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RESEARCH PAPER

Preparation of Polymeric Microcapsules: Formulation Studies

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ABSTRACT

Air-filled microcapsules were prepared by freeze-drying different oil-in-water emulsions containing biodegradable polyester as the wall-forming material. The aim of this work was to find an acceptable formulation with respect to the microcapsule suspension and the stability of the emulsion during the production process. The influence of various formulation parameters (concentrations of mannitol, polymer, and surfactant; pH; oil-in-water phase ratio) was investigated in a factorial design. The results were treated by ordinary least-square (OLS) regression and partial least-square regression (PLSR). In a previous work, air-filled microcapsules were successfully made using human serum albumin as the surfactant in the emulsion (1). In the present work, a new block copolymer based on poly(ethylene glycol) (PEG) was implemented as the surfactant to replace human serum albumin. It was found that the new block copolymer is a suitable replacement for human serum albumin. The concentration of the polymer in water and the concentration of the surfactant in the oil phase and the interaction between these variables had a significant influence on the stability of the emulsion at 60°C. A surfactant concentration of approximately 2% (w/v) in water was necessary when the concentration of the wall-forming polymer was below 5% (w/v) in (-)-camphene. The concentration of the polymer in the oil phase influenced the yield, measured as the volume concentration of particles in suspension per milligram of polymer added and as acoustic effect per milligram of polymer. Low levels of polymer concentration in (-)-camphene (<5% w/v) gave the highest yield. Excess polymer in the oil phase did not form microcapsules, but precipitated in the suspension or was included in the wall of

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the microcapsules. Addition of mannitol protected the microcapsules from being destroyed during freeze-drying and resulted in freeze-dried products with few cracks, little shrinkage, and higher suspension yield.
Key Words: *Emulsion; Formulation; Microcapsules; Statistical design; Ultrasound contrast agent.*

INTRODUCTION

It has been known for many years that air-filled particles are effective contrast agents for ultrasonography (2,3). The challenges of making an effective and safe ultrasound contrast agent are many, and few products therefore are commercially available. Issues such as particle size, acoustic efficacy, biodegradability, toxicity, and pressure stability in the heart must be solved.

In a previous work (1), air-filled microcapsules with polymeric wall material were prepared successfully from oil-in-water emulsions containing human serum albumin as the surfactant. On freeze-drying, the droplets formed hollow and approximately spherical microcapsules with a volume mean diameter of typically 4–5 μm and well-defined walls 150–200 nm thick. In this paper, a new amphiphilic block copolymer based on poly(ethylene glycol) (PEG) was used as the surfactant to replace human serum albumin. The objective of this work was to find an acceptable formulation with respect to the microcapsule suspension and the stability of the emulsion during the production process. The production process was complex and included several intermediates (Fig. 1). The influence of various formulation parameters (concentrations of polymer, surfactant, and mannitol; pH; oil-in-water phase ratio) on the emulsion stability, the freeze-dried product, and the resulting microcapsule suspension was therefore investigated in a factorial design. The results were treated by ordinary least-square (OLS) regression and partial least-square regression (PLSR).

EXPERIMENTAL

Materials

The biodegradable double-ester polymer with ethylidene units, poly[ethyl-1,1-bis(16-oxohexadecanoate)-1,6-dioxohexamethylene], used as the microcapsule wall-forming material, was synthesized by the Department of Process Chemistry, Nycomed Imaging AS, Oslo, Norway (Patent WO 96/07434). The weight-average molecular weight M_w was 40,000 Da, and the polydispersity M_w/M_n (number-average molecular weight) was approximately 2.

The amphiphilic PEG-based block copolymer, α-(16-hexadecanoyloxyhexadecanoyl)-Ω-methoxypolyoxyethylene ester, used as the surfactant in the emulsion, was synthesized at Nycomed, Incorporated (Philadelphia, PA;

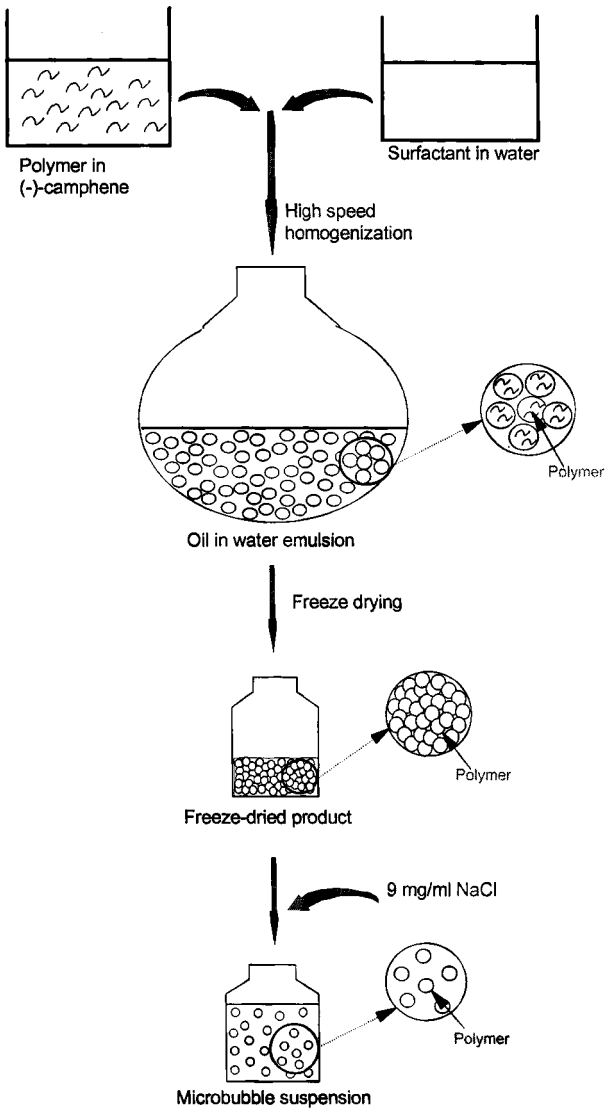


Figure 1. Production of the microcapsule suspension by freeze-drying of the oil-in-water emulsion. Shown is the location of the wall-forming polymer during preparation.

Patent WO 95/06518). The M_w was approximately 10,000 Da, and the polydispersity M_w/M_n was approximately 1.

(-)-Camphene (≈85% purity), with a melting point of 33.5°C–34.6°C was purchased from Fluka Chemie AG, Buchs, Switzerland. NaCl (9 mg/ml) was purchased from Kabi Pharmacia AB (Halden, Norway) and mannitolum ad usum parenterale (Ph.Eur.) was purchased from Norsk Medisinal Depot in Oslo, Norway. Water for injection was produced at Nycomed Imaging AS (Oslo, Norway).

Production Methods

The production method is outlined in Fig. 1. The block copolymer (surfactant) was dispersed in cold water by stirring, dissolved at approximately 80°C for 30 min, and swelled at 4°C over night. The solution was heated to 60°C prior to emulsification. Mannitol was added to the water phase, and the pH was adjusted by addition of HCl or NaOH. The wall-forming polymer was dissolved in melted (-)-camphene during stirring at 60°C. The organic phase was added to the water phase, and 80 ml emulsion was prepared by high-shear stirring for approximately 4 min at 60°C with a Ystral Rotor Stator T1500 (Ystral GmbH, Germany) in round-bottom flasks.

After homogenization, the emulsion was immediately filled as 5-ml samples in 20-ml vials. The emulsions were quenched in dry ice/methanol for approximately 10 min before transferring to a Virtis Genesis Freeze-drier (Virtis Company, NY) with the shelf temperature initially set at −40°C. The nominal freeze-drying cycle is outlined in Table 1.

The microcapsule suspensions were prepared by adding 10 ml of 9 mg/ml NaCl solution to 100 mg of the freeze-dried product. The suspensions were shaken at 250 rpm for 18 hr on an IKA KS 501D shaking table (IKA-Laboratorietechnik GmbH, Staufen, Germany).

Table 1

Nominal Freeze-Drying Cycle

Parameter	
Freezing	−40°C for 1.5 h
Primary drying	−30°C for 20 hr with temperature then gradually increased to +15°C over 15.5 hr
Secondary drying	+20°C for 2 hr
Pressure	Varied from 0.15 mbar to 0.3 mbar

Experimental Designs

The emulsion stability was first investigated in a screening study. The droplet size should not change during dispensing of the emulsion into vials prior to the freeze-drying. The variables screened were the oil-in-water phase ratio and the surfactant concentration in the water solution. The aim of this investigation was to evaluate the level of these parameters before performing a more comprehensive factorial design. The pH in the water phase was not adjusted, and mannitol was not added in this screening study. The concentration of the wall-forming polymer in the (-)-camphene was kept at 5% (w/v).

Based on the results from the screening study, a two-level ½-fractional factorial design (4) was performed with the following parameters: mannitol concentration in the water solution (A), pH in the water phase (B), polymer concentration in the (-)-camphene solution (C), surfactant concentration in the water phase (D), and oil-in-water phase ratio (E). The experimental design is outlined in Table 2. For practical reasons, the experiments were performed in two sample blocks (F), each with two center points, and in random order. In the two-level ½-fractional factorial design, all main interactions were clear of two-factor interactions, and two-factor interactions were clear of each other.

Table 2

Different Levels of the Input Variables

Factor	Level		
	−1	0	+1
A: Mannitol concentration in water (% w/v)	0	2.5	5
B: pH in the water phase	4	6	8
C: Polymer concentration in (-)-camphene (% w/v)	2.5	5.0	7.5
D: Surfactant concentration in water (% w/v)	0.5	1.0	2.5
E: Ratio of oil-in-water phase	0.1	0.25	0.4

Characterization of the Emulsions

The droplet concentration and the size distribution were measured using a Coulter Multisizer Mrk II E model with Accucomp for Windows software, version 1.15 (Coulter Electronics, Ltd., UK), described in a previous paper (1). The volume concentration and the volume mean diameter were used as response parameters. Samples were carefully homogenized by gentle manual agitation immediately prior to analysis.

The emulsion stability was investigated for 24 hr at 60°C in both the screening study and the factorial design. The temperature was above the melting temperature of the (-)-camphene (33.5°C–34.6°C). The stability of the emulsions was assessed as the ratio d_{24hr}/d_0, where d_0 and d_{24hr} were the mean droplet volume diameters at time zero and 24 hr, respectively. Any correlation between the formulation parameters for the emulsions in the factorial design was investigated by OLS regression. In the screening study, the emulsion stability was also investigated after 2 hr (d_{2hr}).

An accelerated stability test using one freezing (dry ice/methanol)/thawing (room temperature) cycle was applied to get a coarse impression of the emulsion stability during stressed conditions. The droplet diameter after this treatment was denoted d_f.

The emulsions were also inspected visually and in a Nikon UFX-II light microscope (Nikon Corp., Tokyo, Japan).

Characterization of the Freeze-Dried Products and the Microcapsule Suspensions

The freeze-dried products were investigated visually. All the samples were investigated during 1 day. The appearance was scaled from 1 to 4, with 4 indicating white cakes with few cracks and little shrinkage and 1 indicating destroyed cakes. Cakes with some shrinkage and little cracks were scaled 2.5. The influence of the formulation parameters on the freeze-dried product was investigated by OLS.

The disintegration of the freeze-dried product was observed visually when 10 ml of a 9 mg/ml NaCl solution was added slowly. The suspension was then manually shaken for 1 min and visually inspected regarding aggregates before being placed on an IKA KS 501D shaking table (IKA-Laboratorieteknik GmbH) at 250 rpm for 18 hr. A sample from the suspension was investigated in a Nikon UFX-II light microscope.

The particle volume mean diameter d_p and particle volume concentration were measured in a Coulter Multisizer Mrk II E with Accucomp for Windows software, version 1.15, as described earlier (1).

The acoustical properties of the suspension samples were characterized in a measurement set described earlier (1). Two transducers with center frequencies of 3.5 and 5 MHz measured the acoustic attenuation (dB/cm) of a sound beam going through a diluted suspension of the sample.

Any correlation between formulation parameters and characteristics of the suspensions was investigated by PLSR (5).

RESULTS AND DISCUSSION

Stability of the Emulsion

The new block copolymer applied as the surfactant is structurally a methoxy PEG chain with M_w 10,000 Da and is linked to palmitoyl palmitate through an ester bond. The relatively large PEG unit in the molecule makes the block copolymer soluble in water because of its dominating hydrophilic group. The palmitoyl palmitate provides the molecule with the hydrophobic properties. The block copolymer could therefore be a suitable emulsifier for oil-in-water emulsions, with the PEG solved in the continuous water phase, and the fatty acid solved in the oil phase. In addition, the block copolymer is nonionic and should be suitable at a wide range of pH; it is also generally more suitable for injection than ionic surfactants due to decreased toxicity (6).

Different concentrations of the surfactant were investigated in combination with different levels of the other ingredients in the product. To find the overall acceptable formulation, these variables were investigated in a statistical design.

The oil-in-water emulsions prepared were of low concentrations and had low viscosity. Emulsions with an oil-in-water ratio of 0.25 have viscosity in the range 1.5–3.2 mPa · s (1). The oil droplets easily flocculated at the top of the emulsion due to flotation (creaming). This may cause the droplets to coalesce too fast if the emulsifier is unsuitable. The oil-in-water emulsion is an intermediate product, and the need for stability is therefore process related. The emulsion should be stable for some hours during a normal process and, in the worst case, overnight if problems during production occur. For the oil-in-water emulsion containing (-)-camphene, elevated temperature must be applied during storage before freezing to avoid solidification due to the high melting temperature of (-)-camphene and subsequent coalescence and increase in

Table 3

Stability of the Emulsions at 60°C and After Freezing and Thawing (Screening Study)

Surfactant in Water (% w/v)	Ratio of Oil-in-Water Phase	d_0	$d_{2\,hr}/d_0$	$d_{24\,hr}/d_0$	d_f/d_0
2	0.25	7.4	1.04	1.02	1.20
1	0.05	5.1	1.13	1.24	1.07
1	0.13	6.2	1.01	1.02	1.08
1	0.25	7.0	1.03	1.04	1.16
0.5	0.13	6.2	1.02	0.90	1.03
0.5	0.25	7.8	0.94	0.94	1.13

d_0, $d_{2\,hr}$, $d_{24\,hr}$, and d_f are the mean volume droplet size of the emulsion at time 0, 2, and 24 hr, and after freezing and thawing, respectively.

droplet size (1). The stability of the emulsion was therefore investigated at 60°C for 24 hr.

The volume mean diameter of the droplets in the freshly prepared emulsions d_0 in the screening study varied in the interval 5.1–7.8 μm (Table 3). The stability of the emulsions was fairly good during 24 hr at 60°C. The change in the mean droplet size in the emulsion after storage d_{24hr}/d_0 was within the interval ±10% (0.9–1.1), except for the emulsion with the lowest oil-in-water phase ratio. A random variation of ±10% in droplet size is acceptable because the emulsions are extensively diluted during Coulter counting. The emulsion with the low oil-in-water phase ratio of 0.05 had nearly a 25% increase in droplet size (d_{24hr}/d_0 of 1.2). The increase in droplet size could be due to creaming and subsequent coalescence. Another mechanism for increase in the mean droplet size could be diffusion of (-)-camphene from the smaller droplets into the water phase and thereafter diffusion into the more stable larger droplets (Ostwald ripening). The presence of the long-chain and nonpolar polyester in the oil phase probably would slow this diffusion by making the organic phase less polar or sterically hinder the diffusion of (-)-camphene (7).

Freezing and thawing of emulsions as a coarse test for stability is a well-known method (8,9). In the process for preparing microcapsules, the emulsion was frozen before the freeze-drying. A freeze/thaw stability test was therefore applied to investigate the effect of large changes in temperature on the emulsion. The mean droplet size increased insignificantly in the emulsions containing a low oil-in-water phase ratio, $d_f/d_0 < 1.1$ (Table 3). The increase was somewhat larger for the emulsions containing the highest oil-in-water phase ratio, with d_f/d_0 of 1.1–1.2. The different amounts of surfactant (0.5–2.0% w/v) in the water phase did not significantly affect the stability during the freeze/thaw cycle (Table 3). The deviation between the stability at 60°C and after freezing and thawing could be attributed to the stress applied to the emulsion during freezing. When cooling the emulsion to the freezing point, ice crystals were formed, forcing the droplets and the surfactant to be distributed unevenly in the water phase. In addition, particles were more concentrated in some areas of the dispersion and easily came in contact to flocculate and coalesce after thawing. Increased amounts of the organic phase increased the number of droplets and promoted these effects, as can be seen in the higher d_f/d_0 for the most concentrated emulsions (Table 3).

The oil-in-water phase ratios investigated in the factorial design were both below and above the highest level in the screening study (Table 2). Due to the poor stability at 60°C, the lowest level, 0.05, was excluded from further investigations. The results from the factorial investigation were evaluated by OLS, and the correlation coefficient describing the correlation between model estimated and measured d_{24hr}/d_0 was 0.91. The concentration of the wall-forming polymer in (-)-camphene (C) and the concentration of surfactant in water (D), as well as the interplay between these variables, were the only parameters that influenced the stability significantly ($p < .05$). The contour plot showing the influence of these parameters on the emulsion stability at 60°C, denoted d_{24hr}/d_0, is outlined in Fig. 2. The influence of the polymer concentration on the emulsion stability is insignificant when the concentrations of the surfactant is kept at a high level, as can be seen as $d_{24hr}/d_0 < 1.10$. When the surfactant concentration is low, a high concentration of polymer in (-)-camphene is needed to obtain d_{24hr}/d_0 below 1.10. This indicates that the wall-forming polyester has an effect as the cosurfactant in the emulsion. In addition, it is possible that the presence of the polymer hinders the diffusion of (-)-camphene from the small droplets into

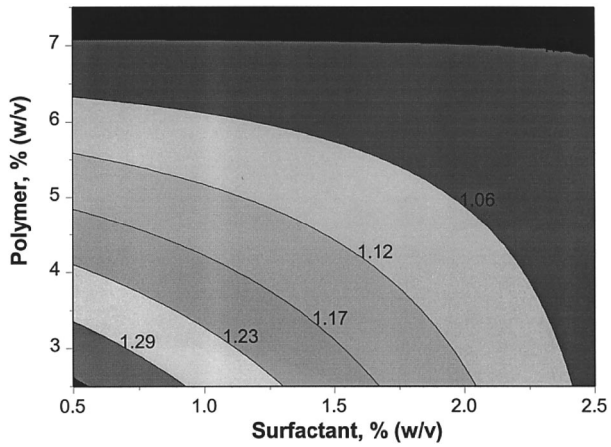


Figure 2. Emulsion stability investigated at 60°C and standstill assessed as the ratio d_{24hr}/d_0 (d_0 and d_{24hr} are the mean droplet volume diameters at time zero and 24 hr, respectively) estimated by OLS. The contour plot shows the influence of the concentration of surfactant in water (% w/v) (C) and polymer concentration in (—)-camphene (% w/v) (D).

the water phase (i.e., Ostwald ripening) (7). At polymer concentrations below 5% (w/v), a surfactant concentration of approximately 2% (w/v) in water was necessary. As could be seen from these results, the block copolymer can successfully replace human serum albumin as the surfactant in the emulsion. The insignificant effect of mannitol showed that the increase in the continuous phase did not prevent creaming to a large extent. The surfactant was suitable in the pH range investigated (pH 4–8), as expected due to the nonionic nature of the block copolymer. This could be seen by the insignificant effect of pH on the fresh emulsion (d_0) and the stability (d_{24hr}/d_0).

A smaller effect of the blocking of the samples (F), expressing a variation in the results between the two sample blocks, was seen. The reproducibility of the emulsification process should therefore be further investigated. The block difference was accounted for in the regression model and did not interfere with the estimation of other effects.

Freeze-Dried Product

Freeze-drying is drying by sublimation of ice from the surface of the product. This sublimation depends on a physically continuous ice phase in the sample to provide access to the surface through a network of ice channels (10). When water vapor has to diffuse through barriers like frozen droplets to reach the surface, cracks and fragmentation can occur in the sample due to generation of high pressure inside the product.

Mannitol is frequently used as an excipient in freeze-dried pharmaceutical products because of its effect as a cryoprotectant by ordering the water structures in the product and as an inert bulking agent (11). Another advantage is that mannitol is approved for inclusion in products for parenteral administration (Ph.Eur., USP).

The freeze-dried products without mannitol all shrank and contained cracks. The products containing the highest concentration of mannitol showed the best appearance, with no cracks and little shrinkage, denoted with the value 4. A further optimized freeze-drying process would probably reduce the shrinkage. The influence of the different formulation parameters on the appearance of the freeze-dried products was estimated by OLS. The mannitol concentration in water (A) and the interaction of the mannitol concentration and the oil-in-water phase ratio (A*E) were found to be the only significant parameters ($p < .05$). The influence of these parameters is shown in the contour plot outlined in Fig. 3. As can be seen, the oil-in-water phase ratio did not influence the visual appearance at high concentrations of mannitol in water (>4% w/v).

Microcapsule Suspension

In addition to its properties as an emulsifier in the oil-in-water emulsion, the block copolymer should act as a stabilizer in the microcapsule suspension to avoid aggre-

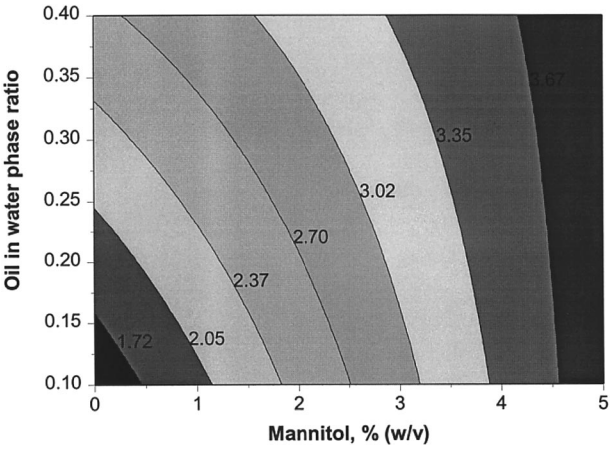


Figure 3. Appearance of the freeze-dried product treated by OLS. The contour plot shows the influence of the mannitol concentration in water (% w/v) and the oil-in-water phase ratio on the degree of cracks and shrinkage described by visual appearance.

gation. The block copolymer used as the surfactant was incorporated into or covered the polymeric surface of the microcapsules during freeze-drying. The PEG chain would then stick into the water, or the molecules would be distributed in the saline, providing an effect of steric stabilization in the suspension. In addition, PEG is surrounded by a large exclusion volume, hindering the contact of other molecules (12).

Lumps were seen in all the suspensions after adding saline to the freeze-dried product and manually shaking the suspensions. Only the most concentrated suspensions contained several lumps after shaking the suspensions at the shaking table for 18 hr. The suspensions from the emulsions containing mannitol and low level oil-in-water ratios appeared most homogeneous. The suspensions from the emulsions with the highest concentrations of the oil phase and polymer were milky white with many microcapsules (2.3–4.1% v/v), but also many lumps and aggregates. When the highest level of oil-in-water phase ratio (0.4) was combined with the highest level of polymer in (-)-camphene (7.5% w/v), the suspensions contained too many lumps above 1 mm for Coulter counting to give reliable results. The suspensions from the less concentrated emulsions were more grayish and contained fewer lumps and aggregates, but also fewer microcapsules (particle volume concentrations in the range 0.5–1.6% v/v).

All the suspensions measured contained air-filled echogenic microcapsules. The characteristics of the suspensions are outlined in Table 4. As described in the previous work (1), the frozen droplets were expected to shrink as the (-)-camphene sublimed and the polymer precipitated. The size of the microcapsules would then be smaller than the size of the corresponding droplets. In

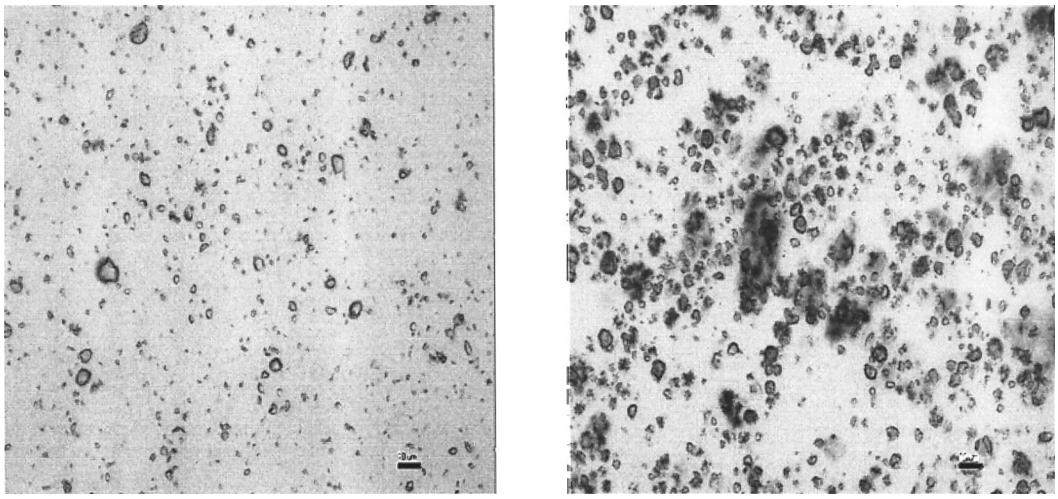
Table 4

Characteristics of the Suspensions from the Factorial Design

A	B	C	D	E	Block	d_0 (μm)	$d_{24\,hr}/d_0$	d_p (μm)	d_p/d_0	Total Particle Volume Concentration (%)	r1 Total Particle Volume Concentration [(%)/mg Polymer]	Normal Attenuation at 3.5 MHz (dB/cm)	r2 Normal Attenuation at 3.5 MHz [(dB/cm)/mg Polymer]
+	−	−	−	−	1	4.8	1.27	5.5	1.15	0.5	0.011	1.0	0.020
−	+	−	−	−	1	4.3	1.19	5.2	1.21	0.4	0.001	0.2	0.001
−	−	−	+	−	2	7.3	1.07	6.1	0.84	0.8	0.008	3.5	0.035
+	+	−	+	+	2	6.0	1.00	6.0	1.00	0.8	0.023	5.8	0.162
+	+	−	+	−	1	6.0	1.13	6.5	1.08	2.8	0.007	6.8	0.017
−	−	−	+	+	1	8.0	1.04	8.9	1.11	3.2	0.018	9.9	0.055
+	+	−	−	+	2	3.7	1.49	6.8	1.84	2.3	0.010	4.5	0.019
−	−	−	−	+	2	7.0	1.09	10.7	1.53	4.1	0.005	15.5	0.020
0	0	0	0	0	1	5.0	1.10	8.0	1.60	3.8	0.013	15.9	0.054
0	0	0	0	0	1	5.6	1.05	7.9	1.41	3.7	0.013	15.5	0.053
0	0	0	0	0	2	4.2	1.19	6.1	1.45	3.2	0.011	13.6	0.046
0	0	0	0	0	2	3.9	1.23	7.2	1.85	2.6	0.009	9.8	0.034
−	+	+	+	−	1	8.6	1.01	8.1	0.94	1.6	0.006	2.6	0.010
+	−	+	+	−	1	8.7	1.04	7.6	0.87	1.6	0.016	3.4	0.034
−	−	+	−	−	2	4.9	1.04	6.7	1.37	1.4	0.002	3.3	0.005
+	+	+	−	−	2	4.9	1.06	10.1	2.06	1.8	0.013	6.4	0.047
+	−	+	−	+	1	10.3	1.01	n.s.	—	n.a.	—	n.a.	—
−	+	+	−	+	1	9.2	0.93	n.a.	—	n.a.	—	n.a.	—
+	+	+	+	+	2	8.6	0.99	n.a.	—	n.a.	—	n.a.	—
−	−	+	+	+	2	8.3	1.01	n.a.	—	n.a.	—	n.a.	—

Variable level outlined in Table 2.

d_p = mean volume particle size of the suspension; d_0 and $d_{24\,hr}$ = mean volume droplet size of the emulsion at time 0 and 24 hr; n.a. = not analyzed due to large aggregates; A = mannitol concentration in water (% w/v); B = pH in the water phase; C = polymer concentration in (-)-camphene (% w/v); D = surfactant concentration in water (% w/v); E = ratio of oil in water phase.



(A) **(B)**

Figure 4. Microscopic investigation of suspensions: (A) from emulsion with 0.5% (w/v) surfactant in water, 2.5% (w/v) polymer in (—)-camphene, and 0.1 oil-in-water phase ratio; (B) from emulsion with 0.5% (w/v) surfactant in water, 7.5% (w/v) polymer in (—)-camphene, and 0.4 oil-in-water phase ratio. Size bar equals 10 μm.

this study, d_p/d_0 was in the interval 0.7–2.1 (Table 4). This variation could be due to aggregates in the suspension increasing the mean particle size.

The particles in the suspensions as well as the aggregates were visualized when the suspensions were investigated in the microscope. Typically, suspensions with high concentrations of particles contained several aggregates above 10 μm compared to the suspensions with low concentrations. Most of the single particles were approximately spherical and below 7.5 μm in size. In Fig. 4, the suspension from an emulsion containing 2.5% (w/v) surfactant in water and 2.5% (w/v) polymer in (-)-camphene with an oil-in-water phase ratio of 0.1 (A) is compared with a suspension from an emulsion containing 0.5% (w/v) surfactant and 7.5% (w/v) polymer with an oil-in-water phase ratio of 0.4 (B).

The formulation variables mannitol, polymer, and surfactant (A, C, and D, respectively) were calculated as amount in the freeze-dried product and denoted A#, C#, and D#, respectively, as outlined in Table 5. The effect of the formulation variables could then be related to the content in the microcapsule suspension. In addition, the yield was calculated as particle volume concentration per milligram polymer (r1) and attenuation at 3.5 MHz per milligram polymer (r2) to be able to separate the effect of increased amount of polymer in the suspension from the degree of utilization of the polymer added (Table 4).

PLSR was used to extract the relevant systematic information from the characterization of the microcapsule suspension (Fig. 5). In PLSR, *Y* is used to achieve a guided decomposition of *X* prior to the estimation of regression coefficients (5). PLSR described the variation of r1 (81%) and r2 (82%) well, with the latter parameter logarithmically transposed to improve its linear relationship to other variables. Insignificant interactions were removed. Full cross validation was applied on the resulting model (13). The amount of mannitol A# and polymer C#, as well as the interplay A#*C#, had the largest effect on the yield, seen by the large regression coefficients in Fig. 5. The high effect of mannitol A# indicates that this ex-

Table 5

Formulation Parameters Related to the Freeze-Dried Product and the Microcapsule Suspension

Factor	Content
A#: The amount of mannitol in the freeze-dried product (% w/w)	0–87
C#: The amount of polymer in the freeze-dried product (% w/w)	5–91
D#: The amount of surfactant in the freeze-dried product (% w/w)	5–90

The total content in the suspensions was always 100%, including all the formulation factors.

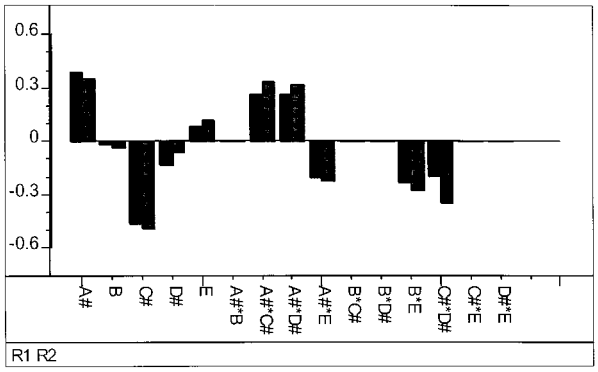


Figure 5. The regression coefficients showing the effect of variables on the yield treated by PLSR. A# is the amount of mannitol (% w/w) in freeze-dried product; B is the pH in the water phase; C# is the polymer amount (% w/w) in freeze-dried product; D# is the surfactant (% w/w) in freeze-dried product; E is the oil-in-water phase ratio; R1 is the particle volume concentration/polymer amount [(% v/v)/g]; R2 is the ln(attenuation/polymer amount) (MHz/g).

cipient protected microcapsules from being destroyed during the freeze-drying cycle. The large negative regression coefficient for C# showed that a low amount of polymer was found to give the highest yield (r1, r2). This is in accordance with previous work (1). The results from the statistical investigations and the visual observations showed that excess polymer in the oil phase did not form microcapsules, but precipitated in the solution or was included in the wall of the microcapsules and thereby formed unwanted aggregates. This is consistent with the results from the previous work, for which 2.5% (w/v), rather than 7.5% (w/v), polymer in (-)-camphene gave the suspension with fewest aggregates and highest amount of encapsulated air per amount of the wall-forming polymer (1).

The main effects of the oil-in-water ratio E and amount of surfactant D# were insignificant when the yield was calculated relative to the amount of polymer in the suspension. Smaller effects were seen from the interplay A#*C#, A#*D#, C#*D#, B*E, and A#*E. The appearance of A# and C# in several of these interplays confirm their importance with respect to the suspension yield.

The correlation between r1 and r2 was indicated by similar regression coefficient patterns with regard to the two responses outlined in Fig. 5. The correlation coefficient for ln(attenuation at 3.5 MHz per milligram polymer) versus volume concentration per milligram polymer was 0.88. This indicates that the concentration of microcapsules strongly influenced the overall echogenic effect of the suspension. In addition, the quality of the microcapsule, like the overall density and elasticity of the polymeric wall, will affect the echogenic effect (14). Non-air-filled microcapsules will contribute to the yield measured as the volume concentration of microcapsules, but will have negligible echogenic effect due to high density.

CONCLUSION

The new PEG-based block copolymer is a suitable replacement for human serum albumin as a surfactant for preparing polymeric microcapsules by freeze-drying of oil-in-water emulsions containing (-)-camphene in the oil phase. Air-filled echogenic microcapsules, with a particle size of approximately 2.5–10 μm were prepared with biodegradable polyester as the wall-forming polymer.

The oil-in-water phase ratio should be kept above 0.05 to give an acceptable emulsion stability at 60°C. A surfactant concentration of approximately 2% (w/v) in water was sufficient to avoid a higher increase than approximately 10% in the droplet size after 24 hr, when the polymer concentration in (-)-camphene was below 5% (w/v).

The yield, measured as volume concentration of microcapsules in suspension per milligram polymer, and as acoustic effect per milligram of polymer, was strongly influenced by the concentration of the wall-forming polymer in the oil phase. A low level of the polymer [$<$5% w/v in (-)-camphene] gave the highest yield. Addition of mannitol to the emulsion protected the microcapsules during freeze-drying and resulted in freeze-dried products with few cracks, little shrinkage, and higher suspension yield.

A correlation coefficient of 0.88 was found for the yield measured as volume concentration per milligram polymer versus ln(attenuation at 3.5 MHz per milligram polymer). This indicated that the volume concentration of microcapsules strongly influenced the echogenic efficacy of the suspension, but that also other factors (i.e., elasticity and density of the microcapsule wall) also influenced it.

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