Early stages of drug crystallization from amorphous solid dispersion via fractal analysis based on chemical imaging

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Abstract

Early stages of crystallization from amorphous solid dispersion (ASD) are typically not detected by means of standard methods like powder X-ray diffraction (XRPD). The aim of this study is therefore to evaluate if fractal analysis based on energy dispersive x-ray imaging can provide the means to identify early signs of physical instability. ASDs of the poorly water-soluble compound, felodipine (FEL) were prepared by solvent evaporation using different grades of HPMCAS, at 50wt. % drug loading. Samples were stored at accelerated conditions of 40˚C. Scanning electron microscopy equipped with an energy-dispersive X-ray spectroscopy (SEM-EDS) was used for elemental mapping of tablet surfaces. Comparative data were generated with a standard XRPD and with more sensitive methods for detection of early instability, i.e. laser scanning confocal microscopy (LSM) and atomic force microscopy (AFM). The SEM-EDS identified changes of drug-rich domains that were confirmed by LSM and AFM. Early changes in drug clusters were also revealed by a multifractal analysis that indicated a beginning phase separation and drug crystallization. Therefore, the presented fractal cluster analysis based on chemical imaging bears much promise as a new method to detect early signs of physical instability in ASD, which is of great relevance for pharmaceutical development.

Keywords: solid dispersion; amorphous drug; phase separation; crystallization; chemical imaging; multifractal analysis

1. Introduction

Amorphous solid dispersion (ASD) has become an established oral delivery technique to formulate poorly water-soluble drugs [1–3]. Given the large number of papers that are published on solid dispersions, it is rather surprising that there are not many more of these products on the pharmaceutical market [4]. One of the main reasons is that ASDs are metastable systems and bear the risk of physical instability during their shelf life. Drug crystallization can occur depending on the history of an amorphous product and on given conditions [5]. Particularly critical is storage at elevated temperature and moisture leading eventually to phase separation and crystallization, which means a loss of biopharmaceutical advantages using ASD [6–8]. The molecular processes of phase separation as well as crystallization from amorphous material are complex and mobility in the glass state plays an important role. Relaxation of amorphous materials take place on different time scales from primary diffusive (α- relaxation) to secondary local relaxation such as Johari-Goldstein relaxations (β-relaxation). While α- relaxation becomes very slow in the glass state, it is mostly the secondary relaxations that are relevant for crystallization from amorphous state [9–11]. Once crystallization starts, it continues to reduce the system's free energy [12,13]. Thus, thermodynamics is the driver for physical change and molecular mobility is a facilitator, while surface effects can act as modulators, and heterogeneities as amplifiers of crystallization [14].

A standard method to detect and characterize crystalline material is X-ray power diffraction (XRPD) but like with other conventional methods such as differential scanning calorimetry, it is challenging to detect small amounts of crystalline material. Such small amounts can be already present following manufacture of an amorphous product or they are generated as initial instability but either ways, such crystallites can negatively affect kinetic stability of ASDs [12,13].

Interesting for detection of crystalline material are imaging techniques that can be based on different physical principles. Since amorphous formulations are typically multi-component systems, chemical image has the advantage that an active pharmaceutical ingredient (API) is differentiated from excipients. Any

chemical imaging involves a sophisticated analytical technique for acquisition of images and spectra that contain the chemical information [15], which typically enables spatial distribution of one or all formulation components [16]. Images can be acquired at the surface and in the bulk by electron microscopy, such as, transmission electron microscopy (TEM) (Yamada et al., 2017) and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS) [17,18]. Important are also vibrational spectroscopic techniques with appropriate optics, such as Raman [15,19,20], near infrared (NIR), or terahertz spectroscopy [14].

It is critical for any imaging technique how the large amounts of data are evaluated. Suitable algorithms such as modern chemometric methods can be applied to extract useful information from otherwise just large and incomprehensible data sets [15]. An algorithmic topic in its own right is how clusters are analyzed in images since a naked eye is not capable of detecting any subtle changes of an imaged microstructure. Promising for any such cluster analysis is fractal geometry. This approach was pioneered by Mandelbrot and does not use classical geometry to describe physical objects [21]. Fractal geometry has been applied in pharmaceutics, for example, to describe a solvent-mediated formation of a drug hydrate [22]. However, a single fractal dimension is often not sufficient to adequately describe a complex heterogeneous system. A more generalized mathematical concept is given by multifractal analysis, which decomposes the self-similar measures into intertwined fractal sets that describe the variations from the average in heterogeneous systems [23].

Recently, the multifractal formalism was introduced in solid dispersion technology to describe the spatial distribution of an inorganic carrier [24]. In a subsequent work, the distribution of different drugs in ASDs was revealed to have a multifractal character [25]. The mathematical formalism was found to model adequately the heterogeneous nature of drug clusters in ASD and a next step would be to study changes over time. The hypothesis of the present work is that multifractal analysis based on chemical imaging can proof utility in analyzing early stages of physical instability. Thus, felodipine (FEL) was used as model drug in solid dispersions with the polymer hydroxypropyl methylcellulose acetate succinate (HPMCAS). The

different grades LF and HF were used with 14-18% and 4-8% of succinoyl substitutions, respectively [26]. ASDs of FEL/HPMCAS were analyzed topographically by SEM-EDS as a chemical imaging technique, which provided the basis for multifractal analysis. To have a comparison with other known sensitive methods, samples were also studied by laser scanning confocal microscopy (LSM) and atomic force microscopy (AFM).

2. Materials and methods

2.1. Materials

Felodipine (FEL) was purchased from Kemprotec Ltd. (Smailthorn, Cumbria, UK) and the different grades of HPMCAS (Shin-Etsu AQOAT®, Type LF and HF) were a generous gift from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). Dichloromethane and methanol (HPLC grade) were procured from Sigma-Aldrich (St. Louis, Missouri, USA). The API chemical structure as well as monomer units of the polymer are given in Fig. 1. Particularly highlighted are chloride atoms regarding their selective detection by energy dispersive X-ray spectroscopy.

2.2. Methods

2.2.1. Preparation of physical mixtures and solid dispersions by rotary evaporation

Initial pretests of varying drug loads suggested that 50% FEL in polymer was rather challenging for amorphous stability, which therefore provided a suitable reference concentration in the ASDs of the main study. Binary mixtures of 50% w/w FEL polymer were then prepared by dissolving drug and polymer in a solvent mixture of 50: 50 (v/v) dichloromethane: methanol. All solvent mixtures were visually inspected to confirm that the drug and the polymer were completely dissolved and the systems formed uniform one-phase solutions. The solvent was then removed at 50˚C under reduced pressure using a rotavapor RE120 (Bünchi Labortechnik AG, Flawil, Switzerland) with a vacuum controller CVC2 (Vacuubrand GMBH + CO, Wertheim, Germany). The obtained solid mass was stored at room temperature overnight to remove any

residual solvents. The ASDs were freeze/milled with SPEX SamplePrep model 6770 (Metuchen, New jersey, USA). For a comparison with amorphous formulations, physical mixtures (PM) were prepared by mixing the powders of FEL and polymer for 5 minutes with a spatula.

Samples of the solid dispersions were compressed to tablets for subsequent surface and image analysis. Thus, powders (100 mg) were manually fed into a hydraulic XP1 press (Korsch AG, Berlin, Germany) and manually compacted. The compacts were flat-faced and round with a diameter of 7 mm.

2.2.2. X-ray powder diffraction

X-ray powder diffractogram (XRPD) were determined for PM as well as for ASD obtained from rotaevaporation. A D2 Phaser diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) was employed that was equipped with a Co-2K KFL diffraction tube configured and a 1D- Lynxeye detector and with a Fe filter. The applied voltage and current were 30kV and 10mA, respectively. Diffraction patterns were obtained using a step width of 0.02˚ with a detector resolution in 2Θ between 6-40˚ and a scan speed of 2s/step at room temperature.

2.2.3. Stability studies

Compacts were stored in hermetically closed glass vials at a temperature of 40˚C using a climate chamber (Binder GmnH, Tuttlingen, Germany). The storage temperature was selected as a realistic but accelerating condition to detect kinetic changes over time (e.g. phase separation and/or drug crystallization). Samples were taken at specific time intervals and analyzed physically.

2.2.4. Scanning electron microscopy and energy-dispersive X-ray spectroscopy

The samples were placed on double-side adhesive carbon tabs and the surface of the compacts was coated with gold under argon vacuum with a Sputter Coater SC7620 (Quorum Technologies Ltd., Kent, UK). These surfaces were then studied by means of scanning electron microscopy (SEM) TM3030 PLUS (Hitachi High-Technologies Corporation, Tokyo, Japan) and micrographs were collected in a mix mode. The microscope was equipped with an energy-dispersive X-ray spectroscopy (EDS) device for elemental mapping. The analysis was based on a Quantax 70 system software (Bruker Nano GmbH, Berlin, Germany) consisting of an X Flash Min SVE signal processing unit, a scan generator and Megalink interface together with an X Flash silicon drift detector 410/30 H (Bruker Nano GmbH, Berlin, Germany). Samples were scanned during 300 seconds at a voltage of 15 kV to map the drug distribution of chloride (Cl) atoms for comparatively higher X-ray scattering intensity. This procedure was performed on all compacts at random location in spot sizes of 113 × 85 μm^2.

2.2.5. 3D-Laser scanning confocal microscopy

Laser scanning micrographs of the sample surfaces were collected by means of a 3D laser scanning confocal microscopy (LSM) VK-X200 (Keyence, Osaka, Japan) using a violet laser (408nm) and a 150x objective lens (Nikon Plan CF Apo, 150x/0.95, WD 0.2mm) . The surface is scanned at high speed in X, Y and Z, allowing image capturing and height measurements with high lateral resolution. Reflected white light and laser light emitted from the focal point are reflected back through the objective lens. The intensity of the laser light that passes through a pinhole is determined by a very sensitive 16-bit photomultiplier. Since the pinhole blocks most of the returning light (except the light from the focal point), confocal LSM delivers much sharper images than conventional microscopy techniques. In addition, a true color image from the integrated second light source is overlaid.

2.2.6. Atomic force microscopy

Atomic force microscopy (AFM) images of compacts were acquired at ambient conditions in dynamic AC mode using a NanoWizard 4 AFM instrument (JPK Instruments AG, Berlin, Germany). Height and phase

images were collected simultaneously using a silicon PPP-NCHR cantilever (Nanosensors AG, Neuchâtel, Switzerland) with a resonance frequency of approximately 320 kHz and 42 N m^{-1} spring constant.

2.2.7. Image processing

The Cl distribution images were exported to Image J software (National Institutes of Health, Bethesda, Maryland, USA) and converted to maximum intensity projection. In a second step, the gray scale images in the bmp format (with 1023 x 766 pixels and a resolution 72 dpi) were resized to the png format with 512 x 512 pixels while keeping the same resolution. The projected images were unsharpened with a radius of 12 pixels and they were binarized using MATLAB software package, version R2017b (The MathWorks, Inc., Natick, USA). In a binary image, a signal pixel is defined as a digital element whose intensity is 1 after thresholding the images of 5 and this conversion to binary format (with a resolution of 96 dpi) was conducted with 10 images per each sample analysis.

2.2.8. Multifractal and statistical data analysis

While classical fractals are mathematical objects with a single fractal dimension, multifractal formalism decomposes self-similar measures into intertwined fractal sets (Gould et al., 2011; Li et al., 2012). A brief review of basic multifractal theory is given in the Appendix.

Box-counting method algorithm was used to cover a 2-D image for determination of fractal dimensions. Using the binary images, boxes (grids of 512 pixel sizes) were counted using at least one pixel of the observed object. A multifractal spectrum was then determined with a customized MATLAB program as proposed and described previously [27].

The formalism of multifractals expresses here a generalized fractal dimension or a “deformation parameter” of variability degrees (D_q) and moment order (q) that is a number within [-∞; +∞] interval extracting characteristics of the cluster distribution [28]. The multifractal spectra or the generalized dimension can be restricted to three values of particular interest D_0, D_1 and D_2. Herein, D_0 is the "classical box-counting dimension" also called the “capacity” dimension and D_1 refers to an information dimension (related to Shannon’s measure of entropy) and characterizes the degree of disorder in a distribution. Finally, D_2 is named a “correlation” dimension so it indirectly marks a degree of clustering [23,27–30].

The obtained fractal dimensions at the different time points were compared statistically by means of an analysis of the variance (ANOVA). STATGRAPHICS Centurion XVI ed. Professional (V. 16.1.15) from Statpoint Technologies Inc. (Warranton, Virginia, USA) was used for all statistical calculations and a p-value < 0.05 was considered as significant. For comparison of the means, Fisher's procedure of the least significant difference (LSD) was calculated for 95% confidence intervals.

3. Results

3.1. Physical characterization

XRPD is a standard method to detect crystalline material based on distinct peaks arising from Bragg scattering from defined crystal planes. The absence of diffraction peaks in the initial analysis of solid dispersions were therefore an indicator of successful amorphization of FEL at a comparatively high load of 50% (w/w). A subsequent storage during four weeks at 40˚C did also not lead to samples in which crystalline drug was evidenced so the formulations were still unchanged at least based on XRPD (Figure 2). By contrast, the analysis following eight weeks of storage (40°C) revealed diffraction peaks of FEL, which was an obvious consequence of re-crystallization from the amorphous solid state. The diffraction peaks in the LF grade appeared to be more pronounced than in case of HF.

3.2. Scanning electron microscopy and energy-dispersive X-ray spectroscopy

A suitable way to analyze the chemical distribution of specific atoms (as markers of molecules) on a surface is facilitated by SEM-EDS mapping. It is a qualitative method of chemical imaging and in this study, chloride was studied as characteristic marker of FEL since this was the only Cl-containing component in the formulation. The SEM-EDS binary micrographs of surface topography are presented in Fig. 3. Images of freshly prepared formulations and of those that show rearrangements of clusters over time (at 40˚C) are shown. The white pixels hold for the chloride (and hence FEL) distribution and it is possible to see some changes in the degree of clustering. However, only qualitative changes are detectable to a limited extent by the naked eye so that a more quantitative analysis is required to study cluster dynamics for which the multifractal formalism is applied.

3.3. Multifractal analysis

Table 1 shows the results of multifractal analysis in terms of the dimensions with q values from zero to two. The different Dq values were pointing to multifractals as a better model than to assume a simpler monofractal cluster distribution, which would entail a constant Dq. This was even more clearly seen when dimensions at any time point were plotted for a broader range of q values, as a typical sigmoidal shape was evidenced with decreasing D_q along decreasing q values (not shown).

The capacity dimension D_0 was clearly below two for the Euclidian dimension but still comparatively high suggesting rather dense fractal structures. Values for D_1 and D_2 were also in a similar range as compared to our previous study [25]. The changes over time were focused on the early stage of stability testing, which did not reveal re-crystallization based on classical XRPD testing. However, Table 1 indicates some changes of Dq over time, which were statistically analyzed. An analysis of the variance (ANOVA) was conducted with time and polymer grade as factors and the generalized fractal dimensions (D_0, D_1, and D_2) were indeed found to be statistically significant to capture the microstructure changes, with significant p-values ($p<$ 0.0001) for a time effect regarding any of the three dimensions studied. By contrast, the factor of HPMCAS grade was not found to be significant with respect to D_0, D_1, or D_2. Fig 4 shows a statistical means plot

246 together with Fisher's 95% LSD intervals. The significant storage effect was similar in extent for all fractal
247 dimensions and is shown in Fig. 4 for D_0 as well as for D_2. The novel approach was obviously capable of
248 identifying microstructural changes even in the early phase of stability testing.

249

250 *3.4. Laser scanning confocal microscopy*

251 LSM provided nondestructive images in a relatively broad microstructural range for the different time
252 points of early stability testing of FEL systems. Fig. 5 shows rather rough surfaces before storage (Fig.
253 5a/b) with hardly any crystals observed. Interestingly, the LSM images after 4 weeks (Fig. 5c/d) suggested
254 generally smoother surfaces with some curved rough regions in the underlying microstructure for both
255 formulations. Either for each system a “blooming” effect was evidenced, which could be interpreted as
256 crystals formed on top of the surface or protruding directly underneath the surface. Crystals and aggrega-
257 tions thereof were seen significantly in the system of FEL/HPMCAS-LF (Fig. 5c), but also from beneath
258 and on top of the surface in the formulation of FEL/HPMCAS-HF (Fig. 5d). In general, LSM suggested
259 occurrence of some crystals at early stability time (4 weeks, 40°C).

260

261 *3.5. Atomic force microscopy*

262 Atomic force microscopy (AFM) is another physical surface analysis method that reaches small fields of
263 view in a submicron range and is therefore complementary to LSM as a reference method of early stability
264 testing.

265 AFM topography (3D height) measurements were carried out to understand the morphology and growth
266 dynamics of the surface before and after storage. The representative micrographs are visible in Fig. 6. The
267 initial images show maximum height values of 100 nm (Fig. 6a) and 160 nm (Fig. 6b), respectively. Brighter
268 islands or domain regions, most likely corresponding to FEL-rich domains surrounded by HPMCAS-rich
269 domains, are indicated by peaks. The round edges of the FEL-rich domains indicate most likely that the
270 aggregated clusters were still coated with polymer; therefore, minor phase contrast can be differentiated

among these surfaces (Fig. 7a, b). These results are in line with previous literature [8]. AFM phase images were recorded to obtain a contrast due to variation in energy dissipation, which is related to the presence of differences in surface adherence and consequently different material properties. This technique also allows detecting localized variations in stiffness, so even more details of morphology can be obtained by phase contrast. Initial samples illustrate that the surfaces have a homogeneous contrast with brighter and darker regions co-existing on the surface (Fig. 7a/b); hence material differences are less pronounced, assuming that the polymer is dominating and/or amorphous domains of the drug prevail upon crystal growth.

On the other hand, after storage at accelerated conditions for 4 weeks, the surface topography (Fig. 6c/d) shows a tendency to generally smoother (the height scale dropped to 14 nm and 30 nm, respectively), but more heterogeneous surfaces in the sense of growing phase separation as seen in the phase contrast images (Fig. 7c/d).

Both AFM modes (topography and phase imaging) strongly support each other and verify the obtained results giving a very detailed insight into the nanoscopic morphology of the specimen.

4. Discussion

The metastable character of ASD is a hurdle for their development because re-crystallization during long-term stability testing is a critical setback on the way to bring a drug product on the market. It is particularly critical when such physical instability is only detected late in pharmaceutical development, whereas an early identification of kinetically unstable formulations is less problematic in a screening phase. Accordingly, there is a tremendous interest in early identification of drug phase separation and re-crystallization from amorphous state. The present work is based on the hypothesis that multifractals can be helpful to early detect instability in amorphous drug formulations. The selected model systems showed some physical changes after four weeks with likely initial phase separation and occurrence of first crystals at the time of four weeks where XRPD still could not detect any changes. It was in line with expectation that LSM and AFM were more sensitive methods than XRPD to capture changes so these reference methods were interesting to compare with the novel multifractal approach based on SEM-EDS imaging.

As a result, differences in the multifractal dimensions D_0, D_1, and D_2 were indeed evidenced after one month compared to the initial analysis. Therefore, multifractals were capable of revealing microstructural changes caused by instability that were otherwise hard to identify from the original images of SEM-EDS and that were undetected by XRPD. Like any chemical imaging technique, SEM-EDS comes with spatial resolution limits and they impact on the determined clusters [31]. Such clusters hold for drug-rich domains and it is not possible to directly infer their physical state. These drug-rich regions can be of different kinds [32], e.g. concentrated drug associated with polymer or it can be separate amorphous drug domains as well as small crystal nuclei. This is important to keep in mind when clusters of drug are considered in the binary images. Changes in these clusters are primarily changes in mathematical objects as captured by the fractal dimensions, D_0, D_1, and D_2. The physical interpretation of these clusters should be always in the context of the applied imaging method. Since the multifractal dimensions provide meaning to cluster distributions they can prove helpful for understanding any early changes in ASD.

The dimension D_0 describes a space-filling capacity [33] and values decreased in the early period of stability testing. This result was not easy to predict because there are different possible processes like drug migration to the surface that may increase the space-filling capacity. An increase could also come from drug that was previously too dispersed and low concentrated to be detected as a drug-rich domain so that local aggregation can lead to new clusters. While these are processes to increase D_0, there are other effects leading to lowered values of this capacity dimension. Some of the drug-rich domains of drug-polymer aggregates may locally become more concentrated in an overall phase separation or drug re-crystallization. The resulting more concentrated clusters would appear still white in the binary images so that overall space coverage could slightly diminish.

The different cluster changes were apparently also leading on the average to a reduction in the information dimension D_1. This dimension reflects the diversity of elements in the system [29,34]. Moreover, D_2 holds

for a correlation dimension [29,30,35] and the evidenced reduction was caused by the microstructural changes. Thinking of the transformation from amorphous clusters to crystals there is of course nucleation as well as growth. Depending on which mechanism prevails, there would be different ways of how the correlation dimension changes. Thinking of the microstructural processes of phase separation, or crystal nucleation and growth, it is possible that different processes affect fractal dimensions in opposite directions, which could entail a loss of discrimination. The sensitivity to detect early physical instability by the multifractal approach is therefore certainly depending on the physical processes that occur as well as on the imaging technique used.

To compare the changes in cluster dynamic with other physical analysis methods, the sample surfaces were also studied by means of LSM and AFM. LSM and AFM are popular microscopic techniques to study surfaces with ultrahigh resolution [36,37]. While LSM can sample comparatively larger surfaces, AFM provides sub-micron images of surface topography and phase imaging.

The initial LSM micrograph profiles (Fig. 5 a/b) of the surfaces were rather uneven and rough and both formulations had rather similar surface texture while hardly no crystals were seen. Compared to the initial rough micrographs it is evident in Figure 5 c/d that there was a structural re-arrangement of the surface suggested likely caused by increased mobility [38]. The surfaces revealed in both formulations flat and smooth areas, and curved rough regions in the underlying microstructure. It is suggested that after the storage at 40˚C, the temperature induced possible re-crystallization and aggregates of drug were formed, as can be observed, small groups of crystals with regular shape grow towards the surface as a result of re-crystallization.

Due to the small area of analysis, AFM was leading to individual view on a sub-micron scale. The initial roughness is confirmed with a continuous matrix where critical spots of drug-rich domains might be occasionally recognized (Fig. 6a/b), while after storage the topography of the surfaces looked generally a bit

smoother (Figure 6c/d). From phase imaging, it is suggested that initially (Fig. 7a/b) the drug and the polymer were remaining both in the amorphous state and showed more or less homogenous contrast in the phase signal. The appearance of a brighter domain in the AFM phase image (Fig. 7c) give evidence of the existence of crystalline domains among partially amorphous and highly dissipating polymer regions, indicating a heterogeneous surface due to the phase separation. These findings seems contradictory at first sight, but under assumption that amorphous regions of the drug are re-crystallizing with accelerating temperature, the polymer needs to reorganize as well and starts to flatten out. This is in agreement with the relaxation phenomenon and mobility in glass state [14].

The orthogonal techniques LSM and AFM would be in line with the assumption that crystalline-amorphous phase separation may have occurred [32]. Based on this mechanism, a larger amount of drug can be uniformly de-mix and segregate in a short amount of time, while nucleation and growth act only locally [39]. The de-mixing was likely to accelerate re-crystallization of drug.

In summary, the finding of the orthogonal methods of SEM-EDS, LSM and AFM suggest that even the freshly prepared solid dispersions had drug-rich and polymer-rich clusters and this heterogeneity was reflected in the binary images obtained from SEM-EDS. The subsequent dynamics of de-mixing and re-crystallization was captured as complex changes in cluster dynamics of the binary images leading to measurable changes in multifractal dimension, LSM and AFM images, whereas in the classical XRPD analysis no changes were observed throughout the same time period.

5. Conclusions

The present work addressed the need for novel tools in early identification of physical instability of solid dispersions. Multifractal analysis was introduced successfully to early stages of stability testing using amorphous solid dispersions. Changes in the fractal dimensions were noted early in stability testing, when no

changes were appreciated based on XRPD analysis. The orthogonal techniques of SEM-EDS, LSM and AFM that are known to be sensitive for microstructural change, suggested that the initial solid dispersions already displayed heterogeneity in terms of drug-rich and polymer-rich domains and de-mixing of the components was likely to precede nucleation of crystalline material.

A decrease of the fractal dimensions D_0, D_1 and D_2 was statistically significant after four weeks of stability testing, while a possible effect of the HPMCAS grade was not revealed. Although the use of multifractals was successful for early instability detection, care is needed to expect the same cluster dynamics in other solid dispersions too. We discussed that different mechanisms of microstructural change can affect clusters and therefore fractal dimensions. However, a clear strength of the presented data evaluation is that this cluster analysis can be based even on more than one physical method of imaging. It could be, for example, also used for imaging based on Raman or near infrared spectroscopy. Moreover, it would be interesting to study different types of solid dispersions. The present work holds much promise but further research is needed to better assess the capability of multifractals to act as early warning tool for physical changes in metastable drug formulations.

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Appendix: Multifractal theory

The fractal dimension is measured by overlaying the binary image with grid of boxes and counting the number of boxes, $N(\varepsilon)$, this is expressed as [23,27]

$$N(\varepsilon) \sim \varepsilon^{-D_0} \quad (1)$$

where D_0 is the fractal dimension, calculated from the following equation:

$$D_0 = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log \frac{1}{\varepsilon}} \quad (2)$$

D_0 is derived by counting the number of boxes with various sizes to cover the image and then estimating the linear region in the log-log plot. However, complex structures may not entirely be described by single fractal dimension, but by multifractal analysis, which considers the amount of mass inside each box, in this way characterize these complex structures. The probability P_i of finding the object pixel in the i^{th} box is determined by

$$P_i(\varepsilon) \sim \varepsilon^{\alpha_i} \quad (3)$$

where α_i is the singularity strength which corresponds to the density in the i^{th} box.

The probability distribution for multifractal measurements is

$$\sum_i [Pi(\varepsilon)^q] \sim \varepsilon^{\tau(q)} \quad (4)$$

Where q is the exponent expressing the fractal properties in different scales of the object. τ(q) can be defined as:

$$\tau(q) = \lim_{r \to 0} [\ln (\sum_i P_i (\varepsilon)^q)] / \ln(1/\varepsilon) \quad (5)$$

The full plot of D_q versus q is representative of the strength of the multifractality of finite measure, and the generalized dimension D_q which is related with q can be expressed as

$$D_q = {\tau(q)}\Big/{(q-1)} \quad (6)$$

Also, the relationship between parameters of $f(\alpha)$ versus α are used to calculate the multifractal spectra:

$$N(\alpha) \sim \varepsilon^{-f(\alpha)} \quad (7)$$

where the number of boxes $N(\alpha)$ for each probability $P_i(\varepsilon)$ has singularity strengths between α and $\alpha + d\alpha$ is found to scale. $f(\alpha)$ against α, in general way it gives the "fractal dimension" $f(\alpha)$ of sets where the measure scales locally with the same exponent α. The multifractal spectrum gives one dimension for each set where the data scales similarly. The variable $f(\alpha(q))$ gives the local fractal dimension at resolution q. $f(\alpha)$ has the same information of generalized information D_q and can be defined as [23,27,28]:

$$f(\alpha(q)) = q\alpha(q) - \tau(q) \quad (8)$$

where α(q) can be defined as:

$$\alpha(q) = {}^{d\tau(q)}/_{dq} \quad (9)$$

In case of monofractal, $D_0 = D_1 = D_2$, whereas different values $D_0 \geq D_1 \geq D_2$ indicate a multifractal system [28].

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Figure captions

Fig. 1. Chemical structures of felodipine (FEL) and HPMCAS.

Fig. 2. Powder X-ray diffraction of FEL in physical mixture (PM) with HPMCAS of different polymer grades and formulated as amorphous solid dispersion (ASD). From bottom to top: ASDs: FEL/HPMCAS-LF, FEL/HPMCAS-HF after 4 weeks stored at 40˚C; PM: FEL/HPMCAS-LF, FEL/HPMCAS-HF (all samples at a drug load of 50 wt% of FEL).

Fig. 3. Results of energy dispersive X-ray spectroscopy (EDS) to obtain two-dimensional binary images of ASDs FEL/HPMCAS-LF, FEL/HPMCAS-HF (50 wt% of FEL), following storage at 40˚C. Drug-rich phase is shown as white domains.

Fig. 4. Statistical means plot of FEL/HPMCAS ASD formulations (50 wt% of FEL) based on a two-factor ANOVA of how D_0 (a) and D_2 (b) are affected by storage time (at 40˚C), and intervals of Fisher's Least Significant Difference (LSD, 95%) are shown.

Fig. 5. Confocal laser microscopy of ASDs before storage (a, b) and after storage (c, d) for 4 weeks at 40˚C. FEL/HPMCAS-LF (a, c), FEL/HPMCAS-HF (b, d), at 50 wt% of FEL. The scale bar is 10 µm.

Fig. 6. AFM topographical images of FEL/HPMCAS ASDs before storage (a, b) and after storage (c, d) for 4 weeks at 40˚C. FEL/HPMCAS-LF (a, c) and FEL/HPMCAS-HF (b, d).

Fig. 7. AFM phase images of FEL/HPMCAS ASDs; before (a, b) and after storage (c, d) for 4 weeks at 40˚C. FEL/HPMCAS-LF (a, c) and FEL/HPMCAS-HF (b, d).

Table 1

Generalized fractal dimensions of felodipine (FEL) solid dispersions over time as based on chemical imaging and conversion to binary pictures.

	Age (week)	**Generalized fractal dimensions**		
		D_0	D_1	D_2
FEL/HPMCAS-LF (50:50)	0	1.92 ± 0.01	1.88 ± 0.01	1.81 ± 0.02
	2	1.92 ± 0.01	1.88 ± 0.01	1.81 ± 0.02
	4	1.91 ± 0.01	1.86 ± 0.01	1.79 ± 0.01
FEL/HPMCAS-HF (50:50)	0	1.93 ± 0.00	1.89 ± 0.00	1.82 ± 0.01
	2	1.92 ± 0.01	1.88 ± 0.01	1.81 ± 0.02
	4	1.91 ± 0.01	1.87 ± 0.02	1.80 ± 0.02