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Experimental design aids the development of a differential scanning calorimetry standard test procedure for pharmaceuticals

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Abstract

Differential scanning calorimetry (DSC) is one of the important thermoanalytical techniques for preformulation studies of pharmaceutical substances. DSC studies can be used to characterize the melting behavior, crystallization, solid–solid transitions and chemical reactions of drugs. The attainment of proper results in a DSC experiment is dependant on a number of factors. The purpose of this study was to establish the appropriate condition for DSC performance. Factorial design and matrix analysis was used extensively in this study. The variables considered in this study were the sample size, heating rates, atmosphere, crucible type and relative humidity (RH). Two model drugs, benzoic acid (typical melting range: 121–123 °C, heat of fusion: 147 J/g) and vanillin (typical melting temperature: 81–83 °C, heat of fusion: 135 J/g) with sample size ranges of 3–5 and 10–12 mg were exposed to heating rates of 2 and 10 °C/min with atmospheres of air and nitrogen. The crucible types evaluated were open crucibles, crimped crucibles without pinhole and crimped crucible with pinhole. The samples were stored under 0, 25, 60 and 100% RH to evaluate the effect of RH on DSC performance. The study in this laboratory established a general protocol for DSC performance, which included the use of a small sample size range (3–5 mg), a low heating rate (2 °C/min), nitrogen as the atmosphere, crimped crucible without pinhole, and storage of the samples in a dry atmosphere (0% RH). Exceptions to the established protocols under various conditions are also discussed.

Keywords: Differential scanning calorimetry; Drug preformulation; Pharmaceuticals; Benzoic acid; Vanillin; Experimental design; DSC standard test method

1. Introduction

Thermal analysis is a term used to describe the analytical techniques that measure the physical and chemical properties of a sample as a function of temperature or time [1,2]. This technique along with other complementary analytical techniques such as spectroscopy, and chromatographic methods is becoming increasingly important for the development of modern day pharmaceuticals [3–8]. A number of thermoanalytical techniques are in use, namely thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and thermal mechanical analysis (TMA), to name a few [3–5]. DSC is the most popular of these techniques due to its wider applicability in comparison to the other methods. In this technique, the difference in heat flow to the sample and the reference is monitored against time/temperature when exposed to a temperature program [2,6,7] DSC techniques provide information regarding the melting point/melting range, heat of fusion, purity, polymorphism, pseudo-polymorphism, glass transition, interaction/compatibility, thermal

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stability, kinetics of decomposition and other results useful for preformulation studies of pharmaceuticals and the subsequent development of a stable and effective dosage form. The performance of DSC is dependent on a number of factors. Some of the important factors to be considered are the sample size, the heating rate, the atmosphere, and crucible type amongst others. The present study employs a factorial design and matrix analysis in evaluating the effect of the above-mentioned factors. In addition, the effect of relative humidity (RH) upon storage of the samples was also evaluated. Benzoic acid, an IUPAC recommended calibration material, and vanillin, a commonly used pharmaceutical flavoring agent were the two replicate samples used in this study. The parameters measured were the melting temperature measured as the extrapolated onset temperature, T_m, the extrapolated peak temperature, T_p and the heat of fusion.

2. Materials and methods

2.1. Materials

Benzoic acid, USP was obtained from J.T. Baker Chemical (Lot No. 1210) and vanillin, USP obtained from Merck (Lot No. 6866). Sodium hydroxide was obtained from Fisher Scientific. The benzoic acid and vanillin samples were studied using a DSC-20 unit from Mettler-Toledo. The 40 μl aluminum crucibles were used for the DSC runs. The purge gases used were compressed nitrogen and air obtained from AGA Gas, Toledo, OH. All DSC curves were analyzed using STAR software (Version 6.10).

2.2. Methods

2.2.1. Sample size, atmosphere and heating rate

The first part of the study evaluated the effect of sample size, atmosphere and heating rate on DSC performance. The sample size ranges under study were 3–5 and 10–12 mg. The atmospheres for the study were air and nitrogen with heating rates or ramp, 2 and 10 °C/min. A 2^3 factorial design was developed for the experiments. The experimentation sequence was determined with the help of a random number table. The flow rate for the purge gas was kept at 50 ml/min 40 μl aluminum crucibles with lid having a pinhole were used in the experiments for this part of the study.

2.2.2. Crucible type

The effect of crucible type was evaluated. Using the optimum conditions developed in Section 2.2.1, DSC runs were performed on vanillin and benzoic acid, using 40 μl aluminum crucibles without lid and with a lid (with and without pin hole).

2.2.3. Moisture sorption studies

The effect of RH upon storage of the samples on DSC performance was evaluated utilizing the optimum conditions developed in Sections 2.2.1 and 2.2.2. Saturated sodium hydroxide solutions were used to create 25, and 60%, RHs in respective humidity chambers. Water was used for the 100% RH chamber. A 500 mg sample of vanillin and benzoic acid were weighed and placed in Petri dishes. The dishes were then placed uncovered along with their respective covers in the above-mentioned humidity chambers and also in tightly closed bottles (0% RH).

3. Results and discussion

The results obtained from the first part of the study, for the evaluation of sample size, atmosphere and heating rate is given in Tables 1 and 2. The melting point (T_m), extrapolated peak temperature (T_p), and the heat of fusion (ΔH_f) for vanillin and benzoic acid were measured. The T_m, T_p and ΔH_f values for vanillin and benzoic acid were positioned at the respective vertices of the matrix (Fig. 1). The average of the differences for each of the factors was calculated in order to determine their effect on the melting temperature, extrapolated peak temperature and the heat of fusion.

From Tables 3 and 4, it is seen that the sample size, atmosphere, and heating rate had a very small effect on the melting temperature and extrapolated peak temperature. However, it had an appreciable effect on the heat of fusion of the samples. Generally, it was seen that the use of a large sample size (10–12 mg), higher heating rate (10 °C/min) and air as the atmosphere results in a positive deviation for the heat of fusion when compared to the literature heat of fusion values for vanillin (135 J/g) and benzoic acid (147 J/g) [2]. Apart from the deviations in T_m, T_p, and ΔH_f, the other factors that were investigated in order to choose

Table 1
Vanillin

Random run	Sample size (mg)	Atmosphere	Heating rate (°C/min)	Melting point, T_m (°C)	Extrapolated peak, T_p (°C)	Heat of fusion, ΔH_f (J/g)
7	11.54	Nitrogen	10	81.99	82.99	140.16
2	3.99	Air	10	81.87	83.09	140.79
3	11.03	Air	2	81.99	83.19	143.96
4	3.92	Nitrogen	2	81.90	82.36	140.25
5	3.95	Nitrogen	10	81.89	82.36	135.71
1	11.75	Nitrogen	2	81.86	82.22	140.10
6	11.28	Air	10	81.98	82.98	143.12
8	3.59	Air	2	81.90	82.12	141.34

Table 2
Benzoic acid

Random run	Sample size (mg)	Atmosphere	Heating rate (°C/min)	Melting point, T_m (°C)	Extrapolated peak, T_p (°C)	Heat of fusion, ΔH_f (J/g)
3	11.54	Nitrogen	10	122.41	123.38	142.48
2	3.99	Air	10	122.31	123.46	140.09
8	11.03	Air	2	121.05	122.71	137.23
5	3.92	Nitrogen	2	122.46	122.09	135.33
6	3.95	Nitrogen	10	122.25	123.36	145.00
1	11.75	Nitrogen	2	121.74	122.26	141.93
7	11.28	Air	10	121.63	122.84	138.92
4	3.59	Air	2	122.42	122.42	127.30

the optimum conditions for DSC performance was the "quality of the DSC curves" obtained. The qualitative parameters considered were the *baseline* and the *resolution* of the curves. This qualitative protocol was accomplished by superimposing the curves obtained and evaluating the results.

3.1. Effect of sample size

From Table 3, it is seen that the sample size does not have a pronounced effect on the T_m and T_p values. However, data in Table 4 indicate that there is 2–3% increase in the heat of fusion, with a sample size range

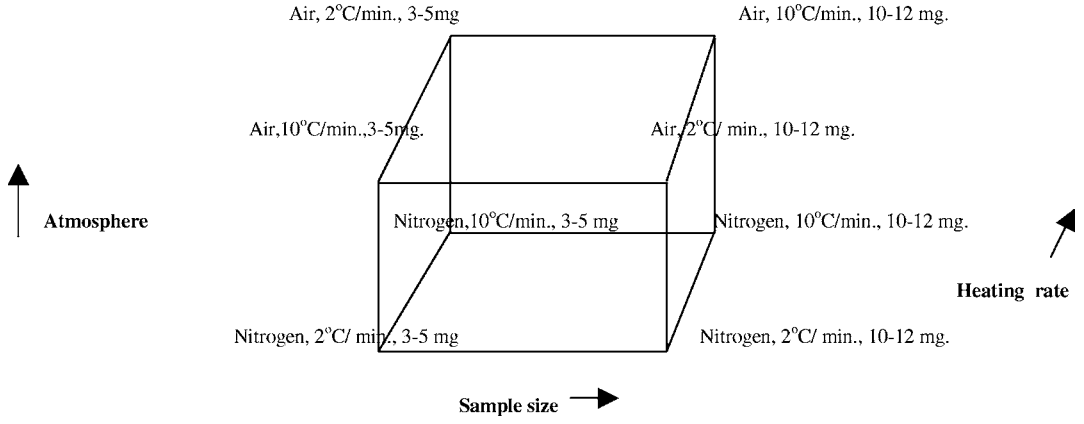

Fig. 1. Matrix for the data.

Table 3
Average of the differences of T_m and T_p for vanillin and benzoic acid

Sample	Average of differences due to the variable parameters		
	Sample size	Atmosphere	Heating rate
Vanillin			
Melting point, T_m (°C)	No effect	−0.08	+0.03
Extrapolated peak, T_p (°C)	−0.02	−0.32	+0.47
Benzoic acid			
Melting point, T_m (°C)	−0.65	−0.36	−0.02
Extrapolated peak, T_p (°C)	−0.70	−0.40	−0.02

of 10–12 mg as compared to the sample size of 3–5 mg. It was observed that there is a broadening of the peak with an increase in sample size, and subsequent decrease in the resolution for the curves for vanillin and benzoic acid. The broadening can be attributed to a number of factors. It is primarily due to a longer time required to melt a large sample, and the development of a temperature gradient within such a sample [2]. The above findings supports the use of a smaller sample size for DSC experiments, typically within a sample size range of 3–5 mg.

3.2. Effect of atmosphere

From Table 3, it is seen that like sample size; the atmosphere does not influence the T_m and T_p values. The heat of fusion varies between a range of −5 to +3% of the actual heat of fusion values for benzoic acid and vanillin samples, respectively. It was observed that for vanillin, the atmosphere does not affect the quality of the curves obtained. For benzoic acid there is, however, a more stable baseline with nitrogen. It can therefore be inferred that atmosphere does not have a significant effect on performance by DSC. If the purpose of a study is to evaluate the oxidative decomposition of a drug and determine its oxidation induction time (OIT) [9] or oxidation onset temperature (OOT) [10] then air or oxygen is the choice of atmosphere. Nitrogen is the choice of atmosphere when inertness is desired.

3.3. Effect of heating rate

Following the same pattern as sample size and atmosphere, the heating rate or ramp was found not to affect the actual values obtained for T_m and T_p (Table 3). The heat of fusion was found to have a positive deviation of 1–6% with higher heating rate. The transfer of heat between the source, such as the furnace and the different parts of the sample or reference material is not instantaneous with a higher heating rate. A thermal lag occurs between different parts of the apparatus [2]. With an increasing heating rate the thermal lag increases accordingly, thus resulting in a less stable baseline. Therefore, a lower heating rate would be preferable.

3.4. Evaluation of crucible type to be used

The values for T_m, T_p and ΔH_f obtained for vanillin and benzoic acid with open and crimped crucibles and under optimum condition-1 are given in Table 5.

The type of crucible did not have any marked effect on melt properties of vanillin, but it did have a marked effect on the heat of fusion of benzoic acid. With an open crucible without lid, the heat of fusion value for benzoic acid was significantly less than the literature value. This is due to extensive sublimation which takes

Table 4
Average of the differences of ΔH_f for vanillin and benzoic acid

Sample	Average of differences due to the variable parameters		
	Sample size	Atmosphere	Heating rate
Vanillin			
Heat of fusion, ΔH_f (J/g)	+2.6 (% deviation = +3)	+3.0 (% deviation = +2)	+1 . 2 (% deviation = +0.83)
Benzoic acid			
Heat of fusion, ΔH_f (J/g)	+3.2 (% deviation = +2)	−5.3 (% deviation = −3)	+6.2 (% deviation = +4)

Table 5
The T_m, T_p and ΔH_f values obtained with different crucible types

Crucible type	T_m (°C)	T_p (°C)	ΔH_f (J/g)
Vanillin			
Open (without lid)	81.65	81.91	139.85
Crimped (with lid)	81.79	82.81	136.84
Benzoic acid			
Open (without lid)	122.44	122.57	95.86
Crimped (with lid)	121.99	122.23	138.09

place in the sample in the absence of a lid, an open environment. The DSC curves obtained for vanillin, and benzoic acid with an open crucible, with crimped crucible with and without pinhole were superimposed on each other in order to evaluate their effect on the *quality of the DSC peaks* obtained.

3.5. Moisture sorption studies

T_m, T_p and ΔH_f values for vanillin and benzoic acid samples stored under 0, 25, 60, and 100% RH under optimum condition-2 is given in Table 6. As seen from the table the T_m, and T_p values obtained for the samples are not affected significantly by RH. However, the heat of fusion deviates significantly as compared to the literature values for vanillin (135 J/g) and benzoic acid (147 J/g) at RHs of 25, 60 and 100%. Values closer to those seen in the literature were obtained for samples at 0% RH. Therefore, it is concluded for preformulation studies which do not address the effect of RH as one of its research question, it is best to store samples at 0% RH.

Table 6
The T_m, T_p and ΔH_f values obtained under different RH of storage

	T_m (°C)	T_p (°C)	ΔH_f (J/g)
RH for vanillin (%)			
0	81.89	82.36	135.71
25	81.75	82.21	120.69
60	81.60	82.16	126.86
100	81.58	82.11	101.22
RH for benzoic acid (%)			
0	122.25	123.36	145.00
25	122.35	122.56	115.22
60	121.94	122.40	121.85
100	122.50	122.92	131.12

4. Conclusion

The purpose of this study was to evaluate the effect of sample size, heating rate, atmosphere, crucible type and RH upon storage, for DSC performance. A general protocol was developed for DSC experiments. However, it should be added that the development of any method would have to depend on the sample being examined and the research objectives of the experiment being addressed. For instance, the standard method developed in this study suggests the use of a small sample size range of 3–5 mg. However, equally important for DSC experiments is that a representative sample should be homogeneous, which may in turn warrant the use of a larger sample size than established.

For heating rates, the user must judge whether it is more important to perform the experiment at conditions as close to equilibrium conditions as possible. In this case a lower heating rate is preferable. If obtaining quick comparative results is desirable, then a larger heating rate can be used (ca. 20 °C/min).

The choice of atmosphere will depend entirely on the research objective. For oxidative stability studies, air or oxygen should be the obvious choice for the atmosphere, but for other purposes nitrogen would be preferable to avoid oxidation of the sample. Crimped crucibles without pinhole should be generally used, exceptions being when oxidative stability studies are performed wherein crimped crucibles with pinhole should be used to expose the sample to the oxidizing atmosphere. This type of crucible would be useful for studies, which involve heating the sample to very high temperatures, where a pinhole would relieve the excess pressure generated.

The effect of RH is often studied in preformulation studies especially to study its effect on polymorphic or pseudo-polymorphic transitions. However, for studies which do not address this factor, this study suggests that 0% RH would be the optimum storage condition for the samples.

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