change. In addition, observation of such a slow change is precluded by the fact that the bound bilirubin concentration in these infants increases by an average of 2 $\mu M$/hr when no treatment is given. Observation of total plasma bilirubin concentrations alone, in consequence, cannot be used for estimating bilirubin-displacing effects of drugs, given to human infants. Measurement of the albumin reserve should be the main tool for this purpose. As shown above, plasma bilirubin and albumin concentrations should be measured in parallel. Unchanged values of all three parameters, observed at a point in time when the drug concentration in plasma is high, constitute evidence against a bilirubin-displacing effect.

These results underline the necessity of using quantitative methods for evaluation of bilirubin-displacing effects. Drugs cannot be rated as displacing or nondisplacing; dosage and plasma concentrations should be related to the displacing effect, expressed in quantitative terms. This method using monoacetyldapsone (I) seems feasible for such studies *in vitro* and *in vivo*.

## REFERENCES


(1) W. A. Silverman, D. H. Anderson, W. A. Blanc, and D. N. Crozier, *Pediatrics*, **18**, 614 (1956).

(2) R. C. Harris, J. F. Lucey, and J. R. MacLean, *ibid.*, **20**, 875 (1958).

(3) L. Johnson, M. L. Garcia, E. Figueroa, and F. Sarmiento, *Am. J. Dis. Child.*, **101**, 322 (1961).

(4) R. Brodersen, in "Intensive Care in the Newborn," vol. 2, L. Stern, Ed., Masson, New York, N.Y., 1978, p. 331.

(5) L. F. Rasmussen, C. E. Ahlfors, and R. P. Wennberg, *J. Pediatr.*, **89**, 475 (1976).

(6) D. Schiff, G. Chan, and L. Stern, *Pediatrics*, **48**, 139 (1971).

(7) R. Brodersen, W. J. Cashore, R. P. Wennberg, C. E. Ahlfors, L. F. Rasmussen, and D. Shusterman, *Scand. J. Clin. Lab. Invest.*, **39**, 143 (1979).

(8) R. P. Wennberg and L. F. Rasmussen, *J. Pediatr.*, **86**, 611 (1975).

(9) R. Brodersen, *Acta Pharmacol. Toxicol.*, **42**, 153 (1978).

(10) R. Brodersen, S. Andersen, C. Jacobsen, O. Sønderskov, F. Ebbesen, W. J. Cashore, and S. Larsen, *Anal. Biochem.*, **121**, 395 (1982).

(11) G. Levy and A. Yacobi, *J. Pharm. Sci.*, **63**, 805 (1974).

(12) S. Øie and G. Levy, *ibid.*, **68**, 6 (1979).

(13) A. F. McDonagh and F. Assisi, *Biochem. J.*, **129**, 797 (1972).

(14) R. Brodersen, *J. Clin. Invest.*, **54**, 1353 (1974).

(15) R. Brodersen and J. Jacobsen, *Methods Biochem. Anal.*, **17**, 31 (1954).

(16) J. E. Mertz and C. D. West, *Am. J. Dis. Child.*, **91**, 19 (1956).

(17) R. Dybkær and H. Hertz, *Scand. J. Clin. Lab. Invest.*, **25**, 151 (1970).

(18) C. B. Laurell, *Anal. Biochem.*, **15**, 45 (1966).

(19) F. Ebbesen, *Acta Paediatr. Scand.*, **70**, 223 (1981).

(20) R. Brodersen, *Crit. Rev. Clin. Lab. Sci.*, **11**, 305 (1979).

(21) J. M. Kaplan, G. H. McCracken, and L. S. Horton, *J. Pediatr.*, **84**, 517 (1974).

(22) G. Nathenson, M. I. Cohen, and H. McNamara, *ibid.*, **86**, 799 (1975).

(23) R. Schmid, I. Diamond, L. Hammaker, and C. B. Gundersen, *Nature (London)*, **204**, 1041 (1965).

(24) W. A. Blanc and L. Johnson, *J. Neuropathol. Exp. Neurol.*, **18**, 165 (1959).

(25) R. P. Wennberg and L. F. Rasmussen, *J. Pediatr.*, **87**, 1007 (1975).

(26) S. Øie and G. Levy, *J. Pharm. Sci.*, **68**, 1 (1979).


## ACKNOWLEDGMENTS


This study was supported by the Danish Medical Research Council (Grants Nos. 512-10626, 512-10767, and 512-15538), Nordisk Gjenforsikrings Selskabs Jubilæumsfond, and by Købmand i Odense Johann og Hanne Weimanns Legat.

The authors wish to thank Professor B. Friis-Hansen for advice and guidance during the study. [$^{14}$C]I was synthesized by C. Jacobsen. Inger Bonnevie, Signe Andersen, and Birthe Lindgaard are thanked for skillful technical assistance, and the staffs of the departments of Neonatology and Clinical Chemistry are thanked for helpful assistance.


# Elementary Osmotic Pump for Indomethacin


**F. THEEUWES *[x], D. SWANSON *, P. WONG *, P. BONSEN *, V. PLACE *, K. HEIMLICH [‡], and K. C. KWAN [‡]**



Received January 22, 1982, from the **ALZA Corporation, Palo Alto, CA 94304* and [‡]*Merck Sharp & Dohme, Inc., West Point, PA.* Accepted for publication April 14, 1982.



**Abstract** □ Based on the principles of an elementary osmotic pump, systems were designed to deliver indomethacin in solution at a constant rate, $Z$, to contain a total amount of drug, $M_t$, and to deliver 80% of their content at time $t_{80}$. To allow selection of the optimal delivery rate into the body, three different prototypes were prepared with respective values for $Z$, $M_t$, and $t_{80}$ of: 7 mg/hr, 85 mg, 11 hr; 9 mg/hr, 85 mg, 8 hr; and 12 mg/hr, 85 mg, 6 hr. These systems were found to deliver 70% of each system's contents at zero-order rates. Delivery rates were independent of pH, method of measurement, and stirring rate. In keeping with these results, the systems in the GI tract of dogs delivered at the same rate as *in vitro*, which qualifies the *in vitro* test as a bioanalogous method predictive of the *in vivo* performance of the dosage forms. Preliminary results in normal volunteers yielded similar urinary recoveries, while plasma profiles were different from each other and distinct from those following conventional capsules.



**Keyphrases** □ Indomethacin—design and preliminary evaluation of an oral osmotic delivery system, zero-order drug delivery □ Osmotic pump—oral, design and preliminary evaluation, indomethacin □ Drug delivery—design and preliminary evaluation of an oral osmotic delivery system containing indomethacin □ Anti-inflammatory agents—indomethacin, design and preliminary evaluation of an oral osmotic delivery system


The concept of continuous drug delivery that maintains the lowest delivery rate and that will elicit a therapeutic effect has much appeal. Intuitively, such a situation should represent the most efficacious use of the drug, while presenting a minimal risk of adverse reactions. Within certain prescribed constraints, theoretical analyses (1, 2) appear to favor dosing patterns that approach a constant *infusion*. However, direct experimental support for this hypothesis is limited (3). In part, this shortcoming may result from a lack of a practical way to deliver a suitable drug chronically and at a constant rate.

The recent development of an oral dosage form, based


*0022-3549/83/0300-0253$01.00/0*

**Table I—Properties of Drug Core-Determining Shape of Release Rate Profile**

| Property | Symbol | Value |
|---|---|---|
| Mutual solubility of indomethacin sodium trihydrate | $S_d$ | 201.2 mg/ml |
| Total osmotic pressure | $\pi_t$ | 140 atm |
| Tablet core surface area | $A$ | 1.6 $cm^2$ |
| Membrane density | $\rho_m$ | 1.3 g/ml |

on the principles of an elementary osmotic pump, permits a close approximation to zero-order drug delivery (4). The present report describes the design and preliminary evaluation of an oral osmotic delivery system containing indomethacin, a potent anti-inflammatory agent used chronically 2–4 times daily in various forms of arthritis. Indomethacin (I) has a relatively short biological half-life (~4 hr) but is well absorbed throughout the GI tract (5–7).

## THEORETICAL

Delivery of any agent in solution from the elementary osmotic pump can be achieved at a rate proportional to the solubility of the agent inside the system ($S_d$) and the osmotic pressure of the formulation inside the system ($\pi_t$). Given a tablet size with surface area, $A$, and given the membrane permeability and thickness, the desired rate can be obtained by incorporating into the core formulation substances that affect either $S_d$ or $\pi_t$. Such a formulation can be called the composite core.

Delivery of potent agents may require the incorporation of formulating agents to permit fabrication of a system of acceptable size. (These agents are also added during the formulation of conventional tablets.) If these agents are water soluble, system performance can be predicted from the knowledge of certain parameters and the theoretical considerations presented here. The zero-order release rate of drug, $(dm_d/dt)_z$, from such a system, assuming a negligible osmotic pressure of the environmental fluid, is then given by:

$$Z \equiv \left(\frac{dm_d}{dt}\right)_z = k\frac{A}{h}\pi_t S_d \qquad \text{(Eq. 1)}$$

where $k$ is the osmotic permeability coefficient of the membrane, $A$ is the membrane area, $h$ is the membrane thickness, and $\pi_t$ and $S_d$ are as defined above. The zero-order rate will persist from time $t = 0$ to $t = t_z$, at which time the solids—drug and osmotic agent—have gone into solution. The nonzero-order rate will decline parabolically as a function of time (4).

**Table II—Average Zero-Order Release Rate and Membrane Weight for Three Lots of EOP-Indomethacin**

| System Designation[a] | Average Zero-order Rate, mg/hr | Rate-controlling Membrane Weight, mg[b] |
|---|---|---|
| 7/85 | 7.2 | 16.9 |
| 9/85 | 9.6 | 11.9 |
| 12/85 | 12 | 8.4 |

[a] Nominal release rate (milligrams per hour)/total drug content (milligrams). [b] Estimated from total solids applied and coating efficiency factor (see *Experimental*).

Equation 1 provides a convenient way of calculating the membrane permeability ($k$) for a set of systems with the same release rate. Alternatively, for systems with different membrane thicknesses and release rates, the slope of the line of the release rate *versus* the inverse of the membrane thickness provides a means of calculating the membrane permeability. Consequently, the release rate can be expressed as a function of membrane weight ($W$), since this weight is related to membrane thickness:

$$W = \rho_m A h \qquad \text{(Eq. 2)}$$

where $\rho_m$ is the membrane density, and $A$ and $h$ are as specified above. By substituting Eq. 2 into Eq. 1, one obtains:

$$Z_d = k\frac{A^2}{W}\rho_m \pi_t S_d \qquad \text{(Eq. 3)}$$

The membrane permeability, therefore, can be obtained from the slope of the line $Z_d$ *versus* $1/W$.

Equation 3 indicates the parameters to which the average zero-order release rate will be sensitive. These parameters are: membrane permeability ($k$), tablet core surface area ($A$), membrane weight ($W$) and density ($\rho_m$), total osmotic pressure ($\pi_t$), and drug solubility ($S_d$). When a composite composition is chosen, $\pi_t$ and $S_d$ become fixed for the zero-order release period. The fixed composition also determines the total surface area ($A$) of the tablet core. When the membrane is chosen and applied reproducibly, values for $k$ and $\pi_m$ are fixed. Therefore, when testing is conducted at a constant temperature, the average zero-order release rate should be a function of the weight of the membrane applied.

To designate the zero-order release rate and total drug content of a system, the following convention was developed. Each system is described by a release rate ($x$) and total content ($y$) of indomethacin and designated as EOP-indomethacin $x/y$. Therefore, a system designed to deliver sodium indomethacin at 7 mg/hr (expressed as equivalents of indomethacin-free acid) from a system that contains 85 mg total (expressed as free

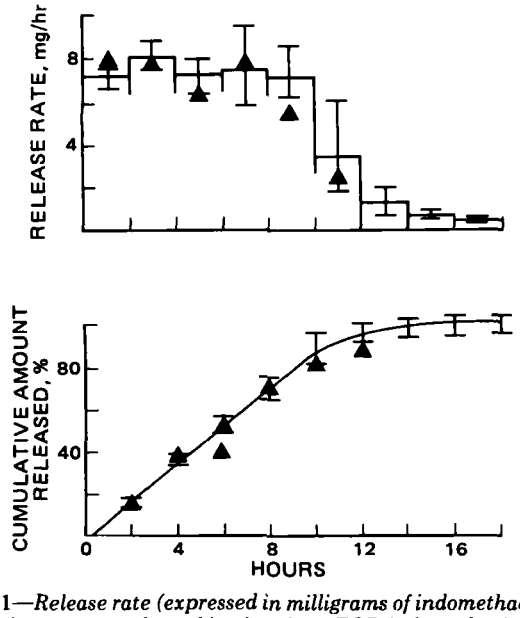



**Figure 1**—*Release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 7/85,* N = 10. *Key: (▲) USP intestinal fluid (I) range (0–4 hr: gastric USP; 4–18: intestinal USP).*

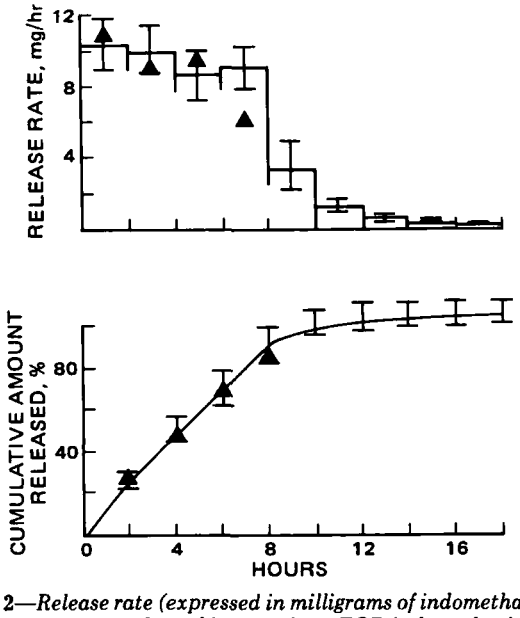



**Figure 2**—*Release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 9/85,* N = 10. *Key: (▲) USP intestinal fluid (I) range (0–4 hr: gastric USP; 4–18: intestinal USP).*

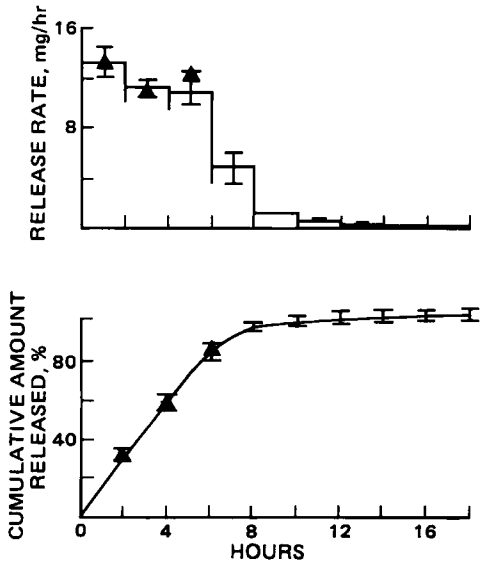



**Figure 3**—*Release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 12/85, N = 10. Key: (▲) USP intestinal fluid (I) range (0–4 hr: gastric USP; 4–18: intestinal USP).*

acid) is designated EOP-indomethacin 7/85. This convention is used throughout the present report for three systems with different release rates but the same indomethacin content (85 mg) designated as EOP-indomethacin 7/85, 9/85, and 12/85.

## EXPERIMENTAL

**Materials**—The tablet, membrane, and laboratory reagents for these studies were USF, NF, or ACS grades and were used without further purification. Sodium indomethacin trihydrate (II) was a gift[1].

Potassium bicarbonate was selected as the osmotic driving agent because of its high osmotic pressure and buffer capacity. In addition, when the dosage form operates in an acid environment, such as the stomach, carbon dioxide bubbles are produced that disperse the indomethacin acid formed. This process produces a finely dispersed drug formulation that readily redissolves and remains available for absorption. Details of the formulation are discussed elsewhere (8).

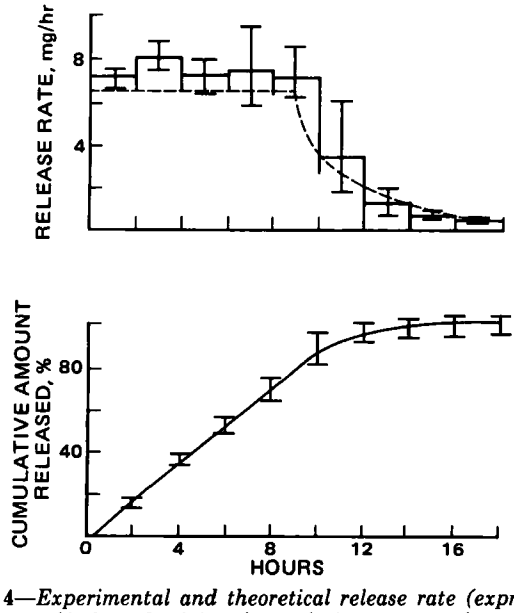
**Determination of Solubilities and Osmotic Pressures**—The solubility at 37° was determined for sodium indomethacin trihydrate in the presence of potassium bicarbonate. Excess amounts of each material were added to water in glass vials; the vials were capped tightly and equilibrated at 37° in a water bath for 24 hr. Aliquots of the resulting solutions were withdrawn from the vials using a preheated syringe and 0.2-μm filter. The sodium indomethacin content was determined by UV analysis after appropriate dilution.

Osmotic pressures of these same solutions were determined[2] at 37°.

**Fabrication of System**—Tablet cores were compressed on a rotary press[3] using 7.9-mm diameter, standard concave (IPT) tooling.

The membranes were applied by an air suspension coater[4], and membrane densities were obtained from volume and weight measurements.

Coated systems were dried in a forced-air oven at 50° until the residual solvent levels were <500 ppm. Systems were then equilibrated at ambient conditions to obtain the equilibrium trihydrate form of sodium indomethacin.




**Figue 4**—*Experimental and theoretical release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 7/85. Key: (- - -) theoretical (I) range.*

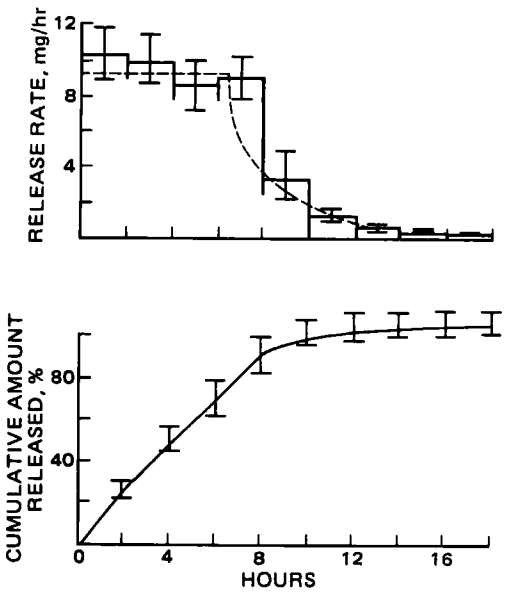



**Figure 5**—*Experimental and theoretical release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 9/85. Key: (- - -) theoretical (I) range.*

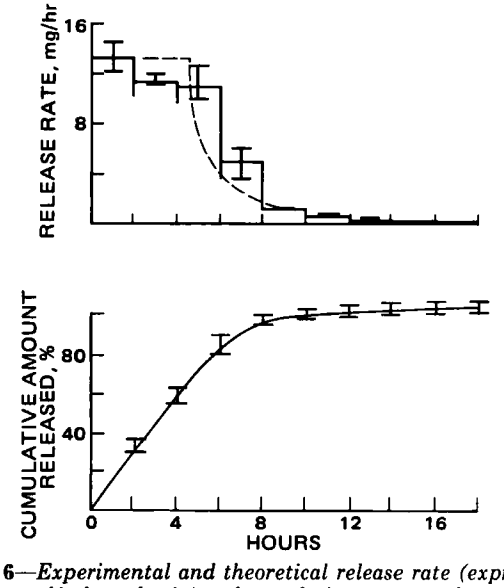



**Figure 6**—*Experimental and theoretical release rate (expressed in milligrams of indomethacin) and cumulative amount released* in vitro *from EOP-indomethacin 12/85. Key: (- - -) theoretical (I) range.*

[1] Merck Sharp & Dohme Laboratories.

[2] Hewlett-Packard model 302B, Vapor Pressure Osmometer.

[3] Manesty D3B.

[4] Wurster.

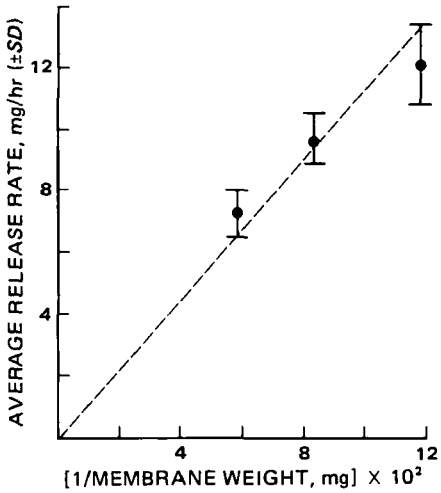



**Figure 7**—*Relation between weight of the membrane coating and average release rate from the EOP-indomethacin.*

Exit ports were drilled in each system by a high-speed mechanical drill or by an automated laser.

**Release Rates**—Differential release rates were determined by placing a finished system in a loose mesh bag and attaching the bag to a glass or plastic rod. The rod was attached to a horizontal arm connected to a vertically reciprocating shaker. The arms containing several systems were then positioned over a water bath (37°) that contained several test tubes. Each tube contained a known amount of release rate receptor media—simulated gastric or intestinal fluid without enzymes with 0.2% polysorbate 20. When the shaker started, the systems were immersed in the release rate media and stirred vertically at an amplitude of 3 cm and frequency of 0.25–0.5 cycle/sec. After 2 hr, the systems were removed from the first receptor container and moved to a second receptor; the stirring then was resumed. This procedure continued until the systems had been tested for 12 hr. Each receptor solution then was analyzed for sodium indomethacin content. The release rate in milligrams per hour for each system for each interval was determined by dividing the amount released in each receptor by 2 hr. The cumulative amount released was determined by taking the sum of the amount released by each system in each interval.

Cumulative amounts released were measured in the USP Dissolution Apparatus 1 (basket) or 2 (paddle); 900 ml of simulated intestinal fluid without enzyme with 0.2% polysorbate 20, pre-equilibrated to 37°, was used. For Apparatus 2, each system was placed in a loose mesh bag weighted with a few small glass beads. Samples were withdrawn from the receptor container at appropriate intervals for analysis, and the amount released was calculated after solution volume corrections were made.

Average zero-order differential release rates for each system were determined by averaging the rates into and including the periods up to the point where 70% of the drug was released.

After the average rate for each system was calculated, an average batch rate was determined using the average rates for all individual systems. The standard deviation obtained from this calculation was the between-system variation. Averaging the rates for all systems in all zero-order intervals provided the same batch average as before but also provided the overall standard deviation. Subtracting the variation between systems from the overall variation provided the variation within systems. This standard analysis of variance is routinely incorporated into all differential release rate determinations.

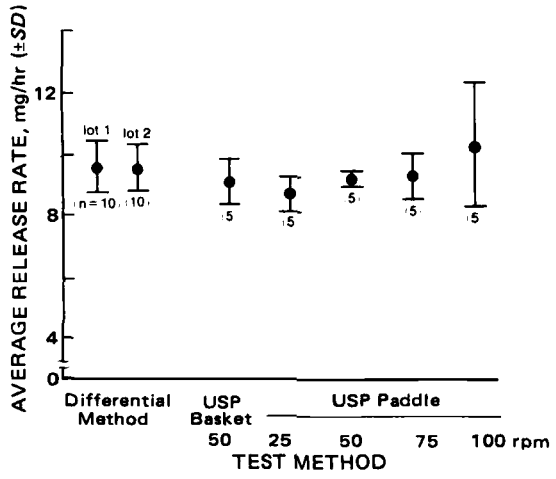



**Figure 8**—*Average release rate from EOP-indomethacin as a function of the test method (SD between-system variation).*

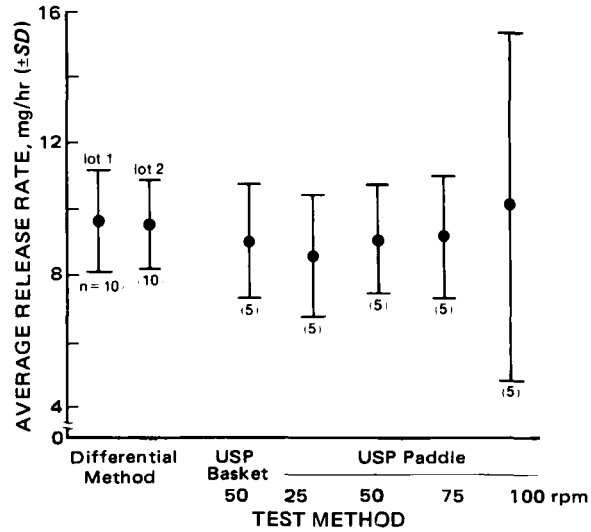



**Figure 9**—*Average release rate from EOP-indomethacin as a function of test method (SD within-system variation).*

Calculations were made as follows:

$$\text{standard deviation between systems} = \sqrt{\frac{\sum_{j=1}^{S}(\bar{Z}_j - \bar{Z})^2}{(S-1)}} \quad \text{(Eq. 4)}$$

$$\text{standard deviation within systems} = \sqrt{\frac{\sum_{j=1}^{S}\sum_{i=1}^{I}(Z_{ij} - \bar{Z}_j)^2}{(N-S)}} \quad \text{(Eq. 5)}$$

$$\text{standard deviation overall} = \sqrt{\frac{\sum_{j=1}^{S}\sum_{i=1}^{I}(Z_{ij} - \bar{Z})^2}{(N-1)}} \quad \text{(Eq. 6)}$$

where $S$ is the number of systems tested, $I$ is the number of zero-order intervals, $\bar{Z}$ is the average batch rate, $\bar{Z}_j$ is the average rate for system $j$, and $N = SI$.

**Cumulative Amounts Released *In Vivo* and *In Vitro***—The release rate performance of these systems *in vitro* was compared with their performance *in vivo* using a dog model. Forty systems were individually weighed and marked with a number in indelible ink. The height (thickness) and diameter of each were measured. These were divided into two groups of 20.

Four mongrel dogs were used after a 3-week quarantine. They were fasted the night before and the day of the study but were allowed water *ad libitum*.

One system was administered orally to each dog 10, 8, 6, 4, and 2 hr before sacrifice, and the time of administration was recorded. Each dog was monitored each hour throughout the study, and all fecal and regurgitated material was examined for the presence of a system. If one was found, the time of recovery was recorded, and the system was washed and stored for analysis. Two hours after the last administration, each dog received an appropriate dose of euthanasia solution[5]. The entire GI tract was removed and opened. Each system was recovered and its recovery location in the tract and time of recovery recorded. All fecal and intestinal material adhering to the systems was carefully rinsed away. Each system was analyzed for its residual drug content.

Concurrent with administration to the dogs, systems were placed in

[5] T-61 euthanasia solution.

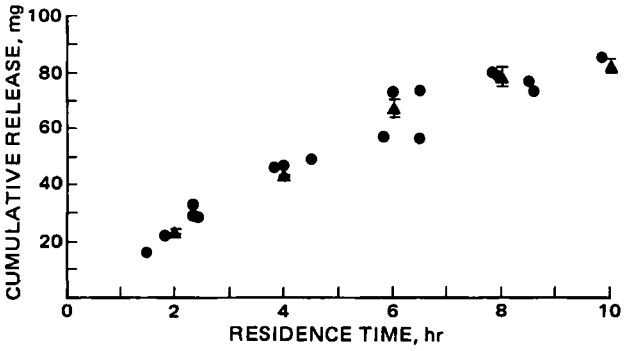



**Figure 10**—*Cumulative amount of indomethacin released by EOP-indomethacin 9/85 (expressed as milligrams of indomethacin)* in vitro *and in the GI tract of dogs. Key: (●) in vivo (dog); (▲) mean in vitro.*

release rate receptor medium. Each system remained in one container of medium for the study. All systems were attached to the vertically reciprocating shaker mechanism. At the end of the study, all systems were removed from the receptor media and each analyzed for residual drug content.

Separately, 10 systems were weighed and analyzed for total drug content; their average fractional drug content ($Q$) was then determined. The parameter $Q$ was then multiplied by the weight of the 20 systems studied *in vivo* and the 20 systems studied *in vitro* to determine the initial drug content of each system. By subtracting the analyzed residual amount from the calculated initial content, the amount of drug released for various time intervals could be determined *in vivo* (dog) and *in vitro*. The data were plotted on the same graph (amount released as a function of residence time) for comparison.

**Preliminary Assessment of Extent of Drug Absorption in Humans**—Two four-way crossover studies were designed to determine the total urinary excretion and temporal pattern of plasma indomethacin concentrations in healthy volunteers. Six fasting subjects received single doses of EOP-indomethacin[6] 7/85, 9/85, 12/85 and indomethacin capsules[7] (3 × 25 mg) in randomized crossover fashion at weekly intervals. In a multiple-dose study, a total of eight doses of the four different formulations were administered at twelve 1-hr intervals to three other subjects. There was a 2-week period between the first dose of each treatment.

During the single-dose study, blood samples were collected at one-half hr preadministration and 1, 2, 4, 6, 12, and 24 hr postadministration; timed urine samples were collected incrementally. During the multiple-dose study, urine samples were collected over 120 hr at 10 equal intervals. The plasma and urine samples were analyzed by HPLC (9).

## RESULTS AND DISCUSSION

Release rate data are expressed as milligrams per hour of indomethacin acid, the pharmacologically active drug moiety. Release rate performance, however, as it relates to the physicochemical constants discussed in *Theoretical*, depends on properties of the drug salt, sodium indomethacin trihydrate, contained in the core (Table I). Molecular weights of indomethacin and its trihydrate salt are 357.8 and 433.8, respectively. The solubility of indomethacin is then obtained from the solubility of its salt, $S_d$, by dividing the solubility (Table I) by the ratio of the molecular weights (1.212). The content of indomethacin per system was selected at 85 mg, which is equivalent to 103 mg of salt.

Table II lists the three dosage forms by their nominal release rate–total drug content and shows their actual zero-order release rates and membrane weights.

**pH Independent Release Rate *In Vitro***—Release rates of three different types of systems (Figs. 1–3) were measured by the differential method from which the cumulative amounts released were calculated and expressed as percent of total drug content. The data, with bars expressing the range of data, were obtained from measurements in gastric fluid for the first 4 hr and in intestinal fluid for the remainder of the test. In a separate experiment, release rates of systems from the same batch were studied in intestinal fluid. Average release rates obtained in this fashion are represented by the triangle. The release rates in either gastric or intestinal fluid were the same.

[6] ALZA Corp.
[7] INDOCIN, Merck Sharp & Dohme.

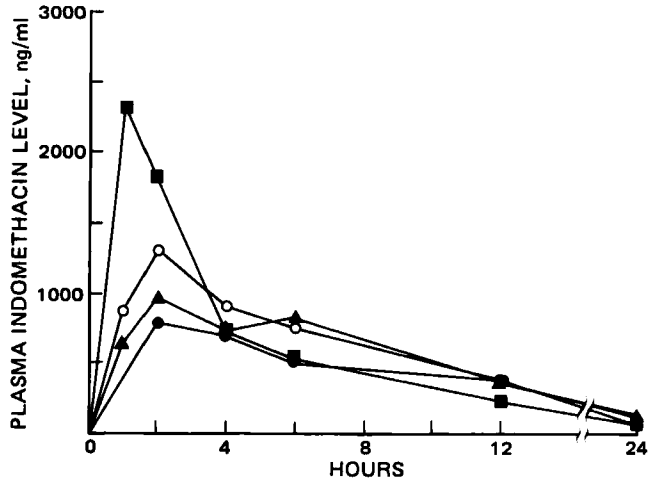



**Figure 11**—*Average (n = 6) indomethacin plasma profiles after single doses of indomethacin capsules (3 × 25 mg) and the EOP-indomethacin 7/85, 9/85, and 12/85. Key: (■) Indomethacin capsules (3 × 25 mg), (●) EOP-indomethacin 7/85, (▲) EOP-indomethacin 9/85, (○) EOP-indomethacin 12/85.*

**Predictable Release Rates**—The relationship between the release rate and membrane permeability for this type of system has been treated elsewhere (4). The purpose of this section, then, is not intended to prove the validity of Eqs. 1 and 3 but to use them as a tool to correlate the data of three sets of experiments on the release rate from systems 7/85, 9/85, and 12/85 (Figs. 4–6). These three systems were coated with the same membrane formulation of which only the thickness and, therefore, weight differed. The average zero order rates ($Z_d$) of these systems can be plotted *versus* $1/W$, the inverse of the average membrane weight (Fig. 7). From the slope of the best straight line through the origin, the membrane permeability constant ($k$) is estimated to be $1.4 \times 10^{-6}$ cm$^3$/hr atm according to Eq. 3.

This estimate is used to calculate the total release rate profile in Figs. 4–6. The theoretical and actual release rates agree to within 10%.

**Release Rate Independent of Stirring Rate**—The effect of stirring rates on the average zero-order rate was studied on a second lot of the 9/85 system using the three different methods listed. Results are shown in Figs. 8 and 9. Average zero-order release rates, calculated as previously described, are the same for lots 1 and 2 of the 9/85 system. Standard deviations between and within those systems, calculated according to Eqs. 4 and 5, are comparable (Figs. 8 and 9). A two-tailed Student's $t$-test of these data indicated no significant differences ($p > 0.1$) between the two lots of systems in rates determined by the differential method. As seen from Figs. 8 and 9, the average rates obtained by the differential method and USP Apparatus 1 (50 rpm) (basket) and 2 (25–75 rpm) (paddle) demonstrate no differences in averages or in deviations from average values. Release rates were measured in artificial intestinal fluid containing 0.2% polysorbate 20.

Comparison of the average release rate for lot 2, determined by the differential method, to the average rates obtained from the USP Apparatus 1 (50 rpm) and 2 (25–100 rpm), showed that no significant differences exist as determined by a Student's $t$-test ($p > 0.1$). When the overall variance for lot 2 is compared to those for the USP methods, only method 2 at 100 rpm is significantly different ($p < 0.05$), as determined by a variance ratio ($f$) test. That deviation was due to rupture of the membrane by the vigorous stirring, making the results at this stirring rate invalid. In all other cases, overall agreement exists between the various *in vitro* methods reported here.

**Release Rate *In Vivo versus* Release Rate *In Vitro***—The release rate *in vivo* cannot readily be measured with the differential method used *in vitro*. Cumulative amounts released *in vivo* were obtained instead and compared (Fig. 10) with cumulative amounts released *in vitro* and also measured directly. The ranges of experimental *in vitro* data are indicated with error bars, while amounts released from individual systems in the GI tract of four dogs are listed as separate data points. The systems studied were of the 9/85 type and were obtained from the batch for which data are given in Fig. 2. The average release rate for each separate system, *in vitro* or *in vivo*, which released an amount $\Delta m_i$ in a residence time $\Delta t_i$ is found as:

$$Z_i = \Delta m_i / \Delta t_i \qquad \text{(Eq. 7)}$$

**Table III—Mean Urinary Recovery and Renal Clearance (±*SD*) of Indomethacin Following Single Doses in Six Subjects**

| Treatment | Renal Clearance, ml/min | Percent Recovered in Urine |
|---|---|---|
| Capsules | 32.4 ± 14.3 | 17.3 ± 12.5 |
| EOP-7/85 | 33.9 ± 11.9 | 22.3 ± 7.7 |
| EOP-9/85 | 35.1 ± 11.4 | 23.8 ± 5.9 |
| EOP-12/85 | 42.1 ± 14.2 | 28.1 ± 7.1 |

The average zero-order rate for systems that delivered for a time period ≤6 hr was found to be:

*In vitro:*

$$\bar{Z}_1 = \frac{\sum_i^{12} Z_i}{12} = 10.8 \pm 9.4\% \qquad \text{(Eq. 8)}$$

*In vivo:*

$$\bar{Z}_2 = \frac{\sum_i^{12} Z_i}{12} = 10.6 \pm 16\% \qquad \text{(Eq. 9)}$$

The *in vitro* and *in vivo* rates are equal and are not significantly different from the rate obtained by the differential method.

**Plasma Concentrations and Extent of Drug Absorption from Two Limited Studies in Humans**—Plasma profiles from the single-dose study illustrated the controlled-release properties of the EOP-indomethacin dosage forms. Compared with the indomethacin capsules, they produced more constant and prolonged plasma levels (Fig. 11); renal clearance and total urinary recoveries of indomethacin and conjugates are shown in Table III. Relative to capsules of indomethacin, which are known to be completely absorbed (5, 7), the bioavailability of the 7/85, 9/85, and 12/85 systems were estimated to be 0.80, 0.84, and 0.88, respectively (10, 11).

Total 120-hr urinary recovery of indomethacin (free and conjugated) for the multiple-dose study was 130.2 ± 26.7, 139.4 ± 8.9, and 147.7 ± 23.1 mg, respectively, following EOP-indomethacin 7/85, 9/85, and 12/85, and was 128.8 ± 39.1 mg following indomethacin capsules. Similar urinary recovery of indomethacin suggests comparable bioavailability.

**Summary**—Preliminary studies in humans showed that the extent of absorption from EOP-indomethacin systems 7/85, 9/85, and 12/85 is similar to that after 75 mg of indomethacin in capsules. Plasma concentration profiles are consistent with the planned differences in drug delivery rate and the anticipated effects of enterohepatic circulation (5–7).

## REFERENCES

(1) S. E. DeJongh and M. Wijnans, *Acta Physiol. Pharmacol. Neerlandica*, **1**, 237 (1950).

(2) A. G. M. Van Gemert and J W. Duyff, *ibid.*, **1**, 256 (1950).

(3) A. C. Wood, G. A. Glaubiger, and T. N. Chase, *Lancet*, **i**, 1391 (1973).

(4) F. Theeuwes, *J. Pharm. Sci.*, **64**, 1987 (1975).

(5) K. C. Kwan, G. O. Breault, E. R. Umbenhauer, F. G. McMahon, and D. E. Duggan, *J. Pharmacokinet. Biopharm.*, **4**, 225 (1976).

(6) J. Alvan, M. Orme, L. Bertilsson, R. Ekstrand, and L. Palmer, *Clin. Pharmacol. Ther.*, **18**, 364 (1975).

(7) K. C. Kwan, G. O. Breault, R. L. Davis, B. W. Lei, A. W. Czerwinski, G. H. Besselaar, and D. E. Duggan, *J. Pharmacokinet. Biopharm.*, **6**, 451 (1978).

(8) P. Bonsen, P. S. Wong, F. Theeuwes, U.S. Pat. 4,265,874 (1981).

(9) W. F. Bayne, T. East, and D. Dye, *J. Pharm. Sci.*, **70**, 458 (1981).

(10) K. C. Kwan and A. E. Till, *ibid.*, **62**, 1499 (1973).

(11) S. Hwang and K. C. Kwan, *ibid.*, **69**, 77 (1980).

# Prediction of Stability in Pharmaceutical Preparations XX: Stability Evaluation and Bioanalysis of Cocaine and Benzoylecgonine by High-Performance Liquid Chromatography

**EDWARD R. GARRETT ˣ and KAZIMIERZ SEYDA**

Received December 7, 1981 from *The Beehive, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.* Accepted for publication April 22, 1982.

**Abstract** □ Specific, sensitive, reverse-phase high-performance liquid chromatographic (HPLC) assays of cocaine (I) and its hydrolysis products, benzoylecgonine (II) and benzoic acid (III), have been devised with analytical sensitivities as low as 15 ng/ml of plasma for I using spectrophotometric detection at 232 nm. Cocaine can be separated from its hydrolysis products by extraction at pH 7.5 with haloalkanes. Benzoylecgonine and benzoic acid can be extracted at pH 3.0 with 1-butanol. The evaporated residues were reconstituted in acetonitrile–water for HPLC assay. The assay was used to determine the stabilities of I and II in aqueous solutions, to establish log $k$–pH profiles at various temperatures, and to evaluate Arrhenius' parameters. Hydrolyses were by specific acid–base catalysis. Cocaine showed hydrogen and hydroxyl ion attack on protonated I with 40 and 90% proceeding through the benzoylecgonine route, respectively, as well as hydroxyl ion attack on neutral cocaine, with only 6% proceeding through the benzoylecgonine route. Cocaine is relatively unstable in the neutral pH range with a half-life of 5 hr in buffer at pH 7.25 and 40°. Similar half-lives were observed in fresh dog plasma at 300 and 30 μg/ml, although one study at 0.5 μg/ml indicated a doubling of the rate.

**Keyphrases** □ Cocaine—stability evaluation and bioanalysis, hydrolysis products, high-performance liquid chromatography □ High-performance liquid chromatography—cocaine, benzoylecgonine, and benzoic acid in buffers in biological fluids □ Stability—prediction and bioanalysis of cocaine and benzoylecgonine in plasma and buffers, high-performance liquid chromatography □ Benzoylecgonine—hydrolysis product of cocaine, stability evaluation and bioanalysis by high-performance liquid chromatography □ Protein binding—cocaine and its hydrolysis products, benzoylecgonine and benzoic acid, stability evaluation and bioanalysis, high-performance liquid chromatography

Cocaine (I) is metabolized *in vivo*, principally to its solvolytic products (1, 2), with an apparent terminal half-life in humans ranging between 40 and 91 min (3). Although available data on the stability of I in plasma *in vitro* is sparse (3), a rough estimate of the half-life in human plasma of 150 min at 25° can be made.



0022-3549/83/0300-0258$01.00/0