International Journal of Pharmaceutics 409 (2011) 62–74

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

New perspectives of ^{19}F MAS NMR in the characterization of amorphous forms of atorvastatin in dosage formulations

Jiri Brus [a,*], Martina Urbanova [a], Ivana Sedenkova [a], Hana Brusova [b]

[a] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky Sq. 2, 162 06 Prague 6, Czech Republic
[b] Zentiva K.S., U Kabelovny 130, 102 37 Prague 10, Czech Republic

ARTICLE INFO

Article history:
Received 13 September 2010
Received in revised form 16 February 2011
Accepted 20 February 2011
Available online 26 February 2011

Keywords:
^{19}F MAS NMR
Factor analysis
Polymorphism
Amorphous solids
Atorvastatin
Pharmaceuticals

ABSTRACT

Despite recent advances in solid-state NMR spectroscopy, the structural characterization of amorphous active pharmaceutical ingredients (APIs) in solid dosage forms continues to be a monumental challenge. To circumvent complications following from low concentrations of APIs in tablet formulations, we propose a new time-saving procedure based on chemometric approach: factor analysis of ^{19}F MAS NMR spectra. Capability of the proposed method is demonstrated on atorvastatin – a typical representative of fluorinated pharmaceutical substances exhibiting extensive polymorphism. Applying the factor analysis on the recorded ^{19}F MAS NMR spectra, unique parameters for every sample were derived. In this way every solid form of atorvastatin was characterized and clearly distinguishable even among various amorphous and disordered forms. The proposed method was also found to be suitable for both qualitative and quantitative analysis of mixtures of various forms of atorvastatin. Reliability of the proposed method was extensively examined by comparing the obtained results with other experimental techniques such as ^{13}C CP/MAS NMR, FTIR and XRPD. As highly linear correlations between the sets of parameters obtained from different experimental data were found, the perspectives of the applied comparative factor analysis to obtain detail structural view on variability of amorphous forms of atorvastatin are also discussed. Although the reported method was tested on atorvastatin, authors expect wider application for any fluorinated compound to give the routine, fast and reliable characterization of amorphous forms of APIs in drug products even at low concentrations (1–5%). Bear in mind that 20–25% of currently developed pharmaceuticals contain at least one fluorine atom in the molecule.

1. Introduction

In pharmaceutical development, amorphous forms of active pharmaceutical ingredients (APIs) currently attract significant attention (Zakrzewski and Zakrzewski, 2006). Amorphous or low-ordered systems show in general higher solubility but less stability in comparison to crystalline, well-ordered systems. Big challenge for solid-state analysis is thus to detect any change of manufactured amorphous form of the API, because the undesired structural changes can affect quality (properties of drugs) and also can lead to complicated patent litigations (Bauer et al., 2001; Blagden et al., 2007; Graeser et al., 2008; Greco and Bogner, 2010).

In general there is a wide range of physical methods suitable for characterization of pharmaceutical solids (Zakrzewski and Zakrzewski, 2006). X-ray powder diffraction (XRPD), ^{13}C cross-polarization (CP) magic-angle spinning (MAS) NMR and vibration spectroscopy are traditional tools to characterize typical well-ordered polymorphs (Brus and Jegorov, 2004; Harris, 2006, 2007; Harris et al., 2007; Husak et al., 2008, 2010), but sophisticated analytical tool to describe subtle differences between different amorphous forms of a particular API is still missing. In particular, in tablet formulations where concentrations of the API are very low and the strong signals of filler compounds dominate, we are balancing on physical and detection limits of conventional analytical methods. In these cases special techniques must be used (Griffin et al., 2007). That is why the characterization of amorphous forms of APIs and their unambiguous identification in tablet formulations is a priority that still remains a challenge.

In this context we see the potential of multivariate analysis of experimental data as a possible way to bridge this gap (Graeser et al., 2008; Heinz et al., 2008, 2009), especially in a combination with ^{19}F MAS NMR spectroscopy (Aso et al., 2009; Harris, 2006). It has become evident that fluorinated compounds have a remarkable record in medicinal chemistry and will play an important role in providing lead compounds for therapeutic applications (Purser et al., 2008). Nowadays approximately 20–25% of drugs in the phar-

* Corresponding author. Tel.: +420 296 809 380; fax: +420 296 809 410.
E-mail addresses: brus@imc.cas.cz (J. Brus), urbanova@imc.cas.cz (M. Urbanova), sedenkova@imc.cas.cz (I. Sedenkova), hana.brusova@zentiva.cz (H. Brusova).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.02.030

maceutical pipeline contain at least one fluorine atom, and in 2007 nearly 50% of 20 best selling pharmaceutical products were based on fluorinated APIs. Consequently, the application of ^{19}F MAS NMR spectroscopy in pharmaceutical research receives growing importance. The increasing interest also follows from high gyromagnetic ratio and 100% isotopic abundance of fluorine atoms (^{19}F). Just this combination makes the ^{19}F isotope to be one of the most sensitive NMR-active nuclei ever. The receptivity for ^{19}F is 83% of that for ^{1}H, and is 4700 times higher than that for ^{13}C. Moreover, almost none of pharmaceutical excipients contain fluorine atoms. Therefore, ^{19}F NMR spectra can be acquired very quickly even for diluted systems without the danger that ^{19}F NMR signals of the API are overlapped by the signals of excipients.

On the other hand, there are still some limitations for fluorine NMR spectra to be generally used in characterizing amorphous solids. Predominantly the number of sites occupied by fluorine atoms in the molecules of common active substances is usually small. ^{19}F MAS NMR spectra also remain poorly resolved even when measured at high spinning rates and at high magnetic fields.

A typical example of such behavior is currently the most produced drug – atorvastatin. This work, however, shows that even the poorly resolved ^{19}F MAS NMR spectra of atorvastatin are sufficient to distinguish different amorphous forms of the given molecule, particularly when factor analysis (FA) is employed. As a consequence, subtle structural differences between different amorphous forms of atorvastatin are revealed and quantitative parameters identifying every form are provided. The ability of the proposed strategy to recognize various mixtures of amorphous forms is subsequently shown and the detection limits of minor components are discussed. Finally, the capability of the proposed strategy to characterize various low-ordered (amorphous) forms of atorvastatin in solid dosage formulations is demonstrated. In parallel, the proposed method is subjected to the extensive evaluation, validation. The results obtained by ^{19}F MAS NMR spectroscopy are compared with structurally highly receptive experimental techniques like ^{13}C CP/MAS NMR, FTIR and XRPD.

Overall, we tested potential of multivariate analysis of ^{19}F MAS NMR spectra in terms of distinguishing different amorphous forms of both atorvastatin as a pure active pharmaceutical ingredient and atorvastatin as a low-concentration tablet formulation. As a result of this testing we suppose that the proposed strategy represents not only fast, routine and reliable tool to characterize amorphous forms of any fluorinated API in solid dosage forms, but also provides a new insight into the systems exhibiting extensive pseudopolyamorphism or polyamorphism for which high-resolution spectroscopic data cannot be obtained.

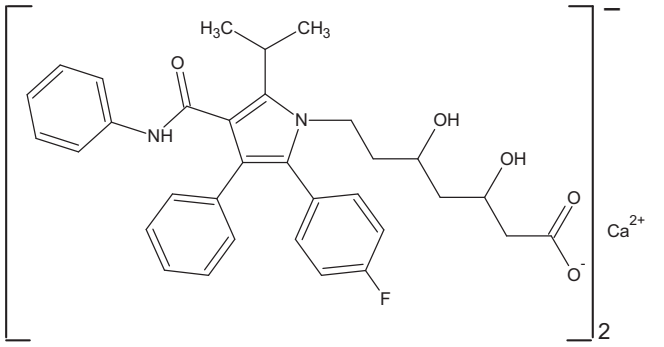
2. Materials and methods

2.1. Materials

Atorvastatin hemicalcium amorphous [$(C_{33}H_{35}FN_2O_5^-)_2$ Ca^{2+}, Scheme 1] by Biocon Laboratories, Bangalore, India; Sucrose and Corn Starch by Sigma-Aldrich were used as received.

2.2. Methods: sample preparation – crystallization

Two spectroscopically well-defined highly crystalline forms and a wide range of amorphous (disordered, semicrystalline) modifications of atorvastatin hemicalcium were prepared according to the procedures described in literature (Aronhime et al., 2006; Ayalon et al., 2008; Briggs et al., 1999; Tessler et al., 2008). Following are the examples of the preparation of atorvastatin hemicalcium Form I, Form X and Form V, respectively: (i) 90 mg of atorvastatin hemicalcium as purchased was dissolved in hot water (9 ml, 70 °C)



Scheme 1. Structural formula of atorvastatin.

and was allowed to stand 1 h at elevated temperature (70 °C) and subsequently cooled down to room temperature. The crystals of the Form I were filtered out (Briggs et al., 1999). (ii) Form X was prepared by dissolving 90 mg of atorvastatin hemicalcium as purchased in a mixture of hot ethanol and water (10 ml, 70 °C, wt. ratio 5:1) and was allowed to stand 2 h at elevated temperature (70 °C) and subsequently cooled down to room temperature. The crystals of the Form X were filtered out (Tessler et al., 2008). (iii) Form V was prepared by dissolving 90 mg of atorvastatin hemicalcium as purchased in the mixture of hot methanol and water (10 ml, 70 °C, wt. ratio 4:1) and was allowed to stand 3 h at elevated temperature (70 °C) and subsequently cooled down to room temperature. The powders of the Form V were filtered out (Briggs et al., 1999). Other modifications of atorvastatin were prepared by similar procedures by dissolving of atorvastatin hemicalcium in various solvents such as methanol, water, ethanol, isopropanol, acetone, acetonitrile and mixtures thereof in varying proportions, at elevated temperature. The prepared solutions were allowed to stand at elevated temperature (70 °C) in the range from 5 min to several hours and subsequently cooled down to room temperature. The obtained powders were filtered out. In all these cases dry powdered samples were used for further spectroscopic analysis without any purification. In summary, approximately 100 crystallization procedures were tested. In this way we tried to prepare as much forms of atorvastatin as possible to obtain a large set of different spectra for factor analysis. From all the prepared systems, the crystalline Forms I, V and X were unambiguously identified based on the ^{13}C CP/MAS NMR and XRPD experimental data (Fig. 1, Briggs et al., 1999; Tessler et al., 2008). In general, however; we did not try to assign the prepared forms of atorvastatin to the products referred in patent literature, because it was not the purpose of our study. The unassigned amorphous modifications of atorvastatin hemicalcium are thus referred as Forms A1–A*n*. The product directly received form the supplier is referred herein as Form A0.

2.3. Models of dosage forms

The models of dosage forms consisting of the prepared forms of atorvastatin (ca. 10 mg) and powdered Sucrose or Corn Starch (ca. 100–200 mg) were prepared by simple physical mixing. The resulting concentration of atorvastatin in the prepared models of low-dose formulations was about 1–10 wt.%.

2.4. NMR experiments

All NMR experiments were performed on a Bruker Avance 500 WB/US NMR spectrometer in 4-mm and 2.5-mm double-resonance probeheads at carrier frequencies 500.18, 470.35 and 125.78 MHz for ^{1}H, ^{19}F, and ^{13}C nuclei, respectively. Standard cross-polarization