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The use of supports in the lyophilization of oil-in-water emulsions

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The results of drying oil-in-water emulsions by lyophilization have shown this process could be applied to emulsions having dissolved in the aqueous phase a solid material which acts as support for the oily phase after the water has been removed. The rate of creaming and the globule size distribution were also investigated. The tests were made before and immediately after lyophilization, and after 20 and 40 days of storage at room temperature (18–20°) at 0° ±1° and at 40° ±1°. The emulsions were coarser and creaming rate was increased after lyophilization.

THE preparation and the stability of emulsions are problems of primary interest (Becher, 1965; Garrett, 1965). The instability of emulsions is mainly characterized by creaming and coalescence, processes arising especially during ageing and storage. In 1960, Richter & Steiger-Trippi examined the drying of emulsions by spray-drying techniques. Drying emulsions so that they can be reconstituted when required may solve some of the problems of ageing and storage. We report a new approach to the drying of emulsions. Some of the properties of the resultant product have also been investigated.

Experimental

PREPARATION OF EMULSIONS

The internal phase was liquid petrolatum (U.S.P. XVII) and the external phase recently distilled water. Sorbic acid was the preservative (50 mg % w/w).

Mixtures of Tweens and Spans were used as emulsifying agents. Previous experiments with these agents showed that a mixture of polysorbate 80 (HLB 15) and sorbitan mono-oleate (HLB 4·3) 62·6 and 37·4% respectively was suitable. This mixture was used at 20% of the oil phase.

USE OF SUPPORTS

Two types of supports were used:

(*a*) *Crystalline.* D-(−)-mannitol (BP 1958), urea (Hopkin and Williams Ltd.), glycine (Riedel), sorbitol (Hopkin & W. Ltd.), glucose (pharmaceutical grade), sucrose (pharmaceutical grade), lactose (Merck Sharp & Dohme).

(*b*) *Colloidal.* Sodium alginate (B.D.H.), polyvinylpyrrolidone, bentonite, acacia, aerosil (Pharmaceutical grade), hydroxyethylcellulose (Cellosize WP-4400 Union Carbamide & Chemical Co.).

The crystalline supports and polyvinylpyrrolidone were used at concentrations of 13·3%; the colloidal type at concentrations of 1 or 2%, except the acacia which was used at 5%. The supports were used singly or in admixture. When the support was of the gel type, it was allowed to swell in water for 12 hr.

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METHOD OF PREPARATION OF EMULSIONS

Both phases of the emulsion were heated separately in a water bath at 70° and premixed with a stirrer at approximately 4,000 rev/min for 5 min. The resulting coarse emulsion was then passed seven times at high pressure through an ultrasonic homogenizer (Minisonic Four Homogenizer "Ultrasonics Ltd.").

Basic formula of the emulsion. Liquid petrolatum, 10%; polysorbate, 1·25%; sorbitan mono-oleate, 0·75%; sorbic acid, 0·05%; support, q.s.; distilled water to make 100 g.

LYOPHILIZATION

Glass equipment was used for the lyophilization (Lyophilizer and Vapour Trap Quickfit, catalogue No. M.F.-45). A high vacuum pump was attached to this equipment. The lyophilization was effected in 500 ml flasks with 80 g of emulsion in each.

The freezing was done by the "shell-freezing" procedure (Calcagno, 1962; Rey, 1960), rotating the flasks at constant speed during 20 min in a freezing bath containing solid carbon dioxide and ethanol at −70°.

Primary drying was done during periods of 8 to 14 hr, using the freezing mixture previously described in the condenser. Secondary drying was effected in a dryer with phosphorous pentoxide at an approximate vacuum of 0·02 mm Hg for 5 days, changing the phosphorous pentoxide, if necessary, every 24 hr.

After the secondary drying, the lyophilized powder was placed in tightly closed and sealed containers of approximately 500 ml. A current of carbon dioxide was passed into the containers before the sealing. They were stored at room temperature, at 0° ±1° and at 40° ±1°.

CONTROLS

The following controls were used:

Moisture. This was determined immediately after lyophilization by drying 2 or 3 g at 105° in an oven for 24 hr.

The following tests were made on the product immediately after lyophilization and again after 20 and 40 days of storage at three different temperatures.

Reconstitution. This test was according to Lachman & Chavkin (1957). To a known amount of powder the corresponding amount of water was added, after 1 min the mixture was shaken for 15 sec. The shaking was alternated with 15 sec periods of rest. If the product assumed the nature of the original emulsion within the first 15 sec of shaking it was considered to be instantaneously reconstituted (I); if 2 to 4 periods of shaking were needed to achieve this state, it was considered good (G); if the emulsion was obtained after 4 periods of shaking, it was considered fair (F) and if the product did not take on the characteristics of the original emulsion, it was considered poor (P).

Creaming rate. To do the test, enough emulsion was placed in tubes 40 cm long to form a liquid column of 30 cm. Three drops of a 1%

solution of amaranth were added to give a better visualization of the process (Appino, Christian & Banker, 1962). The creaming was determined by measuring the height of the separate layer in the upper part of the tubes. It was expressed in cm/24 hr (Peck, De Kay & Banker, 1960). If a separation of a clear and transparent layer in the lower part of the tubes was observed, the creaming rate was related to measurement of the clear layer (Richter & Steiger-Trippi, 1960).

Method of globule measurements. A microscope with built-in light source (Laborlux Ernst Leitz GmBH Wetzlar), a micrometer (Leitz Wetzlar 12·5×), a cavity slide and an immersion lens were used.

A dilution 1/200 with an aqueous solution of propylene glycol at 75% (v/v) was used (Levius & Drommond, 1953; Mullins & Becker, 1956; Peck, DeKay & Banker, 1960), using a micro-calibrated pipette. In each determination, the diameter of 500–1,000 globules was measured. Ten fields were read on each sample, following the order proposed by Richter and Steiger-Trippi. But according to Münzel, Büchi & Schultz (1959) instead of counting 3 globules for each position, all those globules that fell on the scale of the micrometer and its surroundings were counted.

TABLE 1. CHARACTERISTICS OF THE EMULSIONS

Emulsion No.	Petrolatum (%)	Supports	Moisture %	RC	Mean globule diameter (μ) ± s.e.							
					Original emulsion	Reconstituted emulsion	20 days storage			40 days storage		
							R.T.	40° C	0° C	R.T.	40° C	0°C
1	10	Mannitol	0·20	I	1·66 ±0·012	2·47 ±0·026	2·76 ±0·035	2·46 ±0·027	3·10 ±0·028	2·37 ±0·022	2·26 ±0·022	2·35 ±0·017
2	10	Glycine	0·30	I	1·61 ±0·011	2·44 ±0·030	2·08 ±0·028	2·24 ±0·038	2·12 ±0·032	2·56 ±0·047	2·43 ±0·034	2·10 ±0·025
3	10	Urea	0·21	G	1·99 ±0·016	2·04 ±0·027	2·59 ±0·046	•	2·29 ±0·037	3·09 ±0·063	•	2·45 ±0·032
4	10	H.E.C. Mannitol	0·52	F	2·02 ±0·025	2·22 ±0·023	2·71 ±0·039	2·54 ±0·034	2·34 ±0·025	2·69 ±0·033	2·58 ±0·034	2·40 ±0·031
5	10	H.E.C. Glycine	0·57	F	2·27 ±0·040	1·99 ±0·020	2·42 ±0·028	2·90 ±0·032	2·29 ±0·023	2·33 ±0·021	2·79 ±0·050	2·39 ±0·024
6	10	H.E.C. Urea	0·41	G	2·00 ±0·029	2·43 ±0·036	2·57 ±0·035	2·25 ±0·029	2·32 ±0·025	2·45 ±0·035	2·37 ±0·025	2·27 ±0·025
7	10	H.E.C. Aerosil	0·30	P	1·86 ±0·016	2·41 ±0·031	2·65 ±0·040	2·60 ±0·038	2·10 ±0·027	2·72 ±0·040	2·69 ±0·040	2·34 ±0·030
8	10	Sodium Alginate	0·41	P	2·19 ±0·019	2·37 ±0·024	2·26 ±0·025	2·34 ±0·031	2·14 ±0·025	2·44 ±0·032	2·43 ±0·031	2·40 ±0·026
9	10	Aerosil Mannitol	5·30	G	2·28 ±0·047	2·31 ±0·037	2·12 ±0·033	2·30 ±0·037	1·76 ±0·024	2·17 ±0·022	2·22 ±0·031	1·95 ±0·019
10	10	H.E.C. Aerosil Mannitol	2·95	P	2·34 ±0·031	2·46 ±0·035	2·41 ±0·034	2·64 ±0·043	2·23 ±0·025	2·60 ±0·035	2·51 ±0·033	2·34 ±0·019
11	15	Aerosil Mannitol	3·40	G	2·56 ±0·050	3·24 ±0·070	2·54 ±0·054	2·86 ±0·061	2·33 ±0·044	2·62 ±0·039	2·84 ±0·052	2·39 ±0·031
12	20	Aerosil Mannitol	4·87	P	2·19 ±0·051	3·25 ±0·085	3·15 ±0·066	2·69 ±0·049	2·39 ±0·051	2·95 ±0·061	3·85 ±0·088	2·74 ±0·041

R.C. = Reconstitution characteristics. I = Instantaneous. G = Good. F = Fair. P = Poor.
• Melted at storage conditions.
H.E.C. = Hydroxyethylcellulose.
R.T. = Room temperature (18–20° C).

The globules were counted after leaving the preparation resting for 15 to 30 min to obtain stabilization (Levius & Drommond, 1953).

Results and discussion

Table 1 summarizes the results of the lyophilization. Dry products were obtained when the emulsions contained a substance which acted as support.

A series of emulsions containing a mixture of aerosil (2%) and mannitol (13·3%) as supports were examined. The emulsions contained 5, 10, 15, 20 and 25% of liquid petrolatum. With the 5 and 10% concentrations of oil in the emulsion a dry and powderable product was obtained. In emulsions containing 15% of oil, the product was unctuous, and it was even more so when petrolatum was used. Emulsions with 25% of oil phase broke during the lyophilization. Emulsions with a high proportion of oil phase seemed to need either more support or the use of another type.

Results with some supports like mannitol, glycine and urea are summarized in Table 1. Lyophilization failed with the other crystalline supports, the emulsions breaking during the process.

Colloidal materials were used to try to solve some of the negative results obtained with crystalline supports. When sodium alginate was used, a dry, tasteless and yellowish product which could be slowly redissolved in water was obtained. With other colloidal supports, the emulsions broke during the process.

Mixtures of colloidal and crystalline supports were also studied. The results are shown in Table 1.

Creaming rate. The emulsions containing crystalline supports showed a net creaming layer in the upper part of the testing tube. Lyophilization produces an increase in the creaming rate. The behaviour of emulsion 2 is shown in Fig. 1.

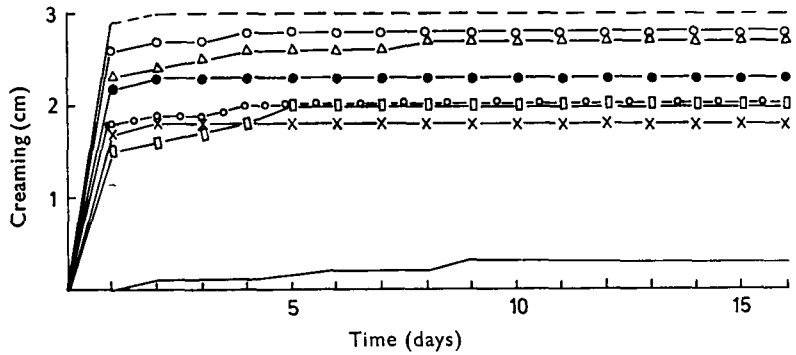

FIG. 1 Effect of time on creaming for emulsion 2 under several conditions of storage. (———) Original emulsion. (– – –) Reconstituted immediately after lyophilization. (-○-○-○-) Reconstituted after 20 days storage at room temperature. (-×-) Reconstituted after 40 days storage at room temperature. (-●-) Reconstituted after 20 days storage at 40°C. (-△-) Reconstituted after 20 days storage at 0°C. (-○-) Reconstituted after 40 days storage at 40°C. (-□-) Reconstituted after 40 days storage at 0°C.

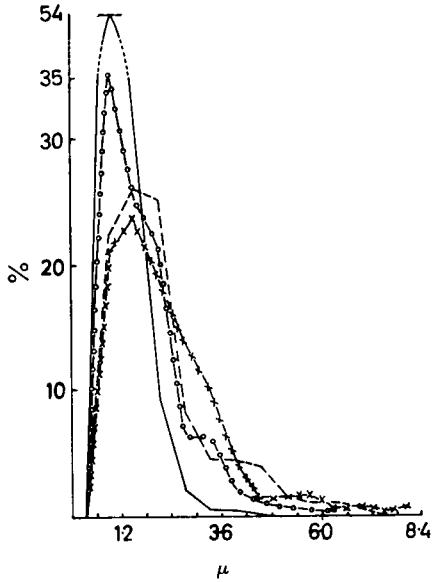

FIG. 2. Globule size distribution of emulsion 2 under several conditions of storage. (———) Original emulsion. (– – –) Reconstituted immediately after lyophilization. (–○–○–○–) Reconstituted after 20 days storage at room temperature. (–×–×–) Reconstituted after 40 days storage at room temperature.

The emulsion containing colloidal supports presented a clear layer at the bottom of the tube, in accordance with the results reported by Richter & Steiger-Trippi (1960), and the lyophilized emulsions showed a higher creaming layer than the unlyophilized preparations. A similar behaviour was observed when the emulsion contained both crystalline and colloidal supports.

It was observed that storage produced changes in the consistency and the colour of the lyophilized product. These effects were enhanced at high temperatures. For example, emulsions stored at 40° and containing hydroxyethyl cellulose greatly increased in consistency. When the support was urea, the product melted. All the emulsions stored at 40° became yellowish with time. These effects may indicate that the lyophilized products should be stored at low temperatures.

PARTICLE SIZE DISTRIBUTION

Table 1 shows the mean diameter of the particles of each emulsion and Fig. 2 shows the particle size distribution of emulsion 2. Both sets of data reveal that lyophilization produced a net increase in the mean diameter of the particle along with a displacement to higher particle size of the distribution curve.

Statistical studies (Bancroft, 1961) using Student's *t*-test showed significant differences in particle size of the original emulsions and those immediately reconstituted after lyophilization except for emulsions 3 and 9.

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