SUMMARY AND CONCLUSIONS

1. On the media defined, enzyme-hydrolyzed casein will inhibit the growth of germinated digitalis seeds.

2. On medium B with complex supplement, the auxins BTOA, 5 p.p.m.; 2,4-D, 2 p.p.m.; NAA, 5 p.p.m.; or appropriate auxin combinations, BTOA, 1 to 5 p.p.m., with 2,4-D, 1 to 2 p.p.m., will cause digitalis seeds or roots to form callus tissues.

REFERENCES

(1) Gautheret, R. J., "La Culture des Tissus Végétaux," Masson et Cie, Editeurs, Paris, 1959.

(2) Audus, L. J., "Plant Growth Substances," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1959.

(3) White, P. R., "The Cultivation of Animal and Plant Cells," The Ronald Press Co., New York, N. Y., 1954.

(4) Nickell, L. G., and Tulecke, W., *J. Biochem. Microbiol. Technol. Eng.*, **2**, 287(1960).

(5) Steward, F. C., Mapes, M. O., and Smith, J., *Am J. Botany*, **45**, 693(1958).

(6) Bergmann, L., *J. Gen. Physiol.*, **43**, 841(1960).

(7) Klein, R. M., *Econ. Botany*, **14**, 286(1960).

(8) French, D. I., and Gibson, M. R., THIS JOURNAL, **46**, 151(1957).

(9) West, F. R., Jr., and Mika, E. S., *Botan. Gaz.*, **119**, 50(1957).

(10) Weinstein, L. H., Nickell, L. G., Laurencot, H. J., Jr., and Tulecke, W., *Contribs. Boyce Thompson Inst.*, **20**, 239(1959).

(11) Wain, R. L., and Wightman, F., "The Chemistry and Mode of Action of Plant Growth Substances," Academic Press, Inc., New York, N. Y., 1956.

(12) Nitsch, J. P., and Nitsch, C., *Am. J. Botany*, **44**, 555 (1957).

(13) Nitsch, J. P., "The Biological Action of Growth Substances," Academic Press, Inc., New York, N. Y., 1957, p. 125.

Radioisotopic Method of Evaluating Dispersed Systems I

Emulsions

By JAMES B. APPINO†, JOHN E. CHRISTIAN, and GILBERT S. BANKER

Liquid petrolatum emulsions were prepared by a standard method and evaluated by a new radioisotopic method. The radioisotopic method required less time and was more sensitive than previously used methods. A radioisotopic creaming rate was determined for each emulsion and can be used to compare microscopically identical emulsions and/or production samples of an emulsion to a previously determined standard. The method is applicable to w/o or o/w emulsions.

THE PRECISE evaluation of emulsions has presented problems to pharmaceutical and other scientists for many years. In an attempt to appraise and control emulsion appearance, uniformity of dosage, and stability, the following emulsion properties have been evaluated: particle size, particle size distribution, viscosity, and creaming rate (1–3). The evaluation of these factors gives some insight into the question, "How long can the product remain pharmaceutically elegant on the pharmacist's shelf?" but may leave the question of uniformity either wholly or partially unanswered.

This study is concerned primarily with an evaluation of the creaming rate and/or phase uniformity of emulsions in less time than is now required by the conventional visual (4) or microscopic (5) methods, or the more tedious though perhaps more accurate chemical or analytical methods (6). Radioisotopes, because of their sensitivity of detection (7), were utilized as tools in evaluating these properties.

Received April 28, 1961, from Purdue University, School of Pharmacy, Lafayette, Ind.

Accepted for publication June 12, 1961.

Presented to the Scientific Section, A. PH. A., Chicago meeting, April 1961.

† Present address: Armour Pharmaceutical Co., Kankakee, Ill.

EXPERIMENTAL

Preparation of Emulsion Samples.—The compositions of the emulsions evaluated in this study are given in Table I. The standard method of manufacture of all emulsion samples involved heating the liquid petrolatum and emulsifiers to 55° and the water and preservatives to 60°. The water phase was then added to the oils with constant agitation and the emulsion mixed until the temperature dropped to 38°. All emulsions were prepared in 1-L. quantities and allowed to deaerate for 24 hours before use.

Approximately 1 mc.[1] of sodium iodide (I^{131})[2] or iodobenzene (I^{131}) (8) per 100 ml. of emulsion was

[1] Gave 8,000–12,000 c.p.m. in end counting and 5,000–8,000 c.p.m. in side counting apparatus. Apparatus described later in text.

[2] Weight of sodium iodide is insignificant.

TABLE I.—EMULSION FORMULAS AND IDENTITY KEY

Ingredients, %	Emulsion Numbers[a] 1	2	3
Liquid petrolatum (heavy)	25.0	25.0	25.0
Span 20	1.2	4.2	1.8
Tween 20	0.8	2.8	1.2
Sodium iodide	..	0.5	..
Paracepts concentrate	1.0	1.0	1.0
Distilled water	72.0	66.5	71.0

[a] Emulsion 1: 1A, NaI^{131} added to finished emulsion; 1A', replication of 1A. Emulsion 2: 2A, NaI^{131} added to finished emulsion; 2B, $C_6H_5I^{131}$ added to finished emulsion. Emulsion 3: 3A, NaI^{131} added to finished emulsion; 3B, $C_6H_5I^{131}$ added to finished emulsion; 3C, $C_6H_5I^{131}$ added before emulsification.

used to label the emulsions studied. These labels were chosen on the basis of their selective solubilities in the water and oil phases, respectively, and insolubility in the other components of the emulsions. One of the following methods was used to prepare any single emulsion sample: (*a*) NaI^{131} was added to the water phase of the finished emulsion, (*b*) iodobenzene (I^{131}) was either added to the oil before emulsification, or (*c*) incorporated in 2 ml. of liquid petrolatum, 0.5 ml. of Span 20,[3] and 0.5 ml. of Tween 20[3] and added to the finished emulsion.

To facilitate detection of any visual changes in phase uniformity of the emulsions, 5–10 drops of a 1% amaranth solution was added to 120 ml. of an unlabeled control sample and to 120 ml. of a labeled control sample of each emulsion.

Evaluation of Emulsion Samples.—Approximately 120 ml. of each emulsion was placed in a separate 18 mm. i.d., constant bore Pyrex cell, 44 cm. in length. Gentle suction was then applied with a water aspirator for approximately 3 minutes to remove any air remaining in the emulsion. The cells were filled to within 3 mm. of the top and one cell was then placed in the counting apparatus and counted. Three other cells containing unlabeled, uncolored; unlabeled, colored; and labeled, colored emulsions, respectively, were stored as controls.

Two counting apparatuses were constructed to determine the ratioactivity in the labeled emulsions (Fig. 1). The first apparatus, number 1, was designed to detect activity through the side of the cell and the second apparatus, number 2, through the ends of the cell. Initially, the side counting method appeared to be the logical method to use in evaluating the emulsions; but due to its insensitivity when the water phase was labeled, the end counting method proved to be superior.

In the end counting apparatus the cells were sealed with Saran[4] wrap and paraffin. The Saran wrap was placed over the end of the cell and paraffin poured around the outer edge to seal it (no paraffin was used at the upper end of the cell). Two Geiger-Müller tubes, type D-33,[5] were used to detect the activity in the cell. The upper tube was placed against a 1/8 inch thick lead plate that had a 1/4 inch diameter hole drilled through it. The lower tube was placed through a plywood platform drilled just to accommodate the tube with the end window against another lead plate which had a 1/4 inch diameter hole drilled through it for collimation.

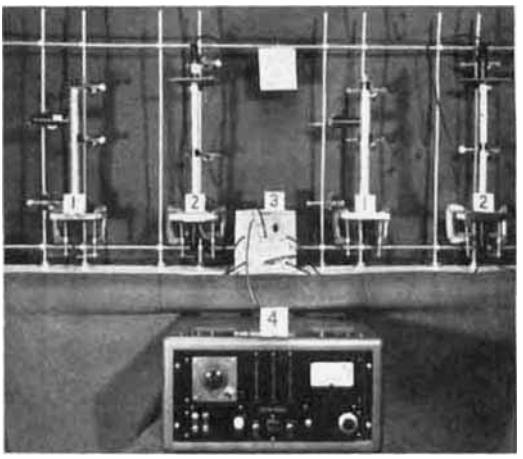

Fig. 1.—Multiple counting apparatus. 1, Side counting apparatus; 2, end counting apparatus; 3, control panel; 4, scaler.

The cell was placed on a 1.5 × 2.5 inch piece of hard rubber to aid in sealing the bottom. The rubber had a 15 mm. diameter hole cut in it and the cell was positioned above this hole. The plywood platform was supported by a support ring attached to a permanent table mounted ring stand. Each Geiger-Müller tube was held in a fixed position by a ring stand clamp.

The cables from six Geiger-Müller tubes were connected to a central control panel (number 3, Fig. 1). The scaler, model 200-S[6] (number 4, Fig. 1), was also connected to this panel and by turning the panel switch from 1 through 6, each of the Geiger-Müller tubes could be put into operation without manually changing any connections.

Viscosity and Particle Size Determination.—A direct microscopic evaluation (9) of the globule diameters was made using a standard microscope equipped with a 10× eyepiece and 43× objective. The eyepiece contained a micrometer with a 5 mm. horizontal scale divided into 100 divisions. A 50% aqueous solution of propylene gylcol was used to dilute one drop of the emulsion three to fivefold to facilitate microscopic examination (10). One drop of the diluted emulsion was placed on a microscope slide, a cover slip placed over it, and then allowed to stand 15 minutes before examination. A sample size of 100 particles was found to be more than statistically adequate using the method of Harris, Horvitz, and Moon (11), with a maximum confidence interval of one unit, a 99% confidence coefficient, and a 95% probability.

The emulsions used in this study were thought to exhibit Newtonian properties (12). This supposition was verified using a Ferranti-Shirley cone-plate viscometer.[7] A Brookfield-Synchro-lectric, model RVF viscometer[8] was thus used in subsequent viscosity determinations using spindle No. 1 at 20 r.p.m. The viscometer was allowed to operate 10 minutes in the emulsion before a reading was taken.

[3] Atlas Powder Co., Wilmington 99, Del.
[4] Dow Chemical Co., Midland, Mich.
[5] Nuclear-Chicago Corp., Des Plaines, Ill.
[6] Radiation-Instrument Development Laboratory, Chicago 21, Ill.
[7] Ferranti Ltd., Manchester 10, England.
[8] Brookfield Engineering Laboratories, Stoughton, Mass.

TABLE II.—STATISTICAL DATA AND RADIOISOTOPIC CREAMING RATES

Emulsion	Correlation Coefficients[a] r_1	r_2	r_3	Radioisotopic Creaming Rate[b]	Last Significant Day[c]	Visual Creaming[d]
1A	0.8772	0.9213	0.9912	0.2047	1	10[e]
1A′	0.9080	0.9550	0.9912	0.2074	1	10[e]
2A	0.8881	0.9358	0.9970	0.3596	4	4[e]
2B	0.9898	0.9750	0.9948	0.8238	7	6
3A	0.9588	0.9513	0.9648	0.0947	1	None
3A Hourly	0.9203	0.9751	0.9992	0.1441	..	None
3B	0.9052	0.9740	0.9920	0.0860	1	None
3B Hourly	0.9626	0.9546	0.9961	0.1382	..	None
3C	0.9542	0.9536	0.9974	0.1506	1	1
3C Hourly	0.9441	0.9023	0.9910	0.2957	..	None

[a] r_1 = Zero order, r_2 = first order, r_3 = log-log. [b] Rate of change of ratio with respect to time, based on log-log plot. [c] Last day significantly different from all other days (Newman-Keuls test). [d] Day phase separation first observed in control emulsions. [e] No distinct phase separation, only a change in color intensity of the controls.

Statistics.—An analysis of variance (13) was used to identify significant differences in phase uniformity of the emulsion samples with time and a Newman-Keuls test (14) was employed to determine the days over which significant changes occurred. The method of least squares (13) was used in the graphical presentations of the data and correlation coefficients (13) were calculated for zero-order, first-order, and log-log expressions to determine the best form of expression of the data.

RESULTS

Radiation Effects.—When the particle size and viscosity data of the various emulsions were statistically analyzed, it was found that the addition of NaI^{131} or $C_6H_5I^{131}$ to the finished emulsion, in the manner described and in the amounts used, had no effect on the emulsion system.

Radioisotopic Evaluation.—The radioisotopic data were recorded as a ratio of counts per minute (c.p.m.) at the top of the cell to c.p.m. at the bottom of the cell. By using this ratio, a form of comparison counting was achieved and corrections for background, efficiency, absorption, and decay were unnecessary. This left only coincidence corrections to be applied to the data. The length of each count was the time calculated to give 1% error or less in counting, usually 5 minutes. The emulsions were counted three times a day at 6-hour intervals until the ratio no longer decreased. The data were then statistically analyzed and plotted. The plots of emulsions 1A, 2A, and 3A are given in Fig. 2. These are typical graphs for emulsions with the water phase labeled. The graphs for the emulsions with the oil phase labeled were of the same type except the slopes were positive.

Two cells of an identical emulsion (1A and 1A′) were used to determine if reproducible results could be achieved on different apparatus. After the counting was completed, an analysis of variance was conducted to determine if day to day differences did exist and a Newman-Keuls test (14) was run to determine which days differed significantly. In both emulsions there were significant day to day differences, but the Newman-Keuls test indicated no significant differences after the first day.

To determine the rate of change of the ratio with respect to time (radioisotopic creaming rate)[9] the

[9] The radioisotopic creaming rate is the rate of phase separation or creaming of labeled emulsions (ratio *vs.* time).

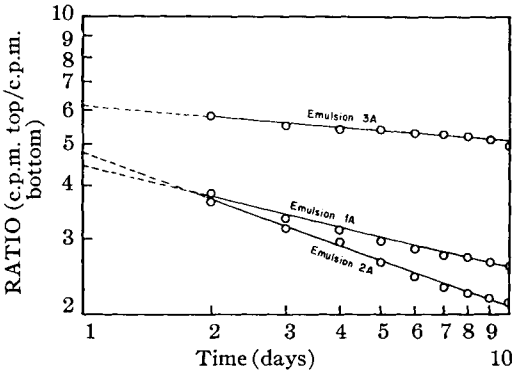

Fig. 2.—Typical log-log creaming curves.

method of least squares (13) was used; and to determine the best linear correlation, the correlation coefficients were calculated. The results of these tests are in Table II. The best linear correlation for emulsions 1A and 1A′, as for the remainder of the emulsions studied, was the log-log correlation. Thus, the mathematical expression was $\ln Y = \ln a + b \ln X$, where Y is the ratio of c.p.m. at the top of the cell to c.p.m. at the bottom of the cell, a is the Y intercept, b is the radioisotopic creaming rate (slope of the line), and X is the time. The general equation would therefore be $Y = aX^b$. From the radioisotopic creaming rates and the day to day variances, it can be seen that the two apparatuses did duplicate the results. The uncolored labeled and uncolored unlabeled controls of emulsion 1 showed no visible signs of phase separation. However, the colored controls showed a change in color intensity on the 10th day (Table II).

Emulsion 2, containing 0.5% electrolyte (sodium iodide), produced a system with more rapid phase separation. The results of this study are shown in Table II as 2A and 2B. The analyses of variance of both emulsions indicated significant day to day changes in the ratio. The Newman-Keuls test (Table II) indicated that emulsion 2B changed for a longer period of time than emulsion 2A. The radioisotopic creaming rates also indicated that emulsion 2, with 0.5% electrolyte, creamed more rapidly than emulsion 1, even though emulsion 2 had 5% more emulsifier. A change in color intensity of the dyed NaI^{131}-labeled control and the unlabeled control emulsions was observed on the 4th day, al-

though no change was observed in the uncolored samples. However, the same basic emulsion labeled with $C_6H_5I^{131}$ started to cream rapidly on the 6th day. This indicated the $C_6H_5I^{131}$ might be causing an acceleration of the creaming rate. To verify this assumption, unlabeled iodobenzene was added to emulsion 2 and again a rapid phase separation was observed.

Emulsion 3 was prepared to verify further the end counting method using NaI^{131} and to find a procedure for incorporating $C_6H_5I^{131}$ without accelerating the rate of creaming (Table I). Because of the rapid phase separation during the first 48 hours, observed in emulsions 1 and 2, emulsion 3 was counted every 2 hours for the first 12 hours, and every 2 hours from the 24th to 36th hour. After the 36th hour they were counted three times a day at 6-hour intervals. The analysis of variance of all three emulsions indicated a significant day to day change in ratio and the Newman-Keuls test showed the first day to be the only one significantly different from all others. In emulsions 3A and 3B there were no visual changes in the labeled or unlabeled controls. However, the labeled colored control of emulsion 3C indicated creaming had started on the first day. The radioisotopic creaming rates (Table II) of emulsions 3A and 3B were very similar, but that of emulsion 3C was greatly increased. From these results it was concluded that the $C_6H_5I^{131}$ added to the oil before emulsification increased the creaming rate, but when added with liquid petrolatum and the emulsifiers to the finished emulsion, had no effect on the emulsion system.

Emulsion 3A-hourly, 3B-hourly, and 3C-hourly are the results of the first 36 hours of counting of emulsions 3A, 3B, and 3C, respectively. As can be seen from Table II, the best linear correlation was again log-log. This indicated the same type of change in the ratios (log-log) from hour to hour or day to day. From these results it is apparent that if two emulsions that cream in a relatively short time are to be compared, an hour to hour isotopic analysis would be preferred.

DISCUSSION

The advantages of the radioisotopic method of evaluation are (*a*) sampling is simplified and subsequent chemical assays are not necessary, (*b*) there is a continuous indication of uniformity and if phase separation occurs, it is readily detected, (*c*) the isotope method approaches the problem of emulsion stability from the most important standpoint of dispersion uniformity, and (*d*) very small amounts of label can be used and thus not affect the emulsion system.

In this study a gamma emitter (I^{131}) was used as the label because it could be readily synthesized into water-soluble or oil-soluble compounds. However, if a beta emitter such as P^{32} were used to label the water phase, it is felt that the sensitivity would be increased due to the greater absorption of the beta particles as the emulsion tended to cream.

Although the end counting method was superior to the side counting method, it is felt that a combination of the two might be advantageous. To use the two methods in the most expedient manner, a precision turn table, that would hold a desired number of cells could be constructed with a Geiger-Müller tube mounted above and another below it. Then as the table turned it would lock a cell into position accurately between the two Geiger-Müller tubes. To side count the cell, another apparatus could be constructed that would slowly move a third Geiger-Müller tube and scan the cell. Thus with only three Geiger-Müller tubes, a scaler, a rate meter, and automatic recorder for the side scanning, a number of emulsions could be evaluated in a short period of time.

The radioisotopic method of evaluating emulsions appears to be applicable to other dispersed systems. By the use of the end counting method, suspensions could be evaluated for rate of sedimentation. The absorption of the label by the solid particles should increase the sensitivity of the apparatus. The radioisotopic method appears to have industrial application in comparing visually and microscopically statistically identical emulsions in a short period of time. Also, once a radioisotopic creaming rate has been determined for a given emulsion system, production samples can be withdrawn, labeled, and rapidly compared to the predetermined standard rate.

CONCLUSIONS

1. A radioisotopic method has been developed for determining the phase uniformity of emulsions and for detecting and expressing ratios of creaming of emulsions that is applicable to w/o and o/w emulsions with either phase being labeled.

2. The radioactivity added to the emulsions had no effect on the particle size or viscosity of the emulsions.

3. For the emulsion system studied, much less time was required for the radioisotopic evaluation than for visual or microscopic evaluations, and great sensitivity was evidenced by the detection of phase separation before it became visible.

REFERENCES

(1) Carter, P. J., *Am. Perfumer Aromat.*, **71**, 43(1958).
(2) Johnson, R. I., *et al.*, "Emulsion Technology, Theoretical and Applied," Chemical Publishing Co., Inc., Brooklyn, N. Y., 1943, p. 88.
(3) Sumner, C. G., "The Theory of Emulsions and Their Technical Treatment," 5th ed., The Blakiston Co., Inc., New York, N. Y., 1954, p. 136.
(4) Peck, G. E., DeKay, H. G., and Banker, G. S., THIS JOURNAL, **49**, 75(1960).
(5) King, A., and Mukherjee, L. N., *J. Soc. Chem. Ind.*, **58**, 243T(1939).
(6) Leviton, A., and Haller, H. S., *J. Phys. & Colloid Chem.*, **51**, 460(1947).
(7) *J. Am. Pharm. Assoc., Pract. Pharm. Ed.*, **8**, 6(1947).
(8) Murray, A., III, and Williams, D. L., "Organic Synthesis with Isotopes," Interscience Publishers, Inc., New York, N. Y., 1958, p. 1229.
(9) Cadle, R. D., "Particle Size Determinations," Interscience Publishers, Inc., New York, N. Y., 1955, p. 105.
(10) Levius, H. P., and Drommond, F. G., *J. Pharm. and Pharmacol.*, **5**, 743(1953).
(11) Harris, M., Horvitz, D. G., and Moon, A. M., *J. Am. Stat. Assoc.*, **43**, 319(1948).
(12) Weltman, R. N., *Ind. Eng. Chem., Anal. Ed.*, **15**, 424 (1943).
(13) Ostle, B., "Statistics in Research," The Iowa Stat College Press, Ames, Iowa, 1958.
(14) Duncan, D. B., *Biometrics*, **11**, 3, 26(1955).