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MDSC parameter optimization for the determination of glass transitions using a Design of Experiments approach

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ABSTRACT

Optimum experimental parameters for modulated differential scanning calorimetry were determined using a Design of Experiments approach. Two different strategies were tested, an isothermal method only varying modulation period and amplitude and a non-isothermal method including an underlying linear heating ramp. Three different test compounds were investigated including a blank of NaCl, a small pharmaceutically active molecule (Valsartan) and a polymer (PVP-VA 64).

Obtained optimum values were found to be identical for each compound tested and shown to be statistically robust for repeated daily experimentation. Optimized signals showed lower glass transition values T_g paired with higher changes in isobaric heat capacity Δc_p. Measured glass transition signals exhibited minimal variation during repeat analysis, and showed significantly better signal-to-noise ratios in comparison to literature data.

1. Introduction

Glass transitions are an important feature of amorphous systems (Wunderlich, 2005; Hutchinson, 2003); however, their determination by thermal analysis is fraught with difficulties. Not only are the transitions weak in comparison to melting events but also their appearance can be significantly influenced by the chosen experimental parameters (Royall et al., 1998; Reading and Hourston, 2006; Hill et al., 1999). Modulated DSC (MDSC) provides a tool to separate reversing from non-reversing events that allows determining glass transitions easier. MDSC provides the user with three adjustable parameters, heating rate *HR*, modulation period *P* and modulation amplitude *A* to optimize their signal (Gill et al., 1993; Reading et al., 1993). Depending which values are chosen, different results for the glass transitions parameters, i.e., temperature T_g and heat capacity change Δc_p are obtained. In addition, the signal can be overlaid with modulation artifacts producing a sine wave pattern in the signal (Thomas, 2006). This can lead to substantially wrong interpretations of the glass transition or in extreme cases to a loss of signal whereby the actual glass transition is no longer visible.

In the literature, several authors have tackled this problem for substances, ranging from small molecules to polymers (McPhillips et al., 1999; Six et al., 2001; Hill et al., 1998). In each case, optimization was apparently performed using a qualitative approach. The modulation parameters were varied until a signal was obtained that either looked pleasing or was defined as the best within the chosen parameter space. Although in all cases the individually 'best' signal was chosen, no attempts were made to mathematically optimize the response to the modulation parameters. Further, all literature 'optima' varied considerably between the compounds studied.

However, the MDSC principle lends itself to an optimization procedure using a Design of Experiment approach, free of qualitative assumptions with regard to glass transition temperature or heat capacity values. In addition, a robust optimum for each compound tested will be obtained, i.e. the result can be reproduced within a 95% confidence interval on a daily basis during routine operations. Furthermore, here we demonstrate that the Design of Experiments approach leads to a single unique parameter optimum for every compound under investigation, in stark contrast to available literature evidence.

2. Materials and methods

2.1. Materials

Sodium chloride was purchased from *Panreac* (Spain), Valsartan was supplied by *Hangzhou Sinolite Industrial* (China), PVP-VA 64 was obtained from *Aldrich* (Germany), HPMC 603 was a generous gift of *Shin Etsu* (Japan), Lactose (as monohydrate), was purchased from *DMV Fonterra* (Germany) and Itraconazole was supplied by

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Sparchem (India). Sapphire (TA-Instruments) and Indium (99.999%, TA-Instruments) were used for instrument calibration. Dry nitrogen (99.999%, Carburos Metálicos) was used as purge gas.

2.2. Methods

All MDSC experiments were performed using TA-Instruments *Q200* Modulated DSC equipped with a refrigerated cooling system *(RSC 40)* and autosampler. Dry nitrogen was used at a flow rate of 50 ml min^{-1} for the DSC cell and at 150 ml min^{-1} for the RCS40 unit. Prior to the study, the furnace was cleaned via an internal pyrolysis routine and recalibrated using sapphire standards and indium according to the manufacturer's instructions.

TA-Instruments *Tzero aluminium hermetic* pans with a self-made pin–hole were used throughout the study. The mass of each empty sample pan was matched to the mass of the empty reference pan to ±0.01 mg for optimization experiments. No pan matching was carried out for the comparison experiments and for the repeatability study. Sample weight was between 2.00 and 7.00 mg for all compounds but NaCl, which weighed between 12.00 and 14.00 mg.

All studies consisted of a preliminary conditioning of the filled pan, which were heated to 40 K above the melting point (for crystalline material) or the T_g (for glassy material) and cooling to 40 K below the T_g, all at 20 K min^{-1}. In isothermal studies, crucibles were reheated to their T_g and modulated isothermally for 30 min, changing amplitude A and period P for each run according to the DoE. Amplitudes and periods varied between 0.01–1.50 K and 40–100 s, respectively. Isothermal temperatures for the three compounds were chosen as the inflection point of the actual glass transition of the compound. For NaCl the temperature was chosen as the arithmetic mean (95 °C) between the glass transition temperatures of Valsartan (80 °C) and PVP-VA (110 °C). Experiments using a linear heating rate ramp were performed similarly but with the addition of heating rates *HR* tested in a very broad range, from 0.1 to 5.0 K min^{-1}. The temperature range for modulation was set from 40 K below to 40 K above the T_g of the compound.

2.3. Modulated DSC

Like conventional DSC, MDSC measures differences in heat flow between sample and reference pan as a function of time by applying a temperature profile. In DSC, this is a simple linear ramp, whereas the innovation in MDSC (Gill et al., 1993; Reading and Hourston, 2006) lies in the introduction of a sine wave of constant amplitude A and period P that modulates the linear temperature profile, as described by Eq. (1):

$$T_{\mathrm{mod}} = T_0 + \frac{\partial T}{\partial t}t + A\ \sin\left(\frac{2\pi}{P}t\right) \tag{1}$$

Thus, the sample is exposed to an oscillating variation of the temperature in time, i.e., to a modulated heating rate as expressed in Eq. (2):

$$HR_{\mathrm{mod}} = \frac{\partial T_{\mathrm{mod}}}{\partial t} = \frac{\partial T}{\partial t} + A\frac{2\pi}{P}\ \cos\left(\frac{2\pi}{P}t\right) \tag{2}$$

In this way, resolution and sensitivity are improved simultaneously. The sinusoidal regime causes larger instantaneous variations of the modulated temperature (larger instantaneous heating rates) providing higher sensitivity to the detection of subtle events, while lower heating rates can be chosen for the linear term, leading to improved resolution of the thermal transitions recorded.

The response to the modulation, i.e., the heat flow from calorimeter to sample is described according to the definition of the isobaric heat capacity, by Eq. (3).

$$H = \frac{\partial Q}{\partial t} = c_p\frac{\partial T_{\mathrm{mod}}}{\partial t} = c_p\frac{\partial T}{\partial t} + c_pA\frac{2\pi}{P}\ \cos\left(\frac{2\pi}{P}t\right) \tag{3}$$

Averaging the modulated heat flow H over one modulation period results in a non-modulated signal (the total signal), which is equivalent to that obtained by conventional DSC. Further deconvolution procedures allow separating the contribution of processes driven by changes in c_p (reversing signal) from that caused by kinetic events (non-reversing signal). In this way, complex transitions can be unraveled and their correct assignment is facilitated. In addition, MDSC allows for accurate heat capacity determination.

An appropriate choice of the modulation parameters A, P and linear *HR* is decisive for a successful modulation experiment and has been extensively studied (Reading and Hourston, 2006). It has been suggested that the parameters are sample dependent and some guidelines for an adequate selection are provided by the developers of the technique (TA Instruments). At least five modulation cycles should occur within a given transition to ensure a good approximation in the signal averaging process and further deconvolution operations. Yet more, the periods should be long enough to optimize heat transfer between the different parts of the experimental set-up. Periods between 40 and 100 s are recommended. Once the period is defined, a suitable heating rate enabling five cycles per transition has to be chosen. The amplitude has to be a compromise between good signal to noise ratio (not too small amplitudes) and a linear response to the heating ramp (not too large amplitudes).

Adequate parameter selections are those that result in a heating profile that the sample can 'follow'. Objective criteria for evaluating a successful modulation experiment are given. First, the profiles of the derivative of the modulated temperature with time $\partial T_{\mathrm{mod}}/\partial t$ and the modulated heat flow H are to be perfectly sinusoidal, i.e., the measured curves follow a standard sine function as shown in Eq. (4)

$$y = a + b\ \sin\left(\frac{2\pi}{c}x + d\right) \tag{4}$$

Second, rapid reaction of the sample to the modulation, i.e., as small phase lag as possible between the stimulus (modulated temperature T_{mod}) and the response (modulated heat flow H). This can be assessed through the corresponding Lissajous figures. Lissajous figures are the result of the orthogonal superposition of two different sinusoidal waves, here H and $\partial T_{\mathrm{mod}}/\partial t$ (French, 1971). The shape of the figures depends on the frequency and phase shift between the two waves. Assuming they exhibit the same frequency, in an ideal case of waves in phase the superposition will give a straight line. Further, in a perfect system in steady state each modulation cycle will be identical to the previous one, leading to a complete coincidence of the Lissajous figures of different cycles. In non-ideal cases, phase shift will result in an ellipse as figure, whereby the length of its minor axis is an indicator of the shift. Non-perfect sinusoidal heat flow will lead to curves with deviations from ellipticity and non-coincidence of the modulation cycles will be apparent as deviation from a mean in the ellipses. The evaluation of the deviations described in the literature is mostly qualitative (McPhillips et al., 1999; Six et al., 2001; Hill et al., 1998). Quantitative analysis of the deviations has been described via elaborate approaches as determining the fractal dimension of the ellipses along the transition (Kawakami and Ida, 2005).

2.4. Design of Experiments and data analysis

Design of Experiments (DoE) is an efficient tool to evaluate the significant influencing factors of an experiment to a single or multiple responses (Montgomery, 2000; Box et al., 1978). In the actual optimization of an MDSC signal, the influencing factors are already known (*HR*, *P*, *A*), however it is uncertain if there are significant interactions between the factors, which could lead to non-linear trends in the responses. The designs used in this work represent response surface models that are capable of describing the changes

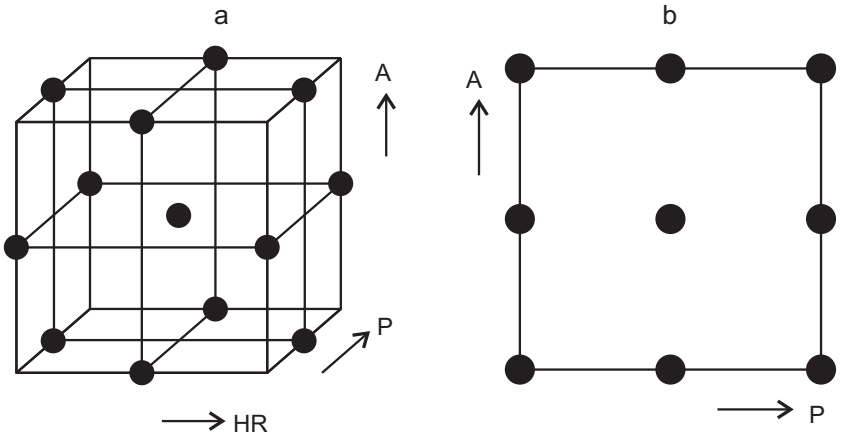

Fig. 1. Design space representation for (a) Box–Behnken and (b) face-centered central composite designs.

of a response using a full quadratic polynomial (Box and Behnken, 1960; Box and Draper, 1987). For three factors x, y and z, i.e., non-isothermal designs (*HR*, *P*, *A*), this results in a polynomial with ten coefficients as shown in Eq. (5).

$$\text{response} = ax + by + cz + dxy + exz + fyz + gx^2 + hy^2 + iz^2 + j \quad (5)$$

whereby *a–c* describe the linear trends in each factor, *d–f* interactions between factors and *g–i* the response surface curvature. In addition, *j* represents the constant term independent of any factor. Isothermal designs, which contain only two factors (*P*, *A*), consequently use a polynomial with only six coefficients, where the terms containing the coefficients *c*, *e*, *f* and *i* are absent.

For the non-isothermal designs a *Box–Behnken* design BB was employed. A BB contains twelve design points at three factor levels with three additional center point repeats (Fig. 1a). A BB was chosen over a three dimensional central composite design to avoid 'extreme' factor combinations leading to data loss due to unusable response values. For the isothermal designs used in this study, a face-centered central composite design CCF was used. A CCF consists of eight design points at three factor levels with three additional center point repeats as shown in Fig. 1b.

Three responses describing the modulation signals were used. In the case of ideal modulation, H and $\partial T_{\text{mod}}/\partial t$ follow a perfect sinusoidal pattern. Hence, the standard error of the regression S_{yx} of a simple sinusoidal wave to H and $\partial T_{\text{mod}}/\partial t$ was used ($S_{yx}(H)$ and $S_{yx}(dT)$). In addition, in an ideal state, every Lissajous curve coincides with each other. Therefore, noise was expressed as standard deviation of the mean heat flow of the Lissajous curve σ_y at the center point of the corresponding ellipse. The three chosen responses are scale dependent. Consequently, they were used as a relative response, whereby the regression errors were scaled by the amplitude of the sine waves and the noise by the ellipse height at its center point.

It should be stressed that the chosen responses solely evaluate the modulation procedure itself, i.e., the sinusoidality of the modulated heating profile and accuracy of heat flow into the sample. Although a 'gold standard' value for Δc_p could be calculated using statistical thermodynamics (Wunderlich, 2005) it is questionable how accurate such a value could be calculated and was therefore dismissed as response to be optimized. Further parameters associated to the phase transition, such as the glass transition temperature or transition width, were also avoided as responses. However, it can be hypothesized that the optimum conditions should lead to as low as possible value for T_g and a as high as possible value for Δc_p, as expected from thermodynamics for an ideal modulation.

Initially, the design space was chosen to be as large as possible, using the manufacturer's recommended maxima and minima for each modulation parameter as factor levels. The limits of the design were deliberately taken broadly to allow for a large variation in response values. DoEs were analyzed using commercially available DoE software. For each design, the main effects and possible interactions between the factors were evaluated. The 'optimum' factor set for each design was determined by minimizing the three individual responses, whereby each response was given equal weight. The optimum solution was obtained using a built-in routine. The optimum was used to create a further smaller DoE design space to fine tune the initial optimum response. This process was repeated until a global optimum response was found.

In addition, for the smallest design space 'robust' optima were calculated to obtain the best values for day-to-day operation that are least influenced by random factor fluctuations. Robustness was checked by repeating the same experiment three times with three different pans. Weights from different pans were not matched; likewise, pans were not matched to the reference crucible to mimic standard daily operations. Results from repetitions were again analyzed with commercially available statistical software.

The obtained parameter set from non-isothermal experiments were used to compare glass transition signals of compounds which previously had been subjected to an 'optimization routine' by other researchers. Transition temperature (T_g), signal width (ΔT_{o-e}) and heat capacity (Δc_p) value were taken as parameters for comparisons.

3. Results and discussion

3.1. Isothermal modulation

Three different compounds were chosen as test substances for the DoEs: NaCl to provide a blank, Valsartan as low molecular weight compound and PVP-VA 64 as polymer. NaCl is a crystalline compound with no phase transitions in the temperature range of the present study and Valsartan and PVP-VA 64 are amorphous compounds exhibiting steep and relatively narrow glass transitions. Their corresponding strength parameters D estimated by thermal methods from the glass transition width (Hancock et al., 1998, Crowley and Zografi, 2001) indicated for both materials a fragile behaviour ($D < 10$).

A two dimensional face-centered central composite design (CCF) was chosen for the experiment. The design spaces for the DoE iterations performed (#1, #2 and #3) are shown in Table 1. Each design consisted of eight experiments plus three additional center point repeats. *P* and *A* were set as DoE factors using $S_{yx}(H)$,

Table 1
Experimental parameter range for Isothermal and non-isothermal designs.

DoE	HR (°C min^{-1})	A (°C)	P (s)
Isothermal designs			
#1	–	0.01–1.50	40–100
#2	–	0.60–1.50	40–100
#3	–	1.00–1.50	55–100
Non-isothermal designs			
#4	0.2–5.0	0.01–1.50	40–100
#5	2.0–5.0	0.60–1.50	50–100
#6	1.6–2.4	1.00–1.50	60–90
#7	1.3–1.9	1.00–1.50	66–100

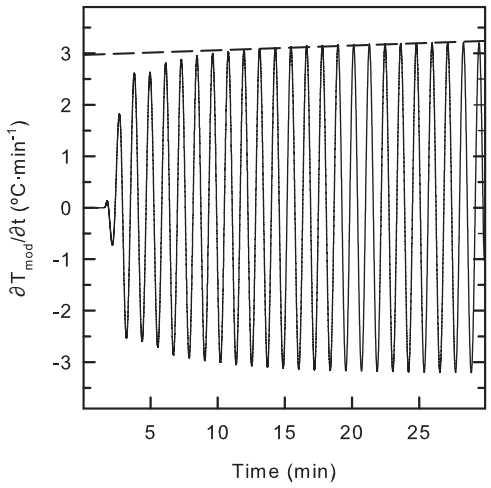

Fig. 2. Profile of the derivative of the modulated temperature with time showing slow equilibration and residual drift (as indicated by the dashed line).

$S_{yx}(dT)$ and σ_y as optimizable responses with the minimization of all three as desired outcome.

After equilibration of the system at T_g, modulation was applied for 30 min. It was observed that after onset of the modulation, a minimum of seven modulation cycles was necessary to obtain a stable signal (see Fig. 2). A stable signal was defined as having an amplitude variation of less than 2%. Even after this initial period, sometimes a slight drift was visible (dashed line in Fig. 2), however was assumed to be due to the choice of modulation conditions or system inefficiencies. Only the last five modulation cycles were used for analysis to ensure that the subsequent analysis was not influenced by non-equilibrium of the modulation conditions.

The largest parameter space (DoE #1) (see Table 1) was tested only with NaCl. Several Lissajous figures representing selected modulation conditions are shown in Fig. 3a and b. At very low modulation amplitudes (0.01 K, Fig. 3a) very noisy signals were obtained. Individual cycles did not overlap and large deviations from a perfect ellipse were visible. Large amplitudes (1.50 K, Fig. 3b) showed a far smoother signal with near perfect elliptical shape, however sometimes deviations from ellipticity were visible as 'kinks'; a sign that the system could not follow the modulation as desired.

No suitable mathematical model could be fitted to the large design using $S_{yx}(H)$, $S_{yx}(dT)$ and σ_y due to excessive variations within the data. Therefore, another DoE with a smaller parameter range was executed. A visual analysis of the Lissajous figures of DoE #1 suggested that a reduction of the modulation amplitude parameter range (from 0.01–1.50 K to 0.60–1.50 K) would likely yield a response variation which could be modeled successfully.

In this second design (DoE #2), the parameter space was curtailed as shown in Table 1, with a 40% variation between parameter limits. With this smaller parameter range, a quadratic model could be successfully fitted for each test compound. $S_{yx}(H)$, $S_{yx}(dT)$ and σ_y percentage values ranged from 0% to 2%, from 1.1% to 3.0% and from 0% to 0.7%, respectively. The global optimum was calculated by simultaneously minimizing the three responses and was predicted to lie at amplitudes between 1.10 and 1.50 K and periods between 70 and 100 s depending on the compound. Although the initial optimum values for the three test systems appeared not to be identical, their closeness suggested that the final optimum might be identical for the three compounds tested.

Another, even smaller design series was executed (DoE #3), whereby only a 20% variation between maximum and minimum in the DoE parameters was chosen. For the third design, only one common design space was defined for the three compounds (see Table 1), to not exceed instrument upper limits for A and P (1.50 K and 100 s).

$S_{yx}(H)$, and $S_{yx}(dT)$ values resulted in even narrower margins than before. The responses were analyzed and optimum modulation parameters for the three test compounds were found to lie between amplitudes of 1.1 and 1.5 K and periods of 85–100 s. The

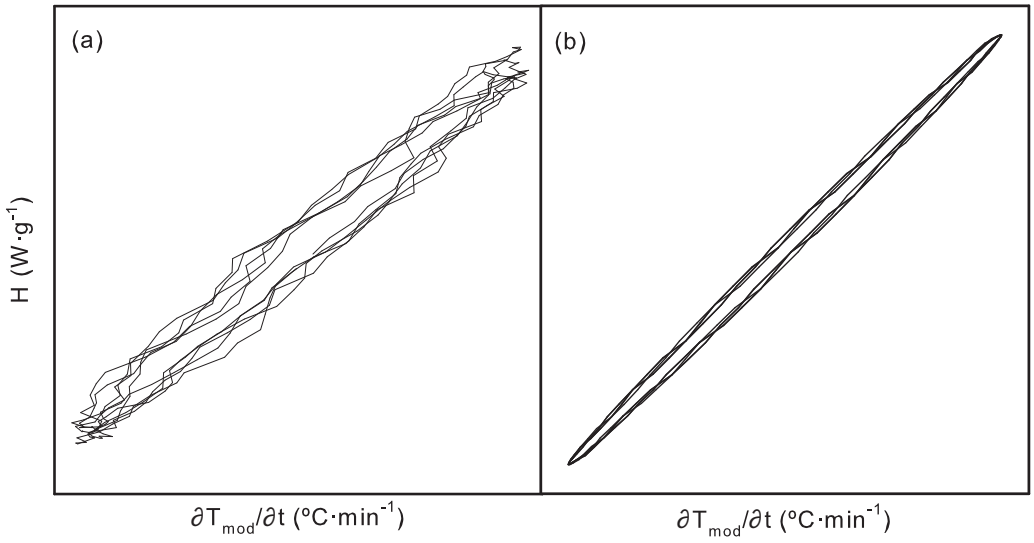

Fig. 3. Representative Lissajous figures (a) for small modulation amplitudes and (b) for large modulation amplitudes.

most important factor of the design was determined to be the modulated period P and the strongest interactions were P^2 and AP.

Identical optima were predicted for Valsartan and PVP-VA 64 (A, 1.50 K; P, 100 s); but the NaCl optimum was slightly different with an amplitude of 1.1 K and a period of 85 s. However, results for the NaCl design showed a relatively flat slope (4%) of the amplitude response surface between 1.10 and 1.50 K. Therefore, it was hypothesized that all three optima were identical within the error of the calculations. Conclusively, the optimum was set as the mean of the three values: $A = 1.40$ K and $P = 95$ s.

It should be noted that a solution found by applying a minimization routine might yield an extreme point in the attainable parameter range and might additionally be sensitive to small changes in the factors. For day-to-day measurements, it is more advantageous to find the most robust factor set giving the least sensitivity to random variations. The robust factor set can be obtained by calculating where the curvature of the individual response surfaces is minimal.

The above procedure was carried out for the response surfaces of $S_{yx}(H)$, $S_{yx}(dT)$ and σ_y, using A and P as noise factors, yielding a single robust optimum of $A = 1.30$ K and $P = 84$ s for all three test systems. From these results, it was concluded that a single robust optimum parameter set exists for all compounds under investigation. The parameters were optimized for fragile glasses with a strong temperature dependence of the molecular motions along the glass transition (Hancock et al., 1998). However, it can also be envisaged that strong glasses with weak temperature dependence might also be able to follow the optimized modulation parameters.

It is possible that the mathematical optimum is identical to the calculated robust optimum. Several experiments were performed using Valsartan to compare both optima responses. Pans were not matched to the reference pan and neither their powder fill mass to mimic day-to-day operations.

One average error was calculated for each response: $S_{yx}(dT) = 1.228 \pm 0.006\%$, $S_{yx}(H) = 1.8 \pm 0.3\%$ and $\sigma_y = 0.4 \pm 0.1\%$ for the true optimum and $S_{yx}(dT) = 1.39 \pm 0.07\%$, $S_{yx}(H) = 1.6 \pm 0.2\%$ and $\sigma_y = 0.6 \pm 0.3\%$ for the robust optimum. A general response error σ_T was calculated as the root of the sum of squares (Eq. (6)) to compare $S_{yx}(dT)$, $S_{yx}(H)$ and σ_y from both optima.

$$\sigma_T = \sqrt{\sigma_i^2 + \sigma_j^2 + \ldots + \sigma_n^2} \quad (6)$$

For both optima an identical value of $\sigma_T = 2.2\%$ was found. In conclusion, the true or the robust optimum can be used for day-to-day performances due to their individual excellent repeatability.

The Lissajous figure using the optimum parameter was expected to represent a near-perfect ellipse. However, as can be seen in Fig. 4, a small kink is apparent near the extremes. Comparison with other Lissajous figures using non-optimal factors showed that all ellipses presented a similar degree of 'imperfection'. It was therefore concluded that the observed residual deviation from ideality was likely due to system deficiencies (overheating) instead of an error in the optimization.

3.2. Non-isothermal modulation

Under isothermal conditions, no actual glass transition signal can be measured. A non-zero heating rate must be added to scan across any possible transition. The isothermal measurements showed that extreme values of the modulated factors created 'uninterpretable' signals. To avoid extremes, a Box–Behnken (BB) response surface design was used for the non-isothermal modulation experiments. HR, P and A were set as DoE factors, using $S_{yx}(H)$, $S_{yx}(dT)$ and σ_y as optimizable responses. Similarly to the isothermal experiments, only the last five modulation cycles of the experiment were used for analysis.

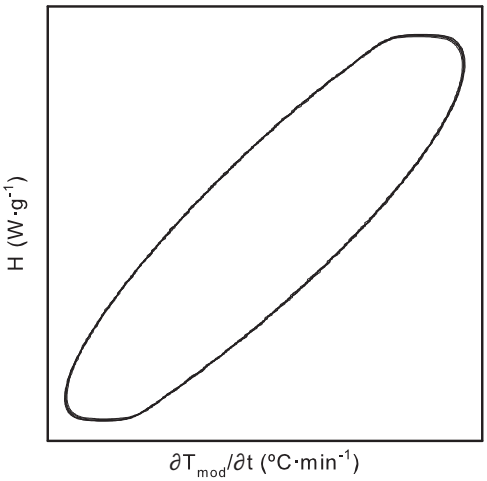

Fig. 4. Lissajous figure at optimum modulation conditions according to the DoE for isothermal experiments.

A similar initial design space (DoE #4) as in the isothermal experiments was used with the addition of a deliberately wide range of heating rates (Table 1). The widest DoE was only executed for NaCl. After equilibration of the system at 80 °C, modulation was performed in a temperature interval from 80 to 160 °C. No model could be adequately fitted for the largest range of modulation parameters. Very noisy Lissajous figures could be discerned at small heating rates, while very large heating rates tended to produce kinks in the signals similarly to the isothermal experiments. Therefore, HR was limited to values of 2.0–5.0 K min^{-1} and A was limited to values above 0.60 K.

A second smaller design (DoE #5) was executed using the parameter space shown in Table 1 with NaCl, Valsartan and PVP-VA. Data could be adequately fitted using a regression model. The global optimum was calculated minimizing the three responses and was predicted to lie at amplitudes between $A = 1.10$ and 1.50 K, periods of $P = 75$–90 s and heating rates of $HR = 2.0$ K min^{-1} depending on the compound. The most important factor in the design was found to be the modulated period P. Surprisingly, no significant interactions between factors were found.

A third design (DoE #6) was executed for the three test compounds (Table 1). Only one common design space was defined for the three compounds not to exceed instrument upper limits for A and P (1.50 K and 100 s). Modulated optimum amplitudes lay between $A = 1.10$ and 1.40 K and periods between $P = 85$ and 100 s. The optimum value for HR was predicted to be $HR = 1.6$ K min^{-1}, which coincided with the minimum of the design space. However, it is not desirable to have one factor optimum value in a corner of the design; therefore, another DoE even smaller (DoE #7) was performed.

A last DoE (#7), using Valsartan was executed (Table 1). The optimization gave a single optimum at $A = 1.50$ K, $P = 96$ s and a $HR = 1.3$ K min^{-1}. Again, HR turned out to be in the minimum value of the design space while A and P had similar values as in DoE #6.

As amplitude and period were unchanged from the previous design, further experiments with constant values for A and P with selected smaller values for HR (1.0, 0.7, 0.4 and 0.1 K min^{-1}) were carried out to find the optimum HR more quickly. Results for these experiments are shown in Fig. 5a. $S_{yx}(dT)$ presented a shallow minimum between 0.6 and 1.6 K min^{-1}. $S_{yx}(H)$ (Fig. 5a) presented two zones which could be fitted with independent linear regression models.

A statistical analysis, showed that values between $HR = 0$ and 0.7 K min^{-1} were identical and any value between those limits could be used to give the same result. Moreover, at HR above

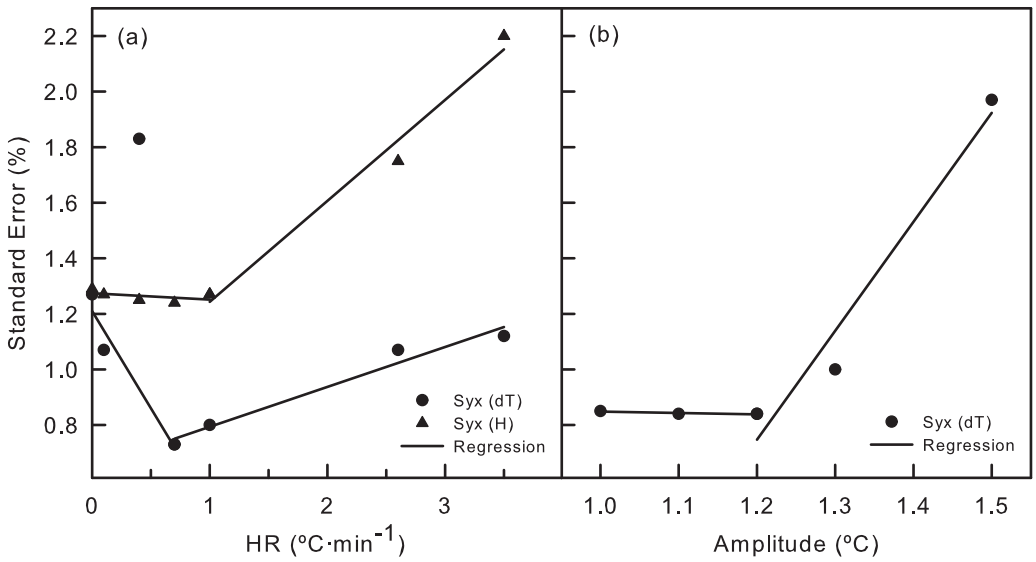

Fig. 5. Standard regression error changes as function of *HR* and *A* for the optimization of non-isothermal modulation conditions. Lines were obtained using piece-wise linear regression of the data points.

0.7 K min^{-1} values for $S_{yx}(H)$ and $S_{yx}(dT)$ were increasing. Therefore, the optimum *HR* was fixed at 0.7 K min^{-1}. The low *HR* obtained as optimum is in agreement with the expectations, as it easily allows the recommended number of modulations along the transition.

For day-to-day measurements, it would be preferable to have short experiment times without the need for the perfect signal. The maximum value of *HR* which could be used without significantly changing the quality of the signal was found to be 1.0 K min^{-1}. This value coincided with the intersection between the two linear regressions mentioned before for $S_{yx}(H)$ in Fig. 5a. Different experiments comparing both *HR* (0.7 and 1.0 K min^{-1}) were studied to probe that the change in *HR* was not relevant. In conclusion, despite 0.7 K min^{-1} is the optimum, increasing *HR* to 1.0 K min^{-1} might be a good alternative to shorten the run time if only a general view is required.

A robustness test was executed and similar periods as in the isothermal experiments were found. A general optimum period was set at $P = 87$ s. Modulated amplitudes varied between $A = 1.20$ and 1.50 K. Due to the relatively small slope (8%) of the response surface of *A* it was speculated that amplitudes in that range would likely not influence the final result. Nevertheless, a single optimum amplitude value was determined using a set of experiments with constant *HR* and *P* and changing *A* (1.00, 1.10, 1.20, 1.30 and 1.50 K).

Fig. 5b shows the data for this set of experiments. $S_{yx}(dT)$ strongly increased from 1.20 K onwards. Amplitudes from $A = 1.00$ to 1.20 K were shown to be statistically the same, therefore, the amplitude mean of $A = 1.10$ K was defined as the optimum. Conclusively, a common optimum was fixed for the three compounds tested: $HR = 0.7$ K min^{-1}, $A = 1.10$ K and $P = 87$ s. As can be seen the final optimum was almost identical to the one reached with the isothermal experiments: $A = 1.30$ K and $P = 84$ s.

3.3. Method validation

The optimum modulation parameters obtained from the DoE exercise were tested by measuring the glass transition of Valsartan and PVP-VA. Three different pans with three different powder masses between 3 and 8 mg were measured three times each, after a conditioning cycle. The T_g and the change in Δc_p were calculated using the system software. The width of the transition signal ΔT_{o-e} was calculated as the difference between the calculated onset and end of the glass transition as determined by the system software. The temperature interval for the signal analysis was 60–95 °C for Valsartan and 100–125 °C for PVP-VA.

Fig. 6a and b shows all repetitions for Valsartan and PVP-VA. Standard deviations of all repetitions were calculated for T_g, Δc_p, and ΔT_{o-e}. Table 2 shows the results obtained for each compound.

Excellent reproducibility could be observed for both compounds with only minor deviations in the extremes. Baseline noise is not discernible. Using an ANOVA test, the differences between pans were shown to be not significant indicating that the observed variation in T_g and Δc_p is almost exclusively due to the deliberate variations in powder fill mass. In conclusion, the calculated optimum modulation parameters result in a superior signal with minimal variation during individual analyses.

3.4. Literature comparison

For HPMC, Itraconazole and Lactose a set of optimum modulation parameter values was available in the literature for comparison with the optimum factor set proposed in this study (McPhillips et al., 1999; Six et al., 2001; Hill et al., 1998). Each compound was analyzed according to their published optimum parameter set and with the optimum parameter set from our DoE analysis. Experimental conditions for the three compounds are specified in Table 3. In the case of HPMC, the literature example uses a slightly different grade of HPMC (E4M) to this study (Pharmacoat 603). However, it was assumed that the literature method would have yielded identical parameters had the authors tested the HMPC grade used in this study.

Fig. 7a–c shows the results for the three compounds. The average T_g, Δc_p and ΔT_{o-e} were determined for HPMC and Itraconazole (Fig. 7a and b) for a set of four repetitions using a single pan. The temperature range for the analyses was 80–170 °C for HPMC and

Table 2
Results for repeated glass transition measurements using the robust design optimum.

	Valsartan		PVP-VA	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)
T_g (°C)	78.0 ± 0.4	0.5	113.1 ± 0.5	0.4
Δc_p (J g^{-1} K^{-1})	0.43 ± 0.01	1.8	0.25 ± 0.01	2.7
ΔT_{o-e} (K)	8.3 ± 0.2	1.9	9.5 ± 0.4	3.8

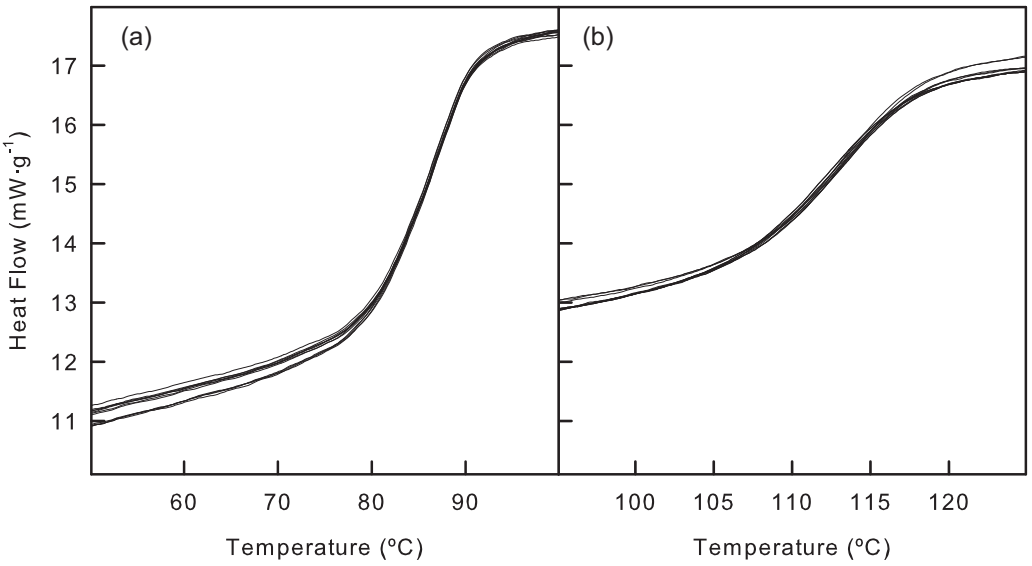

Fig. 6. Repetitions of individual glass transitions measurements under optimum conditions according to the DoE for (a) Valsartan and (b) PVP-VA 64. Signal groups were shifted vertically to coincide in heat flow at the glass transition.

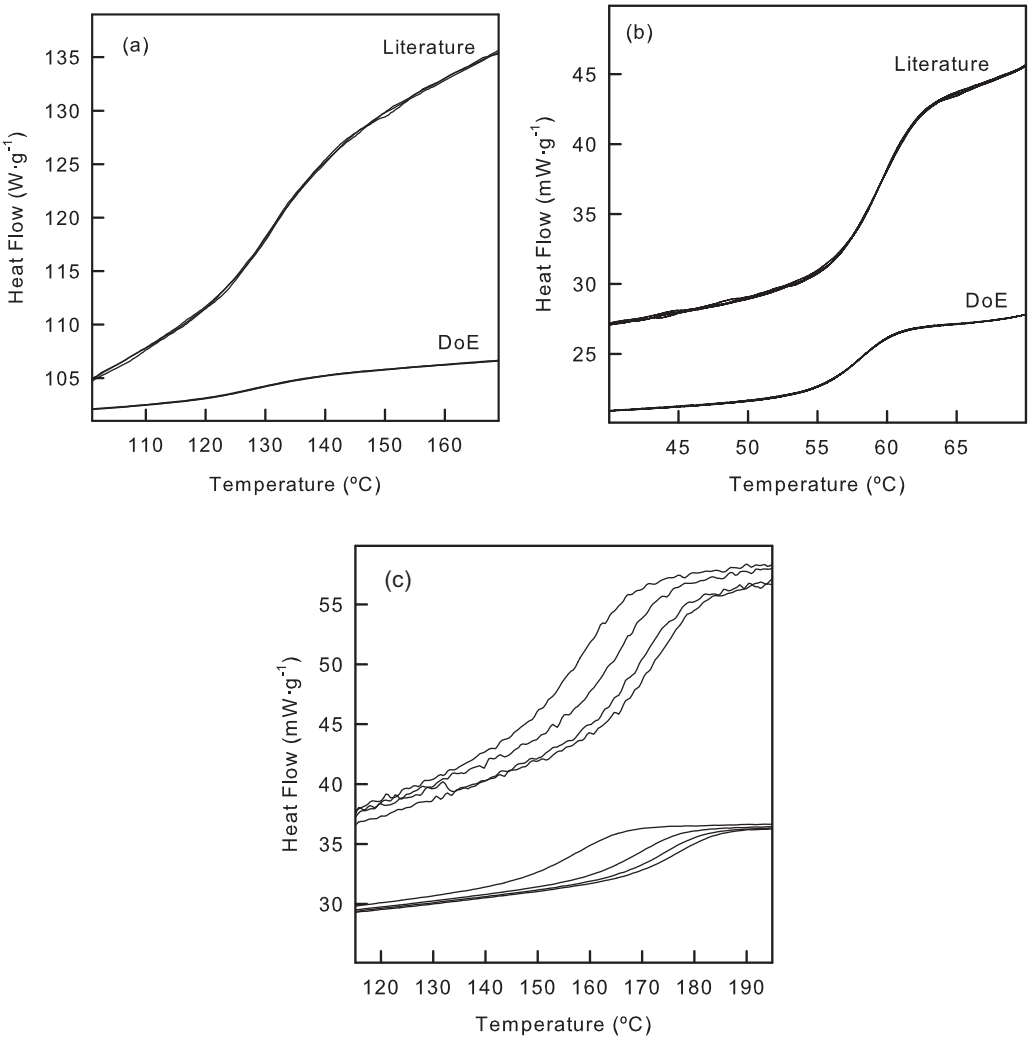

Fig. 7. Literature comparison of glass transition measurements for (a) HPMC 603, (b) Valsartan and (c) Lactose monohydrate.

Table 3
Experimental parameters for the comparison of literature data with the DoE optimum.

	HPMC	Itraconazole	Lactose
Literature optimum			
HR (°C min^{-1})	5.0	2.0	2.0
A (°C)	0.50	0.212	0.16
P (s)	40	40	30
DoE optimum			
HR (°C min^{-1})		0.7	
A (°C)		1.10	
P (s)		87	

40–65 °C for Itraconazole. In addition, the signal-to-noise ratio was taken as a fourth parameter in the comparisons. Table 4 shows the data for both compounds. As can be seen, Δc_p values measured were larger in the DoE optimum conditions than in the literature: 21% for HPMC and 9% for Itraconazole. T_g values were found to be lower in the DoE optimum: 3% for HPMC and 2% for Itraconazole.

From theoretical considerations, it can be hypothesized that if the system behaves ideally during the glass transition and the time scale of the experiment is appropriate, then the change in Δc_p should reach a maximum, reflecting the largest possible input of energy necessary to reach the disordered liquid from the glassy state. Likewise, the measured T_g should be as low as possible.

It can be observed that the optimum conditions have lead to lower T_g and larger Δc_p values in comparison to non-optimized literature data. However, the ΔT_{o-e} was larger using the DoE method parameters for HPMC (8%) but smaller for Itraconazole (5%).

In Table 4, it can also be seen that standard deviations for each glass transition parameter are about one order of magnitude smaller for T_g and Δc_p when using the optimal modulation parameters from this study. In conclusion, the reproducibility of the method is similar for all compounds independent of their molecular nature.

DoE optimum conditions also led to visibly smoother baselines of the signals. Signals were clearly defined in the thermograms despite the smaller step of the transition in the DoE optimum. The signal-to-noise ration was also improved. For HPMC a modest improvement of approximately 10% was seen, however for the small molecule Itraconazole an enhancement of almost 250% was observed.

The lactose was introduced as lactose monohydrate, but dehydrated during the conditioning run in the open pan (pin–hole). The T_g of Lactose was found to constantly shift during repeat analysis for both sets of modulation conditions (Fig. 7c), hence it was not practical to calculate the average and their errors for the three parameters. Therefore, T_g, Δc_p and ΔT_{o-e} were measured only for the first cycle. Nevertheless, the same trend as before was found with a lower T_g using the DoE conditions by 2%, a Δc_p bigger by 3%, paired with a slightly elevated signal width ΔT_{o-e} by 0.5%.

Table 4
Results for the comparison between literature and DoE data.

	HPMC	Itraconazole	Lactose[a]
DoE			
T_g (°C)	127.4 ± 0.9	58.08 ± 0.04	155.6
Δc_p (J g^{-1} K^{-1})	0.24 ± 0.01	0.417 ± 0.004	0.40
ΔT_{o-e} (K)	21.3 ± 0.4	5.7 ± 0.1	19.5
Literature			
T_g (°C)	131 ± 1	59.3 ± 0.4	158.9
Δc_p (J g^{-1} K^{-1})	0.20 ± 0.01	0.38 ± 0.01	0.39
ΔT_{o-e} (K)	19 ± 1	6.0 ± 0.3	19.4

[a] Only the first heating cycle was analyzed.

Moreover, the signal obtained using literature conditions was found to be much noisier than the one obtained using the DoE method. In conclusion, significant improvements using DoE optimum conditions in comparison to published literature procedures were visible.

4. Conclusions

This investigation into the MDSC optimization for the measurement of glass transitions has shown that a unique parameter set exists that is applicable to compounds ranging from small molecules to polymers even those considered as fragile with stronger dependence of the rate of molecular motions on the temperature change during the transition. The determined optimum values for the modulation parameters differed substantially to sets for individual compounds published previously by other authors. The optimum values for heating rate, amplitude and period allowed precise measurement of the glass transition temperature T_g and its accompanying change in isobaric heat capacity Δc_p. The optimum modulation parameters were also shown to be statistically robust for use in day-to-day measurements. Standard deviations of less than 3% for Δc_p and less than 1% for T_g were observed, which are a significant improvement over the coefficients of variation obtained using published literature optima. In comparison with available literature data, it was shown that the optimized parameter set gave consistently better results for a range of chemically different compounds. Baseline noise and signal-to-noise ratio was significantly improved. In summary, the Design of Experiments approach has been proven a useful tool for MDSC parameter optimization.

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