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APPLICATION OF DIFFERENTIAL SCANNING CALORIMETRY IN EVALUATION OF SOLID STATE INTERACTIONS IN TABLETS CONTAINING ACETAMINOPHEN

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Abstract: Differential scanning calorimetry (DSC) is an analytical procedure used to determine the differences in the heat flow generated or absorbed by the sample. This method allows to assess purity and polymorphic form of drug compounds, to detect interactions between ingredients of solid dosage forms and to analyze stability of solid formulations. The aim of this study was the assessment of compatibility between acetaminophen (API) and different types of excipients often used in tablets compression: polyvinylpyrrolidone, crospovidone, pregelatinized starch, microcrystalline cellulose and magnesium stearate by differential scanning calorimetry. The study contains results of thermal analysis of excipients and individually performed mixtures of these substances with acetaminophen before and after compression and after 6 months storage of tablets at different temperature and relative humidity conditions (25 ± 2°C /40 ± 5% RH, 25 ± 2°C /60 ± 5% RH, 40 ± 2°C /75 ± 5% RH) for a period of 6 months. To detect possible changes of API chemical structure, gas chromatography-mass spectrometry (GC-MS) was also applied. GC-MS with electron impact ionization (EI) was employed to determine the fragmentation pattern of API. It was shown that the developed formulations showed excellent compatibility among all excipients used except Kollidon CL. The interaction with Kollidon CL is probably a result of a physical reaction as confirmed by GC-MS analyses. Obtained results revealed that DSC can be successfully applied to evaluate possible incompatibilities between acetaminophen and Kollidon.

Keywords: acetaminophen, DSC, drug-excipient compatibility studies, tablets

A proper choice of the pharmaceutical excipients, which improve physico-chemical properties and bioavailability of drugs, determines the successful formulation of a stable and effective solid dosage form. Even though excipients are considered as pharmaceutically inert, physical and chemical interactions with an active component are possible. Potential interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of medicines and as the result their therapeutic efficacy and safety. Therefore, very important phase in the pre-formulation stage of all dosage forms is examination of drug-excipient compatibility (1-4).

Differential scanning calorimetry (DSC) is a thermoanalytical method described in the European Pharmacopoeia (EP), United States Pharmacopoeia (USP) and Japanese Pharmacopoeia (JP) and is widely used to evaluate physical properties of drugs as well as to study compatibility and stability of the components in pharmaceutical preparations. A possible interactions might be identified by revealing changes in appearance, shift or disappearance of endothermic or exothermic peaks, and/or variations in the corresponding enthalpies of reactions (5-7). Such information is very helpful for analyzing any instability issues during the design and development of new formulations.

The aim of this study was the assessment of compatibility between acetaminophen (API) and often used tabletting excipients with different physico-chemical properties (polyvinylpyrrolidone: Kollidon 25, Kollidon 30; crospovidone: Kollidon CL (crosslinked), Kollidon CL-M (micronized crospovidone); microcrystalline cellulose – Avicel PH 101; pregelatinized starch – StarCap 1500; mag-

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nesium stearate) by differential scanning calorimetry. Experiments were carried out on mixtures of analyzed excipients and acetaminophen before and after compression.

It is well known that temperature and humidity are the most critical factors, which affect the stability of active components, excipients and the quality of final products (8-10). To assess the influence of relative humidity (RH) and temperature, the tablets obtained were stored for 6 months at various temperatures and relative humidity conditions (25 ± 2°C /40 ± 5% RH, 25 ± 2°C /60 ± 5% RH, 40 ± 2°C /75 ± 5% RH) and submitted to DSC analysis. For the identification of possible changes of API chemical structure, gas chromatography-mass spectrometry (GC-MS) with electron impact ionization (EI) was employed to determine the fragmentation pattern of API.

EXPERIMENTAL

Materials

Acetaminophen was received from Sigma-Aldrich (Steinheim, Germany). Polyvinylpyrrolidone was obtained as a gift sample from BASF (Ludwigshafen, Germany). Pregelatinized starch (StarCap 1500) was purchased from Colorcon (Indianapolis, IN, USA). Microcrystalline cellulose (Avicel PH 101) was obtained as a gift sample from FMC Corporation (Brussels, Belgium). Magnesium stearate was purchased from POCh (Gliwice, Poland). Methanol was of GC grade (Honeywell Burdick & Jackson, Morrison, NJ, USA). Mixture of C_7-C_{40} *n*-alkanes in hexane, pyridine and the silylation reagent *N*,*O*-bis(trimethylsilyl)trifluoroacetamid (BSTFA) with 1% trimethylchlorosilane (TMSC) were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals used were of analytical grade.

Methods

Preparation of tablets

Tablets were prepared by using a wet granulation method according to the formulae given in Table 1. Granulation was performed with or without disintegrant (11). A dry blend of acetaminophen and analyzed excipients were mixed in porcelain mortar and then were wetted with a polyvidone aqueous solution as a granulation binder. After incorporation of lubricant, the granules were compressed into tablets in the single punch tabletting press machine (Erweka EP1, Heusenstamm, Germany). Different adjustments of the machine settings were tried. The adjustment giving the similar hardness value was selected and applied to all tablet formulations.

Evaluation of tablets

Physical properties of tablets

Physical characteristics of the tablets were tested according to European Pharmacopoeia 6.0 (12). All tablet formulations were evaluated for weight variation (n = 20), hardness (n = 10) and friability (n = 10). Hardness was determined by using the Schleuniger tablet hardness tester (Dr. Schleuniger Pharmatron Model 5Y, Thun, Switzerland). The friability test was conducted using Electrolab friabilator (EF–1W Electrolab, Mumbai, India).

Drug content determination

Content of acetaminophen was determined by measuring the absorbance of the sample at 243 nm using a spectrophotometer (Hitachi U-1800, Tokyo,

Table 1. Composition of manufactured tablet formulations.

Ingredient (mg/tablet)	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Acetaminophen	500	500	500	500	500	500	500	500
StarCap 1500	170	170	170	170	170	170	170	170
Avicel PH-101	100	100	100	100	100	100	100	100
Kollidon 25*	2.5%	5%						
Kollidon 30*			2.5%	5%	5%	5%	5%	5%
Kollidon CL					20	40		
Kollidon CL-M							20	40
Magnesium stearate	8	8	8	8	8	8	8	8

* water solution

Japan) and comparing the content from a calibration curve prepared with standard acetaminophen in the same medium (phosphate buffer pH 5.8). The exact amount of acetaminophen was calculated from a calibration curve by an analytically validated method (R^2 = 0.9995, repeatability coefficient of variation (CV) = 1.104%).

***In vitro* dissolution studies**

The release rate of acetaminophen from tablets was determined using USP type II dissolution apparatus (Erweka DT600, Heusenstamm, Germany) under the following conditions: 900 mL of phosphate buffer (pH 5.8) at 37 ± 0.5°C and 50 rpm. The absorbance of the solutions was measured at 243 nm.

Stability studies

Tablets were put into Petri dishes and kept inside humidity chambers (Binder, Tuttlingen, UK) at different temperature and relative humidity (RH) conditions (25 ± 2°C /40 ± 5% RH, 25 ± 2°C /60 ± 5% RH, 40 ± 2°C /75 ± 5% RH) for a period of 6 months. After this time, tablets were tested for physical properties, *in vitro* dissolution time and compatibility studies.

Compatibility study

Differential scanning calorimetry (DSC)

DSC measurements were performed by using an automatic thermal analyzer system (DSC TEQ 2000, New Castle, DE, USA). All precisely weighed samples (approximately 4 mg) were placed in sealed aluminium pans. Temperature calibrations were performed using indium and zinc as standard. An empty pan sealed was used as a reference. The entire samples were run at a scanning rate of 10°C/min from 100 to 200°C in nitrogen atmosphere (flow 20 mL/min).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed on Agilent Technologies 5970C VL quadrupole mass spectrometer connected directly to Agilent Technologies 7890A gas chromatograph and to autosampler 7693 (Agilent Technologies, Wilmington, DE, USA). A fused silica capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) from J&W (Agilent Technologies, Wilmington, DE, USA) was used. Aliquots (1 µL) of the silylated extracts were injected in the split (50 : 1) mode. Injector was kept at 300°C. The following oven temperature program was used with helium as the carrier gas at a constant flow rate of 1 mL/min: 2 min at 70°C, then increased to 250°C at the rate of 10°C/min held for 5 min, next increased to 280°C at the rate of 10°C/min. Oven temperature of 280°C was held for 10 min. The electron impact mass spectra were obtained at 70 eV of ionization energy, at the temperature of source and quadrupole 220°C and 150°C, respectively. The mass selective detector was set to scan 40-550 amu.

Sample preparation for GC-MS analysis

For the GC-MS experiments, the grounded tablet powder (0.0500 ± 0.0025 g) was extracted with methanol (5 mL). After 15 min of sonification and 10 min centrifugation at 1200 rpm at 4°C temperature, the 100 µL of upper layer was collected to the vial. The methanol phase was evaporated to dryness, and then 110 µL of pyridine and 40 µL of the silylation reagent *N,O*-bis(trimethylsilyl)trifluoroacetamid (BSTFA) with 1% trimethylchlorosilane (TMSC), (99 : 1, v/v) was added into the vial. The reaction mixture was heated at 80°C during 45 min. All the experiments were performed in triplicates.

Statistical analysis

Quantity variables were expressed as the mean and standard deviation. All studies were performed in triplicate. Statistical analysis was performed using analysis of variance and Tukey's test conducted by using STATISTICA 10.0 software. Differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Acetaminophen, chemically known as N-acetyl-*p*-aminophenol is an effective analgesic and antipyretic drug which is a first step in WHO's pain relief ladder (13). It is classified in the BCS as a class I drug, since it has a high permeability and high aqueous solubility (14, 15). Acetaminophen exists in three polymorphic forms, but only two of them can be successfully isolated. Form I (monoclinic) is commercially marketed and is more stable under ambient temperature conditions than form II (orthorhombic) (16, 17). Physical properties of form I do not enable direct compression without additives. Therefore, to obtained tablets containing form I of acetaminophen, excipients with different functions in the formulation process should be used. In this study, we analyzed excipients often employed in technology of solid dosage forms. The soluble (Kollidon 25, Kollidon 30) and insoluble grades of polyvinylpyrrolidone (Kollidon CL, Kollidon CL-M) of various molecular weight and various particle

Table 2. Physical parameters of prepared tablets.

	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Weight (mg)	797 ± 4.9	801 ± 4.6	802 ± 5.2	799 ± 4.8	808 ± 5.4	831 ± 4.6	812 ± 4.5	829 ± 5.2
Hardness (N)	99.7 ± 0.5	99.8 ± 0.6	114.6 ± 0.3	115.1 ± 0.8	106.9 ± 0.5	107.3 ± 0.6	115.4 ± 0.3	117.3 ± 0.8
Friability (%)	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%	< 1%
Drug content (%)	102.3 ± 1.3	98.9 ± 1.5	98.8 ± 1.2	100.6 ± 0.9	105.2 ± 1.2	101.5 ± 1.8	102.2 ± 1.5	103.5 ± 1.3

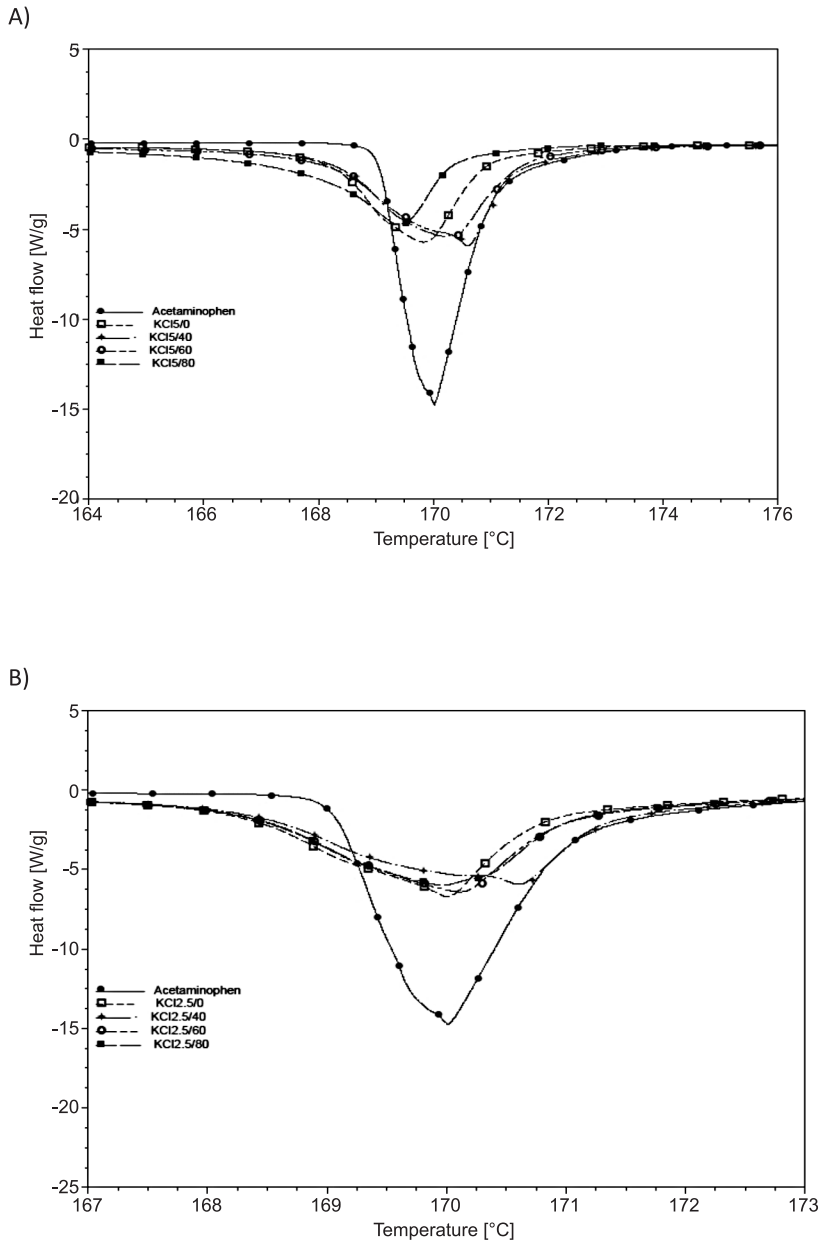

Figure 1. DSC thermograms of pure acetaminophen (●) and acetaminophen in tablets obtained with 5% Kollidon CL (A) and 2.5% Kollidon CL (B) before (▫) and after 6 months storage at different temperature and humidity conditions (+ - 25 ± 2°C/40 ± 5% RH; ❍ - 25 ± 2°C/60 ± 5% RH; ■ - 40 ± 2°C/75 ± 5% RH)

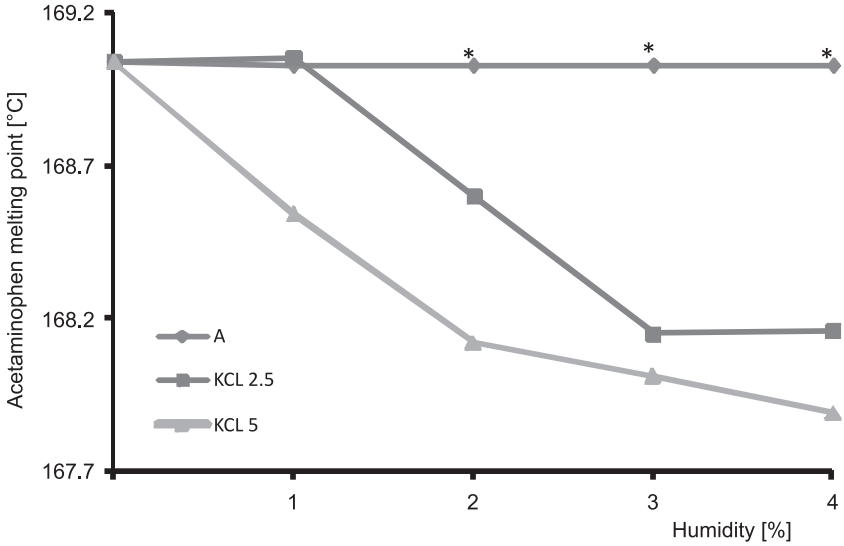

Figure 2. Melting point of pure acetaminophen (A) and acetaminophen in tablets obtained with 2.5% or 5% Kollidon CL (KCL) just after compression process (1) and after 6 months storage at different temperature and humidity conditions: 25 ± 2°C/40 ± 5% RH (2), 25 ± 2°C/60 ± 5% RH (3), 40 ± 2°C/75 ± 5% RH (4), *p < 0.05

size were used. Kollidon 25 and Kollidon 30 are pharmaceutical excipients used as a binder for tablets, especially in wet-granulation process (18), whereas Kollidon CL and Kollidon CL-M (crospovidones) are used for improving the release of active substances from tablets, capsules and granules. Additionally, crospovidones are described in the literature as one of the three "superdisintegrants" (19, 20). Pregelatinized starch and microcrystalline cellulose are the most commonly used diluents and fillers in tablet formulations. Magnesium stearate was used as flow improvement agent. Tablets were prepared by wet granulation method and all the manufactured formulations showed very low weight variation, satisfactory mechanical strength and friability (Table 2), indicating that this method was acceptable for preparing good quality tablets with acetaminophen.

Literature indicates that there are numerous reports of the incompatibilities between polyvinylpyrrolidone and many analgesic compounds (indomethacin, ibuproxam, ketoprofen) (21-23). In order to establish possible interactions between acetaminophen and selected excipients, DSC method was applied. DSC compatibility studies are generally carried out in a binary drug-excipient mixture. Unfortunately, the information obtained by this kind of the study do not necessarily reflect the actual impacts between excipients present in the tablet mass. In real formulations, API and all the excipients are present together at the same time and multiple excipient interactions might occur, also as a consequence of processing technology effects (24). For this reason, in the present study, an assessment of acetaminophen melting point was conducted in complete multicomponent individually performed mixtures with various percentage ratio of different types of Kollidon and constant composition of the other excipients before and after compression process. Tablets were additionally analyzed after 6 months storage under different temperature and humidity conditions.

The thermogram of pure acetaminophen is characterized by the sharp peak at 169.04°C due to the melting of the solid drug (12). It was found that DSC thermograms of mixtures with Kollidon 25, Kollidon 30 and Kollidon CL-M before and after tabletting process and after 6 months storage under various temperatures and humidity conditions did not show significant changes in peak placement in comparison to the peak obtained from pure acetaminophen, suggesting compatibility of the compounds (data not shown). Interestingly, DSC thermograms obtained from mixtures of acetaminophen and 5% Kollidon CL indicate that the peak placement was changed just after compression process

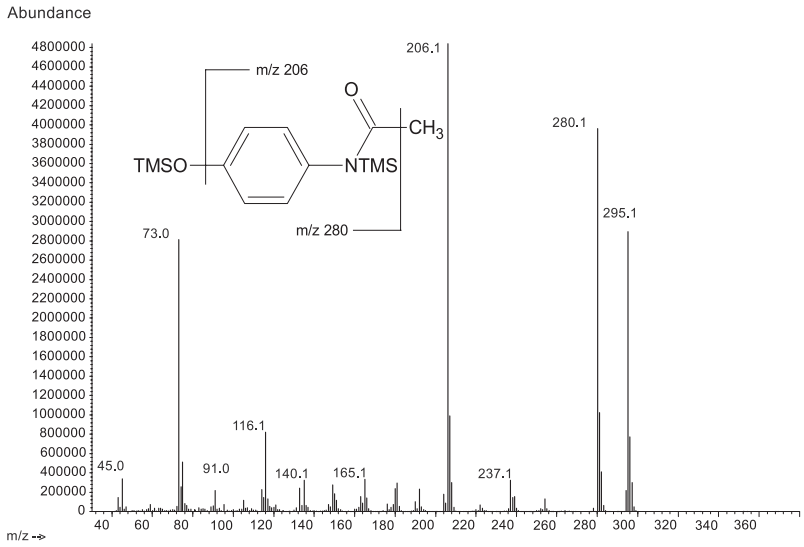

Figure 3. The EI-mass spectra of acetaminophen

and during the storage, with the increase of humidity the changes were also visible in tablets containing 2.5% Kollidon CL (Fig. 1). The greatest shift (T_{onset} = 167.89°C) proved tablets stored at 40 ± 2°C /75 ± 5% RH containing 5% Kollidon CL. The comparison of the onset temperature of the acetaminophen peak obtained from the formulations of Kollidon 2.5% and Kollidon 5% in various humidity conditions was significant ($p < 0.05$) and was shown in Figure 2.

The significant shift towards lower temperature values and broaden of endothermic peak of acetaminophen was probably connected with changes of tablet physical properties. Formulations with Kollidon CL (F5, F6) after 6 months storage at ambient humidity conditions became softer, had lower level of hardness (about 90.7 ± 0.8 N) and higher friability (> 1.3%). However, in this formulations no significant changes in the content of acetaminophen and the *in vitro* drug release profile was observed.

Obtained DSC thermograms might reveal an interaction between acetaminophen and Kollidon CL as a consequence of physico-chemical incompatibility. Physical incompatibilities affect changes in solubility, adsorption or the formation of an eutectic mixture. Chemical interactions are usually due to acid-base, redox, hydrolysis or combination reactions, what greatly increase the possibility of drug degradation (25, 26).

In order to explain the character of interaction between acetaminophen and Kollidon CL, GC-MS method was applied. The electron-impact mass spectra, the fragmentation patterns and molecular ion m/z 295 of acetaminophen are shown in Figure 3. The gas chromatogram of silylated extracts from tablets did not reveal other peaks and linear temperature programmed retention index of acetaminophen was calculated (1648 ± 1). GC-MS study revealed that there were no significant changes of acetaminophen chemical structure, what might indicate that changes observed in the DSC thermograms were the result of a physical reactions mixture. As acetaminophen and Kollidon CL did not have adsorption properties, this interaction could be linked to the eutectic mixture formation. It has been reported that acetaminophen could form eutectic mixtures with some excipients (5, 27). However, in the present study, it was found that the melting temperature of the mixture was not significantly lower than the individual compounds, therefore, the eutectic mixture seems rather unlikely. Crospovidones are hygroscopic and can absorb water during manufacturing, for example, during wet granulation. Probably the high hygroscopic nature of crospovidone is responsible for the softening of Kollidon CL

tablets and shifts on DCS thermograms. Changes in peak placements were observed just after compression process, so the incompatibilities between acetaminophen and Kollidon CL in the tablets could be a consequence of processing technology effects.

CONCLUSION

The interaction between acetaminophen and Kollidon CL is probably a result of a physical reaction (caused by a hygroscopic nature of Kollidon CL) as confirmed by GC-MS analyses. Based on the DSC data, all other tested excipients (Kollidon 25, Kollidon 30, Kollidon CL-M, microcrystalline cellulose, pregelatinized starch, magnesium stearate) were compatible with acetaminophen and could be successfully used as excipients in solid dosage forms. The research shows that DSC is a fast screening test for detection of compatibility/ incompatibilities between acetaminophen and Kollidons as potential constituents of medicinal products.

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