Optimizing the Crystal Size and Habit of β-Sitosterol in Suspension

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ABSTRACT

The aim of this work was to survey how processing parameters affect the crystal growth of β-sitosterol in suspension. The process variables studied were the cooling temperature, stirring time and stirring rate during recrystallization. In addition, we investigated the effect a commonly used surfactant, polysorbate 80, has on crystal size distribution and the polymorphic form. This study describes the optimization of the crystallization process, with the object of preparing crystals as small as possible. Particle size distribution and habit were analyzed using optical microscopy, and the crystal structure was analyzed using X-ray diffractometry. The cooling temperature had a remarkable influence on the crystal size. Crystals with a median crystal length of ~23 μm were achieved with a low cooling temperature (<10°C); however, a fairly large number of crystals over 50 μm appeared. Higher cooling temperatures (>30°C) caused notable crystal growth both in length and width. Rapid (250 rpm), continuous stirring until the suspensions had cooled to room temperature created small, less than 50 μm long (median <20 μm), needle-shaped crystals. The addition of surfactant slightly reduced the size of the initially large crystals. Both hemihydrate and monohydrate crystal forms occurred throughout, regardless of the processing parameters. By using an optimized process, it was possible to obtain a microcrystalline suspension, with a smooth texture.

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INTRODUCTION

Knowledge of crystallization kinetics is important in the scale-up and design of industrial crystallizers. The aim of the crystallization process can be, for instance, the synthesis of single crystals or very small crystals, if a high specific surface area is the desired property. The theory of crystallization is complex and can be thought of as a 3-step process including the development of supersaturation or supercooling, nucleation, and crystal growth.[1,2] Prior to crystal development, embryos must form by collision of molecules of solute in solution.[3] When solute molecules aggregate around each other and nuclei occur spontaneously, nucleation is termed homogenous. Sometimes embryos form by addition of a seed crystal, a dust particle, or by migrated particles from container walls. When nuclei are induced artificially, nucleation is called heterogeneous. Homogenous and heterogeneous mechanisms are also referred to as primary nucleation.[2] The most important variables affecting nucleation are the surface energy, temperature, and supersaturation.[4] In heterogeneous nucleation, the deliberately added solid particles reduce the surface free energy and thus induce nucleation.

Secondary nucleation results from the presence of solute particles in a supersaturated solution.[5] The solute crystals catalyze the nucleation, hence, nucleation occurs at lower supersaturation. Secondary nucleation has been recognized as being the major source of nuclei in many industrial crystallizers.

Recent results based on theory, simulations, and experiments report that crystals obtained from solutions are often not formed via classical nucleation, however, but by much more complex routes.[6] It is not yet clear if each system has to be studied individually or if indeed a general pathway to the solid exists.

The nucleation step in crystallization is followed by crystal growth. The growth of crystals from solution is a 4-step process by which a growth unit passes from the bulk solution to the crystal lattice. At first, the growth unit moves from the bulk solution to the crystal surface, where it absorbs onto the surface. Possible diffusion of the growth units to a more favorable site may

occur. Finally, growth units integrate into the crystal lattice.[7] The rate-limiting steps for crystal growth are generally considered to be the rate of transport of growth units to the crystal surface (diffusion-controlled) and/or integration into the crystal lattice.[1]

Minor changes in crystallization conditions, for example, supersaturation, impurities, or cooling rate can produce significant changes in the particle size, shape, and purity. Increasing the supersaturation because of its effect on the nucleation rate can usually decrease the mean size of the particles.

Surfactants are commonly used in pharmaceutical suspensions in order to lower the interfacial tension between the solid and the solvent facilitating wetting of the solid. They can also be used as colloids to improve the stability of the suspension. Surfactants have been found to promote, to slow down, or to prevent crystal growth in solutions.

The difference in bioavailability is often related to the different dissolution rate of sparingly soluble drugs. The knowledge of dissolution and the factors affecting dissolution behavior should be emphasized in all discussion that concerns the designing of a new dosage form. According to the Noyes-Whitney equation, dissolution rate is directly proportional to the effect of the surface area of the drug. Therefore, a reduction of the particle size may affect dissolution and thus influence drug absorption.[8-10] However, formulations containing micronized drugs do not always result in an improved dissolution rate, since agglomeration is common and a disadvantage.[11,12] In addition, several studies show that the improved dissolution is related only to particles below 5 μm.

β-sitosterol has been shown to reduce plasma cholesterol by blocking the absorption of cholesterol from the gut.[13] In a previous study, the preparation process of the microcrystalline β-sitosterol suspension is described in detail.[14] The suspension has shown to reduce significantly both serum total- and low-density lipoprotein (LDL)-cholesterol levels.[15] Because using sterols as cholesterol-lowering agents in foods results in an undesirable gritty texture, microcrystalline particle size is desirable. This study examines how different process parameters, cooling temperature, stirring rate, and stirring time influence the size and shape of β-sitosterol crystals as well as how the addition of a surfactant influences the behavior of β-sitosterol crystals in suspension.

MATERIALS AND METHODS

Production Process

The suspensions were prepared by heating β-sitosterol, containing ≥78.5% β-sitosterol (414.72 g/mol, $C_{29}H_{50}O$, molecular structure), 8.7% campesterol, 10% β-sitostanol, 1% campostanol (Les Dérivés Résiniques et Terpéniques [DRT], France), and oil (medium chain triglycerides [MCT], SHS International Ltd, UK) up to ~100°C until a clear liquid was formed. After cooling to ~90°C, purified water of the same temperature was added and stirring begun immediately. When studying the effect of surfactant on the crystal size, polyoxyethylene 20 sorbitan mono-oleate (polysorbate 80) (Tween 80, for parenteral use, ICI Surfactant, Germany) was added to the water before combining the water and the β-sitosterol-oil mixture. The batches produced contained 17% (wt/wt) β-sitosterol, 70% MCT-oil, and 13% water. This composition was chosen based on pretests and on previous studies.[14,16] Batches with surfactant contained 1% (wt/wt) polysorbate 80. The effect of cooling temperature was studied by immersing the vessel, in which the suspensions were made, in an ice water bath at 6 different levels of temperature: 0°C, 10°C, 20°C, 30°C, 40°C, and 50°C (±2°C). The temperature range used was based on pretest results. The cooling pattern of the suspensions was measured at all the temperature levels. The temperature was measured every 30 seconds for 4 minutes. After 4 minutes, the suspensions immersed in both 0°C and 10°C ice water baths had reached at least room temperature. At this time the other suspensions had reached the same temperature as their water baths. The initial temperature was assumed to be 90°C the same temperature as the added water. To investigate the effects of stirring time and stirring rate, the batches were prepared with a planetary mixer (Kenwood KM 400, Kenwood Ltd, UK). The metallic bowl was covered with a frozen shell to cool down the suspension as occurred when the beaker was immersed in ice. The rotation speed of the mixer was 0, 60, 120, 200, and 250 rpm. Stirring time was, in this case, 3 minutes. At this point the mass had reached room temperature, and the system appeared equally well agitated. Every 30 seconds the bowl was cleaned on the sides with a spatula. The effect of stirring time was studied at 250 rpm with the following times: 30, 60, 120, 240, and 360 seconds. In these cases also, the bowl was cleaned on its sides every 30 seconds. When preparing suspensions that were cooled in higher temperatures or stirred for a shorter time, the initial temperature was higher. Following preparation, the samples were kept at room temperature for half an hour after which the tempera-

ture was measured (+25°C) and the suspensions were packed. The suspensions were transferred for storage at +4°C. In a prior study of the stability of the suspension, suspensions were stored for 4 months at +4°C without any changes to the crystal size or habit.[16]

Microscopy

The size and shape of the crystals were analyzed by optical microscopy (Leica DMLB, Leica Mikroskopie und Systeme GmbH, Germany). A small amount of the suspension was diluted, because of the high viscosity, with MCT-oil before evaluation. The crystal size of 300 particles was measured manually for each crystallization batch using a measuring rod. Observations regarding crystal habit were defined on a visual basis. Crystal size and habit determinations were carried out on the samples within 1 day of production.

Wide Angle X-ray Scattering Experiments

The crystal structure of the samples was analyzed by wide angle X-ray diffraction (XRD). The experiments were performed with a theta-theta diffractometer (Bruker AXS, D8 Advance, Germany) in a symmetrical reflection mode with Cu K_α 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° with the steps of 0.05°, and the measuring time was 1s/step. The suspensions were analyzed within 1 day of production.

RESULTS AND DISCUSSION

The Effect of Cooling Temperature on the Crystal Size Distribution and Habit

Cooling of a solution is one of the most widely used methods for achieving the supersaturation essential for crystallization. In an earlier study it was noticed that rapid cooling decreased the crystal size of β-sitosterol in a suspension.[16] In the present study, the suspensions were cooled at several different temperatures leading to different cooling patterns of the suspensions. As illustrated in **Figure 1**, both the crystal size and, to some degree, the habit changed with an increase in cooling temperature. The needle-like crystals grew to a certain extent in width, becoming more platy-like crystals. At low temperatures (**Figure 1A**) when the crystals were small, they were even in shape and length, while increased temperatures led to uneven shapes and sizes. Cooling at 50°C created crys-

tals as great as 100 μm, but among these crystals were a great number of crystals only a few micrometers in size (**Figure 1B**). Raising the temperature from 0°C to 50°C clearly increased the median crystal length in suspensions by at least 2-fold (**Figure 2**). At 0°C, the median crystal length was 23 μm, while at 50°C the shape of the particles was more platy-like with the median particle length of 50 μm. When the suspensions were immersed in ice or cold water, the crystallization process started immediately and a viscous suspension was formed within a few seconds.
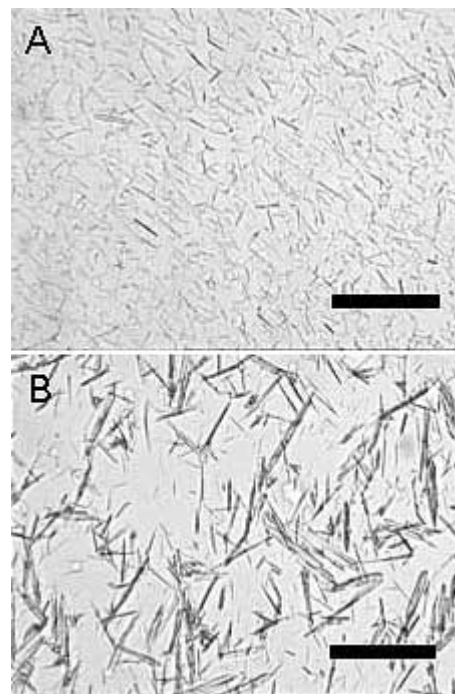

Figure 1. Microscopy pictures of β-sitosterol suspensions prepared by cooling at (A) 0°C (immersed in ice) and (B) 50°C. The pictures were taken 1 day after preparation (bar = 100 μm).

Cooling temperature is known to influence the rate of growth and size of crystals through its effect on supersaturation.[2,4,17] In general, the crystal size decreases at higher levels of supersaturation due to increased nucleation rates. Therefore, a larger number of small crystals can be achieved with low cooling temperatures. A minor factor influencing the nucleation and crystal growth as the temperature decreases is the resulting increase in the viscosity of the oil, causing diminished molecular movement. This, in turn, slows down both nuclei formation and growth rate on the nuclei formed.[2]
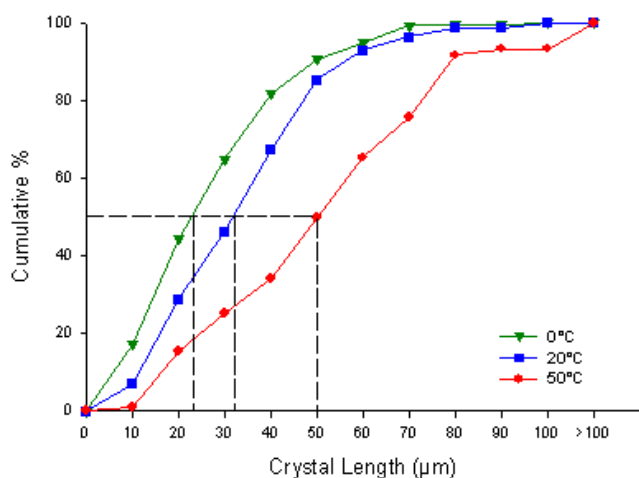
Figure 2. Cumulative crystal length of β-sitosterol crystals (n = 300) in suspensions prepared by different cooling temperatures. Median of each population illustrated with a dashed line.

The Effect of Stirring Rate on the Crystal Size Distribution and Habit

As shown, the crystal size of β-sitosterol clearly decreased with decreasing cooling temperatures during recrystallization. Therefore, further tests were performed at temperatures from 0°C to +10°C. The effect of stirring rate on β-sitosterol crystal size was studied at a ratio of 60 rpm to 250 rpm. Recrystallization at a low (60 rpm) stirring rate produced both needle-shaped and large, flaky crystals. These, mostly flaky crystals are similar to the flaky crystals presented in **Figure 1B**. At 250 rpm, the particles were mainly needle-shaped as in **Figure 1A**. With increasing stirring rates, a greater number of smaller crystals were produced. The results showed that stirring the suspension at 250 rpm produced a nearly 2-fold decrease in the median crystal length compared with those crystals stirred at 60 rpm. The median particle size at 60 rpm was 40 μm, while increasing the rate to 250 rpm produced a median size of 23 μm. Particle sizes produced at different stirring rates are shown in **Figure 3**.

It is known that the stirring rate has a strong influence upon the nucleation rate. While gentle stirring causes nucleation in solutions that are otherwise stable, strong stirring creates an even greater tendency to form nuclei.[18] The reduced crystal size observed with increased stirring rates is therefore likely to result from increased secondary nucleation. Nucleation has been considered as a 2-step process: first, the diffusional transport step, followed by integration of molecules into the crystal lattice.[18,19] Secondary nucleation

is critically dependent on stirring rates. It is well known that as stirring rates increase, so does the secondary nucleation.[2] It is also known that the stirring rate has a strong influence upon the nucleation rate of solutions until a particular stirring rate is reached. After this no increase occurs because solute diffusion is maximized.
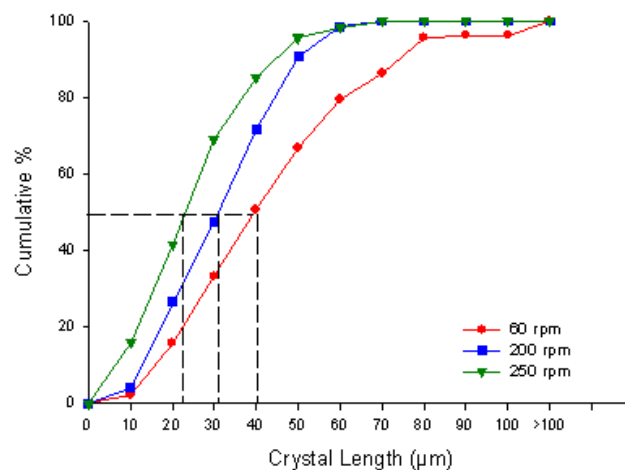

Figure 3. Cumulative crystal length of β-sitosterol crystals (n = 300) in suspensions prepared using different stirring rates. Median of each population illustrated with a dashed line.

The Effect of Stirring Time on the Crystal Size Distribution and Habit

The differences in size and habit between the crystals produced by varying stirring times were notable. A short stirring time (30 sec) created suspensions containing crystals that were in excess of 200 μm in length. These crystals grew not only in length but also substantially in width.

With increased stirring time, the length of the β-sitosterol crystals decreased. When the stirring time was 30 seconds (at 250 rpm, +10°C), the median crystal length was over 38 μm (**Figure 4**). Increasing the stirring time to 120 seconds decreased the crystal length slightly, but the median was still over 34 μm. However, an increase in stirring time to 360 seconds reduced the β-sitosterol particle size, giving a median of clearly less than 20 μm. It is also important to notice that at this point all the crystals were less than 50 μm in length. Thus, we attained the small particle size that was our goal, since the optimum particle size for a so-called creamy product, according to the food industry, lies between 10 μm and 50 μm, and even up to 80 μm if the particles are rounded or flat.[20,21]
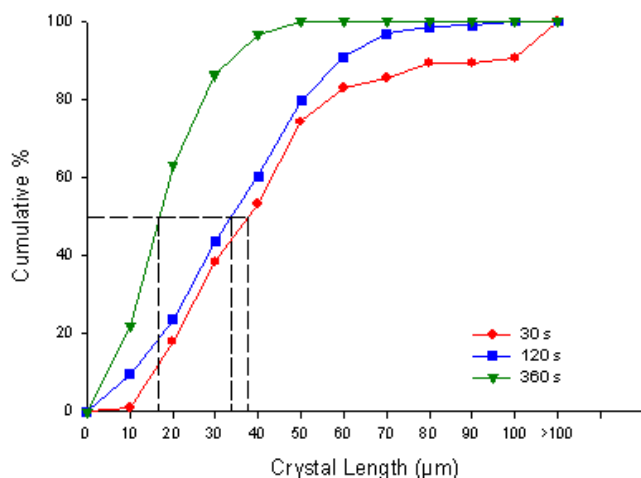
Figure 4. Cumulative crystal length of β-sitosterol crystals (n = 300) in suspensions prepared using different stirring times. Median of each population illustrated with a dashed line.

An explanation as o the difference in crystal length and shape, after different stirring times, is that the suspensions were still at about 60°C after 30 seconds of agitation. The main recrystallization in β-sitosterol suspensions appears at around 60°C. Stopping the agitation at this time leads to continued crystallization without stirring. As the results in the previous chapter showed, the crystals that are produced at low stirring rates are large and flaky. An increase in the stirring time is necessary to prevent this from happening. At 120 seconds, the suspension was still at nearly 40°C, but at 180 seconds, the suspension had cooled to room temperature (+22°C ± 2°C, mean ± SD, n = 3). When stopping the stirring after 360 seconds, the temperature was low enough for crystal growth not to appear, resulting in a smaller final crystal size.

As the crystals take form during recrystallization, a great number of crystal fragments are created during the first minute. After this, attrition of particles becomes almost negligible but increases again after 10 minutes.[22] Agitation must be effective enough to provide complete contact between the crystals and the solvent; however, it must not break the crystals mechanically. The influence of time on crystal attrition is remarkable and affects the number of fragments, their size, and morphology. Since the desired crystal size is achieved after 360 seconds, there is no need to continue the stirring, which could otherwise lead to crystal breakage.

The large differences in the β-sitosterol crystal length and the habit modification produced by various process parameters are likely to have an effect on the bioavail-

ability of the suspension. The surface area of the β-sitosterol crystals, however, was not measured in this study. Based on the results above, it is possible to estimate that the distinction in surface areas is as high as 100-fold.

The effect of improved dissolution is especially emphatic for micronized particles finer than 5 μm, and especially when the particle size is less than 3 μm.[23-25] The particles in this study were approximately 3-μm thick; the length and width varied from a few micrometers up to tens of micrometers.

Role of the Surfactant

In the presence of polysorbate 80, the rate of crystal growth changed only slightly. The size reduction of the β-sitosterol crystals was observed only in the originally larger crystals. The differences in crystal length when surfactant was added could be in excess of 10 μm compared with crystals in suspensions with no polysorbate 80 (**Figure 5**). In this case, when a slight reduction of the crystal size was observed, the suspensions were produced at high temperatures, by slow stirring rate or by short stirring time. By visual examination, it could be observed that some of the crystals grew in width rather than length (**Figure 6**). It should be noted therefore, that the maximum length of the crystals does not describe the actual size of the crystals. In the case where we had already managed to produce desirable small crystals, an addition of surfactant did not have a considerable effect on the crystal length (**Figure 5**). The suspensions were, in this case, made by rapid cooling and rapid stirring for 360 seconds. The maximum difference in crystal length was 4 μm.

It has long been known that the presence of impurities can have substantial effects on the crystal form and the crystal growth rate because of the adsorption of the foreign molecules on the surface of the crystal.[2,26] Polysorbate 80 and other nonionic surfactants are often used as growth retarders during crystal growth.[27-29] A surfactant molecule can adsorb onto a crystal face in either a specific or nonspecific way. It seems that polysorbate 80, in this case, adsorbs selectively onto a crystal face and retards the crystal growth only in length. Similar observations have been made, for example, when crystallizing carbamazepine.[30] Polysorbate 80 changed the growth pattern of carbamazepine crystals, and instead of growing as long needles, the crystals grew in width. Furthermore, carbamazepine crystals seemed to be quite fragile. β-sitosterol crystals seem to have the same property. The flaky β-sitosterol crystals

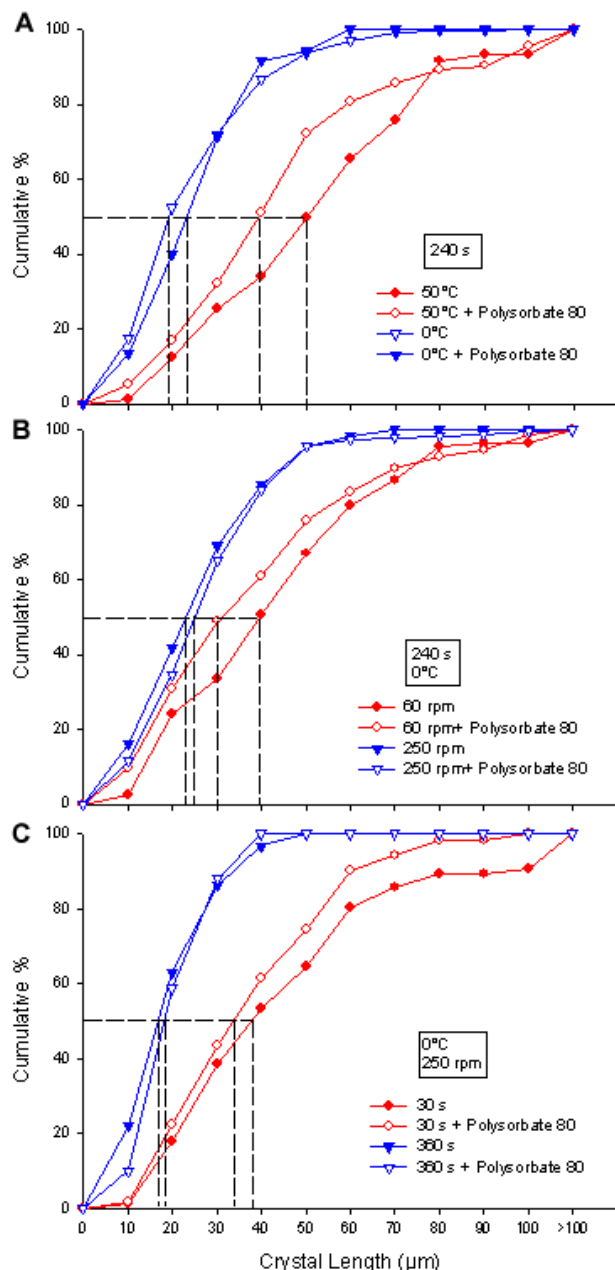were few, but these kinds of crystals did not exist to the same ex-

Figure 5. Cumulative crystal length of β-sitosterol crystals (n = 300) recrystallized containing 1% (wt/wt) Polysorbate 80. Recrystallization (A) at different temperatures (stirring time 240 seconds), (B) at different stirring rates (cooling temperature 0°C, stirring time 240 seconds), and (C) at different stirring times (cooling temperature 0°C, stirring rate 250 rpm).

tent in suspensions crystallized without polysorbate 80. The phenomenon of fragile, flaky crystals appeared only when the length of the crystals was approximately

50 μm or longer. Crystals shorter than these did not behave in the same manner. Usually only a small amount of surfactant is needed to bring changes to crystallization phenomena, but sometimes more than 1% is required to achieve the desired effect.[31] As the surfactant only retarded the larger crystals growth in length, a higher concentration may have been necessary to have an effect on the smaller crystals. With decreasing crystal size the surface area grows and a higher concentration may be needed to cover the surface equally.

Figure 6. Microscopy picture of β-sitosterol suspension recrystallized containing 1% (wt/wt) Polysorbate 80 with a stirring time of 30 seconds. The picture was taken 1 day after preparation (bar = 100μm).

Crystal Form and Crystallinity

In an earlier study, it was observed that β-sitosterol exists in 3 different pseudopolymorphic crystal forms with different water contents: anhydrous, hemihydrated, and monohydrated crystal forms.[14] In this study when water was added during production, the anhydrous form did not exist. The diffraction patterns of the suspensions included reflections, indicating that the crystal of the suspensions is a mixture of hemihydrated and monohydrated form, regardless of the process parameters (**Figure 7**). The addition of surfactant caused the same reflections as presented above. This indicates that the β-sitosterol crystal form remained as a mixture. Quantitative determination of hemihydrated and monohydrated crystals according to the XRD results is difficult because of the preferred orientation of needle-shaped and plate-like crystals. X-ray measurements, however, showed that no crystal form transformation occurred soon after or during 4 months of storage.[16] Further studies should concentrate on the phase stability of these 2 hydrate forms.

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stirring rate as well as an increased stirring time decreased the crystal size. When the stirring was stopped prematurely, the crystals continued to grow, forming large, flaky crystals. The same phenomenon took place at very low agitation rates. An addition of polysorbate 80 only had a slight effect on crystal growth. By using the optimized parameters, we obtained crystals that were all under 50 μm in length, with a median of 17 μm.
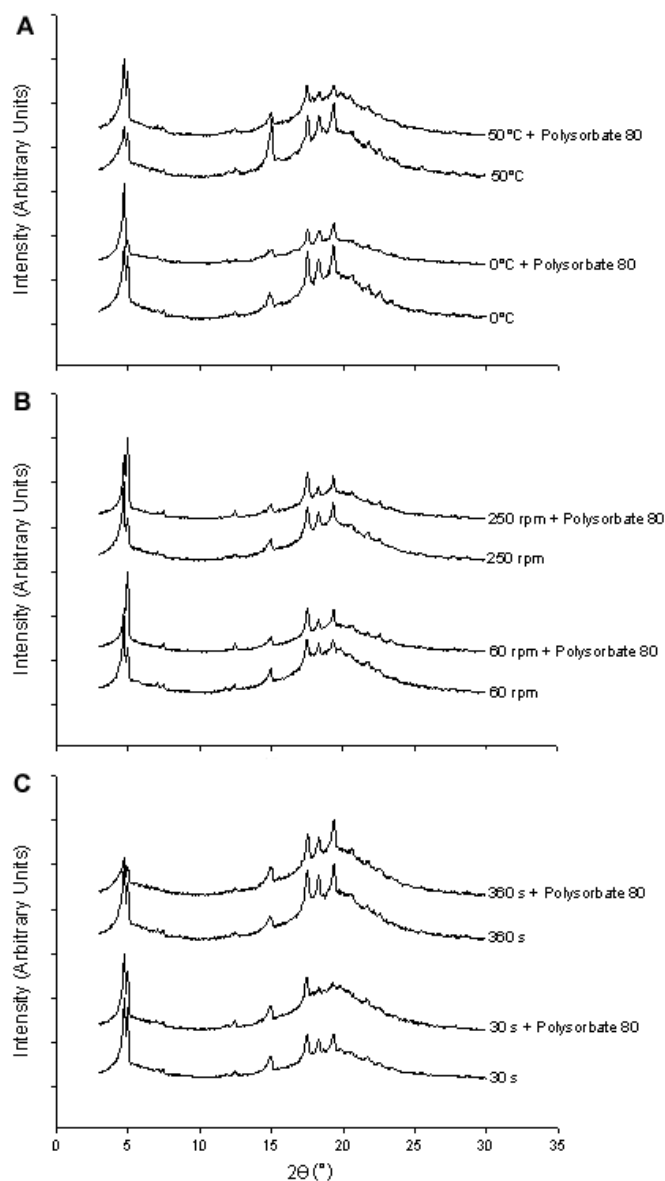

Figure 7. X-ray diffraction patterns of β-sitosterol suspensions both with and without polysorbate 80 (1%, wt/wt). The suspensions were prepared with different (A) cooling temperatures, (B) stirring rates, and (C) stirring times. The samples were measured 1 day after preparation.

CONCLUSION

By optimizing the processing parameters it was possible to obtain a desired β-sitosterol suspension containing small, needle-shaped crystals. Cooling temperature turned out to have a remarkable effect on the crystal size through its effect on supersaturation. An increased

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