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APPLICATION OF FLOW-THROUGH DISSOLUTION METHOD FOR THE EVALUATION OF ORAL FORMULATIONS OF NIFEDIPINE

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ABSTRACT

The drug release characteristics of three oral formulations (one conventional and 2 extended-release) of nifedipine were evaluated using a flow-through apparatus. The experiments were conducted for 4 to 24 hours using water or phosphate buffer (0.05 or 0.1 M; pH 7.4) with or without solubilizing agent, Tween, as a dissolution medium at a flow rate of 12.5 mL/min. The drug concentrations were determined using an HPLC method based on ratios of peak heights corresponding to UV absorbances at 254 nm for nifedipine and nitrendipine (internal standard). Dissolution characteristics in various media correspond to the nifedipine solubility in the medium. Peak nifedipine concentrations with 0.05 M phosphate buffer containing 0.5% Tween were significantly higher than those in the medium without Tween (21.5±1.0 *vs* 8.3±0.2 μg/mL, $p < 0.001$). Using a 0.05 M phosphate buffer with no Tween, the products tested showed distinct dissolution profiles representative of the respective formulation type. The conventional release product (10 mg) showed a higher mean peak nifedipine concentration ($C_{max,d}$) of 49.5±2.4 μg/mL ($p < 0.001$) attained at

($t_{max,d}$) 0.46±0.05 h as compared to those of modified-release products. The corresponding mean values for the modified-release tablets were 8.3±0.2 and 2.6±0.3 µg/mL for $C_{max,d}$, and 0.28±0.03 and 12.0±3.8 h for $t_{max,d}$ for the 20 and 30 mg tablets, respectively. Area under the concentration-time curves ($AUC_{0-t,d}$) for the 10, 20 and 30 mg formulations were 12.3±0.4, 20.5±2.6 and 32.6±3.7 µg.h/mL, respectively ($p < 0.001$). As the dissolution profiles are similar to those of plasma/serum drug concentrations-time profiles obtained from clinical studies, application of this dissolution method, along with the derived *in vitro* drug-release kinetics parameters for potential correlation with *in vivo* parameters are discussed. The results of this study show that, compared to the USP dissolution method using apparatus 1 or 2, the flow-through dissolution system offers a potentially better alternative to assess drug release characteristics for different types of formulations, especially for drugs of low aqueous solubility such as nifedipine.

INTRODUCTION

In vitro drug release testing of pharmaceutical dosage forms, whether in development or in production, is imperative not only to ensure batch to batch quality control both, but also to screen formulations during product development to obtained a optimally effective products (1). Though controversial, since it is not necessarily applicable, the dissolution test is the single most important parameter to assess the *in vivo* drug release characteristics from a formulation. Skelly (2) reported that virtually all products with bioavailability/bioequivalence problems observed by the US FDA during the last 13 years are explainable in terms of poor dissolution on the part of the bioinequivalent product. Therefore, it is important that, where possible, a dissolution test should be designed to mimic as closely as possible the physiological conditions e.g. medium (nature, composition, pH, temperature, etc.).

Generally dissolution studies are performed using Apparatus 1 (basket) or 2 (paddle) as described in the USP (3). Briefly, such studies require monitoring of drug release from a particular product using a recommended medium (e.g., water, buffer) in a volume generally between

500 - 1000 mL for a suggested duration. One of the requirements, is to monitor the drug-release characteristics under a sink condition, generally defined (4) as the maximum expected concentration during an experiment should not be greater than 15% of the solubility of the analyte in the dissolution medium employed. For products with active ingredients of low aqueous solubility or with a large dosage content (e.g. extended-release products) sink condition may be obtained by exploiting the physico-chemical properties of the compound (e.g., use of emulsifiers, hydro-alcoholic media, media with favourable pH, etc.). However, such alterations will often result in non-physiological environments.

A flow-through type dissolution apparatus (5) offers a unique advantage by which large volumes of dissolution medium can be used. By using such a system, use of emulsifiers or organic solvents, to increase the solubility of the analyte, can be avoided. Moreover, compared to the classic dissolution systems, flow-through system more closely resembles the physiological environment of the gastrointestinal tract, with continuous extraction (removal) of drug from the dissolution vessel mimicking absorption into the systemic circulation. Though it is commercially available, applications of the flow-through apparatus to dissolution studies of drug products have been limited. Recently, the USP has accepted (6) a flow-through apparatus as an official compendial method (apparatus 4) for dissolution studies. During a recent USP meeting (7) a general consensus was that application of such a system for the evaluation of different types of drug products is highly desirable.

Nifedipine, one of the commonly used calcium channel antagonist drugs, is indicated for the treatment of hypertension. Chemically, nifedipine belongs to a group of nitro-dihydropyridines and has a very low water solubility. For this drug, in addition to conventional (immediate) release product, extended-released products with higher drug content are also commercially available.

There is a USP monograph describing the dissolution studies for conventional release-form nifedipine capsules. Because of low aqueous solubility of nifedipine, it would not be possible or appropriate to evaluate

the release characteristics of different types of nifedipine formulations using classical dissolution systems with the limited volume of dissolution media. Therefore, comparison of release characteristics for different type of formulations would be difficult. This report describe the application of a flow-through dissolution technique for the evaluation of *in vitro* release characteristics of different types of formulations of nifedipine under the same experimental conditions and its potential use for assessing *in vitro-in vivo* drug release correlation.

EXPERIMENTAL

Materials:

The drug products tested were obtained from the local market. The formulations evaluated were: **A** (10 mg conventional-release capsules) and **B** and **C** (20 and 30 mg extended-release tablets). The drug delivery mechanism of the formulation **C** was based on the principle of osmosis.

Nifedipine and nitrendipine, used as standards, and Tween 80 were obtained from Sigma Chemicals (St. Louis, MO). All other chemicals and solvents were of analytical grade.

Instrumentation:

Dissolution Apparatus: A six cell flow-through dissolution apparatus (SOTAX Dissotest) was obtained from Leap Technologies (NC, USA). The cells of 22 mm diameter used for this study and were kept at 37 °C by circulating heated water. The dissolution medium was pumped through an external pump at a rate of 12.5 mL/min, passing through a heat exchanger to maintain a constant temperature. Eluent samples were collected in a fraction collector by using a 3-way distributor to split the quantity of the eluent.

Chromatographic System: The system consisted of a Beckman HPLC pump (Model 110 A), Waters 440 UV detector system set at 254 nm, an autosampler (WISP, Waters), and a chart recorder set at a chart speed of 0.5 cm/min.

Chromatographic separations were achieved using a 5 μm C_{18} reversed phase column (0.46 x 25 cm) obtained from Supelco Inc (PA, USA). A mixture of methanol and water (75:25 v/v) was used as mobile phase with a flow rate set at 1.0 mL/min. Under the chromatographic conditions used, the retention times for nitrendipine and nifedipine were 5.0 and 7.0 min, respectively.

To avoid degradation of nifedipine, the dissolution studies were conducted under yellow fluorescent lights and all solutions were kept in glassware wrapped in aluminum foil.

Preparation of Standard Solutions:

Nitrendipine (Internal Standard, IS) Solution: A stock solution was prepared by dissolving nitrendipine in methanol to yield a solution having a concentration of 1 mg/mL. A working solution was prepared by dilution of the stock solution with methanol to a concentration of 30 μg/mL. One mL of this solution was used for spiking the study or calibration samples for chromatographic analysis.

Nifedipine Standard Solutions: A stock solution of nifedipine (1 mg/mL) was prepared in methanol and a dilution of this solution was made with methanol: 0.05M (pH 7.4) phosphate buffer (1:1) to yield a solution containing 30 μg/mL of nifedipine. This solution was serially diluted with phosphate buffer containing 50% methanol to yield solutions having the concentrations 15, 7.5, 3.75, 1.87, 0.94, and 0.47 μg/mL. One mL of each of these solutions, after mixing with one mL of the IS solution, was used for injection on the HPLC column to generate the calibration curves. All these solutions were freshly prepared for each set of samples, and kept covered with aluminum foil and protected from light to avoid possible degradation.

Data and Statistical Analysis: The nifedipine concentrations used for calculations or shown in the figures represent mid-point levels calculated as mean concentration over the fraction time. The reported nifedipine concentrations corresponds to the dosage strength and are not corrected or normalized to a particular strength. The area under the drug concentration-time curve to the last quantifiable concentration ($\mathbf{AUC_{0\text{-}t,\ d}}$)

was computed by linear trapezoidal rule. The elimination half-lives ($t_{1/2, d}$) were estimated from the terminal linear portion of the semi-log plot of nifedipine concentration-time curves by least square regression. To differentiate from parameters generally obtained from *in vivo* studies, the derived parameters for the dissolution studies are subscripted with a letter **d**. Parameters were determined for individual tablets or capsules, and mean (n=6) and standard deviations for each parameter were then computed. Statistical evaluations were performed by *Student's t-test* or analysis of variance (ANOVA) using a statistical software package, SAS (SAS Institute Inc., Cary, USA).

RESULTS AND DISCUSSION

Analytical Methodology: Calibration curves were drawn using peak height ratios of nifedipine *vs* IS against nifedipine concentrations (0.236 to 15.0 µg/mL) of nifedipine using weighted (1/peak height ratio) regression analysis. Calibration curves were linear over the range used with high coefficients of determination (R^2=0.999 ± SD=0.0014). The mean (n=12) slope and intercept values (± SD) for the calibration curves were 0.20 ± 0.0178 and 0.0005 ± 0.007, respectively. Coefficients of variation for the predicted (back-calculated) concentrations ranged from 2.2 to 8.6% for the concentrations used. Accuracy of the predicted values, measured as the percent relative deviation from the nominal were more than 97.0 % over the range.

Dissolution Studies: The drug release characteristics were monitored for 4 to 24 h for the formulations using water or phosphate buffer (0.05 or 0.1 M, pH 7.4) as medium. For sampling, after splitting the eluent in a ratio of 1:6, smaller fraction of the continuous stream of eluent was collected for various time intervals. The drug concentrations in individual fractions were determined by the HPLC method, after mixing the 1 mL of test solution with 1 mL of the IS solution. The HPLC conditions used are similar to those described for the USP monograph for nifedipine (8), modified by the use of an internal standard.

Figure 1 shows the release characteristics of formulation **B** using phosphate (0.05M) buffer as the medium with or without Tween (0.5%). It

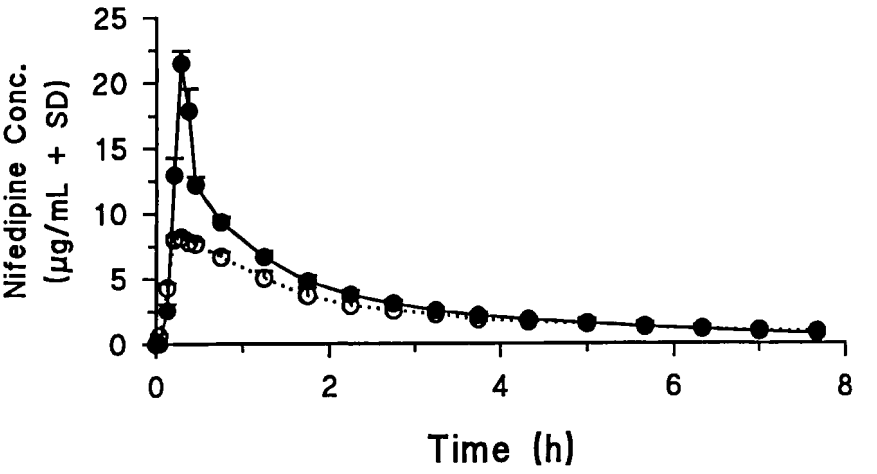

Figure 1

Dissolution profiles of 20 mg nifedipine tablets (formulation B) using 0.05 M phosphate (pH 7.4) buffer with (●) or without (O) 0.5 % of Tween 80.

is apparent from the figure that with the addition of Tween the peak nifedipine concentrations ($C_{max,d}$: 21.5±1.0 *vs* 8.3±0.2 µg/mL) were higher ($p < 0.001$) than in the medium without Tween. The times to reach the peak concentrations ($t_{max,d}$) were similar at 0.29 and 0.28 h for with and without Tween, respectively. As a general rule, use of a solubilizing agent is recommended practice to enhance the solubility in a dissolution medium of low solubility drug. The figure shows that the addition of Tween did enhance the dissolution of the drug, which obviously can be explained by increasing the solubility of nifedipine in presence of Tween. As the physiological system would lack such artificial solubilizing agent, especially in the amounts generally used in *in vitro* studies, such a dissolution test may not be a true representation of *in vivo* release characteristics. Therefore, results obtained without the addition of solubilizing agent may be preferable, as these would reflect drug release characteristics dependent only on the nature of the formulation.

The drug release profiles in different dissolution media are shown in figure 2. Solubilities of nifedipine in water and 0.05 M phosphate buffer are reported (9) to be 11.0 and 9.4 µg/mL, respectively. Apparently, the drug

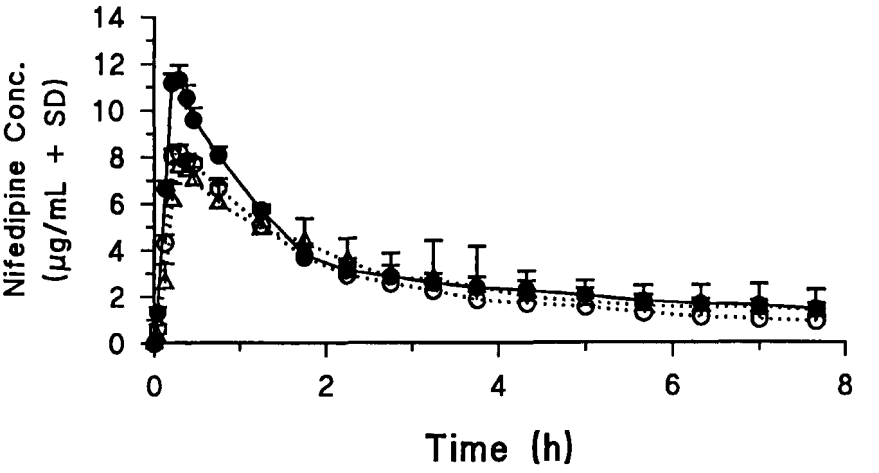

Figure 2

Drug release characteristics of 20 mg nifedipine tablets (formulation I3) in different dissolution media: (●) water; (○) 0.05 M and (Δ) 0.1 M phosphate buffer (pH 7.4).

dissolution profiles correlate well with the solubility of nifedipine in the dissolution media. Although, there were no significant differences between $t_{max,d}$ (0.25±0.05 *vs* 0.28±0.03 h) for the two media, the $C_{max,d}$ values were significantly higher in water than in phosphate buffer (11.4±0.5 *vs* 8.3±0.2 µg/mL, $p < 0.001$). Therefore, the system seems quite sensitive to the solubility of the drug in dissolution medium, which is a highly desirable quality for such studies. Furthermore, in phosphate buffer, the solubility of nifedipine is reported (9) to decrease with the molarity, i.e. 9.4 *vs* 8.5 µg/ml for 0.05 *vs* 0.1 M. Though not statistically significant, the differences in solubilities in the media are reflected in the difference in the $C_{max,d}$ for the *in vitro* release profiles. Compared to the 8.3±0.2 µg/mL in 0.05 M buffer, the corresponding value for $C_{max,d}$ in 0.1 M solution was 7.6±0.4 µg/mL. Although nifedipine has a higher relative solubility in water than in phosphate buffer, the phosphate buffer was chosen to conduct further experiments because of its buffering capacity and also to avoid any potential day to day drift in water pH.

Considering the solubility of nifedipine in 0.05 M phosphate buffer of about 9 μg/mL, a volume of about 22 L of the medium would be required to attain proper sink conditions (15 % of solubility of the drug) for a dissolution study of a single 30 mg tablet using a closed container type dissolution apparatus. For conventional release products, where faster release of drug is generally desirable, dissolution of the drug is increased by the addition of solubility enhancers such as polyethylene glycol or polysorbates etc., to the formulations. Therefore, for dissolution studies, addition of solubilizing agents to the dissolution medium would not be necessary. Presumably, that is why the USP method for dissolution of nifedipine capsules does not require any additional solubility enhancer. Otherwise, in the absence of such solubilizing agents the required USP standard of $Q = 80\%$ dissolved in 20 min from 10 or 20 mg capsules in 900 mL dissolution medium would be impossible to meet. On the other hand, as relatively slower release rates (slow dissolution) are desirable for extended-release products, such solubility enhancers are generally not included in the formulations. Therefore, to evaluate dissolution characteristics of such products in finite and relatively small dissolution volumes, some type of solubilizing agent has to be added to the dissolution medium to achieve the proper sink condition. This not only will create a non-physiological environment, but would not allow comparison of different types and strength of products under similar dissolution conditions.

The figure 3 showed dissolution profiles of the three products evaluated using the phosphate buffer without the Tween. It is apparent that the flow-through apparatus seems quite adequate to conduct comparative evaluation of different types of formulation under similar dissolution conditions. It can be seen that the system differentiates the three types of formulations very well. As expected formulation **A** which is a conventional release product gave the highest $C_{max,d}$ of 49.5±2.4 μg/mL ($p < 0.001$), while corresponding $C_{max,d}$ values for the formulations **B** and **C** were 8.3±0.2 and 2.6±0.3 μg/mL ($p < 0.001$), respectively. The $t_{max,d}$ values for products **A** and **B** were significantly shorter ($p < 0.001$) than that for formulation **C** (12.0±3.8 h).

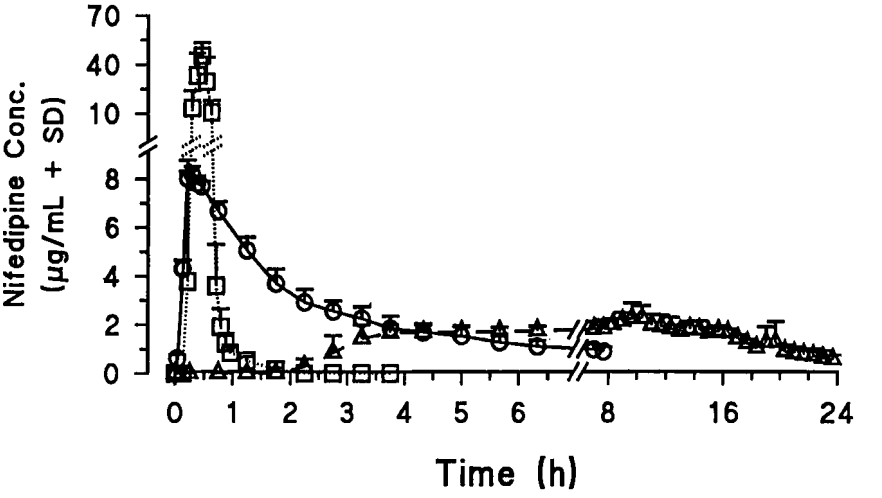

Figure 3

Drug release pattern of nifedipine formulations in 0.05 M phosphate buffer (pH 7.4) using flow-through dissolution apparatus: (□) **A**, 10 mg conventional-release formulation; (○) formulation **B** (20 mg) and (Δ) formulation **C** (30 mg) of extended-release types.

Using the USP apparatuses 1 or 2, the drug dissolution characteristics are usually represented by cumulative percent drug release profiles. Similar profiles can also be obtained using the flow-through apparatus and are shown in figure 4. These profiles were obtained from the data presented in figure 3. The mean (± SD) percent drug released were 92±3, 77±10 and 82±9 for products **A**, **B**, and **C**, respectively.

In vitro-in vivo correlations between physicochemical tests such as dissolution and *in vivo* bioavailability are desirable features for characterization of pharmaceutical products. A suggested approach (10) for such a correlation is the comparison of an *in vivo* dissolution profile based on the percent of drug absorbed or remaining to be absorbed from the GI tract with those of dissolution profiles obtained *in vitro* using USP apparatuses. The technique generally used to obtain an *in vivo* dissolution profile is based on a mathematical technique of deconvolution, in which plasma drug concentration-time profiles of solid oral dosage form and a

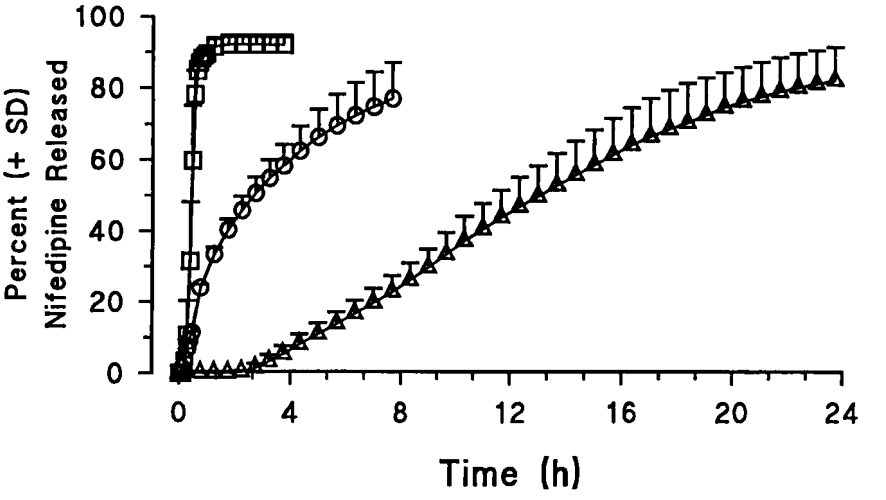

Figure 4

Cumulative percent drug release profiles of three types of nifedipine formulations in 0.05 M phosphate buffer using flow-through dissolution apparatus. Values derived from the data presented in figure 3. (□) **A**, 10 mg Conventional-release formulation; (○) formulation **B** (20 mg) and (Δ) formulation **C** (30 mg) of extended-release types.

solution are compared to extract the dissolution rate attributable to the formulation. The purpose of this approach is to obtain drug release curves similar to the dissolution curve obtained in *in vitro* (11). Application of such techniques are quite common in the literature, e.g. (12). Notwithstanding the usefulness of such a technique, its limitations and complexities are obvious (13).

On the other hand, concentration-time based dissolution profiles using a flow-through apparatus provides drug-release patterns similar to the blood concentration-time profiles from clinical studies. Therefore, it lends itself naturally to *in vitro-in vivo* correlations. As the *in vitro* drug-release profiles using flow-through are similar to that of profiles obtained *in vivo*, dissolution release kinetics parameters such as $C_{max,d}$, $t_{max,d}$, k_d, $t_{1/2,d}$, and AUC_d, akin to the pharmacokinetic data obtained from plasma concentration-time profiles, were calculated and summarized in table 1. The derived *in vitro* release kinetics parameters successfully reflect the release

TABLE 1

In vitro Drug-Release Parameters Derived from the Drug Dissolution Profiles of Different Nifedipine Formulations in 0.05 M Phosphate (pH 7.4) Buffer Using a Flow-Through Dissolution Apparatus.

FORMULATION	$t_{max,d}$ (h)	$C_{max,d}$ (μg/mL)	$AUC_{(0-t),d}$ (μg.h/mL)	k_d (h^{-1})	$t_{1/2,d}$ (h)
A (10 mg)	$0.46\pm0.05^{a¶}$	49.5 ± 2.4^{a}	12.3 ± 0.4^{a}	4.1 ± 0.88^{a}	0.17
B (20 mg)	0.28 ± 0.03^{a}	8.3 ± 0.2^{b}	20.5 ± 2.6^{b}	0.21 ± 0.03^{b}	3.37
C (30 mg)	12.0 ± 3.84^{b}	2.6 ± 3.2^{b}	32.6 ± 3.7^{c}	$0.18\pm0.0.8^{b}$	4.52

¶ Means with the same letter are not significantly different at $p < 0.001$.

characteristics of these formulations. Not only are the release patterns are as expected, AUC values, a measurement of extent of drug release, correspond favourably to the strengths of these formulations. Validation of these *in vitro* results to clinical situations are the subject of future studies, which could be used to develop regulatory standards with appropriate statistical analyses.

In conclusion, compared to the beaker type systems, flow-through dissolution apparatus offers a potentially better alternative for the characterization and comparison of *in vitro* drug release from different types of formulations. As large volumes of dissolution media can be employed, addition of solubility enhancers such as Tween may not be necessary, hence the dissolution profiles obtained using flow-through system are likely to be more representative of the effects of formulations on the drug-release characteristic *in vivo*. Also, because of the similarity of the *in vitro* drug release profile to plasma-time concentration curves in humans or animals, the flow-through technique possibly offers a better alternative for *in vitro-in vivo* correlations assessments.

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