# Evaluation of Various Dissolution Media for Predicting *In Vivo* Performance of Class I and II Drugs

**E. Galia,[1] E. Nicolaides,[2] D. Hörter,[1] R. Löbenberg,[1] C. Reppas,[2] and J. B. Dressman[1,3]**

*Received October 24, 1997; accepted February 20, 1998*

***Purpose.*** In this paper we seek to verify the differences in dissolution behavior between class I and class II drugs and to evaluate the suitability of two new physiologically based media, of Simulated Gastric Fluid (SGF) and of milk for their ability to forecast trends in the *in vivo* performance of class II compounds and their formulations.

***Methods.*** Dissolution behavior of two class I drugs, i.e. acetaminophen and metoprolol, and of three class II drugs, i.e. danazol, mefenamic acid and ketoconazole, was studied with USP Apparatus 2 in water, SGF, milk, Simulated Intestinal Fluid without pancreatin ($SIF_{sp}$) and in two media simulating the small intestinal contents in the fed (FeSSIF) and fasted (FaSSIF) states, respectively.

***Results.*** Class I powders dissolved rapidly in all media tested. Acetaminophen dissolution in milk was slow from one tablet formulation, in all other cases dissolution was more than 85% complete in 15 minutes. The dissolution rate of metoprolol was shown to be dependent on formulation and manufacturing method, and one of the three tablet formulations did not meet compendial specifications (80%/30 minutes). Dissolution behavior of class II drugs was greatly affected by choice of medium. Dissolution from a capsule formulation of danazol proved to be dependent on the concentration of solubilizing agents, with a the 30-fold increase in percentage dissolved within 90 minutes upon changing from aqueous media without surfactants to FaSSIF. Use of FeSSIF or milk as the dissolution medium resulted in an even greater increase in percentage dissolved, 100 and 180-fold respectively. Dissolution of the weak acid mefenamic acid from a capsule formulation is dependent on both pH and bile salt concentration, which leads to an offset between increased bile salt concentration and lower pH in the fed state compared to the fasted state medium. The weak base ketoconazole showed complete dissolution from a tablet formulation in Simulated Gastric Fluid without pepsin ($SGF_{sp}$) within 30 minutes, 70% dissolution in 2 hours under fed state simulated upper jejunal conditions but only 6% dissolution in 2 hours under fasted state conditions.

***Conclusions.*** As predicted, dissolution of class II drugs proved to be in general much more dependent on the medium than class I drugs. With the array of compendial and physiological media available, it should be possible to design a suitable set of tests to predict the *in vivo* dissolution of both class I and II drugs from immediate release formulations.

**KEY WORDS:** dissolution; physiological media; milk; compendial media; acetaminophen; metoprolol; danazol; mefenamic acid; ketoconazole.

[1] Institut für Pharmazeutische Technologie, J.W. Goethe Universität, Marie-Curie-Straße 9, 60439 Frankfurt am Main, Germany.

[2] University of Athens, Department of Pharmacy, Panepistimiopolis, 15771 Athens, Greece.

[3] To whom correspondence should be addressed. (e-mail: Dressman@em.uni-frankfurt.de)

## INTRODUCTION

The use of high throughput techniques for screening new compounds for pharmacological activity is becoming increasingly important (1). As a result, drugs being developed today exhibit an ever wider range of physicochemical characteristics. To assess whether these compounds possess not only the desired pharmacological activity but also the properties necessary for adequate bioavailability following oral administration, additional tests are required. Especially sought after are *in vitro* tests that are capable of predicting *in vivo* performance.

The recently proposed Biopharmaceutics Classification Scheme (BCS) (2) can be used as a guide to define which tests are most suitable for which drugs. According to the BCS, drugs can be divided into four classes on the basis of their aqueous solubility and their ability to permeate the mucosa in the gut from the apical to the basolateral side. Class I drugs are defined as those with high permeability which are able to dissolve readily in aqueous media over the pH range 1 to 8. Since dissolution is not rate limiting to oral absorption of these drugs, a point to point correlation between *in vitro* dissolution and absorption is not to be expected. Instead, a one point dissolution test requiring 85% dissolution within 15 minutes in a mild aqueous medium has been suggested as an indirect measure of bio(in)equivalence of two immediate release formulations of a class I compound (3).

In contrast to class I drugs, the choice of medium is expected to play a very important role in the dissolution of class II drugs. Class II drugs are defined as those with high permeability but whose solubility in aqueous media is insufficient for the whole dose to be dissolved in the gastrointestinal (GI) contents under usual conditions. Since dissolution, for these substances, is the rate limiting step to absorption and since dissolution of a class II drug can depend on a wide variety of factors such as surfactants, pH, buffer capacity, ionic strength and volume available for dissolution, the media used need to closely represent the prevailing conditions in the upper GI tract in order to achieve a meaningful *in vitro/in vivo* correlation (IVIVC) (4). Compendial media often fail for IVIVC of class II drugs because their composition does not take the above-mentioned physiological parameters into account. In an attempt to better predict *in vivo* performance of class II drug formulations, two new media representing the fed and fasted state in the upper jejunum have been developed (5). Milk, 3.5 % fat, and the USP simulated gastric fluid (6) with or without pepsin ($SGF/SGF_{sp}$) were additionally chosen as media to represent fed and fasted state conditions, respectively, in the stomach.

In this paper we seek to verify the differences in dissolution behavior between class I and class II drugs and to evaluate the suitability of the two new "physiological" media, of $SGF_{sp}$, and of milk, for their ability to forecast trends in the *in vivo* performance of class II compounds and their formulations. The class I drugs chosen for our dissolution studies were acetaminophen and metoprolol. Acetaminophen, an analgesic and antipyretic, was chosen on the basis of its high water solubility (14.5 mg/ml (7)), lack of ionization in the physiological pH range (pKa = 9.5 (8)), and favorable absorption properties. The second class I drug chosen was metoprolol, a widely used β-blocker, which has a pKa of 9.7 (8), a log P value of −0.1 (8) an aqueous solubility exceeding 1000 mg/ml (tartrate salt) (9)

0724-8741/98/0500-0698$15.00/0 

and high permeability (10). Our model drugs for class II were danazol, mefenamic acid and ketoconazole. The steroid danazol is a neutral, lipophilic compound (log P = 4.2 (8)) which is practically insoluble in water (aqueous solubility = 1 μg/ml (11)). Mefenamic acid, an NSAID, was chosen on the basis of its low aqueous solubility as a free acid (0.5 μg/ml (12), log P = 5.3 (8)) and the fact that changes in pH can have a profound influence on its solubility (pKa = 4.2 (8)). As an example of a poorly soluble weak base we chose the antifungal ketoconazole, the intrinsic aqueous solubility of which is 4.5 μg/ml (experimentally determined at 37°C). With pKa values of 6.5 and 2.9 (13), the dissolution of ketoconazole also varies greatly with pH in the physiological range. The log P of 4.3 (8) suggests further that ketoconazole could be solubilized by bile salts (14). The dissolution behavior of all five compounds was studied in various media including the two new "physiological" media, milk, $SGF_{sp}$ and in two other media commonly used to evaluate dissolution, namely $SIF_{sp}$ and water.

## MATERIALS AND METHODS

### Materials

Sodium taurocholate 98% pure lot #15H5001 was purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Egg-phosphatidylcholine, Lipid E PC 99.1% pure, lot #12091-1, was a gift from Lipoid GmbH (Ludwigshafen, Germany). Potassium dihydrogen phosphate, sodium dihydrogen phosphate and potassium chloride, all Analytical Grade, were purchased from E. Merck (Darmstadt, Germany). The source of the long life milk, 3.5% fat, was Landesgenossenschaft Ennstal Molkerei-Betriebe (Steinach, Austria).

Panadol® tablets (500 mg acetaminophen, lot #4125) were purchased from Sterling Health (Athens, Greece). Acetaminophen powder (lot #K28457) was a gift from Stada (Bad Vilbel, Germany). Acetaminophen powder (lot #7845993P150) was purchased from Mallinkrodt (Phillipsburg, NJ, USA). Three lots of metoprolol tartrate tablets (100 mg metoprolol tartrate, lot #DF931004, lot #DF931007 and lot #DF 931011) were manufactured at the University of Maryland, (Baltimore, MD, USA) (15). Metoprolol tartrate powder was a gift from Ciba-Geigy, (Basel, Switzerland). Danatrol® capsules (100 mg danazol, lot #M618730) were purchased from Sanofi Winthrop GmbH (Munich, Germany). Danazol powder (lot #64H0209) was purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany). Parkemed® capsules (250 mg mefenamic acid, lot #P521) were purchased from Parke-Davis (Berlin, Germany). Mefenamic Acid powder (lot #25/N) was a gift from Parke-Davis (Ann Arbor, MI, USA). Nizoral® tablets (200 mg ketoconazole, lot #95K27/995) were purchased from Janssen (Neuss, Germany). Ketoconazole powder (lot #10002924) was a gift from Janssen (Beerse, Belgium).

### Methods

For all dissolution tests the USP Apparatus 2 (paddle method) was used, employing 500 ml of dissolution medium at a temperature of 37 ± 0.5°C. The dissolution behavior of the various class I and II drug substances was tested according to the conditions listed in Table I.

Samples of approximately 5 ml were withdrawn at appropriate times, using a 5 ml Fortuna Optima® syringe (Fischer Labortechnik, Frankfurt/Main, Germany) fitted with appropriate tubing to facilitate representative sampling with sample replacement. Aqueous samples were filtered through 0.45 μm filters, chosen in each case for their lack of adsorption of the compound in question. Milk samples were filtered through Whatman No 41 (8 μm) filters. In addition to the different media, different rotational speeds were used to see if there was a significant hydrodynamic effect on the rate and extent of dissolution of the drugs under consideration. All experiments were run in triplicate.

#### *Composition of Various Media*

*Water.* For dissolution tests in water, deionized water obtained from a Labconco "water pro. ps." system (Labonco Comp., Kansas, MO) was used. The pH of this water ranged from pH 5.9 to 7.0.

*$SIF_{sp}$.* $SIF_{sp}$ was composed according to USPXXIII without pancreatin. At the time that these studies were initiated, the recommended pH for this medium was 7.5 (6).

*SGF/$SGF_{sp}$.* SGF, which was composed as described in USP XXIII (6) was used for the metoprolol studies, whereas $SGF_{sp}$, i.e. without pepsin, was used for the ketoconazole studies. Both media have a pH of 1.2.

*FaSSIF/FeSSIF.* Fluids simulating conditions in the proximal small intestine in the fasted state (FaSSIF) and fed state (FeSSIF) were composed according to data from the literature and studies performed at the University of Michigan. Gray and Dressman (3) have summarized pH values, while Bakatselou *et al.* (16) have summarized data for bile salt levels in the upper jejunum for the fed and fasted state. The compositions of the two media are given in Table II.

*Milk.* Bovine milk, 3.5% fat, was purchased from Landesgenossenschaft Ennstal Molkerei-Betriebe Steinach/Austria. This is a homogenized milk treated with ultra high temperature (UHT) to extend the shelf-life and containing no preservatives. It is isoosmotic, has a pH of about 6.5 and a buffer capacity of about 14 mEq/l/pH (17).

#### *HPLC-Analysis*

Three different HPLC/UV analytical systems were used. The first HPLC system (system 1) consisted of a Rheodyne 7161 injection valve (Rheodyne, Cotati, Ca., USA), a SP8800/8810 LC pump (Spectra Physics, San Jose, Ca., USA), a Spectra 100 UV-photometer (Spectra Physics, San Jose, Ca., USA) and a SP4400 integrator (Spectra Physics, San Jose, Ca., USA). The second HPLC system (system 2) consisted of a Bischoff Degaser Unit SDU 2003 (Bischoff, Leonberg, Germany), Bischoff 728 Autosampler (Bischoff, Leonberg, Germany), Rheodyne 7010 Injection valve (Rheodyne, Cotati, Ca., USA) mounted on a Model 732 electronic valve actuator (Micromeritics, Norcross, GA, USA), Bischoff model 2250 HPLC pump (Bischoff, Leonberg, Germany), Bischoff Lambda 1000 UV-detector (Bischoff, Leonberg, Germany) and a Shimadzu CR6A integrator (Shimadzu Europe, Duisburg, Germany). The third HPLC system (system 3) consisted of a WISP 712 autosampler

**Table I.** Media, Type of Dissolution Apparatus and Paddle Rotational Speeds used for Dissolution Studies of the Various Drug Substances

| Drug | BCS | Media | Dissolution tester type | rpm |
|---|---|---|---|---|
| Acetaminophen | I | water, $SIF_{sp}$, FaSSIF, FeSSIF, milk | Pharma Test Type PTW SIII-PTW S3C | 50,100 |
| Metoprolol | I | $SIF_{sp}$, SGF, FaSSIF, FeSSIF | Erweka Type DT6 | 100 |
| Danazol | II | water, $SIF_{sp}$, FaSSIF, FeSSIF, milk | Pharma Test Type PTW SIII-PTW S3C | 50,100 |
| Mefenamic Acid | II | water, $SIF_{sp}$, FaSSIF, FeSSIF, milk | Pharma Test Type PTW SIII-PTW S3C | 50,100 |
| Ketoconazole | II | $SGF_{sp}$, FaSSIF, FeSSIF | Pharma Test Type PTW SIII-PTW S3C | 100 |

(Waters Millipore, Eschborn, Germany), a Multisolvent Delivery System 600 (Waters Millipore, Eschborn, Germany), a Lambda max 481 UV-detector (Waters Millipore, Eschborn, Germany) and a Kontron integration system (Kontron, Milano, Italy).

*UV Analysis*

A Perkin Elmer Lambda 6 Spectrophotometer (Perkin Elmer, Norwalk, Conn., USA) (UV system a) and a Hitachi U-3000 Spectrophotometer (Colora Messtechnik GmbH, Lorch, Germany) (UV system b) were used for UV analysis.

*Acetaminophen Assays*

Samples from dissolution studies run in milk were analyzed by HPLC (system 1). After treating each sample with methanol and evaporating to dryness, 20 µl of acetaminophen and 2-acetamidophenol (internal standard), reconstituted in methanol, were injected onto a Spherisorb S10-ODS2 (4.6 × 250 mm) column and eluted with a mobile phase comprised of 13:87 MeOH:0.02 M acetate buffer (pH 4) at a flow rate of 1.3 ml/min. Acetaminophen and 2-acetamidophenol were detected at 254 nm. Samples from all other dissolution studies were analyzed by UV-spectroscopy at a wavelength of 243 nm. UV systems a and b were cross-verified with dissolution in $SIF_{sp}$.

*Metoprolol Assays*

Samples from all dissolution studies were analyzed by HPLC using system 3. For experiments in FaSSIF and FeSSIF, 50µl appropriately diluted samples were injected onto a Lichrocart 125-4 Lichrospher 60 Rp-Select B (5µm) column with guard column and eluted with a mobile phase comprised of 1:2:7 AcN:MeOH:30 mM $NaH_2PO_4$ buffer (pH 4.5) at a flow rate of 1.5 ml/min. For experiments in $SIF_{sp}$ and SGF, 15µl appropriately diluted samples were injected onto a Lichrocart 125-4 Lichrospher 60 Rp-Select B (5µm) column with guard column and eluted with a mobile phase comprised of 20:47:33 AcN:MeOH:30 mM $NaH_2PO_4$ buffer (pH 4.5) at a flow rate of 2 ml/min. Metoprolol was detected at 223 nm.

*Danazol Assays*

Samples from dissolution studies in milk and water were analyzed by HPLC using system 1. After treating each sample with n-hexane and evaporating the supernatant to dryness, 20 µl of a methanolic solution of danazol and testosterone propionate (internal standard) were injected onto a Spherisorb S5-ODS2 (4.6 × 250 mm) column and eluted with a mobile phase comprised of 65:35 AcN:$H_2O$ at a flow rate of 1 ml/min. Danazol and testosterone proprionate were detected at 270 nm. Samples from dissolution studies in FaSSIF and FeSSIF and $SIF_{sp}$ were analyzed by HPLC system 2. For experiments in $SIF_{sp}$ (100 rpm) and FaSSIF (50 rpm) 100 µl appropriately diluted samples were injected onto a Merck Lichrospher 125-4 5ODS-3 column with guard column and eluted with a mobile phase comprised of 40:30:30 AcN:MeOH:$H_2O$ at a flow rate of 1 ml/min. For experiments in FaSSIF (100 rpm) and FeSSIF (100 rpm), 100 µl of appropriately diluted samples were injected onto a Whatman Partisil 10ODS-3 (250 × 4.6 mm) column with guard column and eluted with a mobile phase comprised of 40:30:30 AcN:MeOH:$H_2O$ at a flow rate of 1.5 ml/min. Danazol was detected at 280 nm. As a cross check, samples from studies in $SIF_{sp}$ were analyzed by both HPLC systems.

*Mefenamic Acid Assays*

Samples from dissolution studies run in milk were analyzed by HPLC using system 1. After treating each sample with cyclohexane and evaporating the supernatant to dryness, 20 µl of a solution of mefenamic acid and flurbiprofen (internal

**Table II.** Composition of the Two, "Physiological Media" used in the Present Study to Simulate Fasted State (FaSSIF) and Fed State (FeSSIF) Intestinal Conditions

| Fasted state simulated intestinal fluid (FaSSIF) | | | Fed state simulated intestinal fluid (FeSSIF) | | |
|---|---|---|---|---|---|
| pH | | 6.5 | pH | | 5.0 |
| osmolality | | 270±10 mOsmol | osmolality | | 635±10 mOsmol |
| Sodium taurocholate | | 3 mM | Sodium taurocholate | | 15 mM |
| Lecithin | | 0.75 mM | Lecithin | | 3.75 mM |
| $KH_2PO_4$ | | 3.9 g | Acetic acid | | 8.65 g |
| KCl | | 7.7 g | KCl | | 15.2 g |
| NaOH | qs | pH 6.5 | NaOH | qs | pH 5.0 |
| Deionized water | qs | 1 liter | Deionized water | qs | 1 liter |

standard), reconstituted in methanol were injected onto a Spherisorb S10-ODS2 (4.6 × 250 mm) column and eluted with a mobile phase comprised of 60:35:0.5 AcN:$H_2O$:acetic acid at a flow rate of 1 ml/min. Mefenamic acid and flurbiprofen were detected at 280 nm. Samples from all other dissolution studies were analyzed by UV-spectroscopy at the wavelength of maximum absorbance of 285, 285, 284.5, 286, 292.5 nm, for experiments in water, $SIF_{sp}$ (system a), $SIF_{sp}$, FaSSIF and FeSSIF (system b), respectively.

*Ketoconazole Assays*

Samples from all dissolution studies were analyzed by HPLC using system 2. For experiments in $SGF_{sp}$ and FeSSIF, 20 μl and for experiments in FaSSIF 100 μl of appropriately diluted samples were injected onto a Merck Hibar Rp 18 125-4 (5 μm) column with guard column and eluted with a mobile phase comprised of 48:55:0.02 AcN:$H_2O$:$(C_2H_5)_2NH$ at a flow rate of 1.2 ml/min. Ketoconazole was detected at 254 nm.

*Data Treatment*

Data at 5 and 10 minutes occasionally showed large coefficients of variation (CV up to 75%), which were attributable to variable disintegration of the dosage forms. Thereafter, the CVs remained in the range of 0.4 to 15%. Representative standard deviations are shown in Figure 6.

## RESULTS

*Acetaminophen.* Figure 1 shows the mean dissolution profiles of acetaminophen from Panadol® tablets in various media at 100 rpm. For experiments in $SIF_{sp}$, water, FaSSIF and FeSSIF the profiles were nearly superimposable. Neither the amount of bile salts and lecithin, nor the pH affected the dissolution of acetaminophen from Panadol® tablets. Only in milk was the dissolution of Panadol® tablets clearly slower and in this case the percent release plateaued after approximately five hours (data not shown).

*Metoprolol.* Figure 2 shows the mean dissolution profiles of metoprolol tartrate from the three lots of tablets studied. Dissolution from lot #DF931004 in various media is summarized in Figure 2a. This formulation was observed to disintegrate quickly into fine particles. Choice of dissolution medium had no influence on the dissolution rate of metoprolol tartrate from this lot. Lot #DF931007 (Figure 2b) also disintegrated into fine particles and fulfilled the USP XXIII requirements of not less than 80% of drug dissolved within 30 minutes (6). The third lot of metoprolol tartrate tablets (# DF931011) exhibited disintegration characteristics different from those of the first two lots (Figure 2c). Tablets swelled over a time period of 10 to 15 minutes, after which they began to disintegrate into particles larger than those from the other two lots. Furthermore, the core of this formulation was still discernible after one hour. The dissolution profiles of tablets from lot #DF931011 reflect the

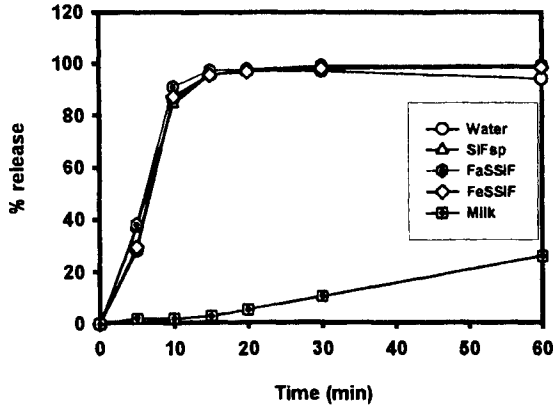


**Fig. 1.** Mean dissolution profiles of acetaminophen from Panadol® tablets in various media at 100 rpm.

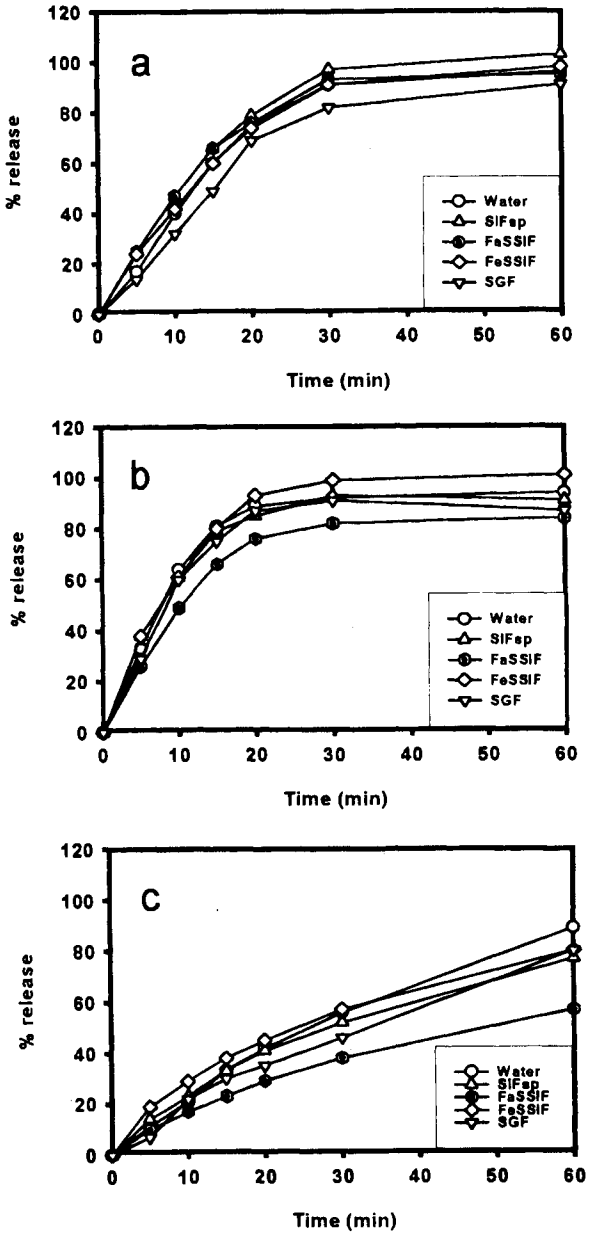


**Fig. 2.** Mean dissolution profiles of metoprolol tartrate in various media at 100 rpm from three different lots of tablets. (a) lot # DF931004; (b) lot # DF931007; (c) lot # DF931011.

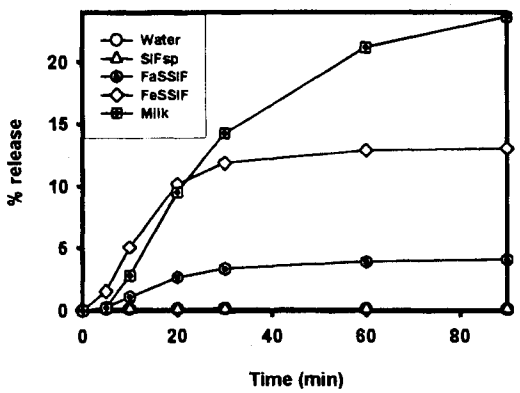


**Fig. 3.** Mean dissolution profiles of danazol from Danatrol® capsules in various media at 100 rpm.

disintegration characteristics of these tablets: dissolution is slow and incomplete even after one hour.

*Danazol.* Figure 3 shows the mean dissolution profiles of danazol from Danatrol® capsules in various media at 100 rpm. In media containing no surfactants, dissolution of danazol was practically nonexistent regardless of the pH of the medium. Changing to a bile salt/lecithin containing medium greatly enhanced the extent of dissolution (30 to 100-fold), but did not produce complete dissolution. Dissolution was even better in milk (180-fold increase in 90 minutes c.f. water). Furthermore no plateau was reached within the study interval.

*Mefenamic Acid.* Figure 4 shows the mean dissolution profiles of mefenamic acid from Parkemed® capsules in various media at a rotational speed of 100 rpm. In $SIF_{sp}$ medium (pH 7.5), the % release of mefenamic acid from Parkemed® capsules was 80-fold higher than in water (pH range 5.9–6.0) (40% versus 0.5%) after 60 minutes. This difference in dissolution behavior of mefenamic acid cannot be attributed solely to the pH difference between the two media. In the case of water, the lack of buffer capacity enables a microclimate pH close to the pKa of mefenamic acid to be established at the dissolving surface of mefenamic acid particles, limiting further dissolution. Comparing the two bile salt-containing media one can see a tradeoff between the solubility enhancement resulting from an increase in bile salt/lecithin concentration and a decrease in solubility resulting from the lower pH when changing from FaSSIF to FeSSIF medium. The favorable dissolution profile of mefenamic acid in milk can be explained by its high lipid content combined with its relatively high pH value (measured to be pH 6.6).

*Ketoconazole.* Figure 5 shows mean ketoconazole dissolution profiles from Nizoral® tablets in various media at 100 rpm. This weak base shows complete dissolution under acidic conditions ($SGF_{sp}$, pH 1.2) within 30 minutes. In the FeSSIF medium dissolution is somewhat slower, with 70% dissolving in two hours, indicating that the less favorable pH in the fed state medium (FeSSIF pH 5 versus $SGF_{sp}$ pH 1.2) is not compensated for completely by the high concentration of surfactants. In the FaSSIF medium the percentage dissolved falls to 6% in two hours. The decrease in bile salt/lecithin concentration combined with a further increase in pH (FaSSIF pH 6.5) drastically impairs dissolution of this weak base.

*Influence of the Rotational Speed on Dissolution Characteristics.* Figure 6a shows the mean dissolution profiles of Panadol® tablets in $SIF_{sp}$ obtained at 50 rpm and 100 rpm. The differences are marginal and within the range of the standard deviation. Danazol dissolution in FaSSIF (Figure 6b) exhibited the largest difference in dissolution behavior between rotational speeds of 50 and 100 rpm. As the scaling in case of Figure 6a is 24 times larger than in case of Figure 6b and as the percentage Danatrol® dissolved after 90 minutes was virtually the same in both cases, it seems that rotational speed influences even the dissolution of danazol only to a minor degree. Results for mefenamic acid as a function of rpm were similar (results not shown), suggesting that when the paddle method is used to evaluate immediate release dosage forms, large differences in dissolution behavior between 50 and 100 rpm are the exception rather than the rule.

## DISCUSSION

Classification of drugs according to the BCS (2) appears to be a valuable tool for the selection of suitable dissolution media. As long as there is fast disintegration of the tablet into

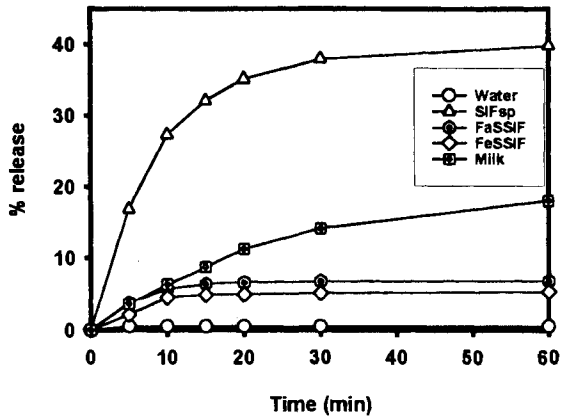


**Fig. 4.** Mean dissolution profiles of mefenamic acid from Parkemed® capsules in various media at 100 rpm.

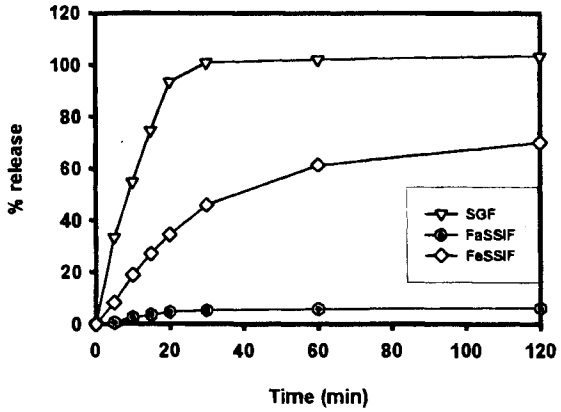


**Fig. 5.** Mean dissolution profiles of ketoconazole from Nizoral® tablets in various media at 100 rpm.

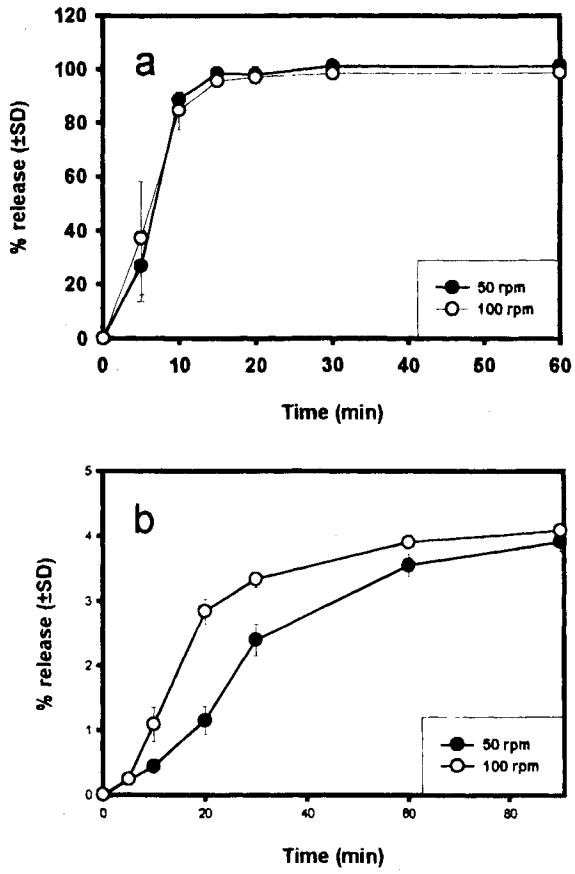


**Fig. 6.** Influence of rotational speed on the mean (± SD) dissolution profiles of (a) acetaminophen from Panadol® tablets in $SIF_{sp}$, and (b) danazol from Danatrol® capsules in FaSSIF.

fine, dispersed particles, dissolution of class I drugs should be rapid in any mild aqueous medium. This was found to be the case for acetaminophen. Only in milk was the dissolution of acetaminophen from Panadol® tablets slow. Experiments with acetaminophen powder and a second, commercial 500 mg acetaminophen tablet were also conducted. In both cases dissolution was complete, either within 15 minutes (powder) or within 2 hours (tablet) (results not shown). These results indicate that the much slower dissolution from Panadol® tablets can be attributed to specific interactions of the excipients with milk components. Panadol® tablets are film-coated whereas the second tablet formulation is not. The general lack of influence of dissolution medium properties on the *in vitro* dissolution performance of class I drugs is reflected by the simplicity of the media suggested for their dissolution tests in the monographs of the USP (6). Examples include enalapril and diltiazem, which are tested in water, and piroxicam and labetalol, for which $SGF_{sp}$ and 0.1 N HCl are used respectively as the dissolution medium.

To further investigate the influence of formulation properties on dissolution and bioavailability of class I drugs, three different formulations of metoprolol tartrate tablets, manufactured at the University of Maryland, were tested *in vitro* and compared with bioavailability data (15). According to these studies, the three lots were essentially bioequivalent, although they exhibited increasing $t_{max}$ values in the order lot #DF931007 <DF931004 <DF931011. The *in vitro* dissolution profiles reflected this tendency among the three lots. Dissolution from tablets from lot #DF931011 was markedly slower than from the other two lots and failed to meet the compendial requirements of 80% release within 30 minutes (6). As experiments with metoprolol tartrate powder in the various media indicated that dissolution is very fast and complete (results not shown), slow dissolution from a metoprolol formulation can be attributed entirely to the excipients and manufacturing process. The poorer *in vitro* dissolution properties of lot #DF931011 did not, however, lead to reduced absorption from this formulation.

The lack of bioinequivalence in conjunction with poor dissolution properties of formulations containing class I drugs throws the test criteria proposed by both the USP (6) and the SUPAC Guidelines (4) into question. One problem with setting a time limit for the dissolution of class I drugs is that factors other than dissolution, especially gastric emptying, can influence the rate of absorption to a large degree. For example, a substantial delay in the $T_{max}$ due to delayed gastric emptying has been observed for acetaminophen when food is coadministered or by retarding gastric emptying using narcotics such as meperidine or pentazocine. When Clements *et al.* (18) administered an acetaminophen solution to eight male subjects, with or without co-administration of meperidine or pentazocine, a substantial delay in $T_{max}$ was observed in the former case. McGilveray *et al.* (19) showed, likewise, that although the extent of absorption of acetaminophen was not influenced by administration of food (orange juice, cornflakes with milk and "Pop Tarts"), the $T_{max}$ was substantially delayed. The FIP defines a formulation as "very fast releasing" when 80% of the drug substance is dissolved in about 20 to 30 minutes (20). This dissolution time window is based on typical gastric emptying times for water in the fasted state. Gastric emptying times for calorie rich fluids (21) and meals can be substantially longer. Depending on when and with what fluid the dosage form is to be administered, it may be appropriate to adjust the duration of the test (4).

It is clear from the large differences in the dissolution profiles among the various media, that in the case of class II drugs, considerable care must be taken in the choice of dissolution medium. As dissolution of these drugs is the rate limiting step to absorption it should generally be possible to establish an IVIVC. However, since the standard compendial media do not simulate the physiological conditions well enough, development of meaningful IVIVCs with these media is seldom possible.

For the quality control testing of drugs showing poor aqueous solubility, the USP (6) suggests a wide range of dissolution media. The medium used in the USP monograph for danazol dissolution consists of 40% 2-propanol and 60% 0.1 N HCl, clearly unphysiological. In other cases of low solubility drugs sodium dodecyl sulfate (SDS) is often added to achieve 100% drug release. Examples are griseofulvin (4% SDS in the case of regular and 0.54% in the case of ultramicronized) and carbamazepine (1% SDS). It is by no means clear whether SDS is a good surrogate for bile components. For example Shah et al. (22) obtained different dissolution rates in SDS than in bile salt containing solutions for two different griseofulvin formulations. Additionally, if dissolution is rate limiting to drug absorption, a medium in which 100% release is achieved within the duration of the test may be unsuitable for prediction of *in vivo* performance purposes (4).

The influence of food on bioavailability of the poorly soluble antifungal drug, griseofulvin, was studied by Crounse *et al.* (23). When griseofulvin was given with a high fat meal its bioavailability was increased. Knowing the physiological conditions in the upper GI tract, it is obvious that solubilization by bile and food components, together with increases in fluid volume, are responsible for the dramatic increase in bioavailability of this lipophilic compound (log P = 2.2 (8)). In vitro studies by Bates *et al.* (24) and de Smidt *et al.* (25) underline the importance of the bile salts for enhanced griseofulvin solubility and hence, its enhanced bioavailability. The same effect is likely to be responsible for the increased bioavailability of danazol, when given with food. In an *in vivo* study carried out by Charman and coworkers (26), plasma concentration time profiles of danazol showed a $C_{max}$ and an AUC in the fed state about three times higher than in the fasted state. Solubility studies of pure danazol in bile salt/lecithin solutions conducted by Naylor (27) showed an increase in solubility of the same order of magnitude. Our *in vitro* dissolution results with Danatrol® capsules also reflect the *in vivo* performance well. As can be seen in Figure 3, the percent release after 90 minutes is three-fold higher when FeSSIF rather than FaSSIF is used for dissolution.

The situation is even more complicated when the solubility of the compound under investigation is also pH dependent. For drugs with pKa values in the physiological range, the apparent solubility may change greatly with changes in pH of the environment. It is interesting to note that the USP (6) provides no information on how to perform dissolution tests with mefenamic acid formulations. The very low solubility of mefenamic acid in its free acid form of 0.5 μg/ml, combined with a dose of 250 mg, results in a dose:solubility ratio of 500 liters. This exceeds the commonly assumed volume of GI fluids of 250 ml by a factor of 2000. TenHoor *et al.* (12) reported the solubility of mefenamic acid under simulated fasted and fed state conditions. They reported that the increased solubility resulting from higher bile salt concentrations under fed state conditions offset the lower pH value of the medium. Our dissolution test results essentially follow this solubility behavior and concur with published bioavailability data. Hamaguchi *et al.* (28) conducted an *in vivo* study to examine the influence of food and water intake on mefenamic acid bioavailability. Their results indicated that bioavailability of mefenamic acid is independent of food intake. $T_{max}$ data from experiments in dogs (29) indicate that absorption continues over a long time period ($T_{max}$ median value: 6.0 h; range: 3.0 to 10 h), when mefenamic acid powder is administered in a capsule. Poor dissolution in the acidic environment of the stomach, rising pH along the small intestine and the excellent permeability of the mucosa to mefenamic acid (30), may account for the extended absorption of this compound. Although our *in vitro* results in FaSSIF, FeSSIF and milk bracket the absolute bioavailability of mefenamic acid in dogs and monkeys (31) further studies with weak acids for which the absolute bioavailability in humans is known are needed to assess the predictive capability of the new media more closely.

Ketoconazole was used as an example of a weakly basic class II drug. For poorly soluble weak bases dissolution in the stomach plays an important role in the overall dissolution, especially when the pKa of the weak base is very low. In the fasted state, gastric pH has been measured to be in the range of 1.5 to 2 for healthy subjects (32). This is more favorable to weak base dissolution than the pH conditions in the stomach after ingestion of a meal, when the pH can be elevated to values above pH 5 (32). Van der Meer *et al.* (33) showed that bioavailability of ketoconazole in the fasted state was dependent on gastric pH. Acidified solutions given with 200 mg ketoconazole showed improved absorption, while 200 mg ketoconazole given two hours after administration of 400 mg cimetidine or with 100 ml of 0.5% bicarbonate solutions showed decreased bioavailability. In the fed state, unfavorable increases in gastric pH can be offset by the favorable effects of higher bile concentrations, especially in the case of substances with high log P, and also by the larger volume of GI fluids available. Daneshmend *et al.* (34) found an improvement in absorption of ketoconazole at doses of 400 and 600 mg when it was taken with a standard breakfast. The dissolution profiles in Figure 5 demonstrate that the effects of acidity and concentration of bile salts on *in vitro* ketoconazole dissolution are consistent with results of clinical studies.

Other poorly soluble weak bases behave similarly to ketoconazole. In a clinical study, Russell *et al.* (35) studied the bioavailability of dipyridamole in fasted elderly subjects with or without achlorhydria and with co-treatment of famotidine or glutamic acid, respectively. In both groups the "low gastric pH treatments" exhibited higher dipyridamole bioavailability. As in the fasted state the stomach may be the most important site of dissolution for weak bases, another very important factor to their bioavailability in the fasted state could be precipitation of the compound when it enters the duodenum, where pH increases to above pH 6. Mithani (36) investigated nucleation phenomena of dipyridamole in various media and found that it is possible for dipyridamole to precipitate in the fasted state. A similar phenomenon has been observed for ketoconazole by Hörter (unpublished work). Change-of-medium experiments, using FaSSIF as the second medium, can be conducted to assess the likelihood of precipitation upon entry in the duodenum.

## CONCLUSIONS

Appropriate recommendations concerning the dissolution test(s), suitable for a meaningful prediction of *in vivo* performance, can be made according to the BCS classification of the drug(s) under investigation.

*Class I.* Simple, mild aqueous dissolution media such as $SGF_{sp}$ are sufficient for class I drug formulations. Use of more complex media such as FaSSIF and FeSSIF is not warranted. Use of milk as a dissolution medium may be a useful tool for detecting specific food/formulation interactions.

*Class II.* In the case of a class II drug formulation, the choice of dissolution test(s) will depend on whether the drug is ionizable or not. In the case of a neutral class II compound, the concentration of solubilizing components is the prime determinant of solubility and hence dissolution behavior. In this case results in FaSSIF should be representative of dissolution under fasted state conditions in the upper GI tract. In the case of a class II weak base formulation, dissolution in the stomach in the fasted state will be of paramount importance. $SGF_{sp}$ can be used to assess this initial dissolution. To assess the possibility of precipitation, a dissolution test, changing conditions from gastric fasted state conditions ($SGF_{sp}$) to fasted state intestinal conditions (FaSSIF), might be suitable. Results obtained under appropriate fasted state conditions can then be compared to dissolution in FeSSIF to help establish whether the formulation should be administered before or after meals. If the formulation

contains a class II weak acid, dissolution under intestinal conditions will be important in the fasted state. This can be modeled well with FaSSIF. $SIF_{sp}$, at a pH of 7.5, cannot be recommended since the high pH results in an overprediction of the dissolution rate of weak acids. As a medium to simulate the fed state, either milk with its composition of lipids and proteins (gastric conditions) or FeSSIF with its high bile salt/lecithin levels (proximal small intestine conditions), can be used. Simulation of dissolution in the stomach under fed state conditions with milk may lead to overestimation of the dissolution of weak acids due to the relatively high pH of milk. FeSSIF is a reasonable starting point for assessing dissolution in the intestine after administration with meals.

In conclusion, with the array of compendial and physiological media available, it should be possible to design a suitable set of tests to predict the *in vivo* dissolution of both class I and II drugs from immediate release formulations. The similarity of these media to physiological conditions should, as in the case of the examples studied, lead to more predictive *in vivo* tests in the preclinical phase.

## ACKNOWLEDGMENTS

This study was presented in part at the 11th Annual Meeting of the American Association of Pharmaceutical Scientists, Seattle, USA, 1996. This work was supported by research grants from GlaxoWellcome, Inc., Janssen Pharmaceutica and the FDA.

## REFERENCES

1. P. Smith. Combinatorial screening approaches for selection of development candidates—experiences within SmithKline Beecham. Fourth International Conference on Drug Absorption, Edinburgh, Scotland (1997).
2. G. L. Amidon, H. Lennernäs, V. P. Shah, and J. R. Crison. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.* **12**:413–420 (1995).
3. A. Rudman and R. Wiliams. Center for Drug Evaluation and Research (CDER). Guidance for Industry. Immediate release solid oral dosage forms. Scale-up and post-approval changes: Chemistry, manufacturing and controls, *in vitro* dissolution testing, and *in vivo* bioequivalence documentation. FDA, Rockville, MD (1995).
4. J. B. Dressman, G. L. Amidon, C. Reppas, and V. P. Shah. Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms. *Pharm. Res.* **15**:11–22 (1998).
5. E. Galia, E. Nicolaides, C. Reppas, and J. B. Dressman. New media discriminate dissolution properties of poorly soluble drugs. *Pharm. Res.* **13**(Supplement):S-262 (1996).
6. The United States Pharmacopeia (USP 23). United States Pharmacopeial Convention, Inc., Rockville, MD (1995).
7. J. E. Fairbrother. Analytical Profiles of Drug Substances (Volume 3), Ed. K. Florey, Academic Press, New York, NY (1974).
8. D. B. Jack. Handbook of Clinical Pharmacokinetic Data, Macmillan Publishers Ltd, Houndmills, UK (1992).
9. The Merck Index (Eleventh Edition), Ed. S . Budavari, M.J. O'Neil, A. Smith, and P. E. Hechelman, Merck & Co., Inc., Rahway, NJ (1989).
10. H. Lennernäs, S. Nylander, and A. L. Ungell. Jejunal permeability: A comparison between the Ussing chamber technique and the single-pass perfusion in humans. *Pharm. Res.* **14**:667–671 (1997).
11. L. J. Naylor, V. Bakatselou, N. Rodriguez-Hornedo, N. D. Weiner, and J. B. Dressman. Dissolution of steroids in bile salt solutions is modified by the presence of lecithin. *Eur. J. Pharm. Biopharm.* **41**:346–353 (1995).
12. C. N. TenHoor, V. Bakatselou, and J. B. Dressman. Solubility of mefenamic acid under simulated fed- and fasted state conditions. *Pharm. Res.* **8**:1203–1205 (1991).
13. T. K. Daneshmend and D. W. Warnock. Clinical pharmacokinetics of ketoconazole. *Clin. Pharmacokin.* **14**:13–34 (1988).
14. S. D. Mithani, V. Bakatselou, C. N. TenHoor, and J. B. Dressman. Estimation of the increase in solubility of drugs as a function of bile salt concentration. *Pharm. Res.* **13**:163–167 (1996).
15. G. Rekhi *et al.* Evaluation of *in vitro* release and *in vivo* absorption characteristics of four metoprolol tartrate IR tablet formulations. *Pharm. Dev. Tech.* **2**:11–24 (1997).
16. V. Bakatselou. Dissolution of steroidal compounds at physiological bile salt concentrations. Doctoral Thesis, University of Michigan, Ann Arbor, MI (1990).
17. P. Walstra, and R. Jerness. Dairy Chemistry and Physics, Wiley-Interscience, New York, NY (1984).
18. J. A. Clements, R. C. Heading, W. S. Nimmo, and L. F. Prescott. Kinetics of acetaminophen absorption and gastric emptying in man. *Clin. Pharmacol. Ther.* **24**:420–431 (1978).
19. I. J. McGilveray and G. L. Mattok. Some factors affecting the absorption of paracetamol. *J. Pharm. Pharmacol.* **24**:615–619 (1972).
20. M. Siewert. FIP guidelines for dissolution testing of solid oral products. *Pharm. Ind.* **59**:760–766 (1997).
21. W. Brener, T. R. Hendrix, and P. R. McHugh. Regulation of the gastric emptying of glucose. *Gastroenterol.* **85**:76–82 (1983).
22. V. P. Shah, J. J. Konecny, R. L. Everett, B. McCullough, A. C. Nooriazadeh, and J. P. Skelly. *In vitro* dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. *Pharm. Res.* **6**:612–618 (1989).
23. R. G. Crounse. Effective use of griseofulvin. *Arch. Dermat.* **87**:176–180 (1963).
24. T. Bates, M. Gibaldi, and J. Kanig. Solubilizing properties of bile salt solutions. *J. Pharm Sci.* **55**:191–199 (1966).
25. J. H. deSmidt, M. Grit, and D. J. A. Crommelin. Dissolution kinetics of griseofulvin in mixed micellar solutions. *J. Pharm. Sci.* **83**:1209–1212 (1994).
26. W. N. Charman, M. C. Rogge, A. W. Boddy, W. H. Barr, and B. M. Berger. Absorption of danazol after administration to different sites of the gastrointestinal tract and the relationship to single- and double-peak phenomena in the plasma profiles. *J. Clin. Pharmacol.* **33**:1207–1213 (1993).
27. L. J. Naylor. Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems. Doctoral Thesis, University of Michigan, Ann Arbor, MI (1993).
28. T. Hamaguchi, D. Shinkuma, Y. Yamanaka, and N. Mizuno. Bioavailability of mefenamic acid: Influence of food and water intake. *J. Pharm. Sci.* **75**:891–893 (1986).
29. C. Reppas, G. Eleftheriou, J. B. Dressman, P. Macheras, and M. Symillides. The effect of elevated viscosity in the upper gastrointestinal tract on drug absorption in dogs. *Eur. J. Pharm. Sci.* (in press).
30. L. C. Dermentzoglou. Changes in upper gastrointestinal pH with aging: Implications for drug absorption. Doctoral Thesis, University of Michigan, Ann Arbor, MI (1989).
31. E. J. Randinitis and A. W. Kinkel. Bioequivalence comparison of mefenamic acid and soluble salts in dog, monkey and human. Personal Communication (1995).
32. J. B. Dressman, R. R. Berardi, L. C. Dermentzoglou, T. L. Russell, S. P. Schmaltz, J. L. Barnett, and K. M. Jarvenpaa. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm. Res.* **7**:756–761 (1990).
33. J. W. M. van der Meer, J. J. Keuning, H. W. Scheijgrond, J. Heykants, and J. van Cutsem. Influence of gastric acidity on the bioavailability of ketoconazole. *J. Antimicro.* **6**:520–524 (1980).
34. T. K. Daneshmend, D. W. Warnock, M. D. Ene, E. M. Johnson, and G. Parker. Influence of food on the pharmacokinetics of ketoconazole. *J. Antim. Ag. Ch.* **25**: 1–3 (1984).
35. T. L. Russell, R. R. Berardi, J. L. Barnett, T. L. O'Sullivan, J. G. Wagner, and J. B. Dressman. pH-related changes in the absorption of dipyridamole in the elderly. *Pharm. Res.* **11**:136–143 (1994).
36. S. Mithani. Dissolution and precipitation of dipyridamole: Effect of pH and bile salt concentration. Personal Communication (1997).