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Effect of β-sitosterol on precipitation of cholesterol from non-aqueous and aqueous solutions

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Abstract

The aim of the present work was to study the solubility and phase behaviour of the β-sitosterol–cholesterol mixed crystals in the presence and absence of water. Cholesterol, β-sitosterol and 3:1, 1:1 and 1:3 mixtures of these were co-precipitated from acetone and acetone–water solutions. Precipitated crystals were analysed using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), optical microscopy and Karl–Fischer titrimetry. The quantification of the sterols in solutions was preformed using GC–MS. The solubility of the sterols was mutually limiting. In the aqueous system, the solubility of both the sterols were significantly lower than in the absence of water, but the decrease in the solubility was considerably greater with the more hydrophobic β-sitosterol. In the aqueous system, the total sterol solubility decreased with the increasing proportion of β-sitosterol. The formation of new crystal structures, solid solutions of cholesterol and β-sitosterol, was observed in non-aqueous as well as in aqueous environments except with the lowest cholesterol proportion in the system, in which case mixed crystals with eutectic behaviour were formed.

Keywords: Cholesterol; β-Sitosterol; Co-precipitation; Solubility

1. Introduction

The absorption of cholesterol by the intestine involves several steps including solubilization of unesterified cholesterol in mixed micelles composed of bile acids, phospholipids and products of triglyceride digestion (Thomson et al., 1993). Mixed micelles carry cholesterol through the unstirred water layer, which is the rate-limiting step in cholesterol absorption. β-Sitosterol, the most common plant sterol, and its saturated form β-sitostanol are known to reduce the absorption of cholesterol in the intestinal lumen. The mechanism is not fully understood, but some proposed mechanisms involve (i) formation of an unabsorbable mixed crystal with cholesterol (Davis, 1955; Karpuj et al., 1982) and (ii) reduction of cholesterol solubility in the oil and aqueous phases in the intestinal lumen (Ikeda and Sugano, 1983; Ikeda et al., 1988).

In the human body, cholesterol may crystallise in pathological deposits such as in the atherosclerotic plaques and gallstones as well as in the intestinal lumen when the solubility of the sterol is exceeded (Bogren and Larsson, 1963; Hsu and Nordman, 1983; Staggers et al., 1990). Deposited cholesterol exists mainly as monohydrate crystals, but the anhydrous form has also been found. Plant sterols are C-28

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(campesterol) or C-29 (β-sitosterol) sterols, differing from cholesterol (C-27) by the presence of an additional methyl or ethyl group in the cholesterol side chain. The increase in the length of side chain decreases the absorbability of sterol and increases the hydrophobicity of the molecule (Heinemann et al., 1993). Despite the low absorption, several plant sterols have been found in small quantities in plasma lipoproteins, in bile, and gallstones (Miettinen et al., 1986). Similarly to cholesterol, high plasma plant sterol concentration has been found to be atherogenic (Glueck et al., 1991). According to Hirsch et al. (1988), a formation of a mixed crystal of cholesterol and its saturated form, cholestanol, has been located in the arteries and nucleus of gallstones, where it seems to act as a seed crystal for nucleation of cholesterol monohydrate. Similarly, the presence of β-sitosterol or other plant sterols can facilitate the crystallization of cholesterol leading to formation of gallstones or atherosclerotic plaques. Thus, the phase behaviour of plant sterols with cholesterol can have an effect on cholesterol absorption in the intestinal lumen as well as on the formation of atherosclerotic plaques or gallstones.

In previous studies, cholesterol and β-sitosterol have been co-crystallized from ethanol or methanol without water (Davis, 1955; Karpuj et al., 1982; Christiansen et al., 2001a), but the effect of the sterol composition on the solubility was not reported. The aim of the present work was to study the effect of β-sitosterol proportion on the solubility and co-precipitation of β-sitosterol and cholesterol in non-aqueous as well as in aqueous environments. Acetone and acetone–water were used as solvents because cholesterol as well as β-sitosterol precipitate from acetone as anhydrous crystals and from acetone–water as monohydrated crystals (Craven, 1976; Garti et al., 1981; Christiansen et al., 2002).

2. Materials and methods

2.1. Materials

Cholesterol was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and β-sitosterol from Merck (Darmstadt, Germany). The chemical structures of cholesterol and β-sitosterol are presented in Fig. 1.

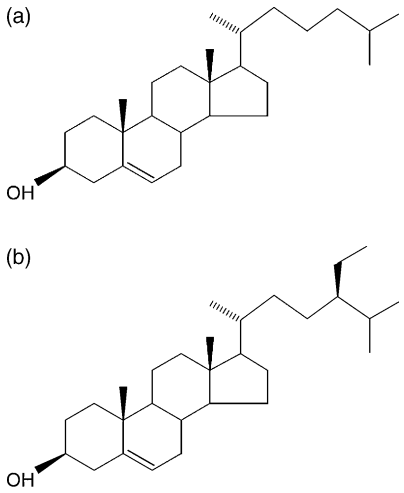

Fig. 1. Chemical structures of (a) cholesterol and (b) β-sitosterol.

The used solvents for crystallization were acetone (Analytical grade, Riedel-de Häen, Seelze, Germany), and acetone–water mixture, in which the proportion of purified water was 5% v/v.

2.2. Crystallization of sterols

Cholesterol, β-sitosterol and 3:1, 1:1 and 1:3 mixtures of these were co-precipitated from acetone and acetone–water solutions. Excess sterol or sterol mixture (500 mg) was dissolved in acetone (40 ml) or acetone–water mixture (50 ml) by heating the mixture until the solid material had dissolved (50–55 °C). The solution was cooled down unassisted at 4 °C and equilibrated for 24 h. The precipitated crystals were filtered through a paper filter. Anhydrous crystals precipitated from acetone were dried for 8 h at 80 °C and stored in desiccators for short periods of time over silica gel at 20 ± 2 °C, corresponding to relative humidity less than 3%. Hydrated crystals precipitated from acetone–water were stored over saturated K_2SO_4 solution at 20 ± 2 °C corresponding to 98% relative humidity (Nyqvist, 1983).

2.3. Solubility of the sterols in acetone and acetone–water

The solubility of the sterols were determined from the clear supernatant after removing the precipitated

crystals by filtering. A 1 ml sample of the supernatant was further filtered through a syringe filter with a 0.2 μm pore size (Whatman Inc., Clifton, NJ, USA) and 200 μl of the filtered solvent was collected for quantification analysis. Evaporated solids from 200 μl supernatant were dissolved into chloroform and derivatised with bis-trimethylsilyltrifluoroacetamide containing 1% of trimethylchlorosilane (BSTFA + TMCS, Pierce, Rockford, IL, USA) at 120 °C for 20 min. The samples were kept at room temperature overnight. The next day, 1 ml aliquots were injected into a Hewlett-Packard (HP) 5890 GC interfaced with an HP-5970A MS detector and equipped with a NB-54 fused-silica capillary column (15 m, 0.20 mm i.d., Nordion, Helsinki, Finland). The oven temperature was programmed from 250 to 285 °C (10 °C/min), the injector and detector temperatures were 285 °C. The compounds were identified by comparing the mass spectral and retention data of pure substances. 5-α-Cholestane was added as internal standard before derivatisation. The solubility results are expressed as mean ± S.D. of three experiments.

2.4. Structural analysis of the crystals

The crystal habits of the sterol crystals were evaluated using optical microscopy (Leica DMLB, Leica Mikroskopie und Systeme GmbH, Germany).

The thermal behaviour of the crystals was analysed using differential scanning calorimetry (DSC). The DSC measurements were carried out using a TA Instruments model 910S differential scanning calorimeter (TA Instruments Inc., New Castle, DE, USA). Two milligrams of samples were accurately weighed and analysed at a heating rate of 5 °C/min from 30 to 160 °C. Crimped aluminium pans were used for anhydrous crystals and crimped pans with a few holes in the pan lid for hydrous crystals. Similar empty pans were used as references. Melting temperatures (as peak values) and enthalpies of melting are expressed as mean ± S.D. of three experiments.

Powder X-ray diffraction (PXRD) patterns of the crystals were measured using a theta–theta diffractometer (Bruker AXS D8 Advance, Germany). The PXRD experiments of the samples were performed in symmetrical reflection mode with Cu Kα radiation (1.54 Å) using Göbel Mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. The angular range was from 3 to 30° (at 2θ) with the steps of 0.05°, and the measuring time was 1 s per step. The experiments were carried out at room temperature. In order to avoid preferred orientation, the samples were prepared by placing the sample powder loosely to the sample holder.

The water content of the crystals precipitated from acetone–water solutions was determined by a Karl–Fischer titrimeter (Mettler DL35, Mettler-Toledo Ag, Switzerland) and the results were expressed as a mean ± S.D. of three experiments.

3. Results

3.1. Solubility of the sterols in acetone and acetone–water

The solubility of cholesterol in acetone was 9.7 ± 0.7 mg/ml which was slightly higher than the solubility of β-sitosterol 7.6 ± 0.3 mg/ml (Fig. 2a). The solubility of the sterols seemed to be mutually limited as the addition of the other decreased the proportion of the other in the solution. The total sterol solubility of sterol mixtures did not differ clearly from that of cholesterol, but were slightly higher than that of β-sitosterol. The solubility of the sterol mixture with the lowest cholesterol proportion seemed to be higher than solubility of the mixtures with higher cholesterol proportions. With this composition, the solubility of β-sitosterol was almost the same as that of β-sitosterol in the absence of cholesterol.

The solubility of cholesterol as well as that of β-sitosterol was significantly lower in acetone–water compared to pure acetone (Fig. 2b). In acetone–water, there was a considerable difference between the solubility of cholesterol (6.2 ± 0.3 mg/ml) and β-sitosterol (1.5 ± 0.1 mg/ml). The total sterol and cholesterol solubility in acetone–water decreased with the increasing proportion of β-sitosterol. The proportion of cholesterol in dissolved sterols was clearly higher than the proportion dissolved due to the lower solubility of β-sitosterol in acetone–water mixture. As in acetone, the solubility of β-sitosterol did not decrease when a minor amount of cholesterol was introduced into the system. With higher cholesterol proportions the solubility of β-sitosterol decreased with increasing cholesterol proportion.

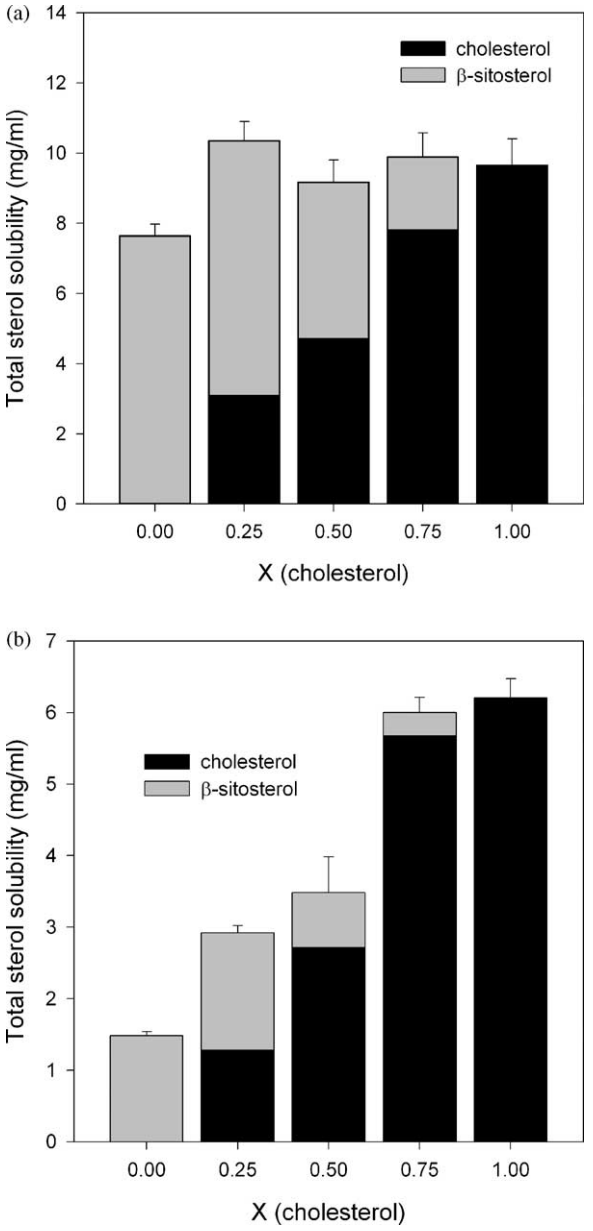

Fig. 2. The solubilities (mg/ml) of sterol mixtures with different cholesterol proportions (X (cholesterol)) in acetone (a) and acetone–water (b). Error bars represent standard deviations for the total sterol solubility values. The average proportions of cholesterol and β-sitosterol in the solutions are exhibited as black and grey colours, respectively.

3.2. Crystals precipitated from dry acetone

3.2.1. Crystal habits

Crystals of cholesterol, β-sitosterol and co-precipitates of these exhibited all rather similar elongated lath-shaped crystals (Fig. 3). Similar crystal habits have been previously reported for anhydrous cholesterol (Garti et al., 1981; Shieh et al., 1977) and for anhydrous β-sitosterol (Christiansen et al., 2002).

3.2.2. Thermal behaviour of the crystals

The thermogram of cholesterol precipitated from dry acetone showed two endothermic peaks: the first peak at $36.4 \pm 0.1\,^{\circ}C$ corresponded to a polymorphic phase transition typical of anhydrous cholesterol and the second peak at $149.5 \pm 0.1\,^{\circ}C$ (with an enthalpy of melting of 75 ± 3 J/g) corresponded to the melting of the sample (Ekman and Lundberg, 1976; Dorset, 1990; Loomis et al., 1979). The thermogram of β-sitosterol crystallized from acetone showed a single endothermic melting peak at $138.1 \pm 0.6\,^{\circ}C$ with an enthalpy of melting of 53 ± 1 J/g.

When cholesterol and β-sitosterol were co-precipitated from acetone, the proportion of cholesterol in the crystals was less than the proportion of cholesterol in the overall system, due to the better solubility of cholesterol in acetone compared to β-sitosterol (Table 1). The co-precipitate in the lowest cholesterol proportion contained only 4% cholesterol. The addition of a small fraction of cholesterol to the crystals depressed the melting point to $136.4 \pm 0.4\,^{\circ}C$ (56 ± 1 J/g). This indicates that a small amount of cholesterol acts as an impurity with eutectic behaviour with β-sitosterol (Van Dooren and Müller, 1984; Giron, 1986). When the cholesterol weight-fraction

Table 1
The mass proportion of cholesterol of total sterols (X_{CHOL}) in the crystals precipitated from acetone and acetone–water solutions with different sterol compositions (mean ± S.D.)

Composition of dissolved sterols (cholesterol:β-sitosterol)	Acetone X_{CHOL} (%-w/w)	Acetone–water X_{CHOL} (%-w/w)
1:3	3.6 ± 4.1	17.1 ± 0.2
1:1	46.4 ± 2.7	34.8 ± 3.6
3:1	59.8 ± 7.4	45.5 ± 2.6

The results are calculated according to quantitative analyses of the solutions.

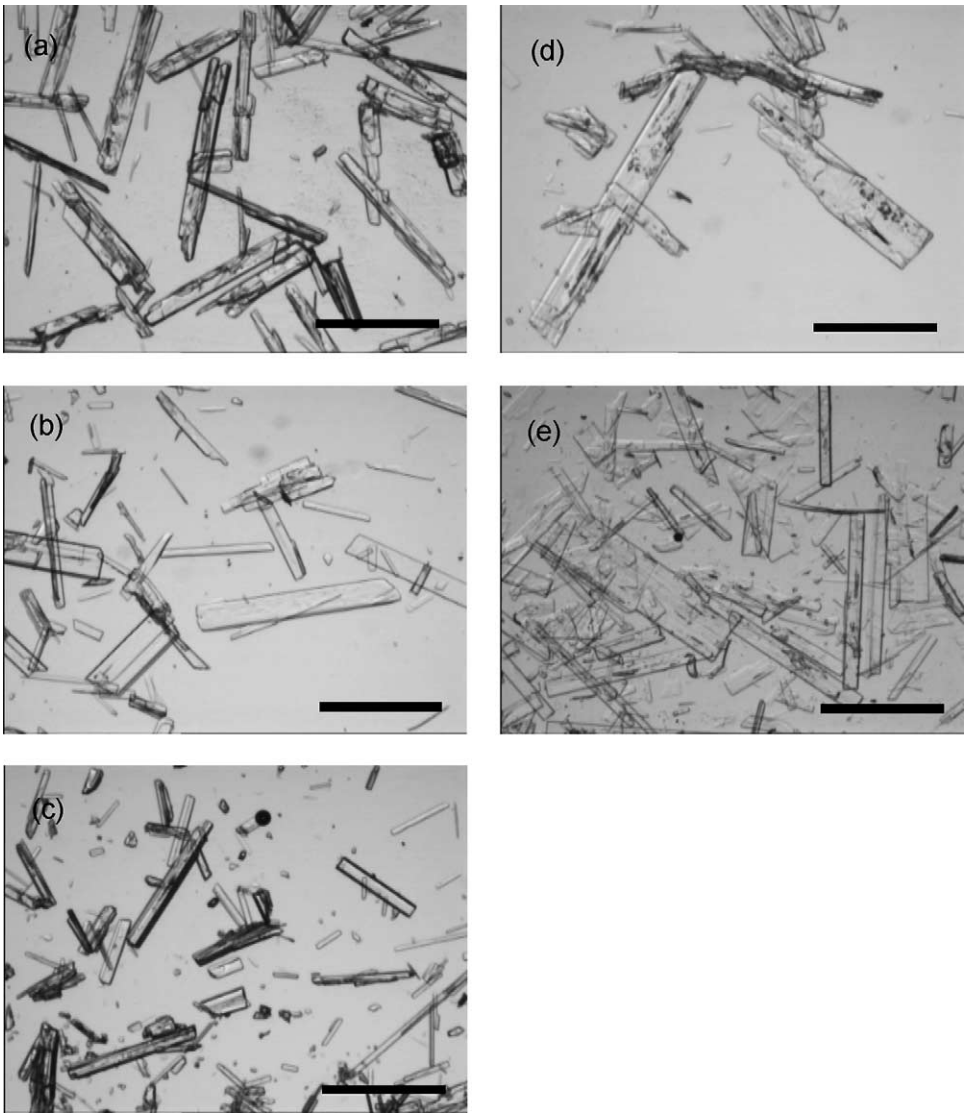

Fig. 3. Microscopy pictures of the sterol crystals precipitated from acetone: (a) cholesterol, (b) 3:1 co-precipitate, (c) 1:1 co-precipitate, (d) 1:3 co-precipitate, and (e) β-sitosterol. Bar represents 500 μm.

in crystals was 46 and 60% the thermograms of co-precipitates showed one endothermic peak corresponding to the melting of the sample at 145.3±0.1 °C (74 ± 2 J/g) and 147.4 ± 0.0 °C (74 ± 1 J/g), respectively. The presence of only one melting point, which is higher than the melting point of β-sitosterol but lower than that of cholesterol indicates the formation of a solid solution with cholesterol proportions of 46 and 60% in the crystals.

3.2.3. Powder X-ray diffraction

The PXRD patterns of cholesterol and β-sitosterol crystallized from dry acetone corresponded to the patterns of anhydrous forms described previously (Garti et al., 1981; Christiansen et al., 2002) (Fig. 4a and e).

In the lowest cholesterol proportion (4%), the PXRD pattern of the co-precipitate resembled closely that of β-sitosterol (Fig. 4d). The X-ray diffraction patterns of the co-precipitates with 46% (Fig. 4c) and

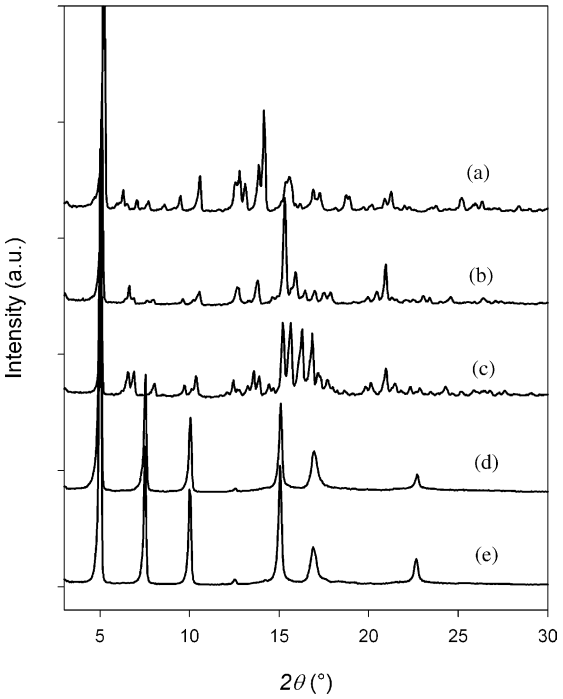

Fig. 4. X-ray diffraction patterns of (a) cholesterol, (b) 3:1 co-precipitate, (c) 1:1 co-precipitate, (d) 1:3 co-precipitate, and (e) β-sitosterol crystals precipitated from acetone.

60% (Fig. 4b) cholesterol differed clearly from those of cholesterol and β-sitosterol indicating the presence of a new structure, a solid solution of cholesterol and β-sitosterol. The crystal structure of the solid solution varied with composition as the diffraction patterns were different. Comparison of the diffraction pattern of the co-precipitate (containing 46%-w/w cholesterol) with the diffraction pattern of a 1:1 physical mixture supported the formation of a new crystal form (Fig. 5).

3.3. Crystal precipitated from acetone–water

3.3.1. Crystal habits

The crystal habit of the crystals precipitated in the presence of water varied with composition (Fig. 6). Cholesterol crystals exhibited platy crystal habit which is typical of monohydrated cholesterol crystals (Garti et al., 1981; Loomis et al., 1979; Narayana Kalkura and Devanarayanan, 1986; Saraswathi and Granam, 1997). β-Sitosterol crystals exhibited similar thin elongated needle-shaped crystals as previously described for β-sitosterol monohydrate (Christiansen et al., 2002; Narayana Kalkura and Devanarayanan, 1989, 1991). The crystal habits of the sterol mixtures varied from elongated flake-like to needle-shaped crystals. A decrease in crystal size and thinning of the elongated crystals were observed with an increasing proportion of β-sitosterol.

3.3.2. Thermal behaviour of the crystals

The DSC thermogram of cholesterol precipitated form acetone–water mixture showed a broad endotherm between 30 and 80 °C, a small endothermic peak at 76.1 ± 0.3 °C and a melting peak at 149.4 ± 0.1 °C (with an enthalpy of melting of 73 ± 1 J/g). The observed thermogram was in accordance with a DTA thermogram of cholesterol monohydrate in an open sample pan in a previous study (Wada et al., 1992). The broad endotherm and the peak at 76 °C represent the loss of water of hydration and formation of anhydrous cholesterol. A small endothermic peak at 122 °C indicates a cholesterol transition from crystalline to liquid crystalline form in the presence of water, which is normally observed more clearly when analysed in closed sample pans (Loomis et al., 1979; Saraswathi and Granam, 1997).

The thermogram of β-sitosterol precipitated from acetone–water showed two wide endothermic peaks below the melting peak at 138.9 ± 0.0 °C (56 ± 3 J/g). The wide endothermic peaks corresponded to the dehydration of β-sitosterol monohydrate which occurs in two separate stages (Christiansen et al., 2002). Below 60 °C, half of the water of hydration leaves the crystal and a hemihydrate crystal structure is formed. At the second dehydration stage, the rest of the water of hydration leaves the crystal below 90 °C. In the DSC experiments, it was observed that both these stages exhibited two overlapping endothermic peaks, corresponding to the dehydration and to the evaporation of crystal water (Suihko et al., 1997).

The DSC thermogram of the co-precipitate with a cholesterol weight-fraction of 17% resembled closely that of β-sitosterol exhibiting two separate dehydration endotherms and a melting peak. The lowering of the melting point to 135.9 ± 0.2 °C (53 ± 3 J/g) reveals the eutectic behaviour of β-sitosterol–cholesterol mixed crystal with this cholesterol proportion. When the cholesterol fraction in the crystal was 35%, the

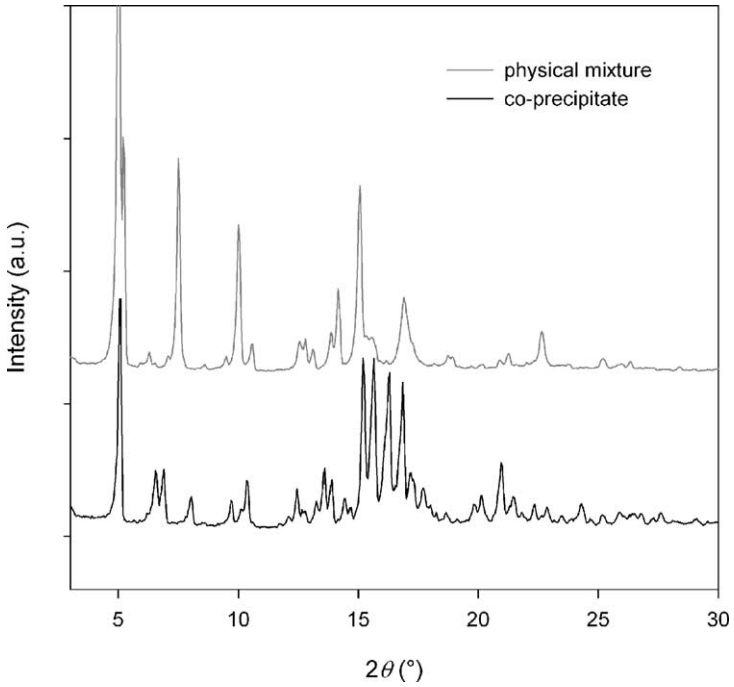

Fig. 5. Comparison of the X-ray diffraction pattern of a 1:1 physical mixture of cholesterol and β-sitosterol precipitated from acetone and the pattern of the cholesterol–β-sitosterol co-precipitate from acetone with cholesterol mass fraction of 46%-w/w.

dehydration also occurred in two stages but the second dehydration endotherm occurred at a lower temperature. Similarly to co-precipitate from dry acetone, the melting point at $140.7 \pm 0.3\,^{\circ}C$ (65 ± 5 J/g) was between those of cholesterol and β-sitosterol indicating a formation of a solid solution. With a cholesterol fraction of 46%, the melting point of the solid solution was $145.3 \pm 0.1\,^{\circ}C$ (68 ± 1 J/g). In this composition, dehydration was observed as rather a broad endothermic peak, where no separate stages were observed.

3.3.3. Powder X-ray diffraction

The PXRD pattern of cholesterol was similar to a previously described pattern for cholesterol monohydrate (Wada et al., 1992) (Fig. 7a). Similarly, the diffraction pattern of β-sitosterol was in accordance with a previous study with β-sitosterol monohydrate (Christiansen et al., 2002) (Fig. 7e).

The diffraction pattern of the co-precipitate with the lowest cholesterol proportion (17%) resembled closely that of the β-sitosterol monohydrate, but the peaks were slightly shifted to left at $2\theta < 17^{\circ}$ and a few extra peaks were observed at $2\theta < 17^{\circ}$ (Fig. 7d). The diffraction patterns with higher cholesterol proportions (Fig. 7b and c) also partly resembled that of β-sitosterol but were clearly different at 2θ values $\geq 17^{\circ}$. The change of the crystal structure as a function of the increasing cholesterol proportion could be explained by a solid solution formation. The comparison of the PXRD patterns of the physical mixture of the sterols precipitated from acetone–water with that of the co-precipitate of the sterols from acetone–water supported the solid solution formation with cholesterol weight-fraction of 46% (Fig. 8).

3.3.4. Water content of the crystals

According to Karl–Fischer analyses crystals precipitated from acetone–water contained 4.5–5% water corresponding to 1–1.2 mol water per mol anhydrous sterol (Table 2). The Karl–Fischer method determines the total water content of the sample, and therefore, do not differentiate between adsorbed moisture and water of crystallization. The hydrated crystals were stored at ≈100% relative humidity in order to prevent

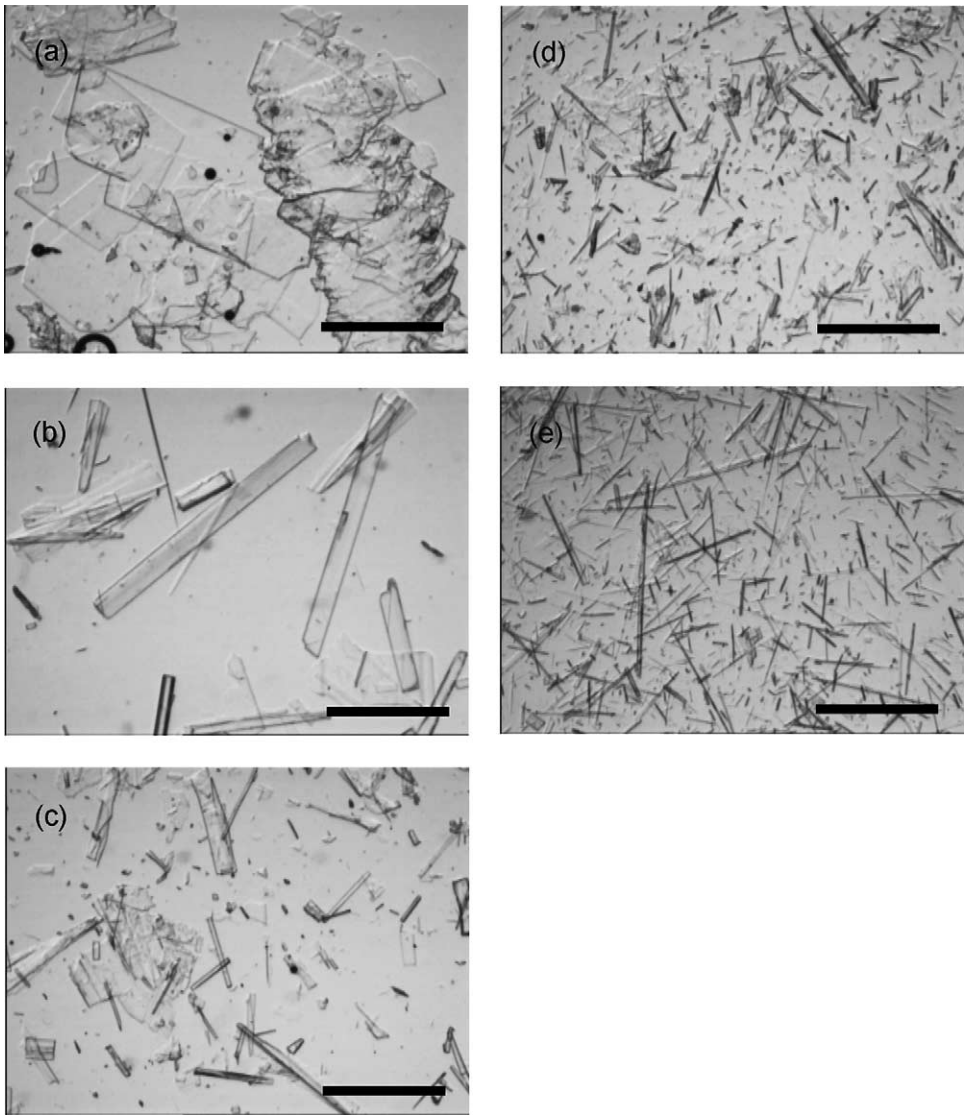

Fig. 6. Microscopy pictures of sterol crystals precipitated from acetone–water: (a) cholesterol, (b) 3:1 co-precipitate, (c) 1:1 co-precipitate, (d) 1:3 co-precipitate, and (e) β-sitosterol. Bar represents 500 μm.

the desolvation. Due to such a high relative humidity some condensation of water on the crystal surfaces takes place. The increase in water content with increasing β-sitosterol proportion was explained by the different crystal habits of the crystals. The more the crystals contained β-sitosterol, the larger their surface area was due to smaller particle size, and thus, the more moisture was adsorbed to crystal surfaces.

4. Discussion

In the present study, cholesterol and β-sitosterol and 3:1, 1:1 and 1:3 mixtures of these were precipitated from dry acetone and acetone–water mixture. In agreement with an earlier study, the presence of water decreased the solubility of cholesterol in a solvent (Jandacek et al., 1977). The additional ethyl group in

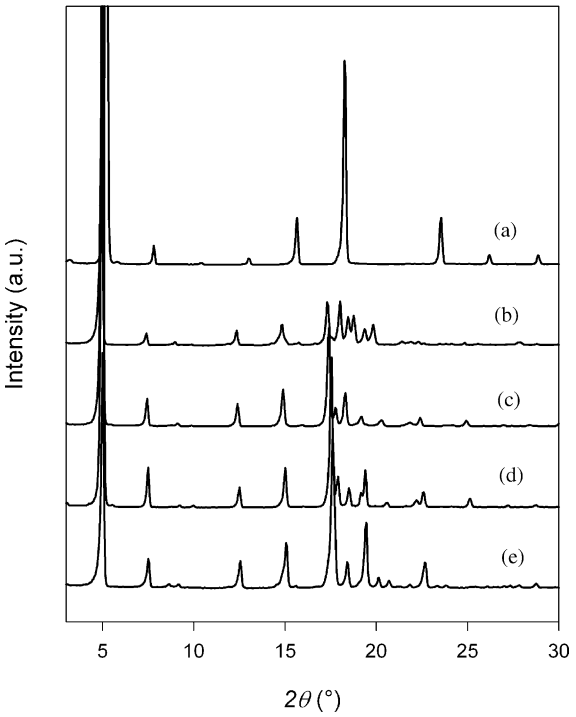

Fig. 7. X-ray diffraction patterns of (a) cholesterol, (b) 3:1 co-precipitate, (c) 1:1 co-precipitate, (d) 1:3 co-precipitate, and (e) β-sitosterol crystals precipitated from acetone–water.

Table 2
The water content of the co-precipitates from acetone–water determined using Karl–Fischer titrimetry

Proportion of cholesterol (%-w/w)	Water content (%-w/w)	Water content (mol H_2O/mol sterol)
0	5.05 ± 0.17	1.23 ± 0.04
17.1	4.89 ± 0.03	1.17 ± 0.01
34.8	4.78 ± 0.17	1.13 ± 0.04
45.5	4.72 ± 0.24	1.11 ± 0.06
100	4.50 ± 0.13	1.01 ± 0.03

the side chain of β-sitosterol molecule makes it more hydrophobic compared to cholesterol (Armstrong and Carey, 1987), and thus, the decrease in solubility of β-sitosterol was greater compared to cholesterol when water was introduced into the system. The solubility of the sterols seemed to be mutually limiting in both non-aqueous and aqueous solutions. In the non-aqueous solution, the solubility of β-sitosterol was slightly lower than that of cholesterol, but differences between the solubility of cholesterol and sterol mixtures with different compositions were not significant, expect with the lowest cholesterol proportion. The total sterol solubility with the lowest cholesterol proportion was clearly higher than with other proportions, and the addition of small amount of cholesterol did not decrease the solubility of β-sitosterol. In the aqueous system, the solubility of β-sitosterol was significantly lower than that of cholesterol and the total sterol solubility of sterol mixtures decreased with the increasing proportion of β-sitosterol. Again, with the lowest cholesterol proportion the solubility of β-sitosterol was not affected by the presence of cholesterol.

In an earlier study, cholesterol and β-sitosterol formed solid solutions with cholesterol weight-fractions ≥20% of total sterols in the crystals (Christiansen et al., 2001a). In that study, sterols were co-precipitated quickly, by evaporating the solvent (ethanol) in a vacuum drier. In the present study, sterols were allowed to precipitate slowly from supersaturated solutions obtained by heating and consequent slow cooling. Due to higher solubility of cholesterol compared to β-sitosterol in the solvents, the weight-fraction of cholesterol in the precipitated crystals was lower than in the overall system. The formation of new crystal structures was observed in both non-aqueous and aqueous environments except with the lowest cholesterol proportion in the system. When the proportion of cholesterol of total sterols was 0.25 in the overall system, mixed crystals with eutectic behaviour were formed. The solubility of the sterol mixture with this eutectic composition in acetone was slightly higher than that of other mixtures. In addition, with this composition the solubility of β-sitosterol either in acetone or in acetone–water was not affected by the presence of cholesterol water unlike with other compositions. The deviation of the sterol solubility with this composition could be due to a thermodynamically less stable crystal structure that is the eutectic mixture of the two compounds.

Anhydrous cholesterol, β-sitosterol as well as mixed crystals were obtained from dry acetone. A new observation was that cholesterol and β-sitosterol behave similarly in the presence of water except that the precipitated crystals were monohydrated with different crystalline structures compared to anhydrate sterols. In addition to a different crystal structure and thermal behaviour, the crystal habits and the

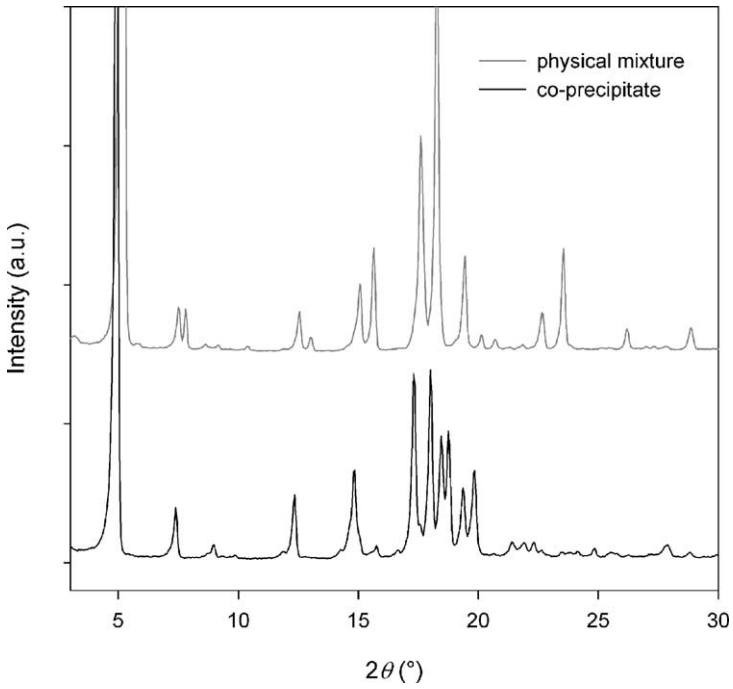

Fig. 8. Comparison of the X-ray diffraction pattern of a 1:1 physical mixture of cholesterol and β-sitosterol precipitated from acetone–water and that of a cholesterol–β-sitosterol co-precipitate from acetone–water with cholesterol mass fraction of 46%-w/w.

solubility of the monohydrated crystals varied with composition.

The present study supports the theory that the restriction of cholesterol solubility is the mechanism by which β-sitosterol reduces cholesterol absorption. Dietary cholesterol and plant sterols are distributed between oil and aqueous phases in the intestinal lumen (Miettinen and Siurala, 1971). Both cholesterol and β-sitosterol crystallize in the similar way from supersaturated oil solutions as from acetone forming anhydrous crystals in the absence of water and hydrated crystals in the presence of water (Jandacek et al., 1977; Christiansen et al., 2002). Thus, co-precipitation could be expected to occur in the oily phase in the intestinal lumen in the same way as in the present study. In the aqueous phase, β-sitosterol is known to restrict the micellar solubility of cholesterol in bile salt solutions (Ikeda and Sugano, 1983; Ikeda et al., 1988, 1989).

Cholesterol crystallization in the human body is associated with several diseases. Cholesterol supersaturation, nucleation and crystal growth in bile lead to the formation of cholesterol gallstones (Strasberg, 1998). On the other hand, crystallizing of free cholesterol plays an important role in a formation of atherosclerotic lesions (Tabas, 1997). Under normal conditions, the β-sitosterol concentration in human serum is only about 1/1000 of serum cholesterol concentration, although typical dietary intake of plant sterols is almost equal to dietary intake of cholesterol (Ling and Jones, 1995; Christiansen et al., 2001b). The consumption of effective doses of β-sitosterol (0.8–3 g per day) causes about a two-fold increase in serum concentration (Westrate and Meijer, 1998). Similarly to cholesterol, high plasma plant sterol concentration has been found to be atherogenic (Glueck et al., 1991). Increased plasma levels of β-sitosterol can occur in individuals who suffer from sitosterolemia. Sitosterolemia is an extremely rare recessively inherited disorder caused by increased intestinal absorption and reduced hepatic plant sterol excretion. Due to different physical properties, i.e. melting point and solubility, a solid solution of sterols can behave quite differently from pure cholesterol in the human body. The significantly lower solubility of β-sitosterol compared to

cholesterol in an aqueous environment results in a proportionally greater amount of β-sitosterol in the crystal phase than in the overall system. The presence of large amounts less soluble β-sitosterol can facilitate the crystallization of cholesterol leading to the formation of gallstones or atherosclerotic plaques.

Acknowledgements

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