Table II—Accuracy and Precision from Matrix Standards: Individual Determinations of I and II [a]

Compound	Value Expected, ng/mL	Mean Found[b], ng/mL	CV[c], %
I	20	23	4.7
	50	50	3.3
	100	100	6.3
	500	503	1.5
	2000	1852	4.5
II	100	100	7.9
	250	250	1.0
	500	515	4.3
	2500	2405	2.0
	5000	5146	3.6
	10,000	9835	3.5

[a] Procedure used for mouse plasma samples. [b] Found calculated from peak height ratio and peak height ratio response factors for I and II, respectively, from four standard curves each run on a different day for each compound. [c] For four determinations.

pounds in pharmacological, pharmacokinetic, and toxicological studies in rats, dogs, mice, monkeys, and rabbits. These procedures had reproducible quantifiable limits of 20 ng/mL for I and 50 ng/mL for II. Separate determinations of I and II were required for animal samples in which ratios of the concentration of II to the concentration of I were large enough to significantly degrade the resolution between the HPLC peaks. These high ratios occurred in plasma samples from dosed rats, mice, and rabbits. The separate determinations were used in these cases; otherwise, the selectivity, sensitivity, and other analytical indicators were the same for the plasma of all species tested.

Results from the dual determination of I and II in plasma from dogs are shown in Fig. 3. Part of these results were generated using the standardization curve data reported in Table I. The animals were dosed orally with one-half of the daily dose of I just after the time zero samples were taken and with the remainder of the daily dose after the 4-h samples. The data were evaluated for peak plasma level times (6–8 h) as well as for relationships between dose and plasma levels (no consistent dose response). The low detection limits for both I and II and the low plasma volume requirements for analysis have made this procedure very useful in multisampling and small animal studies. These points and the versatility of this procedure are amply demonstrated in Fig. 3.

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Collaborative Study of the USP Dissolution Test for Prednisone Tablets with Apparatus 2

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Abstract □ Five lots of prednisone tablets that disintegrate within 5 min were collaboratively studied by 11 laboratories using USP Apparatus 2 under carefully controlled conditions. One lot gave complete dissolution. The reproducibility and repeatability of Apparatus 2 for the four lots still dissolving at the end of the test were 2.6 and 1.6% of label claim, respectively, for the 11 laboratories.

Keyphrases □ USP dissolution test—collaborative study of Apparatus 2, five lots of prednisone tablets, 11 laboratories □ USP Apparatus 2—collaborative study of dissolution of five lots of prednisone tablets, 11 laboratories □ Prednisone tablets—dissolution of five lots with USP Apparatus 2, collaborative study by 11 laboratories

The USP dissolution test for prednisone tablets (1) requires that when 12 tablets are tested, an average of ≥80% of the labeled amount of prednisone must dissolve in 30 min. The tablets are individually tested under experimental conditions which must be carefully controlled if reproducible results are to be obtained.

Four common sources of error associated with Apparatus 2 have been identified: misalignment of equipment (2), nonuniformity of the bottom curvature of vessels (3), excess gases in the dissolution medium (4), and the interaction of the test with slowly disintegrating tablets (4). Equipment, tools, and technique were developed and improved between 1978 and 1980 to control the first three sources of error. Certain products consist of slowly disintegrating tablets that do not always settle at the center of the bottom of the vessels; such variability of tablet position before disintegration can give imprecise results (4). However, rapidly disintegrating tablets gave results of sufficient precision to warrant a collaborative study.

The purpose of this collaborative study, conducted by 11 laboratories in the second half of 1980, was to measure the reproducibility and repeatability of Apparatus 2 under state-of-the-art conditions for prednisone tablets that disintegrate within 5 min. The secondary objectives were to determine whether personnel in many laboratories could correctly adjust Apparatus 2 by following a set of detailed instructions and whether the apparatus would hold the adjustment over an ~2-week test period.

EXPERIMENTAL

Dissolution Test—The instructions[1] to collaborators conformed to the USP conditions for testing prednisone tablets (1) with two exceptions. The collaborators were instructed to drop a tablet down the side of the vessel with the paddle rotating rather than to drop a tablet into the vessel and then start paddle rotation. The collaborators were instructed to position each vessel so that its vertical axis was not more than 1 mm at any point from the axis of the paddle shaft. A 2-mm tolerance is allowed in the USP specifications. If this second

[1] The complete instructions are available from the authors on request.

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Table I—Collaborative Dissolution Results [a] for Tablet 2, 10-mg Prednisone Tablets

Laboratory	Individual Tablet Results, % of label claim						Mean ± *SD*
	1	2	3	4	5	6	
1	41.7	44.1	42.9	37.0	43.7	44.1	42.3 ± 2.7
	43.8	43.3	39.6	40.2	43.8	42.6	42.2 ± 1.9
2	40.2	36.9	37.4	36.9	36.9	39.1	37.9 ± 1.4
	42.0	43.8	38.0	33.4	36.9	38.0	38.7 ± 3.7
3	41.9	36.0	37.6	37.8	44.2	43.1	40.1 ± 3.4
	34.8	33.0	34.2	34.5	32.7	34.7	34.0 ± 0.9
4	33.5	31.7	33.8	35.4	35.0	34.2	33.9 ± 1.3
	41.2	37.8	42.4	41.1	32.0	40.7	39.2 ± 3.8
5	33.7	35.9	41.1	33.3	40.4	37.6	37.0 ± 3.3
	35.1	33.2	39.0	34.6	34.6	37.6	35.7 ± 2.2
6	33.1	35.4	40.8	32.8	36.8	37.3	36.0 ± 3.0
	36.9	33.9	33.8	39.9	32.6	39.7	36.1 ± 3.2
7	32.1	36.4	34.8	38.9	37.6	33.7	35.6 ± 2.5
	33.9	43.2	45.6	34.6	43.9	42.8	40.7 ± 5.1
8	36.7	42.3	31.3	39.4	35.4	39.5	37.4 ± 3.8
	32.0	42.3	36.1	37.4	37.4	41.9	37.8 ± 3.8
9	46.2	44.5	41.5	41.8	39.4	42.1	42.6 ± 2.4
	45.7	49.1	46.4	46.7	44.4	45.8	46.3 ± 1.6
10	42.3	40.2	42.2	44.6	42.9	43.6	42.6 ± 1.5
	39.9	37.8	37.8	41.5	38.0	39.8	39.1 ± 1.5
11	38.8	43.8	31.2	41.1	41.0	39.2	39.2 ± 4.3
	45.6	47.5	39.6	37.0	41.8	39.4	41.8 ± 4.0

[a] Duplicate subsamples of six tablets.

Table II—Collaborative Dissolution Results [a] for Lot A, 5-mg Prednisone Tablets

Laboratory	Individual Tablet Results, % of label claim						Mean ± *SD*
	1	2	3	4	5	6	
1	99.2	102.7	98.6	96.2	103.1	100.7	100.1 ± 2.6
	102.5	98.9	97.3	101.3	101.2	98.1	99.9 ± 2.1
2	96.6	93.3	93.3	94.4	94.4	94.4	94.4 ± 1.2
	99.1	99.1	100.2	99.1	99.1	101.3	99.6 ± 0.9
3	96.2	98.7	96.6	98.4	98.2	97.8	97.6 ± 1.0
	100.0	98.4	95.6	100.7	100.7	98.4	99.0 ± 2.0
4	95.8	96.4	97.7	100.3	94.7	97.1	97.0 ± 1.9
	100.0	98.7	98.4	98.4	97.3	100.2	98.8 ± 1.1
5	98.2	100.2	101.2	100.7	100.2	98.8	99.9 ± 1.2
	100.0	102.1	97.7	101.0	102.3	99.6	100.4 ± 1.7
6	96.5	102.0	101.1	97.2	98.6	103.4	99.8 ± 2.8
	104.9	99.4	98.3	96.4	101.0	101.0	100.2 ± 2.9
7	98.0	96.6	100.8	96.9	96.7	98.6	97.9 ± 1.6
	99.3	100.6	99.2	101.0	96.0	101.7	99.6 ± 2.0
8	98.1	103.8	97.9	102.0	100.6	102.2	100.8 ± 2.4
	99.5	99.2	99.5	97.6	99.5	99.5	99.1 ± 0.8
9	95.9	97.0	93.5	94.1	97.4	96.1	95.7 ± 1.6
	96.0	103.5	97.8	110.6	100.5	103.1	101.9 ± 5.2
10	97.7	102.0	97.3	101.4	102.7	100.6	100.3 ± 2.3
	97.1	96.8	97.4	98.2	99.2	100.2	98.1 ± 1.3
11	103.0	98.9	102.3	98.4	101.4	97.9	100.3 ± 2.2
	95.3	96.4	93.7	94.6	99.1	95.9	95.8 ± 1.9

[a] Duplicate subsamples of six tablets.

requirement could not be met, the dissolution drive was deemed unsuitable for the study.

All laboratories used similar six-spindle dissolution drives[2], paddles[3], vessels[4], and slotted vessel covers[3] without guide bushings. A transparent water bath was specified. The collaborators were required to use a specially designed centering tool (5), a 2.5-cm depth gauge, and a torpedo level with two bubble indicators at right angles to each other. Step-by-step instructions for the setup of equipment were supplied. The volumes of deaerated dissolution medium were measured[5] in volumetric flasks or calibrated graduated cylinders. The medium was preheated to 37°C before it was added to the vessels[6]. After the medium was placed in the vessels, paddle rotation was started and the system was allowed to equilibrate for 15 min.

Each vessel, vessel position, and corrresponding tablet result were assigned the same number. Thus, for each subsample of six tablets tested simultaneously, every individual tablet result was identified with a particular vessel and position.

The tablets were immersed at 1-min intervals to permit the collaborator to draw and filter an aliquot of dissolution medium after each tablet had been subjected to the test for precisely 30 min. A 50-ml aliquot was taken from the same point in each vessel with a syringe equipped with a glass tube. The aliquot was then forced through a 0.8-μm porosity membrane filter[7], and the first 25 mL was discarded to wash the filter free of material that might interfere with the determinative step and to saturate the filter with drug. The filtered aliquots were diluted, if required, and the absorbances of the solutions were measured manually at 242 nm in a 1-cm cell. Portions of the same batch of dissolution medium used for a subsample were also used as the reference solution in the spectrophotometer and as the diluent for the standard.

Collaborative Study—An intralaboratory study was first conducted in this laboratory. Two analysts, one inexperienced with the dissolution test and the other experienced with the test, were able to follow the instructions and obtain similar results from different equipment. Portions of each lot of tablets, the instructions, and standard prednisone powder were then sent to 10 other FDA laboratories. Seven of the eleven laboratories have been conducting dissolution tests for several years and are considered experienced with the test. The others (laboratories 1, 5, 7 and 10) had received their dissolution equipment within 12 months of participating in the study and were considered relatively inexperienced.

Lots Studied—Four of the lots were commercially manufactured for use as drugs and were received under a certification program conducted in this laboratory. The fifth lot subjected to collaborative study was the USP disintegrating calibrator. All five lots were selected because they disintegrated within 5 min, gave means of six-tablet dissolution results that fell within a range of ~4% of label claim when tested in this laboratory, and responded to minor variations in the test to different extents.

One of the lots subjected to study will be referred to as Tablet 2, the name used to designate this lot in previous papers. Tablet 2, a lot labeled to contain 10 mg of prednisone per tablet, has been extensively studied (3, 4) and was provided for practice and to allow a collaborator to test the apparatus after it had been aligned. The collaborators were required to obtain results from six tablets that fell within 30–50% of label claim and whose mean fell within 35–43%. If the results were outside of these ranges, the collaborator was instructed to discuss the results with this laboratory before continuing the study. The disintegrated tablet particles stay on the bottom of the vessel throughout the test and are somewhat affected by misalignment of equipment and nonuniformity of vessel curvature. If excess gases are present in the dissolution medium, the tablet particles are lifted from the bottom of the vessel during the test, and dissolution results range from 50 to 90% of label claim.

Lot A, tablets labeled to contain 5 mg of prednisone, gives complete dissolution of drug content within 15 min. Lot A was used to assess the technique of each laboratory in the determinative steps: aliquoting, filtering, and measuring absorbance. The dissolution results should agree closely with the content uniformity results. When tested for content uniformity in this laboratory (6), 60 tablets gave a mean of 98.7% of label claim with an *SD* of 1.7%.

Lot B, tablets labeled to contain 5 mg of prednisone, gives dissolution results close to 80% of label claim at 30 min[8]. The disintegrated tablet particles stay on the bottom of the vessel throughout the test; lot B is similar to Tablet 2 in this respect. Lot B, though not extensively studied, is sensitive to misalignment of equipment. When tested for content uniformity (6), 60 tablets gave a mean of 95.1% of label claim with an *SD* of 1.5%.

Lot C, tablets labeled to contain 50 mg of prednisone, was selected because it also gives dissolution results of ~80% of label claim at 30 min[8]. The disintegrated tablet particles rise and circulate in the dissolution medium during the test. The tablets, though not studied extensively, appear insensitive to misalignment of equipment. When tested for content uniformity (6), 60 tablets gave a mean of 105.1% of label claim with an *SD* of 2.3%.

Lot D is the USP disintegrating calibrator[9], labeled to contain 50 mg of prednisone/tablet. This lot has been studied extensively in this laboratory (4) and was included in two collaborative studies (7, 8) conducted by the Pharmaceutical Manufacturers Association. The latter studies revealed large differences in results among laboratories. Although the tablets do not respond to the common systematic errors associated with the test (4), they were included as a blind sample for further study. Lot D is similar to lot C in physical

[2] Nine laboratories used the Model 72RL and two laboratories used the Model 72SI.; Hanson Research Corp., Northridge, Calif.

[3] Hanson Research Corp.

[4] Eli Lilly and Co., Indianapolis, Ind.

[5] Five laboratories used 500- and 900-mL flasks marked T.D./T.C.; Kimble Products, Vineland, N.J. Three laboratories used 500-ml. flasks marked T.C. and 1000-mL graduated cylinders. Three laboratories used 500- and 1000-mL graduated cylinders.

[6] One laboratory deviated from these instructions. Deaerated medium was added to the vessels from graduated cylinders at room temperature. The medium was then brought to 37°C.

[7] No. AAWP, 2.5-cm diameter; Millipore Corp., Bedford, Mass., or equivalent.

[8] This is not a "limiting value." If the paddle speed is increased and the test continued, complete drug dissolution is eventually achieved.

[9] USP lot F.

Table III—Collaborative Dissolution Results [a] for Lot B, 5-mg Prednisone Tablets

Laboratory	Individual Tablet Results, % of label claim 1	2	3	4	5	6	Mean ± *SD*
1	72.1	75.0	75.7	74.2	73.0	73.2	73.9 ± 1.4
	76.1	73.7	73.3	70.8	72.3	73.6	73.3 ± 1.8
2	78.1	73.8	82.5	73.8	80.3	76.0	77.4 ± 3.5
	85.2	73.7	71.4	78.4	76.0	76.0	76.8 ± 4.8
3	84.2	86.7	75.1	92.6	92.4	90.6	86.9 ± 6.7
	77.2	79.9	72.8	79.2	83.8	79.7	78.8 ± 3.6
4	70.8	78.0	73.6	71.2	67.0	73.4	72.3 ± 3.7
	68.4	70.7	80.8	78.8	71.2	74.5	74.1 ± 4.9
5	81.4	75.6	81.4	75.4	87.0	82.6	80.6 ± 4.4
	71.4	74.0	76.1	74.7	81.6	83.9	76.9 ± 4.8
6	73.6	78.3	74.0	73.3	73.1	76.5	74.8 ± 2.1
	70.7	72.7	71.4	67.7	69.8	72.5	70.8 ± 1.9
7	71.8	73.5	80.2	72.0	79.5	71.5	74.8 ± 4.0
	71.8	75.2	80.8	72.4	81.8	70.6	75.4 ± 4.8
8	71.4	78.0	70.5	77.4	68.2	74.6	73.4 ± 4.0
	73.5	80.0	68.2	76.4	67.5	76.9	73.8 ± 5.0
9	103.3	75.0	82.9	71.9	71.9	79.3	80.7 ± 11.9
	81.0	78.3	77.4	73.7	69.1	80.1	76.6 ± 4.5
10	80.3	74.5	72.3	79.6	76.4	77.0	76.7 ± 3.0
	80.4	75.0	75.0	74.7	75.9	73.1	75.7 ± 2.5
11	77.6	86.4	73.2	79.5	76.0	71.8	77.4 ± 5.2
	73.0	81.5	79.0	83.5	80.8	70.8	78.1 ± 5.1

[a] Duplicate subsamples of six tablets.

behavior; the tablet particles rise and circulate in the dissolution medium during the test.

Tablet 2 was packaged and labeled in 100-tablet bottles. The tablets from each of lots A, B, and C were tumbled in beakers until each lot was thoroughly mixed. Ten newly purchased bottles of the USP disintegrating calibrator were used as lot D in this study. Lots A, B, C, and D were repackaged in this laboratory by nesting 24 tablets from each lot in cotton in glass bottles possessing metal screw caps. The glass bottles were identified with the appropriate letter designation. Each collaborator was sent one unopened 100-tablet bottle of Tablet 2 and one repacked 24-tablet bottle of each of lots A–D.

Test Sequence—The collaborators were instructed to test a total of 12 tablets from each lot in the following sequence, six tablets being tested at a time: Tablet 2, lots A, B, C, D, A, B, C, and D, and Tablet 2. The study was planned to cover two 5-d work weeks. The first 4 d of the first week were devoted to setting up equipment and testing Tablet 2. The collaborators were then instructed to test six tablets from each of two lots as follows: day 5, lots A and B; day 6, lots C and D; day 7, lots A and B; day 8, lots C and D; and day 9, six tablets of Tablet 2. Laboratories 1, 3, 5, 6, 10, and 11 conformed to this schedule. All laboratories conformed to the sequence in which the tablets were tested. Often, several six-tablet subsamples of Tablet 2 were tested at the beginning of the study; however, only the last six results taken by each laboratory before progressing to lots A–D were used in the statistical analysis.

Table IV—Collaborative Dissolution Results [a] for Lot C, 50-mg Prednisone Tablets

Laboratory	Individual Tablet Results, % of label claim 1	2	3	4	5	6	Mean ± *SD*
1	79.0	78.7	75.8	77.0	77.1	78.9	77.8 ± 1.3
	78.4	79.6	77.7	77.4	77.1	75.4	77.6 ± 1.4
2	69.2	69.2	68.2	67.2	71.2	69.7	69.1 ± 1.4
	75.8	74.7	75.2	74.7	73.7	74.7	74.8 ± 0.7
3	77.5	77.0	75.1	76.4	76.7	75.4	76.4 ± 0.9
	77.2	77.6	77.2	76.3	77.4	75.2	76.8 ± 0.9
4	76.4	76.0	76.2	72.4	75.7	76.6	75.6 ± 1.6
	74.7	72.8	76.1	71.7	73.9	73.6	73.8 ± 1.5
5	78.6	75.9	76.9	76.3	77.9	75.6	76.9 ± 1.2
	77.0	77.8	77.9	77.6	76.4	77.8	77.4 ± 0.6
6	74.1	74.2	75.9	77.0	77.8	77.6	76.1 ± 1.6
	76.3	76.7	74.8	78.5	76.1	77.2	76.6 ± 1.2
7	77.7	77.6	75.8	77.8	78.4	77.5	77.5 ± 0.9
	76.3	75.5	79.5	77.8	77.4	77.0	77.3 ± 1.4
8	81.3	78.6	81.5	80.3	81.5	79.5	80.4 ± 1.2
	78.9	79.2	77.4	77.9	77.2	78.1	78.1 ± 0.8
9	70.1	69.0	72.3	71.9	67.1	70.5	70.1 ± 1.9
	73.3	76.4	75.0	75.7	78.4	78.2	76.2 ± 2.0
10	78.2	79.9	78.2	77.6	79.0	79.1	78.7 ± 0.8
	78.6	79.4	80.1	81.7	80.5	78.8	79.9 ± 1.2
11	77.9	76.4	78.1	76.3	78.8	77.4	77.5 ± 1.0
	79.3	76.5	78.9	79.3	77.7	76.6	78.1 ± 1.3

[a] Duplicate subsamples of six tablets.

Table V—Collaborative Dissolution Results [a] for Lot D, the USP Disintegrating Calibrator Tablets

Laboratory	Individual Tablet Results, % of label claim 1	2	3	4	5	6	Mean ± *SD*
1	67.6	65.3	64.8	66.0	67.2	66.5	66.2 ± 1.1
	64.6	67.8	66.3	65.7	67.7	66.7	66.5 ± 1.2
2	66.6	65.0	64.0	65.5	63.5	65.5	65.0 ± 1.1
	65.5	64.4	64.4	65.0	65.0	63.9	64.7 ± 0.6
3	66.5	63.8	59.8	63.5	64.2	65.6	63.9 ± 2.3
	68.5	66.6	59.2	66.3	65.0	62.2	64.6 ± 3.4
4	65.0	67.3	65.8	65.4	67.2	68.5	66.5 ± 1.4
	62.2	63.6	64.8	62.5	62.7	64.3	63.3 ± 1.0
5	68.2	70.6	69.5	63.2	69.2	68.2	68.1 ± 2.6
	69.2	65.3	66.1	64.1	65.3	68.7	66.4 ± 2.0
6	60.1	65.4	66.5	65.3	67.6	67.8	65.4 ± 2.8
	65.6	66.5	67.9	71.9	68.8	67.7	68.1 ± 2.2
7	64.1	68.5	66.6	68.3	67.8	66.7	67.0 ± 1.6
	68.1	68.2	68.1	68.5	66.4	67.4	67.8 ± 0.8
8	70.2	68.1	64.5	67.6	66.1	70.0	67.8 ± 2.2
	66.0	69.4	66.3	64.8	63.9	70.9	66.9 ± 2.7
9	64.4	61.3	63.6	64.6	57.0	60.4	61.9 ± 2.9
	62.9	65.5	65.6	61.0	63.1	65.3	63.9 ± 1.9
10	70.3	69.7	68.6	69.3	67.3	67.3	68.8 ± 1.2
	68.1	67.1	70.3	68.3	65.2	64.8	67.3 ± 2.1
11	61.0	66.7	66.5	69.7	67.6	65.8	66.2 ± 2.9
	69.7	65.8	68.1	68.1	72.1	68.2	68.7 ± 2.1

[a] Duplicate subsamples of six tablets.

Reported Difficulties—When laboratory 3 first tested Tablet 2, high results traced to excess gases in the medium were obtained; subsequent tests of Tablet 2 were satisfactory. At the beginning of the study, laboratory 4 reported that the mean of six tablet results from Tablet 2 fell slightly below the acceptance range. Since the individual tablet results fell within the specified range, laboratory 4 was instructed to continue the study. Midway through the study, laboratory 6 observed background interference in the UV spectra of the tablets and repeated the second six-tablet tests for lots A and B. The background interference was attributed to dirty spectrophotometer cells. Laboratory 9 obtained high results from Tablet 2 at the beginning of the study. A loose drive belt was found on the apparatus. After the belt was adjusted, satisfactory results were obtained.

Laboratory 11 obtained high results from Tablet 2 at the end of the study. Inspection of the dissolution drive revealed that the base of the unit was warped. After the base had been mounted on a plastic plate to provide additional support, laboratory 11 repeated the study and obtained 12-tablet means that were lower by 3.0, 0.8, 7.0, 1.9, and 1.4% of label claim, respectively, for Tablet 2 and lots A, B, C, and D; these means were significantly ($p < 0.001$) lower for lots B and C. The results from the repeated study were used in the statistical analysis.

The results obtained by laboratory 2 for lots C and D were ~20% of label claim below those reported by the other laboratories. Although an adequate explanation could not be found, the cause of the discrepancy was shown to be related to the laboratory and not to the stability of the tablets. Laboratory 2 was asked to repeat the test for lots C and D, and the results of the repeated tests were used in the statistical analysis.

RESULTS AND DISCUSSION

The individual tablet results for each lot and laboratory are given in Tables I–V. The results of the six-tablet subsamples are grouped in rows of six across the tables and correspond to the tablets tested simultaneously. The first subsample tested by each laboratory appears above the second subsample. The results correspond by number to position and vessel number: the results in the "tablet 1" column were obtained from position and vessel 1, *etc.*

To achieve the purpose of the study, it was necessary that the laboratories conduct the dissolution tests under similar conditions and that the laboratories be able to maintain these conditions with respect to time. Because each lot responds differently to changes in the test conditions, such changes within a laboratory could only be monitored by staggering the times at which the lots were tested. Only Tablet 2 and lot B responded to known systematic errors associated with the test. Thus, Tablet 2 was purposely tested at the beginning and end of the sequence, and tests of lot B were spaced within the sequence.

The possibility of a "settling in" effect was noted in a previous collaborative study of Apparatus 2 (9); *i.e.*, differences among dissolution results with re-

Table VI—Analysis of Variance for 11 Laboratories and Group 1 [a]

Source	Sum of Squares	*DF*	Mean Square	*F* Ratio	*F* (0.95)
Laboratories	115.44	10	11.54	0.89	2.2
Grouped lots	20,694.52	3	—	—	—
Interaction	388.39	30	12.95	3.16	1.7
Means of six	180.20	44	4.10	—	—
Total	21,378.55	87			—
Reproducibility *SD*[b]			2.89		
Repeatability *SD*[b]			2.02		

[a] Six-tablet subsamples; Group 1 is Tablet 2 and lots B, C, and D. [b] In percent of label claim.

spect to time were present in the early days of the study but not later on. In the present study the time between tests and the test sequence were both specified so that all laboratories would be subjected to the same time effects, if present. Several laboratories stated that they could not participate in the study if they were required to observe the exact daily schedule, and these laboratories were instructed to ignore it. However, all laboratories were required to follow the same test sequence.

Reproducibility and Repeatability—The statistical techniques of Steiner, presented in Youden and Steiner (10) were used to assess the reproducibility and repeatability standard deviations of the tablets tested with Apparatus 2. The reproducibility standard deviation measures the agreement of individual results obtained by different laboratories with the same method on identical test material. It may be expressed by:

$$R = (L + I + W)^{1/2} \qquad \text{(Eq. 1)}$$

where L is the error variance among laboratories, I is the error variance of the interaction among laboratories and test material, and W is the error variance within laboratories.

The repeatability standard deviation measures the agreement of successive results obtained by a single laboratory with the same method on identical test materials and conditions. It may be expressed by:

$$S = (W)^{1/2} \qquad \text{(Eq. 2)}$$

The variances necessary to calculate these two standard deviations are obtained from the mean squares derived from an analysis of variance of two crossed classifications, laboratories and test materials, with replication.

The means of the two six-tablet subsamples reported by each laboratory for each lot were treated as duplicate determinations in the step-by-step procedure suggested by Steiner (10) to obtain the reproducibility and repeatability standard deviations. If the subsamples from each lot could be considered identical, the differences among the mean values within and among the laboratories could be attributed to method error. In reality, associated with these subsample means is an inherent variance that is one-sixth the variance of the tablets. The within-laboratory variance contains this source of variance in addition to the within-laboratory error variance. A reduction of this inherent variance would have required a substantial increase in the workload of each collaborator, an impractical approach. Thus, it was important to select lots that gave reasonably uniform dissolution results. The inherent tablet variance is dealt with later in this paper.

Examination of the Reported Means—The six-tablet means reported by each laboratory (Tables I–V) were averaged into 12-tablet means that were then ranked from low to high for each lot. These rank values were summed across the five lots for each laboratory. The rank totals were then tested at the 5% significance level to determine if a laboratory consistently reported high or low results; none did. The ranked 12-tablet means within each lot were then tested at the 5% significance level to determine if any mean within the lot was abnormally high or low [Dixon's test (10)]; none were.

When an analysis of variance is performed on data that can be broken down into classifications of laboratories and lots, the assumption is made that the within-lot variance is constant for the lots. When data are obtained from lots that respond to minor variations in the method to different extents, the within-lot variance can be different from lot to lot. Steiner (10) suggests a statistical test, based on comparison of ranges of laboratory means, to determine groups of lots that have similar variances. The test indicated, at the 5% significance level, that the data from all five lots could not be grouped together. The data from Tablet 2 and lots B, C, and D could be grouped if the data from lot A were removed. Tablet 2 and lots B, C, and D have a common characteristic—they do not give complete dissolution of prednisone at 30 min. Thus, it is logical to treat these lots together in an analysis of variance and to treat lot A separately. When grouped for statistical analysis, Tablet 2 and lots B, C, and D were designated Group 1.

The variances of the six-tablet means reported by each laboratory for each lot in Group 1 were calculated. The ratio of the largest variance to the smallest variance was tested at the 5% significance level and showed that the within-lot variance could be considered constant for the group. An analysis of variance was then performed on this group.

Reproducibility and Repeatability of Group 1—The analysis of variance is shown in Table VI. The *F* ratios indicate that there are no significant differences among the laboratories at the 5% level, but that there is a significant interaction among the laboratories and lots. The significant interaction mean square implies that the lots in this group responded differently to Apparatus 2 from one laboratory to the next. For example, laboratories 2, 3, and 9 reported among the highest results for lot B and the lowest results for lot D; laboratories 6 and 8 reported among the lowest results for lot B and among the highest results for lot D. This interaction was great enough overall to be significant.

The among-laboratory variance, the interaction variance, and the within-laboratory variance were obtained from the mean squares given in Table VI. The reproducibility and repeatability standard deviations were then calculated and are also shown in the table.

Physical Dissolution Characteristics—A tablet whose disintegrated particles stay on the bottom of the dissolution vessel will usually react more to minor differences in Apparatus 2 than tablets whose disintegrated particles are lifted and circulated by the dissolution medium. In this study the former type of product was represented by Tablet 2 and lot B, termed Group 2, and the latter type of product by lots C and D, termed Group 3.

Reproducibility and Repeatability of Groups 2 and 3—Because of their distinctly different physical behavior in the dissolution test, it was of interest to perform an analysis of variance for groups 2 and 3 and to calculate the reproducibility and repeatability standard deviations for each group. Analyses of variance for these groups are shown in Table VII. Group 2 did not show significant differences among laboratories, but did show significant interaction among the laboratories and lots. Group 3 showed significant differences among laboratories, but no significant interaction among laboratories and lots. Thus, lots C and D responded in the same manner to Apparatus 2 in a given laboratory; Tablet 2 and lot B did not. The reproducibility and repeatability standard deviations of each group are given in Table VII. As expected, these terms are somewhat higher for group 2.

Examination of the Individual Tablet Results—A total of 132 results were reported by the 11 laboratories for each lot. Steiner provides a statistical test for rejection of outlying results at the 5% significance level, based on the distance, in standard deviations, that an individual result lies away from the mean Youden and Steiner (10). When one examines a total of 100–200 results, a single result must lie from 3.4 to 3.6 *SD* away from the mean before it can be considered an outlier. The mean and standard deviation for the 132 results for each lot are given in Table VIII. For each of lots A, B, and D, laboratory 9 reported one individual tablet result that was, respectively, 4.4, 4.9, and 3.5 *SD* from the mean of the lot. No cause for these outlying results could be found. The outlier from lot D was borderline and was not far removed from

Table VII—Analysis of Variance for 11 Laboratories and Two Groups [a]

Source	*DF*	Group 2 Mean Square	Group 2 *F* Ratio	Group 3 Mean Square	Group 3 *F* Ratio	*F* (0.95)
Laboratories	10	18.75	1.24	14.46	7.23	3.0
Grouped lots	1	15,393.84		1178.35	—	—
Interaction	10	15.17	2.73	2.00	0.76	2.3
Means of six	22	5.56	—	2.63		
Total	43	368.73	—	32.58	—	
Reproducibility *SD*[b]		3.29		2.33		
Repeatability *SD*[b]		2.36		1.62		

[a] Six-tablet subsamples. Group 2 is Tablet 2 and lot B; group 3 is lots C and D. [b] In percent of label claim.

Table VIII—Statistical Summary of Collaborative Dissolution Results (Percent of Label Claim) for Individual Results from Prednisone Tablets by USP Apparatus 2

Statistics	Tablet 2	Lot A	Lot B	Lot C	Lot D
Mean[a]	38.9	98.9	76.3	76.5	66.1
Total SD[a]	4.2	2.7	5.5	2.9	2.6
S_t[b]	3.0	2.1	4.7	1.3	2.1
S_m[c]	3.2	1.8	3.5	2.7	1.9

[a] $n = 132$. [b] Standard deviation of six tablet results (see text). [c] Standard deviation of means of six tablet results (see text).

results reported by other laboratories. Though the outliers from lots A and B are far removed from the rest of the results for these lots, it is doubtful that including them in an analysis of variance would alter conclusions drawn from the analysis.

Analysis of Variance of Each Lot—Usually, the dissolution test is performed on six tablets at one time, each in its own dissolution environment. Differences in the individual tablet results can be attributed to differences in the tablets only if the dissolution environments affect the tablets similarly. Nonuniform environments, such as differences in liquid velocities generated by misaligned paddle shafts or irregular vessels, may cause individual tablets to disintegrate and dissolve at different rates. With the type of six-spindle dissolution apparatus used in this study, each combination of paddle and vessel may produce a different environment; however, each environment can be reproduced. Thus, if the assumptions are made that there is no interaction between subsamples and positions of an apparatus within a laboratory and that the variance obtained from the positions of the apparatus can be pooled, a two-way analysis of variance can be performed on the data reported by each laboratory for each lot. These sources of variances can then be pooled for all the laboratories.

The analyses for all the lots are shown in Table IX. The sources of variance from subsamples, positions, and the interaction between them are vested with the laboratories (11). The F ratios of the laboratories (the laboratories' mean square divided by the between-subsample mean square) indicate differences among laboratories for lots B, C, and D. The between-subsamples' F ratios (the between-subsamples' mean square divided by the interaction mean square) indicate highly significant differences between subsamples within laboratories for all the lots. The among-positions' F ratios (the among-position mean square divided by the interaction mean square) indicate marginal differences among positions for Tablet 2 and lot D, but the F ratio for lot B is highly significant.

Intepretation of the Mean Squares—The interaction mean squares (Table IX) are residual variances that contain the inherent tablet variance and the within-laboratory error variance of the analytical procedure used to determine the quantity of dissolved drug. The design of the study does not permit these variances to be separated. It is reasonable to assume, however, that the variance of the analytical procedure is constant within a laboratory for all the lots. Because lot A dissolves completely, one would expect its dissolution results to be similar to its content uniformity results. If the standard deviation of the content uniformity results for lot A from this laboratory is converted to a variance, a value of 2.89 is obtained. This variance also contains the inherent tablet variance and the within-laboratory error variance (0.77, as percent of label claim) obtained by a different procedure that was used to determine the quantity of the dissolved drug (6). If it is assumed that this laboratory is typical of those in that collaborative study (6), the within-laboratory error variance obtained from that study (0.77) may be subtracted from the variance of the content uniformity results (2.89) to obtain an independent estimate of 2.12 for the tablet variance of lot A. This value for lot A may then be subtracted from the interaction mean square in Table IX (4.79) to obtain an estimate of 2.67 for the within-laboratory error variance of the analytical procedure used in this study. The latter value indicates that the inherent tablet variance is a relatively small part of the interaction mean square of lot C, whereas it contributes a large portion of the interaction variance of lot B.

The among-position mean square contains the interaction mean square and possibly a mean square associated with the influence exerted by different apparatus positions on the tablet results. The magnitude of the latter mean square depends on the dissolution characteristics of the lot under test and on the extent that the positions differ with respect to the alignment of the paddles in the vessels and the uniformity of the vessels. It has been shown that the results obtained for lots A and C are affected very little by the differences in apparatus positions, but that the results for Tablet 2, lot B, and lot D are influenced to various extents by such differences.

The within-subsample mean square is a pooled variance that measures the dispersion of the results of six tablets tested simultaneously. It is obtained by pooling the interaction mean square with the among-position mean square for each lot and is 9.05, 4.58, 22.50, 1.61, and 4.26, respectively, for Tablet 2 and lots A, B, C, and D. It may be expressed by:

$$MS_w = A \qquad \text{(Eq. 3)}$$

The collaborators verified that the apparatuses met or exceeded USP specifications before they started the study. From a practical viewpoint, therefore, the within-subsample mean square represents the residual variance obtainable for each lot at the current state of the art and was taken to represent the tablet variance.

The between-subsample mean square is a pooled variance that measures the dispersion of six-tablet means within a laboratory. It may be expressed by:

$$MS_b = A + 6B \qquad \text{(Eq. 4)}$$

where B is a variance that measures a dispersion that cannot be attributed to A. The B term may be considered as a pooled within-laboratory error variance.

The laboratories mean square is a variance that measures the dispersion of 12-tablet means. It may be expressed by:

$$MS_l = MS_b + 12C \qquad \text{(Eq. 5)}$$

where C is a variance that measures a dispersion that cannot be attributed to A and B. The C term may be considered as a pooled among-laboratory error variance.

If no error existed within or among laboratories, the three mean squares would, in theory, be the same and the corresponding F ratios would be unity. In practice, the mean squares will almost always be different. If they are the same, the F ratios will fluctuate around unity. To measure the probability that the mean squares are the same, the F ratios are compared with tabulated values that would not be exceeded at that probability level. Equations 3–5 can be used to calculate algebraically the within-laboratory error variance (B) and the between-laboratory error variance (C) for each lot. Since the F ratio may fluctuate around unity if two mean squares are equal, the values calculated for B and C may be negative or positive. The significance of B or C should be judged against the probability of the corresponding F ratios.

Acceptance Ranges—If laboratories that exemplified the 11 laboratories in the study obtained mean results from six tablets tested simultaneously, those means would have a dispersion about the overall mean of a lot that can be expressed by:

$$S_m = [(A/6) + B + C]^{1/2} \qquad \text{(Eq. 6)}$$

where S_m is a standard deviation that may be considered as the standard error about the overall mean result. It is the standard deviation for means of six tablets. If B or C is negative, the value may still be summed algebraically as long as the sum of B and C is positive. If the sum of B and C is negative, the sum is assumed to be zero (10) and

$$S_m = (A/6)^{1/2} \qquad \text{(Eq. 7)}$$

The standard deviation of the six tablets in a subsample is expressed as

$$S_t = A^{1/2} \qquad \text{(Eq. 8)}$$

S_m and S_t for each lot are given in Table VIII.

Table IX—Analysis of Variance for 11 Laboratories and All Lots

Source	DF	Tablet 2 Mean Square	Tablet 2 F Ratio	Lot A Mean Square	Lot A F Ratio	Lot B Mean Square	Lot B F Ratio	Lot C Mean Square	Lot C F Ratio	Lot D Mean Square	Lot D F Ratio	F (0.95)
Laboratories	10	89.42	2.58	12.86	0.46	114.66	3.56	65.56	3.02	32.53	3.50	2.85
Between subsamples	11	34.61	5.00	27.98	5.84	32.33	2.94	21.70	12.19	9.30	2.96	1.95
Among positions	55	11.19	1.62	4.38	0.91	33.99	3.09	1.44	0.81	5.39	1.72	1.54
Interaction	55	6.91	—	4.79	—	11.01	—	1.78	—	3.14	—	—
Total	131	17.33	—	7.18	—	30.36	—	8.18	—	6.84	—	—

Acceptance ranges for each of the lots may be established in a manner similar to that used in the PMA studies (7, 8) to establish the acceptance range for the USP calibrators. An acceptance range of six-tablet means could be defined as mean $\pm 2S_m$ for 132 tablets. The standard deviation of six individual tablets should not exceed $1.97S_t$ (12). Thus, from the data in this study, the acceptance range of means of six tablet results for Tablet 2 would be from 32.5 to 45.3% of label claim; the standard deviation of six tablet results should not exceed 5.9%. The acceptance range of means of six tablet results for lot D, the USP disintegrating calibrator, would be from 62.4 to 69.8% of label claim; the standard deviation of six tablet results should not exceed 4.1%.

Reproducibility and Repeatability of Apparatus 2—The repeatability standard deviations of groups 1, 2, and 3 contain two sources of variance: the inherent variance of the tablets and a within-laboratory error variance. The following relationship exists for the mean square of the means of six given in Tables VI and VII and the between-subsample mean squares for the lots reported in Table IX:

$$MS_m = \Sigma(MS_{bj})/mq = \Sigma(A_j)/mq + \Sigma(B_j)/q \qquad \text{(Eq. 9)}$$

where MS_m is the mean square of means of six results for q lots in the group, MS_{bj} is the between-subsample mean square for the jth lot in the group, m is the number of tablets in a subsample, A_j is the within-subsample mean square for the jth lot in the group, and B_j is the within-laboratory error variance attributed to the jth lot. The term $\Sigma(B_j)/q$ can be considered as the within-laboratory mean-square error for the group.

For group 1 (Tablet 2 and lots B, C, and D) this term is 2.52. The repeatability standard deviation of Apparatus 2 for this group is the square root of 2.52, or 1.59% of label claim. The error from the interaction of the laboratories with the samples and the error among the laboratories remain unchanged. The corresponding reproducibility standard deviation of Apparatus 2 for this group is then 2.60% of label claim. The reproducibility and repeatability standard deviations of Apparatus 2 are, respectively, in percent of label claim: for group 2 (Tablet 2 and lot B), 2.86 and 1.71; for group 3 (lots C and D), 2.22 and 1.46.

Effect of Time and Test Sequence—Table IX shows significant differences between subsamples within laboratories. A difference between subsamples within laboratories might indicate an effect that could be associated with time or the sequence in which the lots were tested. Therefore, the data from the six laboratories that followed the daily schedule were tested. For each lot the six-tablet means reported for the first subsample were grouped and compared with a similar group from the second subsample. A one-way analysis of variance showed no significance difference ($p > 0.25$) between the first and second subsample for any of the lots. The test was then repeated for the data from all laboratories. Again, no significant difference was found ($p > 0.1$). Thus, neither the time of testing nor the order of testing contributed significantly to the results.

Effect of Experience—For each lot, the 12-tablet means of the four laboratories that had the least experience were grouped together for comparison to the 12-tablet means of the seven experienced laboratories. A one-way analysis of variance showed no significant difference ($p > 0.05$) between these groups for any of the five lots. Thus, if a laboratory carefully follows the instructions used in this study, the level of previous experience with the test is not significant.

CONCLUSIONS

The laboratories satisfactorily controlled the critical parameters of Apparatus 2. Of a total of 660 tablet results reported by 11 laboratories, only two were considered outliers. The reproducibility standard deviation of Apparatus 2 for means of six tablet results was found to be 2.60% of label claim for a group of four lots of tablets that disintegrate rapidly, are undergoing dissolution at the time the aliquots are taken, and exhibit different dissolution characteristics. The corresponding repeatability standard deviation was 1.59%. Statistical analysis indicated that the lots in this group responded somewhat differently from one laboratory to the next.

Tablet 2 was useful in several laboratories for identification and correction of problems with technique and equipment. The requirement that each laboratory obtain acceptable results from this difficult lot at the beginning of the study was integral to the success of the study. The results indicate that Tablet 2 was marginally affected by the internal paddle-vessel combinations of the dissolution apparatuses used in the laboratories. The mean of 132 tablet results (38.9%) of label claim, and the standard deviation from the within-subsample (38.9% of label claim), results (3.0%) compare favorably with the mean of 72 tablet results (39.7% of label claim) and the standard deviation from the within-position results (2.7%) reported previously by this laboratory (3).

For lot A, the mean (98.9% of label claim) and the within-subsample standard deviation (2.1%) obtained from this dissolution study, compare favorably with the respective values (98.7 and 1.7%) obtained from the content uniformity results in this laboratory. Thus, the laboratories exercised good control over the analytical procedures associated with the measurement of dissolved prednisone. The standard deviation for means of six tablet results (1.8%) is similar to that expected if several laboratories were to test these tablets for content uniformity.

For lot B, the mean of 132 tablets (76.3% of label claim) indicates that this lot comes within 4% of label claim of passing the USP dissolution requirement. This is precisely the type of tablet product that often leads to disagreement in "pass-fail" decisions in different laboratories. The results indicate that lot B was affected by the internal paddle-vessel combinations of the dissolution apparatuses used in the laboratories. Even though lot B possesses this degree of sensitivity to minor variations in the test, 10 of the 11 laboratories obtained results that showed that lot B failed the USP requirement.

Lot C also comes within 4% of label claim of passing the USP requirement. All of the laboratories obtained results that showed that this lot fails the requirement. Since this lot is not affected by the paddle-vessel combinations of the dissolution apparatus, better agreement among laboratories is to be expected.

For lot D, the USP disintegrating calibrator, the mean of 66.1% of label claim obtained in this study compares favorably with the mean of 66.8% obtained in the Pharmaceutical Manufacturers Association study of 1980 (7). The within-subsample standard deviation (2.1%) compares well with that of the PMA study (2.3%). The standard deviation of means of six tablet results found in this study (1.9%) is considerably smaller than that reported previously (7) (5.1%). Both studies indicate significant differences among laboratories. In addition, this study indicates significant differences within laboratories. However, the magnitude of the differences associated with the laboratories is smaller in this study than in the Pharmaceutical Manufacturers Association study. The results indicate that lot D was marginally affected by the internal paddle-vessel combinations of the dissolution apparatuses. This laboratory, however, has not been able to show a correlation between results obtained with this lot and misaligned paddles or irregular vessels (4).

The analysis of variance for group 3 (lots C and D) indicates that the lots respond to Apparatus 2 similarly within a laboratory and that there is a significant bias among laboratories. Thus, a laboratory that obtains a high or low result for lot C is likely to obtain a correspondly high or low result for lot D. The reproducibility and repeatability standard deviations for this group were found to be 2.22 and 1.46% of label claim, respectively, for Apparatus 2.

The analysis of variance for group 2 (Tablet 2 and lot B) indicates that each lot responds differently to perturbations in Apparatus 2 from one laboratory to the next, but that there are no significant differences among laboratories for the group. That the lots respond differently from one laboratory to the next may be explained by the differing degrees of sensitivity these lots show toward dissolved air in the dissolution medium and internal misalignment of the dissolution apparatuses. The reproducibility and repeatability standard deviations for this group were found to be 2.86 and 1.71% of label claim, respectively, for Apparatus 2. These standard deviations indicate that, at present, tablets whose disintegrated particles stay on the bottom of the vessel cannot be tested with the same precision as tablets whose disintegrated particles are lifted and swirled by the dissolution medium. It is encouraging to note that the differences between the precisions are fairly small.

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Physicopharmaceutical Characteristics of an Oil-in-Water Emulsion-Type Ointment Containing Diclofenac Sodium

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Abstract □ The oil-in-water (o/w) emulsion-type ointment was prepared with food additives containing diclofenac sodium. The oil phase and the emulsifier used were 1,2,3-propanetriyl trioctanoate (caprylic acid glyceryl ester) and sugar wax, and sugar ester, respectively. The emulsion stability of the o/w emulsion-type ointment as well as the diclofenac sodium release profile were investigated and compared with those from conventional ointments. The emulsion stability was evaluated in terms of the viscosity of the emulsion product, the particle size distribution of oil droplets, and the zeta potential of the droplets. It was found that sugar esters have excellent properties as emulsifiers, based on the results of viscosity and zeta potential measurements. The *in vitro* release test revealed that the amount of diclofenac sodium released from o/w emulsion-type ointment was greater than from the hydrophilic and absorptive ointments. Accordingly, it was concluded that o/w emulsion-type bases are suitable for pharmaceutical use in ointment products.

Keyphrases □ Diclofenac sodium—release from various topical ointment bases, oil-in-water emulsions, physicopharmaceutical characteristics □ Oil-in-water emulsions—use as a topical ointment base for diclofenac sodium, release rate, physicopharmaceutical characteristics □ Ointment bases—oil-in-water emulsions, topical release of diclofenac sodium, physicopharmaceutical characteristics

In developing procedures for the design of pharmaceutical products, it is necessary to consider the bioavailability and safety of both the drugs and bases. Potent steroidal agents, which have substantial anti-inflammatory properties, have been used for external application for a long time. However, these steroidal agents have side effects (1), which were found to be directly dependent on the amount of drug applied to the skin. Therefore, various nonsteroidal agents (2, 3) have been developed recently to replace the steroidal agents.

Diclofenac sodium is a potent nonsteroidal anti-inflammatory agent, which has been limited to oral (4) and rectal administrations (5). In this investigation, diclofenac sodium was selected for topical application because no toxicity or topical irritation has been reported.

Although reports on ointment application have been published (6–8), few have dealt with the selection of optimum conditions for the preparation of the ointment. Oil-in-water (o/w) emulsion-type ointment bases offer many advantages over other preparations (9): they permit incorporation of aqueous and oleaginous ingredients, they allow a greater release of many incorporated medicaments, and their rheological properties can be controlled easily. Therefore, the selection of oil base and emulsifier is one of the most important factors in the preparation of o/w emulsion-type bases.

1,2,3-Propanetriyl trioctanoate (caprylic acid glyceryl ester, I) a medium-chain triglyceride, and sugar wax were chosen as the oil phase, since the combination is very stable, solubilizes various drugs, is nontoxic, and does not irritate the human skin (10). Sugar ester is a nontoxic, tasteless, odorless, and nonirritative sucrose fatty acid ester (11). Because it is widely and safely used in food (12), cosmetic (13), and pharmaceutical fields (14), it was selected as an emulsifier in this investigation. It is available in a wide range of hydrophilic–lipophilic balances (HLB) from oil to water soluble and has excellent emulsifying and dispersing abilities. Furthermore, Nobile *et al.* conducted standard tests with the sugar ester, and they reported no irritation to the human skin (11).

Various methods have been reported for the examination of drug release from ointment (15–17); however, no unified and simplified method has yet been established. In view of the

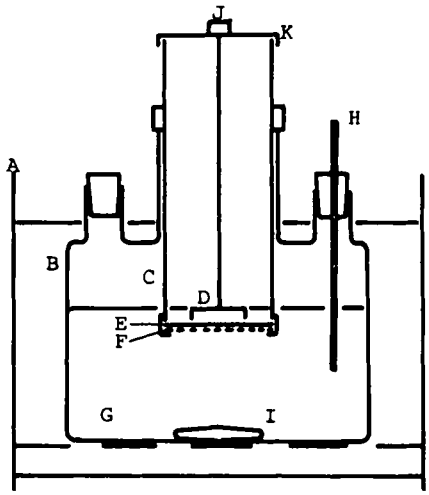

Figure 1—*Cross-sectional diagram of the drug release apparatus. Key: (A) thermostat equipment; (B) releasing fluid glass vessel; (C) inner cylindrical cell; (D) metal dish; (E) membrane; (F) metallic net; (G) releasing fluid; (H) thermometer; (I) stirring bar; (J) stopper; (K) cover.*

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