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INFLUENCE OF EXPERIMENTAL VARIABLES ON CURVES IN DIFFERENTIAL SCANNING CALORIMETRY. PART I. STUDY DESIGN AND RESULTS OF CALIBRATION CHECKS

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ABSTRACT

The extent to which experimental variables may influence the results of quantitative DSC was investigated in factorial designs. In this paper, the first of a series which reports our findings, the study design is presented. The results of the calibration checks, carried out throughout the whole period of testing, are also given.

Calibration was done with indium each day. Temperature values, peak width and shape index of indium peaks were found to be independent of the apparatus adjustment. The peak height appeared to be dependent on the mass of the indium sample and the specific sensitivity E_{In} depended on the apparatus adjustment. Area determinations with a planimeter generally had a lower standard deviation than with the "cut and weigh" method.

INTRODUCTION

Differential scanning calorimetry (DSC) is generally accepted as an important analytical tool in pharmaceutical research [1]. Principal applications are purity analysis [2] and pharmaceutical preformulation studies like the investigation of polymorphism [3] and solid—solid interactions [4—7]. It is also generally recognized, however, that experimental variables can considerably influence DSC results [8]. The most important factors are:

(1) the adjustment of the apparatus: calorimetric sensitivity and heating rate;

(2) the sample: nature and mass, particle size (distribution), packing and porosity, pretreatment and dilution;

(3) the reference material: nature, mass and pretreatment;

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(4) the atmosphere: oxidizing or inert, thermal conductivity, flowing or static conditions.

In our opinion, a thorough knowledge of the influence of these factors is essential to determine thermodynamic values from DSC curves correctly. Care should be taken to ensure that the experimental conditions are well defined before drawing any conclusions. We have not yet found publications on basic studies of the influences of experimental factors on the quantitative data from curves of pharmaceutically interesting substances. We therefore conducted a number of factorial experiments in a statistically justified manner to investigate the main effects of these factors and possible interactions.

This series of papers reports our findings. In Part I, we describe the study design and give the results of calibration checks. In Part II, the results on baseline-related parameters are discussed. Part III gives the results of some peak-descriptive parameters and in Part IV we present the results on temperature characteristics and specific enthalpy.

EXPERIMENTAL

Materials

Test substances

The following compounds were chosen as examples of substances with interesting thermodynamic behaviour.

(a) *Adipic acid,* ($HOOC(CH_2)_4COOH$). This melts without noticeable decomposition. It has been suggested as a reference compound for temperature calibration (literature value of the extrapolated onset temperature 151.0°C). The substance was supplied by Baker Chemicals (The Netherlands) and is described in ref. 9. The mean diameter by weight was 67 μm. Fractions <32 μm and $\geqslant 160$ μm were collected by sieving. Part of the substance was triturated in a mortar with pestle and the fraction <32 μm was also used.

(b) *Naphazoline nitrate,* (nitrate of 4,5 dihydro-2-(1-naphthalenylmethyl)-1H-imidazole). This melts at 167—170°C with decomposition. It is described in ref. 10. Our batch was supplied by the Onderlinge Pharmaceutische Groothandel (The Netherlands). The mean diameter by weight was 91 μm. Sieve fractions <32 μm and $\geqslant 400$ μm were also collected. The triturated sample had diameters <32 μm.

(c) *Potassium nitrate,* KNO_3. This undergoes a solid—solid transformation at 128°C. It is described in ref. 11 and it is recommended by ICTA [12] for temperature calibration in DTA. Our batch was granular and was supplied by Baker Chemicals (The Netherlands). The mean diameter by weight was 255 μm; fractions of <160 μm and $\geqslant 354$ μm were also collected. The triturated sample was <160 μm.

(d) *Sodium citrate dihydrate,* $C_6H_5Na_3O_7 \cdot 2\ H_2O$. This becomes anhydrous at 150—160°C. It is described in ref. 10. Our batch was supplied by the Onderlinge Pharmaceutische Groothandel (The Netherlands). The mean diameter by weight was 320 μm. The sieved samples had sizes between 106 μm and 212 μm, and ≥500 μm, respectively. The triturated sample had a sieve diameter between 106 and 212 μm.

Reference compounds

The following substances were chosen as references or diluents.

(a) *Carborundum.* A sample of technical carborundum was pretreated and purified according to the method described by Barrall and Rogers [13]. The mean diameter by weight was 30 μm.

(b) *Aluminium oxide.* A batch of aluminium oxide 90 aktiv, Merck (neutral, activity level I, for column chromatography) was used. The mean diameter by weight was 90 μm.

(c) *Indium.* For calorimetric and temperature calibration, ultrapure indium (purity >99.999%) was supplied by Mettler (The Netherlands).

Apparatus

The equipment used was a Mettler TA 2000 heat-flux DSC system with a constant supply of gas and a check of the amount of oxygen in the effluent gas (Fig. 1). If an inert atmosphere was required the whole system was evacuated and flushed with the inert gas at least two to three times to reach an oxygen content of less than 1%. The holder lids were pierced with a special

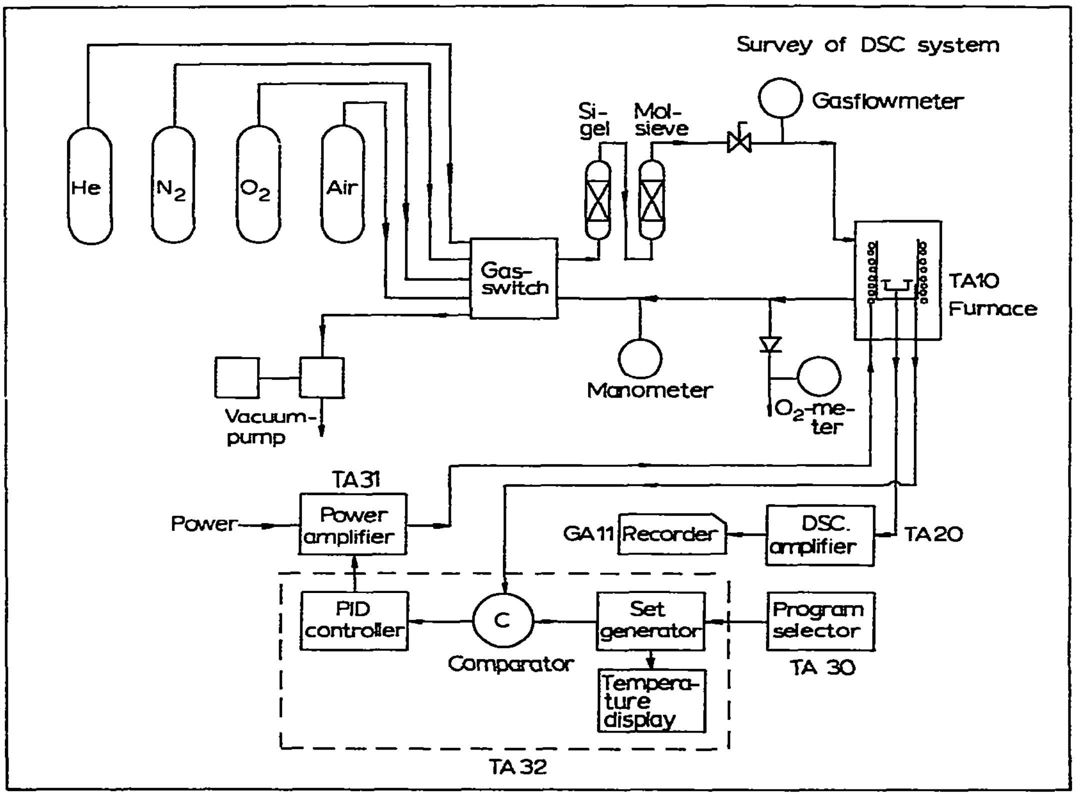

Fig. 1. The DSC system.

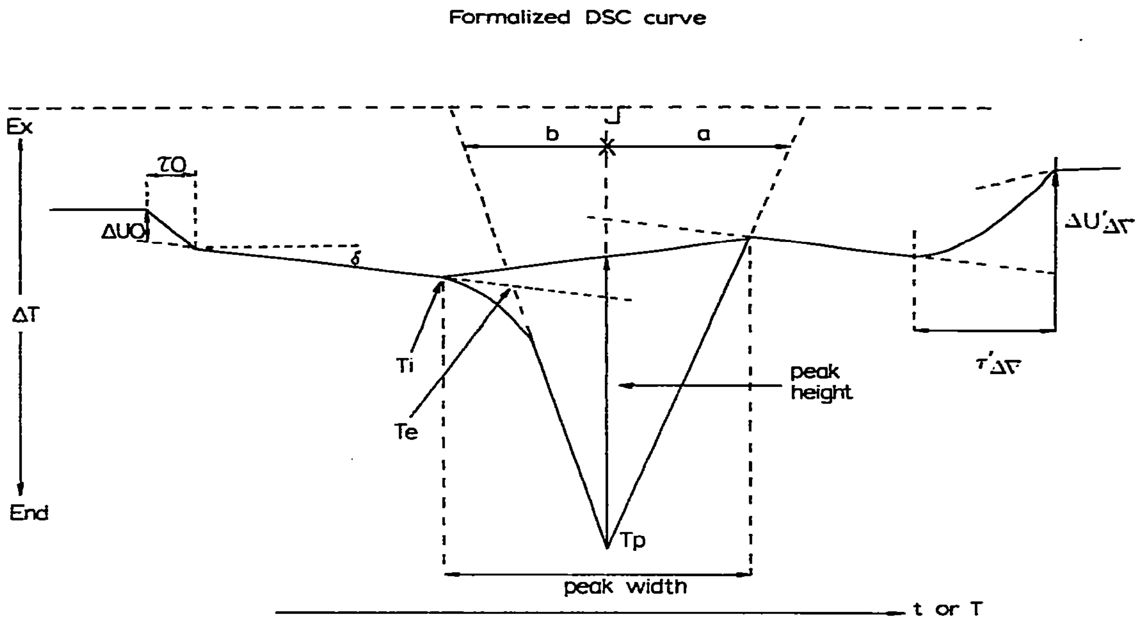

Fig. 2. Formalized DSC curve.

device to ensure reproducible holes and thus reproducible contact of the specimens with the atmosphere. Weighings were done on an electronic Mettler ME 30 microbalance. They were reproducible within 0.004 mg. Where it was found that the sample mass of a compound did not appreciably affect the curve characteristics, the weighings were accurate within 0.040 mg. The holders with sample were weighed before and after the analysis. Sample and reference holders were always positioned accurately in the furnace. An Ott planimeter (type 30139) was used to determine the areas under the curves.

In preliminary experiments it had been found that the amplifier range setting only had a negligible influence on the determination of peak area and that the accuracy of the measured heating rate was better than ±1%.

Characterization of the DSC curves

The evaluation of the DSC curves was done on the following basis (see Fig. 2).

Baseline-related characteristics:

initial baseline deflection (isothermal → heating) ΔU_0 (in μV) (N.B. if after starting the heating programme the baseline deflected to the exothermal side, the ΔU_0 value was given a negative sign);

lag time (isothermal → heating) τ_0 (in sec);

baseline drift angle δ (in μV K^{-1}), after Gäumann and Oswald [14];

baseline deflection $\Delta U'_{\Delta V}$ (heating → cooling) (in μV);

lag time $\tau'_{\Delta V}$ (heating → cooling) (in sec).

Temperature characteristics

onset temperature T_i (in °C);

extrapolated onset temperature T_e (in °C), as defined by ICTA [12];

peak temperature T_p (in °C) (temperature at peak top).

Peak-related characteristics

peak height (in μV), as defined by ICTA [12];

peak width (in K), as defined by ICTA [12];

shape index $s = a/b$, similarly to Kissinger's definition [15];

specific enthalpy ΔH_s in (J g^{-1}), computed from the peak area;

baseline displacement (in μV), being the difference between the extrapolated baselines before and after the peak, measured at the line through the peak top vertical to the time axis.

Calibration of the equipment

The following calibration checks were carried out.

(a) Determination of peak parameters for different amounts of indium on different days to determine which correction should be taken: the value calculated per day and/or mass, or a mean value over a period of time.

(b) Determination of the specific sensitivity (E_{In}) with indium on each day on which DSC curves were recorded. Each indium sample was used for only 1 week.

The peak areas were determined in two ways: (1) by cutting and weighing (after tracing the peak on Diamat polyester film, the weight of which is negligibly influenced by the relative humidity of the environment); (2) with the planimeter (mean of three independent determinations).

Study design

All tests were carried out in random order. The compounds were heated from 47°C to 20 K above the peak temperature and then cooled. A number of different tests were carried out with each compound.

Determination of influence of sample mass

[Experiment No. 1.1 (adipic acid); 2.1 (naphazoline nitrate); 3.1 (potassium nitrate); and 4.1 (sodium citrate dihydrate).]

No reference compound, no dilution.

Sample holders with pierced lids.

Stream of flowing air 24—36 ml min^{-1}.

No pretreatment.

Heating rate 0.08 K sec^{-1}.

Sample masses (mg): 0.100, 0.200, 0.500, 1.000, 2.000, 5.000, 10.000 and 15.000.

Determination of influence of heating rate

[Experiment No. 1.2 (adipic acid); 2.2 (naphazoline nitrate); 3.2 (potassium nitrate); and 4.2 (sodium citrate dihydrate).]

Sample mass 2.0 mg.

No reference compound, no dilution.

Sample holders with pierced lids.

Stream of flowing air 24—36 ml min^{-1}.

Pretreatment: (a) no pretreatment (exp. No. 1.2.1, 2.2.1, 3.2.1 and 4.2.1); (b) trituration with pestle and mortar; sieve fraction of small particles taken (exp. No. 1.2.2, 2.2.2, 3.2.2 and 4.2.2).

Heating rates (K sec^{-1}) 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.495.

Determination of influence of dilution

[Experiment No. 1.4 (adipic acid); 2.4 (naphazoline nitrate); 3.4 (potassium nitrate); and 4.4 (sodium citrate dihydrate).]

Heating rate 0.08 K sec^{-1}.

Sample holders with pierced lids.

Stream of flowing air 24—36 ml min^{-1}.

Dilutions freshly prepared.

References also used as diluting compounds.

Mass of reference the same as mass of diluting compound.

Mass of active ingredient always 2.0 mg.

Variables	Levels
Concentration (%)	10, 30, 50
Particle size distribution of active ingredient	Small particles, complete size spectrum, large particles
Method of dilution	Mixing (whirl mixer), trituration (mortar), dissolution and recrystallization
Reference compounds	Carborundum, aluminium oxide, no reference compound

Determination of influence of atmosphere (in duplicate)

[Experiment No. 1.5 (adipic acid); 2.5 (naphazoline nitrate); 3.5 (potassium nitrate); and 4.5 (sodium citrate dihydrate).]

Sample mass 2.0 mg.

No reference compound, no dilution.

Complete size spectrum.

Heating rate 0.08 K sec^{-1}.

Flow rate of gases 24—36 ml min^{-1}.

Variables	Levels
Sample (and reference) holder	Completely closed, with pierced lid, without lid
Atmosphere	Static air, flowing air, flowing nitrogen (99.98%), flowing oxygen (99.5%), flowing helium (99.995%), vacuum (7500 Pa)

Determination of influences of sample masses, particle sizes, reference masses and test persons

[Experiment No. 1.3 (adipic acid).]

No dilution.
Reference compound: carborundum.
Heating rate 0.08 K sec^{-1}.
Stream of flowing air 24—36 ml min^{-1}.

Variables	Levels	
Sample mass (mg)	2.0	5.0
Particle size distribution	<32 μm, ≥160 μm	Complete size spectrum,
Mass of reference compound (mg)	0.0	2.0 5.0
Test person	Person 1	Person 2

Determination of influences of particle sizes, heating rates and atmospheres

[Experiment No. 2.3 (naphazoline nitrate); 3.3 (potassium nitrate); and 4.3 (sodium citrate dihydrate).]

No reference compound, no dilution.
Sample holders with pierced lids.
Gas flows 24—36 ml min^{-1}.

Variables	Levels
Sample mass (mg)	2.0, 5.0
Particle size distribution	Small particles, complete size spectrum, large particles
Heating rate (K sec^{-1})	0.02, 0.08, 0.32
Atmosphere	Air, oxygen, nitrogen

Statistical analysis

Effects of factors (variables) and their interactions on the various curve characteristics were tested for statistical significance by means of analysis of variance. Means per factor level were obtained in order to facilitate the interpretation of the effects. Furthermore, the standard deviations of the single determinations were estimated for each characteristic.

RESULTS AND DISCUSSION OF CALIBRATION CHECKS

Correction factors for indium peak characteristics

The conclusions to be drawn for the correction factors for the characteristics of indium peaks are derived from the data in Table 1. If extremely accurate work is required, the following corrections should be made:

in case of day effects, take the average of all masses per day;

in case of mass effects, take the average of all days per mass, if possible;

in case of influence of mass and day, make a correction per mass and per day.

TABLE 1

Results of indium determinations (seven masses, each determined on seven separate days)

	E_{In} (μV mW^{-1})	T_i (°C)	T_c (°C)	T_p (°C)	Peak height (μV) *	Peak width (K)	Shape index
Mean values							
m_1 = 4.869 mg	13.476	156.270	156.626	157.091	43.886	2.540	0.2839
m_2 = 5.548 mg	13.593	156.246	156.589	156.956	48.886	2.601	0.2294
m_3 = 6.023 mg	13.647	156.243	156.643	157.206	49.903	2.810	0.2083
m_4 = 6.504 mg	13.624	156.239	156.591	157.250	54.286	2.756	0.2109
m_5 = 6.902 mg	13.521	156.257	156.636	157.180	55.977	2.774	0.1806
m_6 = 7.590 mg	13.516	156.207	156.529	156.919	63.571	2.717	0.1991
m_7 = 7.895 mg	13.543	156.189	156.569	156.951	66.457	2.784	0.2196
Repeatability							
s	0.092	0.061	0.109	0.242	2.62	0.15	0.030
C.V. (%)	0.68	0.04	0.07	0.15	4.8	5.5	13.8
Reproducibility							
s	0.104	0.075	0.129	0.292	3.67	0.196	0.03
Rounding interval	0.05	0.01	0.05	0.1	1	0.05	0.01
Significant effect	Day + mass	Day	Day	Day	Day + mass	Day + mass	Mass

* Peak height increases almost linearly with mass.

It was concluded that except for the responses of peak height and peak width, the differences between repeatability and reproducibility were so slight, that for practical purposes the values may be averaged over days and masses to give mean values and reproducibilities.

Calibration with indium

The results of the calibration tests with indium during the total period of testing are given in Tables 2—5. It can be seen that effects of thermopile sen-

TABLE 2

Specific sensitivity (E_{In}): mean values

	Area determination	E_{In} (μV mW^{-1})	n	s (μV mW^{-1})	C.V. (%)
July 1978—Jan. 1979	Cut/weigh	14.49	97	0.35	2.40
	Planimeter	14.50	97	0.23	1.62
Jan. 1979—April 1979 *	Cut/weigh	14.41	28	0.34	2.38
	Planimeter	14.34	28	0.36	2.51
April 1979—July 1979 *,**	Cut/weigh	13.57	16	0.21	1.54
	Planimeter	13.58	16	0.18	1.33
July 1979—Sept. 1979 *,**	Cut/weigh	14.27	14	0.20	1.41
	Planimeter	14.20	14	0.15	1.06

* New thermopile.

** New sample holders, which give a slightly lower sensitivity, but less holder-to-holder variation.

TABLE 3

Indium determinations, temperatures of melting endotherm

Period	T_i (°C)			T_e (°C)			T_p (°C)		
	Mean	s	C.V. (%)	Mean	s	C.V. (%)	Mean	s	C.V. (%)
July 1978—Jan. 1979	156.17	0.10	0.07	156.50	0.14	0.09	156.75	0.28	0.18
Jan. 1979—April 1979 *	156.32	0.08	0.05	156.65	0.11	0.07	157.00	0.22	0.14
April 1979—July 1979 *,**	156.27	0.10	0.06	156.60	0.11	0.07	156.95	0.25	0.16
July 1979—Sept. 1979 *,**	156.36	0.07	0.04	156.66	0.07	0.04	156.98	0.19	0.12
Mean value July 1978—Sept. 1979	156.22	0.12	0.08	156.55	0.15	0.09	156.83	0.28	0.18

* New thermopile.

** New sample holders.

TABLE 4

Indium determinations, peak width and shape index of melting endotherm

Period	Peak width (K) ***			Shape index		
	mean	s	C.V. (%)	mean	s	C.V. (%)
July 1978—Jan. 1979	2.56	0.20	7.69	0.246	0.058	23.6
Jan. 1979—April 1979 *	2.60	0.19	7.15	0.203	0.024	11.9
April 1979—July 1979 *,**	2.65	0.26	9.82	0.246	0.036	14.7
July 1979—Sept. 1979 *,**	2.72	0.20	7.49	0.239	0.039	16.1
Mean value July 1978—Sept. 1979	2.59	0.21	8.01	0.237	0.052	22.0

* New thermopile.

** New sample holders.

*** Values of masses < 6.0 mg have been discarded.

TABLE 5

Indium determinations, peak heights per unit mass

Period	Peak height per unit mass (μV mg^{-1})	s (μV mg^{-1})	n	C.V. (%)
July 1978—Jan. 1979	9.784	0.784	97	8.01
Jan. 1979—April 1979 *	9.358	0.652	28	6.97
April 1979—July 1979 *,**	8.667	0.894	16	10.32
July 1979—Sept. 1979 *,**	9.348	0.682	14	7.30

* New thermopile.

** New sample holders.

sitivity on T_i, T_e, T_p, peak width and shape index are negligible. Therefore, the values over the whole period of testing (July 1978—September 1979) can be averaged.

Due to the effect of sample mass on peak height, this parameter was rather expressed per unit of mass (Table 5). In the period April 1979—July 1979 the values for the specific sensitivity (E_{In}) and peak height per unit mass were significantly lower than in the other periods. It was therefore not justified to average these values.

The procedure for peak area determination does not affect the values found for the specific sensitivity, as can be seen in Table 2. Generally, however, the method with the planimeter gives a lower standard deviation, and also because of its ease in use this was therefore our method of choice for the area determinations.

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