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Determination of Particle Size Distribution of Fluorocarbon Emulsion by Means of Centrifugal Sedimentation —A Proposal for Specifying the Particle Size Distribution—

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A method of specifying particle size distribution in fluorocarbon emulsion by means of stepwise centrifugation is proposed here.

On account of very large specific density of perfluorinated compounds, a physical calculation was devised for specifying particle size of fluorocarbon emulsion in relation with centrifugal rotation applying Bostock and Stoke's law of sedimentation.

Two samples of perfluorotributylamine (FC43) emulsion were trially prepared with different grade of emulsification and distribution of particle sizes of the emulsions were examined by the proposed method of centrifugal differentiation and electron-microscopic photography. Close coincidence between the findings by the both methods was found.

It is concluded that the distribution of particle sizes in fluorocarbon emulsion can be determined by a simple method of stepwise centrifugal sedimentation.

The particle size of FC43 emulsion trially prepared with Manton-Gaulin homogenizer was found to be very fine and a half or more of the particles were smaller than 0.1 μ in diameter.

The use of perfluorinated compound as a candidate for "artificial blood" has been studied by several workers.[2-5)]

Through our animal experiments on the toxicity and metabolism of perfluorotributylamine (FC43) emulsion injected intravenously, it has been known that toxic effects are closely related to particle size of the emulsion; the larger the particles, the more toxic.

Previous investigations on perfluorinated compound emulsion have concerned almost exclusively with the oxygen transport ability and consequent biological effects, but a method measuring and specifying the particle size has not been studied enough.

The purpose of this study is to establish a method for determining and specifying the distribution of the particle size of FC43 in the emulsion. The size of particles in common emulsion is generally determined by methods of microscopy and sedimentation. Optical or electron-microscopic determination is indispensable for the studies of particle shape and size. But, as the range of the optical microscopic measurement is limited to the particles of about one micron, it is not applied to the measurement of fine particle size of perfluorinated compound in the emulsion. The electron-microscopic analysis is not only a time consuming procedure, but it remains uncertain whether the distribution of particle size observed by electron-microscope on a very small part of the emulsion really represents the whole figure of the emulsion or not. Instead, sedimentation method is common procedure for the analysis of size of particles in common emulsions based on the relationship between the diameter of spherical particle and its rate of sedimentation under gravity (*g*).[6)]

1) Location: *a) Midorijuji, 1-3, Gamou-cho, Joto-ku, Osaka*; *b) Kashima-cho, Higashi Yodogawa-ku, Osaka.*

2) L.C. Clark, Jr. and F. Gollan, *Science*, **152**, 1755 (1966).
3) H.A. Sloviter and T. Kamimoto, *Nature*, **216**, 458 (1967).
4) H.A. Sloviter, M. Petokovic, S. Ogoshi, and H. Yamada, *Fed. Proc.*, **27**, 669 (1969).
5) R.P. Geyer, R.G. Monroe, and K. Taylor, *Fed. Proc.*, **27**, 384 (1969).
6) C.T. Brodnyan, *J. Colloid Sci.*, **15**, 563 (1960).

Though most of sedimentation methods have problems of the effect of convection, diffusion and Brownian motion of particles, the method offered with this article, may be an aid to avoid such difficulties.

Theoretical

Previously, Fujita, Suyama and Yokoyama[7] made a proposal to specify the particle size pattern of FC43 emulsion by means of centrifugation (1971). The present authors have, however, found a certain point of physicomathematical errors in the original report and were led to make a slight correction therein.

As the density of fluorocarbon is larger than that of medium solution, the theory originally developed by Oden[8] and modified later by Bostock[9] is applicable for measuring size and distribution of particles of perfluorinated compound emulsion by means of centrifugal sedimentation.

On an assumption that quantity of perfluorinated compound particles in the emulsion (ΔW) with a diameter D distributes in compliance with the function

$$\Delta W = F(D), \tag{1}$$

the part of the substance in emulsion particles larger than Dt in diameter (Wt) is expressed with

$$W_t = \int_{D_t}^{D_\infty} F(D) dD \tag{2}$$

accordingly, where D is diameter of particle and Wt is the part of the emulsified substance having a diameter larger than Dt as shown in Fig. 1.

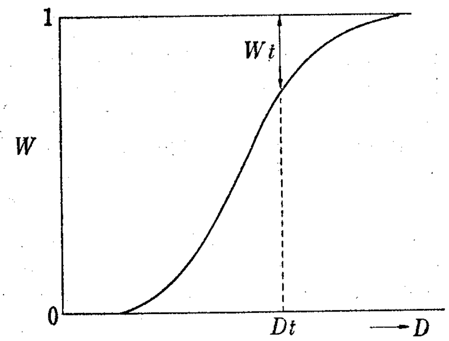

Fig. 1. Wt and Dt

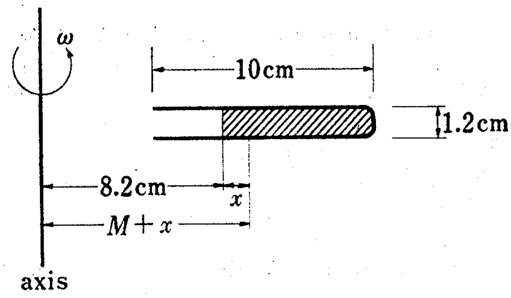

Fig. 2. Centrifugal System

According to the Bostock's theory, the weight percent (P) which has settled out of a certain upper portion in fluid column of a centrifugation tube as shown in Fig. 2 at time t consists of two parts;

$$P = \int_{Dt}^{D_\infty} F(D)d(D) + \int \frac{vt}{x} F(D)dD, \tag{3}$$

here, the first term of the right-hand side in equation (3) consists of all the particles with a larger diameter than Dt which is given by the following equation by Stoke's law:

$$D_t = \sqrt{\frac{18\eta \cdot \ln[(M+x)/M]}{(\rho_e - \rho_m)\omega^2 t}} \tag{4}$$

7) T. Fujita, T. Suyama, and K. Yokoyama, *Europ. Surg. Res.*, 3, 436 (1971).
8) S. Oden, *Kolloid Z.*, **8**, 33 (1916).
9) W. Bostock, *J. Sci. Intr.*, **29**, 209 (1952).

where M is distance between the surface of emulsion and the axis of the centrifuge, and

x = depth from the surface to the bottom of the portion in the fluid column,

ω = speed of rotation of centrifuge,

η = coefficient of viscosity of the medium,

$\rho_e:\rho_m$ = density of the particle and the medium,

the second term consists of particles smaller than Dt which are sedimented out of the portion starting from some intermediate position in the fluid column. If the mean velocity of sedimentation of these smaller particles is v, the fraction of particles of this size that is driven out of the portion is vt/x.

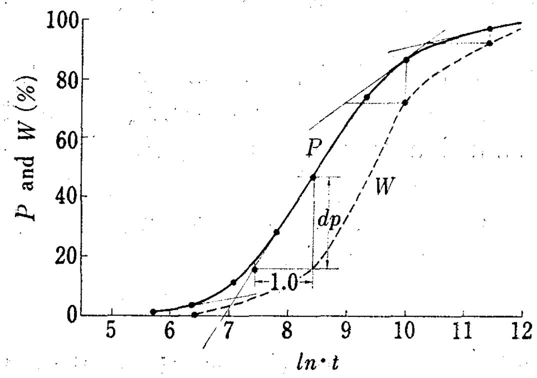

Fig. 3. Calculation of W from P by the Method of Drawing Figure

By differentiating formula (3) by time t and multiplying by t, the following equation is derived:

$$t\frac{dP}{dt} = \int_{D_{\min}}^{D_t} \frac{vt}{x} F(D)dD \tag{5}$$

By substituting (2) and (3) to the equation (5)

$$P = W_t + t\frac{dP}{dt} \tag{6}$$

$$W_t = P - \frac{dP}{d(\ln\cdot t)} \tag{7}$$

If P and t are given, it is possible to determine Wt by means of the formula (7).

An experimental collations were made to scrutinize the practical application of formula (7) as described below.

Experimental

(1) **Preparation of FC43 Emulsion for This Experiment**——The emulsion of FC43 (perfluorotributylamine) in water for trial determination of particle size distribution was prepared as follows:

185 ml of FC43 (supplied by the 3M Company) and 815 ml of 5% Pluronic F-68 (Wyandott Co., Ltd.) aqueous solution were poured into an 1.5 liter cylinder and stirred for 10 minutes at 2000 rpm with a common homomixer (Tokushu Kikakogyo Co., Ltd., Japan). The resultant "coarse" emulsion was further dispersed by passing six times through Manton-Gaulin homogenizer under the 2 nd stage and total pressures of 100 and 500 kg/cm², respectively. This emulsion was named Sample A. Sample B was prepared by passing Sample A further six times under the same condition as described above.

(2) **The Quantitative Analysis of FC43 in the Emulsion by Gas Chromatography**——Gas chromatograph used was SHIMADZU model GC-4BPTF, equipped with flame ionization detector. A glass column, 1.5 m long and 4 mm i.d. was packed with 20% silicone OV-17 on chromosorb W AW (DMCS), 60—80 mesh (Nishio Kogyo Co., Japan).

The column was operated at 50° and the injection port at 175°. The nitrogen flow rate was 25 ml/min with an inlet pressure 0.3 kg/cm². The determination of FC43 was done as follows:

One ml of FC43 emulsion containing 5 to 17 mg of FC43 was added to about 5 ml of methanol in a test tube. The mixture was shaken and allowed to stand for 30 minutes. The emulsion was destroyed by this procedure and FC43 settled down to the bottom of the tube. Three ml of trifluorotrichloroethane (FCC113 Daikin Kogyo, Japan) was added to the mixture to extract FC43. The tubes were tightly closed with glass cap and shaken vigorously and allowed to stand for 5 minutes in an ice-bath. After centrifugation, the lower FCC113 layer was washed twice with about 5 ml of water in order to remove methanol from FCC113 layer completely. After drying the FCC113 layer by adding an adequate amount of anhydrous sodium sulfate, 1 ml of FCC113 layer was transferred to another test tube and 1.0 ml of 1.0% benzotrifluoride (BTF, Tokyo Kasei Kogyo Co.) standard solution in FCC113 was added accurately as an internal standard.

Then, 0.1 to 0.2 μl of these analytical sample was injected with Hamilton microsyringe into the gas chromatograph. The chromatography was repeated at least twice. The content of FC43 was obtained from the standard curve which was related to peak height tatio and volume ratio to the internal standard.

(3) **Centrifugation Procedure and Calculation Therefrom**——A common refrigerated centrifuge model RS-18P-2 (Tomy Seiko, Japan) was used in this experiment.

FC43 emulsion was diluted with 20 volumes of deionized water and then, 10 ml of diluted emulsion was poured into a centrifuge tube (10×1.2 cm i.d.). The tubes were then placed in a swing type rotor and immediately centrifuged for 5, 10, 20, and 40 minutes at 1000 rpm at 20° to handle the particle size of the larger particles in the emulsion and for 10, 20, 40, 80, and 160 minutes at 3000 rpm at the same temperature to handle that of smaller particles. The distance from the center of rotation to the surface of emulsion was adjusted to 8.2 cm as precise as possible. After the centrifugation, the emulsion of upper 2 cm portion from the surface of liquid was carefully sucked out with a syringe and the amount of FC43 in this portion of the emulsion was measured by the gas chromatography.

From the decreased amount of FC43 in the emulsion of upper 2 cm portion under various centrifugal condiftions, the diameter of particles was calculated by the method described below.

An example of the centrifugal system used in this experiment is as shown in Fig. 2. When this system is applied to formula (3), *i.e.*, $\eta=0.01102$, $\rho_m=0.999$, $\rho_e=1.87$, $m=8.2$, $x=2$ and $\omega=2\pi r/60$, Dt is given as follows:

$$D_t = 2\cdot129\sqrt{\frac{1}{r^2t}} \tag{8}$$

where r is rotation frequencies, rpm.

The correlation between the particle size and centrifugal conditions is calculated with the formula (8). The results are shown in Table I. On the other hand, the really eliminated FC43 from the top 2 cm of column by the centrifugation is P in formula (3).

W is derived by subtracting $dP/d(\ln\cdot t)$ from P value on the figure as shown in Fig. 3 where the abscissa is scaled by $(\ln\cdot t)$; on the vertical line from Pn on the P-curve, Wn locates where it is distant by $\ln\cdot t=1$ from the tangent line shot at pn.

Table I. Correlation between Particle Size and Conditions of Centrifuge

Expt. No.	Time for centrifugation (sec.) 1000 rpm		3000 rpm	Dt[a] Calculated particle size (micron)
	sec	min		
1	300	5	—	1.229
2	600	10	—	0.869
3	1200	20	—	0.615
4	2400	40	—	0.435
5[b]	5400	90	600	0.290
6[b]	10800	180	1200	0.205
7[b]	21600	360	2400	0.145
8[b]	43200	720	4800	0.102
9[b]	86400	1440	9600	0.072

a) Dt is particle diameter calculated by the formula (8).
b) The centrifugation of experiment No. 5 to 9 was performed at 3000 rpm and the time for centrifugation was interpreted to the time at 1000 rpm to calculate the particle size at the same condition.

Table II. Correlation between Time for Centrifugation and Reduction of FC43 Concentration (P and W Values) in the 2 cm Portion of Emulsion from the Surface

Time for centrifugation at 1000 rpm, min.	Particle diameter micron	P value % of FC43 Sample A	Sample B	W value % of FC43 Sample A	Sample B
5	1.229	4.0	2.9	0	0
10	0.869	9.6	3.4	2.0	0
20	0.615	15.1	4.1	5.3	0
40	0.435	22.7	6.6	9.5	0.5
90	0.290	33.4	14.7	14.0	0.9
180	0.205	64.1	29.5	27.8	7.1
360	0.145	87.8	49.8	74.7	14.0
720	0.102	98.1	79.2	89.5	43.5
1440	0.072	99.6	96.5	100.0	80.7

P: Measured loss of FC from the upper 2 cm portion.
W: Calculated quantity (percent) of FC43 substance in the emulsion particles with the corresponding diameter.

Cumulative distribution curve with abscissa scaled by diameter of particles as Fig. 4 is derived therefrom by converting $\ln \cdot t$ to Dt with the formula (8).

(4) Collation between Centrifugal Method and Electronmicrograph——Experiments were made with two sorts of emulsions as mentioned above to verify the particle size pattern of these.

(i) With Centrifugal Sedimentation Technic: Table II shows the correlation between the time of centrifugation and reduction of FC43 concentration (d value) chemically determined from the upper 2 cm portion of FC43 emulsion from the surface of liquid in the tubes. W value was calculated from the P value by the method described above.

In case of sample A, the "coarse" emulsion, 22.7% of FC43 was reduced by the centrifugation for 20 minutes at 1000 rpm and 99.6% for 160 minutes at 3000 rpm. On the other hand, for sample B being the "fine" emulsion, the reduction of FC43 concentration under the same condition is remarkably less, *i.e.* 6.6% for 20 minutes at 1000 rpm. The diameter of particles calculated by the formula (8) was determined from the rotation speed and time for centrifugation. The distribution of diameter of particles is shown in Table III. This was concerned with the decreasing rate of FC43 in the 2 cm portion of FC43 emulsion. The particles in the "fine" emulsion, sample B, were in the range smaller than 0.15 μ in diameter. Furthermore, 19.3% of particles expressed by weight was smaller than 0.07 μ. On the other hand, the particles of "coarse" emulsion, sample A, were widely distributed.

Table III. Analytical Data of Distribution of Particle Size in FC43 Emulsion

	Particle size range	Distribution of FC43	
		Sample A	Sample B
1.	>1.229 μ	2.0%	0 %
2.	1.229—0.869	3.3	0
3.	0.869—0.615	4.2	0.5
4.	0.615—0.435	4.5	0.4
5.	0.435—0.290	13.8	6.2
6.	0.290—0.145	46.9	6.9
7.	0.145—0.102	14.6	29.5
8.	0.102—0.072	10.5	37.2
9.	<0.072	0	19.3

Table IV. Differential Distribution of Particle Size in FC43 Emulsion by Electron-microscopy

Particle range Diameter μ	Sample A			Sample B		
	Number of particles	% number	% weight[a)]	Number of particles	% number	% weight[a)]
0.02	—	—	—	4	0.2	—
0.02—0.04	40	2.6	—	161	9.8	0.6
0.04—0.06	155	9.9	0.6	403	24.7	7.1
0.06—0.08	211	13.5	2.1	626	28.3	30.3
0.08—0.10	241	15.4	5.4	215	13.3	22.3
0.10—0.12	243	15.5	9.3	83	5.1	15.5
0.12—0.14	277	17.7	17.5	22	1.3	6.7
0.14—0.16	178	11.3	17.2	13	0.8	6.1
0.16—0.18	103	6.6	14.5	5	0.3	3.5
0.18—0.20	61	3.9	11.9	4	0.2	3.7
0.20—0.22	35	2.3	9.5	2	0.1	2.4
0.22—0.24	11	0.7	3.8	1	0.1	1.7
0.24—0.26	4	0.3	2.1	—	—	—
0.26—0.28	8	0.6	5.4	—	—	—
0.32—0.34	1	—	1.0	—	—	—

a) Percent of weight is calculated from the measured figures by the equation $(nd^3/\Sigma nd^3) \times 100$, where n and d are number of particles and average diameter of particles in the photograph in each particle range, respectively.

(ii) With Electronmicrography: It has now to be collated whether the method hereby proposed to define particle size and its distribution by means of centrifugation and calculation really represents the actuality or not. For this purpose of checking the coincidence, particle size of the same samples of FC43 emulsion was measured by means of electron-microscopy.

Each sample of FC43 emulsion was diluted 5 to 10 times with 0.05 to 0.1% egg albumin solution dissolved in distilled water. One drop of this diluted sample was placed on the sheet mesh previously filmed with carbon-coated collodion membrane. After drying, the specimen was brought under electron-microscope photography (JEM-T7S, Japan Electron Optics Lab. Co., Ltd.) with a magnification of 5000 times, the diameters of each particle in the photographs were measured with a micrometer. Total 1500 particles thus picked up were then grouped by the classes of size to find distribution of particle size.

In Table IV differential number distribution of particle size is shown together with weight distribution.

As seen in Table IV, sample A was in wide range and particles smaller than 0.1 μ were about 8% as weight of FC43, while sample B was found to be of narrower distribution, and about 60% of particles was smaller than 0.1 μ in diameter.

As shown in Fig. 4, the particle size determined from the centrifugal method was compared with that calculated from the electron-microscopic method in terms of cumulative particle distribution. In case of sample B (Fig. 4), cumulative curve derived from the centrifuged method was found to be closely coincident with that obtained by the electron-microscopic method.

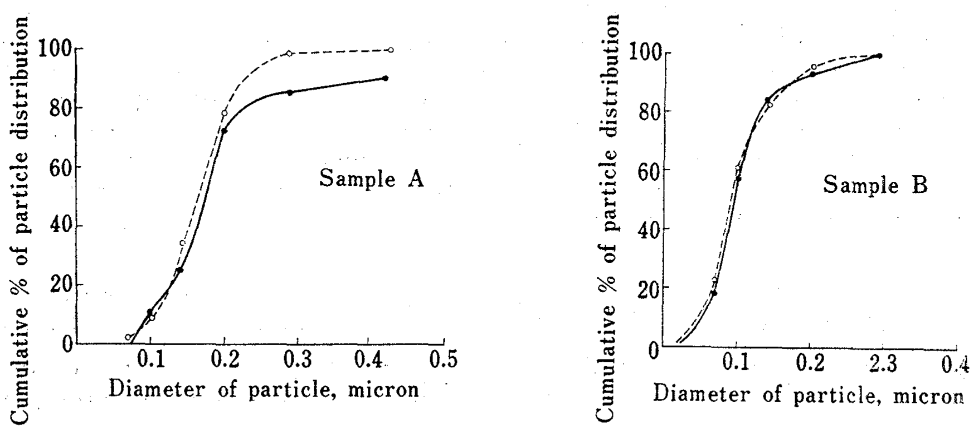

Fig. 4. Particle Distribution determined by the Method of Centrifugal Sedimentation Compared with That by Electron-microscopy

-------: electron-microscopic ———: by centrifugation

Results and Discussion

Due to the importance of particle size of fluorocarbon emulsion closely related with its toxicity, a method for specifying particle sizes and its distribution by means of centrigufation was studied with special regards on collation with conventional electronmicrographic method. It was experienced that the distribution curves derived from the both methods were fairly well coincident so far as the particle size distribution falls in relatively narrow range.

The electronmicrographic method is time-consuming, rather laborious procedure and uncertain whether the finding obtained with a very small part of material really represents the whole figure of the emulsion particles.

It seems to be safe to conclude that the method with centrifugation is a practical procedure to determine the particle size and its distribution of perfluorinated compound emulsions.