Uniformity of Distribution of Cyanocobalamin in Tablet Formulations

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Abstract □ Some of the distribution characteristics of three commercially available, protected cyanocobalamin forms were demonstrated in each of two vitamin formulations of dissimilar particle size. The incorporation of ^{57}Co cyanocobalamin into each of these protected forms permitted rapid, precise measurements of the distribution on individual unit doses. Statistical analysis of the data confirmed the visual observations of improved distribution of the 1% gelatin product.

Keyphrases □ Cyanocobalamin-^{57}Co—formulation distribution □ Distribution characteristics—cyanocobalamin-^{57}Co in tablets □ Wet granulation, direct compression—cyanocobalamin-^{57}Co distribution comparison □ Scintillometry—analysis

Solids mixing has undergone a great deal of scrutiny during the past 20 years, with particular emphasis on rates and mechanisms of the mixing process and on equipment evaluation.

Reviews on these subjects have been made by Weidenbaum (1), Jameson (2), Valentin (3), and Scarlett (4). Few workers, however, have published on the conditions, factors, and properties of the particles themselves or on the particulate system, especially those factors which influence uniform distribution of the active ingredient within a batch and thus affect accurate dosage within each unit dose.

Train (5, 6), in his review articles, considers such factors as particle bed dilation, induced forces, particulate physical properties, and surface forces of particles and their effect on the results of a mixing operation.

He states that particle bed dilation and induced forces are necessary to permit movement of the components in all directions, thus preventing (*a*) dead zones within a system, and (*b*) movement of aggregates as compound entities. In addition, such physical properties as particle size, shape, and density of the various components are key factors in obtaining a uniform mix without segregation tendencies.

The presence of surface-active forces causes aggregation of particles which are difficult to disperse evenly throughout the other components during the normal mixing time.

Rippie *et al.* (7–9) have investigated the properties which contribute to the mixing and unmixing of particulate systems.

The constantly increasing number of high-potency, low-dosage drugs being developed emphasizes the necessity for obtaining proper distribution of particles within a batch.

One of the most potent products of this type in wide use today is cyanocobalamin (vitamin B-12), which is marketed in several protected forms so as to prevent instability of the cyanocobalamin in the presence of certain other active components or their degradation products. The quantity of protected vitamin B-12 necessary to provide the required potency-per-unit dose is extremely low and is usually in the range of 0.05 to 0.15% in relation to the total batch.

Table I—Mesh Distribution of Vitamin Formulations

Mesh	Wet Granulated, %	Dry Blend, %
On 16	4.8	—
On 20	13.4	—
On 30	11.2	0.6
On 40	10.3	5.4
On 60	20.6	15.8
On 80	3.9	12.1
On 100	7.6	7.6
On 140	13.3	8.0
On 200	10.0	11.6
On 325	3.7	6.9
Passes 325	1.2	32.0
	100.0	100.0

The purpose of this investigation is to compare some of the distribution properties of three commercially available, protected forms of vitamin B-12. These properties are examined in multiple vitamin formulations prepared by wet granulation and by direct compression methods.

Presently, there are three methods of assay available for vitamin B-12 in multicomponent tablets: microbiological (10), radiotracer (11), and cyanide measurement (12).

These methods, although valid, do not present a precise picture of the distribution of the vitamin in the final dosage, since the sample required is in excess of a single dose.

The tracer method requires a sample size sufficient to contain 200 to 400 mcg. of the vitamin, thus encompassing as many as 400 dosage units. The result, therefore, is an average potency-per-unit dose.

The microbiological procedure, although not as precise as the previous method, requires a minimum of five doses for proper sampling. Thus, as with the tracer

Table II—Blender Sampling

	Counts/min./mg.[a]					
	1% in Gelatin		1% Resinate		0.1% in Gelatin	
	WG[b]	DB[c]	WG	DB	WG	DB
Top right	15.67	12.11	20.02	13.82	21.12	12.42
Top left	16.91	11.98	20.09	13.05	22.16	12.45
Bottom	16.54	11.94	19.38	13.71	20.82	11.88

[a] Conversion based on sample weight. [b] Wet granulation. [c] Dry blend.

Table III—Distribution of Vitamin B-12 Forms in Wet Granulated Formulation

Counts Per Minute Per Milligram of Tablet Weight								
Beginning[a]			Middle[a]			End[a]		
1% Gelatin	1% Resinate	0.1% in Gel.	1% Gelatin	1% Resinate	0.1% in Gel.	1% Gelatin	1% Resinate	0.1% in Gel.
15.19	18.10	17.05	13.14	16.69	14.65	12.41	15.64	13.74
14.95	17.68	17.74	13.40	15.87	15.18	12.64	15.30	13.93
14.88	17.55	17.37	13.21	16.79	14.37	12.79	15.64	13.91
14.80	17.90	16.51	13.38	16.16	14.36	13.01	14.94	14.26
14.91	17.57	16.71	13.14	17.27	14.58	12.73	15.33	14.16
15.25	18.35	16.91	13.33	16.93	14.71	12.42	15.29	14.87
15.54	17.07	16.86	13.54	17.22	14.20	12.56	15.34	14.29
14.71	17.79	17.21	12.81	15.92	14.62	12.46	16.11	13.96
14.94	18.50	17.35	12.97	16.02	14.97	12.66	15.79	14.44
15.31	17.28	16.57	13.22	16.12	14.65	12.61	15.00	14.97
15.24	17.67	16.72	13.61	16.66	14.69	12.94	15.38	14.38
15.29	17.49	17.25	13.30	16.66	14.42	13.01	15.83	14.43
15.08	18.00	17.07	13.47	16.32	14.57	12.46	14.19	13.92
14.98	17.83	16.94	13.09	17.03	14.86	12.71	14.49	14.66
15.02	18.15	16.51	13.51	16.64	14.29	12.71	14.83	14.08
15.23	16.82	15.85	13.42	16.63	14.70	12.60	15.95	13.99
15.66	18.71	16.86	13.39	16.69	14.57	12.30	15.21	14.27
14.99	17.81	17.02	13.23	16.62	14.46	12.70	15.21	14.00
15.00	17.51	16.77	13.13	16.57	14.77	12.89	16.17	14.83
15.23	18.02	17.40	13.14	16.93	15.08	12.59	15.91	13.96

[a] Portion of compression cycle.

method, only an average potency-per-dosage unit is obtained.

The cyanide measurement method, while being one of the most sensitive chemical methods for cyanocobalamin, is not specific because other nonvitamin materials may also liberate trace amounts of cyanide.

Although the last two procedures could be adapted to single tablet assays, the time required for each determination is excessive.

For this study, the protected cyanocobalamin forms were radioactively labeled, permitting rapid, precise measurements of the vitamin B-12 content on a unit dose.

EXPERIMENTAL

Preparation of Radioactive Materials—Cyanocobalamin-^{57}Co solution, USP,[1] equivalent to 50 and 500 μc., respectively, of radioactivity was used in the preparation of the 0.1%[2] and 1%[3] cyanocobalamin in gelatin products by adding it to the solution prior to the drying process.

Commercially available 1% cyanocobalamin on resin[4] was slurried for 8 hr. with USP ^{57}Co cyanocobalamin solution, equivalent to 500 μc. of the radioactive compound, and dried overnight at 40° in a circulating air oven. The dried powder was passed through an 80-mesh standard sieve to break up any soft agglomerates which formed. Mesh analysis performed before and after labeling revealed that no change in particle size was evident.

Preparation of Basic Formulations—The distributive properties of the cyanocobalamin forms were studied in each of two vitamin formulations: (*a*) a granulated formula, prepared by wet granulation procedures, representing a formulation having diversified particle size; (*b*) a dry blend, directly compressible, representing a formulation of materials in the fine-particle-size range.

The mesh distribution of each of the formulations is shown in Table I.

Mixing Evaluation—The procedure for blending the labeled compounds varied only with respect to the premix step.

In the case of the granulation, 85 g. of finer-than-80-mesh material was removed by screening. The radioactive material was added to the fines, coarsely blended, passed through a 40-mesh screen, and reblended for 5 min. in a rotating bottle.

The premix for the direct compression formulation was prepared by coarsely blending the labeled material with 95 g. of the mixture containing vitamins and diluents normally added after a milling operation, passing this blend through a 30-mesh screen, and reblending for 5 min. in a rotating bottle.

Final blending of all formulations was accomplished by adding the premix to approximately half of the material in a Twin Shell blender,[5] blending for 5 min., adding the remainder of the formula, and blending an additional 10 min.

Samples equivalent to the theoretical tablet weight were then removed for counting from the top and bottom sections of the blender to determine the efficiency of the operation.

Each formulation was compressed on a Rotary B-2 tableting machine[6] at a maximum speed of 700 tablets per minute.

Once the theoretical tablet weight was attained, samples were removed from the beginning, middle, and end of the tableting run. Twenty tablets were selected at random from each of the three samples and individually weighed on a single-pan Mettler balance.

All radioactivity counts were made on a Model SC 530 gamma spectrometer.[7]

RESULTS AND DISCUSSION

The quantities of radioactivity were utilized in the preparation of the labeled forms to provide a minimum of 5000 counts per minute when 5 mcg., plus a 10% excess of cyanocobalamin, were incorporated per tablet.

The degree of mixedness of each of the formulations prior to compression, as shown in Table II, reflects satisfactory blending in all cases.

A radioactive count was performed on tablet samples before and after disintegration in water to ascertain counting efficiency in the dry state as opposed to the wet state. Since the radioactivity count on both samples varied by less than 1%, all future counts were made on intact tablets. Each sample counted in duplicate for 1 min. yielded 6000–7500 counts, with a 10-min. background count of 1000. The average count was corrected for background interference and converted to counts per minute per milligram of tablet weight to provide an accurate determination of the distribution of the vitamin B-12 form on a unit weight basis. The results are shown in Tables III and IV.

[1] Merck & Co., Inc., Rahway, N. J.
[2] 0.1% cyanocobalamin in gelatin, Merck & Co., Inc., Rahway, N. J.
[3] Stabicote, Merck & Co., Inc., Rahway, N. J.
[4] Stablets, Type I, Chas. Pfizer & Co., Inc., New York, N. Y.
[5] Patterson-Kelly, E. Stroudsburg, Pa.
[6] Stokes Division, Pennsalt Chemical Co., Warminster, Pa.
[7] Tracer-Lab, Division of Laboratory for Electronics, Inc., Waltham, Mass.

Table IV—Distribution of Vitamin B-12 Forms in Dry Blend Formulation

Counts Per Minute Per Milligram of Tablet Weight								
Beginning[a]			Middle[a]			End[a]		
1% Gelatin	1% Resinate	0.1% in Gel.	1% Gelatin	1% Resinate	0.1% in Gel.	1% Gelatin	1% Resinate	0.1% in Gel.
11.87	13.85	13.81	12.14	14.05	13.69	12.10	13.61	13.68
11.79	14.86	13.66	12.13	13.23	13.64	12.34	13.91	13.92
12.13	13.84	13.74	12.21	13.85	13.89	12.00	13.44	13.85
11.84	13.53	13.89	11.62	14.38	13.83	12.51	13.69	14.03
12.07	13.92	13.79	11.84	14.19	13.92	12.14	13.26	13.87
11.82	13.79	13.74	11.82	13.84	13.66	12.28	13.64	13.96
11.76	14.35	13.78	11.89	13.92	13.54	12.03	13.41	13.81
12.05	14.02	13.61	12.46	14.12	14.00	11.90	13.86	13.80
11.87	13.20	13.71	11.99	13.80	13.72	12.31	14.08	14.02
12.17	13.66	13.61	12.00	13.43	13.78	12.04	14.23	13.72
12.03	14.52	13.64	12.11	13.54	13.72	12.14	13.44	14.03
12.13	13.93	13.90	11.94	13.10	13.69	12.10	14.03	13.75
12.07	13.69	13.81	12.12	13.56	13.79	12.11	13.69	13.94
12.30	13.94	13.64	12.21	14.36	13.67	12.22	13.48	13.72
11.77	13.78	13.61	11.91	14.47	13.99	11.87	14.50	13.82
12.28	13.96	13.67	11.92	14.37	13.78	12.04	13.40	13.99
12.19	14.52	13.73	12.27	13.85	13.69	11.94	13.48	13.85
12.03	13.57	13.83	11.85	14.10	13.94	11.87	14.08	13.84
12.00	13.61	13.51	11.95	14.09	13.85	12.11	13.67	13.88
11.87	13.41	13.52	11.97	13.34	13.73	12.11	13.88	13.93

[a] Portion of compression cycle.

Table V—Variances by Formulation by Position in Batch

Position	1% Gelatin	1% Resinate	0.1% Gelatin
Wet Granulated Formulation			
Beginning	0.0573	0.2129	0.1709
Middle	0.0408	0.1653	0.0655
End	0.0398	0.2737	0.1275
Dry Blend Formulation			
Beginning	0.0287	0.1640	0.0127
Middle	0.0363	0.1637	0.0158
End	0.0270	0.1060	0.0120

The variances by formulation and position in batch were calculated from the data and are shown in Table V. The analysis of variance for each formulation was made on the logarithms of the individual cell variance and is shown in Table VI. Statistically significant differences in variation were exhibited between formulations. These differences in variability were of such high levels of significance that individual F ratios were computed for the three types of formulations. In all cases, the difference in variability between the 1% gelatin products and the 1% resin adsorbate was more statistically significant than that between the 1% and the 0.1% gelatin products.

The significantly lower variability of the 0.1% product, as compared to the 1% gelatin form in the dry mix formulation, may probably be attributed to a dilution effect caused by the tenfold increase in quantity required to obtain the same potency. This may also be true for the significantly lower variability in both formulations obtained for the 0.1% gelatin product, as compared to the 1% resinate. Table VII depicts the significance levels of the F ratios. These were computed from the ratios of the variances shown in Table V.

Table VI—Analysis-of-variance Calculations on the Logarithms of the Variances

Source of Variation	*DF*	*SS*	*MS*	*F*
Wet Granulated Formulation				
Position of batch	2	0.08139	0.04069	3.30
Formulation	2	0.68376	0.34188	25.65
Pos. X form.	4	0.04932	0.01233	
Total	8	0.81447		
Dry Blend Formulation				
Position of batch	2	0.03191	0.01595	7.03
Formulation	2	1.62227	0.81113	238.22
Pos. X form.	4	0.00911	0.00227	
Total	8	1.66329		

Table VII—Significance Levels of F Ratios

Position	1% Gelatin *vs.* 1% Resinate	1% Gelatin *vs.* 0.1% Gelatin	0.1% Gelatin *vs.* 1% Resinate
Wet Granulated Formulation			
Beginning	99.5%	97.5%	<75.0%
Middle	99.5%	90.0%	97.5%
End	99.9%	99.0%	95.0%
Dry Blend Formulation			
Beginning	99.9%	95.0%	99.9%
Middle	99.9%	95.0%	99.9%
End	99.5%	97.5%	99.9%

It is interesting to note that in comparing the actual variances for both 1% products, the individual position values for the resin product were about 4 to 7 times greater than those for the gelatin product.

SUMMARY AND CONCLUSIONS

Three commercially available protected vitamin B-12 products were labeled with ^{57}Co cyanocobalamin. Each was then blended with both a granulated and a dry mix vitamin formulation and compressed into tablets.

The distribution characteristics for each form were evaluated by measuring the radioactivity of individual tablets obtained from different portions of the compression run.

Statistical analysis of the data showed that in all cases, variations in ^{57}Co labeled cyanocobalamin content throughout both formulations were significantly lower for the 1% gelatin product than for the resin product, indicating that it distributes more uniformly in tablet formulations.

For the granulated formulation, the variation of the 1% gelatin form was also less than its 0.1% counterpart. For the dry blend, however, the variation in the 0.1% product was lower, although the difference was less significant than that found when the two 1% products were compared. A significantly lower variability was also evident in both formulations for the 0.1% gelatin product, as compared to the 1% resinate.

These latter results may possibly be attributed to the fact that the quantity requirement of the 0.1% product is 10 times that of the 1% material.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 31, 1968, from the *Product Development and Service Laboratories, Merck & Co., Inc., Rahway, NJ 07065*
Accepted for publication September 11, 1969.
The authors wish to acknowledge W. Walker, B. Marcus, and E. Eggimann for their assistance in the preparation of this paper.

NOTES

Metabolic Fate of 2,3,5-Triiodobenzoic Acid in Laying Hens

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Abstract □ The whole body retention, distribution, and metabolism of carboxyl-^{14}C-labeled 2,3,5-triiodobenzoic acid (TIBA*) were studied in five laying hens. A single oral dose of TIBA* showed a 22-hr. biological half-life. No organ concentration of TIBA* was noted. TIBA* and seven labeled metabolites were found in excreta collected 6 to 12 hr. after the dose, with TIBA* representing the major end product. TIBA* and four labeled metabolites were detected in excreta collected during the 78 to 90-hr. interval; the metabolites occurred in greater proportion than did TIBA* in this sample.

Keyphrases □ 2,3,5-Triiodobenzoic acid (TIBA), carboxyl-^{14}C-labeled—metabolic fate □ Distribution, metabolism, whole body retention—TIBA, carboxyl-^{14}C-labeled □ Metabolites—TIBA, carboxyl-^{14}C-labeled □ Thick-layer chromatography—separation, identification

Greer and Anderson (1) reported that treatment of soybean plants with 2,3,5-triiodobenzoic acid (TIBA) at the beginning of flowering resulted in an increased seed yield due to two major types of effects. Their studies showed that TIBA caused the plants to change from vegetative to reproductive development more rapidly and also caused morphological changes which permitted more efficient utilization of sunlight by the plants. Since a residue of TIBA remains in soybeans grown from treated plants (2), the potential environmental health hazard of TIBA should be investigated prior to the general usage of the compound in agriculture. For this reason, a study of the retention, distribution, and metabolism of TIBA in laying hens was of interest.

Ice *et al.* (3, 4) employed TIBA labeled with ^{131}I in Position 2 (TI*BA) for metabolism studies in rats and lactating animals. In both studies, a significant thyroid concentration of ^{131}I was noted, and metabolism by deiodination was indicated. In rats and in lactating animals, the whole body retention of TI*BA was characterized by a two-component system.

Ware and Barker (5) administered carboxyl-^{14}C-labeled TIBA (TIBA*) orally to rats and found TIBA* and/or its labeled metabolites in all organs analyzed. Excretion of the compound was primarily through the urine, in which both TIBA and 2,5-diiodobenzoic acid were detected.

The metabolism of TIBA by chickens was investigated by Barker *et al.* (6) who employed TIBA labeled with ^{125}I in Positions 3 and 5. They found that 90% of the orally administered radioactivity was excreted within 48 hr. and that TIBA and 2,5-diiodobenzoic acid were present in the excreta. The same investigators dosed chickens and pigs with unlabeled TIBA and detected, by using gas chromatography, the presence of TIBA, 2,5-diiodobenzoic acid, and 3,5-diiodobenzoic acid in the chicken brains and in the thyroids of both chickens and pigs.

EXPERIMENTAL

Administration of TIBA*—Carboxyl-^{14}C-labeled TIBA (TIBA*) was available from the work reported by Spitznagle (2) and was purified immediately prior to use in this study by a method similar to that reported by Jarboe (7). An ethanolic solution of the impure TIBA* was applied to thick-layer plates (1.0 mm.) of purified silica gel[1] which were then developed three times each (12 cm. per development) in petroleum ether (30 to 60° fraction)–propionic acid (10:1 v/v) (8). The silica containing the pure TIBA*, located on the chromatograms by autoradiography, was removed from the plates and extracted with anhydrous ethyl ether, using a continuous extraction apparatus. The ether was allowed to evaporate at room

[1] Adsorbosil-1 with 10% binder, Applied Science Laboratories, State College, Pa.