(6) V. M. Rosenoer and G. M. Gill, *Med. Clin. N. Am.*, **56**, 585 (1972).
(7) S. S. Brown, *Foreign Compd. Metab. Mamm.*, **2**, 456 (1972).
(8) P. D. Hansten, "Drug Interactions," 2nd ed., Lea & Febiger, Philadelphia, Pa., 1971.
(9) E. W. Martin, "Hazards of Medication," Lippincott, Philadelphia, Pa., 1971.
(10) "Evaluations of Drug Interactions," 2nd ed., American Pharmaceutical Association, Washington, D.C., 1976.
(11) P. L. Morselli, S. Garattini, and S. N. Cohen, "Drug Interactions," Raven, New York, N.Y., 1974.
(12) M. Rowland and S. B. Matin, *J. Pharmacokinet. Biopharm.*, **1**, 553 (1973).
(13) R. J. Lewis, W. F. Trager, K. K. Chan, A. Breckenridge, M. Orme, M. Rowland, and W. Schary, *J. Clin. Invest.*, **53**, 1607 (1974).
(14) W. L. Schary, R. J. Lewis, and M. Rowland, *Res. Commun. Chem. Pathol. Pharmacol.*, **10**, 663 (1975).
(15) M. Rowland, L. Z. Benet, and G. G. Graham, *J. Pharmacokinet. Biopharm.*, **1**, 123 (1973).
(16) R. A. Branch, D. G. Shand, G. R. Wilkinson, and A. S. Nies, *J. Clin. Invest.*, **53**, 1101 (1974).
(17) M. Kristensen and L. K. Christensen, in "Pharmacokinetics and Mode of Action of Oral Hypoglycemic Agents," A. Loubatieres and A. E. Reynolds, Eds., III Capri Conference, Casa Editrice, Milan, Italy, 1969, p. 116.
(18) S. B. Matin and M. Rowland, *Anal. Lett.*, **6**, 865 (1973).
(19) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
(20) P. H. Lenz and A. J. Passannante, *Clin. Chem.*, **16**, 426 (1970).
(21) V. P. Dole, *J. Clin. Invest.*, **35**, 150 (1956).
(22) S. P. Colowick and N. O. Kaplan, "Methods in Enzymology," vol. III, Academic, New York, N.Y., 1957, p. 450.
(23) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(24) H. G. Weder, J. Schildknecht, and A. Kessekring, *Am. Lab.*, **10**, 15 (1971).
(25) G. Wittenhagen, G. Mohnike, and W. Langenbeck, *Z. Physiol. Chem.*, **316**, 157 (1959).
(26) R. C. Thomas and G. J. Ikeda, *J. Med. Chem.*, **9**, 507 (1966).
(27) J. Tagg, D. M. Yasula, M. Tanabe, and C. Mitoma, *Biochem. Pharmacol.*, **16**, 143 (1967).
(28) R. J. Mahler and O. Szabo, *Horm. Metab. Res.*, **2**, 9 (1970).
(29) W. H. Linkenheimer and S. J. Stolzenberg, *Am. J. Vet. Res.*, **26**, 1095 (1965).
(30) E. G. McQueen and W. M. Wardell, *Br. J. Pharmacol.*, **43**, 312 (1971).
(31) E. Jähnchen, L. B. Wingard, and G. Levy, *J. Pharmacol. Exp. Ther.*, **184**, 176 (1973).
(32) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).
(33) W. D. Wosilait, *Res. Commun. Chem. Pathol. Pharmacol.*, **9**, 681 (1974).
(34) R. J. Porter and R. B. Layzer, *Arch. Neurol.*, **32**, 298 (1975).
(35) M. Rowland, S. B. Matin, J. Thiessen, and J. Karam, in "Drug Interactions," P. L. Morselli, S. Garattini, and S. N. Cohen, Eds., Raven, New York, N.Y., 1974, p. 199.
(36) J. R. Gillette, in "Fundamentals of Drug Metabolism," B. N. La Du, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1971, p. 400.
(37) T. Fujita, *J. Med. Chem.*, **15**, 1049 (1972).
(38) G. M. Grodsky, D. L. Curry, L. L. Bennett, and J. J. Rodrigo, *Acta Diabetol. Lat., Suppl. 1*, **5**, 140 (1968).
(39) J. Sehlin, *Acta Diabetol. Lat.*, **10**, 1052 (1973).

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Mathematical Model for Enteric Film Coating of Tablets

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Abstract □ The enteric film coating of placebo tablets, using a methacrylic acid–methyl methacrylate copolymer as the film former in the coating solution, was studied by statistical techniques. The effects of four independent formulation and process variables on the disintegration time of the coated film in simulated intestinal fluid and on the resistance to disintegration of the coated film in simulated gastric fluid were studied. The results of a statistically designed set of experiments were used as the input data. Regression analysis of these data resulted in two first-order polynomial equations. The linear model obtained for the disintegration time of the coating in the simulated intestinal fluid was analyzed by the steepest descent method to determine the most suitable combination of the independent variables.

Keyphrases □ Tablets—enteric film coating with methacrylic acid–methyl methacrylate copolymer, disintegration time, mathematical model □ Film coating, enteric—with methacrylic acid–methyl methacrylate copolymer, tablet disintegration time, mathematical model □ Models, mathematical—disintegration time of tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer □ Disintegration time—tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer, mathematical model □ Methacrylic acid–methyl methacrylate copolymer—enteric film coating of tablets, disintegration time, mathematical model □ Dosage forms—tablets with enteric film coating of methacrylic acid–methyl methacrylate copolymer, disintegration time, mathematical model

Much research in pharmaceutics has concerned the relationship between the controllable formulation and process (independent) variables and the characteristics of the resultant system (response). An empirical relationship can be developed with data from statistically designed experiments. The relationship between the independent

Table I—Levels of Formulation and Process Variables in Physical Units

Variable	Lower Level, −1	Base Level, 0	Higher Level, +1
x_1 = film-former concentration (one experimental unit = 1%)	4.4	5.4	6.4
x_2 = plasticizer concentration (one experimental unit = 0.5%)	1.0	1.5	2.0
x_3 = total spraying time of coating solution (one experimental unit = 20 min)	40	60	80
x_4 = length of drying interval (one experimental unit = 5 sec)	10	15	20

variables can be optimized to obtain the best combination of formulation and process variables. Mathematical models for optimization of pharmaceutical formulation and processing were reported previously (1–3).

The purpose of this study was to develop a mathematical model for the enteric film coating of tablets with a widely used film former, a copolymer[1] based on methacrylic acid and methyl methacrylate, using a spray gun, a drier, and the conventional coating pan.

THEORY

Statistically designed experiments[2] were performed to derive a mathematical model for the optimization of the film-coating technique. The experimental design is dependent on the number of variables. For four independent formulation and process variables (Table I) (4), a half-factorial orthogonal design at two levels with four replicated center points requires 12 experiments (Table II).

The first eight experiments represent a half-factorial design for four factors at two levels resulting in eight (0.5×2^4) trials. The additional four trials represent the replicated center points. The two levels here are represented as +1 and −1. Level zero represents the base level. Experiments 9–12 are included to calculate the experimental error.

The type of predictor equation resulting from such a study was a first-order polynomial having the form:

$$y_j = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 \qquad \text{(Eq. 1)}$$

where y_j = response variable, b_i = regression coefficients for first-order polynomial, and x_i = independent variable. Such an equation can be generated for each dependent variable, relating it to the set of four independent variables.

To estimate the path of steepest descent, the gradient of the linear relation given by Eq. 1 can be written as:

$$\nabla y(x_1, x_2, \ldots, x_k) = \left(\frac{\partial y}{\partial x_1}, \frac{\partial y}{\partial x_2}, \ldots, \frac{\partial y}{\partial x_k}\right) = (b_1, b_2, \ldots, b_k) = b_i \qquad \text{(Eq. 2)}$$

where $i = 1, 2, \ldots, k$. Thus, the change in x_i in the path of steepest descent is proportional to $-b_i$, because it moves in the direction $-\nabla y(x_1, x_2, \ldots, x_k)$. In computations, one variable was selected as a standard and the changes in the other variables corresponding to a λ change in the standard variable (λ is the step size) were computed.

EXPERIMENTAL

The four independent formulation and process variables selected are defined in Table I. The length of the drying interval, x_4, was measured as the time interval the coated tablets were dried in the pan by the drier. The film former was a methacrylic acid–methyl methacrylate copolymer; the plasticizer was dibutyl phthalate[3]. Apart from the film former and the plasticizer, the coating solution consisted of isopropyl alcohol (its percentage was changed with the amounts of film former and plasticizer added), acetone (40% w/w), water (1.50% w/w), and a soluble dye (D&C Orange No. 4, 0.01% w/w).

The placebo tablets were composed of lactose, starch, gelatin solution, and magnesium stearate. The tablets were compressed on 2.65-mm deep concave punches and had an average thickness of 7.50 mm. The tablets were nonfriable with an average weight of 760 mg and an average hardness of 8.0 kg. The coating apparatus consisted of a conventional bench-type copper coating pan with 25-cm diameter and 30-rpm rotational speed.

The spraying unit consisted of a compressed air, hand-operated spray gun, but compressed nitrogen gas was used instead of compressed air. The nitrogen pressure was maintained at 0.4–0.5 atm in all experiments. The spray unit was arranged so that the spray jet met the falling cores in the upper part of the pan. It was fixed rigidly by means of clamps on a stand at the side of the pan opening approximately 20 cm from the surface of the rotating tablet bed. The drying unit consisted of a hair drier giving warm air at approximately 50°. The duration of the spraying and drying cycles was adjusted manually with the help of a stopwatch.

Since the main objective of the coating operation was to envelop the core tablets in a polymeric film, it was decided to determine the quantity of solution that would render the process inoperative. The amount of film-coating solution for a single application of the spray gun that a given weight of tablets could tolerate was determined. The amount of solution was measured as the length of spraying in seconds. The spray rate was kept constant (2.30 g/min) by adjusting the nitrogen gas pressure and

Table II—0.5×2^4 Fractional Factorial Orthogonal Design

Experiment	Levels of Controlled Variables in Coded Form			
	x_1	x_2	x_3	x_4
1	−1	−1	−1	−1
2	+1	+1	−1	−1
3	+1	−1	+1	−1
4	−1	+1	+1	−1
5	+1	−1	−1	+1
6	−1	+1	−1	+1
7	−1	−1	+1	+1
8	+1	+1	+1	+1
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0

[a] Replicated center points.

Table III—Liquid Tolerance of Tablets with Solutions Having Different Concentrations of Film Former and Plasticizer

Formula	Concentrations in Solutions, %		Tolerance, sec/500 g of Tablets	Visual Inspection
	Film Former	Plasticizer		
A	5	1	20	Smooth
B	10	1	25	Not smooth
C	5	2	20	Smooth
D	10	2	25	Not smooth

Table IV—Disintegration Time Results of the Film Coat

Experiment[a]	Disintegration in Simulated Intestinal Fluid, min		Resistance to Disintegration in Simulated Gastric Fluid, min	
	Mean Time	*SD*	Mean Time	*SD*
1	6	0.447	50	8.165
2	14	1.080	175	10.165
3	20	0.913	200	11.402
4	15	0.533	280	9.661
5	13	0.632	180	5.477
6	8	0.730	120	17.512
7	16	0.447	250	12.780
8	28	1.414	420	0.000
9	14	0.913	180	11.547
10	14	0.775	200	10.488
11	15	1.169	220	14.491
12	17	0.816	240	18.074

[a] For each experiment, 16 tablets were tested.

[1] Eudragit L, Rohm Pharma GmbH, Darmstadt, Germany.

[2] A discussion of statistical design methods can be found in W. G. Cochran and G. M. Cox, "Experimental Designs," Wiley, New York, N.Y., 1957.

[3] This plasticizer is recommended in "Eudragit Lacquers for Tablet Coating," Rohm Pharma GmbH, Darmstadt, Germany.

Table V—Path of Steepest Descent and Predicted Responses

Level on Path	Levels of Variables in Units				Coded Levels of Variables				Predicted Responses	
	x_1, %	x_2, %	x_3, min	x_4, sec	x_1	x_2	x_3	x_4	y_1, min	y_2, min
0	5.4	1.5	60	15	0	0	0	0	15.00	209.583
1	5.3	1.483	57.467	14.833	−0.1	−0.034	−0.127	−0.033	13.938	193.791
2	5.2	1.466	54.934	14.666	−0.2	−0.068	−0.254	−0.067	12.874	177.967
3	5.1	1.449	52.401	14.499	−0.3	−0.102	−0.381	−0.100	11.812	i62.175
4	5.0	1.432	49.868	14.332	−0.4	−0.136	−0.508	−0.134	10.749	146.351
5	4.9	1.415	47.335	14.165	−0.5	−0.170	−0.635	−0.167	9.687	130.560
6	4.8	1.398	44.802	13.998	−0.6	−0.204	−0.762	−0.200	8.625	114.769
7	4.7	1.381	42.269	13.831	−0.7	−0.238	−0.889	−0.234	7.561	98.945
8	4.6	1.364	39.736	13.664	−0.8	−0.272	−1.016	−0.267	6.500	83.154
9	4.5	1.347	37.203	13.497	−0.9	−0.306	−1.143	−0.301	5.437	67.328
10	4.4	1.330	34.670	13.330	−1.0	−0.340	−1.270	−0.334	4.374	51.537

the controls of the spray gun. Coating solutions with different compositions were applied to the tablets in the rotating pan until the tablets adhered to the pan and to one another and could not be separated without breaking the deposited film. The length of the spraying interval at this point was taken as the liquid tolerance.

The results of these experiments (Table III) showed that the concentration of film former in the coating solution affected the appearance of the coated tablets greatly. Smooth coatings were obtained with a film-former concentration of 5% but not 10%; a 7% concentration was selected as the maximum. A study of the plasticization of the polymeric materials led to the selection of the 1–2% range for the dibutyl phthalate concentration (5, 6). The total spraying time of the coating solution was varied between 40 and 80 min.

During the liquid tolerance experiments, it was observed that the residual film on the tablets dried between 10 and 20 sec. The length of drying was shorter for solutions with low film-former concentrations and longer for solutions with high film-former concentrations. Therefore, the levels chosen for study were 10 and 20 sec (Table I).

In each experiment, a batch size of approximately 500 g of placebo tablets was coated. The coating solution was sprayed onto the tumbling tablets for 20 sec and then dried with warm air according to the length specified in each experiment. This spray–dry cycle was repeated several times until the total spraying time specified was reached.

Other process and formulation conditions including the spray rate, the temperature of the coating solution and tablet bed, the solvent system, and the amount of additives in the solution were the same for each trial.

After each experiment, samples of coated tablets were evaluated for disintegration in the USP disintegration apparatus (7) by placing the tablets into simulated intestinal fluid (7) and simulated gastric fluid (7). The end-point was taken when the first distinct signs of film disintegration, rupture, separation, or softening were evident.

RESULTS AND DISCUSSION

The mean values of the disintegration time in the simulated intestinal fluid and the resistance time to disintegration in the simulated gastric fluid were taken as the true values of the corresponding response at that experimental point. These values were then used in the statistical analysis to determine the regression equation for each response. The mean disintegration times and the standard deviations of the experiments are shown in Table IV.

Model for Disintegration of Film Coat in Simulated Intestinal Fluid—The fitted first-order polynomial model obtained as a result of the 0.5×2^4 fractional factorial design is:

$$y_1 = 15.000 + 3.750x_1 + 1.250x_2 + 4.750x_3 + 1.250x_4 \quad \text{(Eq. 3)}$$

where y_1 is the disintegration time of the film coat in the simulated intestinal fluid. A lack of fit test assuming a 95% F distribution led to the conclusion that the fitted model was adequate to represent the response function. The hypothesis that $b_i = 0$ for all four independent variables assuming a 95% F distribution was rejected. The ANOVA results showed that all regression coefficients were significant, assuming a 95% F distribution. The model had $R^2 = 94.64\%$, measuring the percent fit of the model.

Model for Resistance of Film Coat to Disintegration in Simulated Gastric Fluid—The fitted first-order polynomial model for this response is:

$$y_2 = 209.583 + 34.375x_1 + 39.375x_2 + 78.125x_3 + 33.125x_4 \quad \text{(Eq. 4)}$$

where y_2 is the resistance time to disintegration of the film coat in the simulated gastric fluid. A lack of fit test assuming a 95% F distribution led to the conclusion that the fitted model was adequate to represent the response function. The hypothesis that $b_i = 0$ for all four independent variables assuming a 95% F distribution was rejected. The ANOVA results showed that all regression coefficients were significant, assuming a 95% F distribution. The model had $R^2 = 89.76\%$.

Determination of Optimum Combination of Factor Levels—After obtaining the first-order regression equations for the two responses, one had to find the optimum combination of factor levels that satisfied the following constraints:

1. The film coat should disintegrate in the simulated intestinal fluid within 10 min ($y_1 < 10$ min). Past experience showed that with this choice, when the active material is inserted into the tablets, the tablets as a whole disintegrate within the desired time limit.
2. The film coat should resist disintegration in the simulated gastric fluid for at least 1 hr (7) ($y_2 > 60$ min). To satisfy these constraints, rather than selecting random values for the x's and finding the corresponding y's, the steepest descent method was employed.

When the coded base levels of the four independent variables (0, 0, 0, and 0) were inserted into the response functions, $y_1 = 15.000$ min and $y_2 = 209.583$ min. Response y_1 had to be decreased to satisfy the constraint of $y_1 < 10$ min. The value of y_2 at the base levels of the x's satisfied the constraint set on y_2.

The steepest descent procedure was applied on the regression equation of y_1. A step size of a 0.1% change in the film-former concentration, x_1, was used. Then the respective x_1, x_2, x_3, and x_4 values were obtained. By inserting the coded values of the x's into the two regression equations, corresponding y_1 and y_2 values at those points were predicted (Table V).

More than one combination of variables satisfied the restrictions set on y_1 and y_2 within the experimental region (Table V). The factor levels on the eighth trial were selected as the most suitable combination, giving the lowest predicted value for y_1 and satisfying the restriction set on y_2 within the experimental region, although the level of x_3 was negligibly below the lower limit (40 min) in the experimental design. An experiment was carried out using the selected levels in the same manner as previous experiments. The values of x_3 and x_4 were rounded to 40 min and 14 sec, respectively, since measuring the time in fractions was quite impossible. The experimental results, as the mean values for the disintegration measurements, were $y_1 = 6.375$ min and $y_2 = 80.000$ min. The predicted values for the two responses were $y_1 = 6.660$ min and $y_2 = 86.623$ min. Applying a two-tailed t test at the 5% significance level to the disintegration in the simulated intestinal fluid, with $n = 16$ and $s = 0.587$, and to the resistance to disintegration in the simulated gastric fluid, with $n = 16$ and $s = 12.712$, showed that experimental and predicted results were in good agreement.

REFERENCES

(1) J. R. Buck, G. E. Peck, and G. S. Banker, *Drug Dev. Commun.*, **1**, 89 (1975).

(2) D. E. Fonner, Jr., J. R. Buck, and G. S. Banker, *J. Pharm. Sci.*, **59**, 1587 (1970).

(3) J. B. Schwartz, J. R. Flamholz, and R. H. Press, *ibid.*, **62**, 1165 (1973).
(4) S. Özdurmuş, M.S. thesis, Boğaziçi University, Istanbul, Turkey, 1975.
(5) K. Lehmann, *Manuf. Chem. Aerosol News*, **May 1973**, 36.
(6) *Ibid.*, **June 1973**, 39.
(7) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 932–934, 1026, 1027.

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Degradation of Carmustine in Aqueous Media

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Abstract □ The degradation rate of carmustine was investigated in buffered aqueous media at several pH values. The buffering agents studied were those with potential use in parenteral formulations of this drug: acetate, citrate, and phosphate. The apparent first-order degradation rate constants were calculated using a linear regression procedure. A pH range over which minimum degradation occurred was ascertained. General acid and specific base catalysis was demonstrated for the degradation of carmustine. From the data at 5, 22, and 37°, the apparent activation energies for carmustine degradation in buffered aqueous media were computed and were strongly pH dependent.

Keyphrases □ Carmustine—degradation rate in aqueous media, effect of pH and temperature □ Degradation rate—carmustine in aqueous media, effect of pH and temperature □ Antineoplastic agents—carmustine, degradation rate in aqueous media, effect of pH and temperature □ Stability—carmustine in aqueous media, effect of pH and temperature

Drugs for the treatment of neoplastic disease have received a great deal of attention. Several thousand chemical agents are systematically screened each year for antineoplastic activity, but only a small percentage exhibit activity and are tested further for clinical utility. Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; NSC 409962] (I) was effective in treating L-1210 leukemia, including cases where the central nervous system was involved (1–4).

Prior to the interest in I as a therapeutic agent, a study indicated that 1-methyl-1-nitrosourea (NSC 23909) (II) was unstable in aqueous solution (5), as was a nitrosamide-containing antibiotic, streptozocin, (6). The kinetics of I degradation were considered, and this compound also was unstable in aqueous media (4, 7).

Because I is often administered *via* intravenous solution (8, 9), further consideration of its stability in various aqueous media suitable for intravenous use is desirable. In addition, knowledge of the temperature dependence of the degradation of I is needed to obtain useful shelflife data and to determine desirable storage criteria. Furthermore, knowledge of acid or base catalysis also may be of value in formulating I.

The few published studies directed toward optimizing a parenteral formulation for I emphasized technological considerations such as lyophilization of drug solutions and dry filling with sterile solids (10–12). A formulation for clinical use was prepared by dry filling vials with sterile solid I and sterile, screened mannitol (11, 12). Alternatively, a solution of I in absolute ethanol was filtered through a 0.25-μm membrane and lyophilized (10, 11). These formulations should be used within 1 and 2 hr, respectively, following reconstitution with ethanol and dilution with saline (10, 12).

The purpose of this investigation was to perform accelerated stability studies on I in various aqueous media suitable for injection.

EXPERIMENTAL

Reagents—Carmustine, an investigational new drug, was obtained[1] as a lyophilized powder. All other chemicals were reagent grade.

Materials and Methods—All buffers and other solutions were prepared in volumetric flasks. Distilled water used for dissolving the basic component of the buffer systems was freshly boiled and cooled. Unless noted otherwise, solutions were stored at ambient temperature. Compound I, 0.025 g (stored at −19 ± 1°), was dissolved in sufficient cold (0°) ethanol to make 25 ml of stock solution. The stock solutions were stored at −19 ± 1° for not longer than 7 days.

Buffer solutions were prepared using published procedures (13, 14). All buffer solutions were checked for conformance to the desired pH using a pH meter calibrated at ambient temperature (20–22°) with a pH standard. When necessary, the pH was adjusted to within 0.05 pH unit of the nominal value with either the acidic or basic component of the buffer.

Degradation Experiments—All solvent systems were preequilibrated to the experiment temperature. For ambient (22 ± 1°) and physiological (37.0 ± 0.5°, forced-air oven) temperature experiments, the media were first allowed to equilibrate for at least 16 hr. For experiments at reduced temperature, the media were first chilled in a ice bath (1.0 ± 0.5°) for at least 15 min.

To a volume (~40–45 ml) of preequilibrated media was added 1.0 ml of I stock solution (1 μg/ml). Then the volume was brought rapidly to 50 ml with the appropriate medium, the mixture was shaken, and the time was recorded. A zero-time sample (0.2 ml) was taken for analysis, and the experimental solution was stored at the appropriate temperature. At designated time intervals, a sample volume (0.2, 0.5, 1.0, or 2.0 ml) was removed for analysis.

The criterion used to determine the sample size was that the apparent concentration should not fall below 1.0 μg/ml or exceed 5.0 μg/ml; the optimum range was 2–4 μg/ml to be well within the linear portion of the calibration curve. The sampling time interval depended in part on a previously observed or expected degradation rate.

Analytical Method—The colorimetric method of Loo and Dion (15) was utilized. The use of any substance that might interfere with the analytical method was scrupulously avoided.

An experimental sample volume not greater than 2.0 ml or estimated to contain between 1 and 5 μg of I/ml was added to a 17 × 150-mm Pyrex test tube. If necessary, sufficient distilled water was added to yield a total

[1] Merck Chemical Co. (through the National Cancer Institute).