

either through the pores or through the lipoprotein membrane, or the base may coat the mucous membrane to delay or minimize absorption.

In the studies to follow, the effect of suppository base and physicochemical characteristics of the drug on absorption from the rectum will be explored.

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## Technical Articles

### Count Loss with the Coulter Counter

By JOSEPH C. SAMYN and J. PATRICK MCGEE

Count loss was shown to occur in samples meeting the suggested size specifications of the Coulter counter. This count loss was related to the size and frequency of the larger particles in the sample. The voltages generated in the process were considered. Amplifier blocking of the smaller voltage pulses due to the increased fall time of the larger voltages appeared to be the explanation for the count loss. Accurate frequency counts for the smaller particles necessitate the removal of the larger particles from the sample. It was calculated that the 100- $\mu$  aperture can accurately measure down to 2  $\mu$ , the suggested lower limit, only in the absence of particles larger than 10  $\mu$ . The two Coulter counters used in this study differed in their count loss behavior. This difference suggests that the performance of each counter is apt to change with age, use, etc. The appearance of the oscilloscope pattern was also noted in these studies. In many instances count loss occurred when the oscilloscope pattern was judged to be satisfactory. The importance of the count loss was considered for an oil/water emulsion. The possible error is largest when the frequency data are used directly, as in a dissolution study.

THE COULTER counter<sup>1</sup> is widely used for particle counting. Its basic operation and

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<sup>1</sup> Distributed by Counter Electronics Industrial Division, Chicago, Ill.

many of its specific uses have been described. This paper discusses count loss which can occur in the use of the Coulter counter due to the larger particles in the sample.

The particles to be measured are supposed to be between 2 and 40% of the aperture diameter

for satisfactory counting. A recent instruction manual (1) states, with respect to the large particles: "The particle size range of the calibration suspension is such that all of the particulate mass is accounted for by the largest count taken and the largest particles (near 0.5 aperture diameter) are relatively few." Aperture clogging seems to be the reason for this statement. A tenting or shielding of the small particles by the large particles was mentioned at a recent meeting (2). The relative sizes of these large and small particles were not defined. The importance of this count loss was not made clear. The authors have also observed count loss of this type with some of their calibration samples.

This study investigates the count loss occurring in samples containing particles in the 2-40% size range as suggested in the operator's manual. It is shown that the particle size at which count loss is first noted is directly related to the size of the larger particles in the samples. Two instruments and two apertures are used in this study. The voltage pulses resulting from particle passages are calculated in order to understand the nature of the count loss better. A dilution procedure illustrates the count loss in a typical oil/water emulsion. A discussion of the importance of this decreased count completes the study.

## EXPERIMENTAL

**Apparatus.**—Two model A industrial Coulter counters were used in this study. Instrument I had recently been updated by Coulter Electronics and was considered in excellent condition at the time of this study (3). Instrument II was obtained on loan.<sup>2</sup>

The apertures and sample volumes are listed with the frequency data in each table. A 0.9% NaCl-0.1% Daxad<sup>3</sup> 11 solution was the diluting solution for the standard samples. Polysorbate 80,<sup>4</sup> 0.1% was used in place of the Daxad for the emulsion study. All solutions were filtered through a 0.5  $\mu$  Reeve-Angel<sup>5</sup> and 0.45  $\mu$  Millipore<sup>6</sup> filters.

**Samples.**—Giant ragweed pollen ( $\approx 19 \mu$  average) and glass beads<sup>7</sup> No. 380 ( $\approx 30 \mu$  average) were used as the standard samples. The oil/water emulsion used was prepared as follows: 100 ml. of mineral oil U.S.P. and 300 ml. of a 6.67% polysorbate 80-water solution were mixed for 10 min. in a Waring blender with cooling. Two milliliters of this emulsion was diluted with the 0.9% NaCl-0.1% polysorbate 80 vehicle to 1 L. From this sample 15-, 10-, 5-, and 2-ml. samples were diluted to 200 ml. with additional diluting fluid at the time of counting. This procedure is the same as in a recent publication

(4) except for the cooling during preparation. An emulsion of finer globule size is obtained without the cooling.

The frequency data in Tables I through VI were not corrected for background or coincidence. Both corrections were small and did not affect the data. The data on the emulsion (Table VIII) are corrected values. Where more than one count is listed for each  $t'/I$  value, it is a duplicate count on the same container of suspension.

Frequency counts in all cases were kept below the maximum allowed by a 10% coincidence correction. In the case of the standard samples only the more dilute systems giving relatively low counts have been reported. The count loss is readily apparent in these data, even though the values are well below the permitted count (based on coincidence). The higher frequency values on the standard samples gave as large or larger count losses.

Diameter values corresponding to the  $t'/I$  values have been included in the tables where they aid in understanding the tables or the text.

## RESULTS AND DISCUSSION

Count fall-off is not usually noted in most samples because of the large number of fine particles present. For this reason, known samples were used in the first part of this study. The samples used have a maximum frequency count within the instrument sensitivities.

**Instrument I.**—The data in Table I demonstrate the counting pattern for a known standard. Counts increase, plateau, and then decrease with increased instrument sensitivity (proceeding down the table to the finer particle sizes). Table II presents similar data for a larger sized sample (30- $\mu$  glass beads). Count loss begins at about 5  $\mu$  ( $t'/I = 10/5$ ) in Table I and at about 8  $\mu$  ( $t'/I = 10/3$ ) in Table II. Count loss is not observed for a sample composed of finer particles with this instrument combination. Consequently, it is seen that the loss in count begins at a larger size when the particles being analyzed are larger. In both cases cited the count loss begins at a size considerably larger than the suggested 2% lower limit.

The data in Table III were obtained with a larger aperture and instrument I on pollen and glass beads. Several points were brought out by this experiment. First, count loss was not re-

TABLE I.—GIANT RAGWEED POLLEN—INSTRUMENT I

Gain = 5; 500 $\mu$ l. vol., 100- $\mu$ aperture		
$d$ ( $\mu$ )	$t'/I$	Freq. Count
29	100/1	33, 26
24	60/1	94, 97
21	40/1	377, 361
	30/1	745, 712
17	28/1	1512, 1492
	24/1	4958, 4915
13	20/1	7308, 7474
11	10/1	7582, 7588
8.4	/2	7485, 7475
	/3	7573, 7359
6.7	/4	7283, 7215
5.3	/5	6558, 6373
4.3	/6	5278, 5055
3.4	/7	3695, 4041

<sup>2</sup> Obtained from Basic Service Corp., Detroit Mich.

<sup>3</sup> Obtained from Dewey and Almy Chemical Corp., Cambridge, Mass.

<sup>4</sup> Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

<sup>5</sup> Obtained from H. Reeve Angel & Co. Inc., Clifton, N. J.

<sup>6</sup> Obtained from Millipore Filter Corp., Bedford, Mass.

<sup>7</sup> Obtained from Minnesota Mining & Manufacturing Co., Reflective Products Division, St. Paul, Minn.

TABLE II.—GLASS BEADS NO. 380—INSTRUMENT I

Gain = variable as indicated; 500 $\mu$ l., vol., 100- $\mu$ aperture		
$d$ ( $\mu$ )	$G/t'/I$	Freq. Counts
	3/100/1	64, 80
	3/60/1	395, 418
	4/80/1	1070, 970
	5/100/1	1846, 2012
	5/80/1	2366, 2422
	60/1	2706, 2837
	40/1	2815, 2964
17	20/1	2983, 3018 (max.)
	10/1	2827, 2999
11	10/2	2713, 2810
8.4	10/3	2289, 2360
6.7	10/4	1814, 2032
	10/5	1369, 1487
	10/6	1586, 1861
	10/7	1460, 1466

TABLE III.—RAGWEED POLLEN AND GLASS BEADS—INSTRUMENT I

200- $\mu$ aperture, gain = 5; 500 $\mu$ l. vol.			
$d$ ( $\mu$ )	$t'/I$	Ragweed Pollen	Glass Beads
82	100/1	0	0
...	20/1	...	24, 31
38	10/1	2, 8	278, 252
30	/2	20, 16	645, 655
24	/3	161, 124	775, 846
19	/4	1661, 1540	812, 842
15	/5	2031, 2043	781, 809
12	/6	2111, 2052	650, 640
9.7	/7	1975, 2033	498, <sup>a</sup> 466
7.9	/8	1957, 1904	341, <sup>a</sup> 365
6.6	/9	1816, 1741	360, <sup>a</sup> 424
5.7	/10	1601, 1609	464, <sup>a</sup> 399

<sup>a</sup> Poor oscilloscope pattern.

stricted to a particular aperture. Second, count loss occurred prior to an oscilloscope pattern that was judged to be poor. For the pollen sample, substantial count loss occurred although the oscilloscope pattern was considered satisfactory throughout the study. Therefore, the observance of the oscilloscope pattern alone does not prevent the collection of poor data.

**Instrument II.**—The data in Tables IV, V, and VI were obtained with instrument II. Table IV shows no loss-in-count through  $t'/I = 10/7$ . This contrasts with the data in Table I for instrument I on the pollen sample. The same plateau ( $t'/I = 40/1$  to  $10/2$ ) is observed for the glass bead sample for the two instruments (Tables II and V). However, instead of a count loss, instrument II shows an increase in count at the higher sensitivities, presumably due to electrical noise. The data in Table VI were obtained with instrument II using the 200  $\mu$  aperture. Count loss was not observed with the pollen sample. This contrasts with the data in Table III for instrument I. Instrument II did show count loss with the combined pollen-glass bead sample listed in Table VI.

Generally, count loss was not observed so clearly with instrument II as with instrument I. A difference in noise level between the two units could be the explanation. Instrument I was considered to be the superior unit based on the recent updating

and correspondence (3). Based on the above studies, differences between counters suggest that a given counter could vary in its performance with age, time, use, etc.

**Voltage Pulses Developed.**—A better understanding of the cause of the count loss is gained through a consideration of the voltages being measured. These voltage pulses are generated by the particles

TABLE IV.—GIANT RAGWEED POLLEN—INSTRUMENT II

Gain = 6, 500 $\mu$ l. vol., 100- $\mu$ aperture		
$d$ ( $\mu$ )	$t'/I$	Freq. Count
	100/1	24, 23
	60/1	99, 95
	40/1	656, 539
	32/1	1059, 947
	30/1	1797, 1708
	28/1	3434, 3357
	24/1	8257, 8196
	20/1	10,776, 10,842
	10/1	11,015, 11,222
	/2	11,108, 11,038
	/3	11,078, 11,174
7.0	/4	11,242, 11,123
5.6	/5	11,286, 11,281
	/6	11,270, 11,218
3.6	/7	11,316, 11,132
	/8	13,866, 12,699
	/9	11,118, 11,357
	/10	14,196, 15,429

TABLE V.—GLASS BEADS NO. 380—INSTRUMENT II

Gain = 6, 500 $\mu$ l. vol., 100- $\mu$ aperture		
$t'/I$	Freq. Count	
100/1	511, 473	
60/	673, 688	
40/	746, 751	
20/	766, 754	
10/1	749, 807	
/2	782, 738	
/3	778, 819	
/4	773, 826	
/5	894, 794	
/6	1236, 1258	
/7	1407, 1696	
/8	1024, 1203	
/9	928, 1103	
10/10	886, 1299	

TABLE VI.—RAGWEED POLLEN AND GLASS BEADS—INSTRUMENT II

Gain = 6, 500 $\mu$ l. vol., 200- $\mu$ aperture		
$t'/I$	Freq. Counts	
	Ragweed Pollen	Ragweed and Glass Beads
100/1	1	
20/1	...	63
10/1	...	288, 333
10/2	9	837, 849
/3	168, 176	1188, 1211
/4	2521, 2576	3267, 3334
/5	3016, 3052	4030, 3030
/6	3174, 3151	4052, 4039
/7	3090, 3132	3975, 3974
/8	3147, 3067	3829, <sup>a</sup> 3930
/9	3123, 3077	3713, <sup>a</sup> 3688
/10	3054, 3155	3390, <sup>a</sup> 3503

<sup>a</sup> Oscilloscope pattern poor.

as they pass through the aperture. The magnitude of the voltage pulses depends primarily on the aperture currents applied and the sizes of the particles.

The aperture currents may be calculated by knowing the aperture size, the aperture settings, and the conductance of the vehicle. The various aperture current settings correspond to different precision resistors in series with the aperture and the constant 300-v. source. The following equation can be used for calculation at various aperture current settings:

$$I = \frac{300}{R_{ACS} + R_E}$$

where  $R_{ACS}$  is the resistance value listed in the electrical schematic for each particular aperture current setting, and  $R_E$  is the measured resistance across the electrodes.

The calculated  $I$  values for the various aperture current settings are not altered significantly by the  $\Delta R$  caused by the particle passage.

The particles cause a change in electrical resistance depending upon their size. The magnitude of this resistance change may be calculated by the following relationship (1, 5):

$$\Delta R = \frac{4d^3}{1.5D^4} P_o(1 - P_o/P)$$

where  $P_o$  is the resistivity of the continuous fluid,  $P$  is the resistivity of the particle,  $d$  is the diameter of the particle,  $D$  is the diameter of the aperture.

Usually  $P_o/P \ll 1$  and may be neglected since  $P$  is of the order of  $10^4$  for nonmetallic particles. Then, with  $P_o$  equal to 62.4 ohm-cm. for 0.9% saline, this equation reduces to:

$$\Delta R = (53.0) d^3/D^4$$

Finally, the product of the resistance change and the appropriate current flow result in the voltage pulse.

As an example of the above calculations, Table VII was constructed based on instrument I and the 100- $\mu$  aperture. The diameters of various particles are listed in column 1. The 40- and 30- $\mu$  particles correspond to the estimated larger and average particles in the glass bead sample. Similarly, the 25- and 20- $\mu$  correspond to the larger and

average particles in the pollen sample. The remaining diameters correspond to the particle sizes being detected at the particular  $t'/I$  values listed. The  $\Delta R$  values calculated for these particles are listed in column 2. The threshold/aperture current settings and the calculated current values are listed horizontally across the top of the table.

The  $\Delta E$  values form the body of the table. Diagonally across the table are a series of 0.15-mv. values. These values correspond to the lowest voltage pulse being detected at each successive  $t'/I$  value. Within each column are the  $\Delta E$  values for some of the larger particles at the same  $t'/I$  setting. Note *Footnotes a* and *b* by the  $\Delta E$  values in the columns headed by  $t'/I = 10/3$  and  $10/5$ . These correspond to the voltage pulses for the larger particle, the average particle, and the particle size at the onset of count loss for the glass bead sample (Table II) and the giant ragweed pollen (Table I). In the  $t'/I = 10/3$  column, the ratios of the 16.0- and 6.7-mv. signals to the 0.15-mv. value are 107 and 45 to 1. Likewise, in the  $t'/I = 10/5$  column the ratios of the signals for the larger and average particle to the particle at the onset of count loss are 100 and 51 to 1. These ratios are in good agreement, although it is recognized that the choice of the "larger" particle is somewhat arbitrary.

It is known that satisfactory performance is obtained with a pulse amplifier when the pulses being monitored are within a limited voltage range and frequency. Blocking of the smaller pulses can occur due to the increased fall-time required for the larger voltages. The resultant loss in pulse count becomes more severe as the pulse ratio becomes greater and the pulses become more frequent.

In the above example, 0.15 mv. is the smallest pulse being detected. At the same time, the larger particles are generating increasingly larger voltages as the  $t'/I$  value is changed. Count loss becomes apparent as the ratio of these large to small pulses increases. Satisfactory data were obtained for the glass bead sample from 40 to 8  $\mu$  when using the 100- $\mu$  aperture. Accurate counts extended down to about 5  $\mu$  for the pollen sample. The suggested 2- $\mu$  lower limit for the 100- $\mu$  aperture could be realized if the larger particles did not exceed 10  $\mu$ . The ratio of voltage pulses for such a sample would be 100 to 1, the same as for the above samples. Thus, although it is true that the 100- $\mu$  aperture

TABLE VII.— $\Delta E$  VALUES FOR VARIOUS-SIZED PARTICLES AT DIFFERENT  $t'/I$  SETTINGS

Particle Diam., $\mu$	$\Delta R$ (ohms)	100- $\mu$ aperture; instrument I							
		$t'/I = 10/1^c$ 0.0117 <sup>d</sup>	10/2 <sup>d</sup> 0.0234 <sup>d</sup>	10/3 <sup>d</sup> 0.0467 <sup>d</sup>	10/4 <sup>d</sup> 0.0928 <sup>d</sup>	10/5 <sup>d</sup> 0.184 <sup>d</sup>	10/6 <sup>d</sup> 0.362 <sup>d</sup>	10/7 <sup>d</sup> 0.700 <sup>d</sup>	10/8 <sup>d</sup> 1.31 <sup>d</sup>
40	340	4.0 <sup>e</sup>	8.0 <sup>e</sup>	16 <sup>a,e</sup>	32 <sup>e</sup>	62 <sup>e</sup>	120 <sup>e</sup>	240 <sup>e</sup>	450 <sup>e</sup>
30	143	1.7	3.3	6.7 <sup>a</sup>	13	26	52	100	190
25	83					15 <sup>b</sup>			
20	42	0.49	0.98	2.0	3.9	7.7 <sup>b</sup>	15	29	55
13	12	0.15							
11	6.4		0.15						
8.4	3.1			0.15 <sup>a</sup>					
6.7	1.6				0.15				
5.3	0.79					0.15 <sup>b</sup>			
4.3	0.42						0.15		
3.4	0.21							0.15	

<sup>a</sup> Based on data from Table II. <sup>b</sup> Based on data from Table I. <sup>c</sup>  $t'/I$  value. <sup>d</sup> Current in ma. <sup>e</sup>  $\Delta E$  in mv.

TABLE VIII.—MINERAL OIL/WATER EMULSION—  
INSTRUMENT I

G'/I	500 $\mu$ l. vol. 100- $\mu$ aperture			
	Dilutions			
	15 ml.	10 ml.	5 ml.	2 ml.
		Frequency	Counts <sup>a</sup>	
4/100/1	0	0	0	0
5/100/1	2	0	0	3
80/1	6	2	0	19
60/1	12	9	9	22
40/1	37	42	33	34
20/1	201	216	213	165
10/1	708	699	657	660
/2	1,533	1,464	1,425	1,530
/3	2,649	2,704	2,754	2,715
/4	4,458	4,369	4,563	4,882
/5	7,190	7,075	7,686	8,182
/6	10,916	10,798	12,093	13,485
/7	16,159	16,048	19,107	22,957
/8	22,314	22,668	28,479	35,902
/9	29,616	30,238	40,392	53,430
/10	36,014	37,054	51,321	70,215

<sup>a</sup> All data corrected to 15-ml. dilution.

can measure 40 and 2- $\mu$  particles, it appears that it cannot accurately measure a sample containing particles of both sizes.

**Oil/Water Emulsion.**—The mineral oil/water emulsion was selected for study because it provided a system of spherical particles, of good stability, and of negligible solubility. The method of preparation and dilution was described earlier. The data are shown in Table VIII. The data for the 15-ml. sample are corrected for coincidence. The 10-, 5-, and 2-ml. entries are coincidence corrected and multiplied by 3/2, 3, 15/2, respectively, to convert to the same level as the 15-ml. dilution.

The data recorded in the 15-ml. column appear to be satisfactory upon initial inspection. Count loss at the higher  $t'/I$  values is not observed. The particles counted are within the 2–40% size range for the aperture used and the maximum count does not exceed the count permitted by a 10% coincidence correction. A conversion of the frequency count to weight already accounts for more than 100% of the sample (another problem area). None of the above considerations would suggest that the counts are low.

Count loss is not directly observed at the higher  $t'/I$  settings at any of the dilutions. The large number of fine particles present in these emulsions obscures the count loss that is occurring. The standard samples possessed a frequency plateau in the counting range, and count loss relative to this value was readily evident. The loss in count for this emulsion is demonstrated by comparing the counts between dilutions. The counts are in good agreement through  $t'/I$  values of 10/4 for the various dilutions. The counts begin to differ at higher  $t'/I$  settings and are in large disagreement in the 10/7 to 10/10 region.

It is believed that the count loss displayed in this series is due to the same factor causing the count fall-off observed with the standard samples, *i.e.*, the shielding and subsequent loss in total count due to the presence of the excessively large voltage pulses being generated by the large particles in the sample. By diluting out the system, the frequency of these large pulses is reduced, and the degree of

interference with subsequent loss in count also is reduced. This thus permits a better measure of the fine end of the distribution.

**Importance of the Count Loss.**—The count loss due to the larger particles has been described for the standard samples and the oil/water emulsions. The subsequent use of this frequency data will, of course, determine the importance of this error. Several possible uses of the oil/water data will be considered.

For a particle size analysis, frequency data are often converted to a percentage based on weight. The 15- and 2-ml. samples in Table VIII were treated in this manner, assuming substantially all of the sample was counted at the highest  $t'/I$  settings. The results are plotted in Fig. 1 on normal probability paper. The average size by weight shifts from 14.6 to 13.6  $\mu$  on inclusion of the fine particles lost by amplifier blocking. This error of less than 10% is relatively insignificant. The error is quite analogous to that of the microscopic method of size analysis. In the microscopic method, the estimate of the finer particles is known to be low, but the conversion to weight per cent minimizes the error.

The use of the Coulter data directly to obtain a frequency average results in a slightly greater error. In the case of the oil/water emulsion, the frequency average changes from 3.8  $\mu$  for the 15-ml. sample to 3.2  $\mu$  for the 2-ml. sample, an error of about 20%. The error would have been greater except for the fact that the added particles are spread over a range of  $t'/I$  values rather than being more concentrated. Therefore, the nature of the count loss self-limits the error introduced.

In some instances, an estimate of the particles below the range of the Coulter counter is necessary. One method consists in measuring the difference between the sample weight added and the weight calculated from the frequency counts recorded.

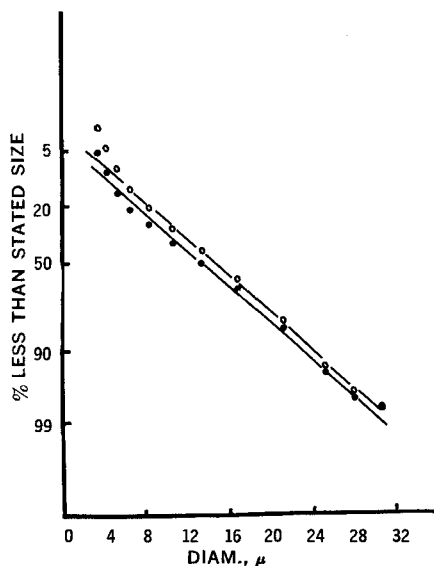


Fig. 1.—Probability vs. volume diameter for mineral oil/water emulsion; Key: O, 15-ml. dilution; ●, 2-ml. dilution. (Data calculated from Table VIII.)

In this procedure, the count loss would lead to a high estimate for the quantity of undersize particles.

Dissolution studies have been carried out with the Coulter counter. In these studies, frequency counts are usually obtained as a function of time at  $t'/I$  settings corresponding to the finer sizes. A large initial count is desired so that subsequent counts are possible as the dissolution proceeds. Larger particles are apt to be present in these systems unless specifically removed. The emulsion data in Table VIII can be used to demonstrate the frequency error, although initial data are seldom obtained in such experiments. The 15-ml. dilution would normally be used since the counts are near the maximum permitted by coincidence correction. At the higher  $t'/I$  settings, the counts are seen to be as much as 50% low when compared to the 2-ml. dilution. A concern with count loss due to the larger particles would appear to be quite important in this type of study. Removal of the large particles or monitoring at the larger sizes or at lower dilutions would seem to be desirable.

### CONCLUSIONS

Count loss has been demonstrated for samples meeting the generally stated size restrictions for the Coulter counter. This count loss was shown

to be a function of the larger particles present in the samples. The counting range for accurate frequency data is thus more restrictive than previously thought.

The two Coulter counters checked were found to differ in their performance. This difference suggests possible changes occurring for a single counter with age, time, use, etc. The appearance of the oscilloscope pattern was also noted in these studies. In many cases, count loss preceded a poor oscilloscope pattern.

The count loss appears to be of greatest importance where the frequency data are used directly, such as in a dissolution study. The error is least important in a size analysis where the data are converted to weight per cent.

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## Characterization of Tablet Colors Obtainable from Some Certified Dyes

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The ISCC-NBS method of designating color was employed to classify and describe the colors formed by varying the concentrations of several dyes and carbon black in compressed tablets. The use of centroid color charts gives the investigator a ready reference for colors already produced and classified. Eventually, after sufficient classification, a library of colors can be developed which aids in compounding of new colors and the understanding of color in the tridimensional sense.

**A**LACK of a suitable color description method exists throughout the pharmaceutical industry. Investigators will often construct a library of colors by saving samples of colored products that have been made in the laboratory. These samples are then used as references when a new color is to be developed. Anyone who has tried this method knows that it is not possible to build any permanence into such a group of samples, since the effects of aging cause eventual changes that render them useless. Furthermore, since the collection is merely a random

group without order, it is not possible to communicate effectively in terms of color nomenclature. A similar problem exists for suitable manufacturing standards of colored products. An initial production sample may be placed aside as a reference for future product comparison. This is a poor procedure since the product itself often has insufficient stability to serve as a reference standard. This initial standard then is often replaced by some future batch causing difficulty in judging color acceptability of a batch because color drift occurred. Second, the use of one batch as a color standard is not satisfactory, since the range of colors that can be produced as inherent color variation of the process is unknown.

Many attempts have been made to character-

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