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Optimally Predictive *In Vitro* Drug Dissolution Testing for *In Vivo* Bioavailability

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Abstract ◻ A systematic method for optimally adjusting the conditions for *in vitro* drug dissolution testing is presented. Although the basic approach also can be applied to other types of dissolution apparatus, a scheme is described using a flow-through dissolution apparatus for the design and implementation of *in vitro* drug release tests. The tests have an optimized capability to simulate and, under predetermined conditions, predict *in vivo* drug bioavailability, blood levels, or pharmacological response *versus* time profiles of appropriate drug products. The apparatus is operated in two (simulative and predictive) stages. First, different dosage forms are used to calibrate the apparatus by operating it in a feedback-controlled mode to find a program for varying the composition, recycle flow, and flow rate of the dissolution medium that provides *in vitro* results that best simulate the different *in vivo* drug release properties of the dosage forms. The variables governing the operation of the apparatus are systematically modified until the differences between the *in vitro* and *in vivo* behavior of the dosage forms become: (*a*) minimized, (*b*) the same for every dosage form, and (*c*) independent of time. Second, predictive tests of the *in vivo* behavior of other drug formulations are performed. The apparatus is now operated without feedback control, using the program determined to be optimal in Stage 1. If necessary, an analog computer is used to vary continuously the composition and recycle flow of the dissolution medium. Mathematical expressions for the performance criteria on which the optimization of the apparatus is based are derived. The optimization procedures are described, and limitations of the method are discussed.

Keyphrases ◻ Dissolution, drug—flow-through-type apparatus described, used to predict *in vivo* bioavailability ◻ Drug release tests—flow-through-type dissolution apparatus described, used to predict *in vivo* bioavailability ◻ Bioavailability—predicted using flow-through-type dissolution apparatus

The advent of potent drugs emphasizes the need to develop pharmaceutical dosage forms that possess optimal effectiveness, safety, and reliability. Although approaches to rationally designing the dynamic bioavailability properties of dosage forms have been described (1–3), it is seldom practical to perform the exhaustive *in vivo* testing required to develop new oral drug products possessing the desired behavior. An inexpensive and rapid *in vitro* method of evaluation is needed.

It has become apparent in recent years that the formulation and manufacturing specifications of different manufacturers, while conforming to USP and NF requirements, can vary and alter the bioavailability characteristics of a drug. Such variations in bioavailability of drug products have often only been detectable by extensive human testing. Consequently, in some cases, products with inadequate *in vivo* drug bioavailability have been marketed and sold for long periods prior to the discovery of their inadequacy. Commonly prescribed drugs continue to be released from patent. Not only does this situation serve as a stimulus for increased generic usage, it also makes it necessary for medical researchers to have a ready means for evaluating the bioavailability of chemical equivalents of these drugs as they enter the market. The compendia should have precise laboratory tests for gauging their bioavailability, but only *in vivo* testing in humans is fully reliable at present.

In the extensive literature on *in vitro* drug release testing (3–17), it is often stated that the problem is quite complicated because a correlation of *in vivo* to *in vitro* release found with a particular test for a particular drug in a particular dosage form may not exist if another drug is substituted or the dosage form is altered. Few attempts have been reported to determine the full extent to which this is the case. The *in vitro* to *in vivo* drug availability correlations found have always been after the fact, as were the single-point correlations of 50% *in vitro–in vivo* release times (7) and the multiple-point correlations (8–11) that linearly related the cumulative amounts of drug released from the dosage form *in vitro* to similar amounts absorbed *in vivo*.

Previous reports from this laboratory (3, 4, 12) sug-

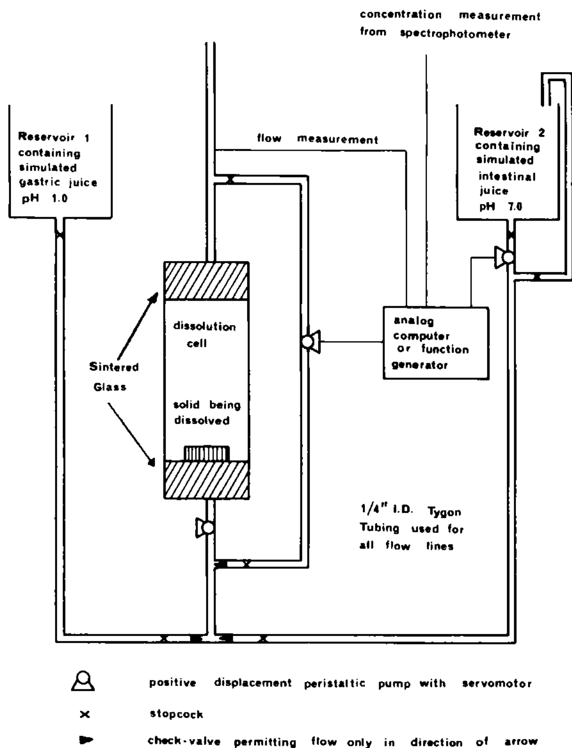

Figure 1—*Schematic diagram of a simple flow-through apparatus with modifications appropriate for use in optimized drug release testing. See text for details.*

gested that *in vitro* to *in vivo* correlations can be improved through a systematic adjustment of test conditions such as agitation or flow rates, composition of the release medium, geometry of the apparatus, solubility volume of a sink, and permeability of membranous barriers. The use of optimization methods was suggested to determine test conditions that would provide uniformly simulative results for drug dosage formulations with different *in vivo* drug release properties. Such a test could be expected to predict the *in vivo* drug release behavior of other formulations with release properties similar to those of the dosage forms for which the test conditions were optimized (4, 17).

The present report describes a methodology employing a flow-through dissolution apparatus similar to that described previously (13, 14). The apparatus is initially operated with closed loop analog computer feedback control of the recycling of the dissolution medium; this phase is followed by an optimized open loop automated operation to predict *in vivo* dosage form bioavailability behavior. It is postulated that this procedure, or a manually operated variation thereof, can provide a relatively simple, economical, and versatile approach to improving the predictive capability of *in vitro* drug bioavailability testing and, therefore, reduce, but not eliminate, the need for *in vivo* bioavailability studies.

EXPERIMENTAL

Apparatus—The use of a single apparatus to test most drug products is theoretically feasible, provided its mode of operation is sufficiently flexible (17). Compared to other types of apparatus, a flow-through dissolution cell, such as that described previously (13, 14), possesses good flexibility and involves no arbitrary and material-dependent parameters (14, 15). Its operating conditions can be well defined and readily standardized.

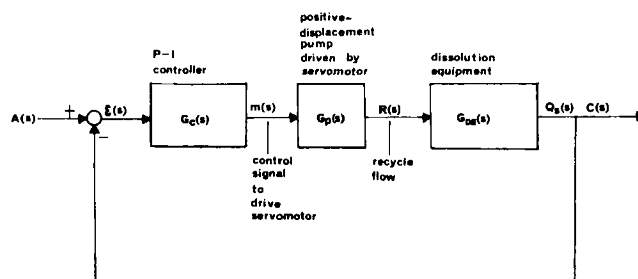

Figure 2—*Block diagram for closed loop feedback control of recycle flow,* R(t), *in the operation of the* in vitro *drug release testing apparatus.*

Figure 1 shows a flow-through apparatus with provisions to change the composition of the dissolution medium and recycle flow through the cell. A change in solvent pH can be used to simulate the *in vivo* change from the stomach to the duodenum. The recycling of solvent through the dissolution chamber allows variable sink conditions to be achieved to simulate existing *in vivo* conditions due to differing barrier properties of drug-absorbing biological membranes. Resistance to biological absorption is simulated by mixing the fresh solvent with the solution leaving the cell. The recycling of solution through the dissolution chamber in this manner decreases the driving force for dissolution.

As shown in Fig. 1, the time-varying recycle flow, $R(t)$, can be controlled to reproduce *in vivo* dissolution rates by using a preprogrammed analog computer. A time-varying mixing of the two reservoirs of dissolution media also can be controlled by an analog computer or a variable diode function generator. The pumps indicated in Fig. 1 are positive-displacement peristaltic pumps with a servomotor. The pumps on the recycle line and second reservoir require a speed modulator to allow the pump to be driven by a signal generated by an analog computer, diode function generator, or analog tape recorder.

Once the pumping rate is set at the outlet of the dissolution cell, the pumping arrangement and use of positive-displacement pumps ensure a constant flow through the apparatus. This is the case despite a time-varying recycle flow and a changing flow from the simulated gastric and intestinal juice reservoirs. The apparatus includes a spectrophotometer or other instrument to measure the concentration of drug in the liquid leaving the cell.

The analog computer is used for either the open loop control of the recycle flow (during a predictive *in vitro* run) or for the closed loop feedback control of the recycle flow (during the calibration or simulation stage of operating the apparatus). During the feedback-controlled mode of operation, the analog computer converts the signal from the spectrophotometer to a voltage representing the drug concentration and then multiplies this voltage with the voltage representing the flow of medium leaving the apparatus. This signal is compared with a signal produced by a diode function generator, programmed to reproduce the *in vivo* bioavailability rate, blood level, or pharmacological response *versus* time profile being simulated. The error signal is formed from the comparison. The error signal then goes to the PID (proportional integral differential) controller, which produces the control signal (usually PI modes are adequate) and drives the recycle flow at the desired rate.

The block diagram for the feedback-controlled mode is given in Fig. 2. When the apparatus is operated to obtain a predictive result, the analog computer produces an open loop control signal which varies the recycle flow without a concentration measurement from the spectrophotometer. The open loop block diagram for this operation is given in Fig. 3.

The dissolution cell (Fig. 1) has a length of 5.0 cm and a diameter

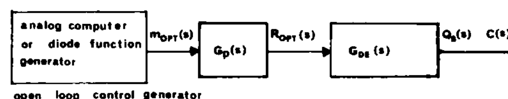

Figure 3—*Block diagram for open loop programmed control of recycle flow,* $\bar{R}_{opt}(t)$, *during operation of the* in vitro *drug release apparatus.*

of 2.5 cm. The volume of fluid held by the chamber can be changed by altering its length or filling the chamber with glass beads. The solid to be dissolved rests on a flow distributor; flow usually is in the laminar range.

The dissolution apparatus in Fig. 1 is in its simplest form and is probably adequate for most drugs and dosage forms. However, the flow-through apparatus can be modified to overcome such possible problems as clogging of the upper sintered-glass filter with undissolved particles or formation of soluble, but *in vivo* nonabsorbable, complexes between the drug and excipients contained in the dosage form. Possible filter clogging might easily be eliminated by filling the chamber with glass beads or replacing the stationary sintered-glass filter with a cone-shaped, rotating filter; such use of spin filters in a dissolution apparatus has been described (18).

A membrane system to simulate biological absorption could be incorporated using a scheme similar to that employed in a commercially available apparatus (19). However, since lipoid membranes are not entirely satisfactory for simulating the transport properties of biological structures (20, 21), membranes having the properties described by Smolen and Hagman (22) or Ling (23) could be applied more successfully. When considered advantageous, other dissolution media including nonaqueous solvents could be used in place of simulated gastric and intestinal juices.

Another possible problem is interferences of dissolved excipients with a spectrophotometric assay for the drug. In this case, discrete sampling techniques may need to be employed. Although this can be done within the context of the described scheme, it would require complicating both the equipment and the computer control of the dissolution process. Obviously, the problems described as possibly necessitating complications in the flow-through apparatus are also common to all other types of continuous sampling drug dissolution apparatus.

General Operation—The operation of the flow-through apparatus consists of two parts. First, the apparatus is calibrated (or adjusted) by operating it in the feedback-controlled mode to find the required recycle flow for two or more dosage forms chosen for having different drug release properties. The operating parameters are adjusted until an optimum combination is found. The optimum set of parameters consists of an optimum flow rate, a program for varying the composition of the dissolution medium, and a single recycle flow function. The relationship between the operating parameters (the process variables) and the dissolution parameters is discussed later. These results are then applied to set the conditions of operation of the apparatus in the optimally predictive open loop mode. In this second stage, the analog computer controls the recycle flow in accordance with the previously found optimal recycle flow function. Predictive tests are then performed on other drug dosage formulations, and the time courses of absorption rates, blood levels, drug effects, or urinary drug recovery rates of the test dosage form are produced. Various performance criteria can be used to optimize the operation of the apparatus by minimizing the differences between *in vivo* bioavailability results and values generated by the apparatus. The performance criteria developed in the *Appendix* also distribute the differences uniformly over time and the different dosage forms used to calibrate the apparatus.

THEORY AND DISCUSSION

Relation between Process Variables (Extrinsic to Drug Product) and Dissolution Rate—The dissolution process often may be described by a simple diffusion layer model (6):

$$\frac{dw}{dt} = \frac{DS}{T}(C_s - C) \qquad \textbf{(Eq. 1)}$$

where:

$\frac{dw}{dt}$ = dissolution rate (milligrams per minute)

D = diffusion coefficient for the solvent and solute under consideration (square centimeters per minute)

S = surface area for dissolution (square centimeters)

C_s = concentration of solute required to saturate the solvent (milligrams per milliliter)

C = actual solute concentration in solution (milligrams per milliliter)

T = effective thickness of the film or diffusion layer (centimeters)

The process variables are:

Q = volumetric flow rate (milliliters per minute)

$Q_A = Q/A_C$ = velocity through the cell (centimeters per minute)

A_C = cross-sectional area of the cell (square centimeters)

R or $R(t)$ = volumetric flow of recycle (milliliters per minute); *i.e.*, it can be a constant or time-varying quantity

τ_1 = time during which all solvent is drawn from the reservoir containing the simulated gastric juice (minutes)

τ_2 = time after which all solvent is drawn from the reservoir containing the simulated intestinal juice (minutes)

Once A_C is chosen, the process variables become Q, R, τ_1, and $(\tau_2 - \tau_1)$. The variables τ_1 and τ_2 can be assumed to be related to gastric and intestinal emptying time, which can vary significantly. However, average values in the neighborhood of 1–2 hr for τ_1 may be initially chosen. In most instances, the experiments would not exceed 2–4 hr, although runs with dosage forms with prolonged-release characteristics could last 12 hr. However, through appropriate time scaling, the actual experimental time can be contracted approximately 10–30-fold.

The solvent solutions can resemble gastric juice (0.1 N HCl) and intestinal juice (a phosphate-buffered solution of pH 7.0). An appropriate surfactant can be added to lower the surface tensions of the solutions into the range for human gastric juice, *i.e.*, from 34 to 50 dynes/cm (24). The rationale for adjusting the surface tension into the *in vivo* range, especially for a hydrophobic drug or dosage form, was demonstrated by Finholt and Solvang (25). They showed that the dissolution of phenacetin was progressively accelerated as the surface tension of the dissolution medium was reduced by the addition of a surfactant, primarily due to the increased wetting of the solid particles. They presented data showing that the dissolution rates obtained using a sample of gastric juice and a solution of hydrochloric acid (0.1 N) adjusted to the same surface tension were similar. A surfactant of the cholate type could also be added to the simulated intestinal fluid.

The relationship between variables in the diffusion layer equation and the process variables are easily seen as given for a fixed value of A_C:

T = a function of Q

C = a function of the volume of the dissolution chamber, V, and the volumetric flow rate, Q, *i.e.*, the residence time, V/Q, and the flow of recycle, R or $R(t)$

To avoid changing the volume of the dissolution chamber by changing its length to change C, this could also be effected by changing the recycle flow.

C_s = a function of the properties of the solvent

Using the simulated gastric and intestinal juices mentioned previously, the process variables to be manipulated here are τ_1 and τ_2, as graphically depicted in Fig. 4. The variables D and C_s are obviously affected by the solvents used; but once the solvents have been specified, D and C_s are reflective of the solid being dissolved.

S = in addition to being a function of Q, a function of the initial amount of drug, m_0, and the physical properties of the solid

Once these variables are fixed, *i.e.*, once a drug and a dosage form are decided upon, the time course of S as the experiment proceeds is reflective of the properties of the drug product.

Automatic Feedback Control to Produce a Time-Varying Recycle Flow and Simulate $\dot{A}(t)$—The objective is to devise an apparatus that will predictively simulate *in vivo* drug availability data. The dissolution data produced by the flow-through apparatus are in a differential form. Using the symbol $A(t)$ (milligrams) for the cumulative *in vivo* availability, the output of the apparatus should reproduce $\dot{A}(t)$ (milligrams per minute), *i.e.*, the rate of bioavailability, blood level, urinary excretion rate, or pharmacological response *versus* time profiles. If $Q_s(t)$ is the volumetric flow rate out of the apparatus to the sink, it is given by $Q_s(t) = Q - R(t)$ (milliliters per minute). The rate of flow of dissolved drug to the sink is then given by $Q_s(t)C(t)$, where $C(t)$(milligrams per milliliter) is the concentration of the stream leaving the dissolution cell. It is then desired to have:

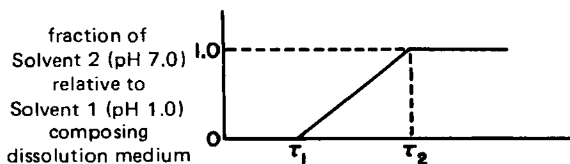

Figure 4—*Relationship between solvent compositions and dissolution rates; τ_1 and τ_2 are described in the text.*

$$\dot{A}(t) = Q_s(t)C(t) \quad \text{(Eq. 2)}$$

or:

$$\dot{A}(t) - Q_s(t)C(t) = \epsilon(t) \quad \text{(Eq. 3)}$$

where, in the ideal case, the error $\epsilon(t) = 0$. It may not be possible to obtain $\epsilon(t) = 0$, but it is possible to produce a small $\epsilon(t)$ with the feedback control scheme indicated in Fig. 1 and described in terms of the block diagram in Fig. 2. Although Q can be held constant with time, $Q_s(t)$ can be changed by changing $R(t)$ by PI control action generated by an analog computer. In fact, all variables needed for the feedback control of $R(t)$, *i.e.*, the calculation of $R(t)$ and $Q_s(t)$, the multiplication of $Q_s(t)$ by $C(t)$ (measured by a spectrophotometer or other appropriate device and fed back to the computer), the comparison of $\dot{A}(t)$ (*e.g.*, produced by a diode function generator) with $Q_s(t)C(t)$ to produce the error signal, and the desired control action to drive the servomotor of the pump on the recycle stream, are all easily accomplished with an analog computer. In addition, the servomotor on the second reservoir can be driven according to the desired τ_1 and $\tau_2 - \tau_1$ quite easily by a function generator or a signal prerecorded on magnetic tape.

The effect of increasing the recycle flow is to lower the amount of drug entering the sink. This effect is produced by a lowering of the volumetric flow to the reservoir as well as by an increase in the inlet concentration of drug in the liquid entering the dissolution apparatus, which lowers the driving force for mass transfer. Furthermore, if the recycle flow is a controlled function of time, $R(t)$, which is prescribed by an experimentally determined differential *in vivo* drug availability rate, $\dot{A}(t)$, it is not considered as an ordinary process variable as is the constant recycle flow, R. It is now reflective of the experimentally obtained *in vivo* data and is, therefore, not an arbitrary process variable extrinsic of the drug product. Instead, it is an intrinsic variable of the drug product and the *in vivo* system from which $A(t)$ was obtained.

Closed Loop Operation for Optimization of $R(t)$ and Subsequent Open Loop Operation of Apparatus with a Programmed Optimal Recycle Flow—The objective of the operation of the *in vitro* drug release apparatus is to obtain results that uniformly reflect the *in vivo* drug availability with optimal fidelity over time and varying drug release behavior of the dosage forms. For any given set of process variables, *i.e.*, Q_A, τ_1, and $\tau_2 - \tau_1$, the closed loop operation of the *in vitro* testing apparatus will produce a function $Q_{s,i}(t)C_i(t)$ for each ith dosage form so that the expression $\dot{A}_i(t) - [Q - R_i(t)]C(t)$ closely approximates zero. A function $R_i(t)$ will be obtained for each dosage form of the drug tested that was chosen to possess different drug release dynamics. The $R_i(t)$ functions can be readout by the computer on magnetic tape or a potentiometric recorder during the closed loop operation of the apparatus.

At this stage, the apparatus merely simulates the $A(t)$ functions determined from *in vivo* experimentation. Analog $R_i(t)$ function signals recorded on magnetic tape for each dosage form can be conveniently processed on an analog computer, or digitized, and their values can be averaged, over dosage forms, at each time to obtain an average, $\bar{R}(t)$, function representing the mean behavior of all dosage forms included in the closed loop operations. A second set of *open loop* runs must then be performed for each dosage form with the $\bar{R}(t)$ function programmed to control recycle flow. The second set of runs is needed because different $C_i(t)$ functions will result, since it may be expected that $R_i(t) \neq \bar{R}(t)$ for any dosage form. The second set of $C_i(t)$ functions are again obtained from the readout of a spectrophotometer or other analytical devices used to monitor continuously the concentration of drug in the dissolution medium recycle stream or the stream flowing out of the apparatus.

An objective function, F_0, defined in the *Appendix*, can be formed from the $\bar{R}(t)$, $C_i(t)$, and $\dot{A}(t)$ functions. A minimal value of the objective function is achieved by systematically adjusting the process variables. A minimal value of the objective function corresponds to optimal open loop operation of the apparatus under determined conditions of Q_{opt}, $\tau_{1\,opt}$, $(\tau_2 - \tau_1)_{opt}$, and $\bar{R}(t)_{opt}$. As mentioned, various means can be implemented to control the recycle flow dynamics to conform to $\bar{R}(t)_{opt}$. The open loop operation of the apparatus with $R(t)_{opt}$ is block diagrammed in Fig. 3.

Procedural Steps—The *in vitro* test apparatus should be operated in the simplest manner that provides acceptable *in vitro* results with regard to *in vivo* drug availability behavior. To determine the magnitude of sensitivity of the fidelity of the test to different operating conditions, the test can be initially performed in four successive phases of increasing complexity and equipment requirements.

Phase I can be performed without automatic control, using fixed, time invariant, values of the process variables, Q and R; R is then a constant recycle flow. An optimal composition and pH of the dissolution medium may be found and thereafter maintained constant; *i.e.*, τ_1 and $\tau_2 - \tau_1$ are not included as process variables.

Phase II can be performed similarly to Phase I but with the inclusion of τ_1 and $\tau_2 - \tau_1$ as process variables.

Phase III can employ an analog computer control of a time-varying recycle flow, $R_i(t)$, and involve the optimization of one process variable, Q. In this case, it will be recalled that $R_i(t)$ is not an arbitrary process variable but is prescribed by the feedback control implemented with the analog computer; *i.e.*, this is a servo problem where $R_i(t)$ is manipulated so that the output from the *in vitro* apparatus is made to follow (or match) closely the experimental *in vivo* dissolution rate data in differential form.

Phase IV is the most complicated case of optimizing three process variables, Q, τ_1, and $\tau_2 - \tau_1$, with feedback control-driven $R_i(t)$.

These four modes of operation can be repeated for different dosage forms of the same drug to obtain the optimal conditions over all dosage forms. One would then have the optimal process conditions, ranging from the simplest arrangement, that would yield Q_{opt}, $\tau_{1\,opt}$, $(\tau_2 - \tau_1)_{opt}$, and $\bar{R}(t)_{opt}$. The simplest mode of operation possessing an acceptable fidelity would then be chosen for future studies with the drug.

Statistical Design—At any stage of the experiment, an objective function may be minimized with minimal experimental effort. The optimization of the process variables and $\bar{R}(t)$ can be accomplished using a fractional two-level factorial experimental design in the process variables (26). A quarter or half, duplicated, replicate experiment can be performed and an objective function, F_0, can be evaluated. To minimize F_0 and, therefore, uniformly minimize the discrepancy between *in vitro* and *in vivo* results, a "path of steepest descent" in the objective function should be followed. This path represents changes in the independent (process) variables so that the most gain (least discrepancy) is made by proceeding in this direction rather than any other.

Optimization is continued by planning another experiment around the best values found along the path of steepest descent. Ultimately, a more extensive experiment such as a three-level factorial or a central composite can be designed to develop a mathematical model of the effects of the process variables on the discrepancies represented by the objective function. The optimum set of conditions can then be determined by differentiating this model and locating the minimum (26).

Possible Limitations and Properties of the Test—The *in vitro* testing apparatus can be calibrated to predict bioavailability rates, $\dot{A}(t)$. These rates can be integrated to provide cumulative amount of drug absorbed profiles, $A(t)$. Alternatively, blood level–time profiles $[C_b(t)]$, urinary excretion rates $[\dot{A}_u(t)]$, or pharmacological response intensities $[I(t)]$, can be predicted. When quantities other than $\dot{A}(t)$ are used as *in vivo* bioavailability criteria, the $C_b(t)$, $\dot{A}_u(t)$, or $I(t)$ values are merely substituted for $\dot{A}(t)$ in all equations in which it appears.

When using pharmacological data (1–3, 27–30), the observed response intensity *versus* time profiles $[I(t)]$ should first be converted to their corresponding biophasic drug level *versus* time profiles $[Q_B(t)]$; the details of the basis and procedures for the interconversion were reported previously (2, 27, 28). Of the various *in vivo* bioavailability criteria, only $\dot{A}(t)$ has a directly corresponding *in vitro* counterpart in $[Q - R(t)]C(t)$ to which it is compared. For this reason, it would theoretically be preferred. However, $\dot{A}(t)$ is computed from blood, urine, or pharmacological data using a pharmacokinetic model (1–3, 28–32). As such, it may be a large step removed from the actual observed $C_b(t)$, $\dot{A}_u(t)$, or $I(t)$ data used for its computation. Since these latter quantities are usually of primary interest, they could be used directly without first converting them to $\dot{A}(t)$.

The direct comparison of $C_b(t)$, $\dot{A}_u(t)$, or $I(t)$ to *in vitro* drug release rates cannot be justified as easily as $\dot{A}(t)$ on mechanistic grounds. However, considering that the pharmacokinetic behavior of most systems can be described by linear compartment models (1), the relationship between $\dot{A}(t)$ and $C_b(t)$, $\dot{A}_u(t)$, or $I(t)$ [converted to $Q_B(t)$] will usually be given by a first- or higher order transfer function (1–3, 6). Therefore, $C_b(t)$, $\dot{A}_u(t)$, or $Q_B(t)$ profiles will resemble and possess the essential features of $\dot{A}(t)$, except for differing from them by the first or higher lag time always imposed in the same way by the transfer functions. The operation of the apparatus should be sufficiently versatile to compensate for this effect.

In vitro drug release results are obviously only of interest in bioequivalency testing when they reflect *in vivo* bioavailability behavior of drug formulations. *In vitro* drug release testing can never obviate performing *in vivo* studies in human subjects. However, the described approach to optimized *in vitro* drug bioavailability testing minimizes *in vivo* experimentation by confining it to what is needed to optimize and verify the predictive operation of the apparatus. Thereafter, the apparatus will optimally simulate and predict the bioavailability behavior of the panel of human subjects who contributed the *in vivo* data used in its calibration. Within the discussed limitations, the calibrated apparatus can then be considered to serve as a substitute for this same panel of subjects who, in theory, are no longer needed to test additional dosage forms.

The results obtained from *in vitro* testing can, of course, be no better than the *in vivo* bioavailability data used to calibrate the apparatus. However, once properly calibrated, the *in vitro* apparatus can provide predictive results rapidly (the *in vitro* dissolution can be accelerated up to approximately 10–30 times the *in vivo* rates), conveniently, and inexpensively. Therefore, it can be used routinely to screen large numbers of generic drug products or preliminary formulations prepared in developing a new drug product. Such large-scale routine screening is not practical with human testing. However, when a particular drug product is brought into question on the basis of *in vitro* testing results, it would generally be prudent also to evaluate it *in vivo*.

The optimized *in vitro* drug release test apparatus is conceived as a drug bioavailability simulator. In principle, the apparatus can be adjusted to predict optimally the bioavailability behavior of any drug-absorbing biological system when it is operated with drug dosage forms possessing drug release dynamics similar to, and within the range for which, the process variables have been optimized. In other words, the dynamics of drug release in the apparatus are optimized to simulate the drug absorption dynamics of the specific *in vivo* system from which the $\dot{A}(t)$ or other bioavailability results are obtained.

If the dynamic behavior of both the *in vitro* and *in vivo* drug transference systems could be assumed to be linear, then, in principle, it would only be necessary to optimize the operation of the *in vitro* drug release apparatus for a single dosage form having any arbitrary drug release properties. However, this situation will not be the general case; two or more different dosage forms will minimally be required to optimize the test. The apparatus will then possess fidelity in directly reflecting *in vivo* drug availability for a range of drug release behavior in the vicinity of that characteristic of the dosage forms for which the apparatus was specifically calibrated.

In *in vivo* bioavailability testing of generic drug products, the objective of the test may be merely to establish whether a given generic formulation is bioequivalent to an innovating drug company's product which is chosen as a standard (33). Similarly, in an *in vitro* test, if only a relatively qualitative determination of bioequivalence is sought, as opposed to a quantitative prediction of the *in vivo* performance of the test formulation, it may not be necessary to calibrate the apparatus optimally. In this case, it may only be required to choose values arbitrarily for the process variables, τ_1, $\tau_2 - \tau_1$, and Q, that permit the apparatus, when operated in the closed loop mode, to simulate an *in vivo* response *versus* time profile for the standard formulation.

In the process of performing this simulation, a recycle flow function, $R(t)$, will be obtained; $R(t)$ can subsequently be used to control open loop dissolution tests performed with the generic products under evaluation. The resulting *in vitro* release profiles for the tested formulations can then be statistically compared (33) with results obtained from the standard to determine whether their profiles fall within the range observed for different, acceptable lots of the standard dosage form. Although this shortcut procedure can provide a rapid and simple means of detecting *in vitro* differences in drug formulations relating to their *in vivo* bioavailability behavior, the direct relationship between *in vitro* and *in vivo* performance obtained when the apparatus is optimally calibrated cannot be assumed.

No *in vitro* dissolution apparatus, however complex and how well it simulates known *in vivo* data, can be fully relied upon to predict *in vivo* performance. Human testing must always be performed, not only to obtain the data required to calibrate the apparatus but also to ensure that the unpredictable biological and/or chemical factors specific for a particular dosage form are not operating to vitiate the predictive capability of the apparatus. The probability of this occurrence may be diminished if the chemical composition of the dosage forms used in its calibration differs only quantitatively from the composition subsequently tested. The determination of the full extent of applicability of a test for any particular drug must always be performed by experimentation.

Conceivably, an extension of the presently described scheme for *in vitro* drug bioavailability testing could provide an approach to the ideal of an ultimately "universal" drug release test. The limits to which optimized *in vitro* drug release testing could be generalized to drugs for which the tests have not been specifically developed may be explored through the determination and subsequent testing of any observed dependencies of optimized fixed process variables on the physical properties of the drugs (*e.g.*, water solubility, diffusion coefficient, pK, and oil–water partition coefficient). Such correlations would obviously require studies with various dosage forms of several drugs possessing physical properties sufficient to permit extrapolations and interpolations.

The accuracy of such estimations of optimal test conditions for a previously unstudied drug may, of course, be expected to increase with the number of drugs for which the *in vitro* test conditions have previously been optimized. The possible occurrence of unpredictable or nonlinear biological influences that could vitiate the predictive capability of tests performed under extrapolatively on interpolatively estimated conditions would obviously always require an *in vivo* verification of *in vitro* test results. The periodic pooling of results reported by different laboratories could serve to accelerate the accumulation of information required to determine the "universality" and reliability of optimized *in vitro* drug bioavailability testing.

APPENDIX: OBJECTIVE FUNCTION

A performance criterion for the *in vitro* test should reflect both the magnitude of deviations from the ideal, as represented by *in vivo* bioavailability results, and the uniformity of deviations. Consider an *in vitro* apparatus operating with a fixed set of process variables and an average recycle flow $\bar{R}(t)$. Nonzero deviations will generally occur between comparisons of *in vivo* bioavailability rates, *i.e.*, $\dot{A}(t)$, and their *in vitro* counterparts, *i.e.*, $Q_s(t)C(t)$. Such differences may be referred to as apparatus errors. For any dosage form, the apparatus produces errors that vary as a function of time over the *in vitro* drug release test process.

It is desired to both minimize the magnitude of the average error for the entire drug dissolution process and to distribute the error uniformly over time. The error tends to be uniformly distributed when the variance of the error at each instant of time from the average error is minimized. An apparatus performance criterion meeting these objectives may be developed as follows. For the ith dosage form, the squared deviation, d_{i,t_j}, of the *in vitro* from the *in vivo* data at each discrete *in vivo* sampling time, t_j, is defined by:

$$d_{i,t_j} = [\bar{\dot{A}}_{i,t_j}(t) - P_{i,t_j}(t)]^2 \qquad \text{(Eq. A1)}$$

The quantity:

$$\bar{\dot{A}}_{i,t_j} = \frac{\sum_{k=1}^{N_i} \dot{A}_{i,t_j,k}}{N_i} \qquad \text{(Eq. A2)}$$

defines the average *in vivo* value at sampling time t_j, N_i is the number of *in vivo* replicate experiments performed with the ith dosage form, and P_{i,t_j} represents the value of the *in vitro* counterpart of $\bar{\dot{A}}_{i,t_j}$. The average value of the squared error, d_i, for the ith dosage form over the entire *in vitro* testing time can be calculated by:

$$d_i = \frac{\sum_{j=1}^{n_i} w_{i,t_j} d_{i,t_j}}{n_i} \qquad \text{(Eq. A3)}$$

where n_i is the number of discrete *in vivo* sampling times; and w_{i,t_j} represents a weighting factor, defined by Eq. A4, which attenuates each sampling time contribution to d_i. As seen here, the degree of

attenuation depends upon the magnitude of the error at each time that is determined from the replicated *in vivo* data:

$$w_{i,t_j} = \frac{q_i}{q_i + q_{i,t_j}} \quad \text{(Eq. A4)}$$

where q_{i,t_j} represents the variance of each *in vivo* result about the average value, *i.e.*, $\bar{A}_{i,t_j}$ at sampling time t_j, as defined by:

$$q_{i,t_j} = \frac{\sum_{k=1}^{N_i} (\dot{A}_{i,t_j,k} - \bar{A}_{i,t_j})^2}{N_i - 1} \quad \text{(Eq. A5)}$$

The variance of the *in vivo* data averaged over the entire experimental time is symbolized by q_i and defined by:

$$q_i = \frac{\sum_{j=1}^{n_i} q_{i,t_j}}{n_i} \quad \text{(Eq. A6)}$$

For each ith dosage form, a measure of the uniformity of the distribution of the error over the experimental run can be expressed as the variance, v_i, of the squared error at the jth discrete sampling time about the average squared error for the entire time of the *in vitro* experiment:

$$v_i = \frac{\sum_{j=1}^{n_i} (d_i - w_{i,t_j} d_{i,t_j})^2}{n_i - 1} \quad \text{(Eq. A7)}$$

Combining Eqs. A3 and A7 results in an objective function, F_i, for each dosage form. This function expresses both the magnitude of the total error and the uniformity of its distribution over time as expressed by:

$$F_i = g_{m,i} d_i + g_{u,i} v_i \quad \text{(Eq. A8)}$$

and:

$$F_i = F_{m,i} + F_{u,i} \quad \text{(Eq. A9)}$$

where $g_{m,i}$ and $g_{u,i}$ represent a magnitude (*i.e.*, average value) weighting factor for the ith dosage form and a uniformity (*i.e.*, variance) weighting factor, respectively; $F_{m,i}$ is the weighted average magnitude of the deviation over time for the ith dosage form; and $F_{u,i}$ is the weighted uniformity in the deviations over time for the ith dosage form.

In addition to the average error and distribution of error over experimental time for a single dosage form, an *in vitro* drug release apparatus operating with fixed process variables may be expected to produce different average errors and error distributions for different dosage forms. Therefore, the optimum set of process variables is one that:

1. Produces the smallest *overall* average error for all dosage forms; *i.e.*, the smallest value of $\bar{F}_M$ as defined by Eq. A10, where p is the number of dosage forms:

$$\bar{F}_M = \frac{\sum_{i=1}^{p} F_{m,i}}{p} \quad \text{(Eq. A10)}$$

2. Produces a uniform distribution of average error between dosage forms. A measure of this is represented as the variance of the average error of the individual dosage forms, $F_{m,i}$, about the overall average error for all dosage forms as expressed by:

$$F_{u,D} = \frac{\sum_{i=1}^{p} (\bar{F}_M - F_{m,i})^2}{p - 1} \quad \text{(Eq. A11)}$$

3. Produces the greatest degree of *overall* uniformity of error over time for all dosage forms. This will be reflected by the value of the *overall average* weighted time variance for all dosage forms as expressed by:

$$F_{M,u,D,T} = \frac{\sum_{i=1}^{p} F_{u,i}}{p} \quad \text{(Eq. A12)}$$

4. Produces a uniform distribution of uniformity of error over experimental time among all dosage forms. A measure of this criterion is provided by the variance of the weighted time variance of the individual dosage forms, $F_{u,i}$, above the overall average weighted time variance, $F_{u,u,D,T}$, as expressed by:

$$F_{u,u,D,T} = \frac{\sum_{i=1}^{h} (F_{M,u,D,T} - F_{u,i})^2}{p - 1} \quad \text{(Eq. A13)}$$

The quantities defined by Eqs. A10–A13 are the average values and variances, over the dosage forms, of the terms in Eq. A9. The overall objective function, F_0, as expressed by Eq. A14, represents both the magnitude and uniformity performance criteria enumerated above:

$$F_0 = G_M \bar{F}_M + G_{u,D} F_{u,D} + G_{M,u,D,T} F_{M,u,D,T} + G_{u,u,D,T} F_{u,u,D,T} \quad \text{(Eq. A14)}$$

The weighting factors, G's and g's in Eqs. A14 and A8, may be chosen with respect to the relative importance of each consideration and the relative magnitudes of each term constituting F_0. Each value of G and g can be defined to constitute a product factor a and G' and a and g', *i.e.*, $G = aG'$ and $g = ag'$. The values of a will compensate for innate differences in the magnitudes of the terms in the objective function; *i.e.*, the ratio of any two terms multiplied by their values of a may be chosen to be unity at the optimum, *e.g.*, $a_M F_M / a_{u,D} F_{u,D} = 1$.

The values of G may be selected on the basis of the relative importance of the reduction of magnitudes of errors and uniform simultaneous convergence to the optimum. If desirable, the complexity of the objective function, as defined by Eq. A14, can readily be reduced by setting weighting factors equal to zero. Obviously, in each case the simplest form of an objective function found to be appropriate should be implemented.

As noted previously, when operating with feedback control of recycle flow, it is necessary first to obtain $R_i(t)$ for each dosage form to determine a $\bar{R}(t)$. A second set of open loop runs using the programmed $\bar{R}(t)$ on each dosage is required to obtain corresponding $C_i(t)$'s that will be different since, in general, $R_i(t) \neq \bar{R}(t)$. The $C_i(t)$ values obtained in the second run of experiments are required for the evaluation of the performance index, as defined by Eq. A14, whose minimization indicates that an optimal set of fixed process variables, *i.e.*, Q, τ_1, and $\tau_2 - \tau_1$, has been determined. The operation of the apparatus with this set of variables and their corresponding $\bar{R}(t)$ values provides an *in vitro* drug release test that is optimally reflective of *in vivo* bioavailability.

An alternative, although somewhat less rigorous, procedure may serve to diminish the amount of open loop duplication of the experimentation for optimization. With closed loop feedback control of recycle flow, the differences between the *in vitro* and *in vivo* dissolution process can be made very small for each individual dosage form. However, since $R(t) \neq \bar{R}(t)$, the error increases when open loop operation with $\bar{R}(t)$ is performed. Therefore, if a set of fixed process variables can be found such that the average deviation between the individual $[R_i(t)]$ and average $[\bar{R}(t)]$ recycle time functions is a minimum, then the average error produced when the individual dosage forms are tested with the apparatus operated with $\bar{R}(t)$ will tend to be a minimum. This set of fixed variables would minimize the quantity M expressed by Eq. A15, where T_i is the length of the experimental run on the ith dosage form:

$$M = \frac{\sum_{i=1}^{p} \frac{I}{T_i} \int_0^{T_i} [R_i(t) - \bar{R}(t)]^2 \, dt}{p} \quad \text{(Eq. A15)}$$

For a finite set of discrete sampling times, M can be approximated by Eq. A16, where n_i is the number of equally spaced discrete sampling times:

$$M_A = \frac{\sum_{i=1}^{p} \frac{1}{n_i} \sum_{j=1}^{n_i} [R_{ij}(t) - \bar{R}_j(t)]^2}{p} \quad \text{(Eq. A16)}$$

The rationale for Eq. A16 may be seen to develop from the consideration that the test conditions producing uniformly accurate results for every dosage form would tend to minimize the quantity $S(t)$ given by:

$$S(t) = \sum_{i=1}^{p} S_i(t) = \sum_{i=1}^{p} \{[Q - R_i(t)] C_{ii}(t) - [Q - \bar{R}(t)] C_{iA}(t)\} \quad \text{(Eq. A17)}$$

where $C_{ii}(t)$ is the concentration of drug entering the sink with the dissolution apparatus operated with the recycle time function, $R_i(t)$, for the ith dosage form, and $C_{iA}(t)$ is similarly defined for a recycle time function, $\bar{R}(t)$. With all process variables fixed in value, the concentration in the effluent stream is a function of only the recycle time function, *i.e.*, $C_{ii} = f[R_i(t)]$ and $C_{iA} = f[\bar{R}(t)]$. Therefore, as $R_i(t)$ approaches $\bar{R}(t)$ for each ith dosage form, then $C_{ii}(t)$ approaches $C_{iA}(t)$ and $S_i(t)$ tends to zero.

It is not considered likely that a set of process variables exists such that $R_i(t)$ will precisely equal $\bar{R}(t)$ for $i = 1, 2, \ldots, p$. However, one set will minimize the sum of the deviations for all dosage forms. One approach to determining an approximation to this set of process variables is to find the minimum value of Eqs. A15 and A16.

This alternative approach is seen to obviate open loop operation runs which would otherwise be required to be performed with $\bar{R}(t)$. It should be noted that with the alternative performance criterion for optimization, as expressed by Eqs. A15 and A16, the influence of the *in vivo* bioavailability data for each dosage form is implicit in $R_i(t)$ by virtue of its use in providing a reference for the closed operation of the apparatus. In any event, this alternative procedure is an indirect approach to the problem of minimizing the deviation of *in vitro* data from *in vivo* data. It may best be considered as a method for more rapidly attaining a set of process variables in the neighborhood of the optimum set. The fidelity of an *in vitro* test may best be gauged from a statistical comparison between drug release results obtained with the apparatus operated with optimal $\bar{R}(t)$ and fixed process variables with *in vivo* bioavailability data using dosage forms previously unstudied *in vitro* and not included in the optimization procedure.

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Subnanogram Assay for Pilocarpine in Biological Fluids

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Abstract □ A method for the determination of pilocarpine was developed in which the imidazole ring of pilocarpine was acylated with heptafluorobutyric anhydride, using triethylamine as a catalyst. After cleanup, the pilocarpine derivative was analyzed using GLC with electron-capture detection. The limit of sensitivity was 25–50 pg of pilocarpine, which had been subjected to the derivatization and cleanup procedures. The method was specific for pilocarpine, with the isopilocarpine derivative eluting prior to the pilocarpine derivative.

Keyphrases □ Pilocarpine—GLC analysis, biological fluids □ GLC—analysis, pilocarpine, biological fluids □ Ophthalmic cholinergic agents—pilocarpine, GLC analysis in biological fluids

Pilocarpine is used extensively in clinical ophthamology as a topical agent for lowering intraocular pressure of patients suffering from open- and closed-angle primary glaucoma. Much interest and analytical development (1) have recently been focused on this drug because of its incorporation into a new dosage form called an ocular therapeutic system (2, 3). This system is placed beneath the eyelid and provides continuous delivery of pilocarpine at a controlled low rate into the tear film, as opposed to intermittent pulses of drug into the tear film by conventional eyedrops.

Colorimetric methods based on the oxidation of the tertiary amino group or on opening the lactone ring with hydroxylamine have been reported (4). Pilocarpine has