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EVALUATION OF FAT EMULSIONS PREPARED BY ULTRASONIC WAVES

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ABSTRACT

The stability of vegetable oil-based emulsions is influenced mainly by emulsifiers and the required hydrophilic-lipophilic balance (HLB) value. Oil-in-water emulsions containing 10% of soyabean, cottonseed, peanut or rapeseed oil were prepared by ultrasonic waves. Egg and soya phosphatidyl choline, bile salts, and polyoxyethylene-polyoxypropylene derivatives were used as emulsifiers and the required HLB values were determined accordingly. As a preliminary indication of stability, each freshly prepared emulsion was evaluated with respect to its organoleptic properties, pH and resistance to sterilization and centrifugation.

INTRODUCTION

Parenteral nutrition is now an essential tool in order to facilitate patient recovery from any situation restricting normal food intake. Fat emulsions included in parenteral nutrition, offer an important source of energy and essential fatty acids (1,2,3,4). Previous investigations indicated that, ideally, perfused fat emulsions should be identified as chylomicrons, so

that they can be transported throughout the blood stream, captured and utilized safely by the tissues (5,6,7,8). Thus, special considerations should be given to the droplet size distribution, the selection of emulsifiers and emulsifying technique, and the stability of the emulsions.

Few reports on special homogenization techniques in parenteral-administered emulsions were cited in pharmaceutical literature. Schurr (9) presented a high pressure homogenization technique for cottonseed oil. The performance of ultrasonic emulsification has been reported by Skauen and co-workers (10). It has been demonstrated that phosphatidyl cholines, as natural emulsifiers, produce well stabilized emulsions (11,12). However, earlier studies showed that their administration to animals produced many toxic or adverse reactions (11,13,14,15). Experiments with bile salts produced emulsions which were unstable during autoclaving (16). Other studies relating to synthetic emulsifiers indicated that polyoxyethylene-polyoxypropylene derivatives[1] (I) are convenient for parenteral use (17, 18).

Very little work has been done or suggested to guide rationally the pharmaceutical formulator in dealing with perfused fat emulsions, particularly in the choice of homogenization techniques and emulsifiers. It is the purpose of this paper to investigate the preparation of fat emulsions using ultrasonic waves, and to evaluate the effect of different emulsifier systems on the emulsion characteristics.

EXPERIMENTAL

MATERIALS

Four vegetable oil-based emulsions were prepared using natural and synthetic emulsifiers. Oils, emulsifiers and other ingredients are given in Table I. They are classified according to the different emulsifying systems studied and the formula of each emulsion. HLB values for all emulsifiers were obtained from the manufacturer (for (I)) or calculated by the following formula:

$$HLB = \frac{\text{molecular weight of the hydrophilic portion} \times 100}{\text{total molecular weight} \times 5}$$

EMULSIFICATION METHOD

Aqueous and oil phases were heated separately to 95°C. The aqueous phase was then added to the oil phase and blended using mechanical stirring for 45 min. Two hundred grams of this pre-homogenized mixture was then submitted to ultrasonic waves[2] in a 400 ml becher. Figure 1 represent a scaled-drawing of the system used for sonification. Previous studies on the sonification conditions indicated that the maximum size reduction, without overexposure to ultrasonic irradiation, was obtained when the pre-homogenized mixture was placed in an ice bath and radiated at 16 kHz for 12 minutes. These conditions were kept constant through all the study. The emulsions were autoclaved[3] at 121°C, 15 psi for 15 minutes.

MEASUREMENT OF ORGANOLEPTIC PROPERTIES

Visual appearance of each freshly prepared and autoclaved emulsion was examined. Emulsion resistance to creaming and coalescence was tested by subjecting the systems to centrifugation[4] at 6000 rpm for 20 minutes in 12 mm diameter tubes. The pH[5] was monitored before

TABLE 1

Emulsifying Systems and Formulas of the Emulsions

Emulsifying Systems	Oils 10%	Formulas
(II)[8]	Soyabean[13] Cottonseed[13] Peanut[13] or Rapeseed[13]	Emulsifiers: 1,5% 3% or 5% Dextrose[14] : 4% Water qs 100% HLB values: 6 to 12
(III)[9]	Soyabean Cottonseed Peanut or Rapeseed	Emulsifiers: 1,5% 3% or 5% Dextrose : 4% Water qs 100% HLB values: 6 to 11,5
(IV)[10]	Soyabean Cottonseed Peanut or Rapeseed	Emulsifiers: 1,5% 3% or 5% Dextrose : 4% Water qs 100% HLB values: 7 to 13
(V)[11]	Soyabean Cottonseed Peanut- or Rapeseed	Emulsifiers: 1,5% 3% or 5% Dextrose : 4% Water qs 100% HLB values: 7 to 13
(VI)[12]	Soyabean Cottonseed Peanut or Rapeseed	Emulsifiers: 1,5% 3% or 5% Dextrose : 4% Water qs 100% HLB values: 8 and 9

and after autoclaving. Droplet size was determined from microphotographs[6] and compared with a commercially available fat emulsion[7].

RESULTS AND DISCUSSION

In formulation studies involving emulsions, a close following-up of the change in organoleptic properties during the different phases of processing offers important informations. Observations on colour,

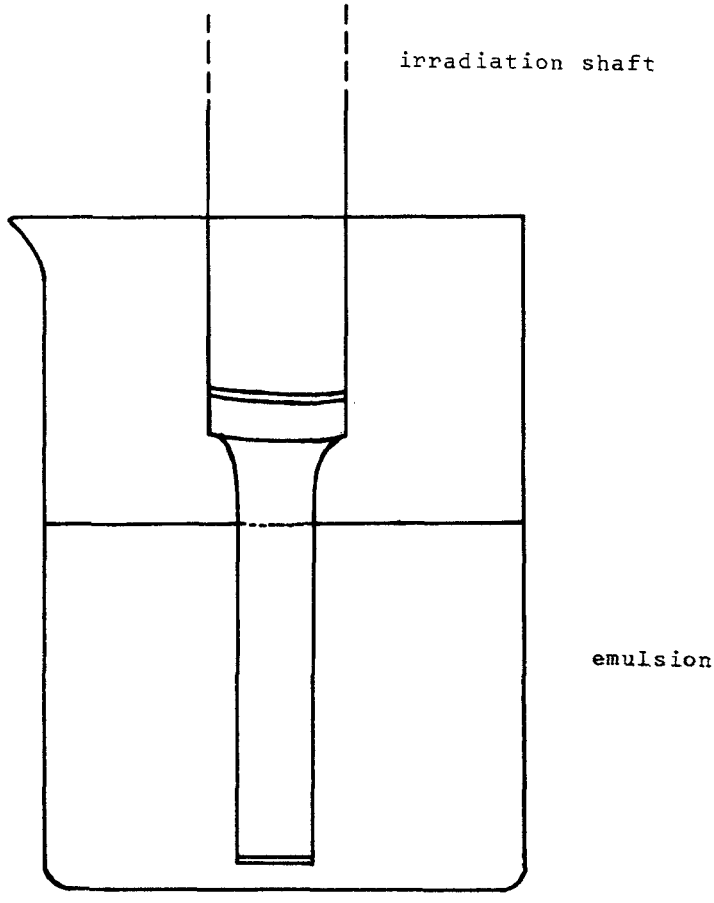

FIGURE 1

Scaled Drawing of the System Used for Sonification

coalescence and separations, and precipitation are essential before making detailed stability studies and stability predictions. Table 2 gives the significance of symbols used in the forthcoming tables which characterize and specify these properties.

In all systems, the white appearance of the emulsions (W) was observed and evaluated in a comparative

TABLE 2

Significance of the Symbols Used in the Tables

A:	Aggregates formed
B:	Slight browning of the emulsion
C-S:	Coalescence and separation
E.S.:	Emulsifying system
F:	Foaming
H:	Homogenous
N:	No change in organoleptic properties
O:	Oil droplets at the surface
P:	Brown precipitate at the surface
S:	Separation of the phases
W:	White appearance

manner as seen in the tables. This visual examination gives in some way, a qualitative indication for the size reduction. From a practical point of view, Table 3 to 8 summarize a certain number of results while comments are made on other trials. Table 3 and 4 represent the results obtained with emulsifying system (II). Table 3 shows the results for soyabean oil, while table 4 illustrates those obtained for peanut and rapeseed oils. In this table, only the most representative HLB values are shown. For that particular emulsifying system, however, no satisfactory emulsion was obtained with cottonseed oil; after sonification, particles of (II) and sometimes oil droplets were present at the surface of these emulsions. In that case, other parameters were not evaluated. Table 5 and 6 show the results obtained with emulsifying system (III) for the four oils tested. However, only three HLB values, 7, 8 and 9, are preseented for peanut, cottonseed and rapeseed oils. Table 7 and 8 illustrate the results for emulsifying system (IV) and (V), for the four oils tested. Again here, only three HLB values, 8, 9 and 10, are presented. It can be pointed out that the critical HLB values differ from

Table 3 . Emulsifying System (II), Soyabean Oil

SOYABEAN OIL: 10%

	HLB / E.S.	6	7	8	9	10	11	12
ORGANOLEPTIC PROPERTIES	1,5%	W^{++} A	W^{++} H	W^{++} H	W^{++} H	W^{+++} H	W F	W O
	3,0%	W^{++} H	W^{++} H	W^{++} H	W H	W H	W^{++} H	W O
	5,0%	W^{++} A^{++}	B H	B H	B H	B H	B H	W O
STERILIZATION	1,5%	O	N	N	N	N	N	-
	3,0%	N	N	N	N	N	N	-
	5,0%	-	P	P	P	P	P	-
CENTRIFUGATION (cm)	1,5%	1,0	1,0	0,8	0,5	0,5	0,5	-
	3,0%	1,0	1,0	1,0	0,5	1,0	1,5	-
	5,0%	-	0	0	0	0	0	-
pH	1,5%	6,65	6,75	6,75	5,75	6,10	6,65	-
	3,0%	6,35	6,55	6,55	6,55	6,60	5,65	-
	5,0%	-	6,40	6,40	5,50	6,45	6,40	-

Table 4. Emulsifying System (II), Peanut and Rapeseed Oils

	10%of:	Peanut Oil			Rapeseed Oil		
	HLB / E.S.	7	8	9	7	8	9
ORGANOLEPTIC PROPERTIES	1,5%	W O	W H	W H	W^{+++} H	W^{+++} O	W^{+++} O
	3,0%	W A	W A	W H	W^{++} O	W^{++} F-O	W^{++} H
	5,0%	B H	B H	B H	B^{+} O	W H	B F-H
STERILIZATION	1,5%	P	P	P	N	O	O
	3,0%	-	O-P	O-P	O	O	N
	5,0%	N	O-P	O-P	O	N	O
CENTRIFUGATION (cm)	1,5%	1,0	1,0	1,0	1,5	1,5	1,5
	3,0%	-	1,0	1,0	1,5	1,5	1,5
	5,0%	S	S	1,0	2,0	1,5	5,0
pH	1,5%	6,65	5,80	6,35	6,75	6,75	6,75
	3,0%	-	6,55	6,05	6,65	6,65	6,65
	5,0%	6,45	6,50	6,05	6,50	6,25	5,50

Table 5. Emulsifying System (III), Soyabean Oil.

SOYABEAN OIL: 10%

	HLB / E.S.	6	7	8	9	9,5	10,5	11,5
ORGANOLEPTIC PROPERTIES	1,5%	W^{+++}	W^{+++}	W^{+++}	W^{++}	W^{++}	W^{++++}	W
		A	A	O	O	O	H	O
	3,0%	W^{+}	W^{+}	W^{+}	W	W^{++}	W^{++}	W
		O	O	O	O	H	O	O
	5,0%	W	W	W	W	W	W	W
		H	H	H	O	O	O	O
STERILIZATION	1,5%	O	O	O	O	O	O	-
	3,0%	O	O	O	O	O	O	O
	5,0%	O	O	N	O	-	O	O
CENTRIFUGATION (cm)	1,5%	1,5	1,5	1,5	1,5	1,0	1,0	-
	3,0%	1,5	1,5	1,5	1,5	1,5	1,0	1,0
	5,0%	S	S	S	S	-	S	S
pH	1,5%	6,65	6,55	6,15	6,15	6,00	6,40	-
	3,0%	6,55	6,45	6,50	6,45	6,45	6,40	6,35
	5,0%	6,55	6,55	6,55	6,55	-	6,50	6,45

Table 6. Emulsifying System (III), Peanut, Rapeseed and Cottonseed Oils

10% of:		Cottonseed oil			Peanut oil			Rapeseed oil		
HLB / E.S.		7	8	9	7	8	9	7	8	9
ORGANOLEPTIC PROPERTIES	1,5%	B O	B O	B O	W O	W H	W H	W++ O	W++ O	W++ O
	3,0%	B H	B H	B H	W++ H	W++ H	W++ H	W++ F-H	W++ F-H	W++ H
	5,0%	B H	B H	B O	W H	B H	B H	B O	B O	B O
STERILIZATION	1,5%	O	O	O	-	N	N	O	O	O
	3,0%	O	O	O	O	O	O	O	O	O
	5,0%	O	O	O	O	O	O	-	-	-
CENTRIFUGATION (cm)	1,5%	O	O	O	-	1,5	1,5	S	1,5	1,5
	3,0%	O	O	O	S	1,5	1,5	1,0	1,0	1,5
	5,0%	O	O	O	S	S	S	-	-	-
pH	1,5%	6,65	6,65	6,25	-	6,25	5,95	6,65	6,55	6,70
	3,0%	6,55	6,55	6,55	6,15	6,45	6,35	6,55	6,55	6,95
	5,0%	6,35	6,25	6,25	6,35	6,45	6,35	-	-	-

Table 7. Emulsifying System (IV), Soyabean, Peanut, Rapeseed and Cottonseed Oils.

	10% of : Soyabean oil			Cottonseed oil			Peanut oil			Rapeseed oil		
	HLB	8	9	10	8	9	10	8	9	10	8	9	10
	E.S.												
ORGANOLEPTIC PROPERTIES	1,5%	W++++			W++++			W++++			W++++		
	3,0%	O			O			O			O		
	5,0%												
STERILIZATION	1,5%	C - S			C - S			C - S			C - S		
	3,0%												
	5,0%												
CENTRIFUGATION (cm)	1,5%	-	-	-	-	-	-	-	-	-	-	-	-
	3,0%	-	-	-	-	-	-	-	-	-	-	-	-
	5,0%	1,3	1,0	0,8	1,3	1,4	1,0	1,4	1,3	1,4	1,5	1,4	1,3
pH	1,5%	-	-	-	-	-	-	-	-	-	-	-	-
	3,0%	-	-	-	-	-	-	-	-	-	-	-	-
	5,0%	4,7	5,3	5,1	4,7	5,2	5,2	5,0	5,0	4,9	4,8	5,1	4,8

Table 8. Emulsifying System (V), Soyabean, Peanut, Rapeseed and Cottonseed Oils

	10% of: Soyabean oil			cottonseed oil			peanut oil			rapeseed oil		
E.S. / HLB	8	9	10	8	9	10	8	9	10	8	9	10
ORGANOLEPTIC PROPERTIES 1,5%	W++++			W++++			W++++			W++++		
ORGANOLEPTIC PROPERTIES 3,0%	O			O+			O			O		
ORGANOLEPTIC PROPERTIES 5,0%												
STERILIZATION 1,5%												
STERILIZATION 3,0%	O			-			O			O		
STERILIZATION 5,0%												
CENTRIFUGATION (cm) 1,5%	1,1	1,5	1,1				1,0	1,0	1,0	1,1	1,0	1,1
CENTRIFUGATION (cm) 3,0%	1,4	1,1	1,5	-			0,8	1,0	0,8	1,1	0,8	0,7
CENTRIFUGATION (cm) 5,0%	1,1	1,9	1,5				0,8	0,8	0,8	1,1	1,2	0,8
pH 1,5%	8,4	8,5	8,6				8,2	8,4	8,4	8,4	8,5	8,6
pH 3,0%	8,4	8,4	8,3	-			8,4	8,4	8,3	8,2	8,3	8,5
pH 5,0%	8,1	8,2	8,9				8,3	8,3	8,3	8,1	8,2	8,3

one unit between the systems. This variation is not significant since the precision of the values is ± 1. The tests for cottonseed oil with emulsifying system (IV) were not carried out since a large quantity of oil was present at the surface immediately after emulsification. No satisfactory emulsion was obtained with emulsifying system (VI); all emulsions, for all the oils, were not homogenous and were brownish. Aggregates of egg yolk phosphatidyl choline and oil layers were observed at the surfaces.

Sterilization by autoclaving is an important step in the preparation of emulsions intended for parenteral use, where no preservatives can be added. Resistance of the emulsion to autoclaving is an important requirement. Emulsions made with soyabean oil and emulsifying system (II), demonstrated high resistance to thermal and pressure effects (Table 3). The same emulsifying system (II), with rapeseed oil, gave relatively satisfactory results. On the other hand, in other emulsifying systems, large droplets of oil were observed at the surface and a complete separation occured with system (IV). Preparations containing dextrose (and similar carbohydrates) and phosphatidyl choline can lead to the formation of a brown precipitate after autoclaving (19). For this reason, observations and recording of such changes were made, and they appear in tables 3 to 8. It can be seen only with 5% of emulsifying system (II), at all HLB values, for soyabean oil, and is more generalized for peanut oil with the same emulsifying system (II). From results obtained, it seems that not only the dextrose - phosphatidyl choline association is responsible for the reaction, but also the type of oil and the relative concentration of the emulsifying agents.

In this study, accelerated creaming, produced by centrifugation has been regarded as a sign of future instability. The best result was obtained for soyabean oil with emulsifying system (II), where the creaming observed ranged from 0,5cm to 1,0cm with one exception. For other systems, higher creaming values were observed and sometimes complete separation took place.

Physiological pH is an important requirement in perfused solutions. Previous investigations showed that synthetic soyabean oil emulsions undergo spontaneous hydrolysis during storage, with the production of free fatty acids (15). This hydrolysis is minimum at pH 7. In this study, with the exception of systems (IV) and (V), the pH was about 6,5. Synthetic emulsifiers alone (systems (IV)), gave a pH of around 5, while systems containing cholate (V), gave values around 8,3. Experiments on the complete characterization of pH-time-dependence are now under study.

Photomicrographs were taken for some emulsions and are presented in figure 2 to 5. Figure 2, 3 and 4 show the result for soyabean, peanut and rapeseed oils respectively. Emulsifying system (II), at 1,5% and HLB value of 8, was selected in all cases. Complete characterization of size distribution is now under study. A picture taken under the same conditions, for a commercialized emulsion, is presented in figure 5.

CONCLUSION

Using ultrasonic wave technique, a series of fat emulsion formulations were prepared. The organoleptic properties and physical changes during centrifugation and after autoclaving were closely observed and recorded. The best emulsifying system is an association of soyabean phosphatidyl choline and polyoxyethylene-poly-

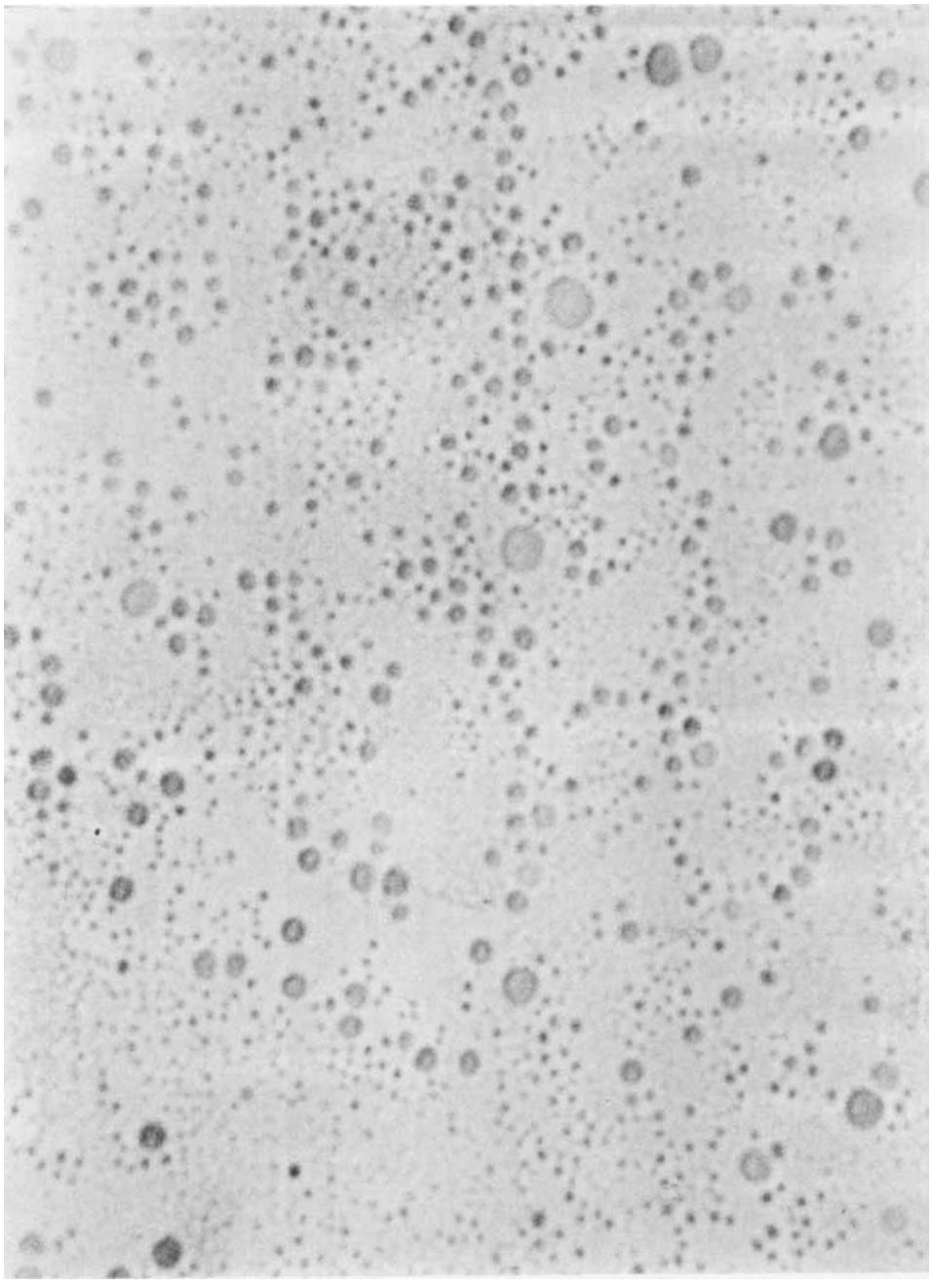

FIGURE 2

Photomicrograph of Soyabean Oil Emulsion with Emulsifying System (II) at 1,5% (original magnification - 1,0mm = 0,385 µm, present magnification - 1,0mm = 0,327 µm).

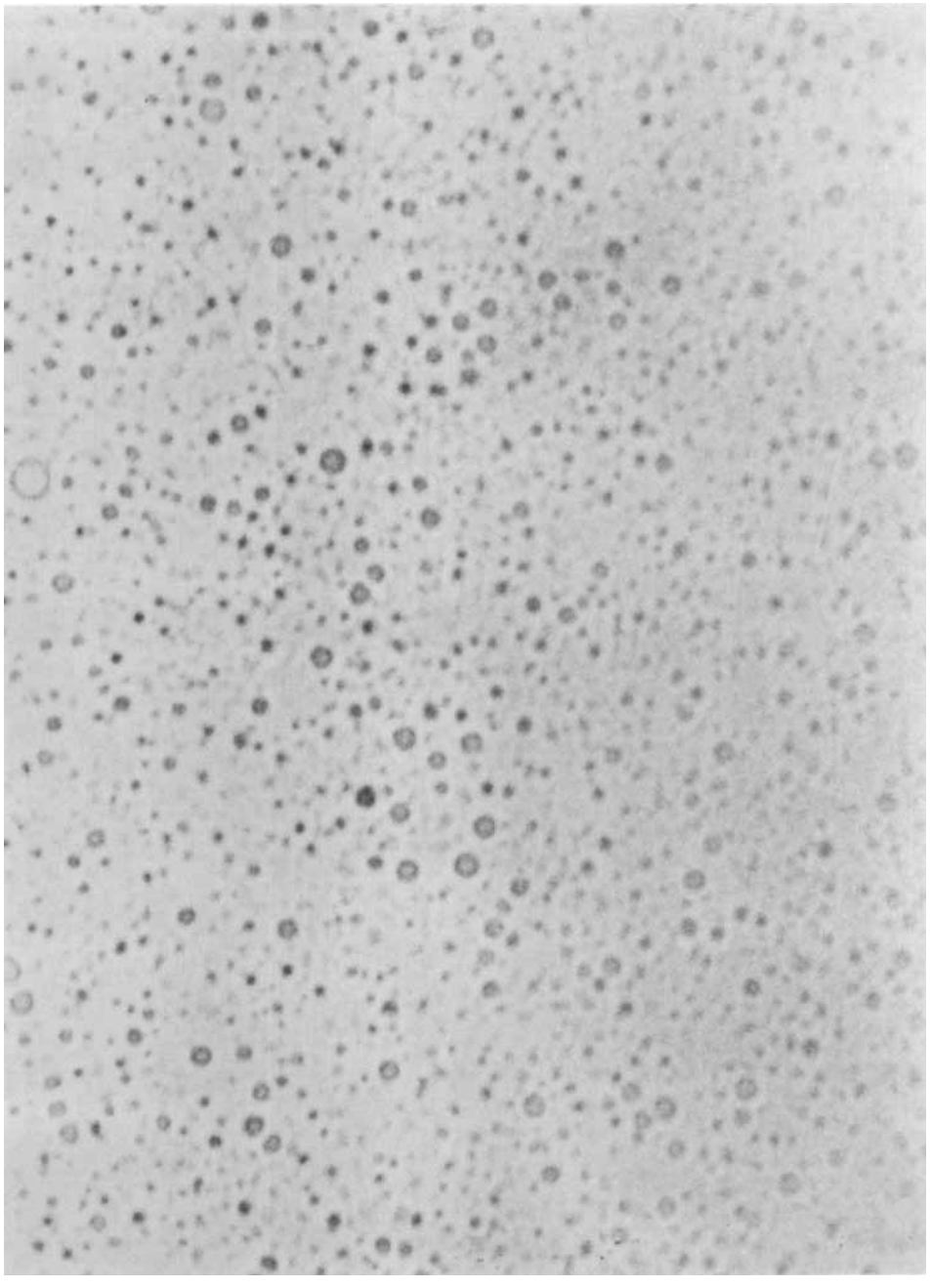

FIGURE 3

Photomicrograph of Peanut Oil Emulsion with Emulsifying System (II) at 1,5% (original magnification - 1,0mm = 0,322 µm, present magnification - 1,0mm = 0,274 µm).

FIGURE 4

Photomicrograph of Rapeseed Oil Emulsion with Emulsifying System (II) at 1,5% (original magnification - 1,0mm = 0,376 µm, present magnification - 1,0mm = 0,320 µm).

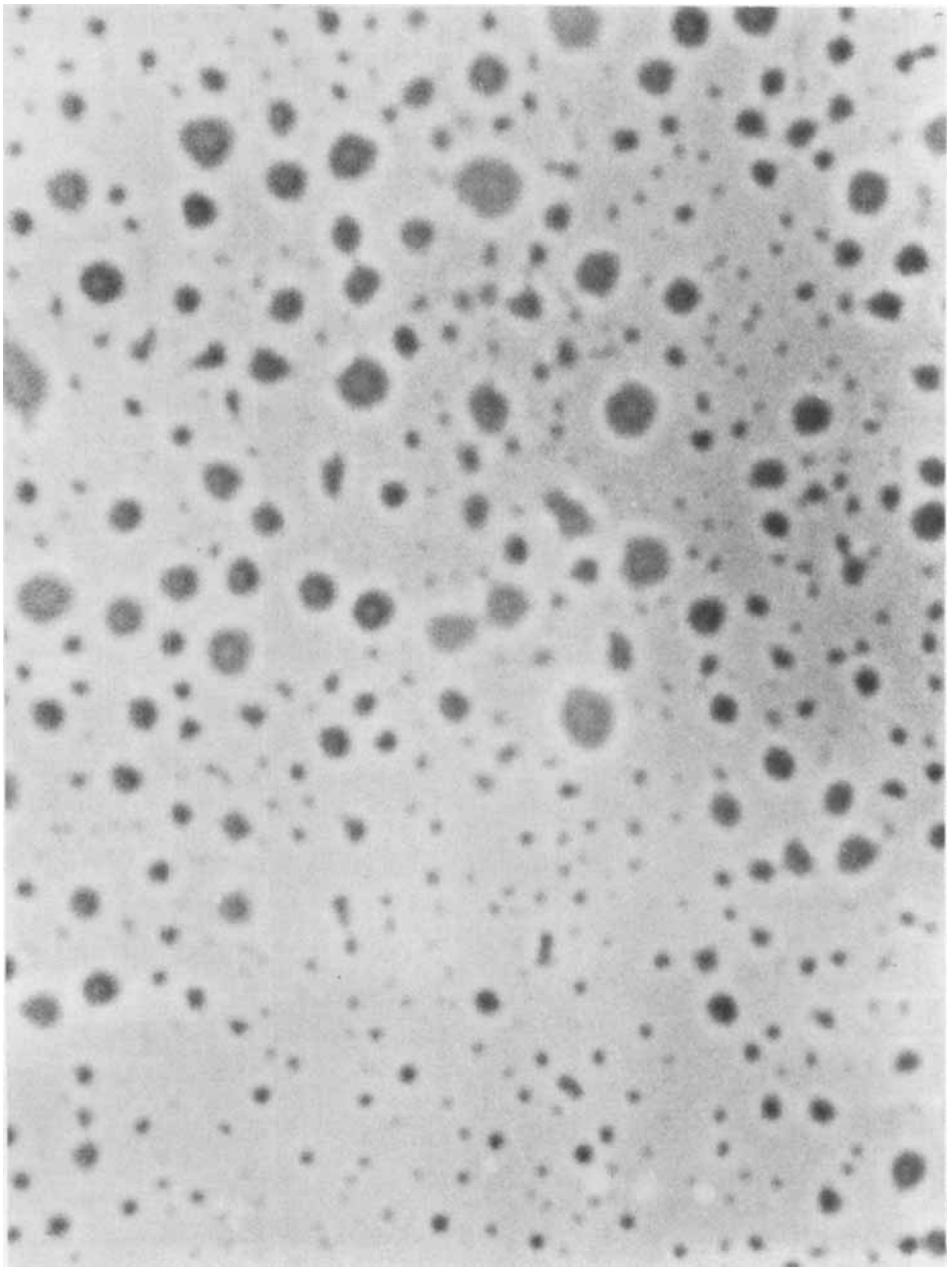

FIGURE 5

Photomicrograph of a Commercialized Fat Emulsion (original magnification ~ 1,0mm = 0,330 μm, present magnification - 1,0mm = 0,281 μm).

oxypropylene derivative. Soyabean oil emulsions prepared with 1,5% and 3% emulsifier were the most satisfactory emulsions obtained for HLB values between 7 and 11. Other emulsifying systems gave less acceptable results or did not yield satisfactory emulsions. These preliminary findings constitute the first part in our program on fat emulsion for parenteral alimentation. Further work to quantitate the effect of formulation factors and processing variables on the stability and compatibility of the systems with other ingredients is underway.

ACKNOWLEDGEMENTS

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FOOTNOTES

1 Pluronics(R), BASF, Wyandotte Corporation, Michigan

2 Sonifier-cell disruptor, model W140D (20 kHz), Branson Sonic Power Co., U.S.A.

3 Barnstead Laboratory Sterilizer, Fisher Sci.,Montreal

4 Sorval Centrifuge, model GLC-1, Ingram & Bell, Ltd, Montreal

5 Metrohm model E512, Fisher Sci., Montreal

6 Photomicroscope II, Carl Zeiss Canada Ltd, Montreal

7 Intralipid(R), Pharmacia Canada Ltd, Montreal

8 Emulsifying system (II): soyabean phosphatidyl choline (as L-alpha-phosphatidyl choline, Sigma Chemicals, St-Louis, U.S.A.) and Pluronic type F_{68}.

9 Emulsifying system (III): soyabean phosphatidyl choline and Pluronic type P_{65}

10 Emulsifying system (IV): Pluronics type F_{68} and L_{62}.

11 Emulsifying system (V): Sodium cholate (as cholic acid sodium salt, Matheson Coleman & Bell, Norwood, Ontario) and Pluronic type F_{68}.

12 Emulsifying system (VI): Egg yolk phosphatides (BDH Chemicals, LTd, Poole, England) and Pluronic type F_{68}.

13 Kindly supplied by Canada Packers, Montreal, Quebec

14 Anhydrous, ACS, Fisher Sci., Montreal

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