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A method to reduce the equilibration time prior to data capture in ampoule-based isothermal microcalorimetric studies

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Abstract

This paper reports a technique for heat conduction isothermal microcalorimeters, which allows, by means of a lowering apparatus, the sample and reference ampoules to be lowered very slowly from the pre-equilibration position to the measuring position. The purpose is to minimize the effect on (total) equilibration time arising from both the pre-equilibration time and the dissipation time (i.e. the time to dissipate the thermal shock which occurs when the vessels are lowered manually). Measurements using 3 ml glass ampoules filled with water, as sample and reference, at 25 and 60 °C, showed that the extent of thermal shock was drastically diminished as the lowering speed decreased, and that the associated heat quantity was reduced to less than 0.2 mJ when lowering occurred over a period of more than 180 s. The dissipation time, the time-period required to dissipate the thermal shock, was also shortened to less than 10 min; the standard manual lowering procedure dissipation time being ca. 25 min. In practice experimental measurements of the imidazole catalysed hydrolysis of triacetin and of the solid state oxidation of ascorbic acid showed that the dissipation time was not shortened when the initial power observed was more than 5 μW, however, it was significantly shortened when the initial power observed was around ≤ 1 μW. The proposed ampoule lowering procedure could be expected to bring about a saving in the total measurement time for reactions with low initial power, such as those associated with long-term stability studies of relatively stable pharmaceuticals. The described procedure also eliminates operator-induced effects associated with manual lowering of ampoules. In principle the device described also would permit automated loading protocols to be developed.

Keywords: Isothermal microcalorimeter; Ampoule; Thermal shock; Long-term stability

1. Introduction

Isothermal microcalorimetry is an important experimental tool in the study of the mechanism of reacting systems. Willson et al. [1] introduced a method for the determination, from power (Φ, in watts)–time curves obtained from this type of microcalorimeter, of both kinetic and thermodynamic parameters (n, the order of the reaction; k, the rate constant; and ΔH, the reaction enthalpy change). This method involved writing a kinetic equation that described the reaction under study, converting it to a calorimetric form and then fitting the calorimetric data using a process of iteration. The method was shown to be applicable to real experimental data through study of reactions such as the solution phase [2] and the solid state oxidation of ascorbic acid [3] and the imidazole catalysed hydrolysis of triacetin [4,5]. Further, Gaisford et al. [6] extended Willson's method of analysis to consecutive reaction schemes and applied it to the acid catalyzed hydrolysis of potassium hydroxylamine trisulfonate (A → B → C → D type). These methods have been extended [7,8] to allow direct calculation of the target parameters, i.e. iteration is no longer required.

A functional diagram of a 2277 Thermal Activity Monitor (TAM, Thermometric AB), a typical heat conduction isothermal microcalorimeter, is shown in Fig. 1 [9]. In a heat conduction isothermal microcalorimeter, a pair of reaction vessels, sample and reference, are inserted into a pair of cylindrical holes surrounded by a metal heat sink, through which heat generated from sample or reference materials is allowed to flow. Thermopiles, positioned between the vessel and the heat sink, respond to the difference in power between the sample and reference vessels through production of a quantitatively equivalent electrical potential. The operating procedures of the TAM require that the sample and

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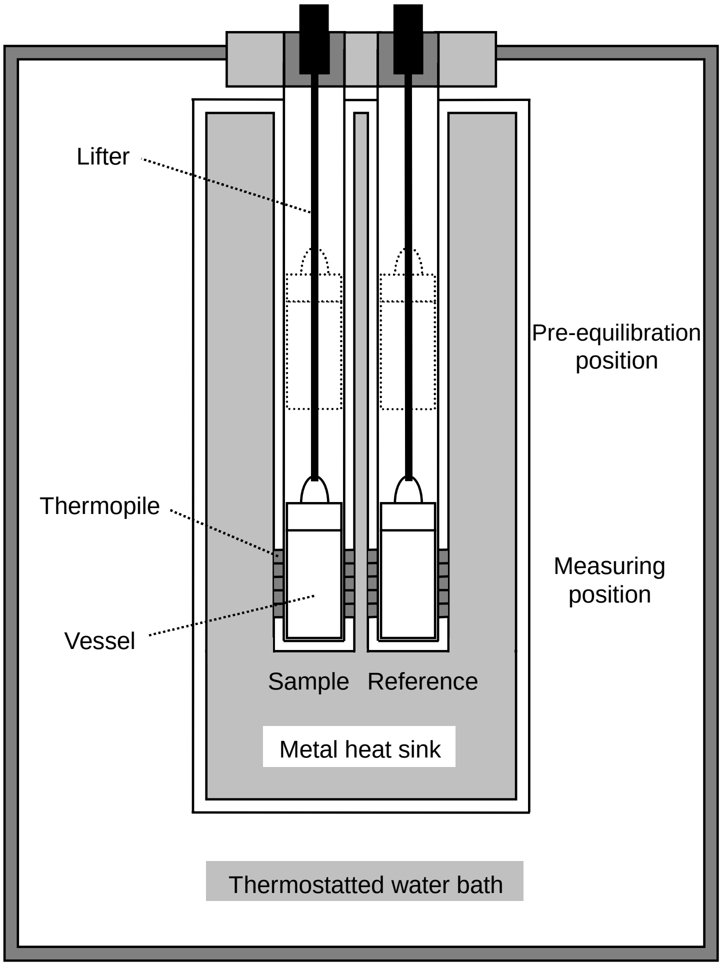

Fig. 1. Schematic diagram of a calorimetric unit in the 2277 Thermal Activity Monitor.

reference vessels are placed at a pre-equilibration position, in the middle of the cylindrical hole, for more than 30 min, to establish thermal equilibrium to the fixed temperature of the calorimeter prior to starting measurement. After thermal equilibrium is reached, the vessels are simultaneously lowered to a measuring position, at the bottom of the cylindrical hole, where the vessels are in contact with the thermopiles, and recording of power starts. However, recorded data for first 60 min (i.e. 30 min pre-equilibration plus 30 min for the dissipation to occur) of a power–time curve must be excluded from analysis. This is because the act of lowering the ampoules into the measurement position itself constitutes a thermal event (“thermal shock”). After this thermal shock has completely dissipated, the desired power–time data can be obtained. The exclusion of data for the first 60 min may be a serious constraint in the study of the reaction mechanisms: unknown reactions initiated as the system for study is assembled; reaction events that can be seen to exist but whose lifetimes are too short to permit quantitative investigation. In addition, it could contribute significantly to time-consuming experiments for the quality control of large quantities of samples.

It is obvious, from every-day experience, that the more slowly the vessels are lowered, the smaller will be the thermal shock delivered to the calorimeter. Therefore, in order to diminish thermal shock, more attention ought to be given to the lowering of the vessels. In this study, we investigated, using a TAM, the influence of vessel lowering speed on the recorded thermal shock, with the objective of proposing a vessel lowering procedure to minimise interference from thermal shock and that will permit earlier data capture. Such a shortening of the time devoted to equilibration would contribute to a better understanding of reaction kinetic and thermodynamic characterisation. For this purpose, a vessel lowering apparatus specifically designed for a TAM was constructed. It can, simultaneously, lower a pair of vessels from the pre-equilibration position or directly from the laboratory conditions, to the measuring position with variable speed. The majority of published data on the kinetics and thermodynamic characterisation of reactions has emerged from this laboratory and hence we take examples from these studies to examine the performance of the device designed. The use of the proposed lowering procedure was thus explored through study of the established test and reference reaction, the imidazole catalysed hydrolysis of triacetin [4,5] and also through study of the solid state oxidation of ascorbic acid [3]. Clearly it is not known if other processes precede data reported and analysed previously [3–5].

2. Experimental

2.1. Materials

Unsieved L-ascorbic acid (more than 99% purity) was purchased from Aldrich and stored in a sealed container in a vacuum desiccator at room temperature. Triacetin and imidazole (more than 99% purity) were purchased from Sigma and Aldrich, respectively. Water used in all experiments was freshly distilled prior to use.

2.2. Apparatus

The isothermal microcalorimeter used in this study was 2277 TAM (Thermometric AB, Järfälla, Sweden), consisting of four independent channels (channels 1, 2, 3 and 4). The microcalorimeter was housed in a temperature-controlled environment (21 ± 0.1 °C) and calibrated periodically using an electrical substitution method. A hand-made vessel lowering apparatus, appropriate for use in any channel of a TAM, is illustrated in Fig. 2. The apparatus consists mainly of an aluminum frame (constructed from workshop-available material), electric motor (Mabuchi Motor Co. Ltd., Chiba, Japan: FA-130, 3.0 V, 500 mA) and pullies (workshop-available and sizes determined by trial and error). The ampoules are suspended on hooks fixed on a plate which is connected to the pullies of the lowering device. The rotation of the pullies allows the plate to be

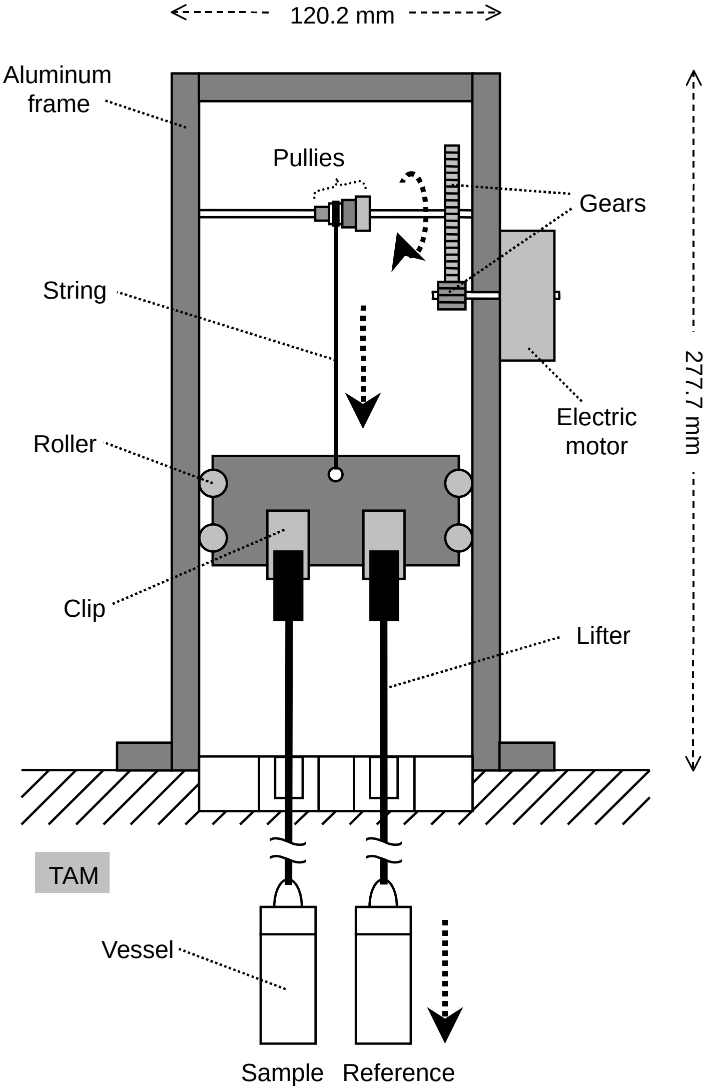

Fig. 2. Schematic diagram of a hand-made vessel lowering apparatus applicable for each channel of the 2277 Thermal Activity Monitor.

lowered vertically by gravity. The lowering speed can be adjusted by using various sizes of pullies.

2.3. Influence of ampoule lowering speed on thermal shock

Three-millilitre-glass ampoules were used, initially, as model reaction vessels. Three milliliters of water were pipetted into a pair of ampoules of sample and reference, respectively, and the ampoules were sealed with crimped aluminum caps, the caps being fitted with teflon sealing disks. These ampoules were thermally equilibrated, prior to the insertion into the calorimeter, in a laboratory environment (ca. 21 °C). The ampoules were placed in the pre-equilibration position of calorimeter for a period of 30 min. They were then lowered to the measuring position (the distance between both positions is about 14 cm) over periods of 60, 90, 120, 180 and 240 s using the vessel lowering apparatus. The ampoule lowering was also performed manually (without the apparatus) over periods of 10, 30 and 60 s at constant speeds as possible. As soon as lowering of the ampoules started, monitoring of the power signal began using the dedicated Digitam (Thermometric AB) software. The extent of the thermal shock caused was evaluated by integrating the power–time curve and measuring the time-period required to dissipate the thermal shock within the instrument specified base line drift (±0.1 μW) [9]. Three repeat measurements were performed for each lowering condition. These experiments were carried out at 25 and 60 °C.

2.4. Minimization of the pre-equilibration time

Measurements were performed according to the procedures described in Section 2.3 (influence of ampoule lowering speed on thermal shock), except that the time at the pre-equilibration position was varied (5, 10, 15, 20, 25 and 30 min).

2.5. Influence on reaction systems

The experimental protocol for the imidazole catalysed hydrolysis of triacetin and the solid state oxidation of ascorbic acid were as described in the previous studies reported by Beezer et al. [4] and Willson et al. [3], respectively. In both experiments, after sample and reference ampoules were thermally pre-equilibrated over a period of 30 min at the pre-equilibration position, they were lowered to measuring position over a period of 240 s using the vessel lowering apparatus or over a period of 60 s without using the apparatus. The experimental temperature was 25 °C.

3. Results

3.1. Influence of ampoule lowering speed on thermal shock

3.1.1. Heat output

Fig. 3 shows the extent of the heat output generated by lowering a pair of ampoules, sample and reference, at various speeds, from the pre-equilibration position to the measuring position in channel 1. Typical power–time curves at 25 °C are also shown in Figs. 4 and 5. As can be seen from the figures, the total number of Joules associated with simply lowering the ampoules was drastically diminished as the lowering speed decreased: the heat output was minimized to less than 0.2 mJ when lowered over periods of more than

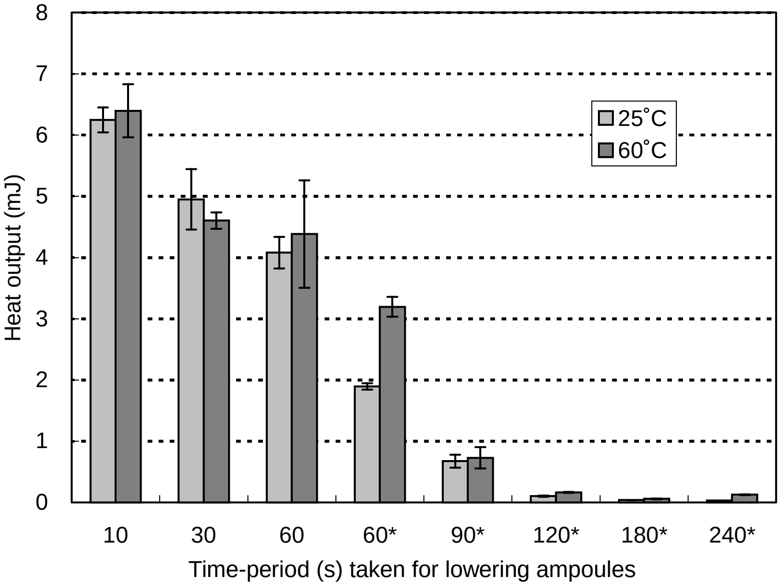

Fig. 3. Influence of ampoule lowering speed on the extent of heat output. Each column represents the mean ± S.D. ($n = 3$). (∗) Using the vessel lowering apparatus.

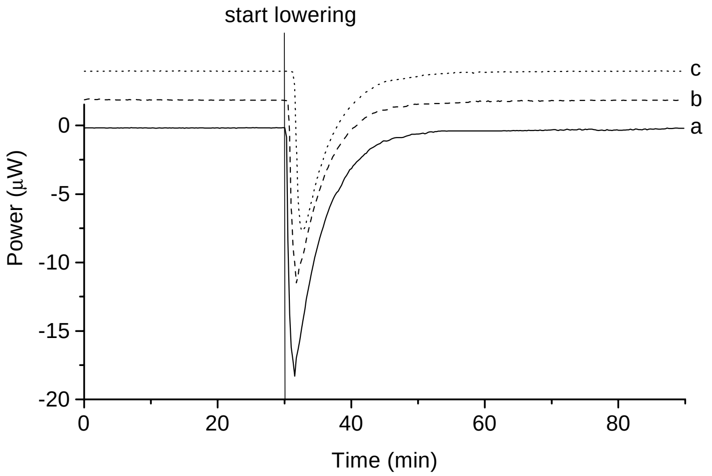

Fig. 4. Typical power–time curves at various lowering speeds at 25 °C. The ampoules were lowered manually over periods of 10 s (a), 30 s (b) and 60 s (c).

180 s. No difference in the influence of lowering speed was observed between 25 and 60 °C. On measurements over a period of 60 s, slight differences in heat output were observed with the use of and without the use of the vessel lowering apparatus (see Fig. 3). This suggests that lowering at an exactly constant speed from start to finish is also one of the factors that contributes to reduction in thermal shock.

Fig. 6 shows the difference in the extent of heat output between different channels at 25 °C. Although slight differences were observed between the response from each channel, the heat output for all channels was drastically diminished with decrease in lowering speed.

3.1.2. Dissipation time

The time-period required to dissipate the thermal shock is an important parameter that will determine the reduction possible in total measurement time. Fig. 7 shows the influence of ampoule lowering speed on the dissipation time; defined as the time-period required to dissipate the thermal shock to within the base line drift (±0.1 μW) [9] including the time-period (10–240 s) required for lowering the ampoule. In the case of manual lowering, dissipation time was not significantly shortened (ca. 25 min) as the lowering speed decreased. However, the use of the lowering apparatus obviously allowed the dissipation time to be shortened (regardless of the experimental temperature) although the effects were not as great as those seen in the decrease of heat output described above. When lowered over periods of more than 120 s, the dissipation time was minimized to less than 10 min, suggesting that thermal shock was completely absent soon after the ampoules entered the measuring position. In addition, no difference in dissipation time was observed between channels at 25 °C (Fig. 8).

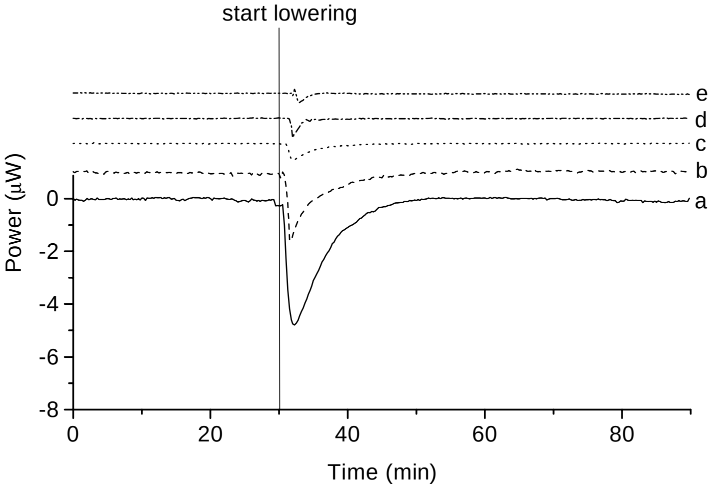

Fig. 5. Typical power–time curves at various lowering speeds at 25 °C. The ampoules were lowered using the vessel lowering apparatus over periods of 60 s (a), 90 s (b), 120 s (c), 180 s (d) and 240 s (e).

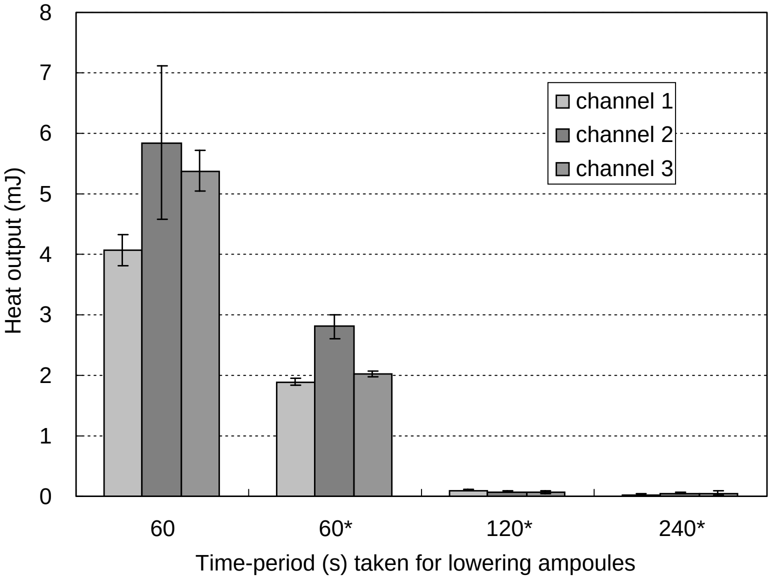

Fig. 6. Influence of ampoule lowering speed on the extent of heat output, in various channels, at 25 °C. Each column represents the mean ± S.D. ($n = 3$). (∗) Using the vessel lowering apparatus.

3.2. Minimization of the pre-equilibration time

Minimizing the time-period allowed for thermal equilibration (pre-equilibration time) at the pre-equilibration position in the calorimeter contributes directly to a saving in the total measurement time. Figs. 9 and 10 show the heat output and the dissipation time when the pre-equilibration time is reduced from 30 to 5 min (note that the pre-equilibration time described in Section 3.1 was fixed at 30 min). When the vessels were lowered using the vessel lowering apparatus (over a period of 240 s), the heat output was still less than 0.2 mJ for pre-equilibration times of more than 25 min but it increased dramatically as the pre-equilibration time decreased (Fig. 9). Fig. 9 shows that thermal equilibrium is achieved within 20–25 min. Dissipation time was also less than 10 min for pre-equilibration times of more than 25 min but increased significantly as pre-equilibration time decreased (Fig. 10); e.g. the dissipation time was more than 140 min when the pre-equilibration time was 5 min.

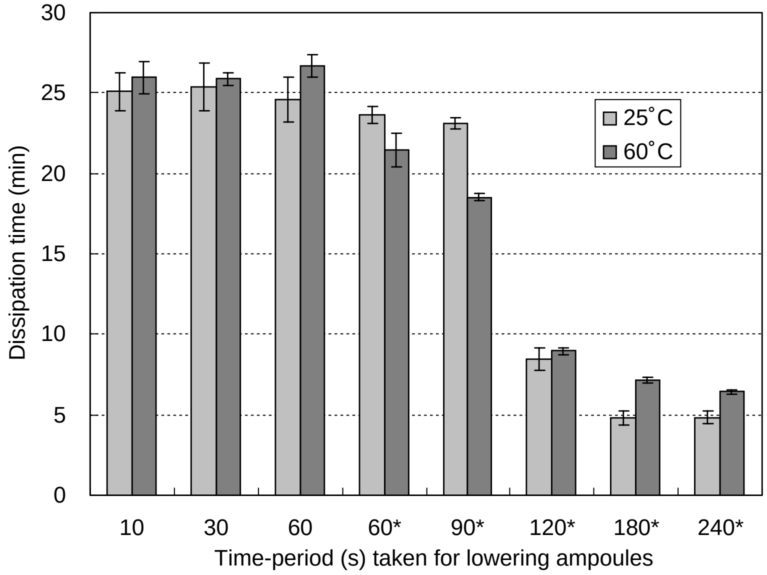

Fig. 7. Influence of ampoule lowering speed on the dissipation time. Each column represents the mean ± S.D. ($n = 3$). (∗) Using the vessel lowering apparatus.

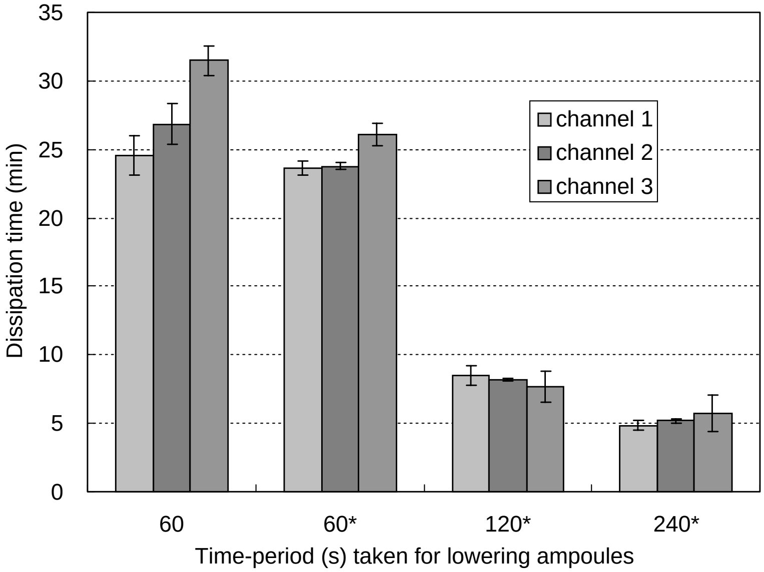

Fig. 8. Influence of ampoule lowering speed on the dissipation time, in various channels, at 25 °C. Each column represents the mean ± S.D. ($n = 3$). (∗) Using the vessel lowering apparatus.

3.3. *Influence on reaction systems*

3.3.1. *Imidazole catalysed hydrolysis of triacetin*

The proposed mechanized ampoule lowering procedure was applied to practical measurements. Triacetin in a buffer solution of acetic acid and imidazole generated an initial power of around 45 μW as is shown in Fig. 11. In contrast to the results described above, the dissipation time was ca. 35 min even when the ampoules were lowered with the apparatus over a period of 240 s. On the other hand, when triacetin solution was prepared without imidazole, the initial power generated was only around 1 μW and the usefulness of proposed lowering procedure was obvious; the dissipation time now was ca. 25 min for manual lowering (over a period of 60 s) against ca. 7 min for lowering with the apparatus (over a period of 240 s) (Fig. 11).

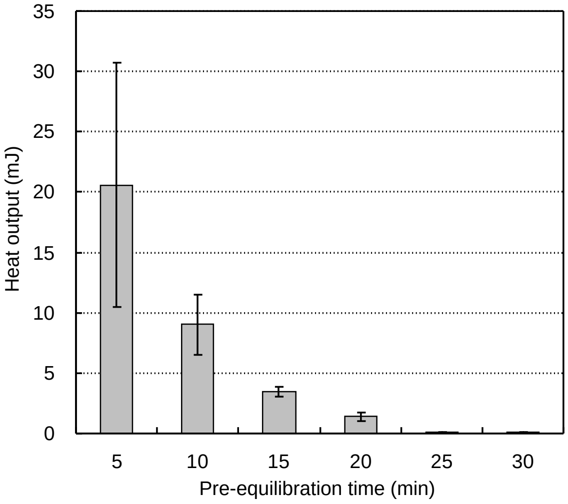

Fig. 9. Influence of the pre-equilibration time on heat output. The ampoules were lowered using the vessel lowering apparatus over period of 240 s.

3.3.2. *Solid state oxidation of ascorbic acid*

The power–time curves for the unsieved ascorbic acid in air are shown in Fig. 12. For measurements with the addition of 30 μl water to 0.5 g ascorbic acid [3] (the initial power generated was ca. 5 μW), almost no difference in the dissipation time was observed between manual lowering and apparatus lowering. However, the dissipation time was quite obviously shortened in measurements without addition of water (the initial power generated was ca. 0.5 μW.).

4. Discussion

The proposed ampoule lowering procedure, for the TAM microcalorimeter, which allows the ampoules to be lowered very slowly (e.g. over a period of 240 s) from the pre-equilibration position to the measuring position, successfully diminished the extent of thermal shock when the initial power observed was very low (e.g. around 1 μW and lower). The dissipation time, normally ca. 25 min with a standard lowering procedure (manual lowering), was shortened to less than 10 min. However, the usefulness of the procedure is not established for measurements in which the initial power observed is more than 5 μW. These results suggest that the procedure could be expected to bring about a saving in total measurement time only for those reactions with low initial power, such as long-term stability studies of relatively stable pharmaceuticals. For additional reduction in total measurement time, the pre-equilibration time at the pre-equilibration position might somewhat be shortened.

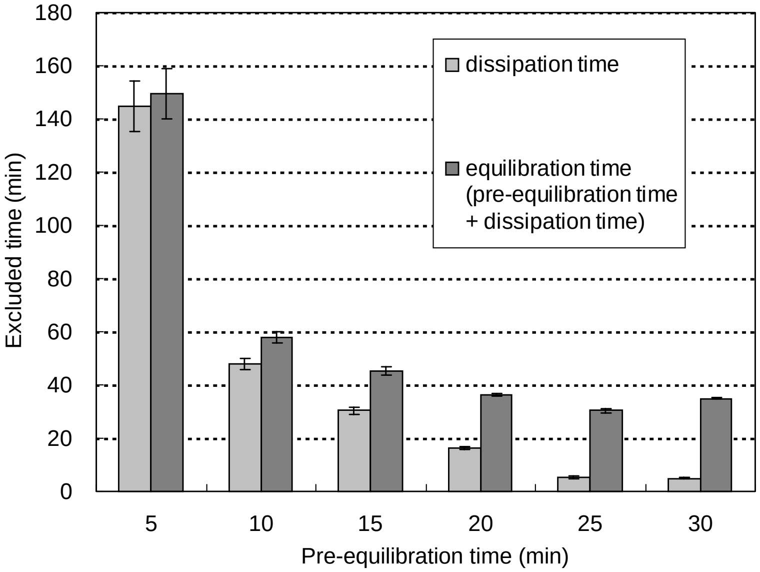

Fig. 10. Influence of the pre-equilibration time on the dissipation time. The ampoules were lowered using the vessel lowering apparatus over period of 240 s.

The minimum pre-equilibration time will depend on the fixed temperature of TAM, the initial temperature of the sample materials (solid or liquid) in the reaction vessels and their heat capacities. It should be noted, however, that careless shortening of the pre-equilibration time may, on the contrary, cause serious increase in the total measurement time (see Fig. 10).

The reason why the proposed lowering procedure is not useful for reactions with initial powers of more than 5 μW is explicable through consideration of the results of the reaction systems measurements. As shown in Figs. 11 and 12, triacetin in a buffer solution of acetic acid and imidazole (Fig. 11(b)) and the solid state ascorbic acid with addition of 30 μl water (Fig. 12(b)) required dissipation times of ca. 35 and ca. 25 min, respectively, even when ampoules were lowered with the apparatus. That is to say, even if the dissipation time for loading can be reduced to around 7 min then the effect will not be observed for reactions that produce significant power (i.e. >5 μW) at early times. These results suggest that it takes a relatively long time (e.g. 35 and 25 min) for the thermopiles to completely convert the difference in power between sample and reference ampoules into electric potential. This consideration is supported as follows; the power obtained from the ascorbic acid study with addition of 30 μl water when lowered with the apparatus (Fig. 12(b)) increased more rapidly, soon after the ampoules

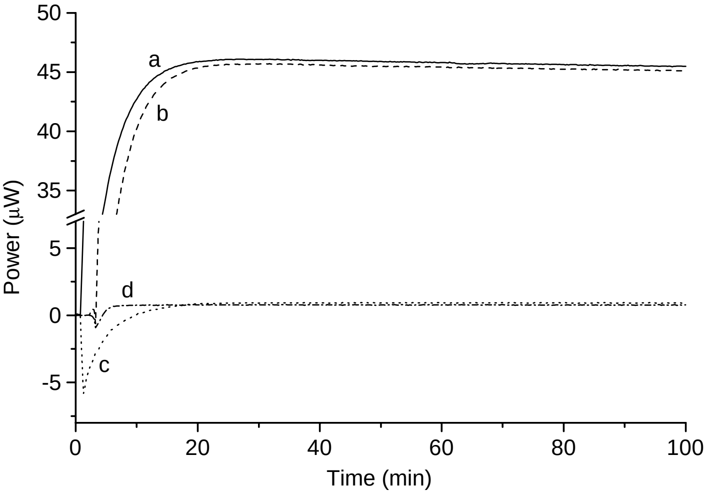

Fig. 11. Power–time curves for the hydrolysis of triacetin in the presence (a, b) and absence (c, d) of imidazole. The ampoules were lowered manually over period of 60 s (a, c) or using the vessel lowering apparatus over period of 240 s (b, d).

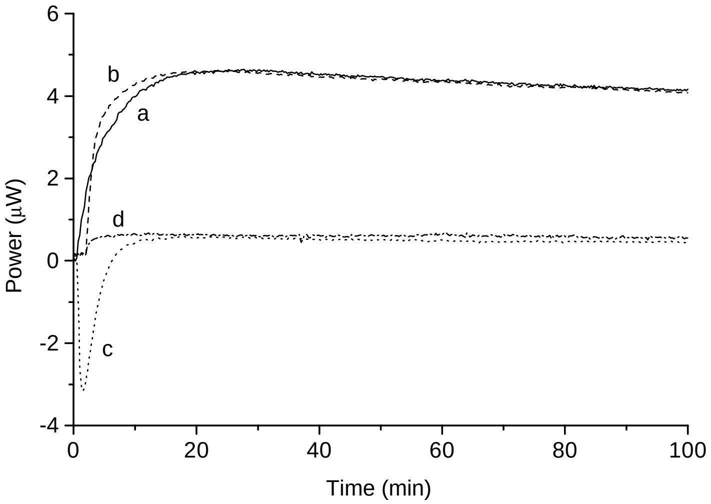

Fig. 12. Power–time curves for the solid state oxidation of ascorbic acid with addition (a, b) and without addition (c, d) of 30 μl of water. The ampoules were lowered manually over period of 60 s (a, c) or using the vessel lowering apparatus over period of 240 s (b, d).

were at the measuring position, than when lowered manually (Fig. 12(a)). This is because there is no influence of thermal shock (endothermic) as is shown in the power–time curves displayed in Figs. 11(c) and 12(c).

In practice, lowering ampoules at as slow and constant speeds as possible is a very difficult for operators to achieve consistently; the critical slow speed should be around 60 s as is demonstrated in this study. The results reported here suggest that since the extent of thermal shock depends upon the manner in which manual loading is done, then we should expect variation in inter- and intra-operation studies. The proposed lowering procedure can not only free operators from the hazards associated with ampoule lowering but also allows greater reproducibility in the derived power–time curves.

In summary the use of this device has both fundamental and practical consequence. Earlier, reliable data capture clearly gives greater confidence to the analysis of calorimetric data over the study lifetime. In practical terms calorimetric experiments should be, as far as loading the calorimeter is concerned, operator independent. Moreover, this simple mechanisation of the procedure to lower ampoules into the calorimeter offers a prospect for automation of ampoule-based studies; particularly for those reactions of low power.

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