col resistant strains of *Shigella*, *Escherichia*, and *Staphylococcus* exhibited such action, but the sensitive strains and all strains of *Pseudomonas*, whether resistant or sensitive, did not.

In the experiments on resistance developed *in vitro* and the inactivation of chloramphenicol, organisms with resistance transferred by the mixed culture method were far more effective inactivators of chloramphenicol than the resistant organisms developed by growth in progressively increasing concentrations of the drug. A direct correlation was observed between the gain and loss of resistance *in vitro* and the increase and decrease of chloramphenicol inactivation in *E. coli* strain 60.

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Determination of Thickness of Walls of Hard Gelatin Capsules by Radioisotopic Means

By GARNET E. PECK*, JOHN E. CHRISTIAN, and GILBERT S. BANKER

A procedure has been developed for the determination of the thickness of the wall sections of hard gelatin capsules. A chlorine-36 source was mounted in the tip of a stainless steel capsule dipping pin and was used to observe the differences in thickness of the wall sections. Twelve series (replications) of 25 determinations each were performed. The measurements taken included the weight, area, calculated weight per unit area, thickness in inches using a micrometer, and beta count rate. The count rates were plotted against the corresponding weight per unit area (mg./cm.^{2}) and thickness (in.). Regression curves were prepared using least squares calculations. A *t* test was used to compare the individual slopes of the regression curves with the mean slope. The applicability of this method of determining the thickness of wall sections of hard gelatin capsules is discussed.

THE FORMATION of hard gelatin capsules involves the use of stainless steel pins which are dipped into a liquid gelatin medium, after which the formed capsules are slowly dried. Not until the drying process has been completed is gauging of the wall sections currently possible. If the thickness of the drying capsules could be gauged early in the drying process, it would be possible to adjust the process more quickly should it produce capsules which were not within control limits. This would result in a saving of time and less loss of product. Furthermore, an isotopic method of evaluating capsule thickness as an in-process control could conceivably result in an automated line operation.

Some of the more important reported uses of radioisotopes in industry apply the principles of penetration and absorption of the isotopic radiations (1). These principles are generally applied to the area of thickness gauging. Those radioisotopes which emit beta particles are most frequently used. Since they are less penetrating than gamma rays, they may be used for measuring the thickness of thin films, paper, and plastic materials (2). One type of gauging system frequently employed is based on the principle of the change of transmission of beta particles through the medium (3). To measure very thin, low density materials—such as paper—carbon-14, a weak beta emitter, has been used (4). Other beta sources that have been used include chlorine-36, krypton-85, strontium-90, and thallium-204 (5–7). Iodine-131 was used to measure the thickness of thin films in the vicinity of 1 μ (8).

The purpose of this study was to select and evaluate a radioisotope to be used for the thickness gauging of wall sections of hard gelatin capsules based on the principle of beta particle absorption.

EXPERIMENTAL

The usual method of expressing the thickness of thin materials by radioisotopic means is in terms of weight per unit area (mg./cm.^{2}). From a study of No. 000 hard gelatin capsules, it was found that the average weight per unit area of capsule sections was 14.12 mg./cm.^{2}. Small pinpoint sources of two pure

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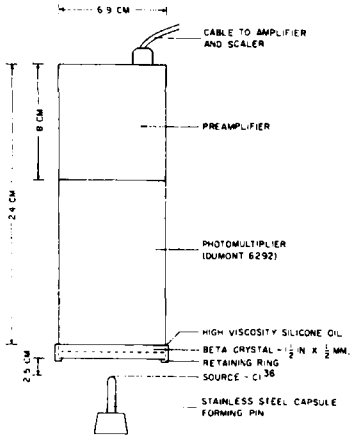

Fig. 1.—Beta detection system and chlorine-36 source.

beta particle sources (phosphorus-32 and chlorine-36) were observed using a series of aluminum absorbers of varying thicknesses expressed in mg./cm.^{2}. An aluminum absorber with a thickness of 13.3 mg./cm.^{2} was the closest available to the thickness of the gelatin sections measured. This aluminum absorber reduced the count rate of the chlorine-36 sample by about 30% compared to the sample counted with no absorber. Since the change in count rate was 19,000 c.p.m., it was thought that adequate sensitivity of detection of small differences in thickness might be achieved with the isotope. The densities of aluminum and gelatin are approximately equal; the change in count rate with gelatin sections with thickness was expected to be of a similar magnitude. The count rate of the phosphorus-32 sample was not appreciably diminished by the same aluminum absorber.

Preparation of Radioactive Pin.—A stainless steel capsule dipping pin, obtained from a producer of hard gelatin capsules, was used to contain the source. A cavity was drilled into the tip of a pin $^7/_{32}$ in. in diameter and $^1/_8$ in. deep. Since chlorine-36 is normally supplied as radioactive HCl, it was necessary to prepare a suitable insoluble salt of the acid. A 4.0% aqueous solution of mercurous nitrate containing 1.0% nitric acid was prepared. The labeled HCl was added to this solution. The mercurous chloride precipitate that formed was heated on a steam bath for 1 hour. The supernatant liquid was then tested for completeness of precipitation, and the volume was reduced to about 1.0 ml. The remainder of the liquid was allowed to evaporate at room temperature, resulting in large crystals of labeled mercurous chloride. The radioactive material was mounted in the tip cavity of the pin and was sealed in place with label glaze.[1]

Gauging System.—The normal thickness gauging system consists of a source, a detector, and an amplifying and recording system (4). The system used in this study may be divided into these parts as: source, chlorine-36 with an average count rate of 73,000 c.p.m. with the detector 2.5 cm. from the source; detector, anthracene crystal with a suitable photomultiplier and preamplifier (see Fig. 1); and amplifying and recording system, (*a*) RIDL model 115 pulse height analyzer,[2] and (*b*) RIDL model 200T scaler.[2]

Since the model 200T scaler was not equipped with a built-in amplifier, it was necessary to use the amplifier section of a pulse height analyzer. This recording system was used for all measurements. In order to observe the measurements visually, an auxiliary system was also used which consisted of a RIDL model 39-1 spectrum scanner-ratemeter[2] and a Minneapolis-Honeywell 0 to 50-mv. recorder.[3] Both recording systems could be operated simultaneously.

Sample Preparation and Measurement.—The gelatin sections used in this study were taken from No. 000 hard gelatin capsules manufactured by Eli Lilly and Co. This size was chosen because of the large sections that were obtained when the capsules were cut into rectangular sections. The capsules were handled with rubber gloves during the cutting and trimming operation. After the sections were cut from the walls of the capsules, they were trimmed so that the corners were right angles. The sections were then weighed and measured with vernier calipers. The weight per unit area of each section was calculated from these data. The sections were placed over the radioactive source and a 3-minute count taken. A 1-minute count was also taken to observe differences in counting time *versus* count rate. After these measurements were made, a hand micrometer was used to measure the thickness of the sections to the nearest 0.0001 in. Twenty-five sections were measured and represented one series. The capsules used in this study contained 7.001% moisture as determined by Karl Fischer titration of 1-Gm. samples.

Table I.—Thickness and Weight per Unit Area Measurements of Gelatin Sections, Series 10

Sample	Wt., mg.	Area, cm.^{2}	Wt./Unit Area, mg./cm.^{2}	Thickness, in.	Beta Count,[a] c.p.m.
1	38.7	2.654	14.243	0.0043	54,500
2	35.8	2.624	13.643	0.0039	56,091
3	34.1	2.448	13.930	0.0042	55,061
4	42.7	2.752	15.516	0.0047	52,612
5	38.0	2.614	14.537	0.0045	53,093
6	37.8	2.639	14.324	0.0043	54,122
7	39.1	2.827	13.831	0.0045	53,260
8	39.1	2.862	13.662	0.0040	56,124
9	39.8	2.995	13.289	0.0042	54,682
10	36.0	2.576	13.975	0.0043	54,070
11	37.5	2.719	13.792	0.0043	54,528
12	39.1	2.690	14.535	0.0043	54,290
13	37.8	2.738	13.806	0.0040	55,416
14	40.7	2.794	14.567	0.0042	54,782
15	46.3	3.181	14.555	0.0045	53,843
16	38.6	2.608	14.801	0.0044	54,166
17	40.2	2.907	13.829	0.0040	55,242
18	37.9	2.924	12.962	0.0039	56,373
19	38.0	2.723	13.955	0.0043	54,605
20	39.1	2.608	14.992	0.0043	53,757
21	36.0	2.693	13.368	0.0041	54,522
22	34.2	2.500	13.680	0.0040	55,548
23	39.1	2.818	13.875	0.0044	54,125
24	38.7	2.854	13.560	0.0040	55,171
25	39.6	2.884	13.731	0.0040	55,108

[a] One 3-minute count was taken for each.

[1] Fisher Scientific Co., Chicago, Ill.

[2] Radiation Instrument Development Lab., Northlake, Ill.

[3] Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.

TABLE II.—EVALUATION OF GELATIN THICKNESS MEASUREMENTS (COUNTS PER MINUTE *versus* WEIGHT PER UNIT AREA)

Series	$\bar{X}$, mg./cm.²	$\bar{Y}$, c.p.m.	Slope, c.p.m./mg./cm.²	Intercept, c.p.m.	σ_x[a]	s[b]	t test[c] for Slopes
1	14.3982	53,759.84	1,177.452	70,712.95	0.90427	319.957	−0.65253
2	14.2453	53,810.92	1,149.247	70,182.02	0.72794	454.216	−0.14401
3	14.3576	53,827.40	1,497.256	75,324.60	0.91856	362.708	−4.63426
4	14.2525	53,999.36	1,117.174	69,922.08	0.72984	392.863	0.13098
5	14.2006	55,250.00	1,066.583	70,396.12	0.80076	549.981	0.47095
6	14.1034	54,601.88	1,224.573	71,872.52	0.90114	489.658	−0.85850
7	14.2530	54,798.08	1,129.886	70,902.35	0.80935	751.861	0.00748
8	14.3250	54,653.40	1,004.431	69,041.87	0.59880	401.806	0.94516
9	14.0901	55,087.00	1,007.208	69,278.68	0.64103	521.677	0.76226
10	14.0383	54,603.64	1,126.230	70,414.00	0.56601	674.186	0.02118
11	14.0035	55,000.68	1,091.325	70,283.05	0.77976	451.454	0.34501
12	13.9992	55,152.84	983.939	68,927.20	0.80347	325.252	1.81982

[a] Standard deviation of X. [b] Standard error of estimate for the universe. [c] Critical region for t test: ± 2.069 for $\alpha = 0.05$ and $n - 2$ degrees of freedom.

RESULTS AND ANALYSIS

Using gelatin sections cut from the walls of the No. 000 hard gelatin capsules, 12 series (replications) of determinations were made, each series involving measurements of 25 different gelatin sections. The measurements taken on each of the 300 sections included weight of the section, area, calculated weight per unit area (mg./cm.²), thickness using a micrometer, and a 3-minute count of the section over the radioactive source. The average count rate for the beta source was 73,288 c.p.m. in air. Representative data as exemplified by series 10 are shown in Table I. With the least squares method, two separate regression curves were determined for each of the 12 series, one in which X (measured thickness) was in mg. per cm.², and the other in which X was in inches (Y in both cases was the corresponding beta count of the section). The necessary calculated values are summarized in Tables II and III. The regression curves of Series 1, 2, 3, and 4 are shown in Figs. 2 and 3. It should be noted that the individual points are not shown because of the great degree of overlapping that would result in showing 100 close packed points. For this reason Tables II and III also include the calculated intercepts for verification of the regression curves.

Nuclear Method Based on Weight/Unit Area.— In Table II the slopes of the various regression curves were summed; the mean slope was determined to be 1,131.275 c.p.m./mg./cm.². The standard deviation of the slopes was calculated using the equation (9)

$$\sigma_x = \sqrt{\frac{n\Sigma X^2 - (\Sigma X)^2}{n}} \qquad \text{(Eq. 1)}$$

and was 130.480 c.p.m./mg./cm.². Only the slopes of Series 3 and 12 did not fall within one standard deviation of the mean slope. A hypothesis was proposed to test the calculated slopes against the mean of these slopes. To test the hypothesis that each slope was either equal or not equal to the mean slope, the following equations were used along with the standard deviation of the X parameter of each series calculated using Eq. 1 (9)

Standard error estimate for the universe

$$s_{yx} = \sqrt{\frac{Y^2 - a\Sigma Y - b\Sigma XY}{n - 2}} \qquad \text{(Eq. 2)}$$

Hypothesis test for the slope

$$t_{n-2} = \frac{b - b_{\text{mean}}}{\sigma_x \sqrt{n}} \qquad \text{(Eq. 3)}$$

Only the slope for Series 3 could reject the hypothesis that the mean slope was not the true slope for an alpha error of 0.05.

Because of the number of measurements involved, it was felt that a composite curve could indicate the ability of the method to detect differences in gelatin thickness. Figure 4 represents the average slope and intercept for the 12 series. Using the mean slope, it would indicate that a change of 1 mg./cm.² would result in a change of 1,131 c.p.m. It must be remembered that these sections were not absolute standards, but samples prepared from market pack-

TABLE III.—EVALUATION OF GELATIN THICKNESS MEASUREMENTS (COUNTS PER MINUTE *versus* MICROMETER)

Series	$\bar{X}$, in.	$\bar{Y}$, c.p.m.	Slope, c.p.m./in.	Intercept, c.p.m.	σ_x[a]	s[b]	t test[c] for Slopes
1	0.004352	53,759.84	334.391[d]	68,312.68	0.000344	290.401	0.00703
2	0.004256	53,810.92	344.068	68,454.53	0.000255	334.217	1.22316
3	0.004300	53,827.40	447.773	73,079.07	0.000309	381.407	−2.89425
4	0.004252	53,000.36	335.684	68,265.55	0.000252	726.896	0.70087
5	0.004180	55,250.00	434.167	73,398.16	0.000219	350.185	−1.81397
6	0.004192	54,601.88	451.118	73,512.73	0.000261	303.279	−3.21956
7	0.004276	54,798.08	356.267	70,032.04	0.000257	354.829	0.71996
8	0.004304	54,653.40	326.981	68,726.68	0.000207	243.802	2.08703
9	0.004152	55,087.00	371.160	70,497.56	0.000200	354.962	0.14162
10	0.004224	54,603.64	409.651	71,907.04	0.000208	357.236	−0.97618
11	0.004176	55,000.68	361.472	70,095.74	0.000252	293.540	0.63119
12	0.004156	55,152.84	341.440	69,343.10	0.000232	312.637	1.28712

[a] Standard deviation of X. [b] Standard error of estimate for the universe. [c] Critical region for t test: ± 2.069 for $\alpha = 0.05$ and $n - 2$ degrees of freedom. [d] Inches $\times 10^{-4}$.

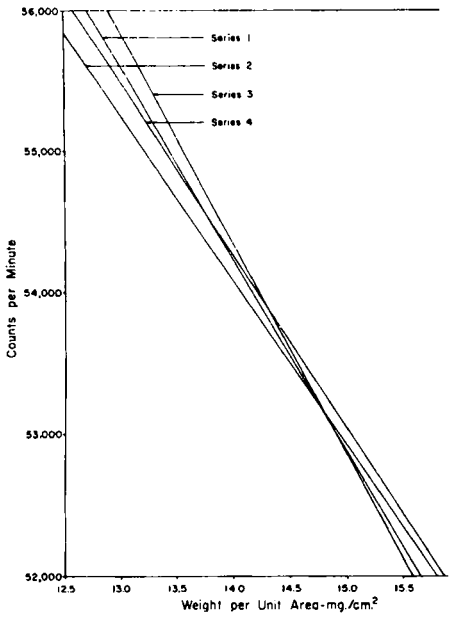

Fig. 2.—Beta thickness measurements of gelatin sections, X in mg./cm.^{2}; Series, 1, 2, 3, and 4.

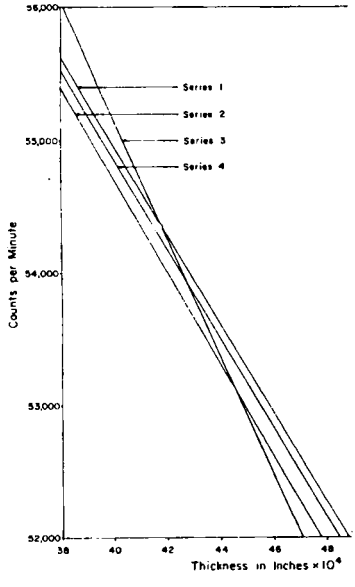

Fig. 3.—Beta thickness measurements of gelatin sections, X in inches; Series 1, 2, 3, and 4.

ages of gelatin capsules, and some degree of variation in thickness from one end of the section to the other end was periodically observed. This would affect the weight per unit area determinations, micrometer readings, and correlation to beta activity. Also, all measurements were not made under a controlled environment. At least a degree of the variation seen in the data may simply be attributed to sample variation as it exists in capsules marketed today.

Nuclear Method Based on Micrometer Thickness.—From Table III the slopes of the various regression curves were summed and the mean slope was 376.181 × 10^4 c.p.m./in. (376.181 c.p.m./1 × 10^{-4} in.). The standard deviation was calculated using Eq. 1 and was 44.635 × 10^4 c.p.m./in. It may be stated that the slopes from Series 3, 5, 6, and 8 did not fall within one standard deviation of the mean slope, but all slopes did fall within two standard deviations. The slopes were tested against the mean slope using the same t test mentioned in the previous section. The slopes that rejected the hypothesis that the mean slope was not the true slope were from Series 3, 6, and 8 for an alpha error of 0.05.

A composite curve was also prepared for this set of 12 series. Figure 5 is the result of the average of the

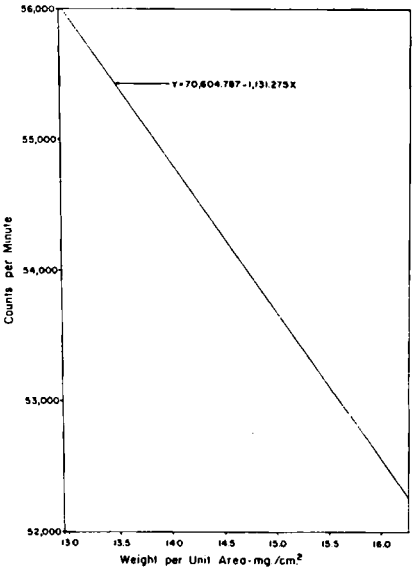

Fig. 4.—Beta thickness measurements of gelatin sections: mean of all series of count rate *versus* weight per unit area.

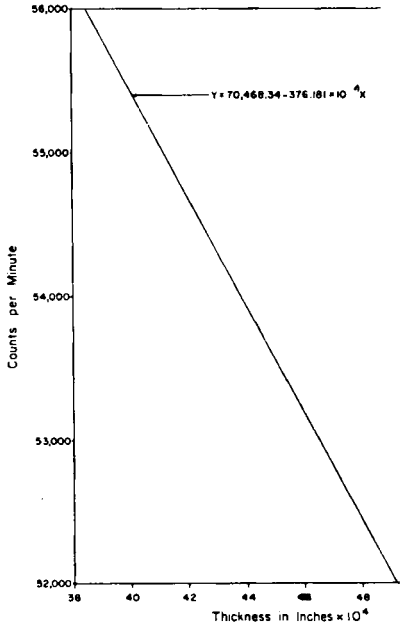

Fig. 5.—Beta thickness measurements of gelatin sections: mean of all series of count rate *versus* micrometer reading.

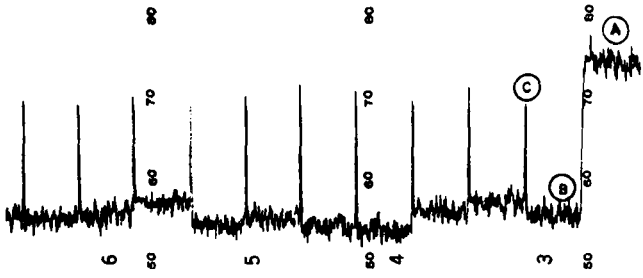

Fig. 6.— Visual recording of beta thickness measurements of gelatin sections, Series, 10. Key: A, source count rate with air; B, count rate with gelatin section over source; and C, sample being changed.

slopes and intercepts. One difficulty in the use of a hand micrometer is that the degree of pressure applied to the gelatin section may vary from measurement to measurement and from day to day. In addition, the micrometer reflects the thickness at only one point on a place surface, which would almost certainly not be the area on the surface measured by the collimated beta beam. However, only Series 6 shown in Table III fell far outside the critical region of the t test.

Visual Observation of Beta Thickness Measurements.—During the normal operation of the radioisotope counting equipment measurements were also recorded on chart paper using a 50-mv. recorder. Figure 6 is an example of a simultaneous measurement obtained with the auxiliary recording equipment of capsule thicknesses of Series 10. This clearly demonstrates the ability of the system to detect differences in thickness of the gelatin sections. Figure 6 also illustrates the possible variation in slopes from one series to the next because of the wide band of counting fluctuation. Although the counting times shown are 4 minutes per sample, a faster recorder speed could be used which would plot the thicknesses easily for shorter counting times. The speed of the recorder was 45 in. per hour.

DISCUSSION

The difficulties in obtaining the standard curves for the beta thickness gauging include the degree of scattering observed within each series of measurements and the lack of perfect reproduction of the slopes of each regression curve between series. The sources of error that may have contributed to the scattering are: (*a*) the small size of the sample which could cause errors in weighing and area determination of the section; (*b*) the variation in pressure when using the hand micrometer from one series to another; (*c*) that all measurements were not conducted in the same working area; and (*d*) slight variations in thickness from one end of the gelatin section to the other. Even though these sources of error were present, it is felt that once a system is in continuous operation many more observations could be made in a short period of time to prove the effectiveness of the method. The visual observation of the measurements through the use of a recorder clearly indicated that differences in the thickness of the gelatin sections could be observed.

The advantages of the thickness gauging of gelatin sections by the use of a beta source of radioactivity are: (*a*) nondestructive testing of a hard gelatin capsule; (*b*) in-process measurement of gelatin capsules or gelatin sheets when it is not possible by other means, resulting in quicker process adjustment should the process be out of control; (*c*) more frequent measurement of a sample with less importance placed on human manipulations of instruments such as a hand micrometer; and (*d*) the in-process beta gauging thickness testing technique might be incorporated into a system of automatic process control. The radioactive source containing pin used in this study could also be used in the routine laboratory testing of thickness of hard gelatin capsules as well as for in-process control. This pin method would be ideal for the thickness measurement of the capsule tips and the capsule walls.

The procedures outlined in this paper could also be applied to the development of isotope methods (isotope selection and procedures design) for thickness determinations of other pharmaceutical materials, such as bandage materials and assorted pharmaceutical films.

CONCLUSIONS

It was possible to detect differences in the thickness of wall sections of hard gelatin capsules by beta absorption measurements from 12 series of 25 measurements each when the measurements are based on weight per unit area or thickness in inches *versus* beta count.

In the measurements based on the weight per unit area, a change of 1 mg./cm.^{2} was indicated by an average change in beta count of 1,131 c.p.m.

A change of 0.0001 inch was indicated by an average change in beta count rate of 376 c.p.m. in the measurements based on thickness in inches.

The ability to detect differences in thickness of the wall sections of hard gelatin capsules was clearly shown by a simultaneous visual observation with a recorder.

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