE 2 **Relationship Between Fraction Absorbed and Time of Immediate-Release Tablets Evaluated at 10 min (○), 30 min (■), and 60 min (◆), and for Theophylline (*). The Various Immediate-Release Tablets Samples Were Designed by CpI Indexed With the Time at Which the Sample was Removed (e.g., $CpI_{10\ min}$ is the Sample Taken at 10 min of the Dissolution Study for the Immediate-Release Tablet).**

Using these values to fit our data to Eq. 3 yields the f_a fraction of dose absorbed at t=∞, which corresponds to the bioavailability in case of no post-absorption, presystemic metabolism (Polli et al., 1996). We obtain $f_a = 0.36$ and $f_a = 0.54$ for the immediate- and controlled-release forms, respectively.

Ginski and Polli (1999) have reported a predicting system for drug absorption taking into account first-order dissolution and permeation rate. They predict that piroxicam, a high permeable low soluble drug, has different behavior whether the drug is included in a fast or in a slow formulation. The absorption was dissolution rate limited for the slow formulation and permeation rate limited for the fast formulation. Using the same approach, Yamashita et al. (2000) successfully developed a predicting system funded on dissolution data and changes of pH in the gastrointestinal tract to study the absorption of albendazole, a poor water-soluble drug.

Figure 4 shows the observed and predicted relationships between absorbed fractions and those dissolved. Because there is a general agreement between the observed and predicted data for both theophylline formulations, these results demonstrate that Eq. 3 has successfully predicted dissolution-absorption relationships. As noted by Polli et al. (1996), the correlation improves with increasing α (i.e., moving toward dissolution-limited absorption).

CONCLUSION

In the present work to compare two forms of theophylline, we coupled permeability coefficient measurements with drug release-dissolution studies using a numerical approach taking into account a

TABLE 3 **Caco-2 Monolayer Permeability and Cumulative Caco-2 Permeation for the Dissolution Samples Investigated**

Dissolution sample	Papp ($\times 10^{-5}$ cm/s)	Cumulative Caco-2 permeation (% of dose)	CV (%) for cumulative Caco-2 permeation
CpI 10 min	4.92±0.25	2.53±0.14	5.54
CpI 30 min	4.45±0.53	4.26±0.59	13.84
CpI 60 min	4.76±0.23	5.82±0.58	9.97
CpCR 120 min	5.05±1.59	2.62±0.40	3.16
CpCR 240 min	4.69±0.41	3.20±0.11	15.27
CpCR 360 min	4.57±0.45	5.12±0.19	3.44
CpCR 480 min	5.28±0.71	5.99±0.63	3.71
Theophylline 10^{-3}M	5.08±0.07	5.38±0.17	10.52

[Each result shows mean±SD (n=3). CpI indices with x min indicates the time at which the sample of immediate-release tablets was removed from experimental medium. Controlled-released tablets are designed by CpCR].

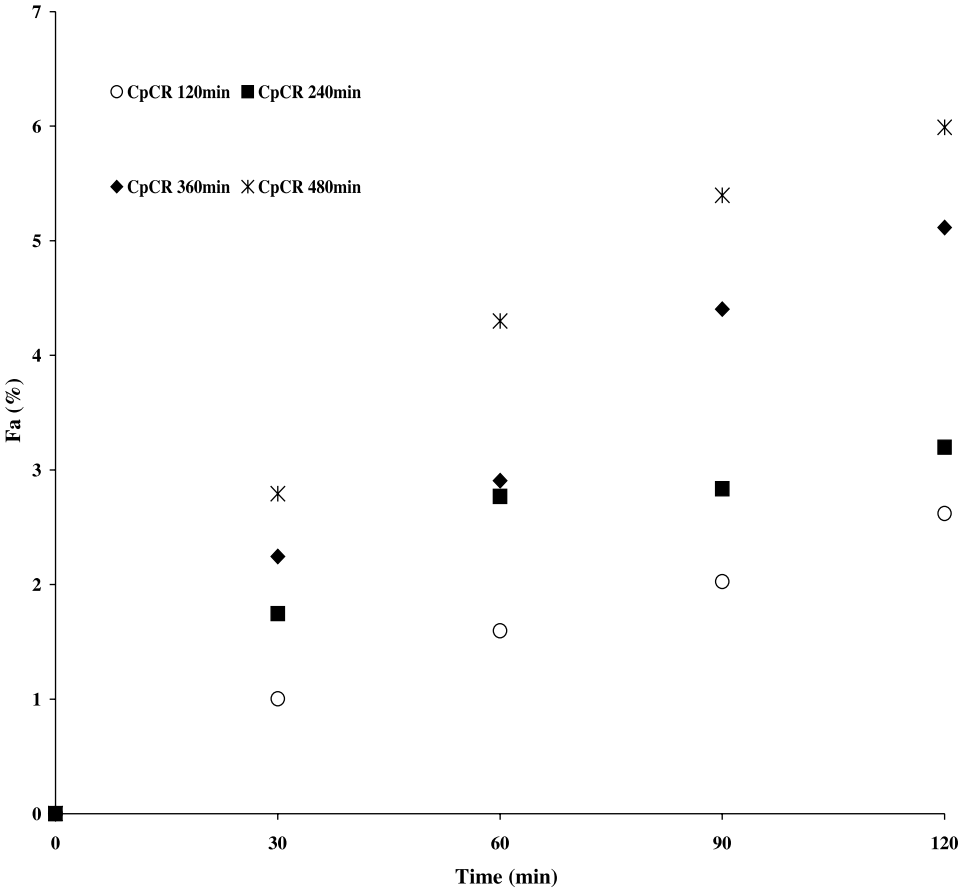

FIGURE 3 **Relationship Between Fraction Absorbed and Time of Controlled-Release Tablets Evaluated at 120 min (○), 240 min (■), 360 min (◆), and 480 min (*). Controlled-Release Tablets Samples were Designed by CpCR Indexed with the Time at Which the Sample was Removed (e.g., $CpCR_{120\ min}$ is the Sample Taken at 120 min of the Dissolution Study for the Controlled-Release Tablet).**

first-order exponential time numerical model. The model has successfully predicted the fraction absorbed from the fraction dissolved, by fitting the bioavailability value to the exponential date, for both immediate- and controlled-release of theophylline. Because the two dosage forms used in this study were formulated in our laboratory to control the technological parameters, future studies may regard factors that affect the established relationship in this study.

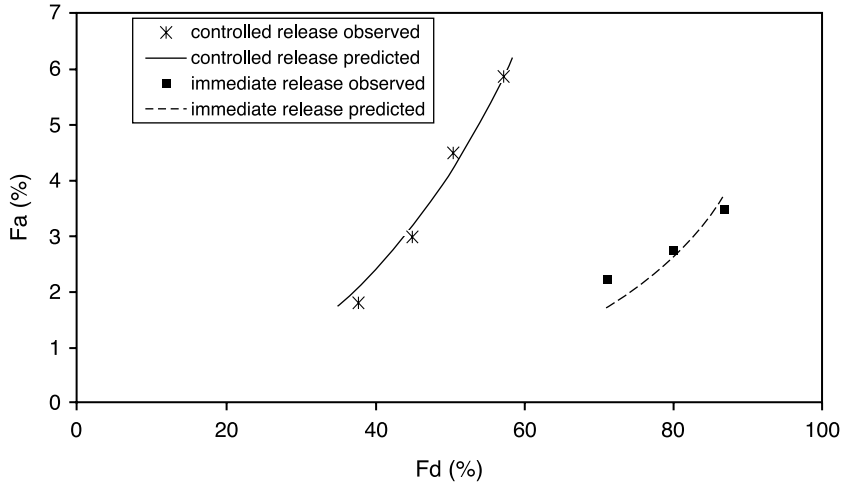

FIGURE 4 **Predicted and Observed Fa/Fd Relationships.**

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