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Recrystallization of waxy maize starch during manufacturing of starch microspheres for drug delivery: Optimization by experimental design

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Abstract

Gelatinized starch/water dispersions with a water content of 70% w/w were studied in order to evaluate the influence of the incubation time at two fixed temperatures (6 and 37 °C) on the recrystallization behavior. The starch material was produced from waxy maize starch by acid hydrolysis followed by a mechanical treatment, i.e. a high pressure homogenization. Empirical models for the recrystallization of the model starch dispersions were investigated by using a central composite circumscribed (CCC) design in two factors involving 11 trials. The rebuilt crystalline structure was estimated by measuring the melting transition parameters with differential scanning calorimetry (DSC). The designed models for the melting enthalpy, the melting transition temperatures, and the melting interval as the responses were reproducible and predictable and the results were analyzed by using a response surface modeling. Operative conditions for the formation of the most thermally stable crystalline structure with the highest possible crystallinity were determined. It was also concluded that the decisive factor for the crystallinity of the given starch material was a prolonged storage at 6 °C, while thermal characteristics of the crystallites could be controlled by storage at 37 °C. An interaction effect was observed between the incubation time at these two experimental temperatures regarding enthalpy values and the melting interval of the samples.

Keywords: Waxy maize starch; Recrystallization; Time/temperature treatment; DSC; CCC-design; MODDE

1. Introduction

Starch is a common excipient with a long tradition within the pharmaceutical industry where it is used as a disintegrant, a binder, or a bulking agent. Starch has been used as a biodegradable polymer in microspheres for nasal delivery of drugs (Björk & Edman, 1988; Critchley, Farraj, & Illum, 1994; Illum, Farraj, Critchley, & Davis, 1988; Illum, Farraj, Davis, Johansen, & O'Hagan, 1990; Shirui, Jianming, Huan, Zhenping, & Dianzhou, 2004; Vivien, Buri, Balant, & Lacroix, 1994), or for the delivery of vaccine given orally and intramuscularly (Heritage et al., 1996; Sturesson & Degling Wikingsson, 2000; Rydell, Stertman, & Sjöholm, 2005). The main part of these starch particles has been produced by polymerization of acryloylated starch in a water-in-oil emulsion (Artursson, Edman, Laakso, & Sjöholm, 1984; Laakso, Artursson, & Sjöholm, 1986; Rydell et al., 2005; Sturesson & Degling Wikingsson, 2000) or by cross-linking soluble starch with epichlorohydrin (Hamdi, Ponchel, & Duchene, 2001; Lindberg, Lote, & Teder, 1984). In a formulation of Biosphere® (SkyePharma AB, Sweden), that is a starch microsphere intended for subcutaneous injections, the colloidal properties of starch

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together with its natural capability to rebuild the crystalline structure on storage were employed instead of chemical cross-linking (Reslow, Jönsson, & Laakso, 2002).

The process of starch microsphere production was described in detail by Reslow et al. (2002) and the same process was used in the manufacture of the starch microspheres characterized by Elfstrand, Eliasson, Jönsson, Reslow, and Wahlgren (2006). Crystalline structure of starch microsphere was mainly formed during one of the steps in the manufacturing, a time/temperature treatment. The time/temperature treatment consisted of an incubation, first at a low temperature and then at a higher temperature; both steps at pre-set definite times. To the time/temperature treatment the starch was subjected being previously dispersed in water upon heating. The aim of this treatment was to allow the starch molecules to recrystallize into a stable crystalline structure making up a solid matrix of the starch microsphere. A better understanding how the production factors affected the formation of ordered structure in the starch matrix was the aim of this work.

In the present study, starch/water dispersions (30/70% w/w) were subjected to a two-step temperature treatment (6/37 °C) in order to mimic the time/temperature treatment step in the microsphere manufacturing process. DSC was used for characterization of the rebuilt structure in the dispersions. The planning of the experiments as well as the evaluation of the effect of the incubation time at these two temperatures on the melting characteristics of the rebuilt structure were performed using design of experiments.

2. Materials and methods

2.1. Starch materials

The starch material used in this study was donated by SkyePharma AB (Malmö, Sweden) where it was produced from a native waxy maize starch (Cerestar SF 04201). The process for the starch material manufacturing and the molecular characteristics of both the native and the modified starch were described previously by Elfstrand et al. (2004). The native waxy maize starch with a molecular weight ($\overline{MW}$) of 4.7×10^7 g mol^{-1} was acid hydrolyzed prior to a mechanical treatment, resulting in a starch material with a reduced molecular weight of 5.1×10^5 g mol^{-1}, and without any granular or crystalline order.

The starch material that had been dispersed in water (30/70% w/w), heated and then incubated at varying time/temperature conditions, was used as a model system for the corresponding time/temperature treatment in the manufacturing of the starch microspheres.

2.2. Experimental design

The objective of the present study was to evaluate the influence of two experimental factors on the response variables. These factors, or independent variables, which were varied over the set of experiments, were the time of incubation of the starch/water dispersion at 6 °C (t_6, h) and the time of the following incubation at 37 °C (t_{37}, h). The measured responses, or the dependent variables, were the onset melting temperature (T_{on}, °C), the melting peak temperature (T_m, °C), the melting completion temperature (T_c, °C), the melting interval (T_{range}, °C, defined as the difference between T_c and T_{on}) and the melting enthalpy (ΔH, J/g), all measured by DSC. By using central composite circumscribed design (CCC) all the factors were investigated at five levels, rendering it possible to estimate the model quadratic terms.

The design was a 2^2 factorial design, augmenting the central composite (CCD) design with a total of 11 experiments (Fig. 1). Such a design consisted of three building blocks, (i) regularly arranged corner (factorial) experiments of a two-level factorial design, (ii) symmetrically arrayed star points located on the factor axes, and (iii) replicated center points. The experimental area according to the CCC design is described in Fig. 1. Three center points were included to give important information on the reproducibility of the experiments (Eriksson, Johansson, Kettaneh-Wold, Wikström, & Wold, 2000). The factor settings in the experimental domain were chosen based on the settings during the manufacturing process of the microspheres. In order to reduce time dependent systematic errors, the run order of the experiments was randomized.

The experimental results were summarized and analyzed with the software MODDE 7.0.0.1. (Umetrics AB). Relations between factors and responses were found by fitting a quadratic model with six terms for each response:

$$y = b_0 + b_{t6} \times x_{t6} + b_{t37} \times x_{t37} + b_{t6\,t6} \times x_{t6}^2 + b_{t37\,t37} \times x_{t37}^2 + b_{t6\,t37} \times x_{t6} \times x_{t37},$$

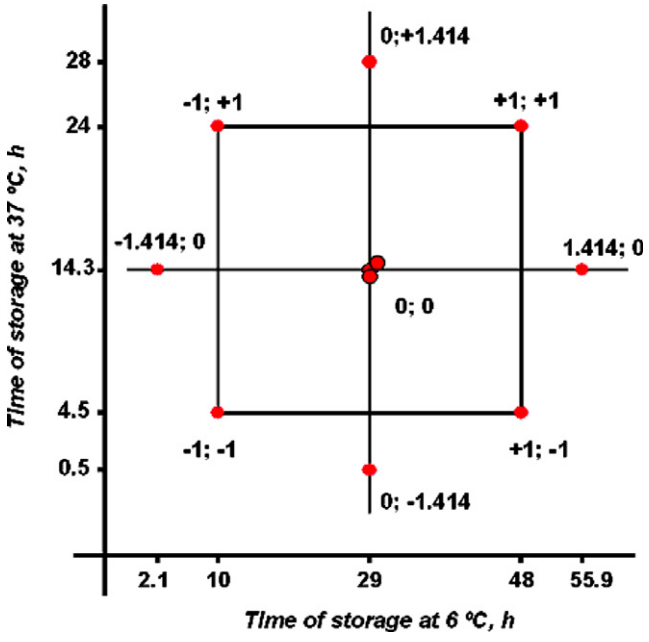

Fig. 1. The experimental area according to the CCC design.

where y was a response, x_{t6} and x_{t37} were input variables, b_0 was a constant term and b_{t6}, b_{t37}, b_{t6t37}, b_{t6t6} and b_{t37t37} were the model parameters.

The goodness of the models, i.e. the correlation between the input and the response data, was evaluated using a *summary of the fit*. This method includes the *goodness of fit* R^2, and the *goodness of prediction* Q^2 where R^2 is an overestimated measure and Q^2 is an underestimated measure of the goodness of fit of the model (Eriksson et al., 2000). The goodness of the model was also evaluated using *ANOVA* (analysis of variance).

Optimal conditions according to the models were determined with the help of the MODDE software, running the Optimizer. The optimizer used a Nelder Mead simplex method with fitted response functions to optimize an overall desirability function combining the individual desirability of each response (Eriksson et al., 2000). The individual desirability for response y was computed as follows: $f(g(y)) = 100 \times (e^{\lambda g(y)} - 1)$ with $g(y) = 100 \times ((y - P)/(T - P))$ where T was the user-desired Target, L the user-defined worst acceptable response value(s), and P the worst response value computed from the starting simplex. λ was a scaling parameter computed as follows:

$$\lambda = -\ln\left(\frac{\frac{100}{(100-\text{Limit})}}{100 * \frac{(L-P)}{(T-P)}}\right),$$

where $\text{Limit} = 90 + 80 \times \text{Log}_{10}(w)$, and w was the weight assigned to each response by the user. The overall desirability was a weighted average of the individual desirability function. An "overall distance to Target", D, was computed for display purpose only according to: $D = \log_{10}\left[\frac{\sum w_i \left[\frac{y_i - T}{T - L}\right]^2}{M}\right]$, where M was the number of responses.

$D = 0$ when all y's were between T and L.

2.3. Measurements of the responses

In the present study the responses, i.e. T_{on}, T_m, T_c, T_{range} and ΔH, were measured by DSC. The samples were prepared by dispersing a 30% (w/w) starch/water mixture (10 g) and heating it in a microwave oven (800 W; LG Electronics Inc., MS-194A, P.R.C.) for 3 periods of 6 s. Samples of approximately 10 mg were weighed into aluminum DSC pans (TA Instruments, New Castle, USA, Ref. No. 900790.901 and 900796.901) within 1 h after their preparation. The pans were hermetically sealed and the samples were incubated according to the experimental plan (Fig. 1). The incubation conditions for every experimental domain could simply be described as t_6 (incubation time at 6 °C, h) and t_{37} (incubation time at 37 °C, h), i.e. t_6/t_{37} (h/h).

In all experiments the incubated samples were analyzed in a DSC 6200 (Seiko Instrument Inc., Shizouka, Japan) with an empty pan as a reference. The melting enthalpy was determined scanning the temperature scan from 30 up to 110 °C at a heating rate of 5 °C/min. The enthalpy was calculated on the dry weight basis of amylopectin. All analyses were performed in at least duplicates. The true amylopectin/water ratio was determined by drying punctured pans at 105 °C overnight.

3. Results

3.1. Crystalline structure variations over the designed region

Melting transition parameters measured by DSC are shown in Table 1 and the melting profiles of the samples can be seen in Fig. 2. By thermal scanning of the incubated samples, the presence of an ordered structure could be detected. With the exception of one sample (Sample 5), the ordered structure was restored in all experiments. Varying melting profiles could be distinguished. Melting peak profiles that were characterized as distinct and deep were observed for the samples incubated at the following conditions (t_6/t_{37}): 48/4.5; 48/24; 56/14.3; 29/28; 29/14.3. Broader and shallower thermogram profiles were registered for samples that were incubated at the conditions 10/4.5; 10/24; 29/0.5 (Fig. 2).

Melting transition temperatures, the melting range and enthalpy values also varied among the samples (Table 1). It is known that crystals with a more perfect structure melt at higher temperatures and over a narrower temperature range than crystals with many imperfections (Mandelkern, 1956; Bassett, 1981). The DSC results from this study revealed variations in the perfection of the crystalline structure over the designed region depending on the incubation time for the two experimental temperatures.

4. Modeling by MODDE

Collected informative data (Table 1) was analyzed by MODDE in order to find a relation between the input variables, t_6 (the incubation time at 6 °C) and t_{37} (the incubation time at 37 °C), and the output variables, i.e. the responses T_m, T_{on}, T_c, T_{range} and ΔH. Evaluation of the raw data showed that the replicate errors were small for all responses. The data distribution for each response was inspected and the metrics of the responses ΔH, T_{on}, T_m, T_c were transformed in order to obtain improved models. Logarithmic transformation was performed for ΔH, and power transformation was carried out for the rest of the responses.

By using ANOVA and *Summary of the fit* methods, the models of the responses were estimated as good, meaning that the responses correlated well with the independent input variables (t_6 and t_{37}).

The relationships between all factors and all responses could be overviewed by displaying the loading plot (Fig. 3). In order to obtain information concerning whether the factors had positive or negative effects on the responses, regression coefficient plots were consulted and interpreted (Fig. 4). Investigation of these plots revealed that the responses T_{on} and T_m were strongly correlated and the

Table 1
The measured responses obtained from the running of the experiments

Experiment number	Factors		Responses				
	t_6 (h)	t_{37} (h)	ΔH (J/g)	T_{on} (°C)	T_m (°C)	T_c (°C)	T_{range} (°C)
1	10	4.5	2.1 (0.6)	49.4 (3.3)	58.4 (1.7)	68.4 (0.2)	19.0 (3.1)
2	48	4.5	4.9 (0.0)	49.8 (0.4)	59.3 (0.4)	66.3 (1.0)	16.6 (1.3)
3	10	24	1.7 (0.2)	52.7 (0.8)	62.4 (0.3)	68.5 (0.6)	15.8 (0.6)
4	48	24	6.2 (0.4)	51.5 (0.1)	61.6 (0.1)	69.1 (0.2)	17.8 (0.1)
5	2.1	14.3	n.d.	n.d.	n.d.	n.d.	n.d.
6	56	14.3	5.6 (0.6)	52.3 (1.4)	60.5 (0.1)	67.4 (0.0)	15.1 (1.4)
7	29	0.5	4.5 (0.3)	45.8 (0.7)	56.0 (1.3)	65.5 (0.5)	19.7 (0.6)
8	29	28.0	4.7 (0.8)	52.9 (1.4)	61.7 (0.2)	68.1 (0.4)	15.2 (1.1)
9	29	14.3	4.7 (0.5)	50.6 (0.7)	60.2 (0.9)	66.7 (0.3)	16.1 (0.5)
10	29	14.3	4.5 (0.2)	51.4 (0.7)	60.8 (0.4)	67.3 (1.3)	15.9 (0.6)
11	29	14.3	4.8 (0.2)	51.8 (1.1)	60.4 (0.6)	66.8 (1.0)	15.0 (0.2)

The onset (T_{on}), peak (T_m) and final melting (T_c) temperatures, the melting temperature interval (T_{range}) and the recrystallization enthalpy (ΔH) obtained from DSC analysis. Standard deviation values are given within brackets.
n.d. not detected.

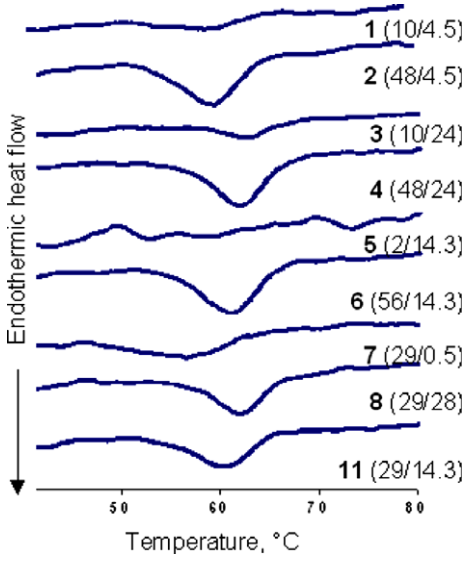

Fig. 2. DSC thermograms of the samples incubated at varying time/temperature conditions given in the brackets (the variables t_6/t_{37}). The numbering of the experimental runs is made according to Table 1.

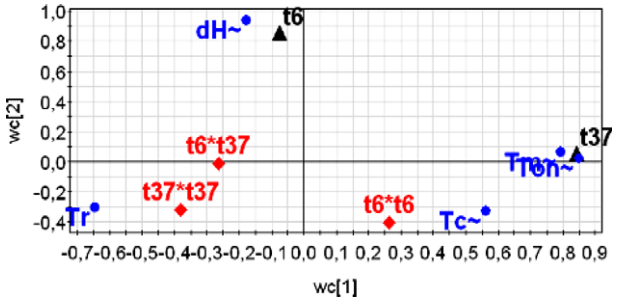

Fig. 3. The loading plot displaying the relation between the factors (▲), the responses (●) and the interaction terms (♦).

dominating factor for T_{on}, T_m and T_c was the incubation time at 37 °C and the factor dominating ΔH was the incubation time at 6 °C. For T_{range} the dominating factor was also found to be the incubation time at 37 °C. This response was influenced negatively by this factor, here leading to a narrowing of T_{range}.

By analyzing the interaction plots (Fig. 5) and the regression coefficients (Fig. 4) the impact of the two-factor interactions could be identified for ΔH, T_c and T_{range}, while for the responses T_m and T_{on} no such interactions could be observed. An interaction effect occurs when a relation between (at least) two variables is modified by other variables (at least one) (Eriksson et al., 2000). In the case of ΔH the effect of incubation time at 6 °C was modified to some degree by the duration of incubation at 37 °C. However, duration of the incubation at 6 °C, in turn, influenced the effect of incubation at 37 °C. At incubation times at 6 °C between 29 and 48 h the enthalpy increased with increasing incubation time at 37 °C. At the incubation times at 6 °C, both below 29 and above 48 h, the enthalpy decreased when the sample was subjected to incubation at 37 °C. In the case of T_{range}, the following conclusions could be made. Holding the sample for longer time at 37 °C decreased T_{range} especially when the incubation time at 6 °C was short. The effect was less pronounced for long incubation times at 6 °C. In the T_c interaction plot the lines of the low and high level of t_{37} almost crossed each other. The results indicated that at low incubation time at 6 °C the T_c was almost uninfluenced by the incubation time at 37 °C. But when the incubation time at 6 °C increased the T_c increased with the longer incubation times at 37 °C.

4.1. Interpretation of the data by Response surface modeling

Impact of the parameters on the responses can be illustrated by a surface in three dimensions, i.e. response surface plot. This method is known as a response surface modeling (RSM) (Eriksson et al., 2000). An alternative graphical method for inspection of the variations of a response surface in two dimensions is a contour plot.

From the contour plots in Fig. 6, the response value variations as a function of t_6 and t_{37}, i.e. incubation times

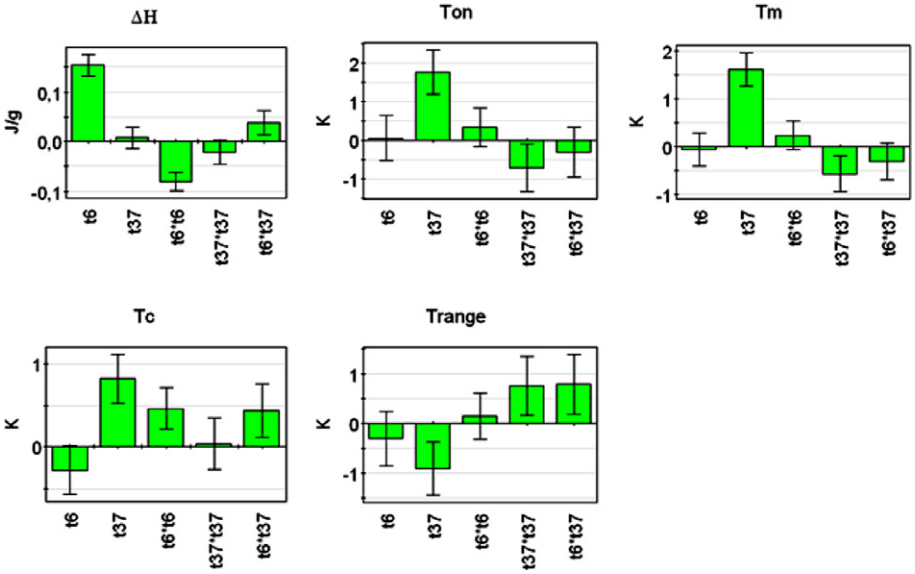

Fig. 4. Coefficient plots for the responses ΔH, T_m, T_{on}, T_{range} and T_c with confidence intervals.

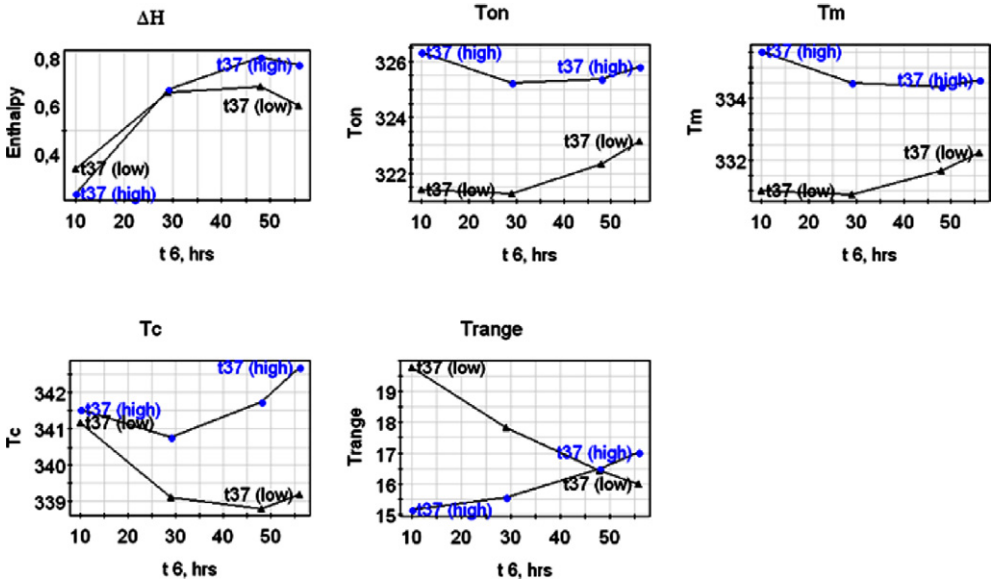

Fig. 5. Interaction plots for $t_6 \times t_{37}$ related to ΔH, T_m, T_{on}, T_{range} and T_c. The low and the high level of t_{37} are symbolized by "▲" and "●", respectively.

at 6 and at 37 °C, could be observed. It became apparent from the figure that in order to obtain as high a ΔH as possible the sample should be incubated for as long as possible at 6 °C as well as at 37 °C. The highest enthalpy value for this set of experiments was obtained when incubation time at 6 °C was longer than 35 h and the incubation time at 37 °C was longer than 10 h. Changes in the incubation time at 37 ° C did not have any effect on the magnitude of the ΔH values if the incubation time at 6 °C was below 30 h. If, on the other hand, the samples were stored at 6 °C for more than 30 h, variations in the incubation time at 37 °C started to influence the enthalpy value. From the T_m contour plot it could be concluded that in order to obtain the highest T_m value one should incubate the samples for as short a time as possible at 6 °C and for as long as possible at 37 °C. To obtain the narrowest T_{range} the sample should be incubated at following conditions: a shorter incubation time at 6 °C (<20 h)/ a longer incubation time at 37 °C (>20 h) (which are the conditions giving the highest T_{on} and T_m for the system, see Fig. 6) or, oppositely, a longer incubation time at 6 °C (>20 h) /a shorter incubation time at 37 °C (<20 h) (Fig. 6). At longer incubation times at these both temperatures the T_{range} became wider again, probably due to the effect of the T_c increase.

By analyzing the collected data (Table 1), it was possible to determine the conditions for obtaining a structure with the highest ΔH or the conditions for obtaining the structure with the highest T_m. The highest enthalpy for this set of experiments was obtained when the sample was incubated for 48 h at 6 °C followed by 24 h at 37 °C. The highest peak

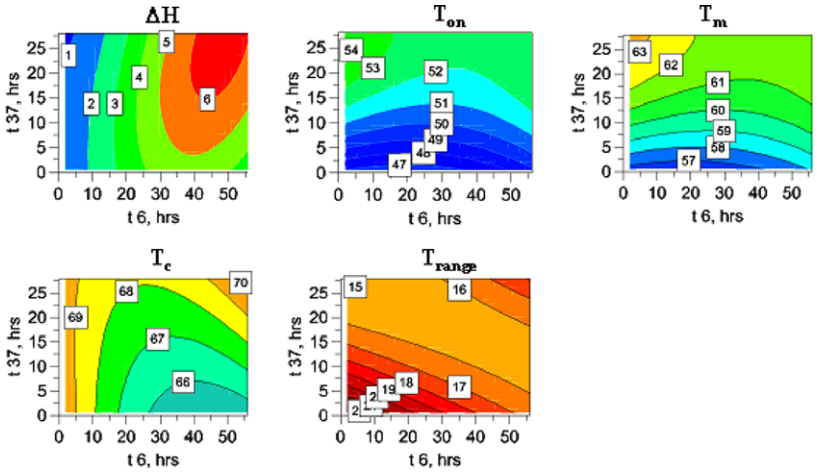

Fig. 6. Contour plots for the responses ΔH, T_m, T_{on}, T_{range} and T_c.

melting temperature was obtained when a sample was incubated for 10 h at 6 °C followed by 24 h at 37 °C; these conditions also gave rise to the lowest enthalpy value. The lowest melting temperature and the widest melting interval was recorded for the sample stored for 29 h at 6 °C and 0.5 h at 37 °C. The narrowest melting interval was obtained for the sample stored at conditions 56/14.3 (t_6/t_{37}).

The optimal production conditions (if the aim was to obtain an ordered structure with high ΔH and high T_m) were found to be the time/temperature treatment consisting of incubation for 55 h at 6 °C followed by 26 h at 37 °C. These conditions were determined by the MODDE software according to the models.

5. Discussion

5.1. Isothermal crystallization

In this work, we have studied the ordering of starch at different temperature conditions using DSC. The results showed that the time/temperature treatment influenced the properties of the ordered structure as measured by DSC. This is in line with results from previous studies that have shown that crystallization process of the starch in starch/water dispersions is influenced by the treatment conditions, the temperature and the duration (Durrani & Donald, 1995; Karlsson & Eliasson, 2003; Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000; Slade & Levine, 1987). DSC technique has previously been reported to be comparable to wide angle X-ray scattering (WAXS) in regards to the detection of crystallinity in stored starch/water dispersions (Durrani & Donald, 1995). However, one should bear in the mind that not all endothermic events observed in DSC of starch systems arise as a result of crystallite melting (Cooke & Gidley, 1992; Jenkins et al., 1994; Tester & Debon, 2000) and that different polymorphic forms of starch might show different DSC results (Donovan & Mapes, 1980; Whittam, Noel, & Ring, 1990). The polymorphic form of the stored starch dispersions have been reported to be of B type (Durrani & Donald, 1995; Radley, 1953; Ring, 1985), although the presence of some additives can influence the crystallinity pattern and cause both types A and C (Hizukuri, Fujii, & Nikuni, 1960). In an ongoing study (not published results) we are combining X-ray diffraction and DSC results on similar samples to the ones used here and so far there are indications that the polymorphic form is not of clean B type. Moreover, there are also indications that the polymorphic form does not change during the time/temperature treatment.

5.1.1. Events at 6 °C: Apparent induction time, nucleation and growth of crystallites

As was concluded during the modeling by MODDE, the factor dominating the ΔH (or the amount of ordered material formed in the stored starch dispersion) was the incubation time at 6 °C. This indicates that the nucleation process that is favored by low temperatures (Mandelkern, 1956; Slade & Levine, 1987) is the process that dominates the formation of ordered structure in the starch/water dispersions.

As could be seen in Fig. 2, no melting transition could be registered for Sample 5, which was the sample incubated for 2 h at 6 °C prior to incubation at 37 °C. When a polymer sample is transferred from a temperature above T_m to the desired crystallization temperature, a well-defined "apparent induction time" occurs before the crystallization process can be observed (Mandelkern, 1956). The samples in this study were subjected to two temperatures; and the DSC analyses were performed after the full two-temperature treatment. It seemed that the "apparent induction time" required for the formation of an observable crystalline structure at the given conditions, should be longer than what was performed when incubating Sample 5. Reminding that the ordered structure in the samples were influenced by both the incubation temperatures another explanation for the lack of the ordered structure in the sample could be suggested, i.e. the crystallite nuclei formed during this short time at 6 °C were small and/or thermally

unstable and melted completely at the following incubation at 37 °C, and the new ones could not be formed at these conditions during this experimental time. This is also in line with the coupling between incubation times at 6 and at 37 °C seen in the model.

For the rest of the samples, an endotherm corresponding to the melting of their crystalline structures could be detected. This suggests that at these conditions the observable ordered structure could be obtained if the samples were incubated at the nucleation temperature (at 6 °C) between 2 and 10 h and that the nuclei formed during this time were stable enough to survive the next step of the treatment.

A longer incubation of the dispersions at 6 °C caused growth of the crystallites, even though mainly lower-temperature-melted material was formed at this temperature. Durrani and Donald (1995) observed that the amount of ordered material that melted at lower temperature increased and the temperature of the transition moved to lower temperatures when the incubation time increased, for starch/water dispersions that were stored at 4 °C for varying durations. In the experiments from the present study, all samples were incubated at two experimental temperatures. It was therefore not possible to draw conclusions on the effect of only one temperature on the crystalline structure of the samples. However, the lowest melting, onset and completion temperatures, and the widest melting interval were recorded for Sample 7 stored at the conditions t_6/t_{37} 29/0.5, with the shortest time at 37 °C, which was obviously not enough to considerably affect the less thermally stable crystallites of the sample.

5.1.2. Events at 37 °C: Molecular reorganization during annealing

As was concluded during modeling by MODDE, the incubation time at 37 °C was a controlling factor for T_m, i.e. the melting peak temperature of the ordered structure reformed in the stored starch dispersions. The samples were brought to this higher temperature after being stored at the lower temperature. The events occurring in the samples could be described in terms of annealing (Jacobs & Delcour, 1998). When the crystallite nuclei are already formed in the sample by incubation at a low temperature, annealing at higher temperatures but below T_{on}, leads to a physical reorganization of the crystallites. Several parallel events take place in the starch dispersions: melting of less thermally stable material, its reformation, and growth of more perfectly ordered structures. A prolonged incubation at 37 °C on samples that were previously incubated 29 h at 6 °C displayed an effect on the ordered structure formed in the samples. Samples 7, 11 and 8, with incubation times of 0.5, 14 and 28 h at 37 °C, respectively, were compared (Fig. 2, Table 1). As a result of this incubation, T_{on} increased for Samples 11 and 8 as compared to Sample 7. This could be explained by melting of the less thermally stable material. By investigating the melting profiles (Fig. 2) and the melting intervals, T_{range}, these conclusions were confirmed since a narrower melting interval was observed for Samples 11 and 8 that were incubated longer at 37 °C, and the T_m and T_c were also found to increase. However, enthalpy values of Samples 11 and 8 did not decrease as compared to Sample 7. This indicated that the melted ordered material reformed into the more thermally stable form.

5.2. Effect of a combined incubation 6/37 °C on the crystalline structure

The crystalline structure obtained during incubation at two fixed temperatures was a result of combined effects of events occurring at these conditions. Variations in the crystalline structure concerning its thermodynamic properties were achieved. Distinct and deep melting peaks on the DSC thermograms with a narrow melting interval (Fig. 2) were recorded for samples incubated for a total incubation time of more than 40 h and with $\geqslant$29 or more hours of incubation time at 6 °C (Samples 2, 4, 6, 8 and 11).

It is well known that when exposed to annealing conditions, i.e. to temperatures between the glass transition temperature (T_g) and crystallite melting temperature at an appropriate water content, starch undergoes the process of recrystallisation where α-glucan chains form double helices that arrange themselves into crystallites (Slade & Levine, 1987). But, as aforementioned, the process of nucleation proceeds extremely slowly if the temperature is just below the melting temperature. As seen from our results to speed up this process, the temperature should be lowered, as was done in this study by incubating the samples at 6 °C.

It could be observed that the duration of the low-temperature incubation, influenced the effect of the high-temperature incubation, on the enthalpy growth (Fig. 7). A longer incubation time at 37 °C gave higher ΔH values if the samples were previously stored for 48 h at 6 °C (Table 1, Samples 2 and 4; 4.5 and 24 h at 37 °C, respectively). When the samples were previously incubated for 29 h at 6 °C, the duration of incubation at 37 °C did not have any effect on the amount of crystallinity (Samples 8 and 11; 28 and 14 h at 37 °C, respectively), while longer incubation times at 37 °C reduced the amount of crystallinity formed in the starch/water dispersions incubated at 6 °C for only 10 h (samples 1 and 3; 4.5 and 24 h at 37 °C, respectively), the latter probably due to melting of ordered material of low thermal stability.

Thus, to obtain an increase in ΔH values in the sample during the time/temperature treatment, a certain level of ordering should first be obtained in the sample at the lower temperature, so that the level of ΔH of the sample does not decrease when the temperature is increased, but stays at the same level or even increases. It seems likely that during the first 10 h at the low temperature many small, heterogeneous, unstable and imperfect crystallites are created (Sample 1). If such a short incubation time at 6 °C is followed by incubation at 37 °C the most unstable crystallites melt and

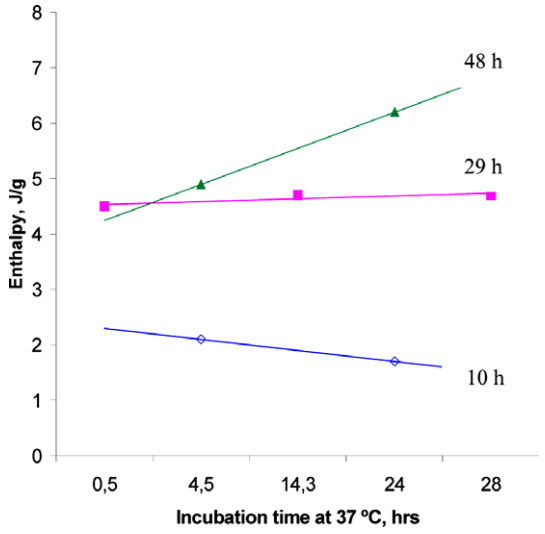

Fig. 7. The melting enthalpy as a function of the incubation time at 37 °C when the incubation time at 6 °C is held constant at 10, 29 and 48 h.

disappear, and the experimental time has not been enough for reorganization to more thermally stable crystallites to compensate this loss.

From the experiments in the present study it could be concluded that melting properties and features of recrystallized material in starch dispersions subjected to incubation gradually changed with the incubation time, i.e. the crystalline structure was in a developing process. The final crystalline structure of the annealed sample was dependent on the level of crystallinity that developed in the sample at lower incubation temperatures, which was also illustrated by our experiments.

6. Conclusions

MODDE software was shown to be a useful tool for the optimization of production conditions, i.e. the time/temperature treatment of the starch dispersions. By using this tool the effect of the two experimental temperatures, 6 and 37 °C, on the recrystallization process of modified waxy maize starch was evaluated. Variation of the incubation time at these temperatures induced differences in thermodynamic properties of the reformed ordered structure in the starch samples. The duration of incubation at 6 °C was a decisive factor for the amount of rebuilt ordered structure as expressed by melting enthalpy values. The incubation time at 37 °C was a determinant for thermal stability of the crystalline structure expressed by the melting peak temperature. Prolonged incubation times at 37 °C were required in order to push the melting peak temperature values up to higher levels.

The duration of incubation at 37 °C influenced the enthalpy values of starch dispersions previously subjected to an incubation at 6 °C. In order to prevent the enthalpy levels from decreasing as a result of the incubation at 37 °C, the samples should be incubated for at least 29 h at 6 °C prior to the incubation at 37 °C.

The formation of an ordered structure with desired properties such as the level of ordering or the level of thermal stability could be controlled by variation of the incubation time at different temperatures.

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References

Artursson, P., Edman, P., Laakso, T., & Sjöholm, I. (1984). Characterization of polyacryl starch microparticles as carriers for proteins and drug. *Journal of Pharmaceutical Science, 73*(11), 1507–1513.

Bassett, D. C. (1981). *Principles of polymer morphology*. Cambridge: Cambridge University Press.

Björk, E., & Edman, P. (1988). Degradable starch microspheres as a nasal delivery system for insulin. *International Journal of Pharmaceutics, 47*, 233–238.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Critchley, H., Farraj, D., & Illum, L. (1994). Nasal absorption of desmopressin in rats and in sheep. Effect of bioadhesive microsphere delivery system. *Journal of Pharmacy and Pharmacology, 46*, 651–656.

Donovan, J. W., & Mapes, C. J. (1980). Multiple phase transitions of starches and Nägeli amylodextrins. *Starch/Stärke, 32*, 190–193.

Durrani, C. M., & Donald, A. M. (1995). Physical characterisation of amylopectin gels. *Polymer Gels and Networks, 3*, 1–27.

Elfstrand, L., Frigård, T., Andersson, R., Eliasson, A-C., Jönsson, M., Reslow, M., et al. (2004). Recrystallisation behaviour of native and processed waxy maize starch in relation to the molecular characteristics. *Carbohydrate Polymers, 57*, 389–400.

Elfstrand, L., Eliasson, A-C., Jönsson, M., Reslow, M., & Wahlgren, M. (2006). From starch to starch microspheres. Factors controlling the microspheres quality. *Starch/Stärke, 58*, 381–390.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wikström, C., & Wold, S. (2000). *Design of experiments – Principles and applications*. Stockholm: Learnways, AB.

Hamdi, G., Ponchel, G., & Duchene, D. (2001). Formulation of epichlorohydrin cross-linked starch microspheres. *Journal of Microencapsulation, 18*(3), 373–383.

Heritage, P. L., Loomes, L. M., Jiang, J., Brook, M. A., Underdown, B. J., & McDermott, M. R. (1996). Novel polymer-grafted starch microparticles for mucosal delivery of vaccines. *Immunology, 88*, 162–168.

Hizukuri, S., Fujii, M., & Nikuni, Z. (1960). The effect of inorganic ions on the crystallization of amylodextrin. Short communications. *Biochimica et Biophysica Acta, 40*, 346–348.

Illum, L., Farraj, N. F., Critchley, H., & Davis, S. S. (1988). Nasal administration of gentamicin using a novel microsphere delivery system. *International Journal of Pharmaceutics, 46*, 261–265.

Illum, L., Farraj, N. F., Davis, S. S., Johansen, B. R., & O'Hagan, D. T. (1990). Investigation of the nasal absorption of biosynthetic human growth hormone in sheep-use of a bioadhesive microsphere delivery system. *International Journal of Pharmaceutics, 63*, 207–211.

Karlsson, M. E., & Eliasson, A.-C. (2003). Effect of time/temperature treatments on potato *(Solanum tuberosum)* starch: a comparision of isolated starch and starch *in situ*. *Journal of the Science of Food and Agriculture, 83*, 1587–1592.

Jacobs, H., & Delcour, J. A. (1998). Hydrothermal modifications of granular starch, with retention of the granular structure a review. *Journal of Agricultural and Food chemistry, 46*(8), 2895–2905.

Jenkins, P. J., Cameron, R. E., Donald, A. M., Bras, W., Derbyshire, G. E., Mant, G. R., et al. (1994). In situ simulations small and wide angle X-ray scattering: a new technique to study starch gelatinization. *Journal of Polymer Science: Part B: Polymer Physics, 32*, 1579–1583.

Laakso, T., Artursson, P., & Sjöholm, I. (1986). Biodegradable microspheres IV: factors affecting the destribution and degradation of polyacrilic starch microparticles. *Journal of Pharmaceutical Science, 75*, 962–967.

Lindberg, B., Lote, K., & Teder, H. (1984). Biodegradable starch microspheres – A new medical tool. In S. S. Davis, L. Illum, J. G. McVie, & E. Tomlinson (Eds.), *Microspheres and drug therapy* (pp. 153–188). Amsterdam: Elsevier.

Mandelkern, L. (1956). The crystallisation of flexible polymer molecules. *Chemical Review, 56*, 903–956.

MODDE 7.0.0.1, software, Umetrics AB.

Radley, J. A. (1953) (3rd ed.). *Starch and its derivatives* (Vol. II). London: Chapman & Hall Ltd, p. 71.

Reslow, M., Jönsson, M., & Laakso, T. (2002). Sustained-release of human growth hormone from PLG-coated starch microspheres. *Drug Delivery Systems and Sciences, 2*(4), 103–109.

Ring, S. (1985). Observations on the crystallization of amylopectin from aqueous solution. *International Journal of Biological Macromolecules, 7*(4), 253–254.

Rydell, N., Stertman, L., & Sjöholm, I. (2005). Starch microparticles as vaccine adjuvant. *Expert Opinion on Drug Delivery, 2*(5), 807–828.

Shirui, M., Jianming, C., Huan, L., Zhenping, W., & Dianzhou, B. (2004). Intranasal administration of melatonin starch microsphere. *International Journal of Pharmaceutics, 272*(1–2), 37–43.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., & Åman, P. (2000). The effects of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. *Carbohydrate Polymers, 42*(2), 175–184.

Slade, L., & Levine, H. (1987). Recent advances in starch retrogradation. In S. S. Stivala, V. Crescenti, & I. C. Dea (Eds.), *Industrial Polysaccharides. The impact of biotechnology and advanced methodologies* (pp. 387–430). New York: Golden and Breach Science Publishers.

Sturesson, C., & Degling Wikingsson, L. (2000). Comparison of poly(acryl starch) and poly(lactide-co-glycolide) microspheres as drug delivery system for a rotavirus vaccine. *Journal of Controlled Release, 68*, 441–450.

Tester, R. F., & Debon, S. J. J. (2000). Annealing of starch – a review. *International Journal of Biological Macromolecules, 27*, 1–12.

Vivien, N., Buri, P., Balant, L., & Lacroix, S. (1994). Nasal absorption of metoclopramide administered to man. *European Journal of Pharmaceutics and Biopharmaceutics, 40*, 228–231.

Whittam, M. A., Noel, T. R., & Ring, G. (1990). Melting behaviour of A- and B-type crystalline starch. *International Journal of Biological Macromolecules, 12*(6), 359–362.