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Recrystallization of waxy maize starch during manufacturing of starch microspheres for drug delivery: Influence of excipients

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Abstract

The formation of ordered structure, such as crystallites, in starch was studied by means of differential scanning calorimetry (DSC). The influence of time/temperature treatment and additives such as polyethylene glycol (PEG), bovine serum albumin (BSA) and a carbonate buffer on the formation was investigated. The experiments were planned with a CCC (Central Composite Circumscribed) design. For all three investigated systems it could be concluded that the incubation time at 6 °C was the decisive factor for the amount of ordered structure obtained during the incubation, while the incubation time at 37 °C was the decisive factor for the thermal stability of the crystallites as expressed by T_{on}, T_m and T_c. The additives seemed to mainly affect the nucleation phase of crystallization process. The additives decreased the time required in order to obtain a certain level of ordering in the incubated starch samples. The carbonate buffer decreased the amount of ordered structure in starch as judged by DSC enthalpy values, while increasing the melting temperature of these structures. The additives PEG and BSA lowered the melting temperatures of the starch in the systems but increased the enthalpy values. By optimization procedure a specific amount of ordered structure with desired thermal characteristics could be predicted.

Keywords: Starch; PEG (polyethylene glycol); BSA (bovine serum albumin); Sodium carbonate; Recrystallization; Time/temperature treatment; Drug formulation

1. Introduction

Starch is a common excipient in drug formulations. Traditionally it has been used in tablets but lately there has been a growing interest in starch microspheres for delivery of active substances through several different routs (Harris, Gauden, Fraser, Williams, & Parker, 2002; Huang, Mehta, & DeLuca, 1997; Morise et al., 2006; Teder, Johansson, d'Argy, Lundin, & Gunnarsson, 1995). The use of starch microparticles has been investigated for several different active substances but peptides and proteins have gained the largest interest (Pereswetoff-Morath, 1998).

The background to the work presented in this article is that during the investigation of quality and production of Biosphere®, a starch microparticle, it was observed that the physical and molecular properties of the starch material in combination with additives, such as PEG, bovine serum albumin (BSA) and buffer, influenced the quality of the microspheres (Elfstrand, Eliasson, Jönsson, Reslow, & Wahlgren, 2006).

The Biosphere® starch microparticles are prepared by emulsifying starch in an aqueous two-phase system, containing polyethylene glycol and then stabilizing the microspheres by crystallization of the starch matrix. The manufacture of Biosphere® has been described in detail by Reslow, Jönsson, and Laakso (2002). During the manufacturing of the microspheres the protein, i.e. the active substance, is encapsulated into the starch matrix.

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The quality of the starch matrix, such as amount of crystallites and ordered structure, and the thermal stability of the ordered structure are influenced by holding the starch emulsion at different times at preset temperatures.

In order to investigate how incubation at two fixed temperatures (6 °C followed by 37 °C) affected formation of the ordered structure in starch/water dispersions one set of experiments (11 trials) was run using a Central Composite Circumscribed (CCC) design in two factors (Elfstrand, Eliasson, Jönsson, Reslow, & Wahlgren, 2007). The amount of the ordered structure and its melting characteristics were measured by DSC (differential scanning calorimetry) and effect of these two temperatures on DSC parameters was evaluated using software MODDE (Umetrics AB). This two-temperature treatment was performed in order to mimic the corresponding step in the microsphere manufacturing process. It was concluded that varying the incubation time at the two temperatures could control the amount of ordered structure obtained and the level of thermal stability of these structure. Thus, an optimal structure could be obtained by subjecting the material to the proper incubation conditions. Furthermore it is likely that the structure of the matrix might influence the release of a drug from the microsphere. Thus it would be beneficial to be able to produce microspheres with different degrees of crystallinity.

The purpose of the present study is to investigate how the crystallization process of gelatinized starch is influenced by different types of additives common in the microsphere production. However, some of these additives (protein and buffers) are also common in other pharmaceutical applications i.e. as binders in tablets, and in food applications, where starch crystallization plays a role for product quality. The three additives that will be investigated in the present study is carbonate buffer, PEG and bovine serum albumin (BSA).

The influence of ions on starch recrystallization has been investigated previously (Hizukuri, Fujii, & Nikuni, 1960; Morsi & Sterling, 1963; Russel & Oliver, 1989), and it was found that the addition of a variety of buffers to starch gels significantly affected the kinetics of the rheological and thermal changes during ageing. The effect depended on the type of salt as well as on salt concentration, but also on the temperature of incubation. It is thus likely that the salt will influence the starch crystallization but, to our knowledge, the effects of temperature treatment of the type used here have not been previously investigated.

BSA is often used as a model protein because it is cheap, available in large quantities and well characterized (Foster, 1977). BSA–starch interactions have been studied at low moisture contents (Chevallier & Colonna, 1999) and in excess of water (Muhrbeck & Eliasson, 1991). The authors concluded that there was no evidence of BSA affecting the starch gelatinization. However, to our knowledge, the recrystallization of starch in the presence of BSA has not been studied and neither has the influence of PEG on the recrystallization behaviour of starch.

Three systems of increasing complexity were investigated in the present study. The first system consisted of starch dispersed in a carbonate buffer, the second one was obtained by addition of a PEG solution to the starch/buffer system and, finally, for the third system BSA was added. Samples of these three systems were incubated at two fixed temperatures, 6 and 37 °C, for predefined periods of time. The melting characteristics of the recrystallized starch were recorded by differential scanning calorimetry (DSC). The design of the experiments and the set of experimental runs were the same as for the starch/water system in a previous study (Elfstrand et al., 2007). The ambition was to understand to what degree the additives change the crystallization process compared to the previously studied starch/water system.

2. Materials and methods

The starch material (an acid hydrolysed and mechanically treated Cerestar SF 04201 waxy maize starch) was donated from SkyePharma AB (Malmö, Sweden). The material is identical with the S3m starch previously described by Elfstrand et al. (2004) and has an average molecular weight of around 500 000 g/mol. Polyethylene glycol (PEG, 20 000 g/mol) was provided by VWR International Ltd. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich. Sodium carbonate buffer (50 mM, pH 8.0) was used for the preparation of the dispersions. All chemicals used for the buffer were of the grade extra pure and came from VWR (Germany).

2.1. Sample preparation

The starch/buffer dispersion was prepared by dispersing the starch in the carbonate buffer and heating it in a microwave oven (800 W, LG Electronics Inc., MS-194A, P.R.C.) for 3 periods of 6 s. The heated dispersions were transparent and visually homogeneous. In all the systems the starch to buffer ratio was 30/70% by weight. The samples were taken within 1 h of the preparation.

For the preparation of the starch/buffer/PEG system, the starch/buffer dispersion was cooled to 50 °C and a PEG solution (38% polymer by weight in carbonate buffer at 28 °C) was added to the starch/buffer mixture at the ratio 0.063:1 (wt). This ratio was experimentally decided by adding PEG to the starch dispersion until a visible phase separation was observed at which the transparent starch/buffer solution turned into a turbid mixture. After addition of the PEG solution, the mixture was stirred at ambient temperature at a low rate for 15 min. It was then kept at ambient temperature without stirring for 30 min. Samples were taken during the following 15 min.

For the preparation of the starch/buffer/PEG/BSA system, the BSA solution (1 g of 3.7% by weight BSA in carbonate buffer, 28 °C) was added to the starch/buffer (45% by weight) dispersion (50 °C) giving a final BSA concentration of 1.3 wt%. The starch/buffer dispersion for this

system was prepared with a starch ratio rendering a starch to buffer ratio in the final mixture of 30/70% by weight; just as for the other two systems. As a final step, the PEG solution (28 °C) was added to the starch/buffer/BSA mixture at a ratio of 0.063:1. The mixture was stirred at a low rate for 15 min and kept at ambient temperature for the following 30 min. Samples were then taken during the next 15 min.

All samples were weighed into DSC pans, immediately sealed and incubated according to the pre-designed experimental plan that can be seen in Fig. 1. According to this plan, the samples were subjected to a two-step incubation consisting of a first incubation step at 6 °C followed by a second incubation step at 37 °C. The time for the incubation steps varied during the investigation.

2.2. Differential scanning calorimetry (DSC)

The thermal transitions of the crystalline structure of the samples were investigated by DSC. Samples of approximately 10.5 mg were weighed into aluminium pans (TA Instruments, New Castle, USA, Ref. no. 900790.901 and 900796.901). The pans were then hermetically sealed and stored at the predefined time/temperature conditions (Fig. 1). The samples were analyzed in a DSC 6200 (Seiko Instruments Inc., Shizouka, Japan) with an empty pan as a reference.

The melting enthalpy was determined by scanning the temperature from 30 °C up to 110 °C at a heating rate of 5 °C/min. All analyses were carried out in at least duplicates. To obtain the true dry matter value the punctured pans were dried overnight at 105 °C. The starch content of BSA containing samples was calculated by subtracting the amount of BSA from the sample and the ΔH values were based on the dry weight of starch.

In this work we have considered the contribution to the enthalpy from denaturation of BSA as insignificant. This amount of BSA used here (1.3%) would cause a mere 1.1 mJ contribution to the enthalpy value of the samples; the value based on the reported denaturation enthalpies for BSA (Chevallier & Colonna, 1999; Paulsson, Hegg, & Castberg, 1985).

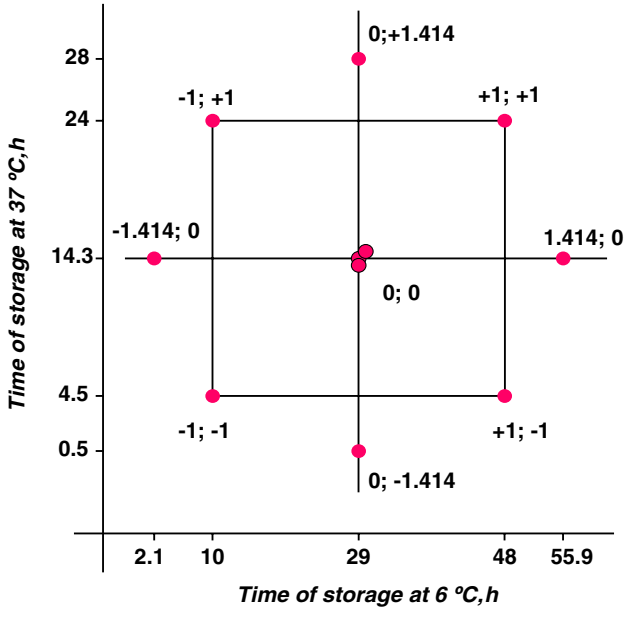

Fig. 1. The design region of the CCC designs in two factors for the model systems.

2.3. Experimental design

The experiments in the present study were designed and performed using a CCC (Central Composite Circumscribed) design (Fig. 1). The experimental details of this design have been previously described by Elfstrand et al. (2007), for a model system consisting of starch and water. The input variables were the duration of incubation of the samples at 6 °C (t_6, h) and the duration of the following incubation at 37 °C (t_{37}, h). The measured responses in the study, 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. The present study used the same settings in the experimental domains as in the previous study (Elfstrand et al., 2007). All the factors were investigated at five levels, rendering it possible to estimate quadratic terms. The design included three replicated center points which give information on the reproducibility of the experiments and, thus, allow for the estimation of the lack of fit in the model (Eriksson, Johansson, Kettaneh-Wold, Wikström, & Wold, 2000).

The experimental results were analyzed with the software MODDE 7.0.0.1. Relations between factors and responses were obtained by fitting a quadratic model consisting of 6 terms for each response.

$$y = b_0 + b_{t_6} \times x_{t_6} + b_{t_{37}} \times x_{t_{37}} + b_{t_6 t_6} \times x_{t_6}^2 + b_{t_{37} t_{37}} \times x_{t_{37}}^2 + b_{t_6 t_{37}} \times x_{t_6} \times x_{t_{37}},$$

where y was a response, x_{t_6} and $x_{t_{37}}$ were input variables, b_0 was a constant term and b_{t_6}, $b_{t_{37}}$, $b_{t_6 t_{37}}$, $b_{t_6 t_6}$ and $b_{t_{37} t_{37}}$ were the model parameters. The goodness of the models, i.e. the correlation between input and response data, was evaluated using summary of the fit and ANOVA (analysis of variance) methods.

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 $g(y) = 100 * ((y - P)/(T - P))$

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 $\text{Limit} = 90 + 80 * \log_{10}(w)$, 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.

The modelling results were presented in so called contour plots, where the input variables t_6 and t_{37} were put on the X and Y axes, respectively, and the response was displayed as different coloured fields where the numeric value for each field was presented on the boarder of the field.

3. Results

3.1. Evaluation of data from the chemometric model

The model systems in the present study, i.e. starch/buffer, starch/buffer/PEG and starch/buffer/PEG/BSA, were investigated using a CCC design. The experimental data (partly presented in Table 1) was analyzed by MODDE in order to determine a relation between the input variables, the factors t_6 and t_{37}, and the output variables, the responses T_m, T_{on}, T_c, T_{range} and ΔH, in the three systems. The evaluation of the raw data showed that the collected data fulfilled the criteria for good data to work with as defined by chemometrics.

The data distribution of each response was inspected and metrics of the responses were transformed according to the same principles as for the starch/water system (Elfstrand et al., 2007). Logarithmic transformation was performed for ΔH, whereas power transformation was employed for the rest of the responses. In all the systems the evaluation of the goodness of the models indicated a good fit between data and models.

The recrystallization behaviour of the starch material used for the preparation of our model systems in the presence of 70 wt% water has been described previously (Elfstrand et al., 2004). An incubation of this previously gelatinized starch material during 20 h at 6 °C was recorded on the level of 3.1 (±0.3) J/g; a result that is in line with the enthalpy value predicted by the modelling of the starch/water system (Fig. 6A). Thus there is some

Table 1
Summarized DSC results of the model systems

Storage conditions, t_6/t_{37} (h/h)	ΔH (J/g)				T_m (°C)			
	Starch/water	Starch/buffer	Starch/buffer/PEG	Starch/buffer/PEG/BSA	Starch/water	Starch/buffer	Starch/buffer/PEG	Starch/buffer/PEG/BSA
10/4.5	2.1 (0.6)	1.7 (0.3)	1.8 (0.1)	1.7 (0.4)	58.4 (1.7)	60.6 (0.8)	61.1 (0.3)	60.3 (0.5)
48/4.5	4.9 (0.0)	4.7 (0.0)	5.7 (0.4)	4.8 (0.2)	59.3 (0.4)	60.6 (0.4)	60.7 (0.1)	60.4 (0.1)
10/24	1.7 (0.2)	1.4 (0.4)	3.5 (0.1)	3.6 (0.1)	62.4 (0.3)	64.2 (0.5)	64.2 (0.1)	63.5 (0.0)
48/24	6.2 (0.4)	4.9 (0.2)	6.6 (0.3)	6.2 (0.2)	61.6 (0.1)	63.4 (0.1)	63.2 (0.2)	62.4 (0.3)
2.1/14.3	–	–	–	–	–	–	–	–
55.9/14.3	5.6 (0.6)	4.7 (0.5)	6.5 (0.4)	5.9 (0.5)	60.5 (0.1)	62.4 (0.3)	62.3 (0.0)	61.8 (0.1)
29/0.5	4.5 (0.3)	3.9 (0.2)	4.6 (0.3)	4.8 (0.1)	56.0 (1.3)	57.7 (0.5)	58.7 (0.1)	57.9 (0.0)
29/28	4.7 (0.8)	4.1 (0.5)	5.8 (0.7)	7.0 (0.6)	61.7 (0.2)	63.8 (0.2)	63.2 (0.4)	62.9 (0.1)
29/14.3	4.7 (0.5)	3.8 (0.2)	5.2 (0.4)	5.6 (0.2)	60.2 (0.9)	62.4 (0.3)	62.1 (0.4)	61.4 (1.0)
29/14.3	4.5 (0.2)	3.8 (0.4)	4.6 (0.7)	5.3 (0.4)	60.8 (0.4)	62.7 (0.4)	62.4 (0.7)	61.7 (0.4)
29/14.3	4.8 (0.2)	4.0 (0.4)	4.0 (0.8)	5.2 (0.2)	60.4 (0.6)	62.5 (0.6)	62.9 (0.1)	61.5 (0.7)

The peak melting temperature (T_m) and the endothermic heat (ΔH) of melting, obtained from DSC analysis. Standard deviation values are given within brackets. Results regarding the starch/water system are obtained from Elfstrand et al. (2007).

indication that the model can predict independent obtained results.

3.2. General observations

For all three investigated systems it could be concluded that the incubation time at 6 °C was the decisive factor for the amount of ordered structure obtained during the incubation, while the incubation time at 37 °C was the decisive factor for the thermal stability of the crystallites as expressed by T_{on}, T_m and T_c. This is in line with what has previously been observed for the starch/water system (Elfstrand et al., 2007). Thus, the additives did not change the general crystallization behaviour of starch gels but rather the kinetics of the crystallization or the amount of structure formed. However, differences between the four systems in the melting temperatures and the enthalpies were observed after different incubation times.

T_{on}, T_m and T_c increased with the incubation 37 °C which was interpreted as the longer incubation time at this temperature having a positive effect on the perfection of the crystallite population. The melting interval (T_{range}) also became smaller as the incubation time at 37 °C increased, i.e. the crystallite population became more homogenous, which further substantiate this interpretation.

The enthalpy is considered to be an indirect measurement of the amount of ordered structure. This structure could be formed by both, by crystallites and by double helixes in non-crystalline regions. This fact that the low incubation temperature in all cases controls the enthalpy value is an indication that nucleation, and not propagation and reformation of the structure, is the factor that dominates amount of the ordered structure as measured by DSC.

No crystallinity could be seen in the samples of any of the systems when they had been stored at conditions t_6/t_{37} (h/h) 2.1/14.3. For all the other storage conditions ordered structure could be detected by DSC. This finding was in line with what was reported previously for the starch/water system (Elfstrand et al., 2007) and indicated that for the experimental interval studied here the additives did not markedly influence the time needed for the development of a sufficient degree of crystallinity to be detectable by DSC, or, in other words, they did not influence the "apparent induction time" (Mandelkern, 1956).

The modelling results of the starch/buffer, starch/buffer/PEG and starch/buffer/PEG/BSA systems were represented by response contour plots for each response and for all systems. These plots can be seen in Figs. 2–6. Response contour plots of the starch/water system (Elfstrand et al., 2007) are also included. Two variables, t_6 and t_{37}, represent the X and Y axes, respectively, whereas the contour level is represented by a third variable corresponding to one of the responses (T_m, T_{on}, T_c, T_{range} or ΔH). The plots predict the response values based on the experimental data. Below we will go into the details of how the responses change with the additives.

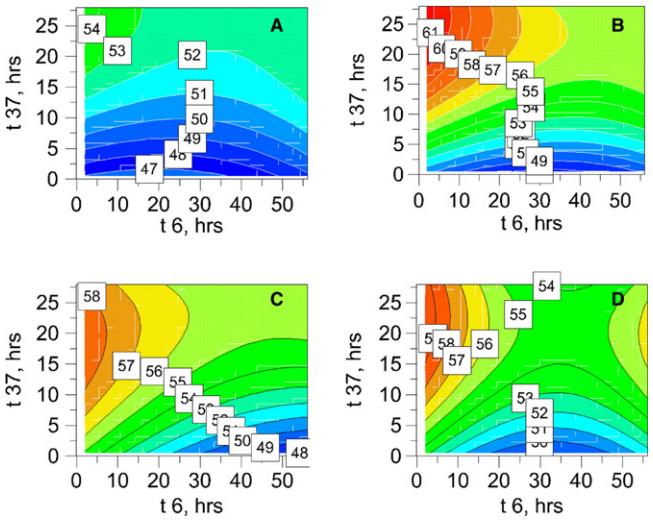

Fig. 2. Response contour plots for T onset in the model systems. (A) Starch/water; (B) starch/buffer; (C) starch/buffer/PEG; and (D) starch/buffer/PEG/BSA.

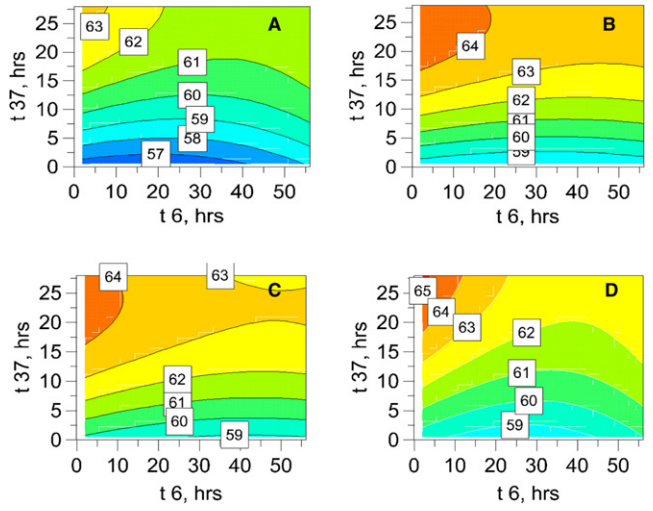

Fig. 3. Response contour plots for T melting in the model systems. (A) Starch/water; (B) starch/buffer; (C) starch/buffer/PEG; and (D) starch/buffer/PEG/BSA.

3.3. Variation of melting temperatures as influenced by the additives

The contour plots corresponding to the melting temperatures are shown in Figs. 2–4. The investigation of the temperature contour plots revealed that the T_{on} and T_m values for the three systems (Figs. 2 and 4B–D) were higher than that for the starch/water reference system (Figs. 2 and 4A). This could be interpreted as that more thermally stable structures were obtained in the presence of the additives indicating formation of more perfect crystallites.

The increase in T_{on} was not the same as increase in T_m within the complete time/temperature interval. The effect of additives on T_{on} were most pronounced at short incubations times at 6 °C followed by long incubation times at

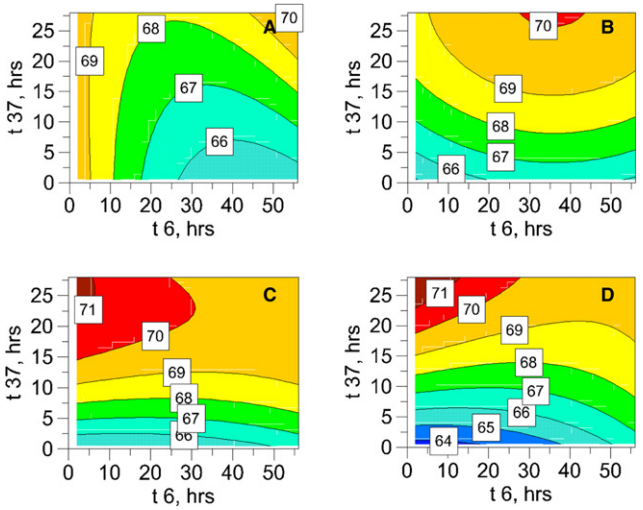

Fig. 4. Response contour plots for T completion in the model systems. (A) Starch/water; (B) starch/buffer; (C) starch/buffer/PEG; and (D) starch/buffer/PEG/BSA.

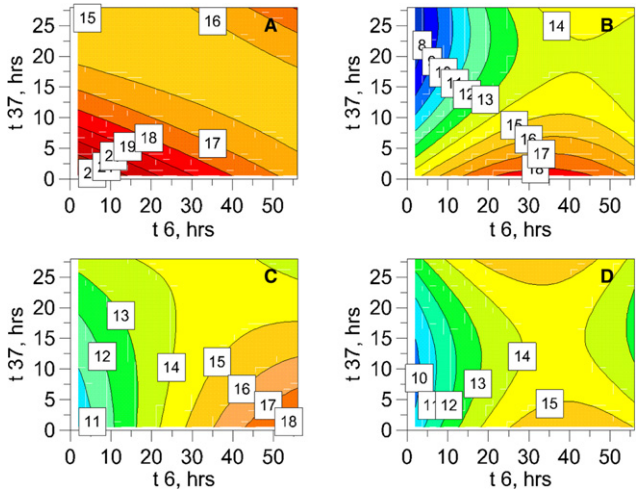

Fig. 5. Response contour plots for T range in the model systems. (A) Starch/water; (B) starch/buffer; (C) starch/buffer/PEG; and (D) starch/buffer/PEG/BSA.

37 °C. No such general trends could be seen for T_m. When studying Fig. 3, it could be observed that, although the starch/water system had the lowest T_m values, a variation of T_m occurred within a 5–6 °C interval for all the systems, i.e. between 57–63 °C for the starch/water systems and between 59–65 °C for the other systems. In contrast, the intervals for the T_{on} values (Fig. 2) varied for all the systems from 7 °C (47–54 °C) for the starch/water system to 13 °C (48–61 °C) for the starch/buffer system. It was also observed that variations of the incubation conditions had a larger influence on the T_{on} values than on the T_m values.

It was obvious that the major increase in T_{on} and T_m was obtained by the addition of the carbonate buffer while further addition of PEG and the protein only affected the results to a minor degree. However, further addition of PEG and BSA reduced the amplitude of the T_{on} variation,

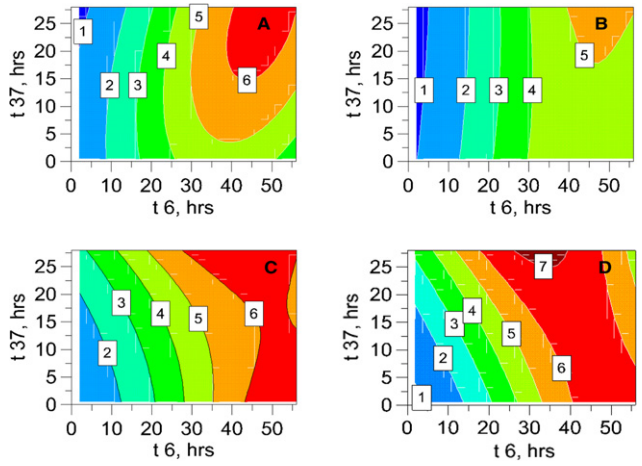

Fig. 6. Response contour plots for enthalpy in the model systems. (A) Starch/water; (B) starch/buffer; (C) starch/buffer/PEG; and (D) starch/buffer/PEG/BSA.

decreasing the difference eliminating the highest T_{on} values (for the starch/buffer/PEG system) and omitting the lower T_{on} values (for the BSA containing system) compared to the starch/buffer system.

The completion temperature, T_c, shown in Fig. 4 did not follow the same pattern as T_{on} and T_m. The variation in T_c with incubation time was usually smaller than for the other temperature responses. In difference from the other temperature responses the T_c values of the starch/buffer system (Fig. 4B) were lower than those of the PEG and BSA containing systems and were within the same temperature interval as the starch/water system. An overview of the contour plots revealed that every additive considerably changed the pattern of the T_c plot. The use of a buffer changed the curvature of the response compared to the starch/water system and moved the optimum (conditions for receiving of the crystalline structure with the highest T_c for the system, in this case 70 °C) on the contour plot towards lower incubation times at 6 °C. The addition of PEG to the system further changed the curvature and the level of 70 °C could now be obtained at even shorter incubation times. The BSA addition decreased the lower limit of the T_c values, thus widening the temperature interval toward lower values (64–71 °C) and lengthening the incubation time at 37 °C in order to obtain the level of 70 °C.

The T_{range} (i.e. T_c–T_{on}) contour plots are shown in Fig. 5. It was clear from the modelling that T_{range} was influenced by both the factors t_6 and t_{37} and that this was similar for all the systems. The widest melting interval was observed for the starch/water system mainly due to the lower T_{on} values for this system. For the starch/buffer system the T_{range} was considerably smaller than that for the starch/water system for the most part of the experimental setup. The addition of PEG influenced the response by eliminating the lowest T_{range} values and thus shortening the variation amplitude. However a melting interval as narrow as for the starch/buffer system could not be obtained for the PEG containing system. The addition of BSA

further decreased the amplitude of T_{range} as compared to the previous system. Thus, for the BSA containing system, T_{range} varied within 5 °C, which was the lowest among the systems studied. This could be interpreted as the most homogenous crystallite population, regardless of incubation conditions, being obtained in the presence of BSA.

3.4. Variation of enthalpy values as influenced by the additives

The DSC melting endotherm indicates the presence of an ordered structure in the sample, and the enthalpy values were used here as an indirect measure of the amount of this structure in the samples. As can be seen from Table 1, the enthalpy values are both affected by the incubation conditions and the type of additives used. The contour plots of the enthalpy value as a function of t_6 and t_{37} for all four systems are shown in Fig. 6.

The lowest enthalpy values were observed for the starch/buffer system (Fig. 6B). Thus, by using a buffer, lower ΔH values were obtained as compared to the starch/water system. The addition of PEG to the starch/buffer system seemed to increase the ΔH values again and the addition of BSA led to a slight further increase.

As stated before the enthalpy value is mainly influenced by the time at 6 °C (or t_6). This was especially obvious for the starch/buffer system. Here the ΔH values were almost independent of variations in t_{37} (Fig. 6B) until t_6 reached 40 h and ΔH was at a level of 4.4 J/g (this level is not shown on the contour plot). It is interesting to note that also for the starch/water system variations in t_{37} started to affect the enthalpy values firstly after that ΔH reached level of 4.4 J/g. However, this enthalpy level was obtained already after 26 h for the starch/water system, whereas it took an additional 14 h in the case of the starch/buffer system.

For the starch/buffer/PEG and starch/buffer/PEG/BSA system longer time at 37 °C led to an increase in enthalpy. This indicated that additional ordered structure was formed during the prolonged holding time at 37 °C. As was previously concluded (Elfstrand et al., 2007), a certain level of crystallinity should first be obtained at the lower temperature in order to enable a positive impact on the level of crystallinity in the sample during the incubation at 37 °C. This way, the level of crystallinity in the sample would not decrease when the temperature was shifted to higher ones, but would stay on the same level and might even increase. This stable low-temperature structure for the starch/water system was obtained after 48 h as reported in the prior study (Elfstrand et al., 2007). It seemed like in the presence of PEG and BSA more stable structures could be obtained after shorter incubation times.

3.5. Optimal conditions

Using the model it is possible to predict the time/temperature treatment that will give a desired outcome considering temperature stability or enthalpy of the starch gel. Optimal conditions were calculated for all the systems with respect to obtain the highest enthalpy and the highest melting temperature by running Optimizer. Firstly, T_m was maximized regardless of ΔH, then ΔH was maximized regardless of T_m, and finally both T_m and ΔH were maximized. The results of the optimization are shown in Table 2.

As can be seen from the results, the samples should be incubated at similar conditions for all systems in order to obtain the maximum T_m. The highest T_m could be obtained at conditions that gave low enthalpy values and which were characterized by a short incubation time (2 h) at 6 °C and a long incubation time (>20 h) at 37 °C, giving a short time for formation of nucleation points for crystallites probably favouring formation of the most stable structures. As the condition to obtain these stable structures are the same for all samples they are most likely not influenced markedly by the additives.

Varying incubation conditions were required in order for the systems to obtain maximum values of ΔH. The conditions were very similar for the starch/water and starch/buffer systems but the enthalpy value was lower for the system that contained buffer. The addition of PEG did not shorten time of incubation at 6 °C but gave quite a short incubation time, only 0.5 h, at 37 °C to obtain the optimum ΔH. It seemed that incubation at 37 °C for longer time than 0.5 h was unnecessary for obtaining the optimum in the system in the presence of PEG, and even more, that a longer t_{37} would influence the maximum amount of ordered structure in the samples negatively (Fig. 6C). In

Table 2
Optimal conditions for the model systems predicted by MODDE running Optimizer

Model systems	Incubation time (hours) at		ΔH (J/g)	T_{on} (°C)	T_m (°C)	T_c (°C)	T_{range} (°C)
	6 °C	37 °C					
Both ΔH and T_m are maximized							
Starch/water	55	26	6.3	52.3	61.5	69.8	17.8
Starch/buffer	42	25	5.1	55.8	63.4	69.9	14.1
Starch/buffer/PEG	33	26	6.1	55.6	63.2	69.9	14.2
Starch/buffer/PEG/BSA	25	28	6.9	54.4	62.8	70.1	15.7
MaxΔH							
Starch/water	48	26	6.4	52.3	61.4	69.0	16.9
Starch/buffer	48	28	5.4	55.2	63.2	69.9	14.7
Starch/buffer/PEG	50	0.5	6.7	48.2	58.9	66.0	17.9
Starch/buffer/PEG/BSA	33	28	7.3	53.9	62.4	69.6	15.7
MaxT_m							
Starch/water	2	25	0.9	54.3	63.3	69.4	15.0
Starch/buffer	2	25	0.8	61.3	64.9	68.8	7.4
Starch/buffer/PEG	2	24	2.4	58.5	64.5	71.2	12.7
Starch/buffer/PEG/BSA	2	28	3.3	59.3	65.3	72.0	12.7

the presence of BSA, the incubation time at 37 °C needed for the optimum was on the same level as for the starch/water and starch/buffer systems, while the incubation time at 6 °C was shortened by 16 h. It seemed as though BSA influenced the initial recrystallization of starch in such a way that a stable crystalline structure could be formed faster.

Optimal conditions for obtaining a crystalline structure with both ΔH and T_m on the maximum level could be obtained at different conditions for all four systems. It is noteworthy that the incubation time at 37 °C was almost the same for all of the model systems (25–28 h), but considerable differences could be seen between the systems regarding the incubation time at 6 °C. The presence of buffer reduced the incubation time at 6 °C by 13 h, further addition of PEG to the system reduced it with another 9 h and, finally, the addition of BSA reduced it with an additional 8 h resulting in a suggested incubation time at 6 °C values for optimal conditions only an half of that for the starch/water system. This fact that the incubation time at 6 °C has been changed with increasing of the model system complexity indicates that it is likely that the additives influenced nucleation more than propagation of the crystallites.

3.6. The impact of buffer

The main affects of addition of buffer are a decrease in the enthalpy and an increase in the onset melting temperature. One possible interpretation is that the addition of buffer hinders the formation of structures with low thermal stability, but also that propagation of structure formation during the incubation at 37 °C occurs slower.

There are a few studies concerning the effect of salt on structure formation in starch dispersions (Baker & Rayas-Duarte, 1998; Bello-Perez, 1995; Hizukuri et al., 1960; Morsi & Sterling, 1963; Russel & Oliver, 1989). In line with the results seen here Baker observed that 5% sodium chloride decreased the amount of structure formed in starch gels both at low and high temperatures (Baker & Rayas-Duarte, 1998). Also, Russel and Oliver (1989) reported that the addition of sodium chloride to starch gels significantly affected the kinetics of rheological and thermal changes during ageing.

Morsi and Sterling (1963) investigated the recrystallization of starch as a function of electrolyte composition and concentration, and concluded that the effects of both anions and cations tended to follow the lyotropic series, which caused retrogradation to decrease in the approximate descending order of the series. Thus, kosmotropes (structure-makers) decreased retrogradation while chaotropes (structure-breakers) favoured retrogradation. In our study, ions of both sodium (Na^+) and carbonate (CO_3^{2-}) were present in the dispersions. Both Na^+ and CO_3^{2-} are known as structure-makers (kosmotropes) while HCO_3^- acts as a structure-breaker (chaotrop). At pH 8.0, the molecules of the carbonate buffer are in a dissociated state and there are portions of chaotropes and kosmotropes; to what extent is decided by the pH value. However, the results indicated that it might be the structure making effects that dominated in our systems.

The gelatinization of starch granules differ from the melting of starch gels in many ways for example by the water transport in the system. The phenomena seen here might be partly explained from previous works on starch gelatinization and there are several authors who have investigated the effects of salts on gelatinization (Evans & Haisman, 1982; Jane, 1993; Lai, Karim, Norziah, & Seow, 2002). Evans and Haisman (1982) points out that the effect seen of many solutes on starch gelatinization can be approximately described by the derived relationship between initial gelatinization temperature, water activity of the system, and volume fraction of water in the granules. Thus also in our system water activity might play a role for the observed results. It is likely that these types of interactions for example influences on free and bound water might be attributed to the results seen in our study, substantiating the earlier discussion about water structure breaking or water making properties of the ions that play a major role in the results observed. However, several other authors have shown that the gelatinization of starch is affected by the interaction between starch polymers and the ions (Chiotelli, Pilosio, & Le Meste, 2002; Jane, 1993; Lai et al., 2002; Lai, Tomasik, Yen, Hung, & Lii, 2001).

3.7. PEG impact

The addition of PEG to the starch/buffer system was found to increase the amount of ordered structure, while the melting transition temperatures mainly were on the same level as for the starch/buffer system.

The increase in enthalpy is seen already at short incubations times at 37 °C but longer incubation times at 37 °C increased the amount of ordered material. The interpretation of this observation is that addition of PEG influences both the nucleation phase and the propagation phase. It seemed that there is a general trend towards easier formation of ordered structure in the presence of PEG. Another reason could be that the presence of PEG increases the formation of nucleation sites and increased the stability of these nucleation sites so that upon further incubation at 37 °C the sites did not melt but allowed for further propagation of starch structure.

It is noteworthy that the PEG influences crystallization process of the system although the concentration of PEG present in the starch/buffer/PEG system is very low. Thus it cannot be ruled out that PEG interacts directly with the starch helix formation. It has been speculated in the literature that cyclodextrines might form complexes with PEG (Horský, 1998). However, to our knowledge there are no data in the literature showing that also starch can form such complexes and the obtained results can also be explained by several other mechanisms. Presences of PEG

in the system could alter the distribution of both water and ions within the starch gel. The presence of ions can as seen in the buffer system affect the formation of ordered structure. Another factor could be that although the amount of PEG present in the starch matrix is low it could induce local phase separation that works as nucleus for crystallization.

3.8. Impact of BSA

The difference between the system containing PEG and the system containing PEG and BSA is much smaller than the difference between the other studied here systems. There is an indication that addition of soluble proteins at moderate concentrations as was used here seems does not have any major effect on the formation of ordered structure in the starch system used.

However there are some observed effects and that is according to the optimization calculations the incubation time at 6 °C is further decreased by the addition of BSA. It is obvious that the low-temperature step which is probably linked to the nucleation process is the step that is most sensitive to the additives and that even an additive as BSA that have low overall effect on structure formation might influence this step.

4. Conclusions

The presence of carbonate buffer, PEG and BSA in starch/water dispersions was found to influence the recrystallization process of starch. For all the systems investigated, the incubation at 6 °C was the decisive factor for the amount of the ordered structure (expressed by ΔH values) in the starch dispersions and the incubation at 37 °C the determinant for thermal stability of the ordered structure (expressed by T_m).

The additives seemed to have larger effect on the processes proceeding during the incubation at 6 °C than those proceeding during the incubation at 37 °C. Thus, the nucleation phase of formation of ordered structure seemed to be more influenced by the presence of additives than the propagation phase.

The presence of a carbonate buffer depressed the development of the ordered structure but enhanced the thermal stability of the structures compared to a system only containing water and starch.

The addition of PEG to the starch/buffer system was found to increase the amount of ordered structure, while the melting transition temperatures mainly were on the same level as for the starch/buffer system.

Addition of BSA to the starch/buffer/PEG system did not induce any large changes in the formation of ordered structure. The most pronounced impact of BSA on the starch crystallization was expressed by a shortened low-temperature incubation phase necessary to obtain stable starch structure.

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