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APPLICATION OF MICROCALORIMETRY TO THE FORMULATION STUDY

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Isothermal microcalorimetry was used to evaluate excipient compatibility of solid dosage form. Oxybutynin hydrochloride and cefaclor were used as model drugs for compatibility test with excipients. The calorimetric data for compatibility test were compared with those of HPLC data. Evaluation of compatibility between drug and excipient of solid dosage form might be possible to use isothermal microcalorimetry instead of conventional method. By using microcalorimetric method, the evaluation of the compatibility between drug and excipient could be successfully performed with a simple operation in a short time. The application of the isothermal microcalorimetry would be useful for the screening test of the drug compatibility with excipients.

Keywords: *cefaclor, drug-excipient compatibility, formulation, isothermal microcalorimetry, oxybutynin hydrochloride*

Introduction

Pharmaceutical products are required to be stable for long period of time. So, the stability and compatibility tests are very important during the development of pharmaceuticals. The stability of pharmaceuticals are related many steps, such as selecting new candidate on the basis of stability, optimal formulation study on the basis of stability. However, these stability tests are time consuming. The conventional stability test is done by using accelerating study. Also the screening for formulation is done performing series of compatibility for mixtures of drug and expecting excipients at elevated temperature and the undegraded drug is determined as a function of time. However, expecting combinations of drug to excipients in formulation are huge and it takes a long time. Consequently rapid and rational methods to perform stability testing would be desired [1–4]. The isothermal microcalorimetry is one of analytical methods with high sensitivity allowing detection of minute thermal heat. The decomposition of drug caused by incompatibility between a drug and an excipient evolves minute heat, therefore, microcalorimetry has a potential to detect heat of the decomposition of a drug due to the incompatibility between a drug and an excipient [5–9].

The purpose of this study was to estimate the compatibility between a drug and an excipient with microcalorimetric method, instead of conventional HPLC method.

Experimental

Materials

Oxybutynin hydrochloride was purchased from Sigma (St. Louis, MO). Cefaclor was obtained from Shionogi Chemical Co., Inc. (Tokyo, Japan). As excipients microcrystalline cellulose (MCC) (Asahi Chemical Co., Inc.), stearic acid (Pure Chemical Co., Inc.), calcium stearate (SYNKEM), magnesium stearate (Kosou Chemical Co., Inc.), talc (Nikkou Pharmaceutical), *D*-mannitol (Kosou Chemical Co., Inc.), lactose (CBC Co., Inc.), corn starch (SYNKEM), sucrose esters of fatty acid (SEFA)(Mitsubishi Chemical Food Co., Inc.), light anhydrous silicic acid (LASA) (Y.K.F.) were used.

Preparation of samples

All materials used for compatibility measurements were used after passing through 80 mesh sieve fraction. The binary physical mixture of a drug and a excipient was prepared by mixing equal mass of drug with each of the aforementioned excipients using V type mixer (inner volume of vessel is about 150 mL) with 36 rpm for 10 min.

Instrumental methods

Measurement of compatibility by microcalorimetry

The microcalorimeter used in this study was the 2277 Thermal Activity Monitor (TAM, Thermometric, Sweden). A mixture of drug and excipient was

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placed into the 20 mL stainless steel vessel. About 5~10 g of mixture was placed in the stainless steel vessel. These measurements were performed at 40°C, RH 75%. The heat flow generated by decomposition of drug was obtained by accumulating the heat flow for 5 days after the baseline became stable condition ($\Delta H_{5\ \mathrm{days}}$). Each measurement was done triplicate.

Measurement of compatibility by HPLC

The compatibility test sample was also stored for 3 months under controlled temperature and humidity conditions at 40°C, RH 75% and remaining amount of drug was examined by HPLC. HPLC assay was carried out using a Shimadzu chromatograph (SPD-AV detector, MCC-6A pump, SIL-6B autosampler, C-R5A chromatographic data analyzer, Japan). For the compatibility test of oxybutynin hydrochloride [10], the decomposed substance of 2-cyclohexyl-2-phenylglycolic acid was assayed using C_{18} reversed phase column (Devlosil ODS-UG-3) with mobile phase of acetonitril/triethanolamine adjusted at the pH of 3.5. For the compatibility test of cefaclor [11], remaining amount of cefaclor was assayed using C_{18} reversed phase column (STR ODS-II) with mobile phase of acetonitril/100 mM of KH_2PO_4 solution.

Simultaneous measurement of XRD-DSC

XRD-DSC measurement was performed using Rigaku XRD-DSC II system (Rigaku, Japan). Sample were placed in open aluminum sample pans whose size was 7 mm×7 mm×0.25 mm. The inside of the DSC unit was purged by nitrogen gas at a flow rate of 50 ml min^{-1}. The heating rate of the DSC run was 5°C min^{-1} and X-ray diffraction was measured simultaneously. CuK_α radiation, a graphite monochrometer was used for XRD measurements. The line shape X-ray source was a RIGAKU/RINT-Ultima system with operating conditions of 2θ =5–35° at 50 kV and 40 mA.

Tukey's HSD test

Tukey's HSD test [12] is multiple comparison test performed by calculating HSD (honestly significant difference). HSD is critical value to judge whether there is any significant difference.

HSD is compared with the absolute value of the average value of each sample, and when the difference of average value is larger than HSD, it is judged that there is significant difference.

$$HSD = q_{(\mathrm{p,k,d,f})}\sqrt{\frac{MSE}{n}} \qquad MSE = \frac{S_\mathrm{E}}{k(n-1)}$$

where $q_{(\mathrm{p,\ k,\ d,\ f})}$: critical value of studentized range at significance level (p) with number of degrees, freedom (d, f) and number of sample, MSE: mean square root, S_E: error sum of square, n: number of data in each sample

Results and discussion

Evaluation of compatibility of oxybutynin hydrochloride by microcalorimetry

The baselines of heat flow of excipients are important to evaluate the compatibility test by microcalorimetry. Figure 1 shows the heat flow–time curves of excipients used in this study. The heat flow time curves were reproducible within normal statistical fluctuation limits and the stable baselines were obtained for stearic acid, calcium stearate, magnesium stearate, talc, *D*-mannitol, lactose, SEFA and LASA. On the other hand, high exothermic heat flow of excipients was observed in the case of MCC, corn starch and PVP-K30. The latter excipients usually contains more than several percents of adsorbed water, so, the large exothermal signal observed for these excipients might be due to the heat of absorption of water caused by storing vessel at high humidity condition. Before microcalorimetric measurements, MCC and corn starch were stored at 40°C, RH 80%, which is higher humidity than measurement conditions for one week and three weeks beforehand to equilibrate the water contents for these excipients, respectively. After equilibrium of water contents for MCC and corn starch, heat flow of MCC and corn starch have become considerably stable as shown in Fig. 2. This pretreatment has enabled to measure stable baselines for MCC and corn starch. However, it was impossible to measure the baseline of PVP-K30 because the continuous deliquescence was observed during the storage at 40°C, RH 75% in appearance, so the deliquescence might be hindered the measurement of microcalorimetry. Therefore, it was found that hygroscopic excipients were not suitable for the compatibility evaluation by microcalorimetry and some

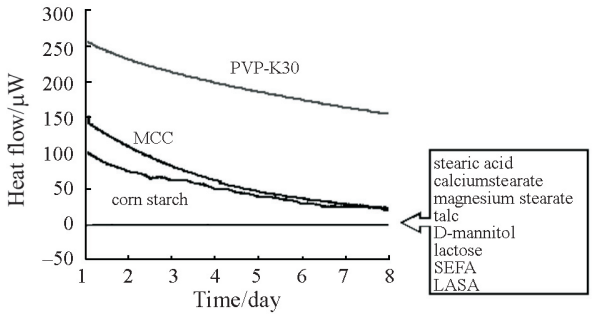

Fig. 1 Heat flow-time curves for various excipients obtained by microcalorimetry at 40°C, RH 75%

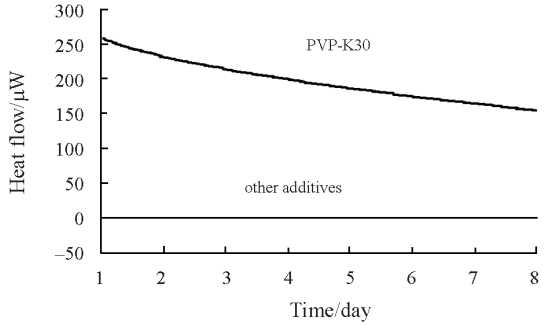

Fig. 2 Heat flow-time curves for corn starch and microcrystalline cellulose pretreated at 40°C, RH 80% for 1 and 3 weeks, respectively. Including other mixture with excipients obtained by microcalorimetry at 40°C, RH 75%

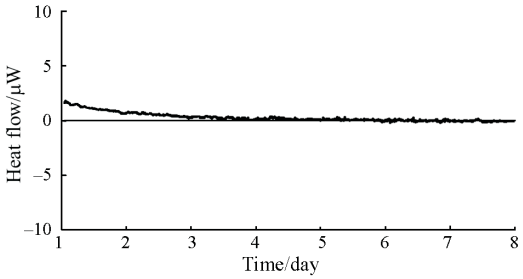

Fig. 3 Heat flow-time curves for oxybutynin hydrochloride obtained by microcalorimetry at 40°C, RH 75%

improved design is required for the measurement. Figure 3 shows of heat flow of oxybutynin hydrochloride by microcalorimetry at 40°C, RH 75%. The heat flow has attained stable state after several days and evolved heat was 0.2 μW over period of 8 days. This indicated that oxybutynin hydrochloride was considerably stable at the measurement condition.

Figure 4 shows the heat flow–time curves of mixture of the oxybutynin hydrochloride with excipients used in this study. The significant exothermic heat was observed for the mixture with magnesium stearate. Hence, it was supposed that oxybutynin hydrochloride incompatible with magnesium stearate. On the other hand, in the case of the mixtures with stearic acid, calcium stearate, talc, *D*-mannitol, lactose, MCC and corn starch, oxybutynin hydrochloride would be compatible with these excipients because

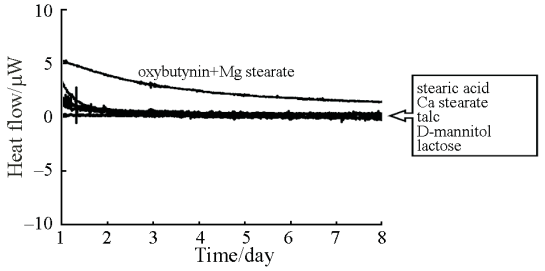

Fig. 4 Heat flow-time curves of mixture of oxybutynin hydrochloride with excipients obtained by microcalorimetry at 40°C, RH 75%

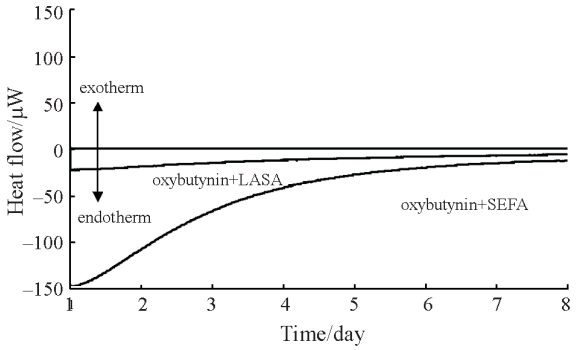

Fig. 5 Heat flow-time curve of mixture of oxybutynin hydrochloride and SEFA or LASA at 40°C, RH 75%

the evolved heat from the mixtures with these excipients was less than 0.3 μW over period of 8 days.

Figure 5 shows the heat flow–time curves of mixtures of oxybutynin hydrochloride with SEFA or LASA. In the case of mixtures with SEFA or LASA, the endothermic heat flow was observed. These phenomena suggested that some interactions other than decomposition of oxybutynin hydrochloride might be occurred. To reveal these phenomena, the simultaneous measurements of PXRD and DSC were performed for the physical mixtures of oxybutynin HCl and SEFA or LASA. As shown in Figs 6 and 7, the polymorphic transition of oxybutynin HCl was occurred at about 40°C. It was found that the endothermic heat flow in the compatibility measurement was confirmed to be the heat generated by the polymorphic transition.

Comparison of compatibility of oxybutynin hydrochloride between microcalorimetric method and HPLC method

Table 1 shows the decomposed amounts of oxybutynin hydrochloride after storage at 40°C, RH 75%

Table 1 Compatibility between heat of decomposition and decomposed amount of oxybutynin hydrochloride assayed by HPLC

Sample	Amounts of decomposed HCl/%
Oxibutynin HCl + MCC	ND
Oxibutynin HCl + stearic acid	ND
Oxibutynin HCl + Ca stearate	ND
Oxibutynin HCl + Mg stearate	0.50±0.04
Oxibutynin HCl + talc	ND
Oxibutynin HCl + *D* -mannitol	ND
Oxibutynin HCl + lactose	ND
Oxibutynin HCl + corn starch	ND
Oxibutynin HCl + SEFA	0.08±0.03
Oxibutynin HCl + LASA	ND
Oxibutynin HCl + hydrocloride	ND

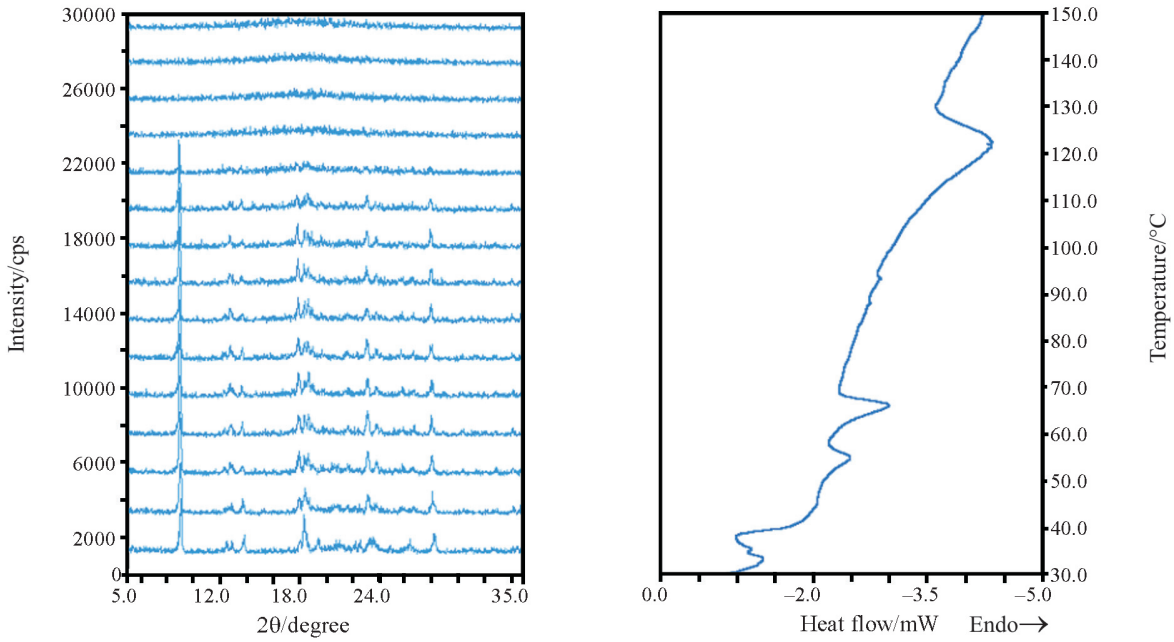

Fig. 6 Simultaneous measurement of XRD-DSC for mixture of oxybutynin hydroxychloride and SEFA

for 3 months by HPLC method. A small amount of oxybutynin hydrochloride was hydrolyzed by the mixing with magnesium stearate or SEFA. The decomposition for another mixtures with excipients was below detection limits. The heat of decomposition due to mixing with excipients was evaluated by the accumulation of generated heats for 5 days ($\Delta H_{5\ \mathrm{days}}$) after the baseline was completely stabilized, cf. MCC (from 10 to 14 days), corn starch (from 15 to 19 days), another excipients (from 5 to 9 days). Figure 8 shows the comparison between the heat of decomposition ($\Delta H_{5\ \mathrm{days}}$) by microcalorimetry and actual decomposition of oxybutynin hydrochloride by HPLC measurement. In the cases of magnesium stearate and SEFA, the decomposed amounts of oxybutynin hydrochloride was 0.21 and 0.04%, and $\Delta H_{5\ \mathrm{days}}$ were 280 and 4379 mJ g^{-1}, respectively. In the case of mixtures of oxybutynin hydrochloride and MCC, stearic acid, Ca stearate, talc, *D*-mannitol, lactose and corn starch, the decomposition amounts were below detection limits

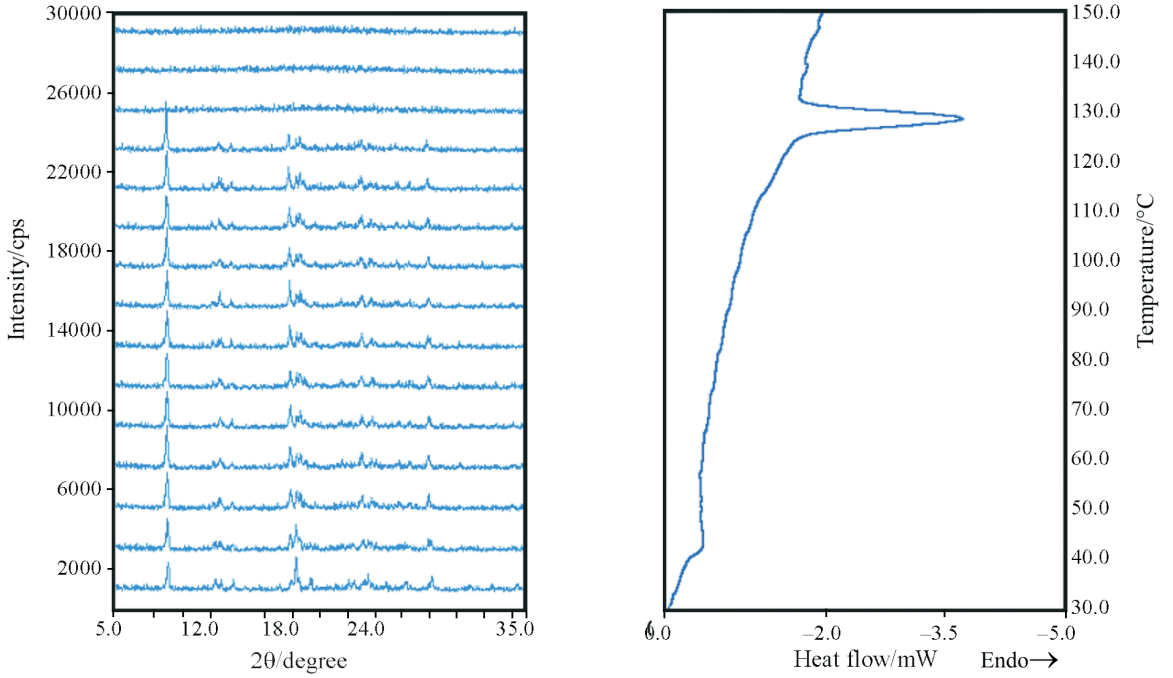

Fig. 7 Simultaneous measurement of XRD-DSC for mixture of oxybutynin hydroxychloride and LASA

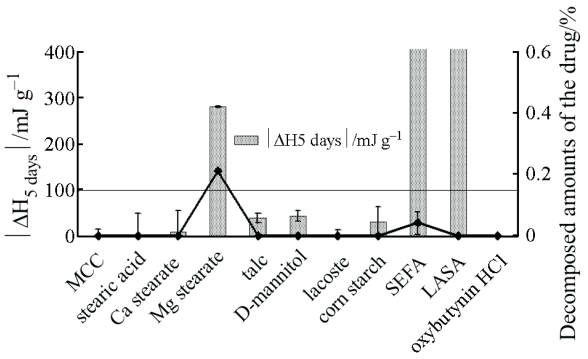

Fig. 8 Relationship between accumulated heat quantities generated for 5 days and decomposed amounts of oxybutynin hydrochloride mixed with excipients at 40°C, RH 75%Each column and point is the mean ±S.D. (n=3)

by HPLC and $\Delta H_{5\ days}$ was less than 44 mJ g^{-1}. The generated heats were statistically analyzed using Turkey's HDS test, judging value for significance was determined as 100 mJ g^{-1}. The generated heats observed by the mixture with magnesium stearate and SEFA were regarded as significant by Turkey's HDS test. Another mixtures were insignificant. For the mixtures with these excipients, the microcalorimetric method was well correlated with conventional HPLC method. However, in the case of LASA, though the decomposition of oxybutynin hydrochloride was below the detection limit by HPLC, $\Delta H_{5\ days}$ was significant value as 4383 mJ g^{-1}. As mentioned before, polymorphic transition of oxybutynin hydrochloride was observed by the mixing with LASA. So, the discrepancy was that the microcalorimetry had detected the heat of polymorphic transition, though the mixture is chemically stable.

Evaluation of compatibility of cefaclor by microcalorimetry

Figure 9 shows heat flow of cefaclor by microcalorimetry at 40°C, RH 75%. The heat flow attained

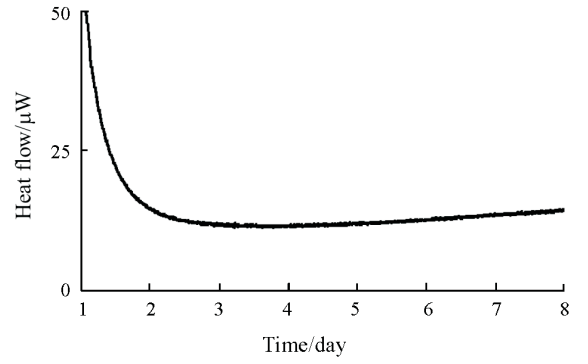

Fig. 9 Decomposed amount of oxybutynin hydroxychloride mixed with excipients assayed by HPLC

stable state after 2 days of storage, however, the evolved heat was about 15 μW, which is about fifty times higher value compared with that of oxybutynin hydrochloride. Figure 10 shows the heat flow–time curves of mixture of cefaclor with excipients used in this study. The significant exothermic heat was observed for the mixture with LASA. Hence, it was supposed that cefaclor was incompatible with LASA. The heat flow of mixtures with other excipients, such as stearic acid, Ca stearate, Mg stearate, talc, *D*-mannitol, lactose, MCC and corn starch, was almost the same as cefaclor only after the baselines were stabilized. So, the excipients other than LASA were supposed to be compatible with cefaclor on the basis of microcalorimetry.

Comparison of compatibility of cefaclor between microcalorimetric method and HPLC method

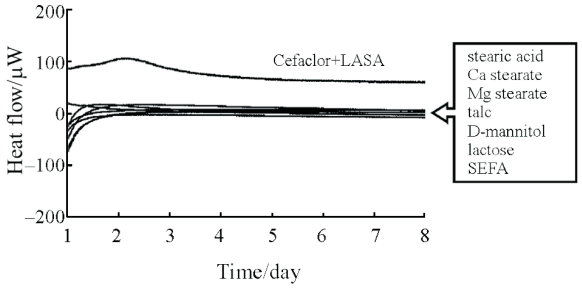

Fig. 10 Heat flow–time curve of cefaclor obtained by microcalorimetry at 40°C, RH 75%

Table 2 shows decomposed amounts of cefaclor for the mixtures with excipients stored at the conditions of 40°C, RH 75% for 3 months. Cefaclor alone decomposed about 7.6% after three months. The greater decomposition was observed for the mixture with LASA compared with that of cefaclor alone. Figure 11 shows the comparison between the heat of de-

Table 2 Compatibility between heat of decomposition and decomposed amount of cefaclor assayed by HPLC

Sample	Amounts of decomposed cefaclor/%
Cefaclor + stearic acid	7.5 ± 0.8
Cefaclor + calcium stearate	4.0 ± 2.3
Cefaclor + magnesim stearate	8.7 ± 1.1
Cefaclor + talc	9.4 ± 2.0
Cefaclor + *D*-mannitol	9.0 ± 0.9
Cefaclor + lactose	8.9 ± 1.0
Cefaclor + SEFA	1.5 ± 0.5
Cefaclor + LASA	18.3 ± 1.5
Cefaclor	7.6± 0.7

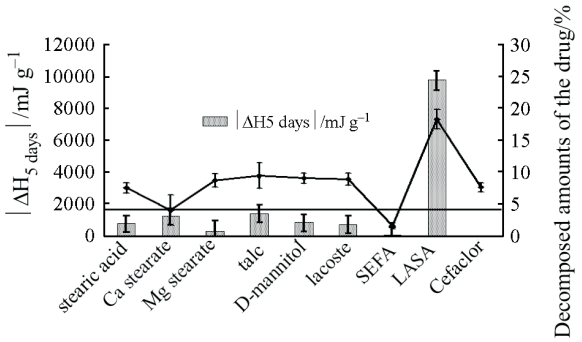

Fig. 11 Heat flow-time curve of mixtures of cefaclor and excipients obtained by microcalorimetry at 40°C, RH 75%

composition ($\Delta H_{5\ \mathrm{days}}$) by microcalorimetry and actual decomposition of cefaclor by HPLC measurement. The generated heats were statistically analyzed by Turkey's HDS test. The HDS values of 95% confidence levels were determined as 1598 mJ g^{-1}. The generated heat observed by the mixture with LASA was 9762 mJ g^{-1}, which was regarded as significant by Turkey's HDS test. Another mixtures were found to be insignificant. In the case of cefaclor, the microcalorimetric method was well correlated with conventional HPLC method.

Conclusions

This investigation demonstrated that the compatibility test using isothermal microcalorimetry would be substituted for the conventional excipient compatibility test under the acceleration conditions. However, the calorimetric method is a nonspecific technique, not only chemical decomposition but also other physical change was detected as heat flow. To use the microcalorimetric method for the compatibility test, it is important to follow the thermal behavior of mixture with other analytical methods.

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