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Abstract: Current pharmaceutical research encounters with the problem of low solubility of many active ingredients (APIs). To overcome this limitation the poorly water-soluble drugs are formulated as solid dispersions in matrices of hydrophilic polymers. In these new dosage formulations, besides the notoriously discussed polymorphism and a bit mysterious pseudopolyamorphism, precise structural characterization of APIs is complicated by their interactions with macromolecules of excipients. In this communication a time-saving method for identification of various forms of pharmaceutical substances in solid polymer dispersions is introduced. The method is demonstrated on a moderately-sized active pharmaceutical ingredient (Atorvastatin, C33H35FN2O5) exhibiting extensive polymorphism and pseudopolyamorphism. The API was incorporated in the matrix of polvinylpyrrolidone (PVP) forming uniformly dispersed nanosized domains. Size of domains, approximately tens of nanometers, was estimated by measuring T1(1H) and T1□(1H) relaxation times. The proposed strategy of structural characterization of the API in PVP is based on the processing of the 19F MAS NMR spectra of various polymeric formulations by multivariate analysis (singular value decomposition algorithm). It was found out that molecular arrangement of APIs in the nanosized domains dispersed in polymer matrices is affected by the interactions with neighboring macromolecules and new non-crystalline forms of the API are created. It is demonstrated, that 19F MAS NMR spectra reflect the changes in the extent of interactions with polymer matrix, and by using factor analysis the different non-crystalline modifications of the API in solid polymeric dispersions can be distinguished and identified. On the other hand, extreme attention must be paid to correctly assess the impact of electrostatic forces or differences in magnetic susceptibility of the polymer matrix on the pattern of 19F MAS NMR spectra of the API. Dramatic changes in 19F MAS NMR spectra of well-defined forms of the API induced by different filler compounds were observed. The factor analysis of the recorded spectra, however, can eliminate and separate these effects. Consequently the subtle structural differences in the molecular arrangement of the API in the nanosized domains dispersed in polymer matrices can be traced. The proposed strategy thus provides a powerful tool for the analysis of new formulations of fluorinated pharmaceutical substances in polymer matrices.

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Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy
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Manuscript: "Characterization of Solid Polymer Dispersions of Active Pharmaceutical Ingredients by ^{19}F MAS NMR and Factor Analysis"

Authors: Martina Urbanova, Jiri Brus, Ivana Sedenkova, Olivia Policianova, Libor Kobera

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Dear Prof. Alexander A. Kamnev,
please enclosed find our manuscript titled

"**Characterization of Solid Polymer Dispersions of Active Pharmaceutical Ingredients by ^{19}F MAS NMR and Factor Analysis**"

by ***Martina Urbanova, Jiri Brus, Ivana Sedenkova, Olivia Policianova, Libor Kobera***

which we wish to submit to the journal ***Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.*** The manuscript is intended for the publication as a short communication for **CSI XXXVII Special Issue**.

Current pharmaceutical research encounters with the problem of low solubility of many active ingredients (APIs). To overcome this limitation the poorly water-soluble drugs are formulated as solid dispersions in matrices of hydrophilic polymers. In these new dosage formulations, besides the notoriously discussed polymorphism and a bit mysterious pseudopolyamorphism, precise structural characterization of APIs is considerably complicated by the variable extent of interactions of the active substances with the macromolecules of excipients. In the manuscript we present a time-saving method for identification of various forms of pharmaceutical substances in solid polymer dispersions. The proposed method is based on the analysis of ^{19}F MAS NMR spectra. Nowadays approximately 20-25% of drugs in the pharmaceutical pipeline contain at least one fluorine atom. Moreover, there is no danger of ^{19}F NMR signals to be overlapped by the signals of common excipients. That is why we believe the proposed strategy could find wide application. Furthermore, we are convinced that this approach can be extended to the analysis of NMR spectra of other high-sensitive nuclei like ^{31}P, ^{23}Na, ^{11}B etc. While absent in filler compounds these nuclei are common components of many API.

In short, our manuscript contains five central messages:

a) Molecular arrangement of APIs in nanosized domains dispersed in polymer matrices is affected by the interactions with neighboring macromolecules. Polymer matrix can

induce rearrangement of the molecules of the API in these nanosized domains, and new non-crystalline forms of the API can be created.

b) The resulting changes in the molecular structure of APIs in these domains are reflected by the specific spectral features detected in the corresponding ^{19}F MAS NMR spectra.
c) As these spectral features are relatively weak and the ^{19}F MAS NMR spectra of the resulting non-crystalline forms of the API are poorly resolved the analysis of molecular rearrangement of the API requires sophisticated processing based on factor analysis.
d) It is demonstrated that the applied factor analysis possesses the same ability to distinguish various modifications of the API in solid polymeric dispersions as it has for pure APIs without filler compounds.
e) Additionally, factor analysis has the ability to eliminate effects of electrostatic forces or differences in magnetic susceptibility of the polymer matrix that can induce changes in the pattern of ^{19}F MAS NMR spectra.

We believe that the concepts behind these experiments are relevant to all chemists who work with semicrystalline, amorphous-like and disordered organic solids, and use, or consider the use of solid-state NMR for their characterization. We also believe that our work will be of interest to a broad readership, as the principles introduced in our work provide the basis for the design of a whole new class of NMR experiments for solid materials.

Moreover, the work has, in part, already been presented at the ***CSI XXXVII* 2011** conference in Brazil. On this occasion, it has attracted considerable interest among researchers, in particular with respect to future applications of solid-state ^{19}F MAS NMR experiments and comparative factor analysis. Since then, many colleagues of mine keep asking me for a paper about our novel concept.

Therefore, we kindly ask you to consider publication of our work as a communication in the journal ***Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, manuscript is intended for the CSI XXXVII Special Issue***.

Yours sincerely,

Martina Urbanova

For our manuscript, the following researchers could potentially act as reviewers:

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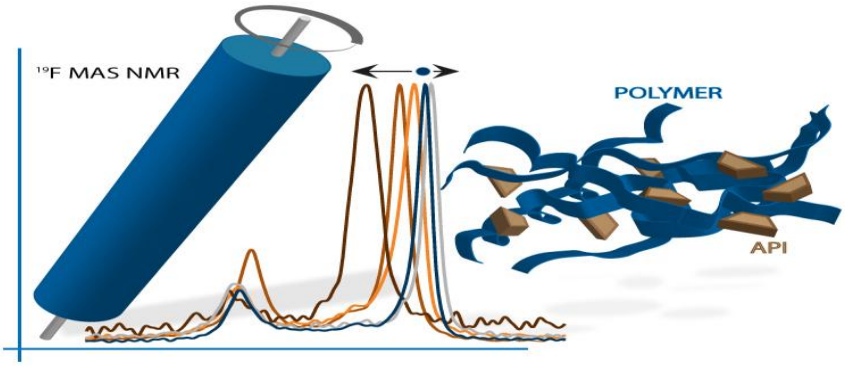
Graphical Abstract



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***Highlights**

- New non-crystalline forms of APIs are created in solid dispersions.
- Structural changes of APIs are detected in ^{19}F MAS NMR spectra.
- ^{19}F MAS NMR spectra reflect the extent of interactions with polymer matrix
- Factor analysis can distinguish different forms of the API.
- Factor analysis eliminates susceptibility effects.

Characterization of Solid Polymer Dispersions of Active Pharmaceutical Ingredients by ^{19}F MAS NMR and Factor Analysis

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ABSTRACT: Current pharmaceutical research encounters with the problem of low solubility of many active ingredients (APIs). To overcome this limitation the poorly water-soluble drugs are formulated as solid dispersions in matrices of hydrophilic polymers. In these new dosage formulations, besides the notoriously discussed polymorphism and a bit mysterious pseudopolyamorphism, precise structural characterization of APIs is complicated by their interactions with macromolecules of excipients. In this communication a time-saving method for identification of various forms of pharmaceutical substances in solid polymer dispersions is introduced. The method is demonstrated on a moderately-sized active

pharmaceutical ingredient (Atorvastatin, $C_{33}H_{35}FN_2O_5$) exhibiting extensive polymorphism and pseudopolyamorphism. The API was incorporated in the matrix of polvinylpyrrolidone (PVP) forming uniformly dispersed nanosized domains. Size of domains, approximately tens of nanometers, was estimated by measuring $T_1(^1H)$ and $T_{1\rho}(^1H)$ relaxation times. The proposed strategy of structural characterization of the API in PVP is based on the processing of the ^{19}F MAS NMR spectra of various polymeric formulations by multivariate analysis (singular value decomposition algorithm). It was found out that molecular arrangement of APIs in the nanosized domains dispersed in polymer matrices is affected by the interactions with neighboring macromolecules and new non-crystalline forms of the API are created. It is demonstrated, that ^{19}F MAS NMR spectra reflect the changes in the extent of interactions with polymer matrix, and by using factor analysis the different non-crystalline modifications of the API in solid polymeric dispersions can be distinguished and identified. On the other hand, extreme attention must be paid to correctly assess the impact of electrostatic forces or differences in magnetic susceptibility of the polymer matrix on the pattern of ^{19}F MAS NMR spectra of the API. Dramatic changes in ^{19}F MAS NMR spectra of well-defined forms of the API induced by different filler compounds were observed. The factor analysis of the recorded spectra, however, can eliminate and separate these effects. Consequently the subtle structural differences in the molecular arrangement of the API in the nanosized domains dispersed in polymer matrices can be traced. The proposed strategy thus provides a powerful tool for the analysis of new formulations of fluorinated pharmaceutical substances in polymer matrices.

KEYWORDS: solid-state NMR, factor analysis, ^{19}F MAS NMR, API, solid dispersions, magnetic susceptibility.

1. Introduction

No matter what reasons are, either searching for structure-property relationships in material science or production of drugs of consistent quality in pharmaceutical industry, the possibility of solid state

existing in different modifications with unique properties still requires development of new methods for their characterization.[1]

In pharmaceutical science, amorphous and semicrystalline forms of active pharmaceutical ingredients (APIs) attract significant attention due to their enhanced dissolution rates compared with commonly used crystalline modifications. Unfortunately, these disordered solids exhibit low thermodynamic stability. This fact can result in polymorphic changes that can affect physicochemical properties[2] of the produced APIs or can lead to complicated patent litigations. Generally thus the enhancement of oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development.[3,4] The problem is further multiplied by the fact that, depending on the preparation technique, different amorphous (non-crystalline) forms of pharmaceutical solids can exist.[5-8] Moreover, in many cases the differently prepared amorphous forms of the API exhibit significantly different physicochemical properties. A typical example of such behavior is amorphous simvastatin that as prepared by cryo-milling rapidly crystallizes, whereas the quench-cooled samples show no sign of phase transformation.[7] Similarly the amorphous indomethacin prepared by melt quenching shows a significant dissolution rate enhancement over the crystalline γ-form, while cryoground amorphous indomethacin undergoes rapid back crystallization to stable γ-form.[8] Significant differences in physico-chemical behavior were observed also for atorvastatin that is still under extensive consideration.[9] Quite recently it has been reported that the intrinsic dissolution rates of different amorphous forms of this API considerably differ form 0.183 to 0.252 $mg.min^{-1}.cm^{-2}$.[10] Therefore the experimental approaches of exact structural characterization of these amorphous pharmaceutical solids are still a subject of enormous scientific effort.

Recent development of solid dispersions of APIs as a practically viable method to enhance bioavailability of the poorly water-soluble drugs overcame many limitations associated with salt formation, solubilization by cosolvents, micronization and/or mechanical amorphization. The term "solid dispersion" refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or

amorphous, and among other compounds various synthetic polymers such as polyvinylpyrrolidone (PVP) or polyethyleneglycol (PEG) are frequently used. The drug can be dispersed in the polymer matrix on molecular level, can form nanosized amorphous or crystalline particles or clusters, and/or can form relatively large domains. These domains can be again either crystalline or amorphous. In some cases if there are specific interactions between the molecules of APIs and polymer matrix the highly ordered composites or complexes exhibiting long-range periodic arrangements can be formed.[11] Consequently, despite the recent advances in structural analysis, the characterization of these multicomponent systems and precise recognition of structural state of the API continues to be a monumental challenge.

X-ray powder diffraction (XRPD), ^{13}C cross/polarization (CP) magic-angle spinning (MAS) NMR and vibration spectroscopy are traditional tools to recognize different solid forms of APIs.[12-15] In addition, the combinations of Raman or infrared spectroscopy with multivariate analysis have been successfully used to probe subtle variations of semicrystalline solids.[5] However, in solid dispersions where concentrations of API are very low, and the strong signals of polymer compounds (excipients) dominate we are balancing on physical limits of these experimental approaches. Therefore characterization of the structural state of APIs and their unambiguous identification in solid polymer dispersions is a priority that still has remained a challenge.

In this context ^{19}F MAS NMR spectroscopy is a promising technique the special strategy of its application is followed herein. Due to a high gyromagnetic ratio and 100% isotopic abundance the isotope ^{19}F gives solid-state NMR spectra within extremely short time even for diluted systems. Fluorine atom is also relatively frequent component of many pharmaceutically active molecules, and there is no danger of ^{19}F NMR signals to be overlapped by the signals of component of polymer matrices. Moreover, previously it has been demonstrated by us that ^{19}F MAS NMR spectroscopy combined with factor analysis offers the possibility to unambiguously identify various crystalline and disordered (non/crystalline and amorphous) forms of fluorine-containing APIs in pure formulations.[16] Extensive testing also confirmed that ^{19}F MAS NMR spectra reflect changes in amorphous phase of fluorinated

compounds in similar extent as provided by the more informative techniques like ^{13}C CP/MAS NMR, FTIR and XRPD.

On the other hand, the characterization of APIs in solid polymer dispersions brings new problems that have to be thoroughly discussed. At first, in these new dosage formulations, besides the well-known polymorphism and a bit mysterious "pseudopolyamorphism", the precise structural characterization of APIs is complicated by their interactions with the macromolecules of excipients. Second, considerable attention must be paid to assess the impact of electrostatic forces or differences in magnetic susceptibility of polymer matrices on ^{19}F MAS NMR spectra. Bear in mind that ^{19}F is highly receptive nucleus. Generally, in tablet formulations the unpredictable electrostatic potentials (charging of the particles of APIs) or changes in magnetic susceptibility produced by filler compounds can be so strong that dramatic changes in the pattern of ^{19}F MAS NMR spectra can be induced. In this communication we discuss all the above mentioned issues with respect to reliability of characterization of APIs in solid polymeric dispersions using ^{19}F MAS NMR spectroscopy. In our best knowledge this is the first attempt to apply ^{19}F MAS NMR spectroscopy combined with multivariate analysis to characterize active pharmaceutical ingredients formulated as solid polymer dispersions.

2. Experimental

2.1. Materials. As a model active compound atorvastatin hemicalcium [$(C_{33}H_{35}FN_2O_5^-)_2Ca^{2+}$ amorphous] produced by Biocon Laboratories, Bangalore, India; was used as received. As a model polymeric excipient polyvinylpyrrolidone K 90; M_w=360000 produced by Sigma Aldrich was used.

2.2. Methods: Sample Preparation. Different forms of pure API was prepared according to patent literature by recrystallization under various conditions from different solvents (acetone, acetonitrile, water, ethanol, methanol, dimethylforamide, *n*-heptane).[16] Recrystallization was performed from solutions with completely dissolved atorvastatin or from saturated solutions with applied mechanical grinding.

Solid dispersions were prepared by free evaporation or lyophilization of the prepared solution of atorvastatin with PVP. These systems were prepared by mixing of solution of atorvastatin and solution of PVP. Solvents of API and polymer were nontoxic and biodegradable such as water, ethanol and *t*-butanol. In all cases the traces of organic solvents were removed from the resulting products by vacuum evaporation. The absence of solvent was checked by ^{13}C and ^{1}H MAS NMR spectroscopy. Total composition of the prepared solid dispersions was always as following: 85 wt% PVP and 15 wt% API.

2.3. Methods: Solid-state NMR. ^{19}F MAS NMR spectra were measured using a Bruker Avance 500 WB/US NMR spectrometer in 2.5-mm double-resonance probehead. A rotor synchronized Hahn-echo pulse sequence was used to measure ^{19}F MAS NMR spectra at MAS frequency of 31 kHz. The length of 90° (^{19}F) pulse was 2.5 μs. The ^{19}F NMR scale was calibrated with PTFE (-122 ppm).

The ^{13}C-detected $T_1(^1H)$ a $T_{1\rho}(^1H)$ relaxation experiments were used to determine homogeneity and the extent of dispersion of the API in polymer matrix. The range of size of domains that could be probed by these relaxation experiments is ca. 1-100 nm. The experimental scheme with a variable spin-lock time in the range 0.1–10 ms after the proton signal excitation followed by constant contact time was used in $T_{1\rho}(^1H)$ measurements; the proton spin-locking field in frequency units was 80 kHz. $T_1(^1H)$ values were measured using the combination of cross-polarization and saturation recovery pulse sequence. Details of the applied experiments can be found in the recently published papers. [17,18]

2.4. Methods: Optical microscopy. The research-grade Leica DM LM microscope with an objective magnification ×50 was used to probe homogeneity of the sample in the range of size of domains > 1 μm.

2.5. Methods: Factor analysis (FA). Factor analysis using the singular value decomposition (SVD) algorithm was performed to extract information from the experimental data obtained by ^{19}FMAS NMR and to visualize differences between different predominantly amorphous forms of atorvastatin. Processing of spectral data was performed in Matlab program package. The following spectral range was subjected to the factor analysis: ^{19}F NMR: from -80 to -140 ppm. All spectra were base-line corrected and normalized. Preparation and processing of a moderately sized data set containing ca. 40 spectra took about 10 min.

3. Result and discussion

3.1. Homogeneity of the systems

Before the analysis of molecular structure of the API we characterized homogeneity of the prepared solid polymer dispersions, i.e. size of domains of the API in polymer matrix was estimated. At first we used optical microscopy, and the obtained quite featureless micrographs (not shown here) indicated that the prepared solid dispersion were homogeneous in the micrometer scale. To probe homogeneity of the prepared systems in the nanometer scale we measured ^{13}C-detected $T_1(^1H)$ and $T_{1\rho}(^1H)$ spin-lattice relaxation times. Previously, on a wide range of two-component and multi-component polymer systems, it has been demonstrated that differences in ^{1}H relaxation times between individual components usually indicate heterogeneous character of the systems. This rule follows from the fact that ^{1}H-^{1}H spin diffusion, that is generally very fast in organic solids, is not able to equilibrate magnetic properties of all ^{1}H atoms. Typically ^{1}H magnetization is transferred over a distance of about 1.1-1.2 nm during 1 ms [19]. In the case of measurements of $T_1(^1H)$ spin-lattice relaxation times the relevant times of ^{1}H spin diffusion are in the range of several seconds. Consequently ^{1}H magnetization can be effectively transferred over ca. 100-200 nm. Therefore if the $T_1(^1H)$ spin-lattice relaxation times of both components are different the two-component system is heterogeneous with the size of domains larger than ca. 100-200 nm. A similar approach applies also to the measurements of $T_{1\rho}(^1H)$ spin-lattice relaxation times. In this case, however, the ^{1}H-^{1}H spin diffusion times are in the range of milliseconds. Therefore the ^{1}H magnetization can be effectively transferred over several tens of nanometers. A multi-component system with the uniform $T_{1\rho}(^1H)$ relaxation time thus can be considered to be homogenous with the size of domains less then several nanometers. Details of the applied procedure can be found in recently published papers [17,18].

Table 1. $T_1(^1H)$ and $T_{1\rho}(^1H)$ spin-lattice relaxation times obtained for pure API, PVP and selected solid dispersions representing typical preparation procedures.

System	solvent	procedure	$T_1(^1H)$, s API	$T_{1\rho}(^1H)$, ms API	$T_1(^1H)$, s PVP	$T_{1\rho}(^1H)$, ms PVP
Pure API	---	---	1.36	5.8	*NA*	*NA*
Pure PVP	---	---	*NA*	*NA*	2.5	28.2
API-PVP (1)	water	free evaporation	1.38	13.5	1.40	22.4
API-PVP (2)	ethanol	free evaporation	1.42	15.7	1.37	23.2
API-PVP (3)	tert-butanol	free evaporation	1.48	14.3	1.31	25.1
API-PVP (5)	water	lyophilization	1.28	17.5	1.46	20.1
API-PVP (6)	ethanol	lyophilization	1.32	18.9	1.35	22.4
API-PVP (7)	tert-butanol	lyophilization	1.25	15.3	1.21	23.6

Table 1 summarizes $T_1(^1H)$ and $T_{1\rho}(^1H)$ spin-lattice relaxation times obtained for pure API, PVP and selected solid dispersions representing typical preparation procedures. From the obtained data it is clear that $T_1(^1H)$ spin-lattice relaxation times are equilibrated indicating that all the prepared systems are homogenous in the scale of hundreds nanometers. On the other hand, the differences in $T_{1\rho}(^1H)$ relaxation times reflect the existence of domains of the AIP the size of which is ranging between ca. 1-10 nm. Precise measurement of domain size is under the current investigation. As both components are not intimately mixed on molecular level the molecules of the API in the domains can preserve original molecular arrangements typical for the pure state (amorphous or crystalline). On the other hand new molecular packing in these domains induced by the interaction with PVP macromolecules can be also expected.

3.2. ^{19}F MAS NMR spectroscopy

Subsequently ^{19}F MAS NMR spectra were measured for a range of the prepared solid polymer dispersions and various modifications of pure API including many amorphous forms and three crystal modifications (I, V, and X; identified previously according to XRPD and patent literature [16]). In the recorded ^{19}F MAS NMR spectra the highly-ordered crystalline forms exhibit clear differences from each other as well as from the amorphous ones (Fig. 1). Quite distinct is the broadening of ^{19}F MAS NMR signals of pure amorphous modifications of the API and the shift toward the high frequency region. The obtained experimental data also reflect slight structural variations of the amorphous products. This is indicated by the presence of high-frequency shoulders (left-handed humps).

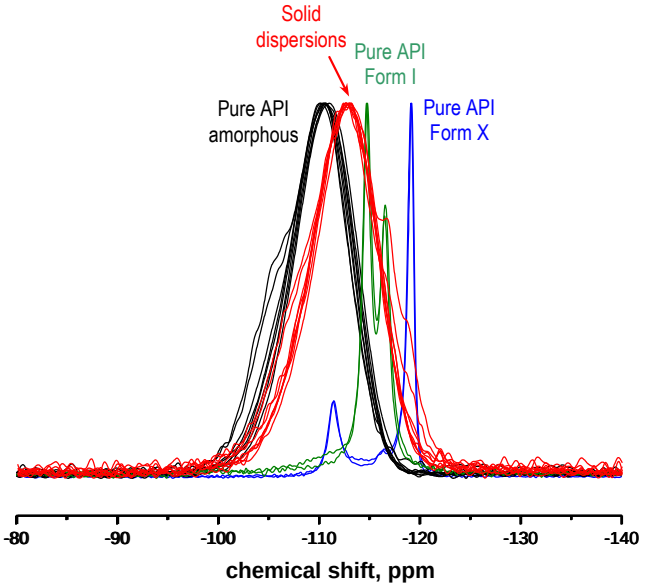


Figure 1. Overlay of ^{19}F MAS NMR spectra of selected formulations of atorvastatin ($C_{33}H_{35}FN_2O_5$). The black lines represent spectra of semicrystalline and amorphous forms the API in pure state; the red lines represent spectra of solid dispersions of the API; the green lines correspond with the spectra of pure crystalline Form I; and the blue lines spectra of pure crystalline Form X of the API.

In contrast, the ^{19}F MAS NMR signals of solid polymer dispersions are systematically shifted back toward the low-frequency region and broadened on both left- and right-handed sides. As demonstrated

in our previous research the formation of high-frequency shoulders can be attributed to the presence of certain motifs with medium-range molecular order and/or nano-sized nearly crystalline molecular fragments/domains [16]. The low-frequency shoulders that are observed only in the spectra of the solid dispersions can indicate formation of new structural fragments the arrangement of which is induced by the polymer matrix. An alternative explanation can operate with the presence of traces of the most frequent crystalline forms I and/or X because the resonance frequency of shoulders is very close to their typical ^{19}F MAS NMR resonance frequencies. However, the presence of these crystalline fractions was not confirmed by ^{13}C CP/MAS NMR spectra (Figure 2). No clear match was found.

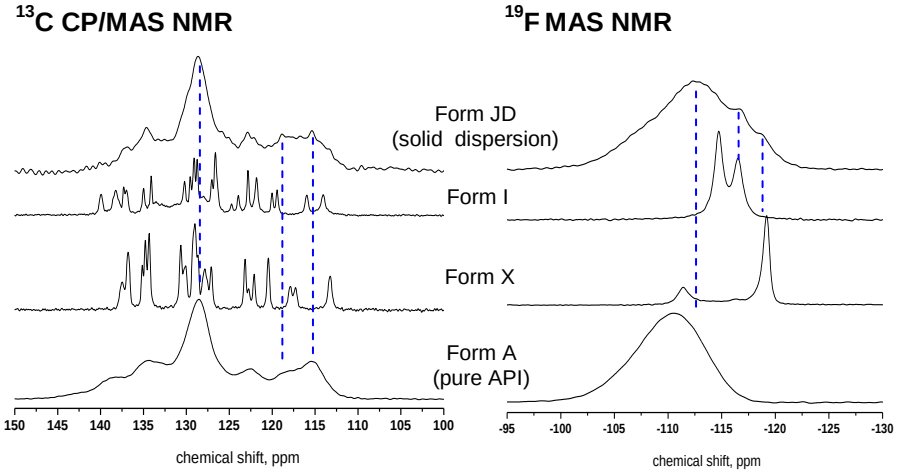
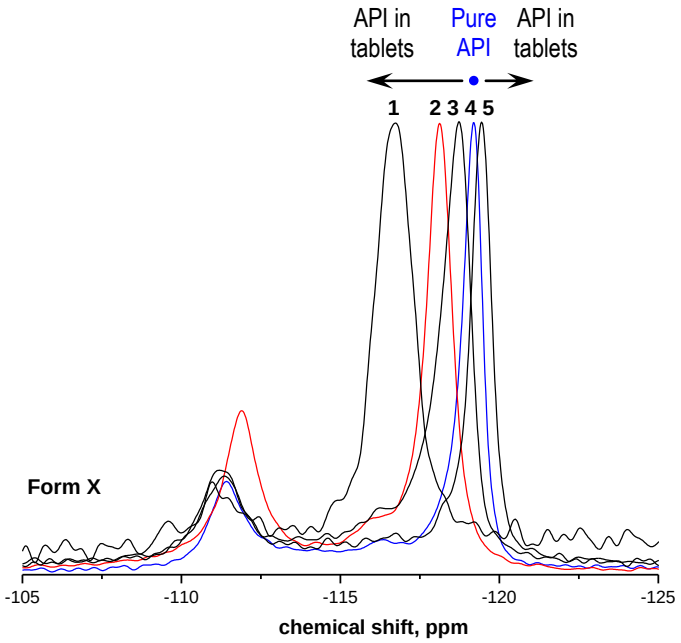


Figure 2. ^{13}C CP/MAS and ^{19}F MAS NMR spectra of typical solid polymer dispersion of the API exhibiting considerable low-frequency shoulder, pure Form I, pure Form X, and amorphous Form A.

On the other hand, the observed systematic low-frequency shift of ^{19}F MAS NMR signals of solid dispersions is a bit surprising and its thorough interpretation requires extensive experimentation that is beyond the scope of this contribution. According to our preliminary test this phenomenon can be explained either by the charging of API particles or by the change in the isotropic bulk magnetic susceptibility of the sample. Polymer segments closely associated with the API induce a different local field and contribute to an off-resonance term of the free induction decay. Consequently the NMR signals can be shifted from the expected regions. Particularly, as ^{19}F is a highly receptive nucleus, significant changes in the patterns of ^{19}F MAS NMR spectra can be expected.



230

231 **Figure 3.** ^{19}F MAS NMR spectra of crystalline Form X of atorvastatin ($C_{33}H_{35}FN_2O_5$) with different
232 excipients: 90 wt. % of dry corn starch 1); 90 wt. % of cellulose 2); 90 wt. % of wet corn starch 3); pure
233 Form X 4); 90 wt. % of sucrose 5).

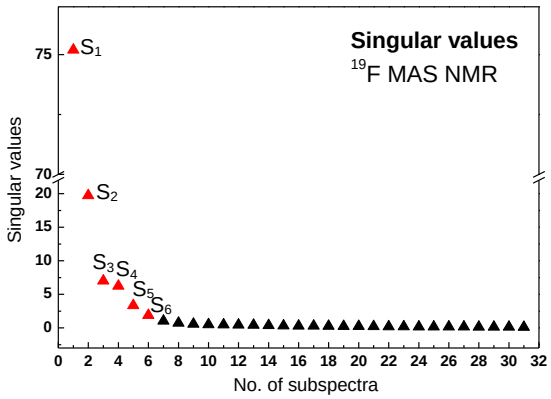
234

235 In order to probe the observed phenomenon in detail we prepared a set of physical mixtures of the
236 Form X of atorvastatin and various excipients. We used wet corn starch, dry corn starch, cellulose, and
237 sucrose. Total composition of the prepared physical mixtures was 90 wt% of excipient and 10 wt% of
238 the API. Figure 3 then demonstrates significant changes in the position and splitting of asymmetric
239 doublets that are the typical feature of the Form X of atorvastatin. As no significant changes in ^{13}C
240 CP/MAS NMR spectra and XRPD patterns were detected no phase transitions occurred during the
241 mixing of the API and excipients. Crystal Form X is still present in the prepared samples. This indicates
242 that the observed changes in ^{19}F MAS NMR spectra (Figure 3) reflect some kind of physical interaction
243 between the particles of API and filler compounds. The observed changes in 19F MAS NMR spectra are
244 rather complex, and their extent depends on the type of excipients. While the macromolecular excipients
245 such as dry corn starch or carboxymethylcellulose induce high-frequency shift and the decrease in
246 splitting of the doublet, relatively low-molecular weight sucrose causes the low-frequency shift and the

247 increase in the doublet splitting (Figure 3). In this case, considering that the dry corn starch has much
248 stronger effect than wet corn starch, we suggest the effect of electrostatic forces or charging of API
249 particles. The dielectric properties of the sample can be significantly changed so the resonance
250 frequency can be slightly shifted. In the case of polymer dispersions prepared from neutral solutions the
251 charging of API particles, however, cannot be expected. Rather we suppose that the observed systematic
252 shift of ^{19}F MAS NMR signals of solid dispersions can be attributed to the susceptibility effect. This
253 finding indicates that the observed susceptibility effects can additionally complicate reliable analysis of
254 ^{19}F MAS NMR spectra. Bear in mind that differences between the amorphous modifications of the API
255 are very subtle even in pure state and visual comparison of these spectra is not enough. As more than 50
256 almost-amorphous modifications of atorvastatin have been described the correct interpretation of the
257 observed spectral variation requires a special statistical processing such as factor analysis.
258
259 **3.3. Factor analysis**
260 In general, factor analysis (FA) provides a versatile tool to explore complex changes in large sets of
261 experimental data. Specifically, the experimental spectra Y_i are converted into the set of orthonormal
262 subspectra S_j (Eq. 1) using Singular Value Decomposition (SVD) algorithm.

263 $$Y_i = \sum_{j=1}^{n} w_j V_{ij} S_j \qquad (1)$$

264 The calculated subspectra S_j are linear combinations of the experimental data and *vice versa* the
265 experimental data can be given as the linear combination of the subspectra. Each subspectrum S_j
266 represents a specific spectral feature that is typical for a given type of analyzed samples. The statistical
267 importance and hence the order of each subspectrum S_j is expressed by the corresponding singular value,
268 w_j. The ability of a particular subspectrum S_j to describe the experimental spectrum Y_i is then expressed
269 by the normalized coefficient V_{ij}. Consequently the coefficients V_{ij} (i.e. scores) represent quantitative
270 parameters reflecting spectral differences between the analyzed samples. In this way any modification of
271 a particular API can be unambiguously identified *via* the set of V_{ij} coefficients.



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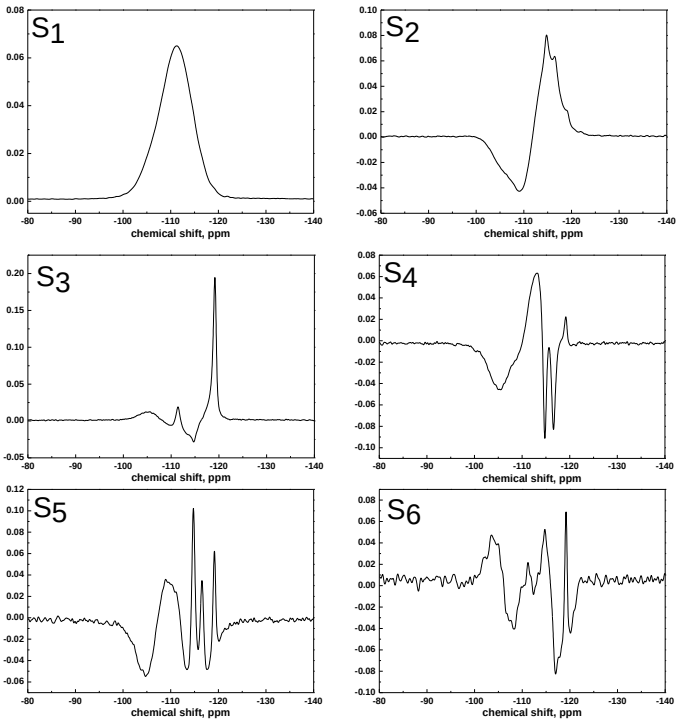
273 **Figure 4.** Singular values w_j calculated from the set of ^{19}F MAS NMR spectra. The highest singular
274 values (red triangles) indicate the most significant subspectra (S_1-S_6).

275

276 In our particular case singular parameters w_1 to w_6 reach reasonable values (red triangles in Figure 4).
277 This indicates that the corresponding subspectra S_1 to S_6 (Figure 5) entirely describe the analyzed set of
278 samples. The first-rank subspectrum S_1 corresponds to the superposition of signals dominating the set of
279 ^{19}F MAS NMR spectra and resembles the typical signal of amorphous forms of atorvastatin. The
280 second-order subspectrum S_2 then demonstrates the most significant spectral deviations found in the
281 analyzed data set. In our case this is the difference in resonance frequency of ^{19}F MAS NMR signals in
282 pure forms of atorvastatin and its solid dispersions. Characteristic markers of crystalline forms I and X
283 are displayed in the third- and fourth-rank subspectra S_3 and S_4. The fourth-rank subspectrum also partly
284 reflects certain changes in ^{19}F MAS NMR chemical shifts between the pure APIs and APIs in polymer
285 solid dispersions. The spectral differences between amorphous modifications are particularly highlighted
286 in the fifth-rank and sixth-rank subspectra S_5 and S_6. In total, the above-mentioned subspectra S_1-S_6
287 explain about 95 % of the spectral variation observed in the set of the recorded ^{19}F MAS NMR spectra.



288

289 **Figure 5.** Subspectra $S_1 - S_6$ calculated from the set of ^{19}F MAS NMR spectra of various modifications
290 of the API and API in solid dispersions.

291

292 To explicitly quantify this spectral variation the normalized coefficients V_{ij} can be used. In addition, as
293 these factors are attributed to each sample, these coefficients unambiguously identify any modification
294 of the API. As follows from the above-mentioned interpretations of S_j subspectra, primary identification
295 of individual samples can be derived form the correlation plot of V_{i1} and V_{i2} coefficients in which four
296 well-separated clusters are clearly apparent: 1) crystalline Form I; 2) crystalline Form X; 3)
297 noncrystalline forms of the API in pure state; and 4) solid dispersions of noncrystalline forms of the API
298 (Figure 6). This finding also indicates that the effects of variable susceptibility of the analyzed samples
299 are entirely described by the coefficient V_{i2}. In other words this means that with high probability there
300 are other coefficients clearly describing structural differences between different noncrystalline forms of
301 the API regardless it is in pure state or in solid dispersion. For instance, structural differences between
302 the crystalline forms of atorvastatin can be explicitly expressed by the factors V_{i3}, while the subtle
303 differences between the non-crystalline (amorphous) forms in pure state as well as in solid dispersions

are preferably described by V_{i5}. In a graphical representation (one of the most suitable representation seems to be 2D correlation plot of V_{i1} and V_{i5} coefficients, Fig. 6; or 3D correlation plot of V_{i1}, V_{i3} and V_{i5} coefficients, Figure 7) the crystalline Forms I and X are clearly separated in horizontal dimension, while the amorphous forms are vertically sorted. The observed systematic array of V_{i5} coefficients clearly reflects differences between the amorphous non-crystalline forms of atorvastatin. As the array of V_{i5} coefficients is a continuous function without any abrupt change (jump), the structurally insignificant susceptibility effects are removed out. Coefficients V_{i5} as well as V_{i6} (the corresponding correlation plots are not shown here) thus seem to be independent on global changes in susceptibility of the analyzed systems. Moreover, the considerably wide range of V_{i5} values covering the interval form -0.3 to 0.3 confirms the high structural receptivity of this parameter.

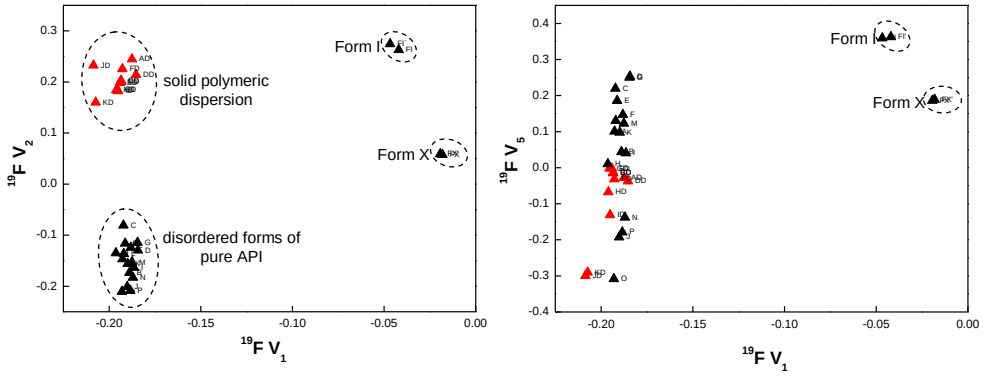


Figure 6. Correlation plots of V_{i1} and V_{i2} coefficients (left), and V_{i1} and V_{i5} coefficients (right). The parameters reflecting solid dispersions of atorvastatin are marked as red triangles, while the factors calculated for pure forms of the API are marked as black triangles.

In general, the observed systematic variations of the scores V_{i5} obtained by multivariate analysis of ^{19}F MAS NMR spectra of non-crystalline products of the API can result from many reasons including presence of impurities (traces of traditional crystalline forms of the API), systematic changes in molecular conformation, variation in molecular short-range order, presence of "embryonic" nanocrystallites etc. In this context, in our previous research [16] we extensively examined the results of

^{19}F MAS NMR spectroscopy and compared them with other experimental methods such as ^{13}C CP/MAS NMR, FTIR and XRPD. Among others, we found out that asymmetric broadening of ^{19}F MAS NMR spectra (formation of the low intensive high-frequency shoulders) is accompanied by the sharpening of originally diffusive broad X-ray reflections. This indicated that structural changes occurring in the amorphous phase of the API is associated with molecular rearrangement leading to the formation of certain structural motifs with medium- or long-range periodic order.

In our current work the recorded ^{19}F MAS NMR spectra of both pure API and the prepared solid dispersions assorted in the descending order according to V_{i5} scores exhibit similar inhomogeneous broadening. In case of the prepared solid dispersions, however, besides the left-hand shoulders, the right-hand ones appear as well (Figure 7). In analogy with our previous findings we assign this broadening to a systematic molecular rearrangement and formation of partially-ordered nanosized domains. The low-frequency shoulders indicate new molecular assemblies the formation of which is probably induced by the matrix of PVP. Detail structural investigation of these structures is currently under investigation.

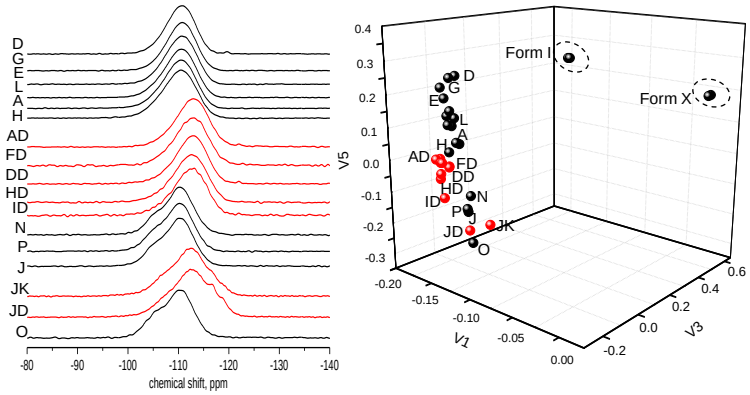


Figure 7. 3D correlation plot of V_{i1}, V_{i3} and V_{i5} coefficients (left), and the corresponding ^{19}F MAS NMR spectra of non-crystalline forms of the API (right). The ^{19}F MAS NMR spectra are sorted in ascending order by V_{i5} coefficients. The spectra corresponding to pure API are in black, the spectra reflecting solid dispersions are in red.

In general there are several explanations of structural variability of amorphous phase of organic compounds.[20,21] One of them operates with the existence of rigid amorphous fraction (RAF) that is believed to be an intermediate between the crystalline and amorphous phase.[22,23] Another concept introduces „crystalline mezophase“, which combines properties of a crystalline phase (long-range order) with properties of an amorphous phase (e.g. glass transition). Alternatively, in some cases the term “pseudopolyamorphism” is used to describe this phenomenon although true polyamorphs exhibiting a first-order transition between them have never been seen in any organic substance.[24] Moreover, the amorphous phase can be contaminated by various nanosized crystal nuclei and traces of crystalline domains that can differ in their number, size, shape, distribution etc. All the above-mentioned phenomena, however, have only slight impact on the overall mean molecular structure of the amorphous phase. For instance, the crucial processes like released molecular dynamics usually occur only at interfacial regions. Therefore the differences between the different amorphous forms of a given compound can be hardly recognized using conventional physical and spectroscopic techniques. Over all these problems ^{19}F MAS NMR spectroscopy combined with factor analysis has sufficient potentiality to identify different amorphous (non-crystalline) forms of the API in solid polymer dispersion with a high degree of reliability. Although ^{19}F MAS NMR spectroscopy cannot provide complete and detail description of structural changes occurring in amorphous phase of the API formulated in solid polymer dispersion the combination with factor analysis provides the way how to rapidly control quality of the produced products.

4. Conclusion

Owing to the success of fluorinated compounds in medicinal chemistry, it may be predicted that the number of fluorine containing drugs will continue to increase. Similarly we can expect growing interest in the formulation of APIs in solid dispersions for which traditional high-resolution spectral data can be hardly recorded because the amounts of the active compounds is very low usually less than 5%. In the

presented communication it is demonstrated that ^{19}F MAS NMR spectroscopy combined with factor analysis (SVD algorithm) offers a fast and reliable tool to distinguish various amorphous forms of the fluorine-containing API in solid polymer dispersions. Specifically, it is shown that the relatively poorly-resolved ^{19}F MAS NMR spectra can be used to detect subtle structural changes in molecular arrangement of nanosized domains of the API induced by the polymer matrix. On the other hand, ^{19}F MAS NMR spectra are rather sensitive on the global changes in susceptibility and/or charging of the analyzed samples. Therefore careful attention must be paid to the interpretation of changes in the spectral pattern. Fortunately, the applied factor analysis of the recorded spectra eliminates these effects. Ultimately, the proposed strategy thus provides a powerful tool for the fast analysis of new formulations of fluorinated pharmaceutical substances in polymer matrices.

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Graphical Abstract

Characterization of Solid Polymer Dispersions of Active Pharmaceutical Ingredients by 19F MAS NMR and Factor Analysis

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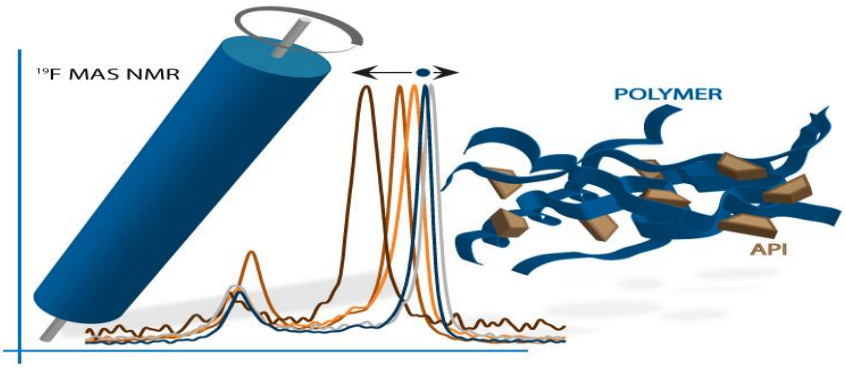

Factor analysis of ^{19}F MAS NMR spectra demonstrates fast and reliable method of characterization of amorphous modifications of solid pharmaceuticals in solid polymer dispersions.

Figure1

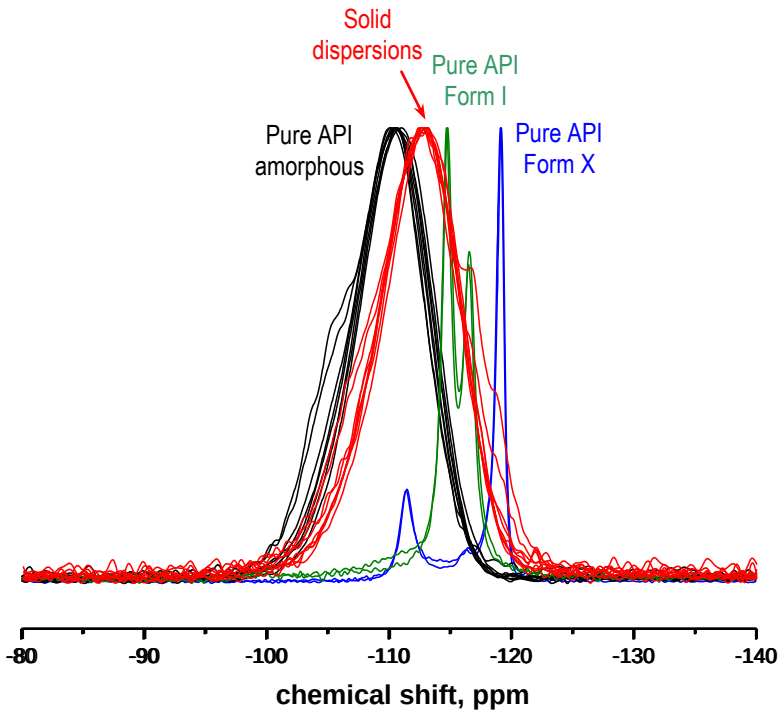
Solid
dispersions
Pure API
Form I
Pure API
amorphous
Pure API
Form X
-80
-90
-100
-110
-120
-130
-140
chemical shift, ppm

Figure2

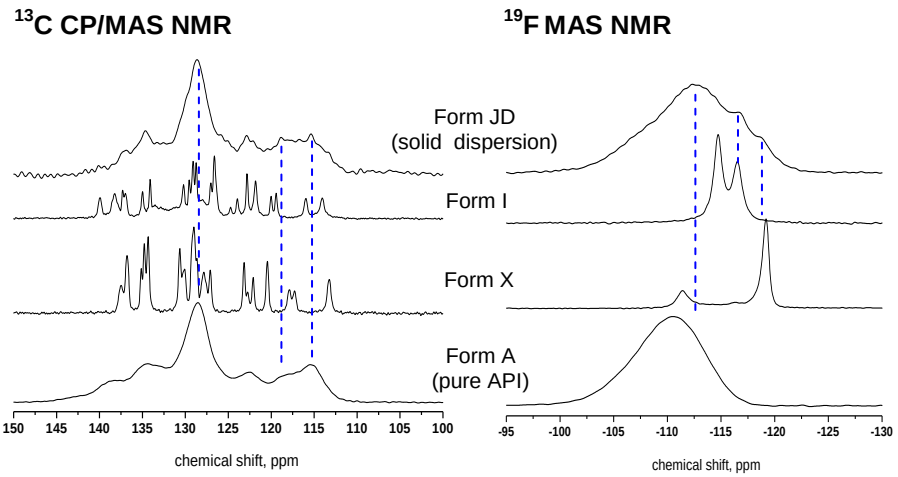
13C CP/MAS NMR
19F MAS NMR
Form JD
(solid dispersion)
Form I
Form X
Form A
(pure API)
150 145 140 135 130 125 120 115 110 105 100
chemical shift, ppm
-95 -100 -105 -110 -115 -120 -125 -130
chemical shift, ppm

Figure3

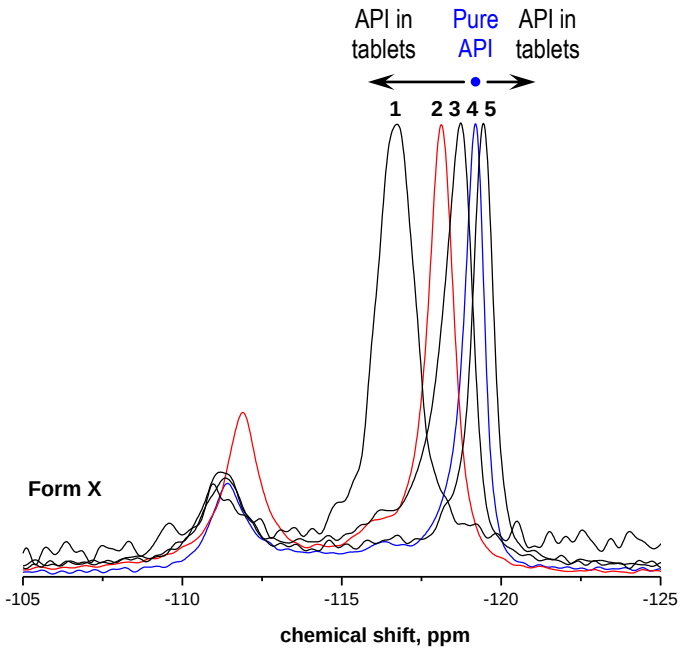
API in tablets
Pure API
API in tablets
1
2 3 4 5
Form X
-105
-110
-115
-120
-125
chemical shift, ppm

Figure4

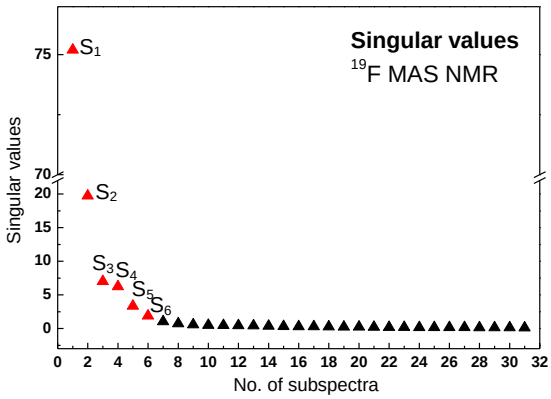
Singular values
^{19}F MAS NMR
S1
S2
S3
S4
S5
S6
Singular values
No. of subspectra
75
70
20
15
10
5
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32

Figure5

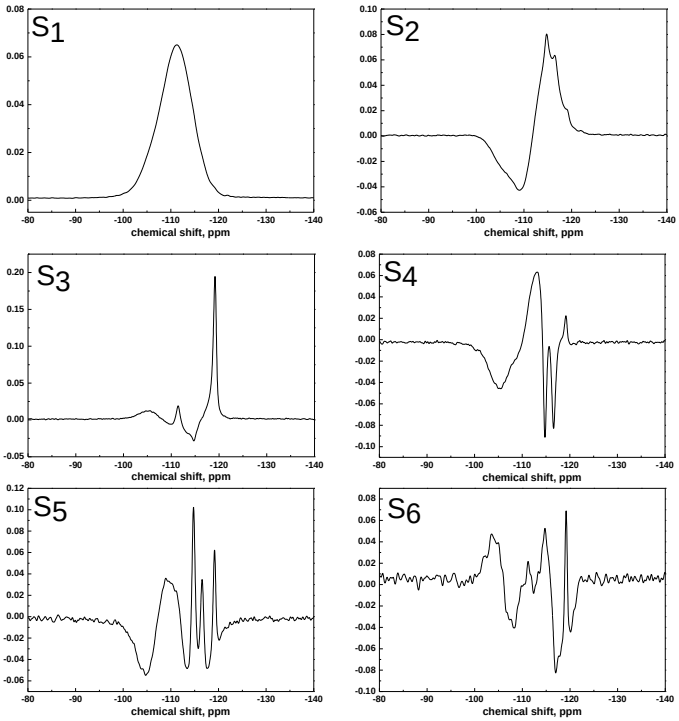

Figure6

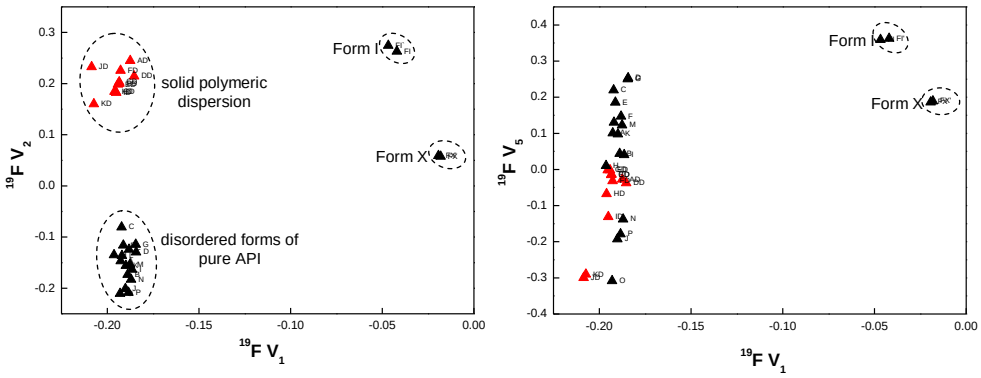
Form I
Form X
solid polymeric dispersion
disordered forms of pure API
$^{19}F\ V_2$
$^{19}F\ V_1$
$^{19}F\ V_5$
$^{19}F\ V_1$

Figure7

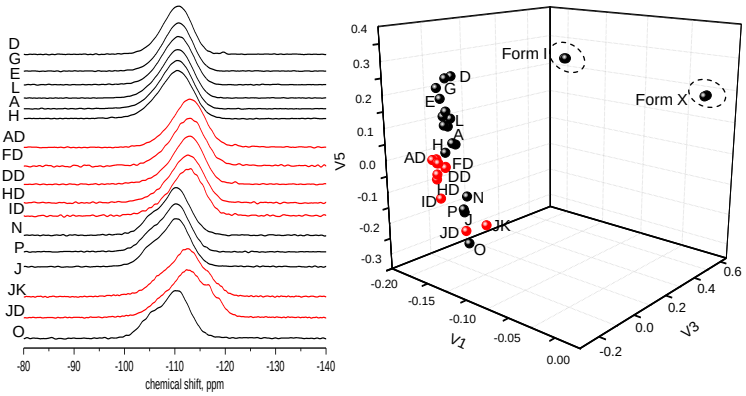
D
G
E
L
A
H
AD
FD
DD
HD
ID
N
P
J
JK
JD
O
-80
-90
-100
-110
-120
-130
-140
chemical shift, ppm
Form I
Form X
V5
V1
V3

Table1

System	solvent	procedure	$T_1(^1H)$, s API	$T_{1\rho}(^1H)$, ms API	$T_1(^1H)$, s PVP	$T_{1\rho}(^1H)$, ms PVP
Pure API	---	---	1.36	5.8	*NA*	*NA*
Pure PVP	---	---	*NA*	*NA*	2.5	28.2
API-PVP (1)	water	free evaporation	1.38	13.5	1.40	22.4
API-PVP (2)	ethanol	free evaporation	1.42	15.7	1.37	23.2
API-PVP (3)	tert-butanol	free evaporation	1.48	14.3	1.31	25.1
API-PVP (5)	water	lyophilization	1.28	17.5	1.46	20.1
API-PVP (6)	ethanol	lyophilization	1.32	18.9	1.35	22.4
API-PVP (7)	tert-butanol	lyophilization	1.25	15.3	1.21	23.6