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PERFLUOROCTYL BROMIDE EMULSIONS AS RADIOPAQUE MEDIA

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ABSTRACT

Brominated perfluorocarbon compounds are a new and unique type of contrast agents. These compounds are biologically inert and chemically stable. Highly concentrated (8:1 to 10:1 by volume) oil in water emulsions have been found to be stable and non-irritating and useful in bronchography in humans and animals and in angiography in animal studies. The physical properties of these unusual emulsions have been investigated. The emulsions have non-Newtonian properties, low interfacial tensions and a broad distribution of particle size.

The emulsion, as well as the pure compounds, has been the subject of an IND application with the Food and Drug Administration. The investigations on the use of the emulsion in bronchography and the pure compounds in alveolography and gastroenterography are on going and are in Phase II of the IND.

OBJECTIVES

The object of this paper is to report on an investigation on the use of perfluoroctyl bromide (see Figure 1) emulsion as a radiopaque medium some physical properties and method of preparation.

Brominated perfluorocarbon compounds are a new and unique type of contrast agents.

INTRODUCTION AND BACKGROUND

Early this year, Long and co-workers, in cooperation with the University of Illinois, Manufacturing Pharmacy Service, filed an investigational drug application for L1913, perfluoroctyl bromide for specific use as radio contrast medium in gastroenterography and alveolography, and the emulsions made from it, in bronchography.

The application was approved and human investigation is now in progress and is in Phase II of the IND.

Perfluoroctyl bromide is a 1-bromo substituted member of the family of fluorocarbons which have been investigated in the last decade for applications based on its oxygen dissolving capacity. Clark and co-workers (1, 2) have shown

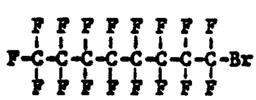


Figure 1. Perfluoroctyl bromide, L1913
($C_8F_{17}Br$)

that pure fluorocarbons which are perfluoro homologs of alkanes, cycloalkanes and some aromatic hydrocarbons, dissolve enough oxygen to support the respiration of entire animals and of isolated organs. They studied the various pure compounds as well as O/W emulsions with Ringers Solution, 5% Glucose, or Water as aqueous phase; and Wyandotte's Pluronic F68, Allied Chemical's ASA, or albumin as emulsifying agents. In Table I, we show the solubility of oxygen in various materials--we note that oxygen dissolves to the extent of 3 ml/100 of plasma; 20 ml/100 ml in whole blood, but 50 ml/100 ml of fluorocarbons. Note that oxygen is very soluble in haemoglobin itself, but after dilution with plasma, the solubility of oxygen drops.

Based on the establishment by Clark and co-workers of the safety in the use of perfluorocarbons, Long and others (3) obtained from the 3M Company seven bromosubstituted compounds as shown in Table II. The bromine atom was introduced to provide the radiopacity. Note that the chainlengths

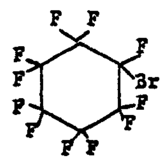
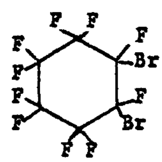
TABLE I

SOLUBILITY OF OXYGEN IN VARIOUS MATERIALS

Material	ml Oxygen/100 ml
Salt water or plasma	3
Vegetable oils	10
Whole blood	20
Silicone oils	20
Fluorocarbons	50
Human haemoglobin	134

TABLE II

BROMINATED FLUOROCARBONS INVESTIGATED FOR POSSIBLE USE AS RADIOPAQUE AGENTS

Code Number	Formula
1913	$CF_3(CF_2)_6CF_2BR$
2529	$CF_3(CF_2)_4CF_2BR$
2501	$BR(CF_2)_4BR$
2499	$BRCF_2CF_2BR$
2351	 $C_6F_{11}BR$
2502	 $C_6F_{10}BR_2$
2500	$CF_3CFBRCF_2BR$

vary from 2 to 8. There are monobromo and dibromo derivatives; of the dibromo derivatives, some are contiguous.

THE INVESTIGATION

Long and co-workers (4) studied these various brominated perfluorocarbons and found that whereas all of them were radiopaque, the toxicity of some of the compounds prevented their usefulness as radio-contrast media. In Table III, we

TABLE III

MORTALITY AFTER ORAL ADMINISTRATION OF RADIOPAQUE FLUOROCARBONS IN NON-ANESTHETIZED ALBINO RATS

Code Number	LD_{50} (ml/Kg)
L-1913	> 64
2351	4-16
2499	16-32
2500	< 4
2501	≅64
2502	< 4
2529	>64

Toxicity: 1913 and 2529 < 2501 < 2499 < 2351 < 2502-2500

show the 50 per cent lethal dose after oral administration of the various compounds to non-anesthetized albino rats. We note that the most toxic is compound 2500 and 2502 with an LD50 of less than 4 ml/Kg body weight of albino rats. The L2500 and L2502 are the 1,2-dibromo perfluoropropane and 1,2-dibromoperfluoro cyclohexane. This suggests that the compounds with contiguous dibromo-substituents are the most toxic.

The safest appeared to be L1913 and L2529, which are the perfluoroctyl bromide and perfluorohexyl bromide respectively, with LD50's of greater than 64 ml per Kg. As it turned out, however, the hexane derivative was much too volatile and tended to vaporize in the body.

The choice then, of working with perfluoroctyl bromide becomes clear after the foregoing data on toxicity. This compound, perfluoroctyl bromide which has a boiling point of 141° C, a density almost twice that of water, 1.928 when measured at 25° C and an interfacial tension against air of 18.2 dynes per centimeter, has been reported on by Rosen (5). The pure liquid was quite heavy and "creeps." It was shown to be useful in gastrointestinal and alveolar roentgenography in animals. An approximately 10:1 oil in water emulsion, with a markedly changed rheological properties when compared to the pure compound, was used in bronchographic studies, also in animals.

Since this study is principally focused on the emulsion prepared from perfluoroctyl bromide and an aqueous phase consisting of 6% Pluronic F68 in Sodium Chloride Injection, some of the physical properties studied are reported.

INTERFACIAL TENSION

Interfacial tension measurements, using a Cenco-duNois tensiometer were made and are shown on Table IV. The interfacial tension of L1913 vs air of 19.6 dynes/cm when measured in a DuNois tensiometer at 27.2° C, and the value of 35.6 dynes/cm when measured against water, suggests that large amounts of energy would be required to break up the bulk fluorocarbon for dispersing as an emulsion. The value of 5.2 dynes/cm however, which was obtained when measuring the interfacial tension between the fluorocarbon and 6% Pluronic F68 in saline showed that much less energy would have to be required for emulsification. The addition of the 6% Pluronic to the aqueous phase dramatically dropped the interfacial tension.

TABLE IV

SURFACE TENSION MEASUREMENTS: L1913 FLUOROCARBON

Phases	T (dynes/cm)	Temp. (° C)
A. L1913 - air	19.6	27.2
B. L1913 - water	35.6	27.8
C. L1913 - 6% Pluronic F68	<5.2*	28.0
D. 6% Pluronic F68 - air	39.3	28.0

* 5.2 dynes/cm probable upper bound

METHOD OF PREPARATION OF THE EMULSION

The sterile emulsion was prepared by filtration of the fluorocarbon and the aqueous phase through a pre-sterilized 0.20 micron Gelman filter using a 42mm Millipore holder with the aid of a vacuum pump in a Class 100 vertical laminar air flow environment. A 2:1 nucleus was then prepared by milling while cooling in a Gifford-Wood homomixer for about 2 hours at 70% power. A white opaque emulsion was obtained. Higher concentrations, up to 10:1, were prepared by carefully mixing additional fluorocarbon to the nucleus emulsion in a sterile beaker with the aid of a sterile spatula. Other methods for preparing fluorocarbon emulsions have been reported by Clark (6) and Geyer (7).

VISCOSITY

As a further effort to standardize the emulsions, viscosity measurements were made on a Haake Rotovisco apparatus for which we thank the DeSoto, Inc., of Desplaines for making one available. A 2:1 emulsion using an MV(I) reducer with a

#500 head and an 8:1 emulsion using an MV(III) reducer with a #500 head were studied. The results are shown in Figure 2.

The apparent viscosity of a Newtonian fluid is not affected by the shear rate. The results showed that the apparent viscosity of the emulsions studied were influenced by the rate of shear and, therefore, were non-Newtonian. In a less obvious way, the results suggest that the emulsion becomes less Newtonian as the concentration increases.

In the 8:1 emulsion, the apparent viscosity drops from 150,000 to 2,000 centipoise when the shear rate is increased from 0.16 to 26 per second. In the 2:1 emulsion, the apparent viscosity decreased from 13,000 to about 440 when the shear rate is increased from 0.8 to 137 per second.

DENSITY

The real ratio of fluorocarbon to aqueous phase in the completed emulsion was determined from density measurements. This ratio could not have been determined from the amount of starting materials owing to the volatility of the fluorocarbon and to unavoidable processing losses. As Table V shows, calculating the actual ratio from density figures obtained by pycnometry allows more precise ratios than nominal figures to be obtained.

Among the parameters studied to establish reproducibility in the preparation of the emulsions, was particle size distribution. This was accomplished by obtaining aliquots which are diluted with distilled water and stained with methyl green. A

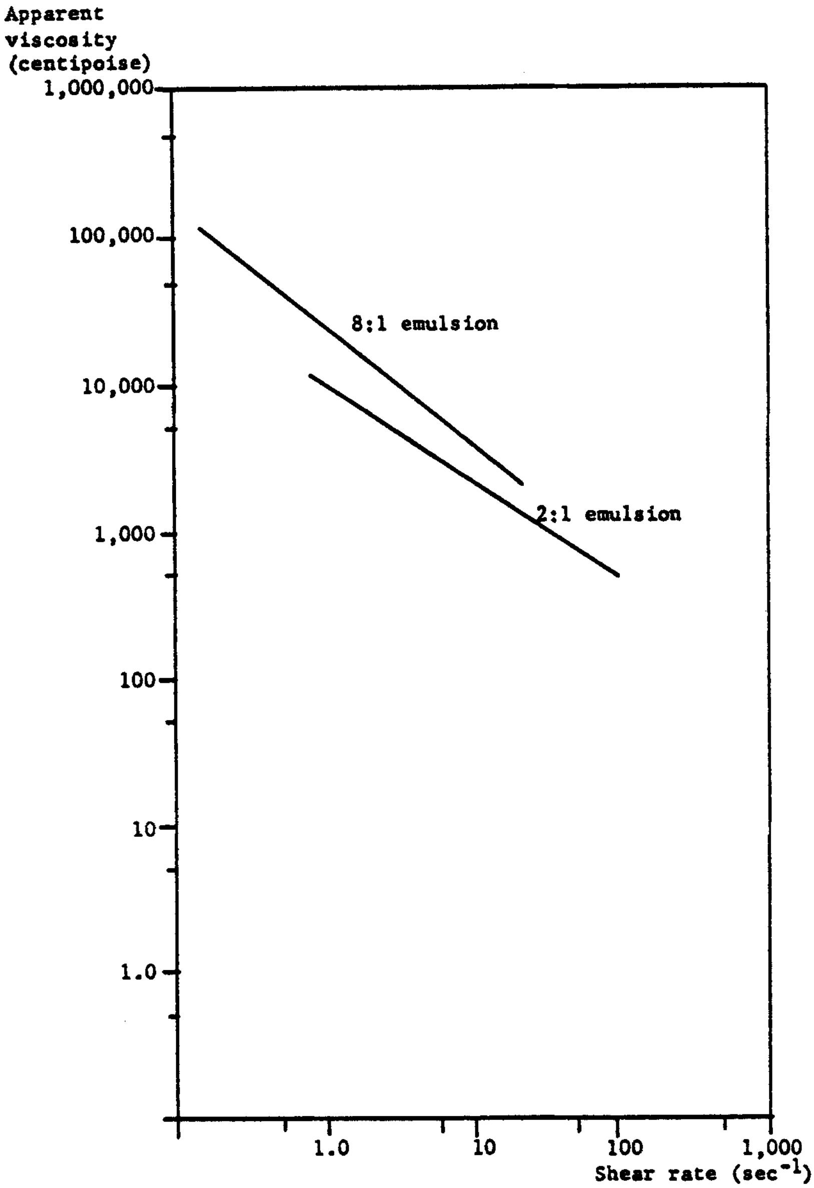


Figure 2. Viscosity of perfluoroctyl bromide emulsions.

TABLE V

DENSITY OF VARIOUS EMULSIONS OF L1913

Material	Density	Temp. C	Composition (Calculated)
L1913 (pure)	1.928	25	100
6% Pluronic F68 (saline)	1.014	25	100
2:1 (nominal composition)	1.167	24.2	1.94:1
6:1 " "	1.796	22.2	5.93:1
8:1 " "	1.823	21.6	7.80:1
10:1 " "	1.929	23.2	9.36:1

portion is then placed on a slide, a cover slip put in place, and then viewed under an oil-immersion objective of a phase microscope.

A typical particle size distribution of a 2:1 "nucleus" emulsion is shown on Table VI. We note that about 65% of the particles counted are under 0.7 microns (700 Nanometers).

TABLE VI

TYPICAL PARTICLE SIZE DISTRIBUTION OF A 2:1 (O/W) EMULSION OF L1913

Microns	%
$<$0.7	65.40
0.7-1.4	23.90
1.4-2.8	7.80
2.8-4.2	1.90
4.2-5.6	0.39
5.6-7.0	0.50
$>$7.0	0*

*Whereas the table shows no particles of this diameter, the method does not allow obtaining particles of large sizes in the sample.

Particles between 0.7 and 1.4 microns (700-1400 Nanometers) make up nearly 24%. The rest of the particles counted are between 1.4 and 7.0 (1400 to 7000 Nanometers).

The radiographic quality of emulsions of perfluoroctyl bromide after administration in animals and in man are shown in Figures 3, 4, and 5.

Figure 3 shows a radiophotograph of a rabbit after intravenous administration of the emulsion. This plate was taken 4 hours after slow administration of a 6:1 emulsion at a dose level of 5 ml/Kg. The spleen is clearly opacified. This usually occurs within 30 minutes and remains for 4 weeks. Histologic examination shows that opacification is due to reticulo-endothileal removal of the fluorocarbon particles. The liver does not pick up any fluorocarbon.

Figure 4 shows a bronchogram of a dog. The contrast agent is an 8:1 emulsion. This bronchogram was taken 10 minutes after endotracheal injection. We believe that the excellent detail is due to the resistance of the emulsion to flow and to its high coating tendency. Fourth and fifth generation branching are demonstrated.

Figure 5 shows a bronchogram of a human patient with a lung abcess in the middle lobe. Twenty-two ml of a 10:1 emulsion of L1913 was administered after informed consent was obtained. Topical anesthesia of the upper airway and tracheobronchial tree was obtained and a catheter was passed into the trachea under fluoroscopic control. Note the definition of the bronchial tree in the upper lobe which is shown to

Figure 3. Rabbit - four hours after slow administration of 6:1 emulsion 5 ml/Kg.

be normal. The middle lobe shows atelectasis. There is contrast mixing in the abcess which is situated anteriorly in the middle lobe. The air-fluid can be seen at the middle level of the abcess.

Figure 4. Dog - ten minutes after endotracheal injection of 8:1 emulsion.

CONCLUSION

This investigation has provided some insight into aspects of radiographic investigative activities in addition to radiography itself including the selection of a compound, the preparation of a dosage form, and the establishment of its physical properties.

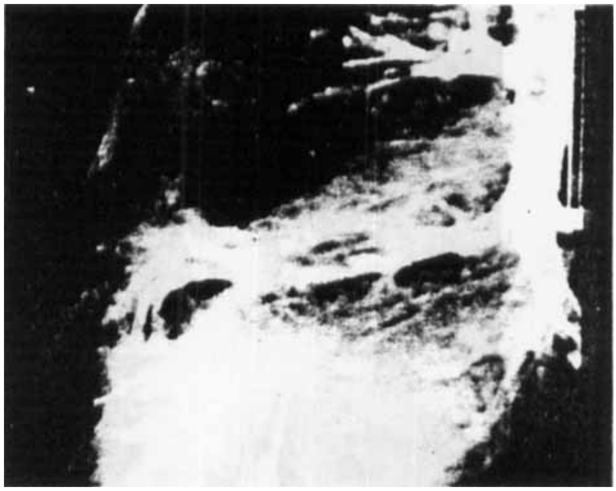


Figure 5. Man - after endotracheal administration of 22 ml 10:1 emulsion.

ACKNOWLEDGEMENTS

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