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Thermal Analysis in the Evaluation of Solid Lipid Microparticles in the Form of Aqueous Dispersion and Fine Powder

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Abstract: In the presented study, an attempt was made to investigate the most important attributes of solid lipid microparticles (SLM) using thermal analysis (DSC/TG) in order to determine the importance of this technique in the research and development of lipid microparticles. Particularly interesting in our studies were drug–lipid interactions and modifications of the SLM matrix structure induced by the production method (the hot emulsification method) and further processing (e.g., spray drying), as well as changes occurring during the stability studies. Cyclosporine A, indomethacin and spironolactone were used as model active substances incorporated into SLM. The conducted research demonstrated the significant potential of DSC/TG, especially for the analysis of SLM in the form of fine powder. The method of sample preparation, consisting of evaporation of water at room temperature, turned out to be crucial for the DSC/TG analysis of SLM dispersion. In the case of the tested SLM, the basic and usually the only observed thermal transformation in the DSC spectrum was the endothermic peak associated with the lipid forming a microsphere matrix. This peak is the main source of information about the properties and stability of the tested SLM. The obtained results show that glyceryl behenate (Compritol) is a significantly better lipid for forming lipid microparticles than stearic acid. Although thermal transformations of the incorporated drug substances are not directly visible in the DSC spectra, their impact on the SLM properties can be assessed indirectly, based on changes in the lipid melting point and the shape of the DSC and TG peaks and curves. DSC/TG studies confirmed the lack of an effect of the spray drying process on the properties of drug-loaded SLM with Compritol. Studies have also shown up to a 2-year stability of SLM with CsA.

Keywords: DSC; TG; solid lipid microparticles; cyclosporine; indomethacin; stability

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1. Introduction

The biocompatibility and biodegradability, as well as the possibility of administering them in various dosage forms by multiple routes [1–7], make solid lipid microparticles (SLM) a promising carrier for many drug substances. Moreover, solid lipid microparticles, due to the combination of the active substance and the lipid in the SLM matrix, are being studied for various applications, such as prolonged action, taste masking, protection of the active substance or reducing irritation of the gastrointestinal tract. Lipid microspheres can be produced by various techniques, from simple and cheap one-stage processes to more complex ones requiring advanced equipment [7,8]. SLM produced in the form of powder or liquid dispersion are often further processed into the final dosage form. The most common techniques for the preparation of SLM dispersions are the melt dispersion technique (also called the hot emulsification method), the solvent evaporation or diffusion method and the microchannel emulsification technique. Meanwhile, SLM in powder form can be produced by spray congealing, spray drying (from an organic solution) or cryogenic micronisation.

The aqueous SLM dispersion can also be transformed into its dry form using the spray drying process or the lyophilization process [5,9,10].

Thermal analysis is a generic term used to describe analytical techniques that examine the behavior of a sample and the changes occurring in a material under the influence of temperature [11]. Different techniques are used, depending on the type of physical changes being analyzed when the sample is heated or cooled.

The most popular methods for thermal analysis are differential scanning calorimetry (DSC) and thermogravimetry (TG). In the DSC method, the difference in the heat flux flow between the test substance and the reference material, as a function of temperature, is recorded. Meanwhile, in the TG method, the change in the mass of the sample is recorded as a function of temperature. The combination of both methods (DSC and TG) enables simultaneous thermal analysis (STA), the advantage of which is obtaining the comprehensive thermal characteristics of the tested sample [12]. In addition, in the case of simultaneous thermogravimetric analysis, both signals are recorded simultaneously on the same apparatus, and the prevailing conditions during the test (atmosphere, pressure, heating rate, the same measuring vessel, etc.) are identical for TG and DSC signals, which is important for the quality of the obtained results.

This thermal analysis is a widely used method for studying not only pharmaceutical active substances and excipients, but also structured carriers and dosage forms. DSC and TG curves provide important information regarding the physical properties of various pharmaceutical compounds and formulations [11]. DSC is a powerful technique to determine the crystallinity of substances [13]. It is also used to test the physicochemical interactions of the drug and excipients and to exclude their potential incompatibilities [14].

It is known that both lipids and active substances are susceptible to polymorphic transitions induced by the production process (temperature changes, high shared forces, solvent evaporation and others) or occurring during storage [15]. These changes have a significant impact on the properties of the obtained drug form, such as the degree of incorporation of the drug substance into the lipid matrix, as well as on the stability of the dosage form (the risk of expulsion of the encapsulated drug from the lipid matrix) [15]. That is why it is so important to evaluate and control the properties of lipid microspheres using thermal analysis at various stages of the manufacturing process and during stability tests, as well as during quality control of the production batch.

In this study the method of thermal analysis, including DSC and TG, was used to examine solid lipid microparticles in the form of liquid dispersion or fine powder.

The aim was to assess the usefulness of DSC/TG in the analysis of SLM in various forms, at various stages of formulation development and processing, and to identify difficulties, e.g., in preparing a sample of lipospheres for analysis. Therefore, the properties of SLM dispersions and spray-dried SLM powders with selected drug substances (cyclosporine A, indomethacin and spironolactone) were evaluated. Thermal characterization of the placebo SLM and physical mixtures was also performed. Particularly interesting in our studies were the drug–lipid interactions and the structure modifications of the SLM matrix induced by preparation (the hot emulsification method) and spray drying. The polymorphic transformation and storage stability of the dosage forms were also investigated.

2. Materials and Methods

2.1. Materials

Cyclosporine A (CsA) was obtained from LC Laboratories (Boston, MA, USA), Indomethacin (Ind) from Fagron (Kraków, Poland) and Compritol 888 ATO (glyceryl behenate) from Gattefossé (Saint-Priest, France). Spironolactone (SPIR), stearic acid and Tween 80 (polysorbate 80) were purchased from Sigma-Aldrich (St. Louis, MO, USA); polyvinylpyrrolidone (PVP) and maltodextrin (Mx) were from BASF (Ludwigshafen, Germany). All other chemicals used were of analytical reagent grade. High-quality water was obtained from a Milli-Q system (Millipore, Milford, MA, USA).

2.2. Preparation and Characterization of SLM Dispersions

SLM dispersions, with and without the active pharmaceutical ingredient (API), were prepared using the hot emulsification method [16]. The drug substances CsA, Ind and SPIR were used in different concentrations. The lipid phase was Compritol or stearic acid at a concentration of 10% (*w*/*w*). The lipid phase and aqueous phase were mixed together at 80 °C using a high-shear Ultra-Turrax mixer (T25 Janke-Kunkel, IKA Labortechnik, Staufen, Germany) and then cooled in an ice bath, as was previously reported [16]. To produce lipid microparticles with API, the drug substance was added to the molten lipid before it was dispersed in a surfactant solution. The final dispersions were stored in the refrigerator. The composition of all prepared formulations (placebo and API-loaded) is presented in Table 1. The particle size in SLM dispersions was determined using the laser diffraction method (Beckman-Coulter LS 13 320, Indianapolis, IN, USA) with the Universal Liquid Module and Polarization Intensity Differential Scattering function. The SLM dispersions were also observed under a microscope.

Table 1. The composition of the investigated SLM dispersions.

Formulation	The Composition of Active Substances and Excipients (*w*/*w* %)					
	CsA	Ind	SPIR	Compritol	Stearic Acid	Tween 80
F1	-	-	-	10.0	-	3.0
F2	-	-	-	-	10.0	3.0
F3/F4/F5/F6	0.1/1.0/2.0/5.0	-	-	10.0	-	3.0
F7/F8	0.1/1.0	-	-	-	10.0	3.0
F9	1.0	-	-	10.0	-	5.0
F10/F11	-	0.2/1.0	-	10.0	-	3.0
F12/F13	-	-	0.1/0.5	-	10.0	3.0

CsA: cyclosporine A, Ind: indomethacin, SPIR: spironolactone.

2.3. Preparation and Characterization of SLM Fine Powder

To obtain fine SLM powder, the SLM dispersions were spray dried. Liquid dispersions were dried directly or, before spray drying, they were mixed in equal parts (1:1) with a PVP or Mx solution (5% *w*/*w*). Spray drying of the SLM formulations was performed using a Buchi Mini Spray Dryer B-290 (Buchi Labortechnik AG, Flawil, Switzerland). The process parameters were adjusted to the type of lipids in the formulations, as was previously described [17]. Depending on whether the lipid matrix was made of Compritol or stearic acid, the SLM dispersions were dried at 90 °C (feed rate 2.4 mL/min) or at 80 °C (feed rate 3 mL/min), respectively. Air was used as the drying gas. The obtained spray-dried powders were stored at room temperature. The particle size of the spray-dried powder was determined by the same laser diffraction method, but with a Tornado Dry Powder System (DPS) module, which was connected to the same device (Beckman-Coulter LS 13 320) as the ULM attachment. The SLM powders were also observed under a microscope for comparison with the dispersions.

2.4. Preparation of Physical Mixtures

The physical mixtures of the lipid with the active substance were prepared in a mortar. The ingredients in the appropriate ratio were weighed and then mixed until a homogeneous mass was obtained.

2.5. Thermal Analysis

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) measurements were performed with a Mettler-Toledo DSC 821^e instrument (Mettler-Toledo GmbH, Greifensee, Switzerland), at the same time and under the same conditions. The samples were heated steadily from 25 °C to 500 °C in a non-hermetically sealed aluminum pan at a heating rate 10 °C/min. The mass of the samples was approximately 10 mg, and measurements were performed in a nitrogen atmosphere at a flow rate of 70 mL/min. Both TG and DSC signals obtained as a result of the measurement were recorded as a function

of temperature, and when both are presented, they are represented on one graph as a thermogravimetric curve and a DSC curve.

Indium and zinc were used for calibration. To check the temperature and heat flow accuracy of the DSC modules, the indium standard (In, purity 99.999%) was selected for the low temperature and the zinc standard (Zn, purity 99.999%) was selected for the high temperature. The checks were within the defined limits.

The DSC/TG analysis was performed on the following samples: placebo SLM, drug-loaded SLM, physical mixtures (binary mixtures of lipid and API), bulk solid lipids, APIs and other excipients. The SLM were tested both in the form of aqueous dispersion and powder obtained by spray drying. Liquid dispersions were prepared for thermal analysis: (i) by evaporation of water at room temperature under compressed air, (ii) by evaporation under the same conditions but preceded by centrifugation of the SLM dispersion or (iii) analyzed directly (without prior sample preparation). The lipid microspheres were also examined after shaking in a vortex for 5 min with methanol and centrifuging at 3500 rpm. This process, usually carried out during distribution studies, allowed for the dissolution and determination of the active substance, located on the surface of the microspheres, without dissolving the lipid matrix of the microspheres.

Thermal characterization of the SLM was also performed after one or two years of storage (in the refrigerator or at room temperature) to evaluate the stability of the SLM dispersions and powders. Two parallel examinations were carried out for all samples. The obtained results were evaluated using STAR^e SW 16.30 Software.

3. Results

When SLM are tested directly as a liquid dispersion, the DSC curve shows one large conversion peak, mainly related to water evaporation, within which it is difficult to isolate a small lipid melting peak (Figure 1). According to the DSC curve, the TG curve can distinguish two stages of decomposition with mass loss, which correspond mainly to the loss of water and also to the decomposition of the other components of the dosage form (Figure 1).

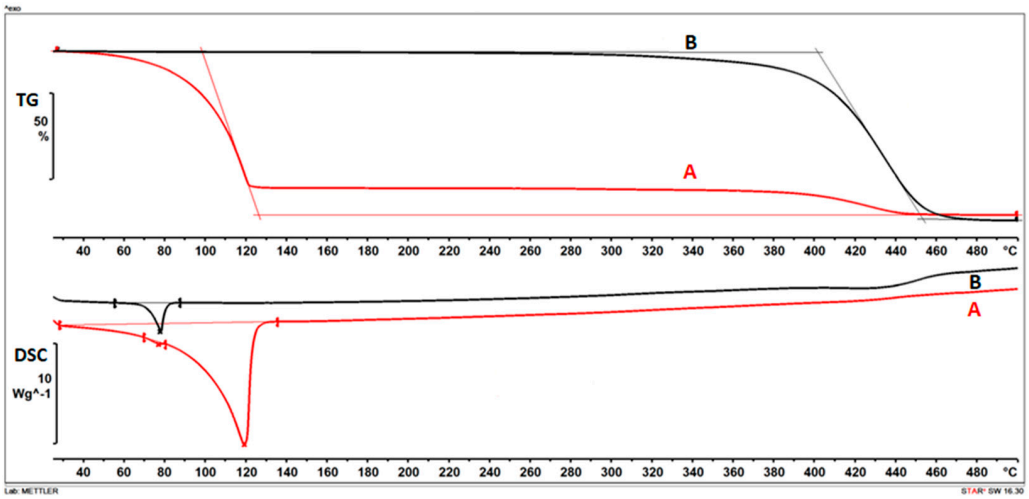


Figure 1. DSC and TG curves of SLM dispersions with Compritol: (A) without water evaporation and (B) after water evaporation, but before thermal analysis.

When SLM liquid dispersions are tested after removing water (in dry form), a single endothermic transition stage can be distinguished in the DSC curves (Figure 1 and Figure S1, presented in Supplementary Materials), without mass loss in the TG curves, which corresponds to the melting process of lipid-forming SLM matrices (for example, an endothermic transition in the SLM with Compritol matrix: onset temperature 71.9 °C, peak 78.5 °C).

Three different therapeutic substances were used in the development studies of solid lipid microspheres: cyclosporine A (CsA), indomethacin (Ind) and spironolactone (SPIR). The melting endotherms of pure drug substances are shown in Figure 2. They confirm the crystalline nature of Ind and SPIR, while the DSC thermograms and thermal behaviors of pure CsA, confirm the amorphous form of the drug substance.

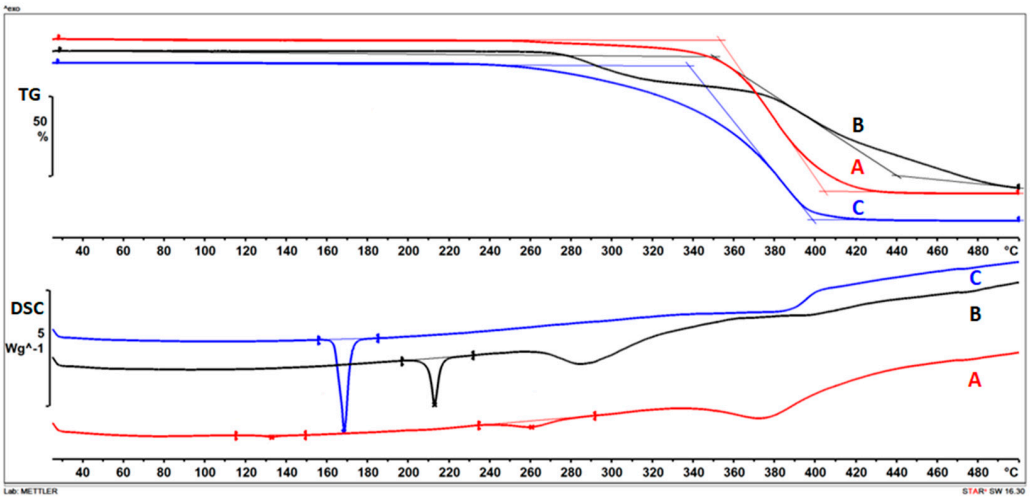


Figure 2. DSC and TG curves of the tested drug substances: (A) cyclosporine A, (B) spironolactone and (C) indomethacin.

In general, the drug-loaded SLM DSC curves do not show particular variability relative to the placebo SLM curves. In the DSC curve of drug-loaded microparticles, only one strong endothermic peak around the melting point of the lipid was found, and the melting peak of the drug substance did not appear. The melting point of the lipid is slightly lower while the intensity of the peak remains almost unchanged.

The lack of any endothermic CsA peaks in all tested SLM formulations, as seen in Figure 3, suggests a molecular interaction between the CsA and lipids or other SLM components and a molecular dispersion of the active substance in the lipid.

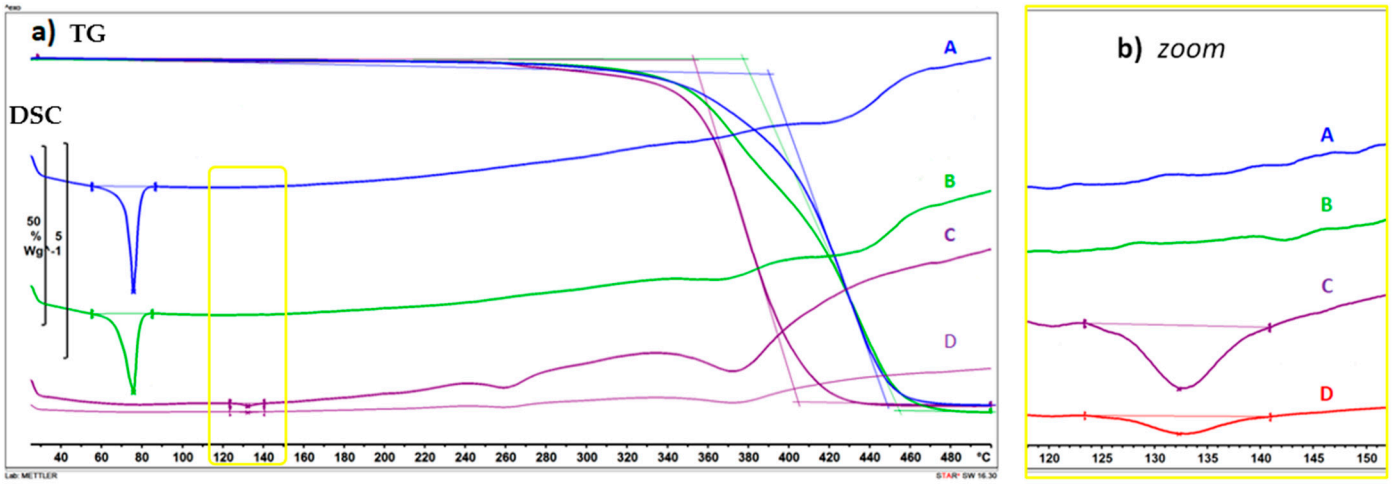


Figure 3. (**a**) DSC and TG curves and (**b**) the zoom of: (A) F5-SLM dispersion with 2% of CsA, (B) F6-SLM dispersion with 5% of CsA, (C) the bulk CsA and (D in figure (**a**) and D in figure (**b**)) the bulk CsA peak converted to a CsA concentration in F6-SLM with 5% of CsA.

Lipid microspheres (both with active substances and placebo SLM) are characterized primarily by the presence of an endothermic peak in the temperature range close to the melting point of the lipid forming the microsphere matrix (Figures 1–3). In SLM with

Compritol, this is a sharp peak with a high intensity in the temperature range of 75–79 °C, while in SLM with stearic acid the intensity, shape and temperature range of the peak can be very different, depending on the tested formulation. If the melting peak of stearic acid is not deformed and shifted, it occurs at about 75 °C.

Figure 3a shows the peak melting temperature plots of lipids in SLM with CsA obtained from the DSC experiments. All CsA-SLM thermographs show a decrease in the melting point of Compritol from 78.5 °C in placebo SLM to 76.1 °C in SLM with 1% of CsA and to 75.8 °C in SLM with 5% of CsA. The same effect was observed by Wong et al. [15] in research on casts with glyceryl behenate and ibuprofen. This phenomenon was also observed in SLM with Ind (Figure 4a), where the lipid melting point decreased by 1 °C (from 78.5 to 77.5 °C) in SLM with 0.2% of Ind and in SLM with stearic acid.

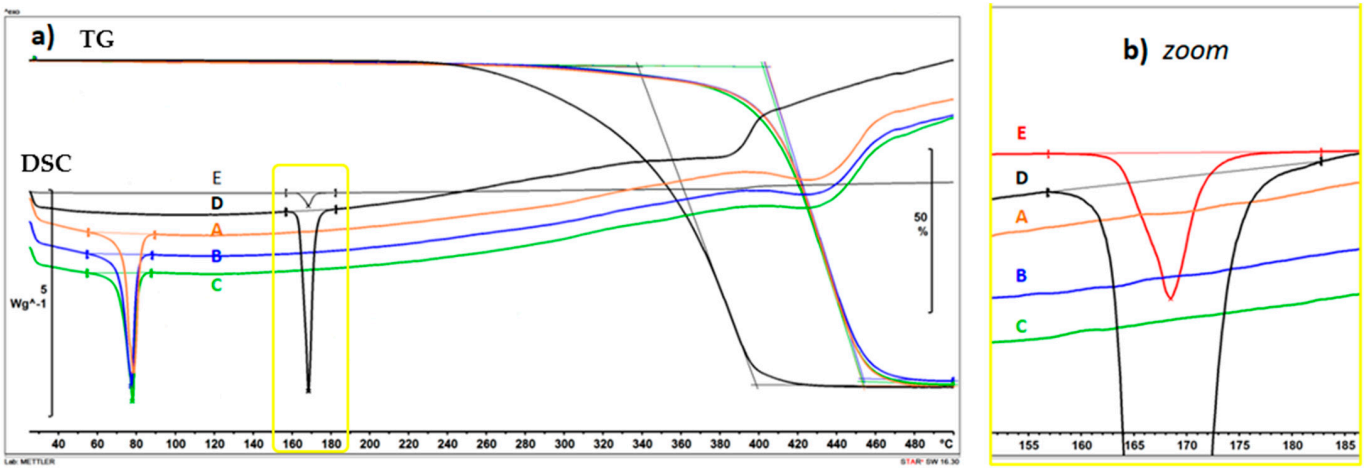


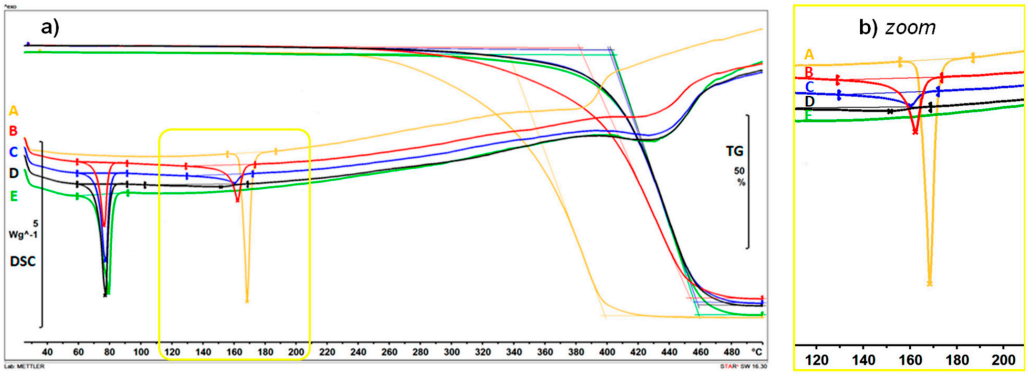
Figure 4. (**a**) DSC and TG curves and (**b**) the zoom of: (A) F1-SLM placebo dispersion, (B) F10-SLM dispersion with 0.2% of Ind, (C) F11-SLM dispersion with 1% of Ind, (D) the bulk Ind and (E in figure (**a**) and E in figure (**b**)) the bulk Ind peak converted to Ind concentration in the F11-SLM with 1% of Ind.

Thermal decomposition of the Ind and SLM with Ind takes place in one stage (Figure 4a). The difference between the temperature ranges corresponding to the decomposition of the pure drug and the dosage form results from and is dominated by the properties of the lipid in the SLM. Therefore, the pure Ind decomposes (96.6%) in a temperature range of 336–397 °C (onset-endset temperature), while in the case of the Ind-loaded microparticles there is mass loss (95.9%) in the range of 401 °C and 452 °C. The small difference that is observed between the onset temperature in the case of Ind-SLM and bulk Compritol (97.4% loss of mass with onset temperature 403 °C), is almost unnoticeable when placebo-SLM without Ind are tested (96.9% loss of mass with onset temperature 403 °C). The same effect was also observed in the tested formulations with other active substances (e.g., CsA, Figure 3a).

Figures 3 and 4 also show the hypothetical peak sizes of drug substances (CsA and Ind, respectively), determined on the basis of the conversion of the pure substance peak into the peak corresponding to its concentration in the tested formulation. As can be seen under magnification (Figures 3b and 4b), there is no drug substance peak in any of the tested drug-loaded SLM.

In the next step, physical mixtures of the Ind and Compritol were tested. When the Ind is present, the physical mixtures show typical endothermic peaks corresponding to Compritol and the second peak of Ind, which is also endothermic (Figure 5). The aspect that should be noted is the shift of the Ind peak towards a lower temperature, which is the greater the lower the concentration of Ind is in the mixture. This fact is confirmed by all parameters characterizing the peak: onset, peak temperature, endset (see Figure 5). In addition to the shift, the width of the Ind peak also changes in a characteristic way, becoming narrower and sharper with increasing Ind concentration. Moreover, none of the

Ind peaks, regardless of their concentration in the physical mixtures, show an intensity proportional to the peak of bulk Ind, although the increase in peak intensity corresponds to the increasing concentration of the active substance in the physical mixtures.

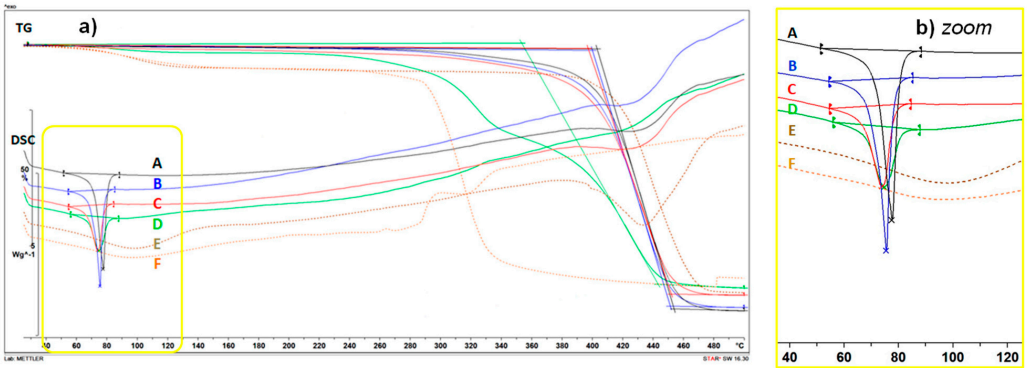


Formulation	Onset	Peak	Endset	Width
Ind	164.60 °C	168.50 °C	171.82 °C	4.29 °C
PM (9:1) 10% Ind	132.10 °C	151.67 °C	163.67 °C	39.02 °C
PM (3:1) 25% Ind	148.13 °C	160.33 °C	165.40 °C	11.25 °C
PM (1:1) 50% Ind	156.09 °C	162.33 °C	166.45 °C	5.90 °C

Figure 5. (**a**) DSC and TG curves and (**b**) the zoom of: (A) the bulk Ind, (B) the physical mixture (PM) of Compritol and Ind (1:1), (C) the physical mixture (PM) of Compritol and Ind (3:1), (D) the physical mixture (PM) of Compritol and Ind (9:1), (E) the bulk lipid Compritol. The parameters of the DSC curves characterizing the Ind peak are also listed below the graph.

Since during SLM dispersion tests, the distribution of the drug substance is determined by shaking the microspheres with methanol [18], DSC/TG tests were also performed before and after this process. The DSC curves, after evaporation of the water–methanol mixture in the distribution test, do not differ in any way from those obtained before the dissolution of the surface-located drug substance fraction (Figure S2).

Spray-dried SLM dispersions with or without the addition of auxiliary substances (e.g., PVP) were also tested (Figure 6).



Formulation	Onset	Peak	Endset	Width
A	71.25 °C	77.50 °C	80.29 °C	4.87 °C
B	70.24 °C	75.50 °C	77.71 °C	4.23 °C
C	65.86 °C	73.83 °C	78.12 °C	7.11 °C
D	67.19 °C	74.67 °C	80.87 °C	8.04 °C

Figure 6. (**a**) DSC and TG curves and (**b**) the zoom of: (A) F10-SLM dispersion with Compritol and 0.2% of Ind, (B) F10-SLM after spray drying, (C) F10-SLM after spray drying with PVP, (D) F10-SLM after spray drying with Mx, (E) the bulk PVP and (F) the bulk Mx. The parameters of the DSC curves characterizing the lipid peak are also listed below the graph.

The obtained thermograms indicate that there is no significant effect of the drying process on the properties of the dried formulations with Compritol (regardless of the tested active substance: the results of SLM with Ind are shown in Figure 6, and the microspheres with CsA and SPIR have been described previously [19]). The melting temperature of the microspheres slightly decreased (from 77.5 °C to 75.5 °C), as did the decomposition temperature in the TG curve (onset from 402 °C to 399 °C). Some changes in the shape and intensity of the lipid melting peak were only observed as a result of the addition of auxiliary substances such as PVP or maltodextrin immediately before the drying process. This resulted in a decrease in the sharpness and intensity of the melting peak, while lowering the temperature to 73.8–74.6 °C, depending on the excipient. A change in the TG curve (acceleration of the degradation process) was observed only in the presence of maltodextrin. When the microsphere matrix was formed by stearic acid, changes in the DSC peaks were unrepeatable at various stages of preparation and further processing of SLM dispersions, as was already described in previous reports [19].

As already mentioned, the lipid melting peaks on the DSC of the drug-loaded SLM were mainly characterized by a slight shift towards lower temperatures, while maintaining the intensity and characteristic sharp shape of the peak. Some differences were observed only in the formulations with the stearic acid, although as can be seen in Figure 7, often stearic acid peaks in SLM also retained their properties, with no changes compared to the placebo microspheres and the bulk lipid.

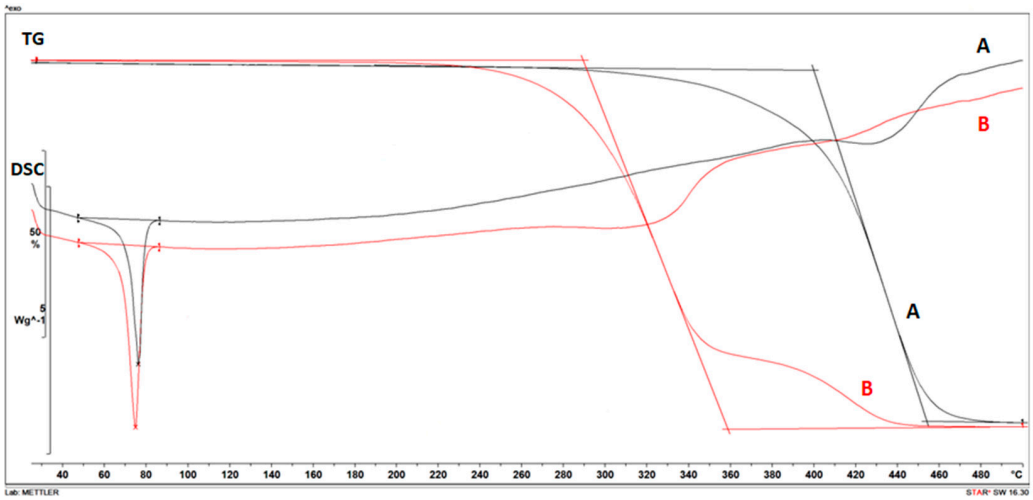


Figure 7. DSC and TG curves of CsA (1%) SLM dispersions: (A) F4-SLM with Compritol and (B) F8-SLM with stearic acid.

Figures S3 and S4 show the thermograms of SLM with 0.2% Ind and 2% CsA in a Compritol matrix freshly prepared and stored (12 months in the refrigerator). In the stored dispersion, the main melting peak of the lipid slightly changed its intensity, but no shift was observed. Both the DSC and TG curves confirm the stability of the tested formulations, which has also been demonstrated in other studies [18,20]. The same results were obtained when testing SLM with 1% CsA and Compritol in the form of an aqueous dispersion and fine powder, stored for 2 years in the refrigerator and at room temperature, respectively (Figure S5). The stability of the formulations discussed in this manuscript has also been confirmed in other studies. Immutability has been demonstrated in terms of the stability of the particle size parameter or zeta potential, as well as the content of the active substance (100 ± 5%) and its distribution in the individual phases of the SLM dispersion or SLM powder.

The stability of lipid microspheres with stearic acid was also investigated. The observed transformations occurred already during the manufacturing or spray-drying stage, not during storage, and are varied in different formulations and their repetitions.

4. Discussion

The thermal characteristics of the placebo and drug-loaded SLM (Table 1) in the form of a liquid dispersion or a spray-dried powder were investigated. Physical mixtures were tested for control purposes. The tested SLM formulations contained from 0.1 to 5.0% of the drug substance, depending on the type of substance. The content of the active substance, determined after preparation, was in each case in the range of 100.0 ± 5%, which was considered the desired value. The detailed distribution of active substances in the individual phases of the SLM formulation has been discussed and characterized in previous reports [18,19].

Carrying out a DSC measurement of SLM dispersion requires proper sample preparation, because the presence of water in the sample during the analysis caused the DSC and TG curves to show a characteristic profile of water evaporation followed by thermal decomposition with carbonization. Only the DSC analysis of dry samples makes it possible to distinguish events related to lipid melting. Of course, this does not mean that the DSC/TG method is not suitable for testing SLM obtained in the form of liquid dispersions. Such microspheres are tested as a dry residue, and sample preparation becomes crucial. The most important thing is that the method of drying the sample must not affect the properties of the microspheres studied at the analysis stage. Pre-heating the sample (e.g., a few minutes at 80 °C) and then cooling it down again before analysis is one of the recommended methods [13,21]. However, it should be taken into account that this process, taking place at a temperature above the lipid melting point, will certainly affect the properties of the formulation. Another method is to lyophilize the dispersion before thermal analysis. Different drying processes can lead to significant changes in the properties of the sample as a result of the effect of temperature (both increased and decreased during freeze-drying) [22], not to mention other adverse forces acting on the microspheres during freezing. In order to avoid preheating before the analysis or a time-consuming and demanding lyophilization process, it was proposed to evaporate the water at room temperature under compressed air (Figure 1), without or after ultracentrifugation of the dispersion (Supplementary Material Figure S1). The thermal transformation peaks of the lipids obtained in both cases were very similar (Figure S1), although in the formulation subjected to centrifugation the peak had a slightly less regular shape, lower height and greater width. Considering the small mass of microspheres used for DSC analysis, it is enough to evaporate water from a small volume of dispersion. Bearing in mind the effect of centrifugal forces during centrifugation, which may also affect the properties of the microspheres, it was ultimately considered beneficial to abandon the centrifugation step. As the final and most advantageous solution, it is therefore proposed to use only the evaporation of water from SLM dispersion under compressed air at room temperature before analysis.

When pure drug substances were tested, cyclosporine exhibits no thermal events at 115 °C, characteristic for a crystalline drug [23], because the endothermic peak is shifted to 128 °C (Figure 2). The peak observed at this temperature probably results from the solid-to-liquid transition of the drug substance at temperatures above 120 °C. The same phenomenon was observed by Onoue et al. [23] and Jiang et al. [13], when they studied the crystalline and amorphous form of CsA. Indomethacin, as a commercial bulk material, usually occurs in the most thermodynamically stable γ form, although it can also exist in the α polymorphic form as well as in the amorphous solid form. The DSC thermograms reported in the literature [24], for both polymorphs and amorphous indomethacin, are characteristic, with the sharp endothermic peak above 160 °C being attributed to the γ form, as in our case. The obtained spironolactone DSC curves show a single sharp endothermic peak at the melting temperature of 213 °C, confirming the purity and crystallinity of the SPIR [25].

Secondly, a characteristic feature of thermal studies of drug-loaded SLM is the absence of the drug substance peak in the DSC spectra. The melting peak of any of the tested drug substances (Figure 2) was not observed in thermograms of SLM (Figures 3 and 4), regardless of their concentration in the formulation. One possible hypothesis is that the disappearance

of the drug substance melting peak in the drug-loaded SLM could be explained by the fact that, at a low drug concentration, the melting point of the drug substance has been lowered and broadened so much that the peak became undetectable by DSC. However, the peak is not visible even when the ratio of CsA to lipid was 1:2 in the F6 formulation (Figure 3b). These observations are consistent with the results of Wong et al. [15], who obtained the melting peak of ibuprofen in a drug–lipid cast with glycerol behenate only at a concentration of 70% and above.

The absence of melting endotherms of the tested drug substances in the DSC thermographs of all drug-loaded SLM preparations (Figures 3 and 4) may therefore result from a drug concentration in the SLM too low to be detected by the DSC method. However, comparison of the SLM thermograms with Ind and a physical mixture with Ind (the same Ind content, Figure 5) also suggests drug–lipid interactions and API incorporation, or at least some kind of interaction of the drug substances with other components of the dosage form. According to one possibility, the disappearance of the peak of the active substance in the microspheres may result from the dissolution of the drug substance in the molten lipid during the manufacturing process. Such explanations appear, for example, in the study of microspheres with theophylline [26]. Similar results (the lack of an endothermic peak for CsA) in solid dispersions were also obtained by Onoue et al. [23].

The thermal behavior of SLM dispersions was very similar, regardless of the type and concentration of the incorporated drug substance (Figures 3a and 4a). First of all, as was shown in the example of the tested drug substances, the presence of a solute in the microsphere matrix decreases the melting point of the lipid matrix, which in the DSC spectrum is manifested by a shift of the lipid melting peak towards a lower temperature. Drug substances that are incorporated in the SLM to varying degrees (depending mainly on the properties of the drug and the composition of the carrier) might interact with the lipid matrix of the microspheres. This effect can change the crystalline structure of the lipid and, consequently, the melting point. A decrease in enthalpy generally suggests a lower crystallinity of lipid matrices [14]. Low crystallinity of lipids is desirable in order to create more spaces for drug localization and enhance the encapsulation efficiency of drug substances [14]. The shift of the melting peak of the lipid matrix in different lipid particles to a lower temperature with respect to the bulk lipid was explained in published studies through various reasons, such as the small size of the lipid particles or the incorporation of a drug substance or interactions between the lipid and the surfactant, which results in a less ordered structure of the lipid (Kelvin effect) [27,28]. Therefore, a certain degree of melting point depression of the lipid peak in drug-loaded SLM can be attributed to the dissolution of the drug substance in the lipid matrix.

The size of microspheres in SLM dispersions is in the range of several micrometers, with the largest particles not exceeding 15 µm. Dispersions with Compritol are generally characterized by a monomodal, and with stearic acid, a bi- or polymodal particle size distribution. The addition of the active substance, especially at a concentration above 1%, affects the SLM, causing a slight increase in the size of the microspheres. The particle size measured by the dry method (DPS) in powders is larger (10–20 µm, most particles below 50 µm) because particles in dry powders tend to agglomerate, which affects the obtained results. However, this process is reversible, as demonstrated by the redispersion tests, and the microspheres in the agglomerates formed after the drying process retain their integrity (shape and size), which was confirmed by the microscopic analyses [17,19]. The issue of particle size and the change in this parameter in detail (particle size distributions, optical microscope and SEM images) have already been discussed in previous publications describing research on the properties of SLM dispersions and spray-dried powders [17,19].

Moreover, thermal transformation of API is not observed regardless of the distribution of API in the SLM. It is not visible even in the case of incomplete incorporation of the drug substance into the lipid microspheres. During development studies of lipid microspheres, one of the important elements is to determine the distribution of the active substance in various phases of the SLM dispersion, such as the core of lipid particles, the surface of the

microspheres or the aqueous phase of the dispersion (as described in previous reports [18]). For this purpose, in one of the stages, the drug substance localized on the surface of the lipid microspheres is dissolved while shaking the dispersion with methanol (without disturbing the structure of the microspheres and extraction of the drug substance incorporated in the lipid matrix). In the distribution studies, this fraction is isolated and quantified [18]. In the DSC study (conducted after such a process) the active substance, extracted from the particle surfaces, after evaporation of the solvent, should precipitate/crystallize outside the microspheres. Despite this, no change or drug peak was observed in the DSC curve (Figure S2).

When the molecularly dispersed drug substance in the solid matrix of SLM exceeds its solubility limit, it is not fully incorporated into the SLM during the manufacturing process, and it precipitates or crystallizes out in the liquid dispersion. An example of such a formulation in our research is the SLM dispersion with 1% Ind (F11). Despite incomplete incorporation, no traces of API were found in the DSC spectrum (Figure S2). Unfortunately, DSC/TG tests do not make it possible to identify incomplete incorporation of the drug substance in the lipid matrix of the microspheres, and other techniques must be used for this purpose.

On the one hand, it can be assumed that the undetectability of the drug substance in DSC tests of lipid microspheres results from low concentrations in the formulation, which is also confirmed by data from the literature [15]. On the other hand, the lack of a peak for the active substance may indicate its incorporation into the lipid matrix in the form of a molecular dispersion and interaction with the components of the dosage form (mainly lipid, but also a surfactant stabilizing the microspheres). Finally, the interaction with the lipid is confirmed by the study of physical mixtures, in which the peak intensity of the drug substance was already reduced (Figure 5). However, taking into account the fact that the active substance is not detected also in those systems, in which incomplete incorporation of the drug substance was observed, it can be assumed that the above-mentioned processes coexist. Taking into account all of the obtained results, it should be concluded that the DSC method is not the optimal tool for evaluating SLM formulation in terms of the content or incorporation of the active substance. Therefore, as already described, the presence of API and its impact on SLM properties in thermal studies can only be inferred indirectly, based on a decrease in the peak temperature associated with lipid melting (DSC) or the subtle change in the decomposition temperature (TG).

As the results indicate, the spray drying process did not have any impact on the thermal properties of the tested microspheres with Compritol (Figure 6). Minor changes in the DSC and TG curves were mainly observed due to the use of additional excipients (like PVP or maltodextrin) in the spray drying process. However, the lipid forming the matrix of microspheres had a significant impact on the properties of the tested formulations. Compritol turned out to be resistant to the conditions of the drying process and stable during storage, in contrast to the less stable stearic acid, which sometimes undergoes unfavorable changes even at the initial stage of microsphere production [17,19]. As in the case of the SLM dispersions, no peak of any of the tested active substances (CsA, Ind, SPIR) appeared in the DSC spectra of the spray-dried SLM.

Comparing the DSC spectra of the microspheres with Compritol and stearic acid after spray drying, it can be concluded that stearic acid sometimes occurs in a less crystalline form in these samples (the intensity of the stearic acid peaks after spray drying was much weaker). Sometimes a similar effect in stearic acid was already observed at the stage of microsphere preparation. A lower degree of the crystallinity of the lipid matrix in SLM may be associated with higher encapsulation efficiency (which, however, was not observed), but also with a higher risk of instability.

It is therefore possible to obtain microspheres characterized by unchanged thermal properties compared to placebo microspheres not only with Compritol, but also with stearic acid as a lipid forming the matrix of microspheres (Figure 7). The only problem in the production of SLM with stearic acid is the limited reproducibility of the process.

The aforementioned shift in the melting point may be caused primarily by the interaction of active substances with lipids and other components of the dosage form, but also by polymorphic transformations of the components, the addition of other excipients or even the particle size.

As it has been shown in earlier studies [19], the preparation of lipid microspheres using the hot emulsification method can proceed without or with a significant impact on the melting point of the matrices and their degree of crystallinity. This is mainly dependent on the lipid forming the SLM matrix, which has a major impact on the polymorphic transformations of the lipid matrix and the stability of lipid particles [29]. These results are consistent with those reported by Silveira et al. [21], which showed the stability of cetyl palmitate (used to obtain the SLM) even after the heating process. The obtained results suggest that Compritol remains stable, while in SLM with stearic acid, changes are observed following melting and heating during the preparation of the SLM.

DSC studies (Figures S3–S5) also confirm the previously described stability of SLM dispersions, in which the lipid matrix is composed of Compritol [20]. Certainly, the stabilizing effect of polysorbate on the properties of the lipid is not without significance. A similar effect has already been reported for polymer additives such as poloxamer, polyvinyl alcohol or polyvinylpyrrolidone, which may affect the polymorphism of the matrix [15].

Microspheres with stearic acid behave differently, they are not necessarily less stable during storage, but much more susceptible to changes occurring under the influence of production processes. Stearic acid, like other fatty acids, can exist in different polymorphic forms and also creates lipid structures with high crystallinity [30]. The difference in melting points of stearic acid (76.5 °C), SLM with this acid (75 °C) and the enthalpy suggest the presence of different polymorphs, as well as interaction with the API, similar to lipospheres with Compritol. The significant decrease in enthalpy is due to the less ordered structure, which requires much less energy to melt than in the case of crystalline substances that have to overcome lattice forces. Compared to data from the literature [30], these events seem to correspond to phase transitions that occur at different stages in the production of SLM, from homogenization during the emulsification process to spray drying. In the case of stearic acid, the limited stability is not improved even by polysorbate. This proves the key importance for the stability of the formulation of the type of lipid forming the microsphere matrix.

In stability studies of SLM with Compritol stored for 12 months in the refrigerator in the form of a liquid dispersion (Figures S3 and S4), no changes were found in the DSC/TG tests, regardless of the type of active substance. It is therefore not surprising that the same formulations are stable when stored in the form of a dry powder (obtained in the spray drying process) at room temperature. In SLM formulations with CsA, stability was confirmed even for a period of 24 months, regardless of the liquid or solid form (Figure S5).

Due to the described problems with the repeatability of the results of the production and testing of SLM with stearic acid, it is not possible to formulate final conclusions regarding the stability of these formulations, as they are ambiguous and require further research.

5. Conclusions

The conducted studies demonstrated the significant potential of DSC/TG for the analysis of multi-compartment, lipid-based formulations such as SLM, especially in the form of a fine powder, but also in the form of an aqueous dispersion, under the condition that the water has been previously evaporated at room temperature.

A recurring feature of DSC studies of lipid microspheres is the lack of thermal transformations characteristic of drug substances incorporated in SLM. The presence of API and its impact on SLM properties in thermal studies can be inferred indirectly, based on a decrease in the peak temperature associated with lipid melting (DSC) or a subtle change in the decomposition temperature (TG).

The DSC/TG studies confirmed the lack of effect of the spray drying process on the drug-loaded SLM with Compritol. SLM studies with other lipids forming the microspheres

matrix, especially stearic acid, certainly require continuation. In our study, thermal analysis was successfully applied not only to assess the properties but also the stability of the lipids forming the microsphere matrix.

Based on the obtained results, we concluded that DSC/TG analysis techniques can be a complementary and useful tool for the evaluation of specific properties of SLM, both in the form of powder and lipid microparticle dispersions (prepared for analysis by the evaporation of water), after production, under further processing (e.g., the spray drying of an aqueous dispersion) or during storage.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app132413282/s1. Figure S1. DSC and TG curves of SLM dispersions: (A) without water evaporation, (B) after water evaporation and (C) after centrifugation and water evaporation; before thermal analysis. Figure S2. DSC and TG curves of SLM dispersions: (A) F11-SLM dispersion with 1% of Ind and (B) F11-SLM dispersion with 1% of Ind after shaking with methanol and evaporation. Figure S3. DSC and TG curves of F10-SLM dispersion with Compritol and 0.2% of Ind: (A) after preparation and (B) after one year of refrigerated storage. Figure S4. DSC and TG curves of F5-SLM dispersion with Compritol and 2% of CsA: (A) after preparation and (B) after one year of refrigerated storage. Figure S5. DSC and TG curves of F4-SLM dispersion with Compritol and 1% of CsA: (A) in the form of aqueous dispersion after two years of refrigerated storage and (B) in the form of spray-dried powder with PVP after two years storage at room temperature.

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