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Use of microcalorimetry in determination of stability of enalapril maleate and enalapril maleate tablet formulations

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Abstract

The stability properties of enalapril maleate (EM) and of different tablet formulations including EM were studied by isothermal microcalorimetry and by high performance liquid chromatography (HPLC). It was shown that water content of the sample and elevated temperature have a high impact on stability properties of the substance itself and of the formulations including this substance. The degradation is more extensive at higher water content and at elevated temperature. The type of the tablet formulation (5 or 20 mg EM tablet formulation) also has an impact: the 5 EM tablet formulation is the less stable one. The heat output of individual tablet formulations was used to evaluate the enthalpy changes and to calculate the difference in the amount of degraded EM between various samples. These results agreed satisfactorily with those obtained by HPLC. Isothermal microcalorimetry proved to be a fast and predictive method that could be used in preformulation studies to accelerate the pharmaceutical development and shorten the time before launching the product to the market.

Keywords: Isothermal microcalorimetry; Chromatography; Enalapril maleate; Stability; Active substance; Formulation

1. Introduction

Stability studies are an integral part of the drug development program and have a very important role in the registration documentation for each individual pharmaceutical product and drug substance. According to International Conference on Harmonization (ICH) note for guidance on stability testing: stability testing of new drug substances and products (CPMP/ICH/2736/99) long term and accelerated stability studies have to be carried out to prove the stability of the marketed product and to ensure its shelf life.

When developing final formulation, which needs to be tested according to ICH stability testing guidelines, stability properties of various preformulation mixtures and early formulations have to be closely monitored by rapid and simple techniques. Techniques that give enough information for the selection of substances or formulations with good stability properties are crucial for correct and fast decisions during the development process. These techniques must distinguish between substances, excipients or formulations with better stability properties in comparison with the non-promising ones. Isothermal microcalorimetry is one of such promising techniques (Gaisford and Buckton, 2001).

Isothermal microcalorimetry is non-specific thermoanalytical method that is used by pharmaceutical industry especially to determine the stability, compatibility and amorphicity properties (Phipps and Mackin, 2000). Calorimetry produces two types of data: heat output Q in J (thermodynamic data) and heat flow $\Phi = dQ/dt$ in W (kinetic data). The technique measures in principle the heat conduction into, or out of a measuring cell, from, or to, a heat sink (Buckton, 1995). The heat flows relating to exothermic processes can therefore be positive or negative depending on the instrument used. The isothermal microcalorimetry was shown to be applicable to track slow reactions that occur in real drug systems (Beezer et al., 1999). An essential difference in comparison with conventional techniques is that a variable of processes are examined at the same time and that the method is non-specific. Both could be understood as disadvantages of the method, but in preformulation studies and when rapid and selective

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decisions have to be taken it is important to be able to select from the whole range of possible formulations one or two that give the best results. At a later stage of the development it is necessary to investigate also the origin of thermal events. For this purpose additional specific methods should be used (Selzer et al., 1998).

Several authors have shown the applicability of this method for studying various aspects of stability (Roškar and Kmetec, 2005; Schmitt et al., 2001; Zaman et al., 2001a,b; Jakobsen et al., 1997; Wilson et al., 1995; Buckton, 1995; Pikal and Dellerman, 1989) but no use of microcalorimetry for a widely used drug substance enalapril maleate (EM) could be found.

Prodrug EM is an ethyl ester of a long-acting angiotensin converting enzyme inhibitor, enalaprilat (ET). It is indicated and worldwide used for the treatment of essential and renovascular hypertension. EM is known to be a very stable chemical compound. The compound when stored at room temperature (amber glass) for 4 years shows no evidence of degradation as determined by HPLC analysis. Less than 2% of degradation can be induced by storage at 80 °C for 3 weeks and less than 1% of decomposition by storage at 40 °C and 75 RH (open vial) for 16 weeks (Ip and Brenner, 1987). Two main degradation products of EM are ET and diketopiperazine (DKP).

EM can easily be destabilised in the presence of a tablet matrix and degradation can rapidly occur when the selection of excipients is not closely monitored. For that reason extensive studies of stabilisation of pharmaceutical dosage form with EM are necessary (Rotar, 2000).

In our survey of the literature we did not find any use of microcalorimetry in the development of EM formulations. The purpose of this work was to investigate whether microcalorimetry can be used in the early stages of drug development for distinguishing between more and less stable substances or tablet formulations. First we performed studies with the drug substance itself and afterwards with two developed formulations that have already obtained Marketing Authorisations in various countries. We induced instability of these formulations by the addition of water to the sample because it has been shown that water content has an influence on stability of EM itself and also on EM formulations (Stanizs, 2003; Rotar, 2000; Shiromani and Bvitz, 1986). The purpose of water addition to the samples was also to study the sensitivity of microcalorimetry in connection with changed stability of the sample induced with different amounts of added water.

2. Materials and methods

2.1. Materials

EM was Ph. Eur. grade, batch no. 25371202, produced by Krka, d.d., Novo mesto.

Other ingredients used in studied formulations of EM 5 and 20 mg tablets are sodium hydrogen carbonate, lactose monohydrate, maize starch, hydroxypropylcellulose, talc, magnesium stearate and are all of Ph. Eur. grade. EM 20 mg tablets contain in addition to previously stated also coloring ingredients color sicopharm red and color sicopharm yellow of pharmaceutical grade in total quantity of 0.2%.

EM 5 mg tablets were batch no. N07247, produced by Krka, d.d., Novo mesto.

EM 20 mg tablets were batch no. N08387, produced by Krka, d.d., Novo mesto.

For high performance liquid chromatography (HPLC) assays, phosphate buffer (0.01 M, pH 2.0) and acetonitrile (Merck) were used.

For Karl–Fisher titration the following reagents were used for the analysis: Hydranal solvent, batch no. 5313A, Hydranal standard—Sodium Tartrate Dihydrate, batch no. 31150 and Hydranal Composite 5, batch no. 5248A. All reagents were produced by Riedel-de Haen.

2.2. Methods

A MicroDSC III (Setaram) calorimeter, operating in the isothermal mode at various temperatures was used together with Hastelloy closed batch vessels. Temperature was maintained with a precision $\pm 1 \times 10^{-4}$ °C. The calorimeter measures the heat conduction out of the sample cell to a heat sink so the output presents exothermic processes as positive heat flows and endothermic processes as negative heat flows.

The calorimeter was calibrated using the Joule effect method (as described in Micro DSC III User Manual by Setaram) in the range from 20 to 80 °C before experiment set.

For the experiments 100 mg of pulverized sample was loaded into the sample vessel. Active substance was already in form of a powder so no preparation was needed. Samples of tablets were prepared by crushing the tablets to obtain powder. The reference vessel was either empty (in case of substance studies) or filled with 100 mg of placebo mixture corresponding to the formulation (in case of formulation studies). The instrument was operated following the manufacturer's instructions. In all experiments the heat flow signal was recorded for at least 60 h.

High performance liquid chromatography (HPLC), area percent method was used for the determination of the contents of EM, ET and DKP. HPLC instrument (Hewlett Packart 1100 Series) with a variable UV detector and column thermostat was used. Analyses were performed under the following conditions: Hypersil ODS, 5 μm particles, 250 mm × 4 mm i.d. column at temperature 70 °C and phosphate buffer (pH 2.0):acetonitrile = 58:42 (V/V) as the mobile phase. Samples were prepared as solutions in phosphate buffer (pH 2.0) in concentrations of approximately 1.0 mg/ml. UV detection was performed at 215 nm.

Karl–Fisher titration was used for the determination of water in intact and in dried samples. Karl–Fisher titrator Mettler DL 35 with 5 ml burette was used.

3. Results and discussion

3.1. Studies of EM drug substance

The first objective of the isothermal microcalorimetry study was to confirm the stability properties of the EM which have

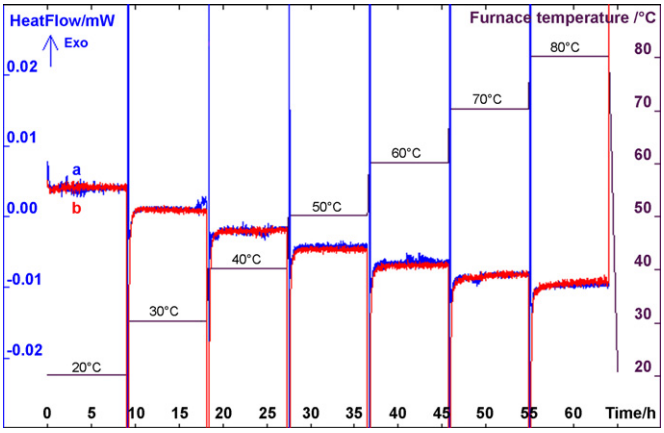

Fig. 1. Two superimposed power–time curves measured at different temperatures and printed on one thermogram: (a) empty cell measurement and (b) EM sample measurement.

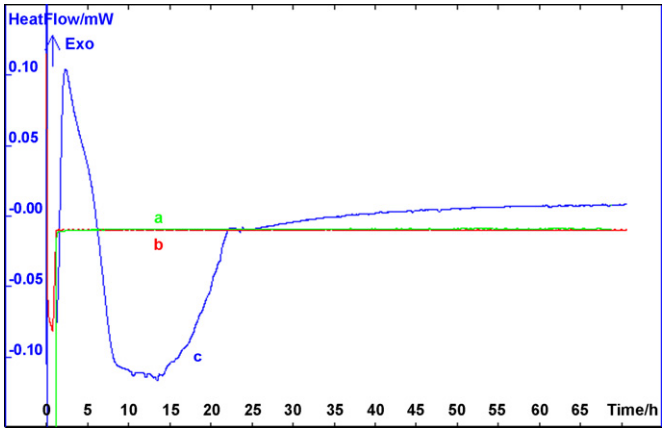

Fig. 2. Power–time curves of EM at 80 °C. (a) Intact sample, (b) stabilised sample and (c) destabilised sample (10 μl of water added); (a) and (b) have the same heat flows.

already been published by several authors (Stanizs, 2003; Rotar, 2000; Ip and Brenner, 1987), but were determined by using analytical methods other than microcalorimetry. We performed isothermal studies of 100 mg of EM versus empty cell at several temperatures in the range from 20 to 80 °C by keeping the substance for 8 h at isothermal conditions. The difference between the thermograms of empty cell versus empty cell (no chemical or physical transformation) and of EM sample versus empty cell (possible degradation or transformation process) was smaller than the noise of the calorimetric signal which is approximately 1 μW (see thermograms reported in Fig. 1). This indicates that EM is a very stable substance even at elevated temperatures such as 80 °C. HPLC study of the same sample before and after exposure to the described microcalorimetric study showed that the amount of EM before and after exposure to elevated temperatures remained the same. The same holds for the degradation products ET and DKP. This is in accordance with microcalorimetric results and confirms that EM does not degrade even at elevated temperatures.

In additional studies of the drug substance we tried to accelerate the degradation of EM. The data obtained from isothermal microcalorimeter and HPLC were compared. The studies were performed at various temperatures (80, 70, 60 and 40 °C) using both methods. Because water is one of the crucial factors that affect the stability of EM we decided to accelerate the chemical processes by adding different amounts of water (5, 10 and 20 μl). The quantity of water was added on the basis of the molar ratio between EM and water; this ratio was approximately 1:0.5, 1:1 and 1:2, which amounts to approximately 5, 10 and 20% of water in the total amount of sample. On the other hand we also tried to increase the stability of the substance by drying the sample at 100 °C for 3 h as stated in Ph. Eur. test for Loss on drying.

In the following text we use the term intact sample for the non-treated one, stabilised sample for the dried one and destabilised sample for the EM treated with water. Content of water in the intact and in the stabilised sample was determined with the Karl–Fisher titration.

Isothermal microcalorimetry results obtained at 80 °C are presented in Fig. 2. They show heat flows for the stabilised and the intact sample in comparison with the destabilised sample. It is evident that the destabilised EM shows large deviations in heat flows when compared to the other two samples. This implies that different chemical and physical processes are taking place in the sample. During the first 6 h the heat flow positive indicating that exothermic reactions are dominant. Between 6 and 23 h, on contrary, endothermic processes prevail.

In the literature (Stanizs, 2004) the first order kinetics of the degradation of EM was demonstrated. The tablets used in this study had the same qualitative composition as the ones in our paper. This finding lead us to use the first order kinetics in all calculations of EM degradation based on microcalorimetry.

In agreement with the calorimetric signals in Fig. 2, HPLC results of samples that were kept at 80 °C in the microcalorimeter clearly show that in the presence of 10 μl of water the degradation occurs rapidly. After 72 h of exposure of the destabilized sample to 80 °C only approximately 3% of EM remains. By taking into account the first order kinetics (see above) it was estimated that after 6 h approximately 30% of EM has degraded. Because higher degrees of degradation are of minor importance for the pharmaceutical development it was decided to monitor this part of the thermogram (1–6 h) where the heat flow was positive indicating exothermic reactions. At high degrees of degradation several additional reactions are most likely going on and prevent the evaluation of enthalpy value related to EM degradation only.

In the case of the stabilised and the intact sample with a very low content of water no degradation could be observed neither with HPLC nor with microcalorimetry. Because water has influence on stability of EM we performed the Karl–Fisher titration test and determined the water content in both samples. They contained approximately 0.06% of water indicating that EM is not hygroscopic.

The degradation of EM leads through two main degradation products, ET and DKP. At higher degrees of degradation (more than 5%) many other degradation products can be observed in the HPLC chromatograms. HPLC analysis of samples being exposed to 80 °C for different time intervals actually showed a decrease in the amount of ET and DKP at high degrees of EM

Table 1
Results of HPLC analysis of EM and main degradation products (ET and DKP) before and after microcalorimetric study at various temperatures and for various water contents in the sample

Temperature	Added water (μl)	EM (%)	ET (%)	DKP (%)
	Intact sample before study	99.87	0.04	bld
80 °C	0 (stabilised sample)[a]	99.82	0.02	0.06
	0 (intact sample)[a]	99.87	0.02	0.04
	5	9.62	2.13	52.96
	10	3.31	1.70	38.57
70 °C	0 (stabilised sample)[a]	99.69	0.02	0.17
	0 (intact sample)[a]	–	–	–
	10	84.32	1.58	9.79
60 °C	0 (stabilised sample)[a]	99.24	0.02	0.11
	0 (intact sample)[a]	99.89	0.05	0.06
	10	98.63	0.05	0.02
	20	97.81	0.22	0.67
40 °C	0 (stabilised sample)[a]	–	–	–
	0 (intact sample)[a]	99.92	0.03	0.04
	10	99.04	0.04	0.04
	20	99.08	0.03	0.03

– Test was not performed.
[a] Water content of intact and stabilised sample was determined by Karl–Fisher titration and was equal to 0.06%.

degradation. This suggests subsequent degradation of both main degradation products and also leads to the fact that percentages of EM, ET, and DKP (see results in Table 1) do not add to 100%.

The results obtained at 80 °C indicated that the observed qualitative correlation between the thermograms and the extent of degradation in the case of EM drug substance can be used in pharmaceutical development because they offer a possibility to predict differences in stability properties of EM in the early stage of pharmaceutical development. We expected to see some differences in the thermograms of the stabilised and the destabilised sample also at lower temperatures. Thermogram of the sample studied at 70 °C with added 10 μl of water is in Fig. 3 compared with the stabilised sample. The destabilised sample shows higher exothermic heat flows in comparison with the stabilised sample, which is again consistent with higher EM degradation (see

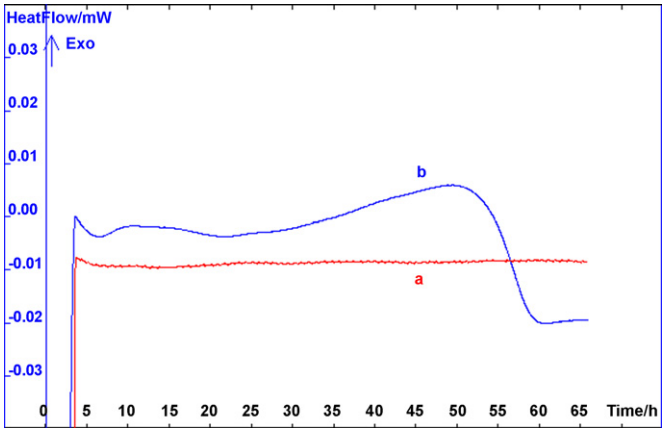

Fig. 3. Power–time curves of EM measurements at 70 °C. (a) Stabilised sample and (b) destabilised sample (with 10 μl added water).

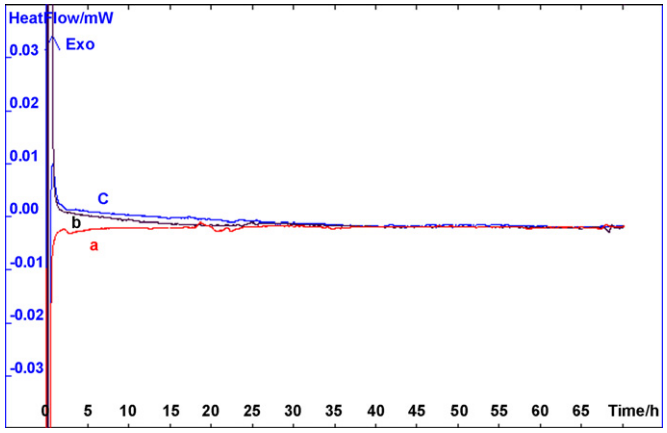

Fig. 4. Power–time curves of the EM at 40 °C. (a) Intact sample, (b) destabilised sample (with 10 μl added water) and (c) destabilised sample (with 20 μl added water).

HPLC results in Table 1). The decrease in the content of EM in the destabilised sample after exposure to isothermal microcalorimetric study at 70 °C is larger than 15%. At the staring point of degradation of EM, when only ET and DKP are formed, exothermic processes are prevailing. At a latter stage, when degradation of ET and DKP starts, the total process becomes endothermic. We may conclude that results at 70 and 80 °C both confirm that higher heat flows in the initial part of the thermogram indicates higher degree of degradation of EM.

Microcalorimetric results of samples with different water contents studied at 40 °C (Fig. 4) show that heat flows of all samples are identical and no exothermic heat flow is observed. This suggests that very likely no degradation of EM is taking place. This finding is confirmed also by HPLC results presented in Table 1.

We can conclude that the first part of our study proved that microcalorimetry can be used as a qualitative non-specific technique to study stability properties of EM and that it enables one to distinguish between more and less stable samples of EM.

3.2. *EM finished product formulations*

In the following we have extended microcalorimetry and HPLC study to the finished product formulations. Our aim was to see whether microcalorimetry can be used also to distinguish between stabilised and destabilised formulations containing EM and to show that calorimetric data (the heat output Q) can be correlated with the observed degradation. For these studies we have used marketed formulations of EM tablets containing 5 and 20 mg of EM. Both formulations differ in qualitative and quantitative composition. This could cause different stability properties that we tried to determine. We have performed comparative studies on formulations with induced instability. Afterwards we also compared stability properties of EM 5 mg tablet formulation with the ones of EM 20 mg tablet formulation.

In view of the conclusion from microcalorimetric studies on EM drug substance we expected that the formulation being less stable would show higher heat flows in the first part of the thermogram (where we expect only a few percent of EM

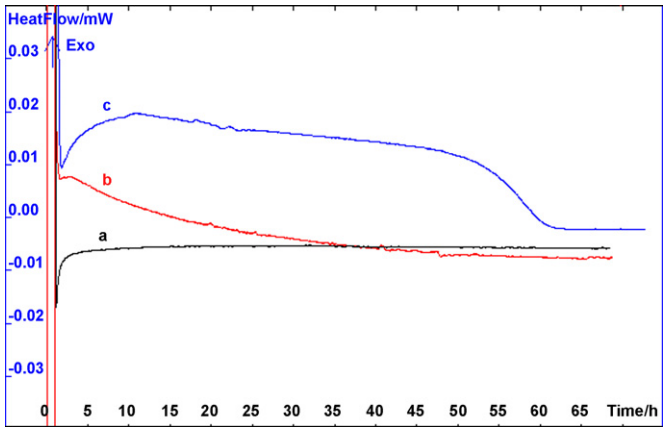

Fig. 5. Power–time curves of EM 5 mg tablets measured at 80 °C with various water contents: (a) stabilised sample (dried), (b) intact sample and (c) destabilised sample (10 μl of added water).

degradation). To show that these exothermic heat flows can be correlated with the observed degradation we have calculated the enthalpy changes for the degradation of EM in different samples and compared them. These values were correlated with HPLC results.

3.2.1. EM 5 mg tablets

We performed three sets of studies with EM 5 mg tablet formulation. In the first set of experiments we added 10 μl of water to 100 mg of the tablet mixture with the aim to accelerate the degradation of the sample. Secondly, we dried the tablet mixture to slow down any possible reactions in the sample induced by water. For the third experiment we used the intact tablet mixture with known stability properties. In the following text we use the same terminology of pre-treated samples as we did for substance samples (intact, stabilised and destabilised). The results of isothermal microcalorimetry study are presented in Fig. 5.

As already discussed above for EM substance itself one can propose that the most stable formulation is the one having the lowest heat flow in the first part of the experiment. Any deviation of the heat flow from the baseline reflects chemical or physical reactions, which in turn indicate instability of the formulation. The curve for the stabilised formulation with minimum water content shows the lowest heat flows and therefore indicates the highest stability. After approximately 5 h, the heat flow is constant and close to the base line at 80 °C (compare with Fig. 1). On the other hand, the curve for the formulation with added water shows higher heat flows in comparison with both the intact and the stabilized tablet mixture and therefore indicates the lowest stability. This was confirmed by HPLC analysis. HPLC results for all samples studied after the exposure to 80 °C in the microcalorimeter are presented in Table 2.

The change in the enthalpy for reactions in the calorimeter can be obtained by evaluating the area under the power–time curves. The heat (Q) evolved in time t is proportional to the number of moles of EM (n) that reacted in this time and to the enthalpy change (ΔH) for the process:

$$Q = n\,\Delta H \tag{1}$$

Table 2
HPLC results of EM and main degradation products (ET and DKP) in the 5 mg tablet formulation after microcalorimetric study at 80 °C for 70 h and with various water contents

Temperature	Added watter (μl)	EM (%)	ET (%)	DKP (%)
80 °C	0 (stabilised sample)[a]	93.97	1.42	1.29
	0 (intact sample)[b]	67.83	15.53	11.41
	10	5.98	59.63	17.60

[a] Water content was 4.46%.
[b] Water content was 5.18%.

Q can be obtained by integrating the power–time curves in an appropriate time interval. In our case, the time interval was chosen in such a way that the total heat output was still exothermic (positive heat flows). In the case of EM 5 mg tablets this time period was between 1 and 38 h. The first hour of the experiment corresponds to the heating of the sample to the working temperature (80 °C), whereas after 38 h we believe that the degradation of ET and DKP are the prevailing reactions. Therefore, heat flows after 38 h were not considered in this calculation.

The heats associated with the degradation of the stabilized and the destabilized samples can be written as $Q_S = n_S \Delta H$ and $Q_D = n_D \Delta H$, respectively, with n_S and n_D the number of moles of EM that degraded in the stabilized and in the destabilized sample. Here, it was assumed that the same reactions are running in the calorimeter in the case of the stabilized and the destabilized sample and therefore the enthalpy change ΔH is the same. The difference between the heat outputs of the two thermograms ($Q_D - Q_S$; the difference between the areas of the corresponding power–time curves) is thus proportional to Δn ($=n_D - n_S$), i.e. the difference in the amount of the degraded EM in both samples:

$$Q_D - Q_S = (n_D - n_S)\Delta H \tag{2}$$

Total enthalpy change of the EM degradation that was calculated on the basis of the above procedure is −734 kJ/mol for the destabilized and −672 kJ/mol for the intact sample. The difference between both ΔH values is less than 10%. It is possible that due to different water content somewhat different rates or ratios of the processes of degradation of EM may be running in the intact and in the destabilized sample, leading to differences in the total enthalpy change. However, the calculated ΔH values show that these differences are not very large. An additional conformation for this conclusion is given by the ratio $(Q_D - Q_S)/(Q_I - Q_S)$, where Q_I is the heat associated with the intact sample. This ratio should be equal to the ratio $(n_D - n_S)/(n_I - n_S)$ (see Eq. (2)) that can be evaluated from the HPLC data. Taking into account the uncertainty of both methods, the calorimetric result for $(Q_D - Q_S)/(Q_I - Q_S)$ (=4.2) is in a good agreement with the HPLC one ($(n_D - n_S)/(n_I - n_S) = 3.8$).

It can be concluded that thermograms of EM 5 mg tablets can be used to distinguish differences in the stability properties between the most stable (stabilised formulation), stable (intact formulation) and the least stable (destabilised) formulation by comparing the heat outputs of different samples.

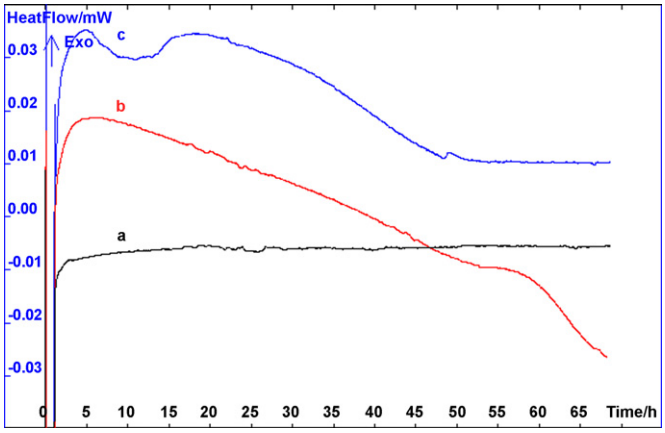

Fig. 6. Power–time curves of EM 20 mg tablets measured at 80 °C with various water contents: (a) stabilised sample (dried), (b) intact sample and (c) destabilised sample (10 μl of water added).

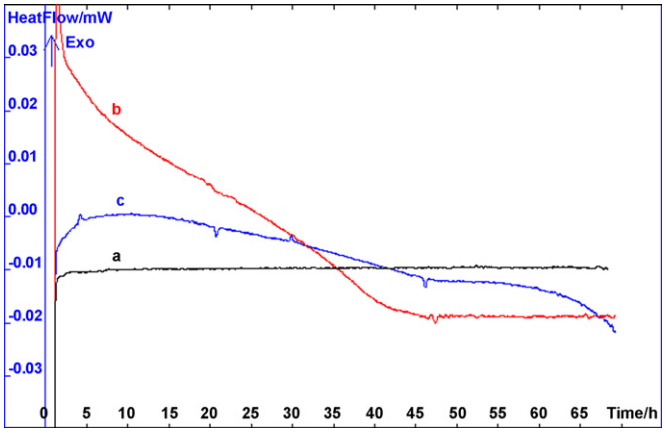

Fig. 7. Power–time curves of: (a) EM substance, (b) EM 5 mg tablets and (c) EM 20 mg tablets, all measured at 80 °C.

3.2.2. EM 20 mg tablets

We have performed studies also with EM 20 mg tablet formulation. Thermograms of the stabilised, the intact and of the destabilised EM 20 mg tablet formulation with added 10 μl of water are presented in Fig. 6. For each measurement 100 mg of tablet mixture was used.

In parallel with microcalorimetry, the HPLC analysis of the samples after exposure to microcalorimetric study at 80 °C for 70 h was performed. Results are reported in Table 3. Clear relation between water content and degree of degradation of EM in the sample can be seen by comparing results in Tables 2 and 3 and also thermograms in Figs. 5 and 6. Higher water content leads to a larger extent of EM degradation.

The calculated enthalpy changes for the degradation of EM in EM 20 mg tablet formulations are −858 kJ/mol for destabilised sample and −749 kJ/mol for intact sample. In this case, the difference between ΔH values is around 15%, whereas $(Q_D - Q_S)/(Q_I - Q_S)$ and $(n_D - n_S)/(n_I - n_S)$ are around 2.3 and 2.0, respectively. Although the discrepancy is a bit higher than in the case of EM 5 mg tablet formulation, these values again show a satisfactory agreement between microcalorimetry and chromatography. These results, in our opinion, justify the use microcalorimetry in the preformulation studies.

The comparison of HPLC and microcalorimetric results for EM tablet formulations shows a higher amount of degradation products (ET and DKP) and a lower enthalpy of reactions in the case of EM 5 mg tablets (see Tables 2 and 3). Consequently, the third set of experiments was performed in order to compare stability properties of EM 5 and 20 mg tablet formulations with microcalorimetry directly.

Table 3
HPLC results of the EM and the main degradation products (ET and DKP) in the 20 mg tablet formulation after microcalorimetric study at 80 °C for 70 h and with various water contents

Temperature	Added water (%)	EM (%)	ET (%)	DKP (%)
80 °C	0 (stabilised sample)[a]	96.79	0.46	0.69
	0 (intact sample)[b]	84.77	1.70	8.38
	10	71.04	6.45	10.66

[a] Water content was 5.04%.
[b] Water content was 5.79%.

3.2.3. Comparison of EM 5 and 20 mg tablet formulations

For comparison reasons we first performed microcalorimetric studies of both intact samples. The quantity of samples for microcalorimetry was calculated in such a way that the amount of the active substance EM was 10 mg in both cases. That implies that the quantity of EM 5 mg tablet mixture was four times higher than the one of EM 20 mg tablets. In order to eliminate excipient–excipient interactions from the heat flows of the formulations we have used placebo matrix in the reference cell and the sample of tablet mixture in the sample cell. The recorded thermograms for both EM tablet formulations are presented in Fig. 7. For comparison reasons thermogram for drug substance EM is also included. All measurements were performed at 80 °C.

One can see that heat flows of both formulations differ from the one of the drug substance itself. The thermograms prove that the stability of EM is influenced by the matrix of the tablet. We observe exothermic heat flows, which indicate that EM is degrading. Comparison of both formulations shows higher deviations from the EM substance curve in the case of EM 5 mg tablets. From this one can conclude that the long term stability of 5 mg tablets is lower than that of 20 mg tablets.

We also performed an HPLC analysis of both samples after being exposed to isothermal microcalorimetric study. The results of HPLC analysis confirm the thesis that 5 mg formulation is less stable. The EM 20 mg tablets were exposed to 80 °C for 72 h and the EM 5 mg tablets for 94 h. Content of EM, ET and DKP for the EM 5 mg tablets calculated according to the first order kinetics for the time 72 h is also included in Table 4. In the case of 5 mg tablets the amount of EM is lower whereas that of ET and DKP is higher than in the case of 20 mg tablets. Higher degradation was expected also from the thermogram on the basis of a higher exothermic heat flow for the EM 5 mg tablet formulation in the time period between 1 and 35 h.

We have evaluated the ratio $(Q_5 - Q_{EM})/(Q_{20} - Q_{EM})$ as described above (where Q_{EM} is the heat output of EM substance which is practically equal to the baseline to the 80 °C; see Fig. 7) and obtained the result $(Q_5 - Q_{EM})/(Q_{20} - Q_{EM}) = 1.9$.

Table 4
HPLC results of samples after being exposed to microcalorimetric study at 80 °C for 94 h (5 mg tablets) or 72 h (20 mg samples)

Formulation	EM (%)	ET (%)	DKP (%)
EM 5 mg tablets (94 h)	52.7	18.6	13.1
EM 5 mg tablets (72 h)[a]	60.9	14.2	10.0
EM 20 mg tablets (72 h)	80.6	6.27	6.24

[a] Calculated with the first order kinetics from results obtained after 94 h.

Table 5
The highest amounts of degradation products during stability study of pilot and production scale batches of three commercial batches of EM tablets in commercial packaging (Al/Al blister)

Degradation products	Conditions (°C/%RH) (storage (months))		
	/ (0)	30/60 (12)	40/75 (6)
EM 5 mg tablets			
ET	0.03	0.08	0.40
DKP	0.03	0.19	0.27
Unknown	Not detected	0.16	0.23
Total	0.05	1.92	1.99
EM 20 mg tablets			
ET	0.02	0.41	0.28
DKP	0.04	0.20	0.19
Unknown	0.03	0.16	0.56
Total	0.11	0.61	1.02

The corresponding value for the ratio $(n_5 - n_{EM})/(n_{20} - n_{EM})$ is 1.8. Both values were calculated for the 35 period. Again, we may conclude that the agreement between microcalorimetry and HPLC is good. Our results prove that microcalorimetry can be successfully used in pharmaceutical development.

Finally, the results of the long term ICH stability studies of both formulations (see Table 5) confirm the above findings on a lower stability of EM 5 mg tablet formulation. HPLC results for both formulations that were stored for 12 months at 30 °C and 60% RH or for 6 months at 40 °C and 75% RH conditions show a higher amount of degradation products in the case of 5 mg tablet formulation and therefore lower stability of EM 5 mg tablet formulations in comparison with to EM 20 mg tablet formulation.

4. Conclusion

We have shown that differences exist in thermograms of EM with or without added water. These differences were compared with the HPLC results and showed that the heat output, which can be evaluated from the thermograms, can be correlated with the extent of EM degradation in the sample as determined by the HPLC.

The studies of two quantitatively different EM formulations have shown that amount of water and excipients have a large influence on their stability properties. We were able to determine with the use of microcalorimetry which of the two formulations shows better stability properties. This result was confirmed by the ICH long term stability testing.

We can conclude that microcalorimetry can be used as an efficient tool in the preformulation studies with the capability of distinguishing between more and less stable drug substances or formulations.

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