THE PHYSICAL STATE OF INDOMETHACIN CONFINED TO NANOMETER DIMENSIONS

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ABSTRACT

Mesoporus silicates are currently widely studied as carriers for controlled drug delivery applications. Although many research groups focus mainly on drug release profiles, there are only a few studies investigating the physical state of confined drugs. However, it is firmly established that the structure of the incorporated drug is directly related to the release kinetics and thus, the biological response of the drug. In order to understand and control the release of the drug, it is crucial to know the physical state of the confined matter. Herein we present some results of our investigations of the structure and dynamics of α, β and γ crystal forms of indomethacin (IMC) and IMC confined in mesoporous silicates using thermal and sorption analysis, XRPD and SS-NMR. Since IMC behaves thermally as a crystalline phase, and at the same time its NMR spectral properties are clearly those of a glass, the physical state of confined IMC cannot be unambiguously defined. The measured proton spin-lattice relaxation times have shown that the dynamical behavior strongly depends on pore size and interactions with the silica walls.

Keywords: physical state, indomethacin, confinement, DSC, NMR

INTRODUCTION

The incorporation of drugs into mesoporous silicates as a means to achieve controlled release has been intensively investigated over the past few years. It was demonstrated that by doping drugs into mesoporous matrices, one can modify the release of drugs in a specific and controllable manner. While the current research is directed mainly towards tailoring drug release kinetics, there are only a few studies focusing on the structure and the physical state of meso-confined phases. It is believed that the physical state of confined drugs is either glassy or liquid-like [1]; some authors even report the existence of a molecular dispersion inside the porous network [2]. It is not rare that one finds explanations of modified release which are based on the altered structure of the confined drug [3]. To explore the effect of spatial confinement on the physical state of the drug, we incorporated indomethacin (IMC) into a very well defined porous structure, such as SBA-15 and MCM-41. These model systems were prepared by impregnation with a sub-saturated solution of IMC followed by a two-step drying process. After determining the loading content, the physical state of confined IMC was probed by a combination of differential scanning calorimetry and solid-state NMR. Dynamical properties were assessed by measuring proton spin-lattice relaxation times.

EXPERIMENTAL

Synthesis of SBA-15 matrix

SBA-15 with an average pore diameter of 9 nm was prepared with a classical hydrothermal synthesis using Pluronic P123 (triblock copolymer, Aldrich) as structure

directing agent and tetraethyl orthosilicate (98 % TEOS, Aldrich) as a silica source. First, Pluronic P123 was dissolved in the mixture of distilled water and hydrochloric acid (37 % HCl, Aldrich). Then, TEOS was added to the Pluronic P123 solution and stirred at 313 K under magnetic stirring. The stirring was continued at higher temperature over night. Then, the silica suspension was transferred into a Teflon-lined autoclave and placed in an oven for hydrothermal treatment at 373 K for 24 h. The obtained white powder was washed with distilled water, dried at 298 K and calcined at 823 K for 6 h in an air flow.

Synthesis of MCM-41 matrix

MCM-41 with an average pore diameter of 3.5 nm was obtained by hydrothermal synthesis using cetyltrimethylammonium chloride (25 % CTACl, Aldrich) as structure directing agent and sodium silicate (Na_2SiO_3, Aldrich) as a silica source. First, 4.4 g of Na_2SiO_3 was added to 27.5 g of distilled water under stirring at room temperature. After 15 minutes, 46 g of CTACl was added dropwise. The pH of the solution was than adjusted to 11 with 0.6 M HCl (Merck) and stirred for 30 minutes before 5 g of distilled water was added. The obtained gel was poured into a Teflon-lined autoclave and placed in an oven for hydrothermal treatment at 373 K for 24 h. After this, the obtained white powder was washed on a filter with distilled water to a pH 7, dried at 333 K for 24 h and calcined at 823 K for 6 h in an air flow to remove the CTACl from the pores.

Drug loading procedures

Drug loading was performed as follows: First sub-saturated solutions of IMC (γ-IMC, Sigma) were prepared in tetrahydrofuran (THF; Sigma-Aldrich). The solutions were than added dropwise to a layer of calcined SBA-15 and MCM-41 powder. Equal amounts of solutions were added to both samples of equal masses. The prepared samples were then dried first at 313 K for 24 h and then at 313 K in vacuum. The samples were denoted SBA-15-IMC and MCM-41-IMC. α-IMC was prepared by recrystallization from ethanol and β-IMC by recrystallization from THF.

Materials characterization

X-ray powder diffraction (XRPD) patterns were recorded on a PANalytical X'Pert PRO high-resolution diffractometer with Alpha 1 configuration using CuK $_{\alpha 1}$ radiation (1.5406 Å) in the range from 0.5 to 5 ° 2θ and in the range from 5 to 40 ° 2θ, respectively. Micrographs were obtained by a Zeiss Supra^{TM} 35VP Scanning Electron Microscope (SEM) operated at 1 keV. Porosity was assessed with nitrogen sorption measurements using Micromeritics ASAP 2020 volumetric adsorption analyzer at 77 K. Before the adsorption analysis, the samples were outgassed under vacuum for 2 h at 473 K. The pore size distributions (PSD) were calculated from nitrogen adsorption data using BJH method. The loading content was determined using Differential Scanning Calorimetry with Thermogravimetric analysis (DSC/TG, Mettler Toledo). Structural and dynamical features of the confined drug were assessed with a combination of Differential Scanning Calorimetry (DSC; Mettler Toledo) and MAS Solid-state Nuclear Magnetic Resonance (600 MHz Varian NMR System quipped with a 3.2 mm CPMAS probehead).

RESULTS AND DISCUSSION

First, we analyzed the prepared crystalline polymorphs of IMC by means of XRPD and SS-NMR. The diffraction patterns and ^{13}C spectra were in agreement with those reported in the literature [4], which confirmed the successful recrystallization. β-IMC crystals are shown in Fig. 1 right. β-IMC is a solvate of THF with a well pronounced crystalline structure and melting transition at 100°C (Fig. 1 left). Upon melting it crystallizes into form α, which melts at 154°C (Fig. 1 left).

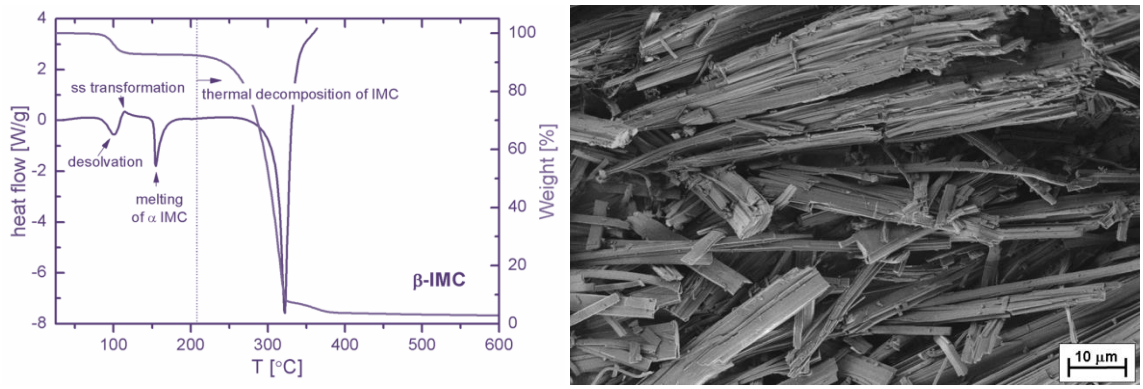

Figure 1: DSC-TG curves (left) and SEM micrograph of prepared β-IMC.

These transitions were used as a fingerprint for the identification of the β-form in case of IMC loaded into SBA-15 and MCM-41. Both silicates were loaded with increasing amounts of IMC solutions. The resulting samples were analyzed with XRPD to detect surplus crystalline IMC particles located outside the pores. Since XRPD is not able to detect crystalline particles as small as the pore size, the sample with highest loading and no observable diffraction peaks was used for further investigations. The DSC curves were clearly those of the β crystalline form, with all thermal events observed in the case of bulk β-IMC (Fig. 2 left) with an expected depression of melting point depending on the pore size. In contrast to this finding, the recorded ^{13}C and ^{1}H NMR spectra of IMC loaded into SBA-15 and MCM-41 exhibited all characteristics of the amorphous form (Fig. 2 right). Thus the range of order in the confined β-IMC is obviously too short to produce crystalline NMR spectra, while it is long enough to produce thermal transitions, such as desolvation, crystallization and melting. As seen from Fig. 2 (left), confined β-IMC crystallizes upon melting into α and γ form. It must be stressed that DSC curves of prepared samples, which were dried using a single step procedure (at ambient pressure or vacuum) exhibited no peaks corresponding to any crystalline form, while some peaks characteristic for free THF (free in terms of not incorporated into the crystal structure) were observed in the NMR spectra. Our experiments with single step drying demonstrate that incomplete drying may completely mask thermal transitions of crystalline phase. Thus if one wants to assess whether the effect of spatial confinement on the physical state has certain generic features, care must be taken during drying. This may also explain apparent differences in the reported structure of confined drug.

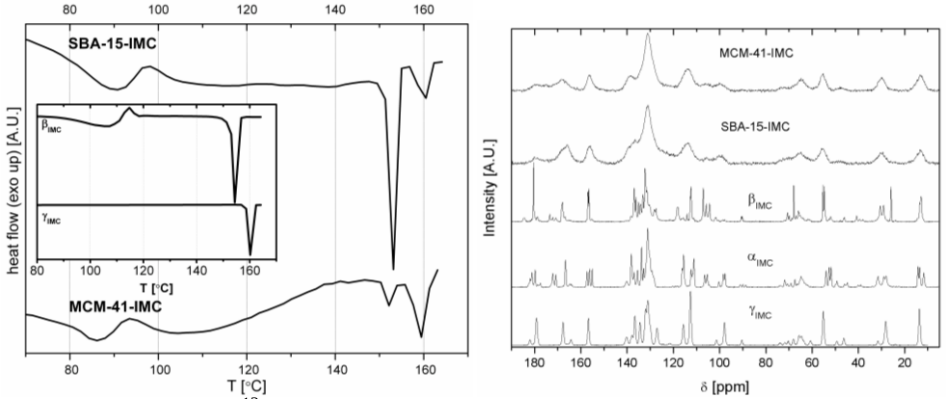

Figure 2: DSC curves (left) and ^{13}C NMR spectra (right) of the prepared samples. The scale and units are arbitrary.

Furthermore, although in both cases (SBA-15 and MCM-41) DSC curves indicate the presence of the β crystalline form, the spin-lattice proton relaxation times (T_1) differ dramatically, and decrease with decreasing pore size (Table 1). T_1 in bulk β-IMC is app. 30%

longer as in the case of SBA-15 loaded IMC and app. 10 times longer than in case of MCM-41 loaded IMC. The dynamics greatly depend on pore size as inferred from T_1, which is established through the coupling with phonons. The enhanced relaxation may be attributed to more efficient coupling of IMC protons with silica matrix phonons, which is more pronounced in smaller pores, due to a higher surface-to-volume ratio. To better understand the form of IMC phases embedded within the mesopores, we also performed ^{1}H-^{29}Si HETCOR analyses of interactions between IMC and the walls of SBA-15 and MCM-41. The interactions between IMC protons and Si atoms appear stronger in the case of MCM-41.

Table 1. Proton spin-lattice relaxation times of bulk IMC polymorphs and IMC loaded MCM-41 and SBA-15.

Sample	T_1 [s]
α-IMC	2.7± 0.2
β-IMC	3.2± 0.2
γ-IMC	3.0± 0.1
MCM-41-IMC	0.3± 0.3
SBA-15-IMC	2.1± 0.1

CONCLUSION

Our findings demonstrate that the physical state of drugs confined to nanometer dimensions cannot be unambiguously defined. From a spectroscopical point of view the drug is in amorphous, i.e. it does not exhibit long-range order, while it behaves thermally as a crystal, i.e. the range of order is long enough to produce thermal transitions, which are characteristic for crystalline phases. Drying can have dramatic effects on the results of thermal analysis, as significant amounts of residual solvent effectively mask all thermal transitions of the confined drug. The proton relaxation dynamics depend strongly on pore size and interactions with the silicate walls.

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