In Vitro Testing of Timed Release Tablets and Capsules

By E. O. KRUEGER and E. B. VLIET

An apparatus, previously described (1), is recommended for *in vitro* testing of timed release dosage forms.

THE INCREASING use of timed release dosage forms (in particular, prolonged action tablets and capsules) was recognized by the Contact Section in the Fall of 1957. A special committee on timed release tablets and capsules was organized in the Spring of 1958 with the following objectives: to develop an apparatus and an *in vitro* procedure that might be generally useful as a routine control method to check the uniformity of drug release from lot to lot.

From the beginning, the committee limited its considerations to *in vitro* control procedures. Specifically, it has not attempted to develop methods that can be used in formulating timed release products. This must be done with appropriate *in vivo* studies.

During the past 4 years the committee has worked towards its objective through a series of collaborative efforts.

Much of the early stimulation and interest was provided by an apparatus and procedure used by Dr. Frank Wiley and his associates at the Food and Drug Administration (2). A description of this equipment and method was sent to interested PMA member firms with the request that they try it on their products. Laboratories that tried the method felt it was somewhat more complex and less convenient than is desired in a routine control method.

The subcommittee agreed that an effort should be made to determine whether the U. S. P. basket-rack assembly (U. S. P. XV, p. 937) might be adaptable as a suitable test apparatus. The first collaborative study using the modified U. S. P. equipment followed the Spring 1958 meeting of the Contact Section. Nine laboratories were equipped with metal cartridges designed to fit inside the tubes of the U. S. P. tablet disintegration apparatus. The cartridges were closed at each end with 30-mesh screens. Two products were tested. Data obtained from this study showed reasonable agreement among the collaborating laboratories.

As a result of comments arising from this work, slight modifications were made in the equipment and a second study was undertaken after the Fall 1958 meeting. In this study, metal cartridges of about the same size as the tubes in the U. S. P. tablet disintegration procedure were used to provide a better flow of the disintegrating fluid, and the 30-mesh screens were replaced with 40-mesh screens so as to retain finer particles better. The cartridges were supported on a basket-rack holder. Three different products were studied. Each of the products contained a single drug component readily soluble in water. The release pattern was determined in two ways: (*a*) by assaying the drug in the disintegrating fluid, and (*b*) by assaying the undisintegrated residue. This study showed good intra- and interlaboratory agreement in results, and established the interchangeability of determining the drug in the test fluid or in the residue. However, it was generally agreed that it was simpler to determine the drug in the residue, since this avoided any interference effects of the test fluid on the analytical procedures. A summary of this second study was published in *Drug Standards* (3).

At that time it was believed that the apparatus and method described in the second study might be generally useful and convenient for laboratory control of lot-to-lot uniformity for most timed release products.

One of the collaborating laboratories then studied this procedure further and compared the results obtained by using it with those by the rotating bottle method previously described in *Drug Standards* (1) and also with those found by using the initial procedure of the Food and Drug Administration laboratories (2). It became obvious that there were marked differences in release rates using the three methods, particularly with products containing sparingly soluble drugs.

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Report of a special committee of the Contact Section of the Pharmaceutical Manufacturers Association, which included C. L. Graham, L. M. Lueck, C. M. Mitchell, A. Nutzul, E. B. Vliet, F. H. Wiley, and E. O. Krueger, chairman.

The rotating bottle method yielded values more usable for demonstrating timed release and completeness of release (hence, more suitable for control purposes) than did the other two methods.

As a result, a third study was set up testing four different products in eight laboratories. One product contained a readily soluble drug, whereas the other three contained drugs that were only sparingly soluble. Each sample was checked in each laboratory by five procedures as follows: (*a*) Rotating bottle procedure with a complete change from simulated gastric fluid T. S. to simulated intestinal fluid T. S. after 1 hour. (*b*) Rotating bottle procedure with a gradual pH change, accomplished by first using simulated gastric fluid T. S. and, at each subsequent time interval, withdrawing one-half of the fluid and replacing it with simulated intestinal fluid T. S. (*c*) Cartridge basket-rack procedure with a complete change from simulated gastric fluid T. S. to simulated intestinal fluid T. S. after 1 hour. (*d*) Cartridge basket-rack procedure with a gradual pH change effected as in (*b*). (*e*) FDA procedure.

The analyses were performed on the residue in methods *a*, *b*, *c*, and *d*, and on the extracting fluid in method *e*. The results from all laboratories on all four samples were in good agreement.

The product containing the freely soluble drug showed by all 5 procedures: (*a*) comparable results at all time intervals, (*b*) almost complete release after 8 hours, and (*c*) no differences in release, irrespective of whether the pH change was abrupt or gradual.

With the other three products the rate of release was greatest (nearly complete after 8 hours) with the rotating bottles; considerably less (from 35 to 65% released after 8 hours) with the cartridges and basket-rack; and least (from 10 to 50% released after 8 hours) by the FDA method. These differences seem to be in line with variation in the rate of flow of the fluids around the tablets or capsules. The flow is greatest in the rotating bottles, considerably less in the cartridges, and is quite restricted in the FDA apparatus. Results using the gradual pH change were, in general, only slightly different than those using the abrupt pH change technique.

It was decided to explore further the use of this *in vitro* rotating bottle procedure on a larger variety of timed release products to determine how extensively it might be applied and to ascertain whether the results obtained in different laboratories would be sufficiently consistent so that the method might be used for routine control testing.

THE NEW COLLABORATIVE STUDY

Scope.—In this study 12 different products were tested; 11 tablets and one capsule. Six had one drug component, while four contained two drugs each, one had three and one had four. There were at least five different types of release mechanisms in this group of samples and the drugs in the products varied from very soluble to sparingly soluble.

Eight laboratories participated in the study. All samples were sent to the laboratories on a coded basis along with the assay techniques to be used. These had been furnished by the firms that submitted the samples and were checked in other laboratories before and during the study. This, and the assays during the tests, entailed a great amount of work, especially in the multicomponent products. The release rates of each sample were generally determined in four different laboratories. One laboratory checked all of the samples.

Apparatus.—The apparatus used was described by Souder and Ellenbogen (1), was referred to in the previous report of this committee (4), and is shown in Figs. 1 and 2. Disintegration of samples is accomplished in 90-ml. bottles containing 60 ml. of disintegrating fluid revolving end over end in a 37° bath at 40–44 r. p. m.

Procedure.—It was realized that, in adapting a routine control testing method to a specific timed release product, the time intervals to be checked and the nature of the extracting fluid to be used would depend upon the release pattern the product was designed to give and upon the nature of the drugs and other components it contains. Nevertheless, it was decided that in this series of tests all laboratories would check the amount of drug released by all samples after intervals of 1, 2, 3.5, 5, and 7 hours. In all cases, simulated gastric fluid T. S. would first be used. At subsequent time intervals this would be replaced by mixtures of simulated gastric fluid T. S. and simulated intestinal fluid T. S. so that after 1 hour the pH would be 2.5, then 4.5 after 2 hours, and 7.0 after 3.5 hours. Simulated intestinal fluid T. S. at pH 7.5 would be used after 5 hours. The analysis would be performed on the residue in all cases.

The laboratory which tested all of the samples also checked each one using an abrupt pH change. Simulated gastric fluid was completely replaced by simulated intestinal fluid after the first hour.

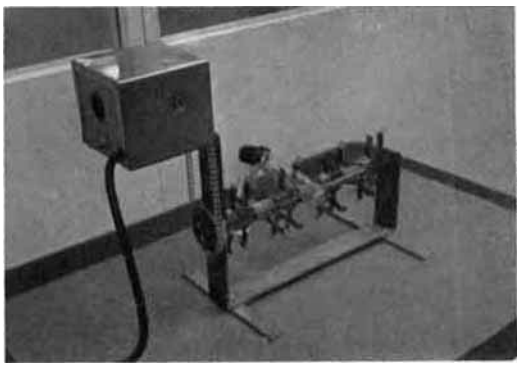

Fig. 1.—Rotating bottle apparatus. (Available from E. D. Menold Sheet Metal Co., 8214 Lisser Ave., Philadelphia, Pa. Bottles are not included.)

TABLE I.—*In Vitro* RELEASE OF DRUG FROM PRODUCT A, REPORTED BY FIVE LABORATORIES

Laboratory No.	Per Cent of Drug Released After Elapsed Times, hr.				
	1	2	3.5	5	7
2	44	50	60	68	78
5	45	54	66	70	82
1	51	58	69	76	84
6	45	50	56	66	68
4	47	60	67	73	82

TABLE II.—*In Vitro* RELEASE OF DRUG FROM PRODUCT B, REPORTED BY FOUR LABORATORIES

Laboratory No.	Per Cent of Drug Released After Elapsed Times, hr.				
	1	2	3.5	5	7
3	30	50	59	63	75
2	37	53	60	68	79
1	35	54	63	72	81
7	46	56	64	74	83

RESULTS

Results obtained in the different laboratories on each sample using the prescribed procedure were in reasonable agreement. When the procedure was modified by abruptly changing the pH of the fluid after 1 hour, the release rates of pH-sensitive drugs were altered, as would be expected.

Of the 12 products tested, only one failed to release its drug as expected. The method, as applied in this study, would seem to be unsuitable for such a product, which depends upon a release mechanism considerably different than those used in the other products studied.

Data shown in Tables I, II, III, and IV, are typical of those obtained in the study and indicate how well results from different laboratories agreed. Results on the other products studied showed the same general degree of agreement.

CONCLUSION

After carefully considering the results obtained in this and the preceding collaborative study, and after communicating with those supplying the samples tested, the special committee made an observation and a recommendation to the PMA Contact Section at its meeting in October 1961. These were unanimously accepted and approved by the membership of the Section and are as follows:

1. The apparatus described in *Drug Standards* (1) and shown in Figs. 1 and 2 is more useful for the *in vitro* control testing of most timed release products now marketed than any other apparatus studied to date.

2. In the event the U. S. P. and N. F. should

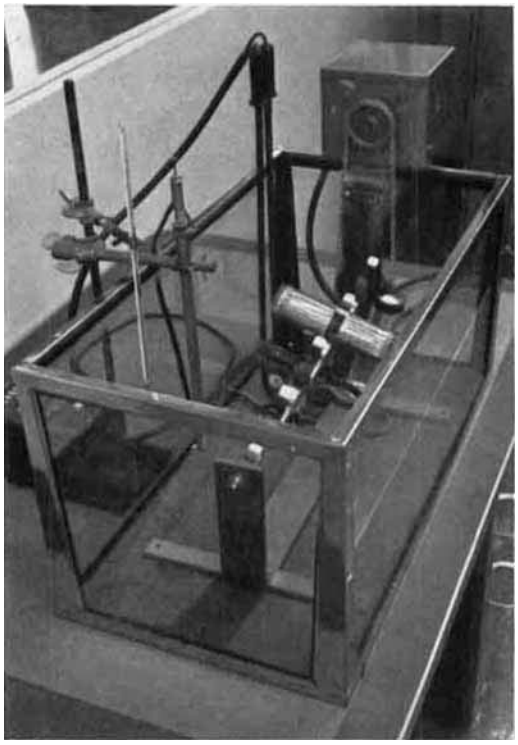

Fig. 2.—Rotating bottle apparatus and accessory equipment: immersion heater, mercury thermoregulator (37°), thermometer, standard relay, constant temperature bath, and bottles ($3^1/_8$ oz. round tablet, Owens-Illinois mold 5676).

TABLE III.—*In Vitro* RELEASE OF TWO DRUGS FROM PRODUCT C, REPORTED BY FOUR LABORATORIES

	Per Cent of Drug Released After Elapsed Times, hr.									
	1		2		3.5		5		7	
Drug component	S	T	S	T	S	T	S	T	S	T
Laboratory No. 1	57	31	79	66	87	84	93	95	99	97
2	61	27	77	46	88	73	91	88	97	97
4	63	29	79	48	88	69	91	85	96	96
3	54	30	73	44	85	68	87	86	94	97

TABLE IV.—*In Vitro* RELEASE OF TWO DRUGS FROM PRODUCT D, REPORTED BY THREE LABORATORIES

	Per Cent of Drug Released After Elapsed Times, hr.									
	1		2		3.5		5		7	
Drug component	X	Y	X	Y	X	Y	X	Y	X	Y
Laboratory No. 1	40	62	53	68	64	77	80	87	90	95
2	34	59	50	67	64	77	76	85	90	95
3	37	56	47	62	67	70	77	81	91	91

include a section on timed release products, it is recommended that this rotating bottle apparatus be considered for use for *in vitro* control testing of such products, with the understanding that the speed of rotation used on certain products may have to be changed to increase the sensitivity of the test for control purposes; and that the time intervals and the pH and other characteristics of the immersion fluids used, must be given individual consideration so as to be appropriate for each product tested.

REFERENCES

(1) Souder, J. C., and Ellenbogen, W. C., *Drug Standards*, **26**, 77(1958).
(2) Wiley, F. H., private communication.
(3) Vliet, E. B., *Drug Standards*, **27**, 97(1959).
(4) Vliet, E. B., *ibid.*, **28**, 113(1960).

Notes

Effect of Ionized Air on Early Growth of Black Mustard Seedlings

By ROBERTSON PRATT

Mammalian systems, bacteria, and fungi have previously been reported to be affected by ionized air. The present paper records an effect of such air on a spermatophyte, black mustard *Brassica nigra* (L.) Koch. Neither positive nor negative air ions influence germination of the seeds, but both types of ions depress the early growth of roots and shoots of the seedlings.

NUMEROUS EFFECTS of ionized air on biologic systems, including human beings, have been reported for more than 200 years, and attempts to apply some of these effects clinically were made as early as 1754. However, most of the reports must be classed as testimonial or, at best, speculative. Within the past decade possibly more reliable, but nonetheless subjective, clinical appraisals have indicated an ameliorating effect of negative air ions and an aggravating effect of positive ions on sinusitis, rhinitis, asthma, pollenosis, and related conditions. The literature up to 1935 has been reviewed extensively (1) and the more recent literature briefly (2).

Among quantitatively measurable effects of air ions on other biologic systems are reduction in succinoxidase content of the adrenal gland of the intact rat exposed to positively ionized air (3); killing of staphylococci by both positive and negative air ions (4); increase in ciliary movement in mammalian trachea, both *in vivo* and *in vitro*, when exposed to negative air ions and the reverse effect, or even abolition of movement, when exposed to positive ions (5–7); and reduction of germination of spores, growth of mycelium and elaboration of penicillin in cultures of *Penicillium notatum* exposed to an atmosphere enriched with air ions, negative ions having a greater effect than positive ones in reducing production of penicillin but positive ions exerting the greater depressing effect on spore germination and mycelial growth (2). The data below, taken from a larger continuing study, record a quantitatively measurable effect of air ons on early seedling growth of a spermatophyte, black mustard (*Brassica nigra*).

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EXPERIMENTAL

Seeds, surface sterilized by immersion for 20 minutes in a dilute solution of calcium hypochlorite, were rinsed several times in sterile distilled water, then were soaked for 1 hour in same, and finally were placed aseptically on moist sterile filter paper strips about 2 cm. wide adhering to the inner surfaces of the germination chambers, consisting of 600-ml. Pyrex beakers. The strips (placed one in each beaker about 6 cm. from the top) were kept moist by a wick which consisted of a "tail" about 1.5 cm. wide that extended to the bottom of the chamber where it dipped into sterile distilled water. Seeds of black mustard are small enough to adhere easily to moist filter paper, even on a vertical surface. Soaking the seeds in water not only removed traces of hypochlorite, it also improved adhesiveness. Experiments were conducted in diffuse daylight; the temperature ranged from 20 to 23°.

A sterile ion generator head,[1] which has been described elsewhere (2), was supported about 1 cm. below the top of each beaker, and then the beaker was aseptically closed with sterile aluminum foil. The details and theory of the ion generators have been reviewed (8–10). At the rectifier voltage employed (860 v.), the atmosphere of the closed beakers was enriched with about 9.5×10^6 ions of appropriate charge/ml./sec. Radiation controls, similar to those run in earlier experiments on *Penicillium* (2), failed to reveal any evidence of direct radiation effects on the seeds or seedlings.

The data in Table I (averages computed from 5 replicate experiments totaling 500 seeds in each of the four environmental conditions) indicate that

[1] The ion generators were kindly provided by the Wesix Electric Heater Co., San Francisco, Calif.