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Freeze-drying of pharmaceutical excipients

close to collapse temperature: influence of the process

conditions on process time and product quality.

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Abstract

The aim of this paper is to investigate the characteristics of a vial freeze-dried product as a function of the temperature at which the primary drying is carried out. The effect of the drying temperature and of the composition on the product appearance and on the final characteristics (apparent density and rehydratability) of pure substances (mannitol, sucrose and dextran) has been studied: the rehydratability results to be faster for the product freeze-dried at lower temperature, when the structure of the product is more porous and softer; higher rehydration rates were also observed for samples obtained from lower initial concentration. In case of binary mixtures (of mannitol and dextran and of sucrose and dextran) the rehydratability gets worse when higher drying temperatures are used, while the relative composition has a smaller effect. Nevertheless, high drying temperatures are required to shorten the process, both in case of pure substances and in case of binary mixtures; thus, it is important to consider the process time-product quality relationship to choose the optimal drying temperature. Finally, we show that the freeze-drying of mixtures can be easier and faster than that of one of the components alone: this is partly a consequence of the higher temperatures allowed, but it is evident that in some cases the drying kinetics can be higher even with the same process temperature.

Keywords

Introduction

Freeze-drying is a technique used to desiccate many kinds of materials, especially biological and heat-sensitive substances, such as pharmaceuticals, due to the low working temperature and to the possibility of carrying out the process in a sterile environment (Liapis et al., 1995; Liapis et al., 1996; Sadikoglu and Liapis, 1997). The process consists of two steps: during *primary drying* the frozen product is maintained at low pressure, thus causing the sublimation of the solvent (heat must be supplied, e.g. using a heating shelf, as the sublimation process in endothermic), while during *secondary drying* the residual solvent, strongly bounded to the partially dried product, is reduced to the target value by desorption (using low pressure and moderate temperature, e.g. +20-40°C).

The drying times required for the freeze-drying process are substantially longer when compared to the conventional evaporative drying methods. It could take a lot of additional time (even days) to freeze-dry a certain product of interest, if the process is not well-designed and/or not optimized (Tang and Pikal, 2004; Sadikoglu et al., 2006). The operating conditions required to obtain a product with the desired characteristics are found mostly by "trial and error": this is a consequence of the impossibility of directly measuring, and thus of controlling, in-line the parameters of interest, namely the product temperature and the residual water content. In some cases the operating conditions are selected by the operator, while in some other cases they are obtained by means of experimental studies (Hottot et al., 2007) or mathematical simulation of the process, with the goal to minimize the drying time (Rene et al., 1993; Lombraña et al., 1993a and 1993b; Boss et al., 2004; Gan et al., 2004; Gan et al. 2005; Velardi and Barresi, 2008).

Beside choosing the optimal operating conditions, a pharmaceutical product prepared by freeze-drying needs to be formulated using adequate excipients to preserve the active principles. The product resulting after the drying should be shelf-stable, good-looking according to the pharmaceutical practice, and it needs to be

almost instantaneously reconstituted in the amount of water required to obtain the therapeutic dose. Although regarded as a soft dehydration process, lyophilization can damage the product as in the various stages, namely freezing, primary and secondary drying, a series of stresses are applied to the molecules of the product, which can be rather labile. Thus, total or partial inactivation during this multistage process is not unusual and the loss of properties can be even of one or more orders of magnitude (in the pharmaceutical world, such activity decay is described in terms of "log-decay", as in the case of vaccines).

The traditional approach to solve these stability problems is to add a stabilizer to the solution containing the active principles. This operation is based on the identification of a potential stabilizer and on the test of the new formulation. Some other components - the fillers - can be added to the product to make it much more resistant to deterioration during the process. Bulking agents, such as mannitol or glycine, are used for increasing the drug volume so that a mechanically more robust product is obtained, thus preventing the so-called "blow-out": this phenomenon may occur in presence of a solution having a very low content of solute, e.g. less than 1% by weight. Other excipients can have a cryoprotective action and are used to prevent the damages caused by the variation of the ionic strength and of the pH of the solution occurring during freezing (Pikal-Cleland et al., 2000; Franks, 2007).

The solutes added to the solution may crystallize or not during freezing, thus remaining in a glassy or amorphous state. The behavior of a solute depends mainly on its chemical structure, on the initial concentration, on the interaction with the other solutes and on the procedure used for freezing. During the freezing of an aqueous solution a complete eutectic phase separation, i.e. solvent and solute that crystallize thoroughly to form a mixture of ice and solute crystals, is rarely observed, and it must be considered that in case of eutectic freezing sensitive biomolecules cannot be adequately protected. Moreover, several solutions tend to supersaturation: water continues to freeze and the temperature drops below the eutectic value; finally, the residual mixture (excluding ice) may undergo the so-called "glass transition"

when the viscosity increases by many orders of magnitude in a narrow temperature interval. The product achieves then the structural form of an amorphous solid and is described according to a glassy phase. The transition from an over-saturated freeze-concentrated aqueous solution of very high viscosity to a glassy solid is characterized by a glass transition temperature T_g' (see, for example, Franks, 2007). Actually, the transition does not occur at a well-defined temperature, but in a narrow range of temperatures, and the observed value depends on the rate of heating or cooling and, thus, on the measurement procedure adopted; moreover, it may depend also on the sample history and is characterized by a change in the specific heat (Blanshard, 1995). If T_g' is exceeded during the primary drying, a collapse of the cake structure can occur: the solute phase next to the frozen layer attains sufficient fluidity to undergo viscous flow at a significant rate and, when the temperature is high enough (and the corresponding viscosity is low enough) to allow the phase to flow over distances of the order of the pore size within the process time scale, the product cake looses its stiff pore structure and undergoes collapse. The temperature at which this is first noted by visual observation is the collapse temperature T_c. This temperature and the T_g' are closely related and the collapse temperature is normally a few degrees centigrade higher than T_g' (even if in practical applications the collapse temperature can be identified precautionarily with the glass transition temperature). Anyway, the distinction between T_c and T_g' is subtle but potentially relevant: in fact, collapse is the result of a glass transition in the dried region formed during the primary drying, while T_g' refers to a glass transition in the amorphous phase in contact with ice. The collapse temperature depends both on the residual water content and on the nature of the solute. It appears to be determined in large part by the molecular weight and by the structural groups of the solute molecules. For example, as far as sugars are concerned, sucrose solutions collapse at lower temperature than raffinose solutions and glucose solutions collapse at even lower temperatures (MacKenzie, 1976).

The collapse causes loss of product neatness, which may vary from moderate deformation and shrinkage to a complete melt and sticking on the vial walls and

bottom. In the extreme case, the vial may appear almost empty. In addition, collapse can lead to an abnormally high residual moisture and to a prolonged reconstitution time; moreover, the degradation of the resulting product can occur. To prevent the occurrence of the collapse, the sublimation interface must be maintained below T_c throughout the primary drying, thus resulting in a higher drying time. The excipients added to the formulation can increase the collapse temperature of the formulations showing a low collapse temperature, but, in some cases, they can make more difficult the drying process because they may be responsible for the structural collapse of the product: in these cases the product must be reformulated, by substituting the solutes with others exhibiting higher values of T_c. Sadikoglu et al. (1998) studied the effect of excipients on the primary drying stage during the freeze-drying of skim milk: they assumed that suitable excipients could be found to increase the collapse temperature of skim milk when they were used in the solution of skim milk and showed, by means of mathematical simulations, that if the melting temperature is increased of 2°C by adding some excipients, then the time required to complete the primary drying stage is reduced by 8.28%, while if the increase of the melting temperature is of 6°C, then this time is reduced by 19.54%.

Structural collapse may also occur during secondary drying, although this step is less hazardous; in fact, as the water content of the amorphous phase decreases during drying, the glass transition temperature increases very sharply.

It is clear that understanding the product response to freezing and drying is essential for defining both the formulation of the organic product and the parameters of the lyophilization cycle.

The product structure and, thus, the apparent density and rehydratability, depend mainly on the freezing step. In fact, freezing determines the shape and the dimension of the ice crystals that form the structure of the frozen product as it has been recently observed and investigated (see, among the other, Kochs et al., 1991; Searles et al., 2001; Nakagawa et al., 2006). The quality of the product must be maintained at an acceptable level during drying, avoiding viscous flow of the dried

5

product and thus the phenomenon of collapse, and this must be accomplished by controlling the product temperature during this step. In the case of pharmaceutical products the requirement about the aspect can be very strict, causing their rejection even in case of limited deterioration, notwithstanding the activity is not reduced, but often microcollapse can be acceptable, and in certain cases (this happens especially for vaccines) even collapsed product can be acceptable. In any case the residual moisture, that depends both on the original structure and on the drying time, and that can be strongly affected by structure modifications during drying, can affect storage stability. For safety reasons, a temperature significantly lower than that at which collapse can occur is generally set, both for difficulties in process control and for the uncertainty on the product behavior around T_g' (and it must be remembered that also T_g' and T_c may be known with an uncertainty of few degrees). Any reduction of the drying temperature has as a consequence a significant increase of the drying time and, thus, it is very important to find the temperature that optimize the process considering both product quality and process time. The goal of our study is, thus, to point out how the temperature at which the process is carried out affects product characteristics (apparent density, residual moisture, and rehydratability) and the drying rate (i.e. process duration). As the structure of the product is determined in the freezing step, in this work we carried out all the tests using the came cooling rate, as it will be discussed in the following, thus focusing on the product quality caused by the working temperature. The influence of the composition of the product composition is also discussed: we considered solutions containing 10% w/w of sucrose, dextran and mannitol, as well as mixtures of sucrose and dextran, with 10% w/w total concentration, and of mannitol and dextran, with 20% w/w total concentration (selected in order to obtain a partly crystalline structure immersed into an amorphous matrix). The behavior of mixtures in comparison to pure substances has received up to now little attention in the literature. It can be remembered that the behavior of low concentration sucrose and mannitol solutions and of a mixture of sucrose and phenylalanine was investigated by Roth et al. (2001): they used a novel

microbalance (Christ) technique to monitor continuously the weight loss of a vial standing on a shelf of a freeze-dryer and evidenced different drying behavior for the sucrose and sucrose/phenylalanine systems, which was attributed to the presence of crystalline phenylalanine in the amorphous sucrose.

Materials and methods

Solutions of mannitol, sucrose (Riedel de Haën, highest analytical grade) and dextran (Fluka-BioChemika, highest analytical grade) and their mixtures in distilled water, either 10% or 20% by weight total concentration, were investigated. Melting temperature (T_m), glass transition temperature (T_g'), and complete solidification temperature (T_{cs}) were measured by Differential Scanning Calorimetry (DSC), using a Perkin-Elmer DSC7 with a heating rate of 2 or 10°C/minute. True collapse temperature, that requires the use of a cryomicroscope, was not measured and T_g' was used as a reference for setting the maximum working temperature in the freeze-drying cycles. The details of the solutions considered and the correspondent characteristic temperatures are given in Table 1.

Mannitol was investigated because, as a pure substance, it can be freeze-dried obtaining a crystalline product, with the only precaution to keep the temperature below that of incipient melting. The behavior of mixtures of mannitol and dextran is different: when the concentration of mannitol is low (below 6% w/w of mannitol in a solution with a total solutes concentration of 20% w/w) Bamba et al. (1995) reported, on the basis of DSC results, that a glass transition occurs during the heating cycle, thus indicating that crystallization was inhibited: a stable glassy system can be obtained up to this limit concentration during the initial freezing and, within this range, the higher is the mannitol concentration, the lower is the glass transition temperature. We have investigated the behavior of mixtures of mannitol and dextran in a wider range of concentration: several aqueous solutions of mannitol-dextran 10%

and 20% w/w total concentration have been prepared and freeze-dried in vials at -30°C and at a total pressure of 10 Pa, after a freezing step using a freezing rate of about 1°C/min. The freeze-dried product has been analyzed by means of X-Ray Diffraction (XRD) in order to investigate how the structure of the solid matrix changes as a function of mannitol fraction; the X-ray powder diffraction patterns were collected with a Philips PW1710 diffractometer using CuK α radiation and a graphite secondary monochromator. The different polymorphs were identified by comparison with reference scans (Burger et al., 2000). The results obtained both at 20% (see Figure 1) and at 10% (not shown) total solute concentration indicate that the threshold concentration above which crystalline mannitol is consistently detected in a freeze-dried solid where a non-crystallizing sugar (dextran) is present is about 30% w/w, in agreement with Kim et al. (1998); some of the obtained matrices are shown in the followings, evidencing the presence of the crystalline structure in the amorphous phase. It can be observed that the monoclinic δ-mannitol is the prevailing phase obtained in case of mixtures with dextran, accompanied by some orthorhombic polymorph, while in the case of pure mannitol, in the tested conditions (and with no annealing), a mixture of the two orthorhombic polymorphs, α-mannitol, that is the most abundant one, and β-mannitol, is formed, with no evident traces of the monoclinic one, in agreement with the results of Kim et al. (1998) and of Hottot et al. (2008). This result may be a confirm of the cryoprotective effect of hydroxyl compounds such as dextran: they prevent injurious freezing of biological systems by trapping the crystallization agent, mannitol, into an highly viscous, or glass-like, phase.

Dextran and sucrose were selected because, as pure substances, give amorphous products, but they have very different glass transition temperatures. The aim of the experimental investigation is thus to point out the behavior of pure substances and of binary mixtures of dextran with mannitol and sucrose.

In all the tests the solutions were freeze-dried in vials (with an internal diameter of $14 \cdot 10^{-3}$ m), each containing 1 ml of product, in a Lyovac GT2 freeze-drier

(Leybold). The freezing step was carried out *in situ*. Three cycles were generally carried out for each formulation in order to investigate the influence of the drying temperature on the freeze-drying process and on the characteristics of the product. In the first cycle (coded as T_1 in the following paragraphs), after the freezing step, the temperature set point was chosen in order to maintain always the product temperature below T_g'. Another cycle (T_3) was partially performed at a temperature higher than the glass transition temperature (potentially causing microcollapse or even collapse): in this case the cryostat set-point was set at 25°C till the end of the process. Finally, a cycle (T_2) was performed at an intermediate temperature, close to the glass transition temperature, but slightly lower than that: this should avoid collapse, even if localized microcollapses are possible if the glass transition temperature is locally trespassed. The cryostat-set points for the cycles at T_1 and T_2 were different for every formulation, depending on the characteristic temperatures, and were set taking into account the dynamics of the apparatus and the heat transfer coefficient, on the basis of preliminary runs. Some examples are given in Table 2. In all cases, at the beginning of the operation the cryostat was set at a low temperature (-50°C), thus obtaining a product cooling rate of about 0.5°C/min from 25°C to -30°C, and of about 0.1°C/min from -30°C to -40°C: good reproducibility of these values in all the experiments was observed. After the freezing step, the temperature of the plate was increased. When the collapse temperature was very low, as for the 10% sucrose solution or for the 8% sucrose - 2% dextran mixture, the whole cycle was carried out at the lowest possible temperature (setting the temperature of -50°C) in order to be sure to avoid collapse. In some cases, e.g. for the mixture 6% mannitol - 14% dextran shown in Table 2, the cryostat set-point was set higher than T_g' in order to speed up the heating, and thus the drying, of the product (the temperature increase was slow due to the presence of the capacitive balance): in any case the product temperature never overcame the T_g'.

In all the runs secondary drying was carried out rising the set point temperature at 25°C (see Table 3 and 4 for the duration of the primary and of the

9

secondary drying stages).

A capacitive balance was used to monitor the variation of the weight of the samples during the cycle (Carullo et al., 1998; Rovero et al., 2001); the presence of the balance affects the heat transfer from the shelf to the vials, but this effect is the same for all the runs and has been taken into account in the selection of the set-point temperatures. The balance yields the cumulative water loss as well as the drying rate vs. time; this allows to detect the endpoint of primary drying: in fact it is possible to calculate the total value of the sublimated mass and, thus, to detect the time instant when almost all the water of the frozen product has sublimated and the sublimation flux is almost constant (at a value of about zero). The use of weighing devices for monitoring the primary drying has been recently discussed by Barresi et al. (2009).

A typical plot of the time evolution of the temperature of the product is shown in Figure 2. The product was frozen at a temperature lower than its complete solidification temperature and then the temperature was increased to the value required for the primary and, then, for the secondary drying. Product temperature is measured by a thermocouple in close contact with the bottom of the vial and it can be noted that it rose much less and more slowly than the plate temperature as a consequence of the presence of the capacitive balance.

The characterization of the freeze-dried product was carried out by evaluating the apparent density (by weighing the dried product and estimating the volume with the caliper), the residual moisture (by weighing the sample before and after the process) and the crystallinity (by XRD). The aspect of the product (including the presence of cracking, vitrification, etc.) was also considered and special attention was paid to shrinkage. The rehydratability is one of the most important properties in pharmaceutical applications, but the small quantity of product available and its high solubility ruled out the use of standard methods (McMinn and Magee, 1997). In fact, the dissolution of the product was very fast if water was added and the vial shaken. In order to allow for quantitative comparison between the products freeze-fried in different conditions, a procedure that allows to slow down the operation was

developed. The freeze-dried product was firstly drawn from the vial (by sectioning it) and then the product cake was divided into four pieces and weighed; this allowed to carry out four measurements (each one after a different rehydration time) on each sample. The exact amount of water required for the rehydratation was added, taking note of the beginning and of the ending time. Then, at a prefixed time, the liquid concentration was measured, drawing all the liquid in presence of the solid with a syringe supporting a 0.02 ppm filter in order to suck the solid. Then the liquid was shaken and analyzed for concentration. Other procedures were tested, but the one described was used because it gave the best and more reproducible results. The possibility of sampling a fraction of the solution was also investigated, but the results come out to be function of the sampling position and they were quite erratic as the characteristic diffusion time was longer than the dissolution time, thus resulting in high concentration gradients in the solution. The concentration was measured by the Refraction Index (RI), using a refractor-meter; all the measurements were carried out at 25°C. Calibration plots were drawn for each component using solutions at various and known concentrations (4, 6, 8 and 10 % by weight). A linear relationship resulted, being the correlation for the three substances very similar because of the close molecular weights. For the binary solutions it was not possible to relate the RI to the concentration of each component; in this case the refractive index $A(t)$ of the solution was made dimensionless using the final RI of the solution (once the solid was completely dissolved). This index was a good indication of the total concentration vs. time, although it did not provide information on the dissolution rate of the individual components.

Results and discussion

As it was stated in the Introduction, the occurrence of collapse phenomena depends on the temperature at which the process is carried out. The effect of the drying

temperature on the freeze-dried product characteristics will be thus discussed in this paragraph for aqueous solutions containing both single components and mixtures of two components. Moreover, the effect of the working temperature on the process duration and on the rate of solvent removal will be investigated.

Effect of the drying temperature on the product appearance.

The products freeze-dried at the lowest temperature (cycle T_1), showed a very porous structure; they did not stick to the vial and no shrinkage occurred.

A vitreous layer appeared on the product surface when the collapse temperature was approached during the cycles carried out at higher temperature (see an example in Figure 3a): this film can be explained by a partial collapse of the structure. A typical collapsed structure was generally macroscopically evident in the product from the runs carried out according to the T_3 cycle; some of these samples, observed at the scanning electron microscope, also showed a particular structure, as shown for example in Figure 3b.

Some examples of the typical structures observed for the different mixtures in runs carried out at a safe temperature are also shown in Figure 3: images d), e) and f) show the detail of the typical crystalline structure of mannitol embedded in the amorphous phase, for different relative concentrations of the two species, compared with that of the pure crystalline mannitol (h), while images c) and g) show, at a larger scale, the pore structure obtained with two different mixtures (completely amorphous and with crystals in the amorphous matrix).

Final characteristics of pure substances.

It was specified that mannitol gives a crystalline structure, while dextran and sucrose form a stable glassy system which remains amorphous. After freeze-drying, the apparent density of the pure substances can vary significantly with the process temperature as reported in Table 3; this is a consequence of significant shrinkage that occurs at the highest temperatures as a consequence of product collapse.

The rehydratability of all the samples, independently of the cycle type actuated, was always very prompt when the solution was stirred or shacked. Due to the methodology adopted and previously described, which aimed to evidence differences between the products, high dissolution time are measured. Figure 4 shows that the rehydratability is faster for the product freeze-dried at lower temperature (T_1), when the structure resulted to be more porous and softer; higher rehydration rates were also observed for samples obtained from lower initial concentration (resulting in a higher porosity and, thus, in a lower apparent density). For example, in the case of sucrose, at the T_1 conditions a complete dissolution was obtained in about 15 minutes, while the T_2 condition originated a product that required about 20 minutes to be completely dissolved; the T_3 conditions gave origin to a product not completely dissolved in the time interval used for the analysis.

In Figure 5 a comparison of the behavior of different substances is presented. Even though the differences are rather small, it can be noted that the mannitol (crystalline structure) dissolved very quickly at the beginning: this is a consequence of its structure (see Figure 3h), with high surface and fine crystals which accelerate initial dissolution. On the contrary, an initial more difficult dissolution for sucrose and dextran is compensated by the bound weakness typical of an amorphous structure. If the cycle is carried out at higher temperature (T_3 cycle, not shown in the Figure) the dissolution is slower, due to the presence of a collapsed structure.

Final characteristics of binary mixtures.

Two different types of mixtures were investigated, namely mannitol-dextran and sucrose-dextran, at different concentrations. In Figure 6 the rehydration curves for the binary mixtures of sucrose-dextran and of mannitol-dextran, freeze-dried with the three different cycles previously described, are shown. Generally, the rehydratability gets worse when higher drying temperatures are used, while the relative composition has a smaller effect. In the T_3 cycle the structure was collapsed: the apparent density increased and the residual moisture was higher, as reported in

Table 4.

In Figure 7 the effect of the relative concentration of the excipients on the rehydratability is shown for the sucrose-dextran mixtures processed with the various cycles. In some cases the mixture dissolves at a higher rate than the respective components. This behavior can be explained by considering that a solid constituted of two amorphous constituents can have more weak bounds than a solid constituted by the same species: it is, thus, easier to dissolve a mixture of two components than a pure substance.

In the case of the mannitol-dextran mixtures a different behavior is observed for the samples freeze-dried at T_1 and at T_3, as it is shown in Figure 8. At T_1 the dextran has a higher rehydratability than the mixture, while in the case of the cycle T_3 the curves of the mixture and of the dextran are very close (it can be remembered, to this purpose, what observed before about different rehydration rate of amorphous and crystalline substances).

In Figures 9 the effect of the temperature on the apparent density and on the residual moisture of sucrose-dextran mixtures at different concentration is shown. Other data are shown in Tables 3 and 4, together with the duration set for the secondary drying stage. As concerns the residual moisture, this parameter was not investigated in detail, but even if the final values are also affected by the total duration of the cycle, it can be observed that for the conditions investigated the cycles carried out at higher temperature, i.e. the T_3 cycles, where collapse occurred, have generally higher residual moisture in the product, as it can be expected when collapse of the dried cake occurs. For both quantities the effect of the temperature is small, but the properties of the dried product depend on the sucrose fraction in the mixture in a non-monotone way.

Effect of the drying temperature on the process duration and drying rates.

The freeze-drying process is expensive, thus the drying kinetics is very important as it affects the duration of the process. In general, we have seen that a higher drying

temperature means a worse freeze-dried product, with lower rehydratability and possibly a bad appearance, but these negative effects are partly compensated by a shorter process. The occurrence of collapse can be responsible of a strong reduction of the sublimation flow rate, due to the higher resistance of the collapsed cake; in some cases microcollapse can occur, resulting in an increase of the sublimation flow rate. This mechanism was described by Milton et al. (1997) and by Overcashier et al. (1999): when product starts to collapse, holes appear in the structure of the dried material, apparently as thin spots, as the surface tension forces become active when the viscosity of the dried material decreases. If the temperature is further increased gross collapse of the dried structure occurs, with clearly observable cake shrinkage.

In Figure 10 examples of the weight loss measured by the capacitive balance as a function of the process time are shown with the values of sublimation rate that can be calculated from these data. Obviously, the freeze-drying process is shorter at higher temperature both for pure substances and for binary solutions; here we focuses on the primary drying duration (determined by means of the balance) at different temperatures. Some data are compared in Tables 3 and 4: the higher the temperature, the lower the time required to complete the drying and, thus, it is of outmost importance to carry out the cycle at a temperature just below the limit value to avoid the collapse of the product.

Figure 11 compares the sublimation rates of sucrose-dextran binary mixtures and those of the constituents during primary drying for different thermal cycles; the product temperature measured by a thermocouple in close contact with the bottom of the vial in the various runs is also shown in the left column. As it can be expected, the higher is the working temperature the higher is the sublimation rate, thus decreasing the time required to complete the primary drying. The time evolution of the sublimation rate as a function of the progress of primary drying, given by the ratio between the sublimated water and the initial total water content, is also shown in the l.h.s.: in all the T_1 cycles the sublimation rate vs. the progress of the primary drying is almost constant (or it does not exhibit sharp changes) through all this step;

for certain solutions the value of the sublimation rate is higher due to the higher shelf temperature (compare the left graphs). It can be seen that the freeze-drying of mixtures can be easier and faster than that of one of the components alone; this is partly a consequence of the higher temperatures allowed, but it is evident that even with the same process temperature the drying kinetics can be higher. At higher temperature, where collapse can occur during drying, the sublimation rate increases sharply in the first part of the cycle (due to the high shelf temperature) and then, when the system approaches the end of the drying, the sublimation rate falls to zero. A similar behavior is observed in Figure 12 for the sublimation rate of dextran and a mannitol-dextran mixture (at the same concentration) for different thermal cycles.

Conclusions

The influence of the temperature at which the primary drying is carried out on the process duration and on the product characteristics has been investigated in this work, as well as the role played by the composition of the solution. Apparent density, product rehydratability and residual moisture are the properties evaluated in order to characterize the product obtained, both in the case of one solute and in the case of binary mixtures of solutes. Results show that primary drying should be carried out at lower temperature in order to get faster rehydratability and to avoid collapse of the product; nevertheless, in order to reduce the duration of the process it is required to work at higher temperature, near to the maximum allowable value. In case of mixtures (of sucrose and dextran and of mannitol and dextran) the freeze-drying results to be easier and faster than that of one of the components alone: this is a result of the higher temperatures allowed in case of mixtures, but even with the same process temperature the drying kinetics can be higher. In any case it has been shown that the reduction of the porosity and rehydration rate is very limited even when the drying has been carried out at the glass transition temperature or for some

time just above it.

Optimal results, in terms of process duration and product quality, can thus be achieved when the primary drying is carried out as closer as possible to the maximum temperature allowed by the product: this requires a reliable system to monitor product temperature and a control system that maintains the product at the desired temperature. This is also one of the targets of the Guidance for Industry PAT (Process Analytical Technology) issued by US Food and Drugs Administration in 2004: in fact, it encourages manufacturers to use the latest scientific advances in pharmaceutical manufacturing and technology so that quality is no longer tested into products, but is built-in or is by design.

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Notation

A	refractive index
C	concentration
t_{95}	time for 95% of final concentration in reconstitution test, min
T	temperature, °C
T_c	collapse temperature, °C
T_{cs}	complete solidification temperature, °C
T_g'	glass transition temperature, °C
T_m	melting temperature, °C
T_1, T_2, T_3	identifier of the set-point for primary drying temperature

Subscripts and superscripts

max   maximum value, corresponding to complete dissolution

Abbreviations

DSC   Differential Scanning Calorimetry

XRD   X-Ray Diffraction

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List of Tables

List of Figures

Figure 4   Influence of the process temperature on the rehydratability of single excipients. Upper graph: 10% sucrose solution; lower graph: 10 % (filled symbols) and 20% (open symbols) dextran solution. ●, ○: T_1 cycle; ▲: T_2 cycle; ■,□: T_3 cycle.

Figure 5   Rehydratability of pure substances: ●, sucrose; ▲, mannitol; □, dextran. T_1 cycle, 10% solution.

Figure 6   Rehydratability of binary mixtures of sucrose-dextran and of mannitol dextran freeze-dried with different cycles. ○: T_1 cycle; Δ: T_2 cycle; □: T_3 cycle.

Figure 7   Comparison between the rehydration rate of the sucrose-dextran binary mixtures and that of the constituents. Open symbol: pure components (○: dextran 10%; □: sucrose 10%); filled symbol: mixtures (◆: 2% sucrose – 8% dextran; ▲: 8% sucrose – 2% dextran). Upper graph: T_1 cycle; middle graph: T_2 cycle; lower graph: T_3 cycle.

Figure 8   Comparison between the rehydration rate of a mannitol-dextran mixture and that of pure dextran. ○: 20% dextran; ▲: 6% mannitol – 14% dextran. Upper graph: T_1 cycle; lower graph: T_3 cycle.

Figure 9   Variation of the apparent density (upper graph) and of the residual moisture (lower graph) with the sucrose concentration in the sucrose-dextran mixture at different process temperatures ○: T_1 cycle; Δ: T_2 cycle; □ : T_3 cycle.

Figure 10   Plate temperature (upper graph), weight loss (middle graph) and

sublimation rate (lower graph) for a binary mixture (2% sucrose – 8% dextran) with different drying cycles. —: T_1 cycle; ⋯⋯: T_2 cycle; -----: T_3 cycle.

Figure 11   Comparison between the temperatures of the product (graphs A1, A2 and A3), the sublimation rates of sucrose-dextran binary mixtures and those of the pure components as a function of time (graphs B1, B2 and B3) and as a function of the progress of primary drying (graphs C1, C2 and C3) for different thermal cycles. Open symbol: pure components (O: dextran 10%; □: sucrose 10%); filled symbol: mixtures (◆: 2% sucrose – 8% dextran; ▲: 8% sucrose – 2% dextran). Graphs A1, B1 and C1: T_1 cycle; graphs A2, B2 and C2: T_2 cycle; graphs A3, B3 and C3: T_3 cycle.

Figure 12   Comparison between the temperatures of the product (graphs A1, A2 and A3), the sublimation rates of mannitol and mannitol-dextran mixture vs. time (graphs B1, B2 and B3) and vs. the progress of primary drying (graphs C1, C2 and C3) for different thermal cycles (O: dextran 20%, ●: 6% mannitol – 14% dextran). Graphs A1, B1 and C1: T_1 cycle; graphs A2, B2 and C2: T_2 cycle; graphs A3, B3 and C3: T_3 cycle.

Table 1

w/w %	T_{cs}	T_m	T_g'	structure
Mannitol 10%	-38°C	-21.5°C	n.a.	crystalline
Dextran 10%	-20°C	n.a.	-9°C	amorphous
Dextran 20%	-24°C	n.a.	-9°C	amorphous
Sucrose 10%	-36°C	n.a.	-33°C	amorphous
Mannitol 2% + Dextran 18%	-42°C	n.a.	-13°C	amorphous
Mannitol 6% + Dextran 14%	-34°C	n.a.	-15°C	amorphous matrix including crystalline mannitol
Sucrose 2% + Dextran 8%	-32°C	n.a.	-18°C	amorphous
Sucrose 8% + Dextran 2%	-34°C	n.a.	-28°C	amorphous

n.a. = not available

Table 2

	T_1 cycle		T_2 cycle	
	Set point T, °C	**Duration, h**	**Set point T, °C**	**Duration, h**
Sucrose 10%	-50	3	-50	3
($T_g{}' = $ -33°C)	apply vacuum		apply vacuum	
	-50	25	-32	15
	25	20	25	5
Mannitol 6%	-50	2	-50	3
Dextran 14%	apply vacuum		apply vacuum	
($T_g{}' = $ -15°C)	-20	10	-12	16
	-10	17	25	20
	25	24	25	20
Sucrose 2%	-50	5	-50	5
Dextran 8%	apply vacuum		apply vacuum	
($T_g{}' = $ -18°C)	-25	15	-16	18
	-20	2	25	6
	25	24	25	6

Table 3

Pure substance		Apparent density, g cm^{-3}	Residual moisture, %	Rehydratability t_{95}, min	primary drying, h	secondary drying, h
Sucrose	T_1	0.097	0.29	10	20	20
10%	T_3	0.134	0.12	20	6	9
Dextran	T_1	0.142	0.10	4	7	15
10%	T_3	0.190	0.18	15	5	7
Dextran	T_1	0.406	n.a.	10	12	26
20%	T_3	0.432	0.30	25	5	13
Mannitol 10%	T_1	0.092	n.a.	8	15	20

n.a. = not available

Table 4

Binary solution		Apparent density, g cm^{-3}	Residual moisture, %	Rehydratability t_{95}, min	primary drying, h	secondary drying, h
Man-Dex 6-14%	T_1	0.186	0.18	10	n.a.	n.a.
	T_2	0.210	0.14	16	9	10
	T_3	0.240	0.9	n.a.	7	9
Sucr-Dex 2-8%	T_1	0.151	0.19	5	12	28
	T_2	0.198	0.35	15	6	8
	T_3	0.238	0.5	20	5	13
Sucr-Dex 8-2%	T_1	0.314	0.28	2	n.a.	n.a.
	T_2	0.338	0.3	5	9	11
	T_3	0.391	0.9	18	8	10

n.a. = not available

Figure 1
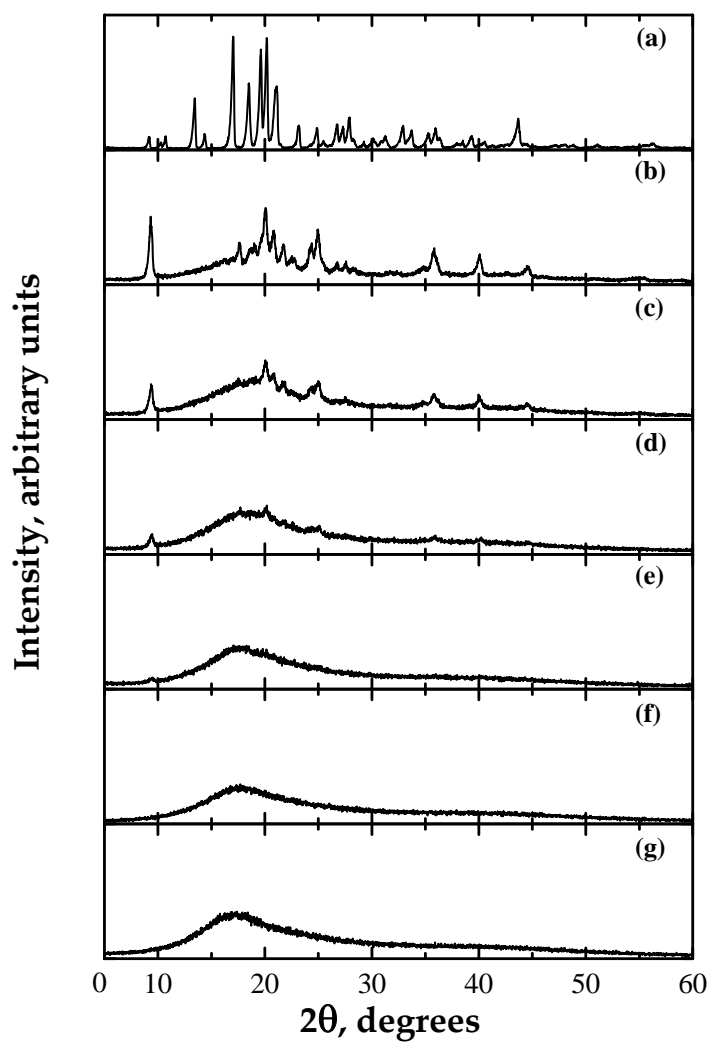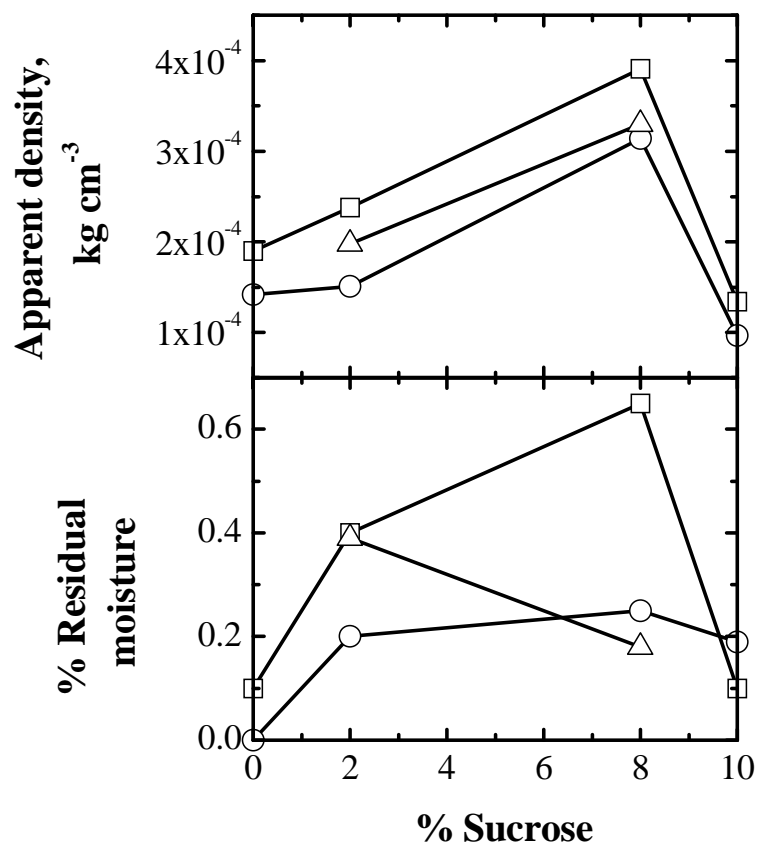
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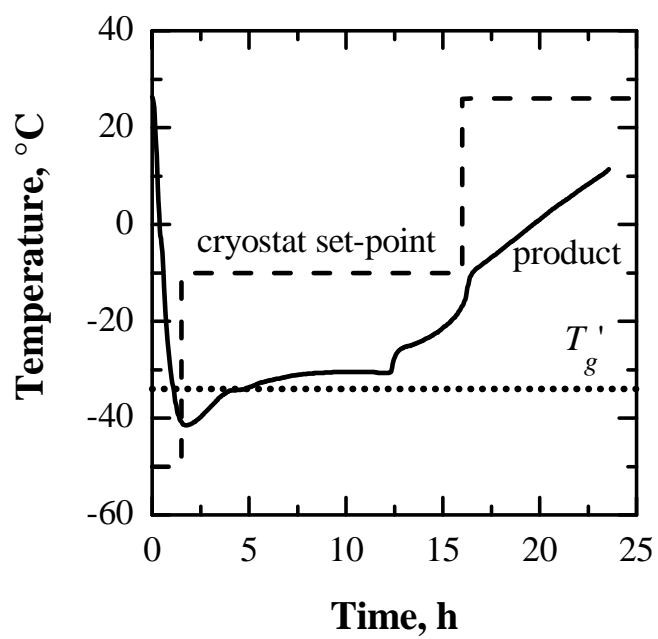
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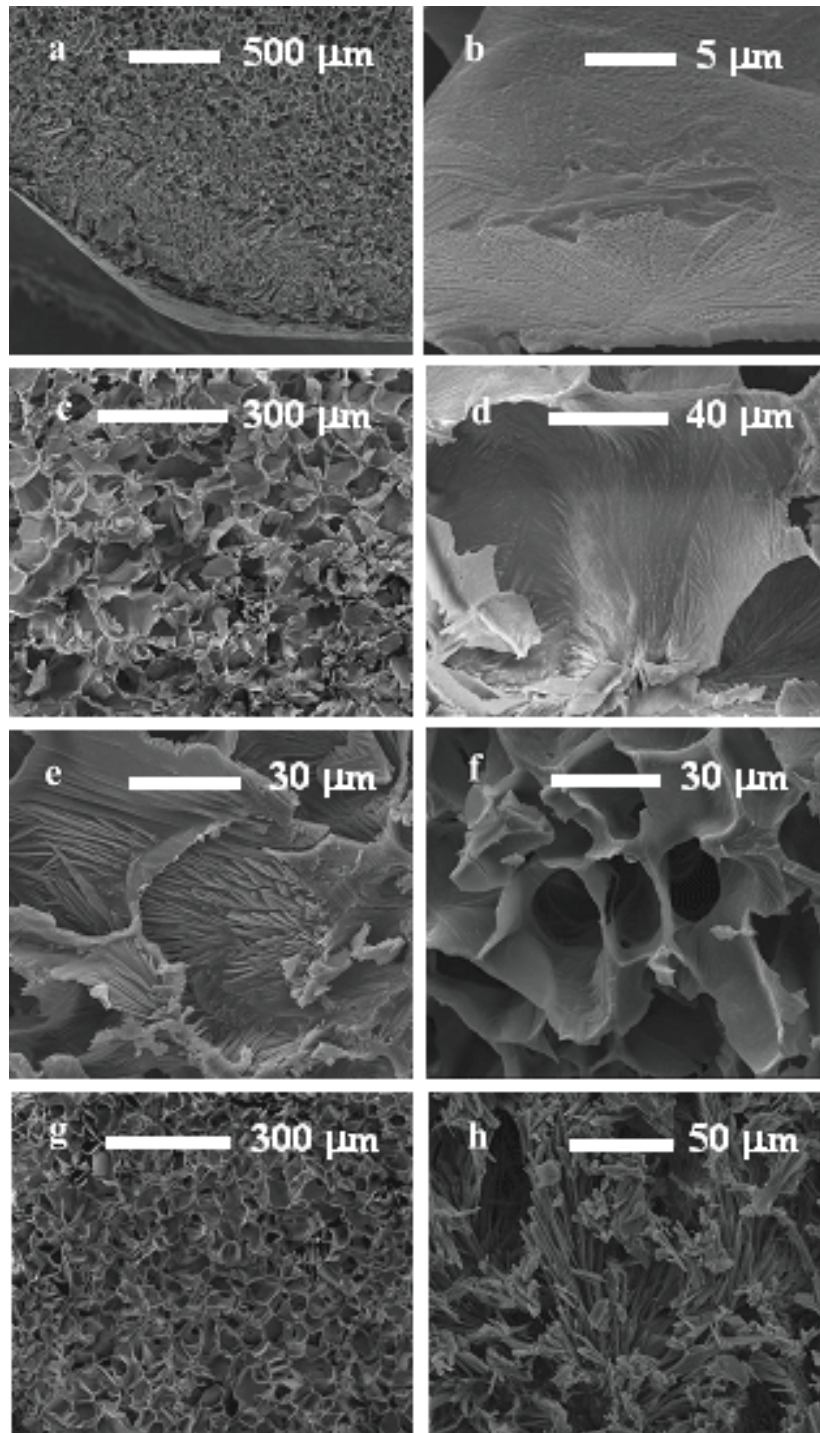
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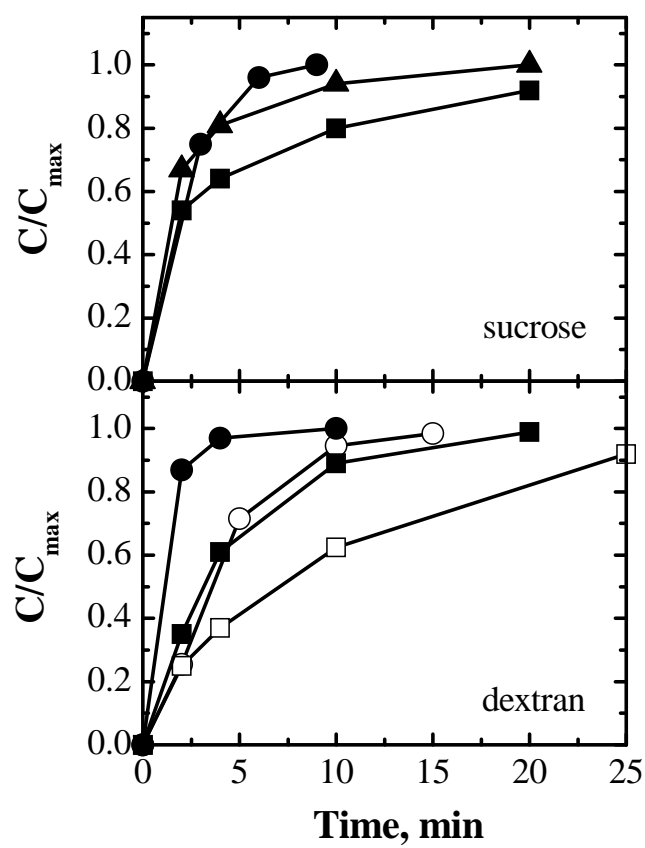
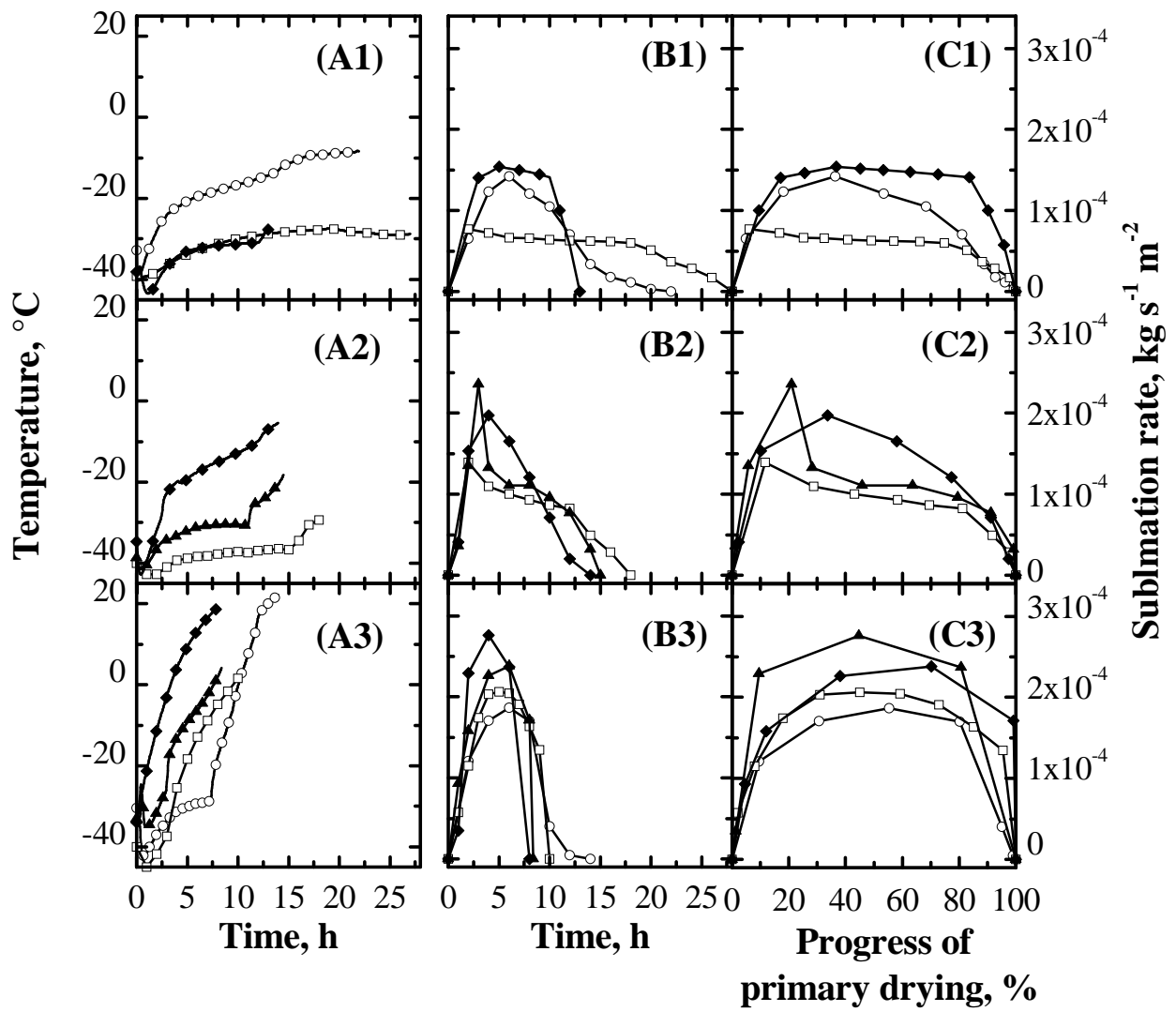Figure 5
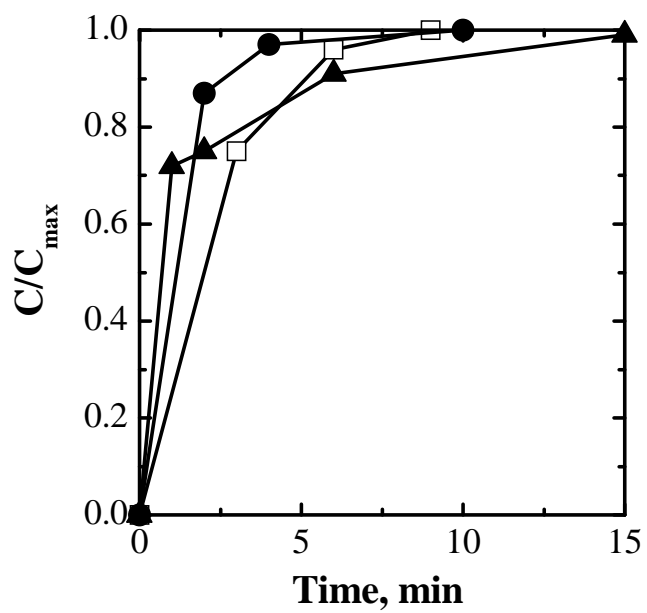
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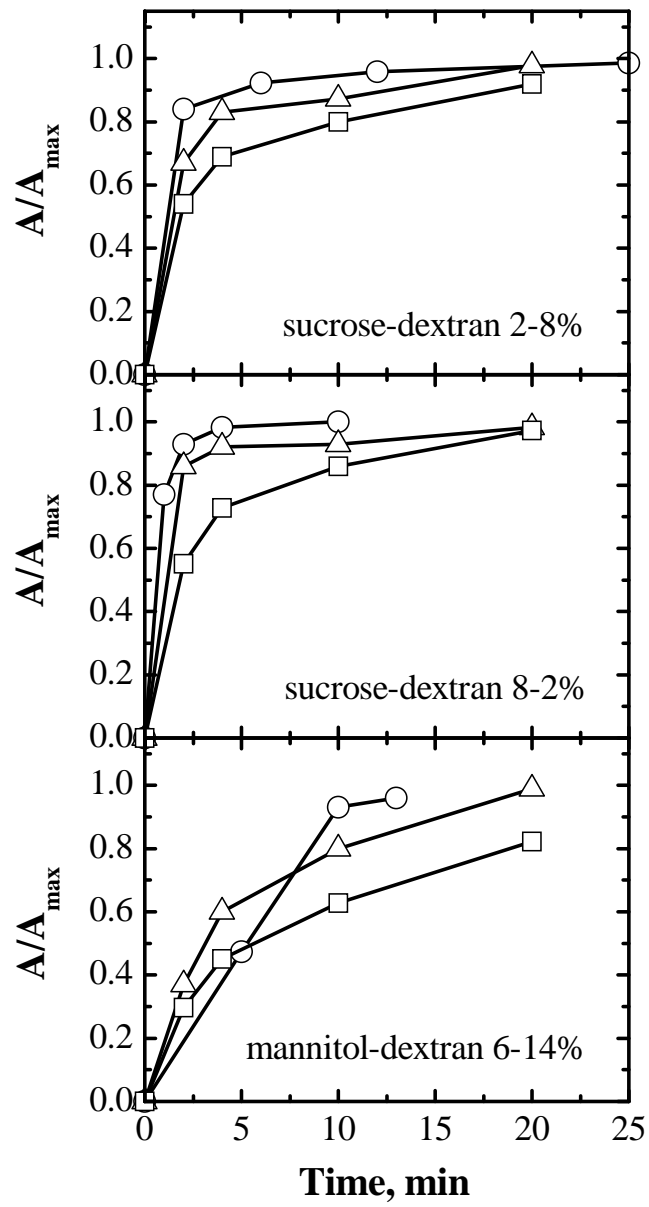
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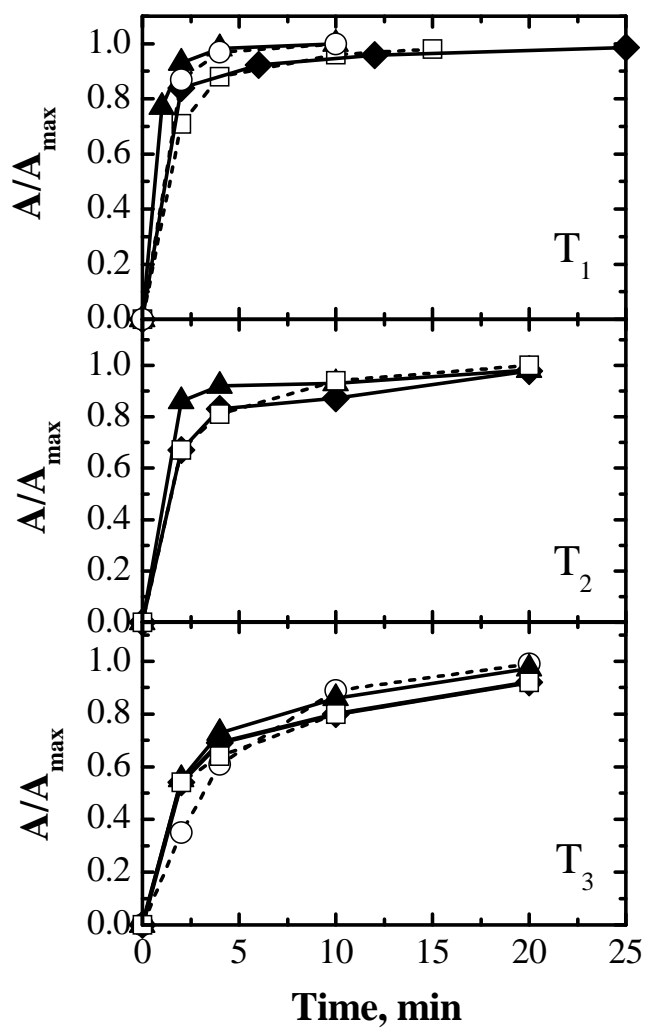
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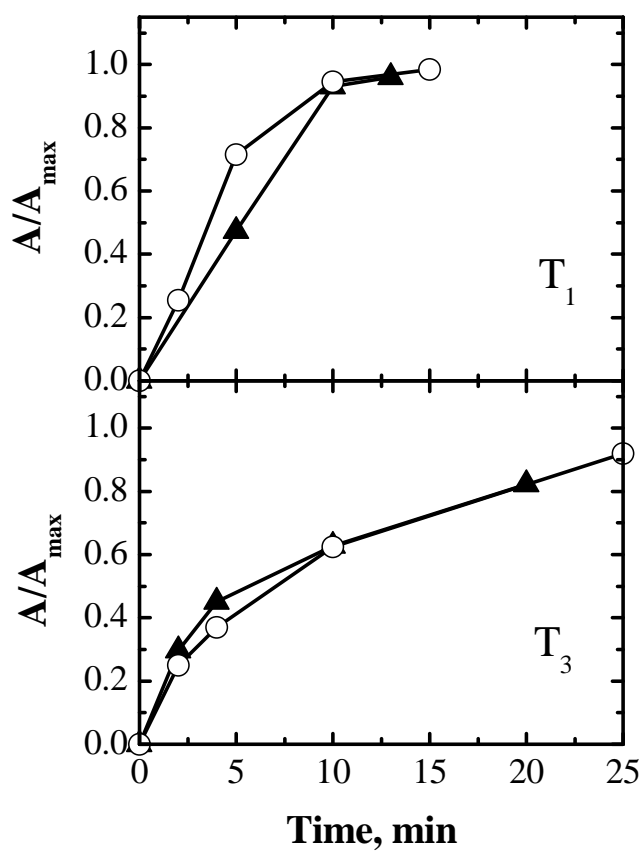
Figure 9

Figure 10

Figure 11

Figure 12