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USE OF ISOTHERMAL MICROCALORIMETRY IN PHARMACEUTICAL PREFORMULATION STUDIES Part III. Evaluation of excipient compatibility of a new chemical entity

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Abstract

Excipient compatibility of a new chemical entity was assessed using an isothermal microcalorimeter. Mixtures of an active pharmaceutical ingredient with a primary amine group and excipients were prepared in a 1:1 ratio and compatibility monitored by exposing to 50, 60 and 70°C in presence of 200 μL of water. The new chemical entity, a primary amine, reacted with reducing sugars such as lactose and resulted in a brown discoloration. This reaction is the Maillard type condensation reaction between amines and reducing sugars. The rate of reaction was dependent on the temperature with rapid degradation at higher temperatures. No other incompatibility was apparent between the primary amine and other excipients

Keywords: browning reaction, compatibility, excipients, Maillard reaction, microcalorimetry, primary amine, reducing sugars

Introduction

In addition to the active pharmaceutical ingredient (API), pharmaceutical formulations contain a number of pharmacologically inert ingredients referred to as excipients. The excipients, however, are capable of interacting physically or chemically with the API [1]. Such interactions may have a profound impact on the stability and shelf life of the formulation. Hence, prior to formulation development it is essential to assess compatibility of the API with excipients. Traditional compatibility studies involve preparation of mixtures of API and excipients and placing them at various stability conditions. These mixtures are periodically assayed for API and compatibility is assessed over the time of storage. Such conventional methods are specific to the API and allow degradation products to be easily identified at an early stage in formulation development. However, the traditional approach tends to be exhaustive and resource intensive. Individual samples have to be assayed

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by HPLC using validated methods at an early stage of drug development, consuming scarce resources.

In order to be less resource intensive a number of recent studies have focused on development of rapid, non-specific methods for excipient compatibility screening during early stages of the development process. These rapid analytical techniques are based on thermal analysis and often use differential scanning calorimetry (DSC) or isothermal microcalorimetry (IMC) or both. The thermal methods tend to be rapid and less resource intensive, than traditional compatibility analysis methods. In general, DSC has been the technique of choice for compatibility studies and in certain cases provides rapid information on excipient compatibility of the API [2, 3]. Recently, a number of reports have demonstrated the utility of IMC for quick assessment of excipient compatibility of the API [4–6]. Advantages of IMC over DSC include higher sensitivity (about 10,000 fold), which is useful in monitoring extremely slow reactions under isothermal conditions at lower temperatures. IMC consists of a small closed reaction vessel in contact with a heat sink such that the reaction vessel is at a constant temperature. A heat flow sensor, located between the sample cell and the heat sink, measures the heat generated due to a reaction between the components present in the sample vessel [7]. The thermal power $P = \mathrm{d}Q/\mathrm{d}t$ due to the reaction is recorded and monitored as a function of time [7].

The objective of the present study was to evaluate the utility of thermal analysis techniques of DSC and IMC for rapid assessment of excipient compatibility of APIs. An API under development was used as a model compound. The compound is a primary amine and is administered orally in a solid dosage form. The compatibility of the API with common solid dosage form excipients was assessed by thermal analysis.

Experimental

Materials

The API was used as received. Particle size analysis results showed a volume median diameter of approximately 90 μm. The API exists as two enantiotropic polymorphs. Polymorph I, stable at temperatures less than 140°C, was used in this study. Excipients were used as received from the manufacturer. The excipients used in this study and their use categories in solid oral dosage forms are provided in Table 1.

Sample preparation for DSC analysis

For DSC analysis a TA Instruments, DSC2290 was used. All samples were analyzed by using crimped aluminum pans. The API and excipient mixtures were prepared in the sample pan. Materials were weighed directly into the pan in roughly equal proportions and mixed using a spatula. Necessary precautions were taken to minimize material loss during sample preparation. Samples were heated from room temperature to 260°C at 10°C min^{-1}. Sample masses ranged from 2–10 mg with mixture masses being higher than pure components.

Table 1 List of excipients used for excipient compatibility analysis

Excipient designation	Excipient (Category)	Solid-state of the excipient
A	Cal. Phos. dibasic dihydrate (filler)	Crystalline
B	Colloidal silicon dioxide (glidant)	Amorphous
C	Hydroxy propyl methyl cellulose (binder) (HPMC)	Amorphous
D	Lactose, anhydrous (filler)	Crystalline
E	Lactose monohydrate (filler)	Crystalline
F	Mag. Stearate (lubricant)	
G	Microcrystalline cellulose (filler) (Avicel PH-302) (MCC)	Partially crystalline
I	Polyvinyl pyrrolidone (binder)	Amorphous
J	Pregelatinized starch (binder)	Amorphous
K	Croscarmellose sodium (disintegrant)	Amorphous
L	Sodium starch glycolate (disintegrant)	Amorphous
M	Stearic acid (lubricant)	Crystalline

Sample preparation for IMC analysis

A Thermal Activity Monitor (TAM) Model 2277 (Thermometric AB, Sweden) was used for compatibility analysis. TAM consisted of four calorimetric units equipped with standard amplifiers. The four calorimetric units were immersed in a constant temperature bath of 25 L with a temperature constance of less than $\pm 2 \cdot 10^{-4}$ K and a sensitivity of 0.1 μW. For analysis 3 mL crimp top glass vials were used, which were sealed with Teflon coated butyl rubber disc and an aluminum cap. Each unit is capable of detecting differences in heat flow and heat output between a sample and reference as a function of time. Before initiation of a series of experiments all calorimetric cells were calibrated using a two-point static electrical calibration at 0 and 300 μW.

For sample preparation approximately 500 mg (for materials with large bulk volumes the sample mass was limited to less than 250 mg) of the material was weighed into a vial to which 200 μL of deionized water was added to accelerate the reaction process, if any. Vial contents were mixed with a glass Pasteur pipette, and the tip of the pipette was left in the vial to avoid material losses [5]. Excipient and API mixtures were prepared by weighing equal amounts of the ingredients. The materials were mixed using a spatula on a mass paper and the mixture transferred to the sample vial. Water (200 μL) was added to the mixture and the contents were mixed with a Pasteur pipette and the tip was left in the vial to avoid material losses. The reference vial was prepared by weighing an amount of talc equal to the sample mass to balance heat capacities. Water (200 μL) was added to the sample vial and contents of vials were mixed as described above.

Results and discussion

Solid-state aspects of API and excipients

The API is a small molecule primary amine, which is highly soluble in water. It is crystalline and exists as two enantiotropic polymorphs. The polymorphic transition was observed to occur at 131°C with an enthalpy of transition (ΔH_{trans}) of 13.6 J g^{-1} (Fig. 1). Polymorph I was used for compatibility studies and was used as received. Excipients were used as received. They were amorphous, partially crystalline or crystalline and the solid-state details are provided in Table 1. The DSC curves of all pure excipients are shown in Fig. 1. The initial broad endotherms (<100°C) apparent in DSC curves of amorphous excipients were attributed to loss of absorbed water (Fig. 1). Crystalline excipients showed endotherms associated with dehydration (lactose monohydrate) or melting (anhydrous lactose) events.

Excipient compatibility analysis by DSC

The DSC curves of API-excipient mixtures are shown in Fig. 2. The DSC curves of non-interacting mixtures are usually a sum of curves of the two individual components. However, any interaction will be apparent in the DSC curve, either as an endothermic or exothermic event or lack of an event apparent in pure components. In order to assess compatibility by DSC, the ΔH_{trans} value associated with the polymorphic transition event of the API in mixtures was obtained. An interaction was assumed to result in a decrease in expected ΔH_{trans} value. The theoretical and experimental ΔH_{trans} values of API in physical mixtures were normalized to its mass and the values are provided in Table 2. A good correlation between the theoretical and experimental enthalpy values was observed (Table 2) and was considered to be a proof of non-interaction between API and excipients. An overlap of the polymorphic transition endotherm of the API with thermal events of an excipient resulted in ΔH_{trans} being lower or higher than the theoretical value. Such an overlap made it difficult to draw any conclusions regarding compatibility with the excipient in question. Also, absence of the polymorphic transition endotherm was considered to be sufficient evidence for incompatibility as observed in the DSC curve for stearic acid and API mixture. However, stearic acid melts at a very low temperature and it is possible that the melt obscured the endotherm associated with the polymorphic transition of the API (Fig. 1, curve 13). It is also necessary to point out that the mixtures were dry and results obtained by DSC are not predictive of an incompatibility in presence of moisture.

Excipient compatibility analysis by IMC

Assessment of incompatibility in presence of moisture or under wet conditions was analyzed by IMC. IMC experiments were preformed isothermally at 50, 60 and 70°C. Deionized water was added to all sample vials at a concentration of 40% *v*/*w* to study the effect of water on compatibility [4, 5]. Due to high solubility of the API in water, any incompatibility observed is more likely to be in solution state rather than in

Table 2 Expected and actual enthalpy values for polymorphic transition of API in presence of excipients by DSC analysis

Materials	Mass of NCE / mg	Mass of excipient / mg	Proportion of NCE	Expected ΔH J g^{-1} for polymorphic transition of NCE	Actual ΔH J g^{-1} for polymorphic transition of NCE
NCE			1.00	13.58	13.58
NCE/ATAB	3.84	2.86	0.57	7.78	Overlapping peaks
NCE/HPMC	1.84	1.94	0.48	7.49	7.46
NCE/Lactose anhydrous	1.86	1.96	0.49	5.22	5.25
NCE/Lactose monohydrate	1.95	1.99	0.49	6.72	Overlapping peaks
NCE/PVP	1.74	2.44	0.42	3.74	3.66
NCE/Microcrystalline cellulose	1.51	1.19	0.56	7.24	7.24
NCE/Sod. starch glycolate	1.90	2.52	0.43	4.01	3.57
NCE/Croscarmellose sodium	2.12	1.42	0.60	7.66	7.78
NCE/Pre-gelatinized starch	4.67	3.78	0.55	7.58	7.24
NCE/Colloidal silicon dioxide	3.09	0.58	0.84	13.42	13.73
NCE/Stearic acid	2.41	2.58	0.48	4.74	No event observed
NCE/Magnesium stearate	2.67	1.47	0.64	8.14	Overlapping peaks

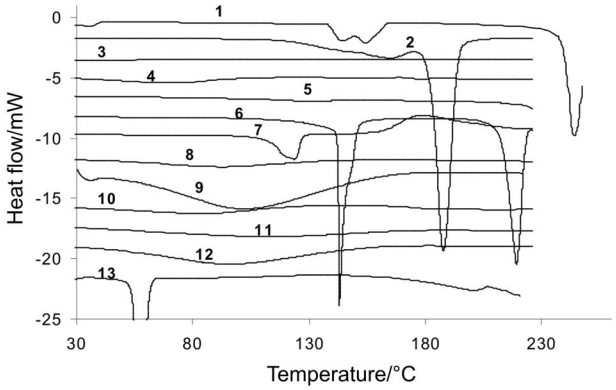

Fig. 1 DSC curves of pure components. 1 – API; 2 – Cal. phosphate dibasic dihydrate; 3 – Colloidal silicon dioxide; 4 – Hydroxypropyl methyl cellulose; 5 – Lactose anhydrous; 6 – Lactose monohydrate; 7 – Mag. stearate; 8 – Microcrystalline cellulose; 9 – Pregelatinized starch; 10 – Polyvinyl pyrrolidone; 11– Croscarmellose sodium; 12. Sod. starch glycolate; 13. Stearic acid

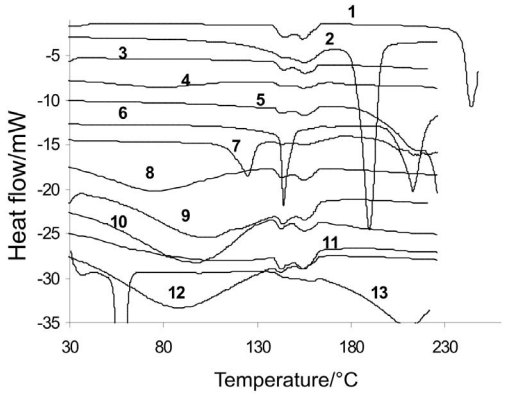

Fig. 2 DSC curves of API-excipient mixtures. 1 – API; 2 – API-Cal.phos.dibasic; 3 – API-Colloidal silicon dioxide; 4 – API-HPMC; 5 – API-Lactose, anhydrous; 6 – API-Lactose monohydrate; 7 – API-Mag. stearate; 8 – API-Microcrystalline cellulose; 9 – API-Pregelatinized starch; 10 – API-polyvinyl pyrrolidone; 11 – API-Croscarmellose sodium; 12 – API-Sod. starch glycolate; 13 – API-Stearic acid

solid-state. However, an incompatibility in solution was assumed to predict incompatibility in the solid-state especially at high humidity and temperature conditions. In general, IMC produces two types of data:heat output Q in J (thermodynamic data) and heat flow $\phi = dQ/dt$ in W (kinetic data). Heat flow is positive for an exothermic process ($\phi>0$) and negative for an endothermic process ($\phi<0$) [7]. Heat flow curves of pure API and excipients, and those of mixtures of the

API and excipients were used for assessing compatibility. Data analysis was performed by DIGITAM for Windows version 4.1 provided by the manufacturer. Calorimetric output is presented as power-time curves in μW. Power-time curves were obtained for API alone in presence of water, excipient alone in presence of water and API – excipient mixture in presence of water. Based on power time curves of pure components a theoretical non-interaction curve, p_{theor}, was generated based on the proportion of components in the mixture. The p_{theor} curve was subtracted from the experimental curve for the mixture p_{mix} to yield a difference curve p_{diff}.

Initially the ability of TAM to detect a Maillard type condensation reaction between a primary amine and lactose monohydrate was assessed by analyzing a mixture of ε-amino-*n*-caproic acid and lactose monohydrate at 50°C in presence of water [5]. The heat flow curves p_{theor}, p_{mix} and p_{diff} associated with Maillard type interaction, are shown in Fig. 3. The difference curve showed an average heat flow of approximately 60 μW at the peak of the curve. This substantial heat flow relative to theoretical non-interaction heat flow was indicative of an interaction between the components. Additional confirmation was obtained when the sample vials were examined visually which showed a brown discoloration characteristic of Maillard reaction (Fig. 3).

Heat flow difference curves (p_{diff}) of API and various excipient mixtures at 50 and 60°C are shown in Figs 4 and 5. At 50°C, difference curves for API and excipient mixtures had heat flow values of less than 10 μW (Fig. 4). These heat flow values were low and indicative of compatibility between API and excipients [5]. Significantly, the difference heat flow curve for a mixture of API and lactose monohydrate also showed a value of less than 10 μW. Moreover visual examination of the sample vial after 70 h at 50°C did not reveal any brown discoloration suggestive of Maillard interaction. Based on the chemical structure and high water solubility of the API, a Maillard type condensation reaction was anticipated to occur

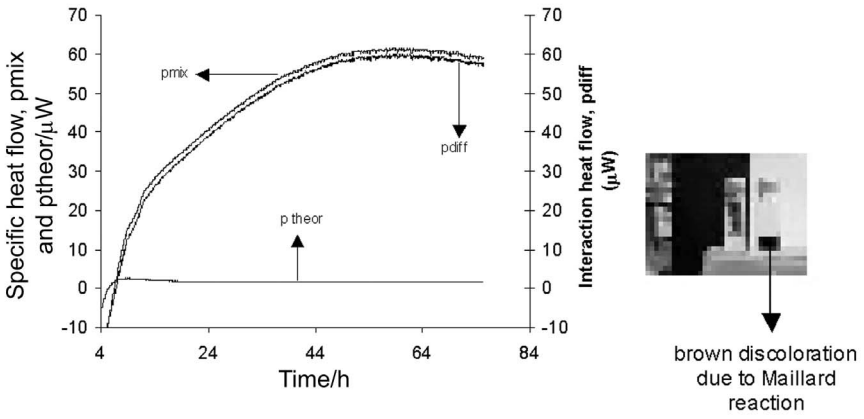

Fig. 3 Microcalorimetric heat flow curves of mixture of ε-amino-*n*-caproic acid and lactose monohydrate

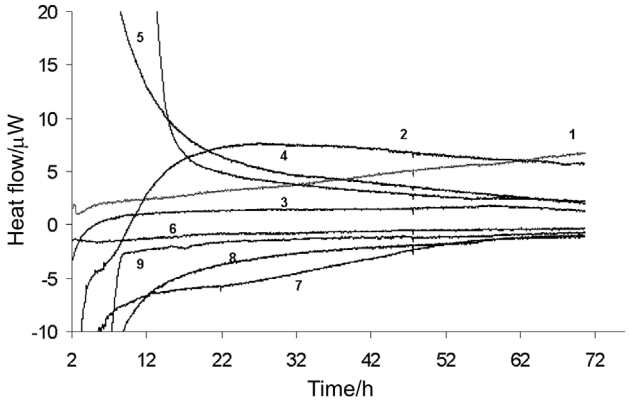

Fig. 4 Microcalorimetric heat flow curves of API-excipient mixtures at 50°C. 1 – Lactose monohydrate; 2 – HPMC; 3 – Microcrystalline cellulose; 4 – Mag. stearate; 5 – Polyvinyl pyrrolidone; 6 – Cal.phosphate dibasic; 7 – Croscarmellose sodium; 8 – Pre-gelatinized starch; 9 – Stearic acid

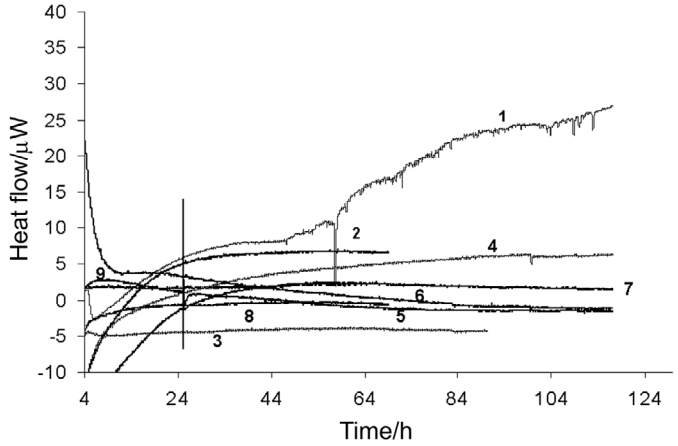

Fig. 5 Microcalorimetric heat flow curves of API-excipient mixtures at 60°C. 1 – Lactose monohydrate; 2 – HPMC; 3 – PVP; 4 – Pre-gelatinized starch; 5 – Croscarmellose sodium; 6 – Stearic acid; 7 – Microcrystalline cellulose; 8 – Cal. phosphate dibasic; 9 – Mag. stearate

at 50°C. It is possible that the rate of reaction between API and lactose monohydrate is slower at 50°C and that a reaction may be detected at higher temperatures.

The temperature dependence of the reaction rate of Maillard interaction was explored by analyzing the API-lactose monohydrate mixture at higher temperatures of 60 and 70°C. The difference heat flow curves at 60°C of API and excipient mixtures are shown in Fig. 5. All API-excipient mixtures with the exception of API and lactose did not show any substantial increase in heat flow than observed at 50°C. Moreover, the heat flow output values were less than 10 μW for all API excipient

mixtures except with lactose monohydrate. The heat flow curve for lactose mixture was exothermic, with a peak value of 36 μW. Visual examination of the sample vial after TAM analysis revealed a brown discoloration similar to discoloration shown in Fig. 3. A similar interaction was observed at 70°C, with the peak apparent at a much shorter time than observed at 60°C (data not shown). The increase in rate of reaction with temperature appeared to follow Arrhenius law. The heat flow curves for mixtures of API and lactose in presence of water appeared to be consistent with an auto-catalytic rate law [8]. The heat flow curves also revealed a maximum value (Q_{max}) in μW g^{-1}, at which point 50% of the reactant has been consumed.

Early prediction of an interaction between the excipient and API is useful in rational formulation development. Most often the excipient compatibility assessment is constrained by limited availability of API and resources. Hence techniques for rapid and predictive assessment of compatibility of API with excipients are of considerable benefit. Several studies have shown the utility of thermal analytical techniques of DSC and IMC for rapid and predictive assessment of API-excipient compatibility [2–5]. These two techniques were utilized for assessment of excipient compatibility of an API with a primary amine group. Of the two techniques, IMC was predictive of incompatibility of API with reducing sugars such as lactose under wet conditions. The higher sensitivity of IMC also allowed its use at temperatures closer to routine stability analysis temperatures (for example 40°C/75%RH). Based on the compatibility assessment, alternative fillers to lactose were used in formulation development.

Conclusions

Isothermal microcalorimetry and DSC were utilized for screening an API having a primary amine group functionality for excipient compatibility. Rapid assessment with least resources was possible by the two thermal techniques. The API undergoes a Maillard type condensation reaction with reducing sugars such as lactose, which was detected by IMC. The rate of reaction was dependent on temperature and showed an Arrhenius type of relationship. Incompatibility with other common solid oral dosage form excipients was not detected. Analysis by DSC was not predictive of an incompatibility with lactose presumably due to lack of an interaction in solid-state. Thermal analytical techniques are rapid and predictive and can be used to screen gross incompatibilities at an early stage of drug development.

References

1 A. T. Serajuddin, A. B. Thakur, R. N. Ghosal, M .G. Fakes, S. A. Ranadive, K. R. Morris and S. A. Varia, J. Pharm. Sci., 88 (1998) 696.
2 A. A. S. Araujo, S. Storpirtis, L. P. Mercuri, F. M. S. Carvalho, M. dos-Santos and J. R. Matos, Int. J. Pharm., 260 (2003) 303.
3 G. Bruni, L. Amici, V. Berbenni, A. Marini and A. Orlandi, J. Therm. Anal. Cal., 68 (2002) 561.

4 A. Marini, V. Berbenni, S. Moioli, G. Bruni, P. Cofrancesco, C. Margheritis and M. Villa, J. Therm. Anal. Cal., 73 (2003) 529.
5 X. Tan, N. Meltzer and S. Lindenbaum, Pharm. Res., 9 (1992) 1203.
6 E. A. Schmitt, K. Peck, Y. Sun and J. M. Geoffroy, Thermochim. Acta, 380 (2001) 175.
7 L. Wadsö, A. L. Smith, H. Shirazi, S. R. Mulligan and T. Hafelich, J. Chem. Educ., 78 (2001) 1080.
8 R. D. Vickery and M. B. Maurin, Utility of microcalorimetry in the characterization of the browning reaction. Journal of Pharmaceutical and Biomedical Analysis, 20 (1999) 385.
9 L. D. Hansen, E. A. Lewis, D. J. Eatough, R. G. Bergstrom and D. DeGraft-Johnson, Pharm. Res., 6 (1989) 20.