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Keyphrases

Tetracycline antibiotics—biosynthesis review
Biosynthetic processes—tetracyclines
Polyketide theory—tetracycline biosynthesis
Structure, basic—tetracyclines
Pretetramids—tetracycline precursors
4-Ketoanhydrotetracyclines—dearomatization
4-Aminoanhydrotetracyclines—tetracycline intermediates
Oxidation, tetracyclines—final biosynthetic step

Research Articles

Nuclear *In Vitro* Method of Continuously Evaluating Release Rates of Solid Dosage Forms

By WILLIAM J. McCLINTOCK*, DANE O. KILDSIG, WAYNE V. KESSLER, and GILBERT S. BANKER

A nuclear *in vitro* continuous release rate measuring method has been developed and evaluated which permits determination of the release rates of ^{14}C-labeled materials from solid dosage forms. The nuclear apparatus consisted of a scintillation flow cell and a liquid scintillation spectrometer which recorded count rates automatically. The nuclear method was compared with a spectral method in measuring the release rates of ^{14}C-labeled caffeine and carrier caffeine from three different tablet systems. The two methods were equally precise and there was no statistical difference in the release rates determined by the two methods of analysis for any of the tablet systems studied. The nuclear method may be more convenient and less sensitive to the presence of interfering substances than a spectral method. The nuclear method can be employed as a continuous and automated methodology to reflect the effect of formulation or physical changes on the release rate of dosage forms.

CONCERN WITH maximum drug availability and effectiveness has resulted in the establishment of various dissolution tests as *in vitro* indicators of availability. Often the comparison of the apparatus used in one laboratory and that used in another yields significant differences in the results of dissolution tests on the same dosage forms and drugs. The rotating-bottle method (1–3), the modified USP disintegration testing basket attached to a Gershberg-Stoll apparatus (4, 5), and the rotating disk (6–8), involve the periodic removal of samples followed by some form of quantitative analysis. Automated methods of evaluating *in vitro* release rates have been developed which employ continuous spectrophotometric analysis (9, 10). However, the use of radionuclides as labels on drug molecules affords a direct method of determining dissolution rates regardless of the drug's spectral characteristics.

Several investigators have substituted radionuclide detection for chemical analysis in the mea-

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sure of drug release, but have retained the sampling methodology (11–13). Other workers, employing nondrug labeled systems, have investigated continuous flow methods and various flow cells. Schram and Lombaert (14) used a spiral of polyethylene tubing filled with anthracene powder, inserted into a flat plastic vial, and placed on the window of a multiplier phototube to measure the radionuclide concentration of a continuously flowing eluate. Rapkin and Packard (15) used a cell packed with crystals of anthracene and placed between two multiplier phototubes. The counting efficiencies for ^{14}C using anthracene crystals were similar to those found in counting vials, according to Rapkin and Packard (15), which indicated that continuous flow measurements appeared to be a practical method of determining concentrations of ^{14}C-labeled materials in solution.

In a previous investigation (16), a method of continuously measuring the release characteristics of γ-labeled drugs was developed. The purpose of this research was to develop a system which would continuously and quantitatively measure the concentration of ^{14}C-labeled drugs in solution. Such a method would be independent of the physical and chemical properties of the other components of the dosage form, the spectral properties of the drug, and eliminate interference problems often found in spectrophotometric analyses.

EXPERIMENTAL

Apparatus and Methods—The nuclear system was composed of a Packard scintillation flow cell,[1] a Packard model 3314 liquid scintillation spectrometer,[1] and a flow system designed to maintain the dissolution medium at 37 ± 1°. The Packard flow cell was a plastic cylinder containing a U-shaped channel for anthracene packing and an aluminum cap. To provide greater flow rates and to ensure leak-free operation, the cap was modified (Fig. 1) from the original manufacturer's model by boring two threaded holes, 0.8 cm. in diameter in place of the approximately 0.8 mm. ($^1/_{32}$ in.) inlet-outlet openings of the manufacturer's model. Two cylindrical brass fittings were constructed (Fig. 1) with a 0.625 cm. i.d. for threading into the top of the cell. The fittings were securely tightened into the cell with the aid of rubber O-rings to assure a water-tight fit. The cap had a groove cut around the circumference to allow the use of another O-ring which sealed out light when the cell was in the spectrometer.

The cell was firmly packed with 1.8 g. of flaked anthracene crystals[1] and each tube opening covered with a plug of glass wool and an 80-mesh monel metal screen. The flow cell, which had a 4-ml. volume when empty, had a 1.2-ml. liquid volume when filled with the flaked anthracene crystals.

[1] Packard Instruments Co., Inc., Downers Grove, Ill.

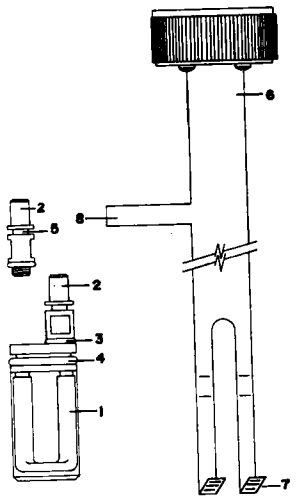

Fig. 1—The scintillation flow cell. Key: 1, flow cell; 2, brass-threaded fittings for inlet and outlet hose connections; 3, rubber O-ring (leak seal); 4, rubber O-ring (light seal); 5, flange for positioning handle prongs; 6, positioning handle; 7, positioning handle prongs; 8, location bar.

After packing the cell the brass fittings were securely tightened in place and the inlet and outlet tubing was connected.

A steel handle was fabricated (Fig. 1) with prongs which could slide into flanges provided in the brass fittings of the flow cell. The handle was employed to accurately position the flow cell in the scintillation counter insuring reproducible counting geometry and to remove the cell from the counter. A location bar on the handle further facilitated positioning of the flow cell in the scintillation well.

The liquid scintillation spectrometer was operated in the repeat mode and with a preset time of 5 min. and a preset count of 900,000 counts. An optimum gain for ^{14}C was used. A background count was established by pumping the solvent, 400 ml. of water, through the cell during a 15-min. period prior to a release study.

Preparation of Dosage Form—Three tablet formulations were prepared as shown in Table I. The

TABLE I—TABLET FORMULATIONS OF THE THREE TABLET GROUPS

Group	Percent Composition by Weight—Caffeine[a]	Magnesium Stearate	Starch
A	40	60	
B	40	40	20
C	40	20	40

[a] Labeled caffeine, approximately 1 μc./200 mg., was used.

caffeine-^{14}C was solvent distributed in the carrier caffeine using isopropanol and the resulting labeled caffeine had a specific activity of approximately 1 μc./200 mg. Accurately weighed samples of the formulations were compressed on a Carver laboratory press[2] under 2,000 lb. of force to yield tablets weighing approximately 500 mg.

[2] Fred S. Carver, Inc., Summit, N. J.

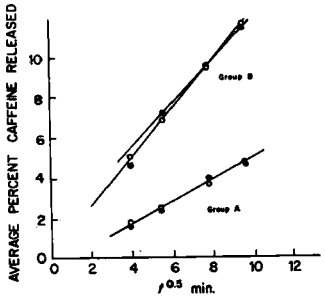

Fig. 2—Average percent caffeine released from Group A and Group B tablets with time as measured by the scintillation and spectral methods. Key: O, spectral; ●, nuclear.

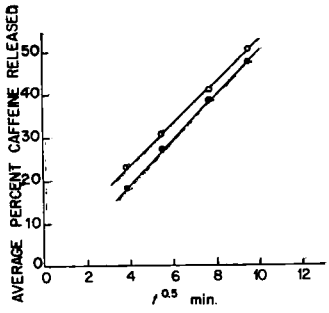

Fig. 3—Average percent caffeine released by tablet Group C as measured by scintillation counting and spectrophotometry. Key: O, spectral; ●, nuclear.

Method of Operation—The tablet was placed in a weighted 60-mesh monel metal screen enclosure which was pyramidal in shape. The enclosure was attached to the arm of a Vanderkamp[3] tablet disintegration apparatus and suspended in the dissolution vessel. The spectrometer was set to print cumulative counts at 5-min. intervals. During the 10-sec. time period required for printing the cumulative count, the pump was stopped and 10-ml. samples were taken for spectrophotometric analysis. After appropriate dilution and filtering, the caffeine was assayed at 273 mμ using a Beckman DU-2 spectrophotometer.[4]

both methods of analysis tablet Group C released caffeine nearly 10 times faster than Group A and more than four times faster than Group B. The release rates for a particular tablet group, as determined by the nuclear and spectral methods of analysis, were compared by two special t tests described by Brownlee (17) to determine whether parallelism existed and to determine whether the two lines represented a single coincident line.

The rates of release as determined using the two methods of analysis, within each tablet group, were first compared for parallelism using a two-tailed

TABLE II—MEAN PERCENT CAFFEINE RELEASED AND STANDARD DEVIATION VALUES FOR TABLET GROUPS A, B, AND C AT GIVEN TIME INTERVALS

	Scintillation				Spectrophotometry		
Group	n[a]	Minutes	Y %[b]	SD[c]	n	$\bar{Y}$ %	SD
A	6	15	1.68	0.23	6	1.79	0.19
	4	30	2.54	0.067	5	2.57	0.23
	6	60	3.93	0.73	5	3.68	0.17
	6	90	4.73	0.65	5	4.72	0.32
B	5	15	4.56	0.63	6	5.02	0.76
	4	30	7.21	0.46	6	6.79	0.83
	6	60	9.63	1.24	5	9.40	0.39
	4	90	11.44	0.42	4	11.59	0.34
C	4	15	18.42	3.36	4	23.16	3.70
	4	30	27.36	4.05	4	30.76	5.18
	4	60	38.67	4.84	4	40.94	4.63
	4	90	47.54	4.71	4	50.44	5.64

[a] n = number of data points used to calculate mean values. [b] $\bar{Y}$% = the mean percent caffeine released. [c] SD = the standard deviation of the mean.

RESULTS AND DISCUSSION

Release Rates—The comparison between the nuclear and spectrophotometric methods of measuring the release of caffeine from three different tablet formulations is shown in Figs. 2 and 3. The release of caffeine from the tablets was linear with square root of time as predicted by Higuchi (18) for a leaching process and as shown in a previous investigation (16) of similar formulations. The mean percent caffeine released at any time period was calculated from at least four determinations, and standard deviation is shown in Table II, for each tablet group.

The release rates were calculated from their regression line slopes (Table III). According to t test and the hypothesis $b_1 - b_2 = 0$ with the critical regions of rejection being set at 0.25. The observed t values (Table IV) were less in every case than the table values for t. The rates of release within each group of tablets for each method of analysis may therefore be regarded as parallel or equal.

TABLE III—REGRESSION LINE SLOPES OF RELEASE PERCENTAGES *versus* SQUARE ROOT OF TIME (AVERAGE PERCENT CAFFEINE RELEASED/MIN.$^{1/2}$)

Group	Scintillation	Spectrophotometry
A	0.553	0.518
B	1.223	1.147
C	5.204	4.844

[3] Van-Kel Industries, Inc., Livingston, N. J.
[4] Beckman Instruments, Inc., Fullerton, Calif.

TABLE IV—RESULTS OF *t* TESTS FOR PARALLEL AND IDENTICAL REGRESSION SLOPES

Group	Parallel	Identical
A	t (a = 0.25) = 0.6038 table t = 0.681	t (a = 0.10) = 1.266 table t = 1.303
B	t (a = 0.25) = 0.6649 table t = 0.681	t (a = 0.05) = 1.445 table t = 1.684
C	t (a = 0.25) = 0.4427 table t = 0.683	t (a = 0.025) = 1.994 table t = 2.045

The rates of release within each tablet group were next compared statistically to determine if they were identical as well as parallel. Brownlee (17) describes the lines as being identical when the two lines can be regarded as a single coincident line. A null hypothesis that the two lines within each tablet group were identical could be accepted (could not be rejected) at a critical rejection region of 0.10 or less (Table IV). The rates of release of each tablet group, as measured by either analytical method, could therefore be regarded as producing a single coincident line.

The variances of different sources of error with each method of analysis were also analyzed statistically. The scintillation method of measurement tended to yield slightly higher variance values than the spectrophotometric method. However, in no case were these differences significant. In general, when the variance of one analytical method was high, the variance of the other method for the same tablet group was also high.

SUMMARY

Many new drug substances are being routinely ^{14}C-labeled early in the drug product development sequence to facilitate illucidation of metabolites and metabolic pathways. It is suggested that the availability of ^{14}C-labeled drug might facilitate drug property characterization, and dosage form design, especially according to the preformulation research protocols recently implemented in many pharmaceutical laboratories. The use of ^{14}C-labeled drug, when available, could expedite preformulation research and drug design related to dissolution rate and other determinations. The necessity of extracting drug from dissolution media containing enzymes of other interfering components of the media, or containing polymeric materials or other interfering dosage form excipients, may be avoided using a nuclear method. The necessity, sometimes encountered, of developing special assay methods or assay modifications during product design can be avoided. In addition, continuous and automated analyses may readily be established for static determinations or for dynamic tests such as continuous dissolution rate analysis.

A method has been described in this investigation by which these objectives could be accomplished, employing ^{14}C-labeled materials and utilizing a scintillation flow cell and scintillation spectrometer. The nuclear method was found to be equally precise and much more convenient than a sampling technique and a spectral analytical method.

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Keyphrases

Release rates—solid dosage forms
Nuclear *in vitro* method—continuous evaluation, release rates
Apparatus—nuclear *in vitro* release rates
UV spectrophotometry—analysis
Scintillation counting—analysis
Caffeine-^{14}C—test compound