DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 9, pp. 925–930, 2004

# A Rapid Micro Quantification Method of Paracetamol in Suppositories Using Differential Scanning Calorimetry

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## ABSTRACT

This study adopts Differential Scanning Calorimetry (DSC) to analyze the thermal properties of samples (2.5–4.0 mg) from the tip, middle, and base sections of individual paracetamol suppositories, which were sampled carefully using a stainless steel scalpel. The contents of paracetamol present in the samples obtained from these sections were determined from the enthalpies of fusion of paracetamol and expressed as % w/w paracetamol to allow comparison of the amount of paracetamol found in each section. The tip, middle, and base sections contained 10.1±0.2%, 10.1±0.2%, and 10.3±0.2% w/w paracetamol, and are statistically similar (One-way anova; $p>0.05$). This indicates that the preparation technique adopted produces high quality suppositories in terms of content uniformity. The contents of paracetamol in the 120-mg paracetamol suppositories determined by DSC and UV spectrophotometry were statistically equivalent (Students's t-test; $p>0.05$), 120.8±2.6 mg and 120.8±1.5 mg, respectively, making DSC a clear alternative method for the measurement of content of drug in suppositories. The main advantages of the method are that samples of only 2.5–4.0 mg are required and the procedure does not require an extraction process, which allows for the analysis to be completed rapidly. In addition, it is highly sensitive and reproducible, with the lower detection limit at 4.0% w/w paracetamol, which is about 2.5 times lower than the content of paracetamol (10% w/w) present in our 120-mg paracetamol suppositories and commercial paracetamol suppositories, which contained about 125 mg paracetamol. Therefore, this method is particularly suited for determination of content uniformity in individual suppositories in quality control (QC) and in process quality control (PQC).

*Key Words:* Content uniformity; DSC; Paracetamol; Suppository; Thermal analysis.

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DOI: 10.1081/DDC-200034991
0363-9045 (Print); 1520-5762 (Online)

## INTRODUCTION

In the production of suppositories, uneven distribution of the drug in individual suppositories may arise due to separation of suspended drug particles during the solidification phase of the fatty base, especially of high density drug particles. This may lead to an undesirable absorption profile of drug through the rectum into the body; therefore, it is pertinent to determine drug uniformity in individual suppositories.[1–3] However, to assay 2.5–4.0-mg samples obtained from different parts of the suppository is tedious and usually involves complex extraction procedures.

The present study uses Differential Scanning Calorimetry (DSC) as a rapid, micro quantification method to determine the uniformity of content of paracetamol in suppositories. The reasons for its choice are firstly, paracetamol is one of the drugs commonly formulated as suppositories; secondly, it has a characteristic and well-shaped calorimetric peak; and thirdly, the other components, such as the suppository base, do not influence the area of this calorimetric peak. The last two criteria, if satisfied, are the basis of quantitation of active ingredients in the drug dosage forms.[4,5] Some examples of quantitative determination of components of drug products such as paracetamol in Doliprane tablets and others have been reported.[4] However, for suppositories including paracetamol suppositories, DSC is mainly adopted to determine the quality and the polymorphic behavior of the excipient[4,6] rather than to determine the drug content. Recently, determinations of diclofenac in pharmaceutical formulations, including suppositories, have been reported. However, this requires an extraction procedure to remove diclofenac from the suppository base before DSC analysis.[5] In this study, we attempted to determine the content of paracetamol embedded in suppository base directly without extraction procedure using DSC.

## EXPERIMENTAL

### Materials

Hydrogenated palm kernel oil (Batch No. 0040933801) and hydrogenated palm kernel stearin (Batch No. 0091420002) were obtained from Cargill (M) Sdn. Bhd., Kuala Lumpur, Malaysia. Stearic acid (Batch No. Tristar149), glyceryl monostearate (Batch No. E01/096), and paracetamol (BP grade, Batch No. 020766) were donated by Hesego Industry (M) Sdn. Bhd., Esterchem (M) Sdn. Bhd., and Raza Manufacturing Sdn. Bhd., Kuala Lumpur, Malaysia, respectively. Other chemicals of analar grade were obtained from either Sigma or Fisher Scientific, U.S.

### Preparation of Suppository Base

The palm kernel oil suppository base was prepared by mixing hydrogenated palm kernel oil and hydrogenated palm kernel stearin (8:2) using a Erweka mixer (Model No. AR 402, 45°C, paddle stirrer speed: 100 rpm), followed by the addition of stearic acid (5% w/w) and glyceryl monostearate (5% w/w). The blend was allowed to solidify at 25°C for 1 week and thereafter kept at 4°C until use.

### Preparation of Paracetamol Suppositories

Paracetamol suppositories (a batch of 60) with the dosage strength of 120 mg each were prepared by melting the suppository base at 40°C and sufficient paracetamol powder incorporated. A double casting method[1,2] was adopted and the paracetamol suppositories were allowed to solidify at 25°C for 60 min before analysis. The average weight of each paracetamol suppository was 1192.1±11.4 mg.

### DSC Studies

To prepare the standard fusion enthalpy concentration curve, paracetamol suppositories (five for each concentration) with 0–75% w/w paracetamol were prepared according to the procedure described previously.

The differential scanning calorimeter (DSC 6, Perkin Elmer, U.S.) was connected to a chiller (C6, Perkin Elmer, U.S.) and a thermal analysis gas station (Perkin Elmer, U.S.) to control the flow of the purge gas, nitrogen, at a flow rate of 20 mL/minute. The DSC

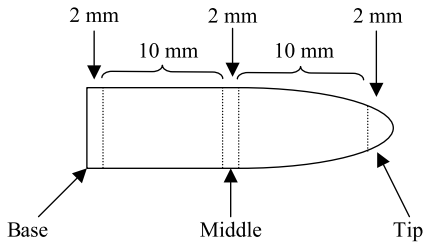


*Figure 1.* Location of sampling done on different parts of the suppository for DSC analysis.

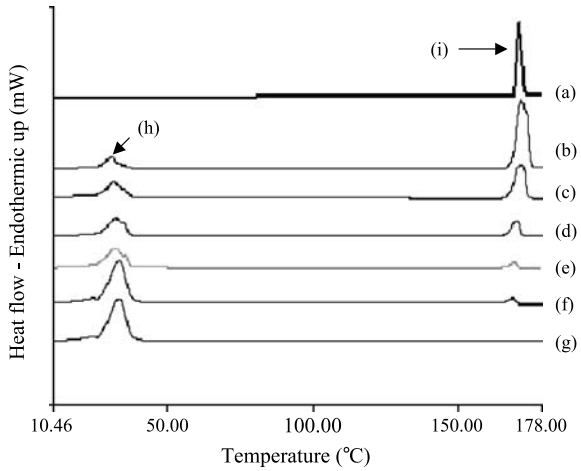


***Figure 2.*** Representative thermograms of different concentrations (% w/w) of paracetamol in paracetamol-suppository base mixtures. (a) 100% w/w paracetamol; (b) 75% w/w paracetamol; (c) 50% w/w paracetamol; (d) 25% w/w paracetamol; (e) 10% w/w paracetamol; (f) 5% w/w paracetamol; (g) 100% w/w suppository base (0% w/w paracetamol); (h) suppository base melting peak; (i) paracetamol melting peak.

pans used were 50-μL closed aluminum pans (with holes), and indium and zinc (Perkin Elmer, U.S.) were used to calibrate the DSC. Each sample weighing between 2.5–4.0 mg (Mettler Toledo UMT2 Microbalance, Switzerland) was scanned from 0°C to 180°C at 5 K/min using DSC. One measurement was performed on each of the five samples at each concentration and the concentrations tested were between 0–100% w/w paracetamol. The standard fusion enthalpy concentration curve was plotted based on the average enthalpies determined from the melting peak of paracetamol and the % w/w content of paracetamol.

To determine the content uniformity, five 120-mg paracetamol suppositories were selected randomly and samples weighing between 2.5 and 4.0 mg were taken from different sections (tip, middle, and base sections) of the suppository using a stainless steel scalpel as depicted in Fig. 1. The samples were then scanned using the conditions described above. The enthalpy change of the paracetamol melting peak was used to determine the paracetamol concentration based on the standard fusion enthalpy concentration curve.

## UV Spectrophotometric Studies

To determine content uniformity, five 120-mg paracetamol suppositories were selected randomly and the content of paracetamol assayed according to the U.S. Pharmacopoeia (USP) method with modifications.[7] Each suppository was weighed and transferred to a separating funnel, followed by addition of 30 mL of hexane to dissolve the suppository. Thirty mL of water was then added and the separating funnel shaken gently and the two phases were allowed to separate. The aqueous layer was transferred to a 200-mL volumetric flask and the hexane layer washed with 30 mL of water twice. The aqueous layer and washings were pooled; thereafter, a solvent consisting of three parts water and one part methanol was added to the pooled aqueous layer to make up to 200 mL. Five mL of the resulting solution was transferred to a 250-mL volumetric flask and made up to 250 mL with the diluent (three parts water and one part methanol) and mixed well. The resulting solution was then filtered through a 0.45-μm filter and its absorbance measured at 249 nm using a Perkin Elmer Lambda 40 uv/vis spectrophotometer. The content of paracetamol was

***Table 1.*** Thermal characteristics of various concentrations of paracetamol (0–100% w/w) and suppository base in paracetamol-suppository base mixtures.

| | Paracetamol | | | Suppository base | | |
|---|---|---|---|---|---|---|
| Paracetamol-suppository base mixtures[a] | Paracetamol (% w/w) | Melting peak (°C) | Enthalpy of fusion, ΔH (J/g) | Suppository base (% w/w) | Melting peak (°C) | Enthalpy of fusion, ΔH (J/g) |
| g | 0 | – | 0 | 100 | 31.7±0.5 [1.53%] | 132.0±0.8 [0.62%] |
| f | 5 | 169.1±0.3 [0.20%] | 5.7±0.3 [6.03%] | 95 | 31.1±0.2 [0.64%] | 127.0±0.7 [0.52%] |
| e | 10 | 169.6±0.3 [0.21%] | 13.1±0.5 [3.98%] | 90 | 31.7±0.4 [0.31%] | 115.1±0.7 [0.57%] |
| d | 25 | 170.0±0.1 [0.09%] | 35.0±1.1 [3.04%] | 75 | 31.1±0.5 [1.71%] | 88.8±0.8 [0.92%] |
| c | 50 | 170.5±0.5 [0.31%] | 72.1±2.2 [3.04%] | 50 | 30.7±0.6 [1.80%] | 56.9±0.8 [3.13%] |
| b | 75 | 170.8±0.5 [0.29%] | 119.0±3.1 [2.63%] | 25 | 30.3±0.4 [1.29%] | 24.0±0.8 [3.31%] |
| a | 100 | 170.8±0.2 [0.09%] | 144.6±2.3 [1.60%] | 0 | – | 0 |

[a]Representative thermograms are as depicted in Fig. 2. Each value was expressed as Mean (n = 5) ± SD. [ ]: RSD.

*Table 2.* Thermal characteristics of various concentrations (0–25% w/w) of paracetamol in paracetamol-suppository base mixtures.[a]

| Paracetamol (% w/w) | Melting peak (°C) | Enthalpy of fusion, ΔH (J/g) |
|---|---|---|
| 0 | – | 0 |
| 1.0 | 168.4±0.4 [0.24%] | 0.8±0.1 [10.84%] |
| 2.0 | 168.2±0.2 [0.10%] | 1.4±0.1 [5.92%] |
| 3.0 | 168.5±0.2 [0.13%] | 2.1±0.1 [4.80%] |
| 4.0 | 168.8±0.3 [0.19%] | 4.8±0.2 [3.98%] |
| 5.0 | 169.1±0.3 [0.20%] | 5.7±0.4 [6.03%] |
| 6.5 | 168.8±0.3 [0.18%] | 8.0±0.4 [4.51%] |
| 8.0 | 168.7±0.4 [0.21%] | 10.7±0.5 [4.47%] |
| 10.0 | 169.6±0.4 [0.21%] | 13.1±0.5 [3.98%] |
| 12.0 | 169.7±0.3 [0.16%] | 15.0±0.5 [3.08%] |
| 13.0 | 169.8±0.4 [0.26%] | 16.5±0.3 [1.98%] |
| 15.0 | 169.2±0.4 [0.24%] | 19.5±0.7 [3.48%] |
| 17.0 | 169.5±0.4 [0.21%] | 22.4±0.9 [3.93%] |
| 20.0 | 169.7±0.2 [0.11%] | 27.6±0.9 [3.30%] |
| 25.0 | 167.0±0.2 [0.09%] | 35.0±1.1 [3.04%] |

[a]Representative thermograms are as depicted in Fig. 2. Each value was expressed as Mean (n = 5)±SD. [ ]: RSD.

then determined from the absorbance using a paracetamol standard concentration curve (1, 2.5, 5, 10, 20, and 30 μg of paracetamol/mL; n=3 for each concentration) and corrected for percentage recovery.

The influence of the suppository base on extraction of paracetamol was assessed by spiking 30, 60, and 120 mg paracetamol to each weighed 120 mg paracetamol suppository or blank suppository (without paracetamol). Each experiment was repeated five times and the extraction and measurement procedures outlined above were followed. The percentage recovery of paracetamol and reproducibility were calculated.

## RESULTS AND DISCUSSION

The DSC thermograms of the paracetamol suppositories consisted of two distinct endothermic peaks at 30°–38°C and 166°–173°C, respectively (Fig. 2). The former is the melting peak of the suppository base while the latter is that of paracetamol, which is consistent with the literature.[8,9] The heat transmission to the crystalline paracetamol embedded in the fatty matrix was influenced by increasing concentration of suppository base as evidenced by the melting peak shift from 170.8° (100% w/w paracetamol) to 168.4°C (1% w/w paracetamol) (Tables 1 and 2). However, the respective calorimetric peaks were characteristic of paracetamol and suppository base, and therefore, the components were unlikely to form eutectic mixtures. Consequently, the area under the paracetamol melting peak represents the enthalpy of fusion and is directly related to the quantity of paracetamol presented in the sample, and is supported by the linear curve of enthalpy of fusion vs. concentration of paracetamol (between 4–100% w/w) (Figs. 3 and 4). For a drug that forms eutectic mixtures with a suppository base, the melting point of the drug will be depressed or lowered significantly, and the depression in melting point depends on the relative concentration of drug to suppository base. In this case, the area under the drug melting peak is no longer directly related to the quantity of drug presented in the mixture and cannot be used for quantification of the drug.

The enthalpy of fusion at 169°–173°C of pure paracetamol and the enthalpy of fusion at 26°–38°C of pure suppository base (n=5) were found to be 144.6±2.3 ([Relative Standard Deviation] RSD, 1.60%) and 132.0±0.8 J/g (RSD, 0.62%) respectively (Table 1). The results also showed that the RSD on melting peak for both paracetamol and suppository base at various

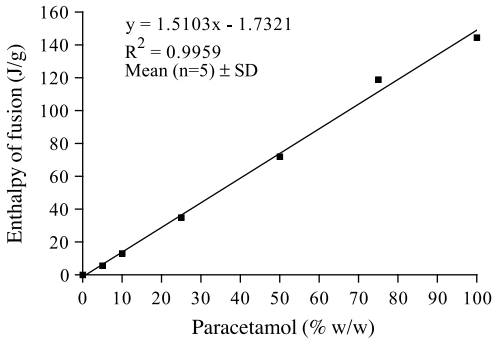


*Figure 3.* Standard fusion enthalpy concentration curve of paracetamol (0–100% w/w) in paracetamol-suppository base mixtures.

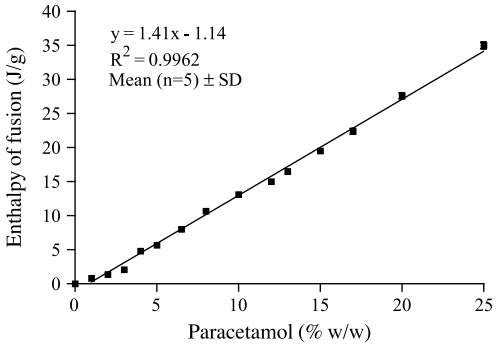


*Figure 4.* Standard fusion enthalpy concentration curve of paracetamol (0–25% w/w) in paracetamol-suppository base mixtures.

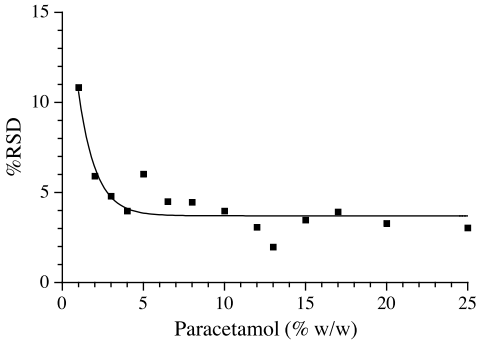


***Figure 5.*** %RSD of enthalpy of fusion vs. concentration of paracetamol (0–25% w/w) in paracetamol-suppository base mixtures.

concentrations were highly reproducible (RSD, below 2.0%) (Table 1). At a high concentration range (0–100% w/w paracetamol), the standard concentration curve of enthalpies of fusion of paracetamol vs. concentrations of paracetamol was linear, giving a correlation coefficient of 0.9959 (Fig. 3). The RSD of each data point was 6.0% or less, suggesting each data point was reproducible (Table 1). However, in the estimation of content of paracetamol in the suppositories, the enthalpy of fusion of paracetamol was in the range of 13.0 J/g, hence, a concentration curve covering the lower concentration range (0–15% w/w paracetamol) was necessary. The standard enthalpy of fusion vs. concentration curve was linear, between 4–25% w/w paracetamol and gave a correlation coefficient of 0.9962 (Fig. 4). However, below 4% w/w paracetamol, linearity was deviated. This clearly suggests that at low concentration both heat transmission and enthalpy of fusion of embedded crystalline paracetamol were affected by suppository base. The RSD of each data point between 2% and 25% w/w paracetamol was 6.0% or less; however, at 1.0% w/w paracetamol, RSD increased to 10.8% (Table 2 and Fig. 5). This suggests the method is reproducible and accurate, and the lower detection limit is at about 4% w/w paracetamol. Therefore, if the % w/w of paracetamol is 10 and above, as in the case of the 120-mg paracetamol suppositories, the use of enthalpy of fusion to estimate the content of paracetamol is reproducible and is suitable as a rapid test.

The main advantages of this method are the utilization of minute quantities of samples, between 2.5 to 4 mg, and the samples can be directly analyzed using DSC without a complicated extraction process. Using the DSC method, we analyzed samples (2.5–4.0 mg) from different parts of individual suppositories, and this allowed the distribution of paracetamol in individual suppositories to be ascertained (Fig. 1). It is observed that the contents of paracetamol from samples obtained from the tip (10.1%±0.2% w/w), the middle (10.1%±0.2% w/w), and base (10.3%±0.2% w/w) sections are similar (one-way anova; $p>0.05$) (Table 3). The results in general support the observation that the uniformity of content in individual suppositories is homogeneous and the techniques involved in preparation of 120-mg paracetamol suppositories is acceptable.

The studies on the influence of suppository base on measurement of paracetamol content showed the mean percentage recovery of paracetamol was 98% and the values were reproducible (RSD, 2.07). This suggests that the UV method to determine the content of paracetamol in suppositories is reliable. Using the enthalpies from the DSC thermograms, the content of paracetamol in the suppositories was found to be 120.8±2.6 mg per suppository compared to the 120.8±1.5 mg obtained from the UV method (Table 3). An independent Student's t-test indicates that the two values are similar ($p>0.05$) and Levene's test ($F=0.487$, $p=0.511$) shows that the variances of the samples are equal. This clearly

***Table 3.*** Content of paracetamol per 120-mg paracetamol suppository determined by DSC and UV spectrometry.[a]

| Section of suppository tested | Enthalpy of fusion for paracetamol (J/g) | Paracetamol (% w/w) | Weight of paracetamol per suppository determined by DSC (mg) | Mean weight of paracetamol per suppository determined by DSC (mg) | Mean weight of paracetamol per suppository determined by UV (mg) |
|---|---|---|---|---|---|
| Tip | 13.1±0.3 | 10.1±0.2 | 120.0±2.1 | | |
| Middle | 13.1±0.3 | 10.1±0.2 | 120.1±2.8 | 120.8±2.6 | 120.8±1.5 |
| Base | 13.4±0.3 | 10.3±0.2 | 122.3±2.2 | | |

[a]Each value was expressed as Mean (n = 5) ± SD. The statistical test on enthalpy of fusion, content of paracetamol, and weight of paracetamol per suppository determined from base, middle, and tip sections of the suppository are not significantly different (one-way anova: $p>0.05$). Independent t-test indicates the mean weights of paracetamol determined by DSC and UV are not significantly different ($p>0.05$).

indicates that determinations of content using DSC and the UV spectrophotometer yield similar results and therefore, DSC can be an alternative to the measurement of content of some drugs in suppositories.

## CONCLUSION

The study supports the use of DSC to determine the content of drugs such as paracetamol that do not form eutectic mixtures with the suppository base from the enthalpy change of the melting peak of the drug. The main advantages of using DSC to estimate content of drug in suppositories are 1) utilization of small quantities of samples (2.5–4.0 mg), and 2) that the method does not require an extraction process and can be completed rapidly.

It is concluded, therefore, that this method is particularly suited for validation of the manufacturing process of suppositories as it allows uniformity of content in individual suppositories, which can be determined rapidly.

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