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Crystallographic investigation of cetylpalmitate solid lipid nanoparticles

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Abstract

Solid lipid nanoparticles (SLN) as alternative intravenous colloidal drug carriers were produced by high pressure homogenisation of the melted lipid cetylpalmitate. The crystallographic properties of the used cetylpalmitate SLN were characterised by small angle X-ray scattering (SAXS) and X-ray diffraction (XRD). It was found that the SLN are available exclusively in a crystalline form. Different cetylpalmitate formulations showed all the same patterns and a uniform crystal lattice was obtained. A partial indexing of the signals of the cetylpalmitate was carried out and the unit cell of the cetylpalmitate was estimated by the Miller indices. A preferred orientation in 00l-direction was observed. This can be explained by an impressive lamellar lattice structure of the cetylpalmitate. The results were compared with the crystal data of cetylpalmitate known from literature. There was no correlation with the monoclinic structure known so far. This could indicate that the SLN consist of crystallites of another modification of the cetylpalmitate.

Keywords: Cetylpalmitate; Solid lipid nanoparticles; Crystallisation; Small angle X-ray scattering

1. Introduction

Solid lipid nanoparticles (SLN) are an alternative carrier system with respect to polymer nanoparticles or liposomes. SLN have the advantage of an improved biodegradability and can be produced on a large scale by high pressure homogenisation. They consist of physiological and biocompatible lipids, which are suitable for the incorporation of lipophilic, hydrophilic and poorly water-soluble active ingredients.

Usually lipids like dynasan, witepsol or compritol are the bases of SLN (Weyhers, 1995; Westesen et al., 1997). There has also been much interest in cetylpalmitate (Lukowski and Pflegel, 1997; Dingler et al., 1998). The wax cetylpalmitate has the advantage of a better in vitro degradation rate and a lower in vivo toxicity in comparison to compritol (Weyhers, 1995). Therefore it seems to be better suited for a fast release of active substances and a frequent application.

The release of active substances from the matrix is influenced by the crystal structure of the cetyl-

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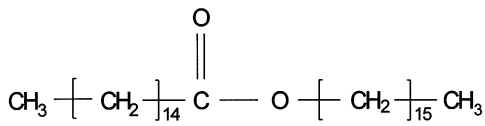

Fig. 1. Structure of cetylpalmitate.

palmitate. However, the interior structure is poorly known up to now (Budavari, 1989). Cetylpalmitate has not yet been described completely by X-ray crystal analysis (JCPDS, 1999). A publication written by Kohlhaas (1938) is the only crystallographic investigation of cetylpalmitate that could be found in the Cambridge Structural Database (1999). Therefore, the aim was the determination of the lattice structure by small angle X-ray scattering (SAXS) and X-ray diffraction (XRD). A comparison of the data with the already admitted crystal data should lead to a better understanding of lattice arrangement in the SLN. Additionally conclusions about the incorporation of active substances into the matrix are possible with the aid of the crystal data of the cetylpalmitate of the SLN.

2. Materials

Cetylpalmitate usually found on the market is a mixture of C14-, C16- and C18-acid and alcohol components (Fig. 1). The batch of cetylpalmitate, which was used for this work, purchased from Merck (Darmstadt, Germany), consists exclusively of the palmitic acid ester of cetyl alcohol. The o/w-emulsifier plantacare 2000 was provided by Henkel (Düsseldorf, Germany). The nanoparticles were produced by high pressure homogenisation of cetylpalmitate dispersed in an aqueous surfactant solution (Müller and Lucks, 1996).

3. Methods

3.1. XRD

XRD patterns were measured using a goniometer of type HZG4-A2 Freiberger Präzisionsmechanik GmbH (Freiberg, Germany) in a Bragg–Bretano arrangement. The X-ray source was a sealed tube with a cobalt anode operating at power settings of 30 kV and 30 mA at a wavelength of 0.1781 nm. The scattering intensities were detected by a proportional detector. The samples were diluted with demineralized water. The analysis of the measured data was performed by the computer program XDAL 3000 developed by the company Seifert (Hamburg, Germany).

3.2. SAXS

The SAXS intensities were measured between $s_{\min} = 0.23\ \text{nm}^{-1}$ and $s_{\max} = 6\ \text{nm}^{-1}$ with the beamline JUSIFA at HASYLAB/Elektronensynchrotron (DESY) in Hamburg. The quantity $s = 4\pi \sin(\Theta/2)/\lambda$ denotes the modulus of the scattering vector $s = k - k_0$, where k_0 and k are the wave vectors of the incident and the scattered wave, respectively, and Θ is the scattering angle. An X-ray wavelength of $\lambda = 1.3793$ nm was chosen. The scattering curves recorded under these conditions contain structural information about all scattering particles in the size range between 1 and 27 nm. The SAXS data were background corrected. The scattering curves show well-defined peaks. With the Bragg equation, the space D can be calculated according to the equation:

$$D = (2\pi n)/s_{\text{m}}, \quad (1)$$

Table 1
Miller-indices for the first three reflexes with their lattice spacings d and scattering vectors s calculated from the small angle X-ray scattering (SAXS)-experiments

Maximum	Miller-indices	$s_{\text{experimental}}$ (nm^{-1})	Lattice spacings $d_{\text{experimental}}$ (nm)
1	(001)	1.52	4.12
2	(002)	3.08	2.06
3	(003)	4.56	1.37

where n is an integer value and s_m is the center position of a detected peak. It is a convention to set $d = D/n$ (Cullity, 1980).

The liquid sample was put into a capillary which was locked air-tight with an appropriate wax and closed with a fast drying two-component adhesive. The sample was examined under vacuum.

4. Results and discussion

The SAXS-investigations of cetylpalmitate-SLN were performed under different experimental conditions. It was found that different cetylpalmitate formulations showed the same sharp peaks. These could also be observed with different energies at the Synchrotron. They are the first detectable reflections and not measurable with a conventional XRD system. Three maxima occur in a repeated manner in all curves. They are characteristic for cetylpalmitate and consequently represent specific orders of the cetylpalmitate. Since the maxima are a multiple of each other in the curve (Fig. 2), it can be presumed that the peaks represent the first lattice spacings in these orders (Eq. (1)). Crystallographically they could present the Miller indices (001, 002, 003), that means the second and the third peak is the double or threefold of the first lattice constants. The arrangement in 00l-direction indicate a lamellar lattice structure (Table 1). The SAXS data were complemented by the XRD data. The s-values of the XRD data were calculated by the Bragg equation (see Section 3) and added to the angles or d-values (Fig. 3). In order to be able to compare the experimental data with the data of Kohlhaas, a conversion was made on the copper Kα-line. On the assumption of the lattice parameters $a = 0.561$ nm, $b = 0.7415$ nm, and $c \cdot \sin\beta = 7.7875$ nm calculated by the work of Kohlhaas, the d-values were calculated (Table 2). The direct comparison of the d-values of the authors' own experimental data with those published in the literature shows no similarity between the data. It is not appropriate to expect the same intensity of the reflections-in the polycrystalline system in comparison to the single crystal system of Kohlhaas. However, no correlation could be found in any reflection. The peaks found actually represent the first lattice spacings of cetylpalmitate and these describe other lattice constants than of Kohlhaas who determined a monoclinic lattice. Therefore, the differences in experimental data could be due to the development of another crystal modification. For the simulation of the experimental data on the assumption of an orthorhombic lattice (Fig. 4) and for the lattice parameter $c = 8.878$ nm (multiple of the lattice spacings), a calculation of the d-values was made. A good conformity with the experimental angles and d-values was found.

5. Conclusions

The cetylpalmitate of the SLN is arranged in a lamellar lattice structure. Crystalline active ingredients could be stored between these layers. In order to make a final indexing with complete crystallographic representation of cetylpalmitate, still further investigations are of great interest and are being prepared at the moment. Additional investigations of cetylpalmitate as a function of temperature will be useful.

Table 2
Main reflections and lattice spacings of the cetylpalmitate solid lipid nanoparticles (SLN) (experimental) and reflections calculated from the literature (Kohlhaas, 1938)

$\text{Angle}_{\text{experiment}}$ 2Θ (°)	Lattice $\text{spacings}_{\text{experiment}}$ d (nm)	$\text{Angle}_{\text{literature}}$ 2Θ (°)	Lattice $\text{spacings}_{\text{literature}}$ d (nm)
2.13	4.12	2.34	3.77
4.29	2.06	4.50	1.96
6.07	1.45	–	–
6.44	1.37	6.78	1.30
8.08	1.11	–	–
9.40	1.09	9.09	0.97
10.09	0.87	11.93	0.74
12.05	0.75	12.75	0.69
15.33	0.58	17.02	0.52
19.51	0.45	20.25	0.43
21.52	0.41	–	–
23.87	0.37	28.70	0.31

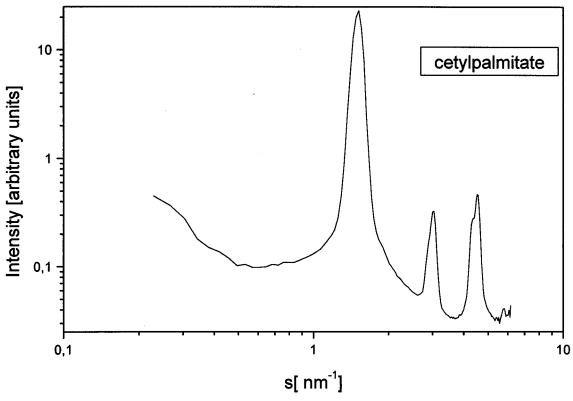

Fig. 2. First scattering peaks of the small angle X-ray scattering (SAXS)-measurement of cetylpalmitate.

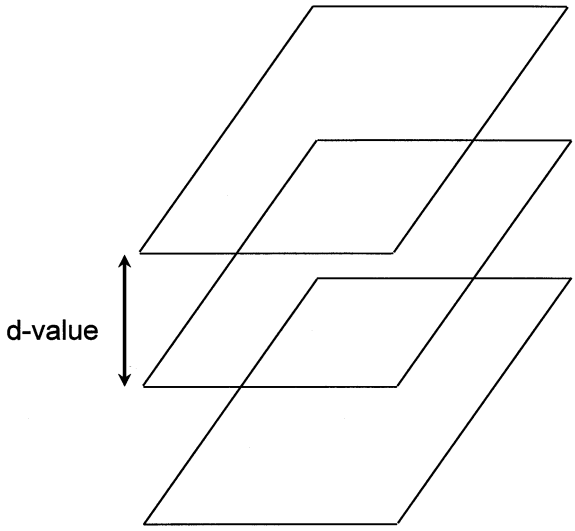

Fig. 4. Model of the orthorhombic lamellar lattice structure of cetylpalmitate.

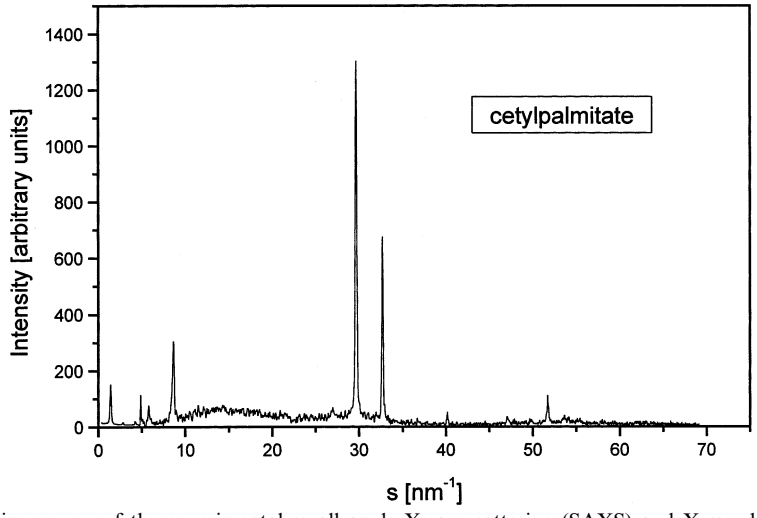

Fig. 3. Scattering curves of the experimental small angle X-ray scattering (SAXS) and X-ray diffraction (XRD) data.

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