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Investigation of aqueous solutions by modulated temperature differential scanning calorimetry

Maggie Aldén*, Anna Hillgren

Department of Pharmaceutical Chemistry, Physical and Inorganic Chemistry, Box 574,
Biomedical Center Uppsala University, S-75123 Uppsala, Sweden

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Abstract

The influence of variables on the observation of thermal events and on the determination of heats of transformation at freezing and thawing in aqueous solutions was investigated using modulated temperature differential scanning calorimetry (MTDSC, ODSC). The investigated systems were pure water and dilute aqueous solutions of polyethylene glycol (PEG) 6000. The influence of eleven combinations of frequency, amplitude and underlying heating rate was analyzed in heating–cooling scans. The selection of variables had a great impact on the shape of the c_p component curve as well as on the amount of heat expressed by the c_p component, ΔH_c, of the thermal event. The conventional ΔH-values were not influenced to the same extent. The ratio $\Delta H_c/\Delta H$ of the melting process might be an expression of the degree of relative crystallinity in frozen aqueous solutions. The requirement of operational setting is an appropriate degree of oscillation in combination with a number of modulations over a distinct transformation temperature. The selection of variables must produce a *heating–cooling* scan.

Keywords: MTDSC; ODSC; Aqueous solutions; Heat of transformation; Crystallinity; Degree of oscillation

1. Introduction

The MTDSC (ODSC) method offers a possibility to study transformations like crystallization, melting and T_g, which are relevant for the characterization of, for example pharmaceutical formulations to be manufactured by the freeze-drying process [1]. As was emphasized by Coleman and Craig in a review of the technique [2], several critical studies are required to explore the full advantage of the technique for applied purposes.

With the MTDSC equipment three variables can be changed: the frequency of the oscillation, the amplitude and the underlying heating rate. The importance of selecting the correct operational parameters in MTDSC has been stressed. The few guidelines that are available emphasize the following. Frequency and overall heating should be selected so as to obtain at least five cycles over the temperature range of the event to be studied [3]. Another possible approach is to give a certain weight to the cyclic component of the overall heating rate. This has been done by Aldén et al. [4] by defining a combination of variables denoted the degree of oscillation and calculated as the product of amplitude (K) and frequency (s^{-1}) divided by the underlying rate ($K\,s^{-1}$). The authors recommend a certain level of this parameter in order to obtain reproducible quantitative data for polymer systems.

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*Corresponding author. Fax: 00 46 18 5517 59.

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The kinetics of the investigated processes influences the range of selections that are possible to use. The effect of modulation of temperature on fast transformations is, on the time scale of the experiment, different from the effect on much slower processes. Additionally, the MTDSC technique includes a Fourier transformation that is based on linearity or the superposition principle. If a process under observation is non-linear the physical meaning of the Fourier transformation should be considered [5].

The recommendations on operational parameters given by Reading [3] or by Aldén et al. [4] refer to semi-crystalline polymer systems. The selection of operational parameters to be used for aqueous systems to examine thermal events and to determine reproducible heat of transformation values might be different and has so far not been systematically investigated. In aqueous diluted solutions the very fast ice nucleation and growth are the dominating process at the crystallization. Also the melting process of ice phase is fast compared to the melting process of a semi-crystalline polymer phase [6].

The objectives of this study were to investigate the influence of the selection of variables on the observations of thermal events at freezing and thawing in pure water and in dilute aqueous solutions of polyethylene glycol (PEG) 6000 and to evaluate the effect of operational parameters on the determination of heats of transformation. The possibility of using the information on the enthalpy changes of the reversible component, ΔH_c , relative to the conventional component ΔH, as an indicator of the degree of crystallinity of a system was also investigated and the prerequisites to obtain significant values elaborated.

2. Theory

The response of an ideal DSC, where no temperature gradients exist can be expressed [3,7]

$$\frac{dQ}{dt} = c_{p,t}\frac{dT}{dt} + f(t,T) \qquad (1)$$

where Q is the amount of heat absorbed by the sample, $c_{p,t}$ is the thermodynamic heat capacity, T is the absolute temperature and t is the time. $f(t,T)$ is a function of time and temperature that governs the kinetic (irreversible, non-reversing) response of a process under steady-state condition. The temperature program can be expressed as

$$T = T_0 + At + B\sin(\omega t) \qquad (2)$$

where T_0 is the starting temperature, A is the underlying heating rate, B is the amplitude of the temperature modulation, ω is the frequency in radians or $\omega = 2\pi f$, where f is the frequency in Hz.

Over a small temperature interval the kinetic process can be approximated as linear. Eq. (1) can be rewritten as

$$\frac{dQ}{dt} = c_{p,t}[A + B\omega\cos(\omega t)] + f'(t,T) + C\sin(\omega t) \qquad (3)$$

where $f'(t,T)$ is the average underlying kinetic function once the effect of the sine wave oscillation has been subtracted and C is the amplitude of the kinetic response to the sine wave oscillation. $A+B\omega\cos(\omega t)$ is the measured quantity dT/dt.

This mathematical background however does not reflect the different responses to oscillation that can be expected in the transformation of materials of different character like for example a polymer system or ice. The thermal events occur with different rates and in a more or less narrow temperature interval. Furthermore the original equation of heat flow rate (1) where $f(t,T)$ represents the non-reversing component assumes a steady-state condition. However this condition is not always fulfilled at crystallization or melting events.

3. Experimental

3.1. Material

Deionized and filtered water (Miller-GS 0.22 μm sterile filter) was used for the samples both in pure form and in solutions. The solutions contained 1% w/v polyethylene glycol (PEG) 6000 (Janssen, Belgium) and 25 μg/ml lactate dehydrogenase (LDH), below denoted by PEG 6000 solutions (ICN Pharmaceuticals, USA).

3.2. Method

The samples were analyzed using a DSC 220C oscillating DSC (Seiko, Japan). The calorimeter was

Table 1
Heat of transformations and peak temperatures for different frequencies in solutions with 1% w/v PEG at heating rate 2.5 K min^{-1} and degree of oscillation 0.96

	Amplitude in K	Frequency in Hz	ΔH in J g^{-1}	ΔH_c in J g^{-1}	T_{peak} in °C	ΔT in °C
Melting	4	0.01	274.3	49.1	3.2	22.2
	2	0.02	287.7	35.8	2.6	22.3
	1	0.04	244.9	51.6	3.8	17.7
Crystallization	4	0.01	−256.4	−23.4 [a]	−14.6	13.5
	2	0.02	−268.7	−20.9 [a]	−15.5	10.9
	1	0.04	−255.3	−38.0 [a]	−17.7	9.0

[a] This value might be an artifact.

temperature and heat-calibrated with indium, tin, gallium and mercury as standards. The samples (20–35 mg) were kept in aluminum pans in an atmosphere of N_2. The samples were analyzed using eleven combinations of variables. All the combinations give both heating and cooling in the temperature cycle. The maximum of one period thus exceeds the minimum of the next period. The number of modulations over a certain temperature were 3–5. The frequency interval was 0.01–0.04 Hz, the heating interval 1–10 K min^{-1} and the amplitude interval 1–5 K. The temperature history included freezing to −60°C, a ramptime at −35°C to −40°C and heating to 30°C.

The variation of frequency was performed at the constant degree of oscillation 0.96 as single experiments; the heating rates, amplitudes and frequencies are presented in Table 1. The variation of heating rate and amplitude were performed at frequency 0.02 Hz; the different heating rates and amplitudes are presented in Table 2. The degree of oscillation was 0.48–4.8. The results are mean values of two or three determinations.

4. Results and discussion

4.1. Measured curves

In the experiments it was assumed, that the transitions occurred with linearity retained and thus that the Fourier transformation was valid. The difference observed for varying operational settings between the modulated heat-flow rate in relation to the modulated temperature indicates, that the assumption is reasonable for the melting events. For the very fast crystallization process occurring at non-equilibrium, the modulated temperature and heat-flow rate are out of phase during longer time, which might give results, that are artifacts.

Raw data from the MTDSC (ODSC) measurements of the samples were separated into the three components, the conventional component (almost identical with the traditional DSC component), the reversing c_p-component and the non-reversing kinetic component. For each combination of variables the energy associated with the melting and crystallization was determined as the conventional enthalpy ΔH and the c_p-enthalpy, ΔH_c. For each combination the peak temperature, T_{peak} was determined in the conventional curve together with melting or crystallization interval. The integration limits were kept constant over the transformation interval.

The typical thermal behavior of a dilute aqueous solution of PEG and of pure water at heating and cooling is illustrated in Fig. 1(a) and (b). It shows an endotherm at about 2°C in the total heat flow rate corresponding to the melting of the system. The reversible c_p and irreversible kinetic components also follow this endotherm. Melting of a stable phase represents a mixture of kinetic and thermodynamic effects and thus manifests itself in both components [8].

On cooling from the melt the phase crystallizes at approximately −15°C showing an exotherm. The thermodynamic crystallization temperature is 0°C, thus the crystallization occurs in an undercooled solution and should thus be an irreversible transformation. Since the modulation results in 'heating–cooling'

Table 2
Heat of transformations and peak temperatures for different solutions with pure water and 1% w/v PEG, respectively at frequency 0.02 Hz

Amplitude in K	Heating rate in K min^{-1}	Degree of oscillation	ΔH in J g^{-1}	SD	ΔH_c in J g^{-1}	SD	$\Delta H_c/\Delta H$	SD	T_{peak} in °C	ΔT in °C
Melting										
H_2O										
2	1	2.4	266.8	28.7	13.8	1.0	0.047	0.003	1.7	13.8
4	1	4.8	287.0	15.5	13.5	0.6	0.046	0.002	2.1	12.1
2	2	1.2	309.1	8.3	28.7	4.2	0.094	0.013	3.4	16.8
4	2	2.4	304.3	9.9	24.6	11.5	0.080	0.037	3.7	21.0
2	5	0.48	298.8	2.0	93.9	8.5	0.322	0.029	5.8	32.0
4	5	0.96	289.5	6.2	49.1	8.1	0.170	0.028	5.8	33.0
1% w/v PEG										
2	2.5	0.96	287.7	8.7	35.8	16.6	0.124	0.054	2.6	22.3
4	5	0.96	300.1	15.0	25.6	3.6	0.086	0.015	4.9	30.6
4	10	0.48	261.5	5.8	51.9	4.5	0.199	0.022	7.2	46.5
Crystallization										
H_2O										
2	1	2.4	−242.1	24.6	−7.9[a]	0.5	0.029[a]	0.002	−14.0	8.5
4	1	4.8	−246.8	12.5	−12.4[a]	7.7	0.046[a]	0.029	−17.3	7.0
2	2	1.2	−256.6	7.5	−18.2[a]	2.3	0.066[a]	0.008	−16.3	12.5
4	2	2.4	−265.2	21.0	−12.5[a]	3.5	0.046[a]	0.013	−14.8	13.0
2	5	0.48	−246.6	14.7	−30.4[a]	0.3	0.126[a]	0.001	−13.3	45.0
4	5	0.96	−230.6	26.5	−20.4[a]	2.9	0.084[a]	0.012	−15.4	47.0
1% w/v PEG										
2	2.5	0.96	−268.7	8.5	−20.9[a]	4.2	0.078[a]	0.014	−15.5	10.9
4	5	0.96	−239.4	15.1	−26.8[a]	19.9	0.111[a]	0.081	−14.2	21.3
4	10	0.48	−200.2	13.7	−43.7[a]	1.1	0.212[a]	0.007	−13.4	37.8

[a] These values might be artifacts.

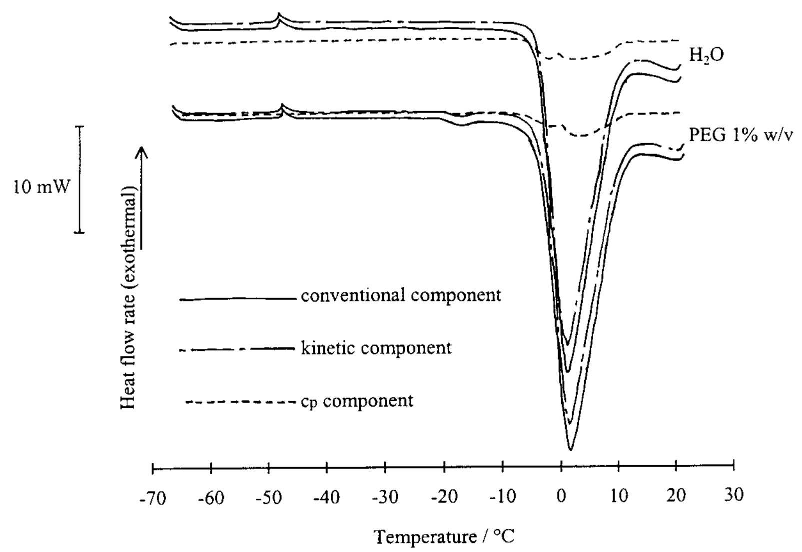
scan some part of the crystals might remelt and crystallize, leading to a signal in the reversing component although the system is far from equilibrium. More likely is, that a non-linearity of the process is reflected. In this case the reversing component might involve an artifact.

At −45°C a small peak appears in the heating process, which seems to be mainly an artifact of the ramptime at the temperature used. It is created when the heat-flow rate is plotted as a function of temperature. The time coordinates of the ramptime are transferred to a single point in the curve and the artificial peak appears. However, some 'real' thermal event is also observed in the interval, which might be connected with the different structures that are possible for ice in this temperature interval. A transition of cubic to hexagonal ice occurs at −47°C if there is an equilibrium in the system [6]. The latter effect is very small 50 μW compared to the artifact effect 1500 μW. In the diluted PEG solutions an additional endotherm, if the heating rate is low or a T_g if the rate is higher, appears at −15°C. It reflects a PEG-ice structure that is transforming [9,10].

4.2. Effect of operational parameters on DSC curves and heat of transformation

4.2.1. Variation of frequency

The effect of selected frequency on the thermal behavior during heating is shown in Fig. 2. All the runs are heating and cooling scans and the weight of the cyclic component, degree of oscillation, is the same in all scans. The total heat flow shows similar features for all frequencies. At crystallization the a



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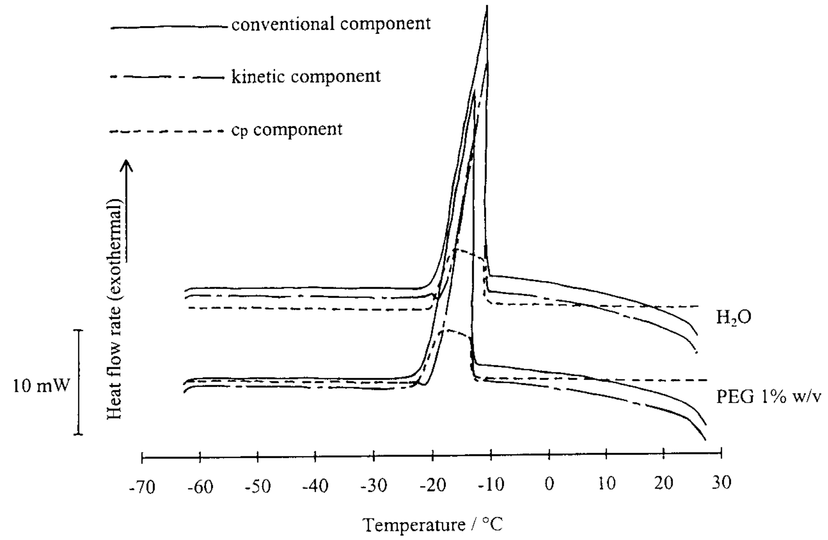

Fig. 1. Measured curves obtained by MTDSC of samples of pure H_2O and 1% w/v PEG 6000. Heating rate 2.5°C/min, amplitude 2°C and frequency 0.02 Hz. (a) Heating in the interval −60°C to 30°C; (b) Cooling in the interval 30°C to −60°C.

longer period at 0.01 Hz gives a much broader peak at −15°C than shorter periods do (Table 1).

For the reversing component of the heat flow rate the overall features observed are the same at different frequencies. The region between −10°C and +10°C shows two overlapping endotherms. At higher frequencies the individual peaks can be identified, while when the frequency is 0.01 the peaks are much less

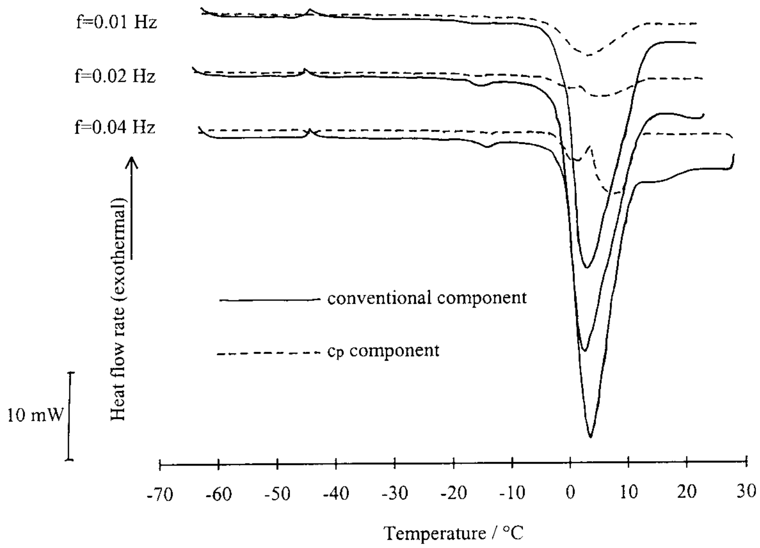

Fig. 2. Heating curves obtained by MTDSC using different frequencies for samples of 1% w/v PEG 6000. Heating rate 2.5°C/min and amplitude 4°C, 2°C and 1°C respectively. Degree of oscillation for all samples 0.96.

distinct. It is possible, that the double peak is an effect of the temperature modulation inducing a phase lag at the melting, if the amount of material is large enough and the period is short. If the period is longer the phase lag might be smaller or at least different. Another explanation might be, that crystals of different sizes are melting and recrystallizing, thereby introducing phases with somewhat different melting temperature. If the period is longer, the number of periods during the melting range are too few to separate the phases and thus the melting appears as a broad and single peak. At crystallization only one peak is observed but it is very broad and probably the sum of overlapping events that are too fast to follow, if the frequency is in the range 0.01–0.04 Hz.

The enthalpies of melting and crystallization as obtained from both the total heat flow curve and the c_p component curve are presented in Table 1 as a function of frequency. The repeatability for the conventional component, ΔH, was fairly good for all samples. The variation of the ΔH_c was however very large. For the crystallization process the values might even be artifacts. The selected frequency thus have a large impact on the shape of the c_p component curve and on the size of the enthalpy that is measured.

4.2.2. Variation of heating rate and amplitude

In Fig. 3(a), (b) and (c) respectively, heating and cooling curves are presented for different heating rates and different amplitudes.

The effect of an increased heating rate on the thermal behavior during melting is shown in Fig. 3(a) and (b). The total heat flow curves have similar features for melting at all rates with the expected broader melting interval at increasing rate as was earlier reported [11]. The reversible component of the heat flow rate shows a double peak in the melting interval, two overlapping endotherms, at all rates. The individual peaks are best identified when the heating rate is high, that is the degree of oscillation decreased. The origin of the peaks is not fully understood. They can reflect different sizes of crystals melting or they can be artifacts of a phase lag, the size of which is depending on amount of material in the sample. From Fig. 3(a) and (b) is observed, that when the amplitude is changed at the different heating rates, it has an impact on the c_p component to a varying degree in different samples. The peaks show less overlapping especially for low heating rates at melting when the amplitude is high. The effect of heating rate on the crystallization process is similar as for melting (Fig. 3(c)). However only one broad peak is observed

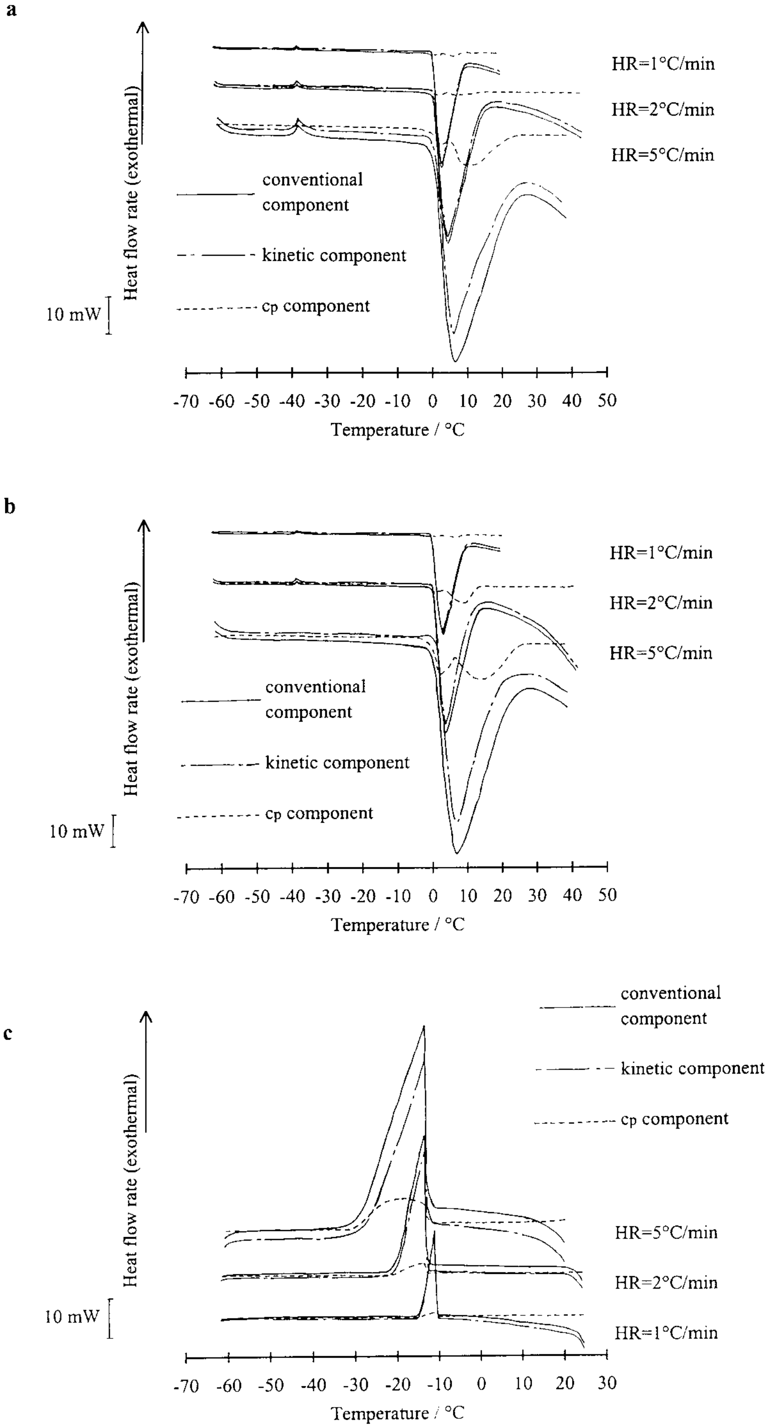

Fig. 3. Measured curves obtained by MTDSC using different heating rates for samples of pure H_2O. Frequency 0.02 Hz. (a) Heating with amplitude 4°C (b) Heating with amplitude 2°C (c) Cooling with amplitude 4°C.

in the c_p component, which might reflect that the very fast exothermal reaction distorts the temperature modulation.

The reproducibility of the conventional heats of fusion was fairly good. The variation of the ΔH_c values was however very large. An enhanced heating rate increased the ΔH_c value if frequency and amplitude were kept constant (the degree of oscillation was decreased), and an increased amplitude decreases the ΔH_c if heating rate and frequency were kept constant (increased degree of oscillation). Also rather low degrees of oscillation gave reproducible results contrary to the results obtained in semi-crystalline systems [4]. Larger absolute values of ΔH were obtained at melting than at crystallization since the crystallization process continues during the whole freezing and thawing process (Table 2). It should be noted, that although the ΔH_c^* values of the crystallization might be artifacts, significant variation between different operational settings were obtained.

4.3. Influence of selected variables on melting and crystallization interval and temperatures

In Tables 1 and 2 the melting interval ΔT and the peak temperatures T_{peak} of the melting endotherm and the crystallization exotherm are presented. As was earlier reported an increased heating rate induces a broader melting and crystallization interval for a sample [4]. The variations can be explained by the fact that the temperature of the whole sample can not follow the changes of the temperature program if the heating rate is too high. Several regions are formed within the sample, each of them in different states of the melting process. At a low heating rate there is time enough for the temperature of the sample to adapt to the programmed temperature homogeneously and the melting interval becomes narrower. No systematic differences were observed when the amplitude was varied.

An increased frequency decreases the melting interval. This effect on the melting interval can be a function of the thermal resistance and the nucleus rate of the sample concerned. At higher frequencies the sample might not form nuclei large enough for crystallization to start before the temperature program raises the temperature above the melting temperature. This means that the melting seems to occur over a relatively narrow temperature interval. At lower frequencies the sample has time enough to melt and crystallize several times and, therefore, a broader melting interval is observed.

The peak temperatures are moved to higher temperature with increased heating rate since the peak temperature corresponds to the point where the inner core of the sample is melting. A variation of amplitude or frequency seemed however, to have no systematic influence on the peak temperatures in aqueous solutions.

4.4. Crystallinity

A tentative evaluation of the MTDSC (ODSC) measurements as a means to determine crystallinity of the frozen aqueous samples was performed. The melting process is an endothermic process which involves both reversible and irreversible events. In the MTDSC measurements the variables can be selected in a way that they involve both cooling and heating in the melting interval and so that the temperature program passes the melting temperature more than once. Theoretically both melting and crystallization can occur in each of the modulations. A material with a high degree of crystallinity should melt and recrystallize in a smaller temperature interval than a semi-crystalline one. The material should in a thermodynamic sense undergo a reversible process during several modulations. This would be observed by a greater reversing component in the ODSC signals. The ratio $\Delta H_c/\Delta H$ might thus be a measure of crystallinity in the system [4].

In Table 2 $\Delta H_c/\Delta H$ values for the melting process are presented for different operational settings. A high degree of oscillation gives a low and constant value of the ratio for pure water, whereas low degrees of oscillation give higher ratios. In the PEG solutions, where PEG is considered to create more amorphous structures (9), there is significant lower values compared to pure water for certain selection of variables. The ratios obtained for the crystallization process of pure water and which might be artifacts, were significantly lower than the melting values. A lower crystallinity is expected, since the crystallization is proceeding during further temperature treatment.

A small amount of perfect crystalline ice should in a heating–cooling scan give a distinct melting process with an almost 100% response to the temperature cycling and thus a large reversing component of the comprised heat flow. A low crystalline amount of ice or PEG/ice might present a more complex melting process where the enthalpy changes also involve a large kinetic component. Our results show, that the relation between the character of the solid phase and reversible or irreversible responses is very complex. The degree of deviation from steady state in the experiment, which is assumed to be at hand in the theoretical consideration for the method, is strongly dependent on the kinetics of the process and the amount of material that is investigated.

4.5. Reversing and reversible

Some attention should be given to the different meaning of reversing and reversible. Reversing signal means in this technique, that the sample temperature follows the modulated temperature without any phase lag. The thermodynamic definition of a reversible process at a certain temperature is, that it is a finely balanced change, the system being in equilibrium with its surroundings at every stage.

In the original equation for heat flow rate (1) $f(t,T)$ is the non-reversing component assuming a steady state condition. Steady state means that the time for the kinetic event considered is almost zero and that the modulation does not influence the event. However this is not always true for a melting or crystallization. The phase lag between the modulated temperature in the heat block and in the sample is zero if a thermal event in the sample is fast and the amount of material is extremely small. In such a case a contribution to the reversing signal is obtained. If there is a phase-lag between the modulated temperature in the block and in the sample there is a contribution to the non-reversing signal. It means that in the function $f(t,T)$ a contribution from the sinusoidal function might be involved. This situation can occur for example if the amount of melting material is rather large.

If the melting is a *slow process*, the temperature of the material follows the modulation of temperature in the block in the melting interval. Reversible processes with no phase lag can thus be observed in that temperature interval. The weight given to the modulation, the degree of oscillation, in the heat flow might then be significant for the size of the c_{p} component. It might well also reflect the sinusoidal function in the kinetic component.

If the melting is a *fast process* in a very narrow interval only few modulations are performed during the event. The effect of modulation on the kinetic component expressed by $C\sin(\omega t)$ is almost zero. The system is then close to steady state; $f'(t,T)$ being a constant. Is there in this case a correlation between reversible processes and ΔH_c-values?

If a heat-*only scan* is performed the effect of modulation on the system is different from the effect of a *heating–cooling scan*. For a phase transition like melting in a material with a distinct melting temperature, a temperature modulation over the melting temperature (interval) means that melting and crystallization follows the modulation if the process is fast enough and if the amount of material is at minimum. However it is not possible to modulate over one distinct melting temperature. If a *heat-only scan* is used the reversing signal cannot be a measure of amount of reversible process. If however a *heating–cooling scan* with several modulations over the certain temperature is performed, the reversing signal or ΔH_c should be correlated to a reversible process also when the system has a distinct melting temperature. The combination of a certain level of degree of oscillation to express the weight of cyclic component in the heatflow together with a requirement of a number of modulations over the transformation temperature may be a good guide of selection in this type of systems. Reading et al. [3] recommended 5–6 modulations during a thermal event, when heat-only scans were considered. In this study we have used 3–5 modulations over one certain temperature in heating–cooling scans.

It must be emphasized that deviations from steady state means lag differences in heat flow phase. Lag differences give non-reversing signal. Thus, if a system is at equilibrium and is melting in a narrow melting interval, the temperature will not follow the modulation as long as there are two phases still left. Especially if *heat-only* scans are used, the amount of material will be decisive for the appearance and size of the non reversing signal.

5. Conclusions

The effect of different selections of operational parameters using MTDSC (ODSC) to investigate thermal events in aqueous systems is greatly influenced by the comparably fast processes in the system. The melting process might for certain operational settings be considered as linear, while the non-equilibrium crystallization is deviating from linearity.

Reproducible results of heats of transformation are obtained for a wide range of degrees of oscillation. The selection of heating rate, amplitude and frequency largely influences the shape of the c_p component curve as well as the amount of heat expressed by the c_p component of a thermal event. The conventional ΔH values are not influenced to the same extent. The selection of heating rate and frequency both influence the transformation interval. The value of the ratio $\Delta H_c/\Delta H$ for the melting process is greatly varying with the degree of oscillation. The ratio might for certain combinations of variables be used as an expression for the relative crystallinity of frozen aqueous solutions. It is however important to select combinations, that produce a special type of scans in this case, heating–cooling scans.

If an amorphous phase with a rather broad melting interval is examined during the melting process the modulations for sample and heating block can be parallel following the variation in c_p of the system. In other words, the reversible process then follows the reversing temperature modulation. A *heat-only scan* can as well as *heating–cooling scans* then give enough modulations over the transformation interval to obtain significant values of enthalpies and crystallinity.

If the melting process is very fast and thus occur at very distinct temperatures like in aqueous systems, the time when the modulation and sample temperatures are out of phase is short, if the amount of material is at minimum. To investigate the extent of reversibility in such processes, it is required, that a modulation is passing the actual transformation temperature a number of times. Thus, in aqueous systems the selection of variables must produce *heating–cooling scans* to obtain reproducible quantitative results on crystallinity. An appropriate degree of oscillation in combination with a number of modulations over a distinct transformation temperature must be selected.

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