Direct compression is the preferred method for the preparation of tablets because it is more economic and ease of manufacture process. Unfortunately, only less than 20% of active components can be compressed directly into tablets (1). Most API requires the addition of suitable excipients, which improve physico-chemical properties and enable compression of the tablet mass (2). Directly compressible adjuvants are the specialty products prepared by chemical or physical modification, spray drying, fluid bed drying or co-crystallization but one of the most widely explored and commercially used method is co-processing (3-5).

Prosolv® SMCC - silicified microcrystalline cellulose (SMCC) - is multifunctional, co-processed excipient consisting of 98% microcrystalline cellulose (MCC) and 2% colloidal silicone dioxide (CSD), combined in a patented co-processed intimate mixture (9). Prosolv® SMCC shows a five-fold bigger surface than microcrystalline cellulose, which provides better compressibility and ensures better flow properties than regular MCC or than traditional physical mixture of MCC with colloidal silicone dioxide. Moreover, in direct compression, SMCC is 10-40% more compactable than regular MCC (10-13).

Starch 1500® (partially pregelatinized maize starch) is a multifunctional excipient that is used in oral solid dosage forms to improve disintegration properties, enhance flow and lubricity (14). In the solid dosage form API is in direct contact with the other excipients used. It might affect the potential physical and chemical interactions, so
an important part of preformulation is assessment of possible incompatibilities between the drug and excipients used. One of the employed techniques in drug-excipient compatibility screening is differential scanning calorimetry (DSC) a thermoanalytical method used to determine the differences in the heat flow generated or absorbed by the sample (15).

The aim of this study was to examine stability of theophylline (API) and other simultaneously used excipients with various physico-chemical properties (silicified microcrystalline cellulose: Prosolv® SMCC 90, Prosolv® SMCC HD 90, Prosolv® SMCC 50®; pregelatinized starch – Starch 1500® and magnesium stearate). Theophylline is a challenge to formulators because it could inconvert between crystalline anhydrate and monohydrate forms as a function of relative humidity (RH) (16-19).

The study presents results of thermal analysis of mixtures of these substances with theophylline, before and after 6 months storage of tablets at various temperatures and humidity conditions (25±2°C /60±5% RH, 40±2°C /75±5% RH). For the identification of possible changes of API chemical structure, gas chromatograph mass spectrometry (GC-MS) with electron impact ionization (EI) was employed to determine the fragmentation pattern of API. Thermogravimetric analysis (TGA) was used to characterize moisture content of the materials. Tablets were also evaluated for thickness, crushing strength, drug content uniformity and dissolution profile.

EXPERIMENTAL

Materials
Theophylline, pyridine and the silylation reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMSC) were obtained from Sigma-Aldrich (Steinheim, Germany). Prosolv® SMCC 50, Prosolv® SMCC 90, Prosolv® SMCC HD 90 were a gift from JRS Pharma (Rosenberg, Germany), Starch 1500® was received from Colorcon (Indianapolis, IN, USA). Magnesium stearate, methanol and chloroform (GC grade) were purchased from POCH (Gliwice, Poland). All chemicals and reagents were of analytical grade.

Methods
Preparation of tablets
Tablets were prepared by direct compression method according to the formulae given in Table 1. Nine formulations of tablets with theophylline (100 mg), containing various types and various percent ratio (10, 50 and 59% by weight of the tablet) of silicified microcrystalline cellulose (Prosolv® SMCC 90, Prosolv® SMCC HD 90, Prosolv® SMCC 50), pregelatinized starch (Starch 1500®) and magnesium stearate. Theophylline is a challenge to formulators because it could inconvert between crystalline anhydrate and monohydrate forms as a function of relative humidity (RH) (16-19).

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Evaluation of tablets
Physical properties of tablets
Physical characteristics of the tablets were evaluated according to European Pharmacopoeia 8.0 (EP) (20). All tablet formulations were tested for weight variation (n = 20), hardness (n = 10) and friability (n = 10). Hardness was applying using the Schleuniger tablet hardness tester (Dr. Schleuniger Pharmatron Model 5Y, Thun, Switzerland). The friability test was determined by using Electrolab friabilator (ER-1W Electrolab, Mumbai, India).

Drug content determination
Content of theophylline in tablets was carried out by measurement of the absorbance of the sample at 272 nm using a spectrophotometer (Hitachi U-1800, Tokyo, Japan) The amount of theo-

Table 1. Composition of manufactured tablet formulations.

<table>
<thead>
<tr>
<th>Ingredient (mg/tablet)</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Theophylline 100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Prosolv® SMCC 90 147.5</td>
<td>147.5</td>
</tr>
<tr>
<td>Prosolv® SMCC HD 90</td>
<td>147.5</td>
</tr>
<tr>
<td>Prosolv® SMCC 50</td>
<td>147.5</td>
</tr>
<tr>
<td>Starch 1500® 22.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Magnesium stearate 2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Phylline was calculated with a calibration curve with an analytically validated method ($R^2 = 0.9989$, repeatability coefficient of variation (CV) = 1.112%).

**In vitro dissolution studies**

To evaluate release profile of theophylline from prepared tablets, the test was performed using USP type II dissolution apparatus (Erweka DT600, Heusenstamm, Germany) under the following conditions: 900 mL of distilled water at 37 ± 0.5°C and 50 rpm. The absorbance of the solutions was measured at 272 nm (USP) (21).

**Stability studies**

Tablets were placed into Petri dishes and exposed inside humidity chambers (Binder, Tuttingen, UK) at various temperatures and relative humidity (RH) (25 ± 2°C /60 ± 5% RH, 40± 2OC /75 ± 5% RH) for a period of 6 months. After this time physical properties, in vitro dissolution and compatibility studies of tablets were tested.

**Compatibility studies**

**Differential scanning calorimetry (DSC)**

DSC measurements were performed by using an automatic thermal analyzer system (TA Q 2000, New Castle, DE, USA). All precisely weighed samples (approximately 4 mg) were placed in sealed aluminium crucibles. Temperature calibrations were performed using indium and zinc as standards. An empty sealed pan was used as a reference. The entire samples were run at a scanning rate of 10°C/min. from 50 to 300°C in nitrogen atmosphere (20 mL/min.). The temperatures range 200-300°C is presented.

**Sample preparation for GC-MS analysis**

The powdered samples of each formulation were dissolved in the mixture of chloroform, methanol (1 : 1, v/v) and sonificated for 5 min. Then, the resultant solution was filtered using 0.45 µm PTFE membrane. Aliquots (100 µL) of filtrate were placed into the vials and the solvents were evaporated to dryness under argon. The derivatization of samples was performed using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMSC), (99 : 1, v/v) reagents. The dry residue was dissolved in 50 µL pyridine and 50 µL of BSTFA was added into the vial. The reaction mixture was sealed and heated during 45 min. at 80°C to obtain TMS derivatives (22). All the experiments were performed in triplicates.

**Gas chromatography - mass spectrometry (GC-MS)**

GC-MS analyses were carried out on an Agilent Technologies 7890A gas chromatograph coupled to an Agilent 5970C VL quadrupole mass spectrometer equipped with an autosampler 7693 (Agilent Technologies, Wilmington, DE, USA). The mass spectrometer was operated using electron impact ionization mode (70 eV). Samples were separated on 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). Aliquots of 1 µL were injected in the split (50 : 1) mode. The injector was kept at 300°C, MS source and quadrupole temperatures were 230 and 150°C, respectively. 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 rate 10°C/min. held for 5 min., next increased to 280°C at rate 10°C/ min. Oven temperature of 280°C was held for 10 min.

**Thermogravimetric analysis (TGA)**

In thermogravimetric studies, the thermogravimetric analyser TGA Q50 (TA Instruments, New Castle, DE, USA) was used. Samples were heated in an open platinum pan from room temperature to 260°C, under nitrogen purge, at a rate of 10°C/ min.

**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 to be significant at $p < 0.05$.

**RESULTS AND DISCUSSION**

Theophylline is a methylxanthine derivative commonly used to treat asthma. It exists as a crystalline monohydrate and four anhydrous polymorphs (I, II, III and IV) (23-25). Theophylline anhydrate (TA) can transform into theophylline monohydrate (TM) at high relative humidity (16, 26). In low relative humidity, TM form has been shown to lose water to produce form II, which is the most prevalent form and has been considered as the only stable form at room temperature (27, 28). Form I is produced by heating form II and is reported to be stable at higher temperatures (24, 29). Form III is a highly metastable and converts easily to form II during storage (25, 30). Form IV has been identified recently, it occurs as a...
result of slow, solvent-mediated conversion from form II, and is now claimed as the most thermodynamically stable anhydrous polymorph of theophylline (31). Debnath and Suryanarayanan found that wet-granulation process induced polymorphic transformation of theophylline, which can result in many difficulties during the compaction (32-34). Therefore, in this study, tablets with theophylline were obtained by direct compression method. All the manufactured formulations showed very low weight
variation, satisfactory drug content uniformity, mechanical strength and friability, indicating that direct compression is appropriate method to prepare proper quality tablets with theophylline.

The physicochemical stability of tablets is important for the quality of pharmaceutical products. Some factors such as heat and moisture accelerate most drug-excipient reactions and might increase the API degradation (35-37). In the present study, an assessment of theophylline melting point was conducted in multicomponent individually performed mixtures with various percentage ratio of different types of Prosolv® (of various particle size and bulk density) and Starch 1500® with magnesium stearate. The thermogram of pure theophylline is characterized by the sharp peak at 272°C due to its melting (270-274°C, EP). It was found that DSC thermograms of tablet mixtures with 59% and 50% content of Prosolv® SMCC 90 (F1, F2), Prosolv® SMCC HD 90 (F4, F5) and Prosolv® SMCC 50 (F7, F8) after tabletting process did not show significant changes in peak placement in comparison to the peak obtained from pure theophylline - suggesting compatibility of the compounds. However, changes were observed in all tablets, with 10% content of Prosolv® (F3, F6, F9) and 49% of Starch 1500® (Fig. 1). Similar differences in peak placement and shape were observed in DSC thermograms achieved from analysis of the tablets.

![Figure 3. DSC thermograms of pure theophylline and theophylline in binary mixtures with Starch 1500®, Prosolv® 90, Prosolv® HD 90 and Prosolv® 50 just after tabletting](image)

![Figure 4. Thermogravimetric analysis of moisture content in tablets containing 10% of Prosolv® SMCC and 49% of Starch 1500®, after 6 months storage at 40±2°C/75±5% RH](image)
after 6 month storage at the room temperature (25 ± 2°C /60 ± 5% RH – data not shown) and in tablets after accelerated storage conditions (40 ± 2°C /75 ± 5% RH) (Fig. 2). Tablets prepared with Prosolv® SMCC at concentrations 50% and 59%, resulted in a proper long term storage conditions.

Pharmaceutical dosage forms are exposed to water present in an atmosphere (during production or storage) or excipients possess a high water content which is able to equilibrate between various components (38). DSC changes in theophylline peak were observed in all cases of tablets containing 10% content of Prosolv® SMCC - that is, with a high content of Starch 1500®. It is known that excipient may possess a high water content (the equilibrium moisture content of starch is about 8-10%), which can lead to a physicochemical change of the tablets and may affect drug stability (39, 40). Therefore, to investigate whether the variation is related to Prosolv or Starch content, mixtures of two component (theophylline and Prosolv® SMCC 90, theophylline and Prosolv® SMCC HD 90, theophylline and Prosolv® SMCC 50, theophylline and Starch 1500®) were prepared. The results of DSC study showed that change

![Figure 5. The dissolution profile of theophylline tablets prepared with 10% of Prosolv® 90 (A), Prosolv® HD 90 (B) and Prosolv® 50 (C) and 49% of Starch 1500® before (○) and after 6 month storage (●) at high temperature and humidity conditions (40 ± 2°C/75 ± 5% RH)](image)

![Figure 6. EI-mass spectra of theophylline](image)
in theophylline peak placement was observed only in the mixture of the drug and Starch 1500® (Fig. 3). The significant shift of melting temperatures and broaden peak of theophylline were also related to changes of the tablets physical and mechanical properties. Formulations containing 10% of all types of Prosolv® and 49% of Starch 1500® (F3, F6, F9) after 6 month storage at high temperature and humidity conditions presented lower hardness (the range from 28 to 36 N) and higher friability (> 1.4%). However, no significant changes in the tablets weight after storage were shown. It suggests that the tablets do not absorb moisture from the atmosphere and probably moisture content in the Starch 1500® is able to equilibrate between the tablets component and lead to drop of physical properties of theophylline (in the case of F3, F6, F9). Additionally, evaluated by TGA water amount in tablets was about 5%, which is related to the water content in the starch (based on the weight of the tablet) (Fig. 4). This phenomenon has been observed by Otsuka et al. - theophylline anhydride changed into theophylline monohydrate at more than 75% RH (27). Sandler et al. demonstrated that only storage at 99% RH lead to theophylline transition from TA to TH (38). The results of the study indicate that water present in Starch 1500® content could induce incompatibilities in theophylline tablets.

Figure 5 showed the dissolution profiles of the theophylline tablets with 10% of Prosolv® SMCC. It was demonstrated that after storage all tablets showed the accelerated release of theophylline compared to tablets just after compression but significant changes in the in vitro release profile was observed in tablets containing 10% Prosolv® (F3, F6, F9). The formulations release the active substances more than two times faster than just after compression. Because dissolution rate of TA depends on the degree of hydration and TH is less soluble than TA, probably theophylline anhydrate does not pass in monohydrate (16, 26).

In order to exclude the effect of chemical decomposition on the change of theophylline DSC peak, prepared tablets were analyzed by GC-MS method. The electron-impact mass spectra, the fragmentation patterns and molecular ion m/z 295 of theophylline are shown in Figure 6. The gas chromatogram of silylated extracts from tablets did not reveal other peaks and linear temperature programmed retention index of theophylline was calculated. GC-MS study revealed that there were no changes of theophylline chemical structure, what might indicate that changes observed in the DSC thermograms were the result of physical reactions.

Changes in peak placements were observed just after tabletting, which may suggest that compression technology accelerate the incompatibilities between theophylline and Starch 1500® in the tablets. The type of interaction will be investigated in future experiments.

CONCLUSION

In the present study, we demonstrated that high concentration of Starch 1500® (49%) in the tablet mass affects stability of the tablets containing theophylline and Prosolv® SMCC. The determining factor is probably water from starch content.

The results confirmed that differential scanning calorimetry, could be used as a quick screening test to evaluate the compatibility between theophylline and Prosolv® SMCC as well as Starch 1500®.

REFERENCES


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