Evaluation of new flavors for feline mini-tablet formulations.

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ABSTRACT

Despite a global interest in companion animal pharmaceuticals, feline peroral medication is still lacking in palatable and voluntarily acceptable drugs of suitable size and attractive taste. As a consequence, treating cats with canine or human medicinal products has weakened patient compliance and treatment commitment resulting in many pet cats going untreated. In the future, the companion animal pharmaceutical business is expected to focus particularly on cats and the development of palatable feline medication. Based on this, the overall aim of this study was to facilitate voluntary drug administration to felines. Specifically the aim was to develop sophisticated and tailor-made feline medicinal products, in the form of mini-tablets, focusing on flavors palatable to felines. Rapid preformulation compatibility and stability screening tests of synthetic flavors were carried out using readily available oral solid form excipients. On the basis that felines are carnivorous, L-methionine, L-leucine, L-proline and thiamine hydrochloride were investigated as possible flavors for improving palatability. These flavors, together with a model substance for a bitter taste, denatonium benzoate, were evaluated for their physicochemical properties, stability and physical compatibility. This evaluation was carried out with the substances alone and in binary combinations of flavors and excipients. Stability and compatibility were examined using differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). The results showed that L-proline and denatonium benzoate anhydrate were hygroscopic. Thiamine hydrochloride was incompatible with talc and sodium stearyl fumarate. The known incompatibility between the amines contained in flavors, and α-lactose monohydrate was used to assess method sensitivity. Overall, this study provided new information on the compatibility of novel flavors with oral solid form excipients. This study also showed the applicability of using XRPD and DSC for the rapid evaluation of instability and incompatibility.

KEY WORDS: Feline medication, excipient, flavor, mini-tablet, formulation, instability, incompatibility, X-Ray Powder Diffractometry (XRPD), Differential Scanning Calorimetry (DSC)

INTRODUCTION

Taste masking represents an additional hurdle in formulating perorally administered tablets

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containing bitter-tasting active substances. To overcome the offensive taste sensation in the mouth, the repulsive flavor has to be concealed in order to support successful patient compliance and treatment commitment (1). A pleasant taste and an appealing shape, odor and mouth feel are exceptionally important when developing companion animal medicinal products, especially for treating ordinary pet cats, which have an individual, extraordinarily selective, and even picky nature (2, 3). Compared to dogs, problems related to taste and acceptability in feline medication have been found particularly demanding. Inadequate taste masking or the repulsive smell of over-sized tablets with an offensive texture can cause salivation, vomiting and a loss of appetite (4). Feline product administration is problematic also because of the susceptibility of these animals to aversion and to both neophobia and neophilia (5). Being selective in nature, felines currently lack palatable medicinal products, especially palatable and reasonably-sized tablets that can be easily administered (6). Thus, although they are the most popular of all companion animals, and despite the fact that the animal health industry has shifted its focus from production animals to companion animals, current feline medicinal products are highly dependent on the existing repertoire of canine and human pharmaceuticals. Therefore, the present study focused on the development of sophisticated and tailor-made feline medicinal products because in the future the overall pharmaceutical business in companion animal medicines is expected to be particularly aimed at palatable feline medicinal products (3, 7).

So far, the simplest way to improve the palatability of peroral meidcation for felines has been to enclose the medicinal product inside attractive animal food kibbles or other delicacies, such as animal fat. However, this is not the most suitable method. In many cases, sick animals suffer from a loss of appetite and may not consume completely the offered dose. Moreover, the offered food consumed together with the medicinal product may affect adversely the bioavailability of the drug or, if administered in a dry form, the kibble may stick onto the mucous walls of the oesophagus and cause salivation, vomiting, or in more severe cases, ulcers. Additionally, attractive food does not always provide protection against the bad taste sensation of the drug, and thus, despite best efforts, the cat may spit out or vomit the product. Following their natural preference for meat products (3), the palatability of canine and feline single oral pharmaceuticals has been improved by combining the drug embodiment with flavoring substances rich in vitamin B and protein, such as yeast and its extracts (yeast hydrolysates) (8). Large amounts of brewer's yeast with a possible combination of commercial meat flavors used in pet feed have also been used in the production of orally administered veterinary products (9). Liver meal as a formulation supplement (10), as well as, yeast, artificial egg, beef, poultry or fish flavor, dairy-based palatability-improving agents, natural herbs and spices or combinations thereof have also been used to increase the appeal of oral medicinal products (1). However, as cats are strict carnivores, the use of herbal substances is unlikely to be successful. In addition, drugs containing naturally derived materials may lack microbiological purity, as well as, suffer from batch-to-batch variation (1). Thus, the use of such substances is expected to incur additional requirements, demands and costs for pharmaceutical manufacturers.

To avoid the aforementioned problems related to natural ingredients in taste masking, synthetic excipients are considered more suitable in the pharmaceutical industry and product development. As these excipients must not interact with the active pharmaceutical ingredient (API), or with each other, during the shelf life of the product, it is crucial to collect compatibility and stability information on the

different formulation components. This is especially important at the very beginning of product development, when the aim is to identify a limited number of model formulations in a short amount of time.

In this study, the compatibility of several flavors, inert tablet ingredients and a model bitter-tasting drug were investigated with the objective to shorten the development time of feline peroral formulations. Following the natural preference that cats have towards meat-based flavors, new candidates from the group of amino acids and B-vitamins for taste-masking excipients of synthetic origin were investigated, as well as, a method for rapidly evaluating their interactions with commonly used tableting excipients. As far as is known, amino acids have not yet been evaluated for their compatibility with common tableting excipients. Certain amino acids have already been used for taste masking in human pharmaceuticals (11, 12, 13). Amino acids and B-vitamins have also been used in food chemistry due to their nutritional contents and their taste-enhancing characteristics. Considering feline dosing difficulties, the focus was on preformulation studies for the components considered for feline tablet formulations. The objective was to rapidly screen possible incompatibilities and instabilities between the chosen flavors and excipients commonly used in tablet formulations. Denatonium benzoate was used as a model substance as a bitter taste for the suggested palatability-improving candidates. In this study, the relevant physicochemical characterization of these substances was performed using scanning electron microscopy (SEM) and laser diffractometry. The palatability-improving substances were also characterized for their density and flow properties. The compatibility and stability of the substances and common pharmaceutical tableting excipients was determined using the rapid techniques of X-ray powder diffractometry (XRPD) and differential scanning calorimetry (DSC).

MATERIALS AND METHODS

Materials

All materials used in this study are presented in Table 1. Denatonium benzoate was used as a model substance representative of a bitter-tasting API. Three amino acids together with thiamine hydrochloride were investigated as palatability-improving flavoring substances.

Methods

Particle size

The particle size distributions of the flavoring substances were determined using laser diffractometry (Malvern 2600C, Malvern, England). Evaluation of the size of the dry powder particles was performed in liquid media, where a small amount of dry powder was loaded into a stirred sample cell containing cooled ether. A focal length of 100 nm and a beam length of 14.3 nm were used to determine the intensity of laser light and calculate the volume diameters d_{10}, d_{50} and d_{90} of the particle size distribution. The experiments were performed in triplicate.

Scanning electron microscopy

The particle morphology (particle shape and surface) of each flavoring substance was studied using a scanning electron microscope (SEM) (Zeiss DSM 962, Karl Zeiss, Oberkochen, Germany). Dry platinum-coated samples were scanned using a voltage of 5–10 kV.

Density and flow properties

The bulk and tapped density, δ_{bulk} and δ_{tap} respectively, for the palatability-improving flavoring substances were determined using the

Table 1 Suggested flavoring substances and tableting excipients of the study

FLAVORING SUBSTANCE	MANUFACTURER	FUNCTION
Denatonium benzoate NF	Sigma-Aldrich, China	Bitter tasting flavoring substance
L- methionine NF	Sigma-Aldrich, Japan	Palatability-improving flavoring substance
L- leucine NF	Sigma-Aldrich, Japan	Palatability-improving flavoring substance
L- proline NF	Sigma-Aldrich, USA	Palatability-improving flavoring substance
Thiamine hydrochloride USP	Hawkins Inc., USA	Palatability-improving flavoring substance
CANDIDATE FOR FORMULATION EXCIPIENT	**MANUFACTURER**	**FUNCTION**
Microcrystalline cellulose NF(Avicel® PH 101)	FMC Corporation, Ireland	Filler
Calcium hydrogen phosphate dihydrate NF (Emcompress®)	Albright & Wilson, Australia	Filler
Lactose monohydrate NF (Pharmatose® 200M)	DMV International, Netherlands	Filler
Mannitol NF (Pearlitol® 160C)	Roquette, France	Filler
Hydroxypropyl cellulose NF (Klucel®JXF)	Aqualon France SA, France	Binder
Povidone PhEur (Kollidon® K30)	BASF Corporation, Germany	Binder
Croscarmellose sodium NF(Ac-Di-Sol®)	FMC Biopolymer, Belgium	Disintegrant
Crospovidone NF (Kollidon® CL-F)	BASF Corporation, Germany	Disintegrant
Sodium stearyl fumarate NF (Pruv®)	JRS Pharma, Spain	Lubricant
Talc PhEur	Imerys Talc S.p.A., Italy	Lubricant

method described in Ph. Eur. (14) using a tapped density tester (Erweka SVM, Apparatebau, Germany). Quantities smaller than those described in Ph. Eur. were used here. Densities were determined as a mean of three measurements. Flow properties of the palatability-improving flavoring substances were assessed using Carr´s index (CI) and Hausner's ratio (HR). The CI (15) and the HR (16) were determined from bulk and tap densities using Equations 1 and 2:

$$CI = \frac{(\delta_{tap} - \delta_{bulk})}{\delta_{tap}} x\ 100 \qquad \text{Eq. 1}$$

$$HR = \frac{\delta_{tap}}{\delta_{bulk}} \qquad \text{Eq. 2}$$

Accelerated stability and compatibility studies

The physical stability of the flavoring substances and chosen excipients was investigated. In addition, their compatibility was evaluated. Samples contained either individual substances or their binary 1:1 w/w physical mixtures. The samples were prepared by gently grinding and mixing the materials manually using a mortar and pestle, and then placing 0.5 g of each sample in a glass sample jar. The samples were then used for stability and compatibility studies before placing them in open bottles in a sealed desiccator at 75% relative humidity. The desiccator was placed in an oven at 40°C for 3 to 4 weeks.

Evaluation for possible changes in stability and compatibility were performed visually, using X-ray powder diffractometry (XRPD) and differential scanning calorimetry (DSC). To obtain a baseline behavior of the materials, the color and consistency, as well as, the thermal and crystal structural behavior were assessed immediately after sample preparation at time

zero (samples hereafter described as 0W). The results were compared with the visual and X-ray data from the same samples stored for 3 to 4 weeks under conditions of high temperature and humidity at 40°C/75% RH (samples hereafter described as 3W or 4W, respectively).

X-ray powder diffractometry

XRP diffraction patterns of scattered intensities were measured using the scintillation counter of a theta-theta X-ray powder diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany). Measurements were performed in a symmetrical reflection mode with Cu Kα radiation (λ = 1.54 Å) at 40 mA and 40 kV using a Göbel mirror. The measured angular range was 5–40° (2*θ*) with steps of 0.05°. The measurement time per step was 1 second. The experiments were performed at room temperature. When applicable, the measured XRPD patterns were compared with theoretical patterns generated from data obtained from the Cambridge Crystallographic Data Centre (CCDC) in the UK.

Differential scanning calorimetry

Powdered samples of 3 to 4 mg were weighed and encapsulated in 40 µl aluminum pans which were hermetically closed with a lid with two pinholes. The samples were heated in an atmosphere of nitrogen gas at a flow rate of 50 ml/min and thermograms were obtained using a Mettler DSC823^e differential scanning calorimeter (Mettler-Toledo AG, Greifensee, Switzerland). Measurements for the thermograms were first carried out by preheating the samples at 25°C for 3 minutes and then continuing at a constant heating rate of 10°C per minute. The measurement end-point temperatures ranged from 250 to 300 °C.

RESULTS AND DISCUSSION

Material properties

The chemical structures and descriptions of the material properties of the chosen substances (appearance, taste and odor, and melting point) based on information from the available literature are presented in Table 2. Scanning electron microscopy (SEM) images for denatonium benzoate and palatability-improving flavoring substances, together with their particle sizes (µm), are shown in Figure 1 (a-e). The SEM images show material differences in particle form and shape. Compared to the needle-shaped particles of denatonium benzoate and L-proline, particles of L-leucine and L-methionine were flat and round. Thiamine hydrochloride consisted of powder-like crystals of variable, irregular sizes.

Density and flowability measurements

Flavor imparting ingredients are incorporated in pharmaceutical dosage forms ranging from 0.025% to 99%, preferably from 0.075% to 50% by weight (1). The density and flowability of these ingredients are therefore expected to significantly influence tabletability. Bulk and tap densities and flowability were therefore determined only for the palatability-improving flavoring substances.

The bulk and tap densities and the parameters for HR and CI describing powder flowability for the palatability-improving flavoring substances are listed in Table 3. Tap density can be generally described as a measure of the particles rearrangement and packing ability. Due to the differences in particle form and shape for the different materials studied, differences in particle flow was expected.

Compared to other palatability-improving flavoring substances, L-leucine which has thick, spherical shape particles had the highest value for bulk and tap densities. The lowest value for bulk and tap density was found for irregularly shaped and formed particles of thiamine hydrochloride. Variations between bulk and tap densities were attributed to particle form and shape. As expected, tap density was shown to increase with spherical particles of regular size.

The Hausner ratio and Carr´s index have been widely used for predicting of powder flowability. Good flow characteristics is expected for materials with a Hausner ratio less than 1.20, whereas a value of ≥ 1.5 is related to poor flowability of powders (16).

Table 2 Properties of denatonium benzoate anhydrate and the palatability-improving flavoring substances

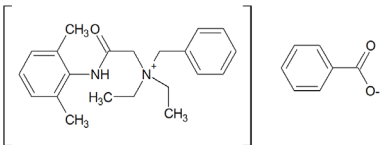
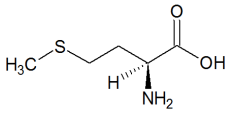
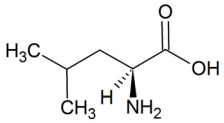
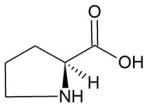
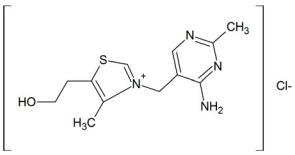
SUBSTANCE	APPEARANCE	ODOR	TASTE	MELTING POINT (°C)	CHEMICAL STRUCTURE
Denatonium benzoate anhydrate	White, crystalline powder (17)	Odorless (17)	Extremely bitter (17)	174-176 (17)	
L-methionine	White crystalline powder or platelets (17)	Slightly characteristic odor (17)	Flat to bitter, possibly sulphurous, meaty or sweet (18)	281 (17)	 α–amino acid
L-leucine	Small white, lustrous plates or a white crystalline powder (19)	Odorless (19)	Slightly bitter taste (19), Flat to bitter (18)	295 (17)	 α–amino acid
L-proline	Colourless or white crystals or crystalline powder (17)	Odorless or a slight, characteristic odour (17)	Slightly sweet taste (19), Possibly complex with salty or sour components (18)	220-222 (17)	 α-amino acid
Thiamine hydrochloride	Small, white to yellow crystals or crystalline powder (17, 19)	Nut-like odor (17), Odor slightly, reminiscent of thiazole (19)	Bitter taste (19)	248 (17)	

Table 3 Powder characteristics of palatability-improving flavoring substances L-methionine, L-leucine, L-proline and thiamine hydrochloride

PALATABILITY-IMPROVING FLAVORING SUBSTANCE	BULK DENSITY (g/ml)	TAPPED DENSITY (g/ml)	HAUSNER RATIO	CARR´S INDEX
L-methionine	0.534	0.591	1.106	9.535
L-leucine	0.677	0.726	1.072	6.681
L-proline	0.400	0.518	1.294	22.626
Thiamine hydrochloride	0.323	0.415	1.288	22.3

Carr´s index values of higher than 20 indicate fair to poor flowability of powders (15).

Of the studied palatability-improving materials, both L-proline and thiamine hydrochloride had a value greater than 1.20 for Hausner's ratio and greater than 20 for Carr´s index. Significantly lower values were obtained for L-leucine and L-methionine indicating excellent flowability for these amino acids compared to L-proline and thiamine hydrochloride. The results were consistent with expectations based on particle shape and form. Regularly shaped spherical particles of L-leucine and L-methionine had better flowability compared to irregularly or needle-shaped particles of thiamine hydrochloride and L-proline. Thus, L-leucine and L-methionine are expected to assist in die filling when compressing mini-tablets.

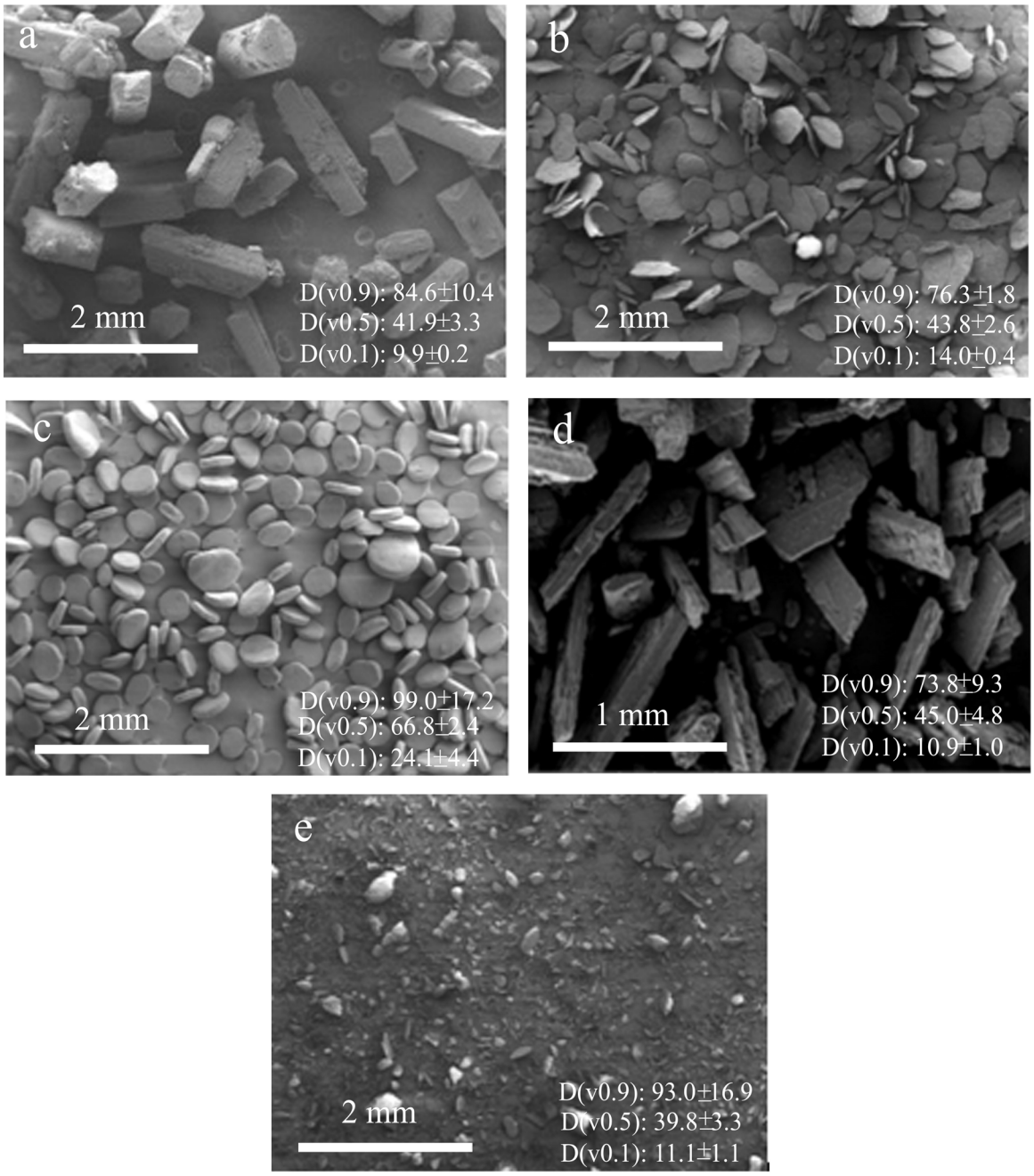


Figure 1 SEM images and particle sizes of (a) denatonium benzoate anhydrate, (b) L-methionine, (c) L-leucine, (d) L-proline and (e) thiamine hydrochloride.

When using thiamine hydrochloride or L-proline, the flowability of the tablet mass could be improved by choosing tableting excipients with good flowability or by increasing the particle size through granulation.

XRPD and DSC measurements for denatonium benzoate and palatability-improving flavoring substances

Screening for interactions between substances can rapidly be determined by studying the

compatibility and stability of binary mixtures in 1:1 compositions. Traditionally, DSC has been used for such studies. During DSC tests, samples are subjected to high temperatures that increase their ability to react. Possible interaction between materials can therefore be seen as a new, disappeared or shifted melting peak in a DSC thermogram. However, the results of such studies must be carefully evaluated before making any final conclusions on possible interaction between materials because they are unlikely to be representative of interactions occurring at temperatures at which pharmaceutical dosage forms are manufactured and stored. Thus, results from DSC should be interpreted in combination with other techniques, such as XRPD.

During XRPD analysis, the samples are characterized for their degree of crystallinity, crystal form and polymorphism. Moisture-induced interactions can be easily detected using XRPD. Interactions between substances can be seen as new, disappeared or shifted peaks in XRPD diffractograms. In the studies carried out here, two substances were deemed to be incompatible if both DSC and XRPD indicated new, disappeared or shifted peaks. However, due to the difficulty in interpreting moisture-induced incompatibilities with DSC, results shown by XRPD were emphasized. It must be stressed, that the objective of this study was to rapidly screen for incompatibility between formulation candidates and flavors. Interactions among ingredients when present at

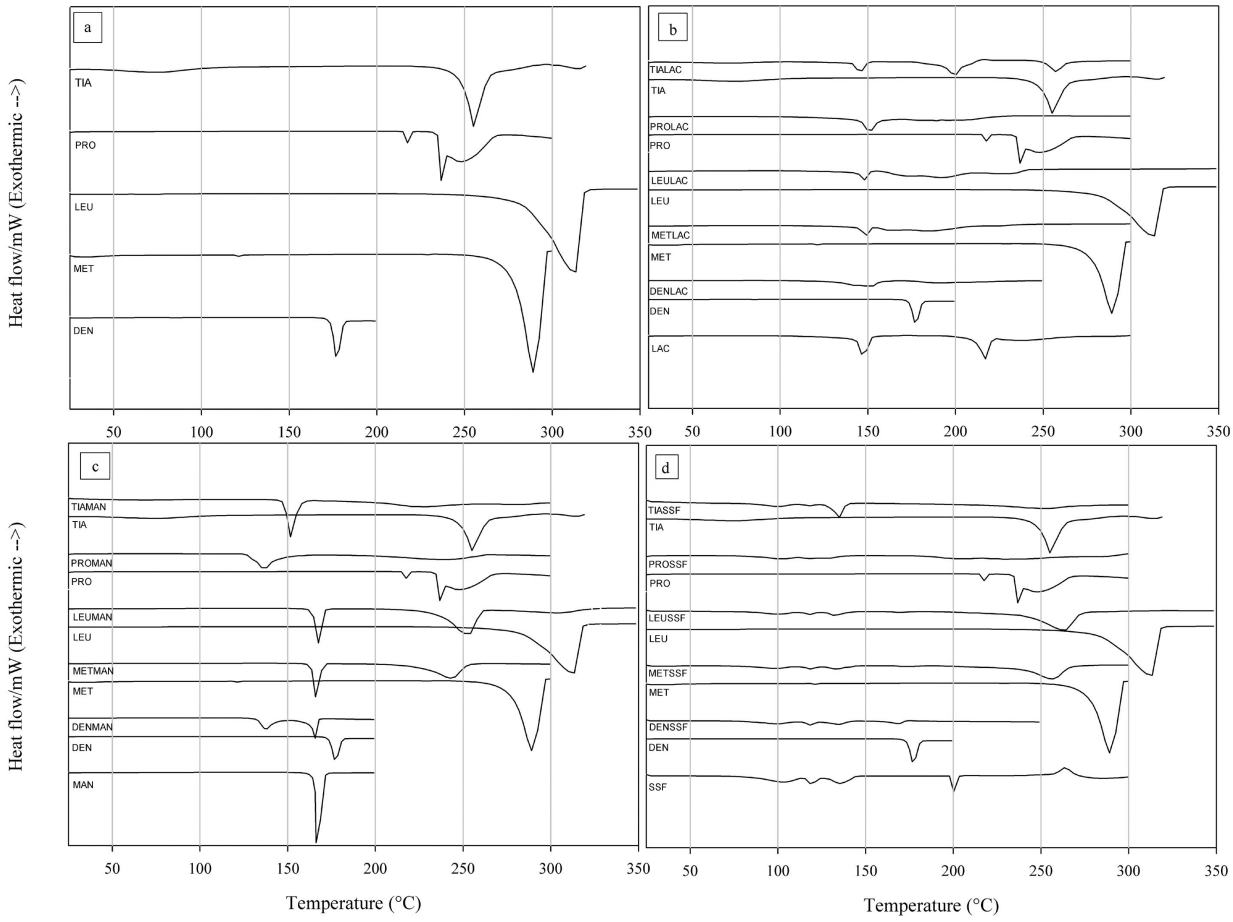


Figure 2 DSC thermograms of (a) denatonium benzoate anhydrate (DEN), L-methionine (MET), L-leucine (LEU), L-proline (PRO) and thiamine hydrochloride (TIA), (b) the 1:1 mixed systems with α-lactose monohydrate (LAC), (c) the 1:1 mixed systems with mannitol (MAN) and (d) the 1:1 mixed systems with sodium stearyl fumarate (SSF).

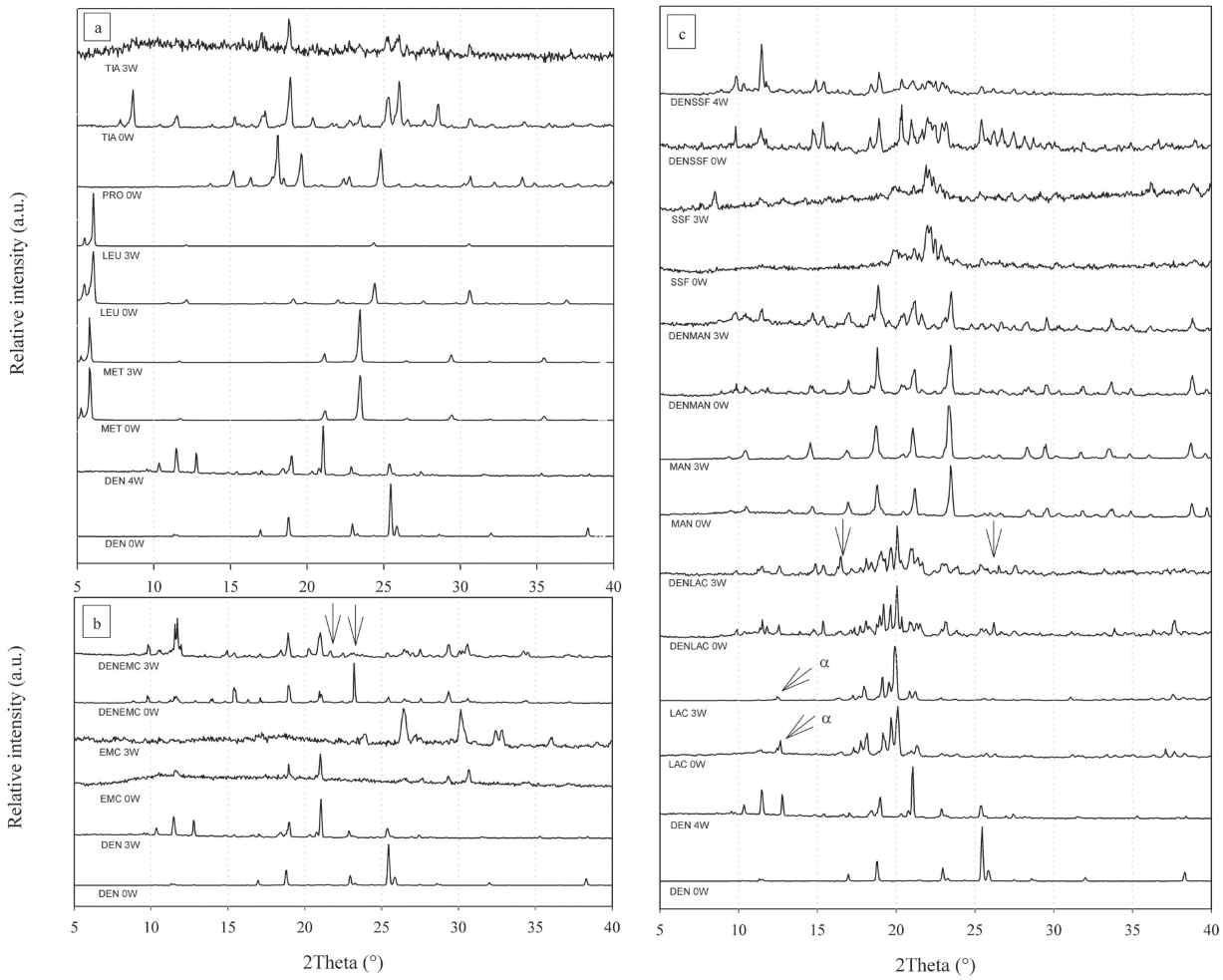


Figure 3 a-c XRPD diffractograms of samples before (0W) and after (3W/4W) storage at 40°C/75% RH (a) for denatonium benzoate anhydrate (DEN), L-methionine (MET), L-leucine (LEU), L-proline (PRO) and thiamine hydrochloride (TIA), (b) for DEN and its binary mixture with calcium hydrogen phosphate dihydrate (EMC) and (c) for DEN and its binary mixtures with α-lactose monohydrate (LAC), mannitol (MAN) and sodium stearyl fumarate (SSF). Changes in sample crystal structures are indicated with arrows for the diffractograms of DENEMC 3W (Figure 3b), and of DENLAC 3W (Figure 3c). α-Arrows in Figure 3c for LAC 0W and LAC 3W indicate peaks typical for α-lactose.

concentrations in the final formulation would therefore still need to be performed at a later phase of formulation development using methods such as high performance liquid chromatography (HPLC).

Pure flavoring substances

Denatonium benzoate is known to exist in both anhydrate and monohydrate forms. In this study, the anhydrous form was used, as indicated by a melting point of 176°C for the pure substance as shown in the DSC thermogram (Figure 2a). The anhydrate was hygroscopic and absorbed moisture during storage at 40°C/75% RH, as indicated by the changes between the X-ray diffractograms of denatonium benzoate anhydrate at 0W and the 4W samples (Figure 3a). No changes were seen during the visual evaluation of the 4W sample after storage under conditions of high temperature and humidity.

Neither L-methionine nor L-leucine showed phase transition after storage at 40°C/75% RH (Figure 3a). This result is consistent with the

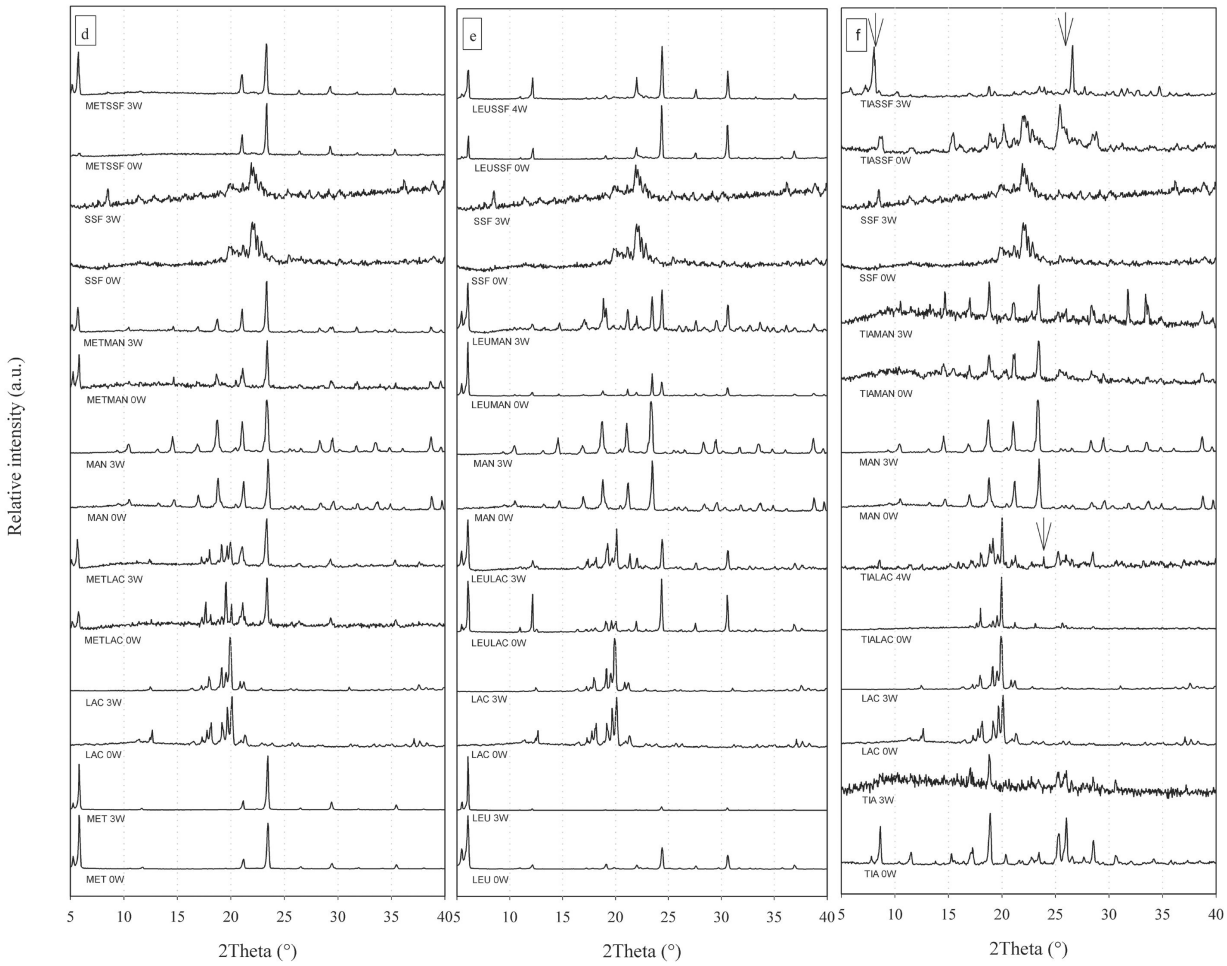


Figure 3 d-f XRPD diffractograms of samples before (0W) and after (3W/4W) storage at 40°C/75% RH (d) for L-methionine (MET) and its binary mixtures with α-lactose monohydrate (LAC), mannitol (MAN) and sodium stearyl fumarate (SSF), (e) for L-leucine (LEU) and its binary mixtures with LAC, MAN and SSF, and (f) for thiamine hydrochloride (TIA) and its binary mixtures with LAC, MAN and SSF. Changes in sample crystal structures are indicated with arrows for the diffractograms of TIALAC 3W and TIASSF 3W (Figure 3f).

findings of Mellon and Hoover (20), who grouped the two substances into non-hygroscopic amino acids. They reported that both L-methionine and L-leucine were able to withstand moisture-induced phase transition up to, and including, 93% RH. However, a strong phase transition-related interaction with moisture and temperature was shown with L-proline. After 3 weeks of storage at 40°C/75% RH, the sample containing pure L-proline had transformed into a clear transparent solution. Thus, it was not possible to produce an XRPD diffractogram for this substance after these accelerated storage conditions.

The behavioral difference between the three studied amino acids has been explained by Mellon and Hoover (20). All three amino acids contain polar groups, indicating the possibility of strong interaction with excess surrounding moisture or water. However, the strength of the interaction depends primarily on the spatial configuration of the polar groups. The polar groups of L-methionine and L-leucine are buried in the crystal structure. The groups that would be able to interact with water are located deep in crystal lattices and voids and thus are not free for interaction. They are either unable to reach the water molecules, or the compact crystal lattices prevent water molecules from

penetrating them. L-proline is different and Mellon and Hoover (20) have reported that L-proline is willing to accommodate as many water molecules as possible. One way it does this is via crystal structure transformation or phase change. For L-proline, no moisture absorption occurs at 31% RH. However, when subjected to 51% RH, L-proline forms a monohydrate that dissolves on standing. The results here indicated that with L-proline, a monohydrate was formed at 40°C/75% RH. In accordance with the results recorded by Mellon and Hoover (20), an excess of moisture in this study was able to penetrate the crystal structure of L-proline, and when doing so, the polar groups interacted with the water molecules, forming hydrated crystals. Later, due to increased sensitivity to moisture these hydrated crystals incurred, a clear solution was formed on standing at 75% RH.

In addition to L-proline, thiamine hydrochloride is known to be humidity sensitive (21). In this study, the effect of moisture exposure was noticed in the changed forms of the XRPD diffractograms between the 0W and 3W for the thiamine hydrochloride samples (Figure 3a). The thiamine hydrochloride polymorphs that are most sensitive to moisture are the anhydrate and nonstoichiometric hydrate. Both forms, when exposed to air humidity (53% room temperature), are transformed into thiamine hydrochloride hemihydrate (22). According to Watanabe *et al.* (23), a similar conversion from thiamine hydrochloride monohydrate into hemihydrate also occurs during prolonged storage in air and humidity (40°C/75% RH). The XRPD diffractograms for thiamine hydrochloride obtained in this study thus indicated a transformation from the monohydrate form to the hemihydrate during storage under the study conditions. The fact that the studied thiamine hydrochloride was in a monohydrate form was later supported by the DSC thermogram for pure thiamine hydrochloride. According to Chakravarty *et al.* (24), the hemihydrate polymorph of thiamine hydrochloride is exceptionally stable under high humidity and even when exposed to high temperatures. The dehydration of hemihydrate begins at temperatures greater than the dehydration of nonstoichiometric hydrate, being initiated when heated to 120°C. In this study, the DSC thermogram of thiamine hydrochloride revealed two endotherms (Figure 2a). The first low-enthalpy endotherm was shown at the early onset of the temperature increase, below 80°C. After this, a sharp melting endotherm with decomposition at a temperature above 248°C was recorded. According to Watanabe and Nakamachi (25) and Wöstheinrich and Schmidt (21), this behavior is characteristic of the nonstoichiometric hydrate form of thiamine hydrochloride. First, this polymorph lost its crystal water at a temperature well below 120°C, characteristic of the dehydration of thiamine hydrochloride monohydrate. Then, its dehydrated water-free form melted with decomposition above 248°C.

Combinations with the chosen excipients

Microcrystalline cellulose

Microcrystalline cellulose was successfully combined with the non-hygroscopic amino acids L-methionine and L-leucine, as no changes in flavor crystal forms or in their thermal behavior were observed (data not shown). Although the 1:1 concentration with the highly hygroscopic L-proline was incompatible and would thus demand a much higher concentration of moisture-absorbing microcrystalline cellulose, compatible binary mixtures were possible in the presence of other studied moisture-sensitive flavors. The results are not presented here, but microcrystalline cellulose was able to capture moisture resulting from the surrounding humid conditions, and was thus able to be combined with denatonium benzoate anhydrate and thiamine hydrochloride. With denatonium benzoate anhydrate, microcrystalline cellulose even seemed able to withstand and protect the moisture-induced phase transition of denatonium benzoate anhydrate, as no

pharmaceutically significant changes indicating flavor instability were observed between the 0W and 3W XRPD diffractograms of the combination. Furthermore, the denatonium-cellulose 1:1 binary combination was confirmed to be compatible, as no changes were revealed in the DSC thermograms of the mixture. For moisture-sensitive thiamine hydrochloride, compared to the 0W sample of the mixture, the 3W XRPD diffractogram revealed a smoother pattern. For this flavoring substance, the surrounding storage moisture apparently caused an increased movement of the nonstoichiometric crystal water, but it appeared that microcrystalline cellulose maintained the thiamine hydrochloride in its monohydrate form even after 3 weeks of storage at 40°C/75% RH, whereas pure thiamine hydrochloride appeared to change into the hemihydrate form after the 3 weeks of storage. As no changes in thermal behavior were seen with the 1:1 physical mixture, thiamine hydrochloride and microcrystalline cellulose were concluded to be compatible.

Calcium hydrogen phosphate dihydrate

The crystal structure of pure calcium hydrogen phosphate dihydrate changed during storage at 40°C/75% RH, as shown by the patterns in the 0W and 3W diffractograms (Figure 3b). This change was probably due to the dehydration of the material under the storage conditions, as the crystal structure of the 3W sample resembled that of calcium hydrogen phosphate anhydrate presented by Kaushal *et al.* (26). Although the excipient is known to be non-hygroscopic and relatively stable at room temperature, it loses its nonstoichiometrically-bound water. Usually, this is initiated under high temperature conditions (27). However, dehydration may also be due to high storage temperatures (28, 29), and may even occur at temperatures below 100°C, e.g., Kaushal *et al.* (26) reported the dehydration of calcium hydrogen phosphate dihydrate at 85°C. This study showed dehydration of calcium hydrogen phosphate dihydrate occurring after 3 weeks of storage at 40°C/75% RH.

In both XRPD and DSC studies on flavor mixtures with calcium hydrogen phosphate dihydrate, samples of non-hygroscopic L-methionine and L-leucine remained unchanged after exposure to moisture and heat-induced pressure (data not shown). However, as expected, slight changes were recorded in the XRPD diffractogram of the denatonium benzoate anhydrate mixture after 3 weeks of storage at 40°C/75% RH (marked with an arrow in Figure 3b). Changes after storage were also observed in the XRPD diffractogram of the 3W mixture containing moisture-sensitive thiamine hydrochloride, and as expected, no XRPD analysis could be performed for the 3W mixture sample containing highly hygroscopic L-proline due to the amino acid rapidly absorbing moisture (data not shown). The XRPD results in this study were unsurprising and conformed with the expectations of the behavior of the materials exposed to moisture and heat. As no relevant changes were revealed by the DSC analyses either, it was concluded that the calcium hydrogen phosphate dihydrate combinations were compatible with the non-hygroscopic amino acids. Denatonium benzoate anhydrate and thiamine hydrochloride were incompatible with calcium hydrogen phosphate dihydrate, unless the moisture-induced changes in their crystal forms could be reduced by combination with another substance having a moisture protecting capacity.

α-Lactose monohydrate

The DSC analysis of pure α-lactose monohydrate showed two endothermic reactions, the first around 140–150°C corresponding to its dehydration (30, 31), and another at 217°C corresponding to its melting (31) (Figure 2b). Compared with the 0W sample, no differences in the α-lactose monohydrate XRPD diffractogram were seen after 3 weeks of exposure to 40°C/75% RH. At 12.6°, a peak typical for α-lactose was observed in both diffractograms (marked with α-arrows in Figure 3c). α-Lactose monohydrate combinations with the flavors studied here were expected to show changes in either XRPD

or thermal DSC analyses, or in both, as the excipient is known to be incompatible with compounds containing primary and secondary amines and with amino acids (32, 33). Often, this reaction causes the discoloration of medicinal products.

The research carried out here on α-lactose monohydrate mixtures with the chosen flavoring substances revealed intense instability-related reactions with the flavors considered hygroscopic. For L-proline, the reaction could even be observed visually. Due to the hygroscopic nature of this amino acid, the 3W sample, in combination with α-lactose monohydrate, yellow-colored with the morphology of slurry, for which no XRPD analysis could be performed. For L-leucine and L-methionine, the non-hygroscopic amino acids, neither sample discoloration during visual characterization, nor differences between their 0W and 3W XRPD crystal patterns were observed (Figure 3d and 3e). For the hygroscopic denatonium benzoate anhydrate, no change in sample coloration was seen after 3 weeks of storage under accelerated conditions. However, minor signal changes corresponding to incompatibility were observed between the 0W and 3W XRPD diffractograms of the combination (marked with arrows in Figure 3c). Between α-lactose monohydrate and moisture-sensitive thiamine hydrochloride, no visible discoloration was noticed after 4 weeks of storage at 40°C/75% RH. As for the lactose mixture containing denatonium benzoate anhydrate, the 0W and 4W XRPD diffractograms of the thiamine–lactose combination also showed only a minor change (marked with an arrow in Figure 3f).

Although the XRPD analysis showed no clear, or only weak signals, of flavor incompatibility with α-lactose monohydrate, a stronger indication of physical interactions was observed when the mixture samples were exposed to dry heat in DSC. None of the mixtures containing amino acids or denatonium benzoate anhydrate were observed to melt in the temperature region typical for dehydrated α-lactose monohydrate at temperatures above 200°C (Figure 2b). However, all showed an endotherm, a clear sign of α-lactose monohydrate dehydration at temperatures below 150°C. In addition, there were no endotherms typical of the melting of denatonium benzoate anhydrate at 176°C, L-methionine at 289°C, L-leucine at 310°C or L-proline starting at 217°C. In the mixtures with thiamine hydrochloride, the melting peak of dehydrated α-lactose monohydrate was decreased from 217°C to 198°C, but no change in the melting point of thiamine hydrochloride was observed (Figure 2b).

The chosen flavors were concluded to be incompatible with α-lactose monohydrate. The interaction leading to mixture instability was most likely due to a Maillard-type base-catalyzed reaction of lactose resulting from a surface pH change from acidic to alkaline (32). The intensity of the reaction has been reported to be dependent on the amine concentration, time and the surrounding humidity. It is therefore often studied in aqueous solutions. The reaction rate can be increased by increasing the amount of reducing sugar and the surrounding temperature. The rate of browning decreases for amino acids when the length and complexity of substituent groups is increased (34). Although some activation energy is usually needed for the Maillard reaction to occur, highly hygroscopic materials are more likely to undergo this reaction with lactose monohydrate, and this sugar may reduce the stability of such materials. Hence, lactose should not be combined with moisture-sensitive substances (35). In addition, although lactose has been widely used in veterinary peroral formulations due to its reasonable price and suitable pharmaceutical properties, its use is not suitable for feline medicinal products, as many adult cats suffer from lactose intolerance (36).

In this study, the intensity of the studied interactions appeared to be mostly moisture dependent. The most extensive interaction was observed when lactose was combined with

highly hygroscopic L-proline. The reaction decreased when exposing the chosen non-hygroscopic amino acid samples to moisture. However, when both the hygroscopic and non-hygroscopic amino acids, in combination with lactose, were exposed to dry heat, a clear and rapid interaction between the lactose and all the chosen flavors was observed either as a decrease or disappearance of the dehydrated α-lactose monohydrate melting point. For amino acids and denatonium benzoate anhydrate, no melting of pure flavor was seen. Maillard-type interactions are well known to occur in mixtures of thiamine hydrochloride and lactose (35, 37, 38). Thus, as an already-known and evident incompatibility between the chosen flavoring substances and lactose exists, it is suggested that this could be used as evidence of the sensitivity of the method used here. The results of the interactions between lactose and substances containing primary/secondary amines demonstrated that the applied techniques of XRPD and DSC were sufficient and adequately sensitive to rapidly detect possible incompatibilities and/or instabilities in such binary compositions. In pharmaceutical research, such a "quick-and-dirty" screening method would aid the overall development of formulations, primarily by excluding incompatible mixtures or establishing the need to stabilize them in the early phase of formulation development. However, data obtained from such studies must be carefully interpreted because the weight ratios used and the temperature at which interaction occur is often unrepresentative of the weight ratios used in a formulation and the temperature of storage of the dosage form. In addition, slower reaction rates, reactions that may be catalyzed by a ternary formulation component, degradation reactions that result in amorphous products or reactions induced by the high pressures encountered during tableting may not be detected.

Mannitol

Bauer *et al.* (39) reported the strong tendency of mannitol to display polymorphism and process-induced transformations. Nevertheless, the XRPD studies carried out here on pure mannitol showed no signs of solid-state changes in the substance after 3 weeks of storage under humid conditions (Figure 3c–3f). The melting of pure mannitol occurred at 166°C (Figure 2c). According to other authors, the onset of melting for the different grades of mannitol polymorphs is 166 ± 2°C, making it difficult to differentiate between the different polymorphs using DSC (40). Thus, based on existing reports for XRPD patterns for the different mannitol polymorphs (41-43), and on work by Walter-Lévy (44) who presented a system for the preparation of, and X-ray data for, α, β and δ polymorphs of mannitol, it was possible to conclude that the chosen mannitol was a mixture of α- and β-polymorphs. This was also consistent with the observed melting point at 166°C, which according to Burger *et al.* (42) could be indicative of the onset of the melting of both α- and β-mannitol.

Mannitol is non-hygroscopic (45) and can be successfully combined with moisture-sensitive materials. In addition, no Maillard reaction has been documented between mannitol and amine-containing substances, rather, mannitol has been reported to retard the degradation of amine-containing thiamine (46). The XRPD studies carried out here, showed that mannitol appeared to be compatible with the non-hygroscopic L-methionine and L-leucine, as no differences between the 0W and 3W diffractograms of the mixtures were observed (Figure 3d and 3e). Even with moisture-sensitive materials such as denatonium benzoate anhydrate and thiamine hydrochloride, no specific changes in the material crystallinity were observed in the XRPD patterns (Figure 3c and 3f). Consistent with reports in the literature, the results obtained here indicated that mannitol could be combined with materials that are moisture sensitive, as the crystal structure of mannitol would not be affected by other substances with a moisture-sorptive capacity. Likewise, the addition of mannitol would not affect the crystallinity of materials used in this study.

However, due to the extensive hygroscopicity and moisture absorption of L-proline, mannitol alone could not be combined with this flavor in a binary 1:1 ratio, as the mixture formed a slurry during storage under accelerated conditions.

In contrast to the XRPD results, studies on the thermal behavior of mannitol mixtures showed several changes (Figure 2c). In the thermogram with denatonium benzoate anhydrate, the disappearance of the melting peak for the bitter substance was observed. However, together with the melting of mannitol at 166°C, a completely new earlier endotherm was noted at 138°C. A similar effect of earlier melting was seen for mannitol combinations containing L-proline and thiamine hydrochloride. For L-proline, the mixture melting pattern showed only two endotherms, the first one clearly at 138°C and a second, wider endotherm, with the highest peak at approximately 240°C. For thiamine hydrochloride, an endotherm at 152°C with a wide right shoulder was observed. Neither of these mixtures showed melting of the pure flavoring substance. In addition, the thermograms did not show the melting of mannitol. In combinations with L-leucine and L-methionine, melting of mannitol at 166°C was shown together with a significantly shifted melting peak for the flavoring substance.

Denatonium benzoate anhydrate is known to be stable up to 140°C. Thus, when the mannitol–denatonium benzoate anhydrate combination was heated to 140°C, it was likely that the changed crystal structure of denatonium benzoate caused differences in the DSC thermal curve at 138°C. However, α- or β-mannitol appeared to maintain the original crystalline structure, as a melting point at 166°C could still be seen in the thermogram for the mixture. An incompatibility between mannitol and L-proline was evident due to the highly hygroscopic nature of the flavor. However, the incompatibility was not evident for the other studied substances due to the inconsistency in the XRPD and DSC results. Incompatibility indicated by DSC seemed to occur only at high temperatures. If such temperatures could be avoided during ordinary product manufacture such as tableting, they could be considered of minor importance. Thus, a conclusion for some compatibility could be drawn.

Povidone

The povidone used in this study was amorphous and highly hygroscopic. When subjected to dry thermal pressure, the DSC thermogram of povidone showed only a wide endotherm indicating material softening near its glass transition temperature below 100°C (data not shown). In 1:1 binary combinations with the chosen flavors, the thermal behavior of povidone remained unchanged, and no changes in the melting points of denatonium benzoate anhydrate, thiamine hydrochloride or L-proline could be observed. Even in combinations of L-methionine and L-leucine with povidone, no significant indications of material incompatibility were seen, although the melting peaks of these amino acids were slightly shifted to the lower temperatures of 256°C for L-methionine and 271°C for L-leucine. However, the presence of increased moisture and temperature showed the behavior of povidone to be consistent with existing knowledge on its strong hydrogen bonding ability (47). Although this povidone interaction with water has been widely used as a component to increase the solubility and dissolution of poorly water soluble drugs (48), it may cause problems through over-wetting in dry product formulations that contain high povidone concentrations and are stored under high moisture conditions. Such difficulties are due to the excess of surrounding moisture that in the povidone molecule is available not only as tightly bound but, also as free water, wetting the material. Thus, in this study, subjecting pure povidone to the high temperature and moisture study conditions of 40°C/75% RH for 3 weeks led to the physical transformation of the sample from solid powder to a liquid-like slurry as expected. Also, subjecting the 1:1 binary povidone/flavor mixtures to the same

conditions led to similar sample wetting, as the povidone slurry formed by an excess of moisture either dissolved or suspended the combined flavor. For such wetted 3W samples, no XRPD analyses could be performed. Although povidone behaved as expected in the studied 1:1 binary mixtures, no conclusions could be drawn on its incompatibility with the chosen flavoring excipients in binary 1:1 combinations. Thus, regardless of the compatibility indicated by the thermal studies, because of the known hygroscopic nature of povidone, factors related to compatibility should be examined with less than 1:1 povidone concentrations and in the presence of moisture. Moreover, in the later phase of product development, incompatibility-related indications should be investigated by HPLC, after the product´s final formulation is completed.

Hydroxypropyl cellulose

The studies carried out here on the mixtures containing hydroxypropyl cellulose, showed no evidence indicating incompatibility between the studied substances (results not shown). The XRPD diffractogram of the sample containing denatonium benzoate anhydrate and hydroxypropyl cellulose revealed a flattened line with some indications of a degree of crystallinity. However, no conclusions on incompatibility could be reached, as the peaks demonstrating denatonium benzoate anhydrate were still clearly seen from the diffractogram. With the rest of the studied materials, excluding the moisture-sensitive L-proline, no indications of either incompatibility or instability were observed. When this information was combined with the findings of thermal studies, the results were suggestive of the compatibility between hydroxypropyl cellulose and the studied substances. In the thermal curves, the endotherms of each material combined with hydroxypropyl cellulose showed no signs of incompatibility.

Crospovidone and croscarmellose sodium

With physical mixtures containing the chosen flavoring substance together with, either crospovidone or croscarmellose sodium, no changes indicating deteriorating stability were observed from the DSC thermal studies. With the exception of L-proline, compatibility with the disintegrants was also confirmed by XRPD results. Based solely on these results, compatibility could be concluded for formulations containing denatonium benzoate, L-methionine, L-leucine and thiamine hydrochloride, and either of the mentioned disintegrants.

Lubricants

Lubricants typically represent only a minor part of the final tablet formulation. Interactions related to them are therefore difficult to predict. However, the 1:1 binary mixtures were also tested for lubricants in this investigation. If any interaction between denatonium benzoate anhydrate or the studied flavoring substances and the chosen lubricants of talc and sodium stearyl fumarate did exist, this would also be observed in these short-term studies. Yet, it should be borne in mind that the magnitude and importance of any such interaction would need to be further investigated after the completion of the formulation.

Talc

Subjecting the binary compositions containing talc to high temperatures, showed no signs indicating incompatibility, as no changes in melting behavior, shifting or disappearance of endotherms were detected (results not shown). However, when the samples containing L-proline or thiamine combinations with talc were subjected to moisture, compatibility results were different from those seen using DSC. Though no XRPD analysis could be performed for L-proline combination with talc, it was concluded that both flavors, L-proline and thiamine hydrochloride, were incompatible with talc. In fact, talc has already been reported to

enhance the hydrolysis of thiamine hydrochloride (49). The XRPD studies carried out here, showed slight differences between the diffractograms of the binary 0W and 3W thiamine samples (results not shown). During the hydrolysis of thiamine, the thiazole ring is opened up caused by the acidic or alkaline pH surrounding it. This is therefore likely to occur when it is mixed with alkaline talc. Thus, thiamine hydrochloride should not be combined with talc or with alkaline substances, as such combinations would in theory increase the degradation of thiamine.

Sodium stearyl fumarate

Sodium stearyl fumarate is known to react with primary amines through the well known Michael reaction. In this reaction, an adduct may be formed between a primary amine and the olefinic double bond of the lubricant's fumarate moiety. The XRPD studies carried out here showed no signs of any interaction between sodium stearyl fumarate and L-methionine, L-leucine or denatonium benzoate anhydrate with a secondary amine structure. Instead, for thiamine hydrochloride, altered crystal forms were seen in the XRPD studies indicated by new and disappeared peaks in the 3W diffractogram of the thiamine hydrochloride and sodium stearyl fumarate combination sample (examples are shown with arrows in Figure 3f). Such incompatibility was also found using DSC. Binary mixtures containing thiamine hydrochloride and sodium stearyl fumarate showed an intense melting peak at 135°C, no melting for pure sodium stearyl fumarate at 200°C and an earlier melting onset for thiamine hydrochloride at 248°C (Figure 2d). Thus, based on both the DSC and XRPD studies, incompatibility between sodium stearyl fumarate and thiamine hydrochloride was concluded. In contrast, based on the XRPD results, it was concluded that the mixtures of sodium stearyl fumarate and denatonium benzoate anhydrate, L-methionine or L-leucine were compatible.

CONCLUSION

With companion animals, the addition of synthetic flavors to bitter-tasting drugs may be the simplest way to improve the palatability of such medicinal products. Though the utilization of nature-derived flavoring excipients is common in the animal industry, it often comes with extra hurdles and costs. In addition, especially for felines because of their unique characteristics, the overall peroral medication of home pets is challenging due to the lack of sophisticated and tailor-made feline medicinal products resulting in the prescription of off-label and/or over-sized, offensive tasting medicinal products. This study presented several new candidates of synthetic flavoring excipients that could be used in feline tablet formulations. Studies for L-methionine, L-leucine, L-proline and thiamine hydrochloride, as well as, the bitter tasting model substance, denatonium benzoate, provided previously unknown information on the stability and compatibility with commonly used solid dosage form excipients.

A rapid start in the preformulation phase is considered beneficial for overall product development. To aid the formulation development in the preformulation phase, a rapid incompatibility and instability screening method has been presented here for the detection of rough physical defects in the suggested 1:1 binary compositions for feline tablet formulations. The suggested combination of simple and cost-effective DSC and XRPD analyses could be reliably used for determining physical stability.

In this study, the majority of the chosen flavors were compatible with the common tableting excipients that were investigated. Problems related to compatibility were either due to the moisture sensitivity of the flavoring substance changing the crystalline structure of the material, or chemical reactivity, most of this owing to the amine content. It must still be kept in mind that these studies were targeted at the rapid screening of compatibility and

physical stability alone. Future studies, for example using HPLC, are needed to examine possible chemical incompatibility and impurity issues.

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REFERENCES

1 K. Kasraian, A.G. Thombre, Palatable pharmaceutical compositions for companion animals, European Patent 1,247,456 A2 (2002).

2 Ahmed I, Kasraian K. Pharmaceutical challenges in veterinary product development. Adv. Drug Deliv. Rev., 54: 871-882, 2002.

3 Thombre AG. Oral delivery of medications to companion animals: Palatability considerations. Adv. Drug Deliv. Rev., 56: 1399-1413, 2004.

4 Boothe DM. Drug therapy in cats: A therapeutic category approach. J. Am. Vet. Med. Assoc., 196: 1659-1669, 1990.

5 Bradshaw JWS, Goodwin D, Legrand-Defrétin V, Nott HMR. Food selection by the domestic cat, an obligate carnivore. Comp. Biochem. Physiol. A Physiol., 114: 205-209, 1996.

6 Boothe DM. Drug therapy in cats: Mechanisms and avoidance of adverse drug reactions. J. Am. Vet. Med. Assoc., 196: 1297-1305, 1990.

7 Riviere JE. The future of veterinary therapeutics: A glimpse towards 2030. Vet. J., 174: 462-471, 2007.

8 R.J. Eichelburg, Yeast hydrolyzate oral ingesta for animals, U.S. Patent 4,118,512 (1978).

9 E.M.J. Jans, P.M.V. Gills, Chewable flubendazole tablets for companion animals, U.S. Patent 5,824,336 (1998).

10 M. von Bittera, H. Voege, R. Bauditz, Medicated animal feed based on liver meal, U.S. Patent 4,283,400 (1981).

11 J.C. Godfrey, Flavor of zinc supplements for oral use, U.S. Patent 4,684,528 (1987).

12 Ogawa T, Nakamura T, Tsui E, Miyanaga Y, Nagakawa H, Hirabayashi H, Uchida T. The combination effect of L-arginine and NaCl on bitterness suppression of amino acid solutions. Chem. Pharm. Bull., 52: 172-177, 2004.

13 G.A. Meyer, T.B. Mazer, Prolamine coatings for taste masking, U.S. Patent 5,599,556 (1997).

14 European Pharmacopoeia. 7th ed. Strassbourg, Council of Europe, 2010.

15 Carr RL. Classifying flow properties of solids. Chem Eng, 72: 69-72, 1965.

16 Hausner HH. Friction conditions in a mass of metal powder. Int J Powder Metall, 3: 7-13, 1967.

17 Ash, M.; Ash, I., Handbook of Flavors and Fragrances. *Synapse Information Resources Inc.*, NY, USA, 2006.

18 Schiffman SS, Engelhard III HH. Taste of dipeptides. Physiol. Behav., 17: 523-535, 1976.

19 Burdoch, G., Fenaroli´s Handbook of Flavor Ingredients. 6th ed., *CRC Press, Taylor & Francis Group*, FL, USA, 2010.

20 Mellon EF, Hoover SR. Hygroscopicity of amino acids and its relationship to the vapor phase water absorption of proteins. J. Am. Chem. Soc., 73: 3879-3882, 1951.

21 Wöstheinrich K, Schmidt PC. Polymorphic changes of thiamine hydrochloride during granulation and tableting. Drug Dev Ind Pharm, 27: 481-489, 2001.

22 Chakravarty P, Govindarajan R, Suryanarayanan R. Investigation of solution and vapor phase mediated phase transformation in thiamine hydrochloride. J Pharm Sci, 99: 3941-3952, 2010.

23 Watanabe A, Tasaki S, Wada Y, Nakamachi H. Polymorphism of thiamine hydrochloride. II. Crystal structure of thiamine hydrochloride hemihydrate and its stability. Chem. Pharm. Bull., 27: 2751-2759, 1979.

24 Chakravarty P, Berendt RT, Munson EJ, Young Jr. VG, Govindarajan R, Suryanarayanan R. Insights into the dehydration behavior of thiamine hydrochloride (vitamin B1) hydrates: Part I. J Pharm Sci, 99: 816-827, 2010.

25 Watanabe A, Nakamachi H. Polymorphism of thiamine hydrochloride. Yakugaku Zasshi, 96: 1236-1240, 1976.

26 Kaushal AM, Vangala VR, Suryanarayanan R. Unusual effect of water vapor pressure on dehydration of dibasic calcium phosphate dihydrate. J Pharm Sci, 100: 1456-1466, 2011.

27 Landìn M, Rowe RC, York P. Structural changes during the dehydration of dicalcium phosphate dihydrate. Eur J Pharm Sci, 2: 245-252, 1994.

28 El-Shattawy HH, Peck GE, Kildsig DO. Aspartame-direct compression excipients: Preformulation stability screening using differential scanning calorimetry. Drug Dev Ind Pharm, 7: 605-619, 1981.

29 El-Shattawy HH. Ampicillin-direct compression excipients: Preformulation stability screening using differential scanning calorimetry. Drug Dev Ind Pharm, 8: 819-831, 1982.

30 Lerk CF, Andrae AC, de Boer AH, de Hoog P, Kussindrager K, van Leverink J. Alterations of α-lactose during differential scanning calorimetry. J Pharm Sci, 73: 856-857, 1984.

31 Casalderrey M, Souto C, Concheiro A, Gómez-Amoza J, Martínez-Pachego R. A comparison of cellactose with two ad hoc processed lactose-cellulose blends as direct compression excipients. Chem. Pharm. Bull., 48: 458-463, 2000.

32 Castello RA, Mattocks AM. Discoloration of tablets containing amines and lactose. J Pharm Sci, 51: 106-108, 1962.

33 Blaug SM, Huang WT. Interaction of dextroamphetamine sulfate with spray-dried lactose. J Pharm Sci, 61: 1770-1775, 1972.

34 deMan, J.M., Proteins, in Principles of Food Chemistry. *The Avi Publishing Company Inc.*, CT, USA, pp 86-134, 1985.

35 Du J, Hoag SW. The influence of excipients on the stability of the moisture sensitive drugs aspirin and niacinamide: Comparison of tablets containing lactose monohydrate with tablets containing anhydrous lactose. Pharm Dev Technol, 6: 159-166, 2001.

36 Ruaux, C.G., Steiner, J.M., Williams, D.A., The gastrointestinal tract, in Chandler EA; Gaskell CJ; Gaskell RM (eds.), Feline Medicine and Therapeutics. 3rd ed., *Blackwell Publishing*, Oxford, UK, pp 397-434, 2004.

37 Hammouda Y, Salakawy SA. Lactose-induced discoloration of amino drugs in solid dosage form. Pharmazie, 26: 181, 1971.

38 Flemming A, Picker-Freyer KM. Compaction of lactose drug mixtures: Quantification of the extent of incompatibility by FT-Raman spectroscopy. Eur J Pharm Biopharm, 68: 802-810, 2008.

39 Bauer H, Herkert T, Bartels M, Kovar K-A, Schwarz E, Schmidt PC. Investigations on polymorphism of mannitol/sorbitol mixtures after spray-drying using differential scanning calorimetry, X-ray diffraction and near-infrared spectroscopy. Pharm Ind, 62: 231-235, 2000.

40 Pitkänen I, Perkkalainen P, Rautiainen H. Thermoanalytical studies on phases of D-mannitol. Thermochim Acta, 214: 157-162, 1993.

41 Hulse WL, Forbes RT, Bonner MC, Getrost M. The characterization and comparison of spray-dried mannitol samples. Drug Dev Ind Pharm, 35: 712-718, 2009.

42 Burger A, Henck J-O, Hetz S, Rollinger JM, Weissnicht AA, Stöttner H. Energy/temperature diagram and compression behavior of the polymorphs of D-mannitol. J Pharm Sci, 89: 457-468, 2000.

43 Yoshinari T, Forbes RT, York P, Kawashima Y. Moisture induced polymorphic transition of mannitol and its morphological transformation. Int J Pharm, 247: 69-77, 2002.

44 Walter-Lévy L. Cristallochimie-sur les variétés cristallines du D-mannitol. C.R. Acad. Sci. Paris Ser. C, 267: 1779-1782, 1968.

45 Debord B, Lefebvre C, Guyot-Hermann AM, Hubert J, Bouché R, Cuyot JC. Study of different crystalline forms of mannitol: comparative behaviour under compression. Drug Dev Ind Pharm, 13: 1533-1546, 1987.

46 Wai K-N, DeKay HG, Banker GS. Stability of vitamins A, B1 and C in selected vehicle matrices. J Pharm Sci, 51: 1076-1080, 1962.

47 Wan L-S, Huang X-J, Xu Z-K. Diffusion and structure of water in polymers containing N-vinyl-2-pyrrolidone. J Phys Chem B, 111: 922-928, 2007.

48 Iwata M, Ueda H. Dissolution properties of glibenclamide in combinations with polyvinylpyrrolidone. Drug Dev Ind Pharm, 22: 1161-1165, 1996.

49 Yamamoto R, Takahashi T. Studies on the stability of dry preparations. III. Decomposition of thiamine by adsorbents. Yakugaku Zasshi, 78: 1242-1245, 1958.