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Solid lipospheres obtained from hot microemulsions in the presence of different concentrations of cosurfactant: the crystallization of stearic acid polymorphs

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Abstract

Solid lipospheres were obtained from a series of six microemulsions prepared at about 70°C, containing increasing concentrations of butanol and fixed levels of stearic acid, Tween 20 and water.

The lipospheres, obtained by dispersion of the microemulsions in cold water, were analysed by X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) in order to examine the influence of butanol on the crystal polymorphs of stearic acid.

Polymorph B, present in three samples, is favoured in the presence of a small amount of butanol. In contrast, polymorph B is not present in mixtures of stearic acid, butanol and Tween 20, which had undergone the same thermal cycle and had the same composition as the above liposphere samples.

Therefore, the droplet microemulsion structure allowed the formation of polymorph B, within the concentration limits of butanol deduced in this study.

INTRODUCTION

Colloidal lipospheres were obtained as carriers of lipophilic drugs such as deoxycorticosterone acetate [1] or of lipophilic complexes of timolol [2] and of anthracyclines [3]. Liposheres [1] were prepared by spraying in water at 2–4°C, a warm oil/water microemulsion whose internal phase was composed of solid lipophilic substances with low melting points (50–70°C), such as fatty acids and triglycerides. Microemulsions are clear, thermodynamically stable dispersions obtained by mixing surfactant, cosurfactant, oil and water [4].

The main aim of the earlier studies mentioned above was to examine the capability of the lipospheres to carry adequate amounts of drug.

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Depending on their crystallization temperature, supersaturation and impurity concentration, long-chain fatty acids may show several crystalline forms. This phenomenon, known as polymorphism, is of interest because of the different stability conditions and crystal structures of the polymorphs.

The main aim of the present work was to study the effect that the method of preparation of the lipospheres has on the polymorphism of pure stearic acid, which was used as the internal phase of the microemulsion. Stearic acid crystallizes in three polymorphic forms [5]: form A (m.p. 46°C) which crystallizes in the triclinic system, and forms B (m.p. 54°C) and C (m.p. 74°C) which are both monoclinic (S.G. $P2_1/a$) [6, 7]. Another aim of our work was to determine if the amount of cosurfactant also affects the polymorphism of stearic acid.

In the present work, we have investigated lipospheres obtained from microemulsions containing stearic acid as the lipophilic phase, in the absence of any drug.

Furthermore, it should be noted that, according to our previous work, the peculiarity of these microemulsions is that they are liquid at high temperatures, whilst at room temperature, the stearic acid is the only crystalline component.

EXPERIMENTAL

Materials

Stearic acid (SA) and *n*-butanol were purchased from Merck (D, Darmstad). SA was purified by four successive crystallizations from methanol, and then characterized by XRPD and DSC. Tween 20 was purchased from Fluka (CH, Buchs).

Instruments

X-Ray diffractometer

The powder diffraction patterns were collected on a Siemens diffractometer (Kristalloflex), using the DIFFRAC programs, and Cu Kα filtered radiation, 1.5418 A, and 2.5 deg $< 2\theta <$ 50 deg.

Differential scanning calorimeter

A differential scanning calorimeter (Perkin-Elmer DSC 7) equipped with an instrument controller (Perkin-Elmer TAC 7/DX) was used. The instrument was calibrated daily with indium ($\Delta H = 28.4$ J g^{-1}, m.p. 156.6°C) for melting point and heat of fusion. A second standard, naphthalene

($\Delta H = 149.0\ J\ g^{-1}$, m.p. 80.3°C) was also used because it has a melting point close to that of stearic acid. A heating rate of 20°C min^{-1} was employed throughout the analyses.

Thermograms were recorded in the temperature range 15–100°C. The enthalpy of fusion was calculated by a computer interfaced with the DSC from the peak area of the melting endotherm. The program estimated the onset temperature for each phase transition by extrapolating the leading edge of the peak back to the baseline.

The thermal behaviour was studied under a nitrogen purge. An empty pan was used as a reference. Triplicate runs were carried out on each sample to check the reproducibility.

Gas chromatograph

A Fractovap 2350 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame-ionization detector was used under the following conditions: stained steel column (2 m × 2 mm), packed with Carbopack C (80–100 mesh, at 0.1% of Sp 1000); injector temperature, 120°C; column temperature, 100°C; carrier gas, nitrogen, at a flow rate of 20 ml min^{-1}.

Methods

Microemulsion and liposphere preparation

A series of microemulsions were prepared with fixed concentrations of water (55 mM), stearic acid (0.7 mM), and Tween 20 (0.4 mM): the moles of butanol, used as cosurfactant, varied from the lowest to the highest amount compatible with the formation of the microemulsion (from 0.765 to 2.513 mM). Stearic acid was melted, and to this was added successively a warm mixture (67–68°C) of water, Tween 20 and butanol. A clear microemulsion was obtained at 65–67°C.

The hot microemulsion (65–67°C) was dispersed in cold water (about 2°C) in a 1:10 ratio, and then gently mixed for 15 min to obtain a dispersion of the solid lipospheres; this was washed twice with water by diaultrafiltration (Amicon TCF2, Grace, USA, Danvers) and then filtered; finally the lipospheres were dried in a desiccator.

Preparation of mixtures with the same composition as the microemulsions

The following mixtures were prepared maintaining the same molar ratio as in the above-mentioned microemulsions: stearic acid (0.703 mM) and Tween 20 (0.407 mM); stearic acid (0.703 mM) and butanol (1.633 mM); stearic acid (0.703 mM), Tween 20 (0.407 mM) and butanol (1.633 mM).

The mixtures underwent the same thermal cycle as the lipospheres. After melting, they were then dispersed in water (1:10) at about 2°C, washed, filtered, desiccated and finally analysed by DSC and XRPD.

Preparation of samples for XRPD, DSC and GC analyses

The samples for X-ray diffractometry were ground gently in an agate mortar and then placed in a standard aluminium sample holder. For DSC measurements, standard aluminium sample pans (Perkin-Elmer) were used. The sample amounts (3–4 mg) were weighed into an empty pan; the pans were then crimped using a standard sample pan crimper press.

For GC measurements, liposhere samples were dissolved in benzylic alcohol and then analysed.

Purity control on stearic acid samples

XRPD patterns and DSC thermograms were obtained on both pure stearic acid (crystallized four times) and stearic acid samples which underwent the same thermal cycle as in the preparation of lipospheres.

The purity of the stearic acid used in our experiments was checked in order to distinguish unambiguously its polymorphic modifications. In fact there are strong variations in the lattice parameters of the C form as a function of the purity reached during successive crystallization steps, as shown in Table 1 [8].

Furthermore, the melting point of the C form varies as a function of the sample purity [9] as evidenced by the DSC thermograms (Fig. 1).

TABLE 1

Variation of lattice parameters of stearic acid (C polymorph) with purity (successive crystallizations from methanol)

Crystal. step	a/Å	b/Å	c/Å	β	Volume/Å^3
I	9.454 ± 0.012	4.999 ± 0.003	50.998 ± 0.026	128.37 ± 0.12	1889.53
II	9.436 ± 0.008	4.989 ± 0.002	50.879 ± 0.018	128.40 ± 0.08	1877.20
III	9.418 ± 0.007	4.992 ± 0.002	50.871 ± 0.016	128.36 ± 0.06	1875.30
IV	9.425 ± 0.008	4.984 ± 0.003	50.801 ± 0.021	128.34 ± 0.08	1871.82

RESULTS AND DISCUSSION

The amounts of stearic acid, water, and Tween 20 were kept constant in the microemulsions in order to understand the effect of the cosurfactant butanol on the crystal structure of stearic acid. The series of microemulsions mentioned above allowed us to determine the limits of the amounts of butanol (0·765–2.513 mmol) between which the microemulsions form. Polymorph C of stearic acid was always present in the lipospheres obtained from these microemulsions.

The series of lipospheres were analysed by GC to determine the residual presence of butanol, if any. No butanol was detected.

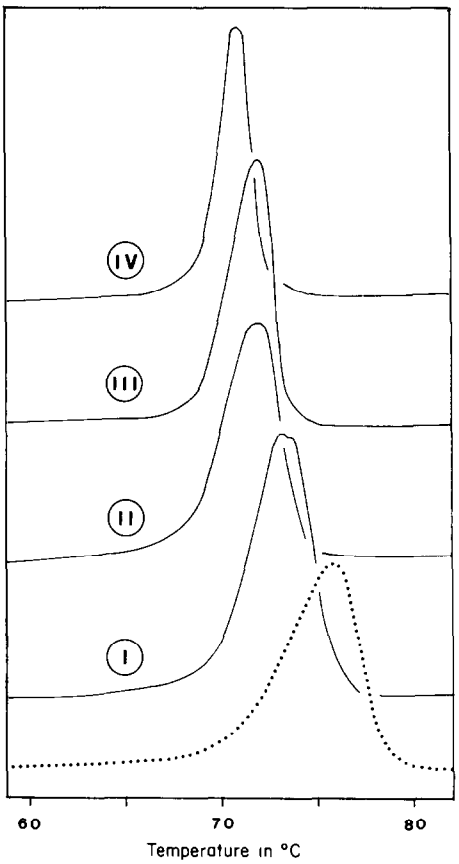

Fig. 1. DSC thermograms of stearic acid (C polymorph) on samples with increasing degrees of purity (from I to IV, Table 1). Commercial stearic acid; dotted line.

The well-defined range of butanol concentrations in the warm microemulsions appears to favour, in addition to the presence of form C, the formation of form B. Polymorph B was present in the lipospheres derived from microemulsions containing butanol in the range 1.65–2.02 mM; the maximum amount of polymorph B was obtained when the amount of butanol reached 1.802 mM (Table 2). Polymorph B was detectable by its

TABLE 2

Presence of stearic acid (polymorph B) in lipospheres prepared with different butanol concentrations

Sample	1	2	3	4	5	6
n-Butanol/mM	0.765	1.420	1.639	1.803	2.022	2.513
B polymorph	None	None	Yes	Yes	Not meas.	None
Enthalpy/J g^{-1}	–	–	0.45	1.32	Not meas.	–

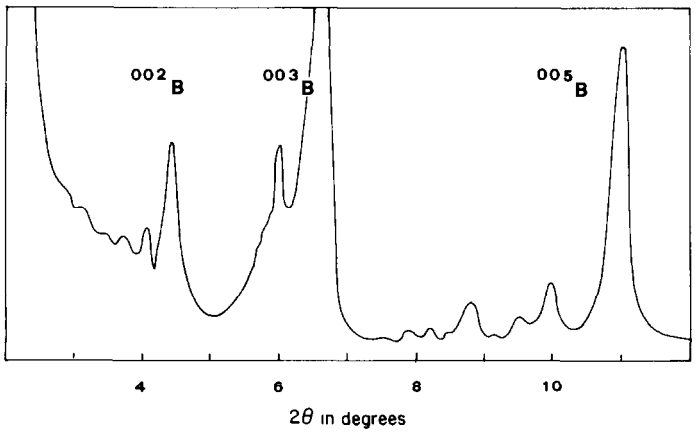

Fig. 2. XRPD pattern of lipospheres at room temperature. Three basal diffraction peaks of polymorph B are clearly evidenced. Higher intensity peaks belong to polymorph C.

characteristic X-ray diffraction pattern and DSC endothermic peaks (see Figs. 2 and 3).

As the ratio of the two polymorphs B/C increases, the peak related to polymorph C in the DSC thermograms tends to split (Fig. 4) and a small shoulder is observed. The splitting is less evident at both the highest and lowest butanol concentrations. On further increasing the butanol concentration, the B peak disappears and only a C peak is observed. Furthermore, the onset temperatures for the melting of the series of

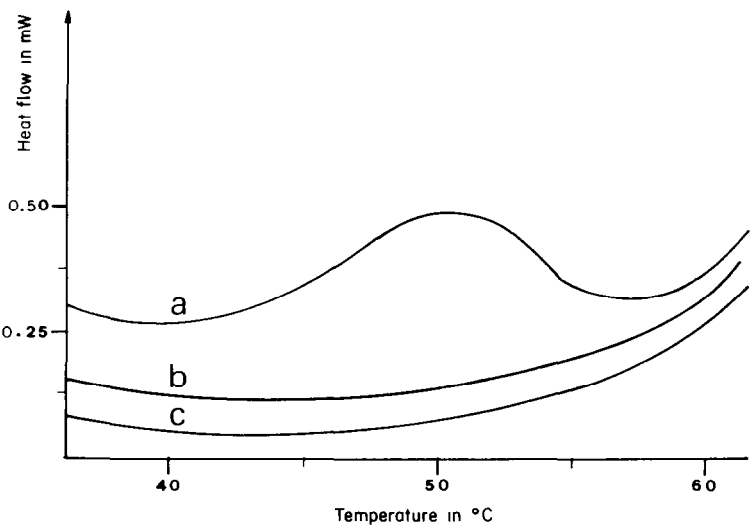

Fig. 3. DSC thermograms revealing the presence of polymorph B of stearic acid in the lipospheres: a, 1.803 mM of butanol in lipospheres (Table 2, sample 4); b, mixture of stearic acid and butanol; c, pure stearic acid.

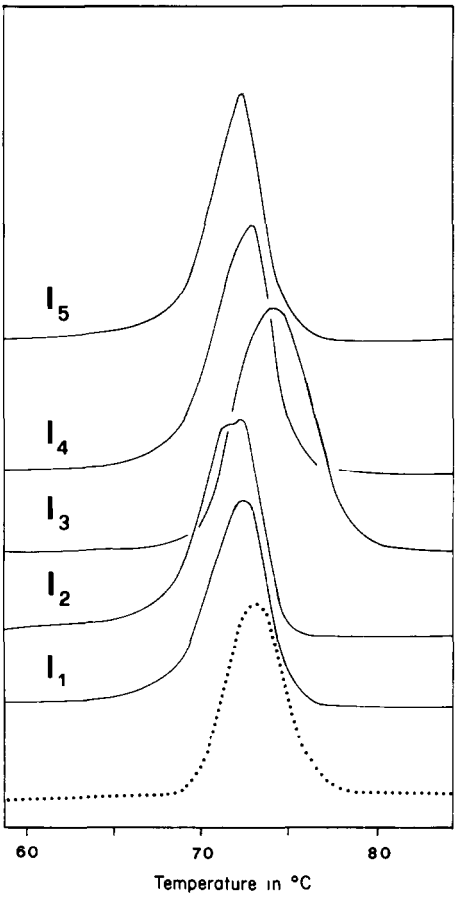

Fig. 4. DSC thermograms of lipospheres (curves I_1–I_5) and stearic acid (dotted line), under the same thermal cycle. Butanol concentration in the lipospheres increases from I_1 to I_5.

lipospheres are lower than for pure stearic acid, as shown in Table 3 and Fig. 4.

The DSC and XRPD analyses of mixtures having the same composition as the microemulsions showed not only that there was no polymorph B

TABLE 3

Enthalpy and melting point of stearic acid (polymorph C) in lipospheres prepared with different butanol concentrations

Sample	1	2	3	4	5	6
n-Butanol/mM	0.765	1.420	1.639	1.803	2.022	2.513
T_m/°C	68.71	68.98	68.42	69.54	68.67	68.70
Enthalpy/J g^{-1}	187.88	182.54	189.15	201.90	193.42	197.27

present but also that there was no splitting of the polymorph C peak. Therefore, the presence of polymorph B cannot be ascribed to the thermal cycle, but to the droplet structure of the microemulsion system from which the lipospheres were prepared.

Comparing the melting enthalpy associated with polymorph C of stearic acid, freshly crystallized stearic acid has a T_m of 68.73°C and a melting enthalpy of 216.60 J g^{-1}, while the recrystallized stearic acid subjected to the same thermal cycle and treatment of lipospheres shows a higher melting point (T_m = 69.80°C) and lower melting enthalpy, 145.08 J g^{-1}. The melting enthalpy of stearic acid in the lipospheres varied as a function of butanol concentration, with a maximum (201.90 J g^{-1}) at 1.803 mM of butanol (see Table 3).

CONCLUSIONS

From the results obtained and discussed above, it can be concluded that polymorph B is favoured by the presence of small amounts of butanol, as has been found for the crystallization of B stearic acid in the presence of polar solvents [5], or with the crystallization of *n*-alkane polymorphs in the presence of linear solvents [10]. In both these cases, the solvent mainly acts as a single molecule placed sideways to the crystallizing chains, forming bonds (temporarily adsorption) perpendicular to the chain development axis (*c*-axis).

In this paper we cannot discuss the details of stearic acid-cosurfactant interaction, because solubility and nucleation measurements are needed to do that, as in the publication by Sato [5]. Nevertheless two experimental evidences should be considered: the effect of butanol on the formation of polymorph B; and the absence of butanol in the washed and dried lipospheres and, more conclusively, in the crystalline phase of the lipospheres.

From these facts and from the above-mentioned literature, we propose the following hypothesis for the butanol-lipospheres interaction. A part of the butanol is placed at the interphase of the microemulsion, the remaining part being partitioned between the continuous and the disperse phase. Some molecules of stearic acid, representing the oil phase, have their carboxylic groups close to the more polar interphase.

During both the quenching and crystallization processes, butanol molecules within the lipospheres act as an impurity, promoting both the lowering the surface tension of polymorph B and its nucleation frequency, without entering the crystal lattice of either polymorph C or B. Washing and drying the lipospheres removes the butanol molecules from their outermost layers.

At present we do not know if polymorph B has its own liposphere population, separate from that of C polymorph, or if polymorph B co-

crystallizes with polymorph C within the same liposphere; moreover we cannot explain why there is only a well-defined range of butanol concentration favouring the occurrence of polymorph B at room temperature.

A parallel study concerning the influence of the preparative method on the crystalline habit of drugs incorporated in the lipospheres should be the object of future studies.

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