New insights into the use of hydroxypropyl cellulose for drug solubility enhancement: An analytical study of submolecular interactions with fenofibrate in solid state and aqueous solutions

Manuel Martin-Pastor | Edmont Stoyanov

1Unidad de Resonancia Magnética, Área de Infraestructuras de Investigación, Universidad de Santiago de Compostela, Santiago de Compostela, A Coruña, Spain
2Chemical Division, Nisso Chemical Europe, Duesseldorf, Germany

Correspondence
Edmont Stoyanov, Chemical Division, Nisso Chemical Europe, Berliner Allee 42, 40212 Duesseldorf, Germany.
Email: stoyanov@nisso-chem.de

Abstract
Hydroxypropyl cellulose (HPC) is a solubility enhancer used for poorly soluble drugs, nano-suspensions and amorphous solid dispersions (ASD). However, the underlying mechanism remains unclear. ASDs of a poorly soluble drug, fenofibrate (FEN), were analyzed using liquid nuclear magnetic resonance (NMR) and solid state NMR (ss-NMR). Liquid NMR revealed interactions between the pyranose ring of the HPC molecule and the diphenylketone from FEN. The water accessibility of the CH3 groups in HPC and FEN is very low, they form a hydrophobic zone in aqueous solution that may sustain the drug nucleation. Moreover, ss-NMR measurements confirmed very low drug crystallinity for HPC-FEN ASDs. Cross-polarization and direct polarization 13C spectra, 13C-CPMAS and 13C-PARIS, distinguished the most rigid and flexible portions in concordance with the ss-NMR proton T1 and T1r relaxation results. Although HPC side chains (hydroxypropoxy) are the most flexible portions, their flexibility is moderate and high rigidity is the predominant. The ss-NMR proton relaxation indicates a rather homogeneous distribution of the components (HPC and FEN) in the solid mixtures. The versatile NMR methodology proposed can be used to study other polymer-drug systems and it may contribute to understand relevant functional aspects such as the rate of drug-delivery and their stability.

KEYWORDS
1D 13C cross-polarization spectra, 1D 13C direct polarization spectra, 1H water ligand observed via gradient spectroscopy, hydroxypropyl cellulose, proton driven spin diffusion, saturation transfer difference 1H spectrum

INTRODUCTION

Pharmaceutical formulations combine active pharmaceutical ingredients (API) with other molecules or polymers used as excipients to improve their bioavailability at the target sites in an organism. Hence, the study of these excipients is valuable in terms of product quality control and evaluation of long-term stability. Furthermore, the precise method of their preparation serves to modulate specific aspects at the molecular level, including the...
miscibility of the components\cite{1} and the strength of interactions between components, either in the solid\cite{2} or aqueous\cite{3} states, thereby enhancing various properties, such as drug solubility in water, which can impact the performance of the drug formulation.

Solid state nuclear magnetic resonance (ss-NMR) is an effective platform for the investigation of phase separation and heterogeneity in solid mixtures of polymers and drugs.\cite{1,4,5} Duan et al.\cite{1} described four types of solid mixtures: (i) physical mixture with no intermolecular interaction between the components; (ii) homogeneous mixture with intermolecular interactions between the components; (iii) phase separated mixture in which each component has intermolecular interactions with itself and only interacts with other components at the surface; (iv) mixed, but inhomogeneous mixture, comprising a combination of the aforementioned mixtures.

Fenofibrate (FEN), a highly water-insoluble drug, belongs to the group “fibrates” and is commonly used to reduce blood cholesterol and triglyceride levels.\cite{6} Meanwhile, hydroxipropylcelulose (HPC) is a derivative of cellulose with both water solubility and organic solubility. As such, HPC is used as a tablet binder,\cite{7} emulsion/suspension thickener,\cite{8} and topical ophthalmic protectant and lubricant.\cite{9} Additionally, low molecular weight HPCs have proven successful as solubility enhancers for poorly soluble drugs, solubilizing agents for nano-suspensions,\cite{10,11} and polymers for amorphous solid dispersions (ASD).\cite{12-14}

However, the mechanism underlying the HPC-drug interactions in aqueous solutions and solid state remains unclear. Hence, understanding the structural arrangement and which components of the molecules are in close proximity and interact at the sub-molecular level, will facilitate proper polymer selection to ensure drug molecules adequately dissolve at their target sites. Indeed, the establishment of favorable HPC-drug interactions has been proposed as a basis to rationalize the miscibility and stability of these formulations in the solid state.\cite{15} Recently, we reported that HPC solubilizing activity in aqueous solutions might be caused by the favorable interplay between HPC chain length and hydration.\cite{16}

In this study we aimed to obtain further insights into the sub-molecular interactions between HPC and a model, poorly soluble drug. Thus, we have applied a combination of liquid NMR and ss-NMR techniques using HPC-based ASDs composed of 15% fenofibrate and 85% HPC as a model drug delivery system. Using liquid state NMR we aimed to identify interactions between HPC and FEN, while assessing the water accessibility of these components in aqueous solutions. ss-NMR was then applied to study the formation of crystalline and amorphous phases in solid mixtures of HPC and FEN prepared under different conditions on the basis that the formation of these phases can impact the long-term stability of FEN embedded in the HPC matrix.

2 RESULTS AND DISCUSSION

2.1 Liquid NMR analysis of HPC-FEN intermolecular interactions in aqueous solution

FEN is a nonpolar small molecule that is barely soluble in water while HPC is an amphiphilic polymer with favorable solubility in water. Proton signal assignment of the pure FEN (sample 9) and HPC (sample 1) samples dissolved in D2O are shown in Figure S1a,b, respectively. The FEN and HPC mixture exhibited favorable water solubility. Although the mixture remained stable for approximately 1 month after preparation, longer storage times at room temperature ultimately caused FEN to precipitate out, which was detected by 1H NMR (Figure S2).

One factor that may contribute to the enhanced solubility of FEN in aqueous mixtures with HPC is the formation of a transient complex between the two molecules favored by the establishment of a number of stabilizing intermolecular interactions. The STD spectra\cite{17} (Figure 1) provided evidence of a HPC-FEN complex in Sample 5. Figure 1B shows the STD on-off spectrum of Sample 5 with on-saturation applied to the aromatic protons of FEN labeled as c and g. The protons of HPC in the region of protons 2 to 8 are visible in this spectrum, an indication of the proximity between FEN and HPC when forming a complex. Further, the STD on-off spectrum, measured with on-saturation applied to the anomic proton J of HPC (Figure 1C), shows the signals of the aromatic protons c, g, b and h and the methyl protons n of FEN. Again, formation of a transient complex in water in which there is close proximity between the diphenylketone moiety and the methyl groups of FEN and HPC, was detected. The analog STD spectra of the aqueous HPC and FEN mixtures with Samples 6, 7 and 8, reach similar conclusions (data not shown), indicating that formation of the transient FEN and HPC complex is relatively independent of the HPC chain length and contribute to enhance the solubility of FEN in water solutions.

The water accessibility in the aqueous HPC and FEN mixtures was studied by WaterLOGSY spectra.\cite{18} For Sample 5 (HPC-UL and FEN 15 wt%) the proton and the WaterLOGSY spectrum are shown in Figure 2A,B, respectively. The WaterLOGSY spectrum shows that protons in regions 1 to 8 of HPC appear with more intensity, indicating that they are more exposed to water molecules with higher residence times in their proximity than protons of the hydroximethylpropyl pendant chains that have low signal 9 intensity. The four aromatic protons c, g, b and h of FEN appear as relative intense broad peaks indicating that this portion of the molecule is accessible.
to water compared to the methyl protons $o$ and $n$, the signals for which are not visible in Figure 2. In the WaterLOGSY spectrum of Figure 2B a broad peak is observed, which is attributed to hydration of water ($H_2O^{\text{hydr}}$); its presence denotes molecules of water with a high residence time in contact with the HPC polymer. The presence of the $H_2O^{\text{hydr}}$ signal in this type of spectrum was previously observed in a previous study of hydrated pure HPC samples.$^{[16]}$ Similar WaterLOGSY spectra were obtained for the aqueous mixtures of HPC and FEN corresponding to Samples 6, 7 and 8 in water (data not shown), indicating that the accessibility of water to the two components of the mixture is relatively independent of the HPC chain length.

Notably, although neither the pendant chain of HPC, nor the methyl protons of FEN, are accessible to water, according to the WaterLOGSY spectra analysis, they could be in close contact with each other forming part of the same hydrophobic core according to the STD spectra of Figure 1. That is, the STD spectrum of Figure 1C, measured with on-saturation over the anomeric signal of HPC transfers saturation to the protons of the pendant chain protons of HPC and the methyl protons of FEN that could be in contact in a hydrophobic core. In fact, this interpretation suggests a plausible mechanism for the precipitation of the HPC-FEN mixture observed in the course of 1 month (Figure S2). Conformational arrangements of the aliphatic chains of HPC could gradually take place to accommodate FEN out of the unfavorable interaction with the water, leading to the enlargement of the hydrophobic core by formation of vesicles and HPC-FEN oligomers. These oligomers would eventually lead to large aggregates and precipitate into a thermodynamically more stable solid form.

### 2.2 WaterLOGSY quantification

The WaterLOGSY spectrum, measured with a mixing time of 50 ms, was quantified to determine the distribution of the water molecules in contact with the solutes of mixtures 5 to 8 (Table 1). In all samples, the pyranoses have the highest hydration contribution ranging from 58.9% to 85.8% of the total water hydration. Moreover, a reduction in the relative intensity of the $H_2O^{\text{hydr}}$ signal is observed that positively correlates with the polymer length (Mw) of HPC. A similar trend occurs for the aromatic signals of FEN, which becomes less exposed to the water.

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**FIGURE 1** 1D saturation transfer difference (STD) spectra of mixture hydroxypropyl cellulose (HPC)-UL (20 kDa) and fenofibrate (FEN) 15 wt% (sample 5) dissolved in D$_2$O. (A) $^1$H STD$^{\text{off}}$, (B) $^1$H STD$^{\text{off-on}}$ with on-saturation over signals c and g of FEN at 7.27 ppm; (C) $^1$H STD$^{\text{off-on}}$ with on-saturation over signal 1 of HPC at 4.45 ppm. The structures are shown on the right with letter and number coding used to identify the atoms. In (A) all protons are identified; in (B) and (C) the arrow indicates the position of the on-saturation, while only the signals corresponding to the saturated proton(s) and the STD intermolecular signal responses are identified [Color figure can be viewed at wileyonlinelibrary.com]
Table 1: Comparison of hydration in the four HPC-FEN mixtures determined from the area of each signal normalized to the full integral in the WaterLOGSY spectrum measured at mixing time 50 ms

| Sample 5 HPC-UL + FEN | Sample 6 HPC-SSL + FEN | Sample 7 HPC-SL + FEN | Sample 8 HPC-L + FEN |
|------------------------|------------------------|------------------------|------------------------|
| Mw (kDa)\textsuperscript{a} | 20 | 40 | 100 | 140 |
| FEN\textsubscript{arom}\textsuperscript{b} | 12.4% | 4.1% | 3.4% | 1.3% |
| H\textsubscript{2}O\textsubscript{hydr}\textsuperscript{c} | 23.2% | 9.3% | 7.2% | 5.4% |
| Pyranoses\textsuperscript{d} | 58.9% | 72.6% | 66.8% | 85.8% |
| Aliphatics\textsuperscript{e} | 5.4% | 13.9% | 22.6% | 23.4% |

Note: The assignment of the signals corresponds to that given in the spectrum of Figure 2B.

Abbreviations: FEN, fenofibrate; HPC, hydroxypropyl cellulose.

\textsuperscript{a}Measured by GPC.
\textsuperscript{b}Signal area corresponds to the sum of the aromatic peaks c, g, b and h of FEN.
\textsuperscript{c}Signal area corresponds to peak H\textsubscript{2}O\textsubscript{hydr}.
\textsuperscript{d}Signal area corresponds to peaks 1 to 8 of HPC.
\textsuperscript{e}Signal area corresponds to peak 9 of HPC.

Figure 2 Study of water interactions in the hydroxypropyl cellulose (HPC)-UL (20 kDa) and fenofibrate (FEN) mixture (Sample 5) dissolved in H\textsubscript{2}O:D\textsubscript{2}O 90:10. (A) \textsuperscript{1}H Noesat (reference spectrum) and (B) WaterLOGSY measured with mixing time of 50 ms. The letter and number codes used to identify the atoms are the same as in Figure 1. In (B) signal H\textsubscript{2}O\textsubscript{hydr} is assigned to water molecules in the first hydration shells of HPC\textsuperscript{[16]} [Color figure can be viewed at wileyonlinelibrary.com]

with increasing polymer length, in contrast with the opposed effect obtained for the aliphatic chains of HPC. This trend for the H\textsubscript{2}O\textsubscript{hydr} signal was previously observed in our study on the hydration of pure HPC of different grades in water\textsuperscript{[16]} and in the case of the mixtures 5 to 8 has a similar interpretation. That is, the trend observed for the aromatic FEN peaks could indicate that the increase in polymer chain length (Mw) interferes with its hydration capability, as a result of the agglomeration phenomena of the HPC moieties\textsuperscript{[16]}

2.3 Solid HPC-FEN mixtures

The solid HPC and FEN mixtures combine the amphiphilic characteristic of HPC with the high hydrophobic nature of FEN. We, therefore, used ss-NMR to investigate solid samples of the pure FEN (Sample 9) and HPC (Samples 1 to 4) components, as well as their HPC and FEN 15 wt% mixture (Samples 5 to 8), to determine the potential formation of phases and heterogeneity occurring at the molecular, or sub-molecular, level. Since the
HPC chain length may promote such heterogeneity, samples of several chain lengths were considered: HPC-UL 20 kDa (Samples 1 and 5), HPC-SSL 40 kDa (Samples 2 and 6), HPC-SL 100 kDa (Samples 3 and 7) and HPC-L 140 kDa (Samples 4 and 8). Additionally, the method used to prepare the solid mixture may impact the heterogeneity. Hence, two methods were considered: (i) spray drying from solvents, such as ethanol or acetone and (ii) spray dried amorphous solid dispersion from ethanol.

2.4 | Solid $^{13}$C NMR spectra

The occurrence of several degrees of rotational mobility and/or conformational flexibility at the molecular or submolecular level in a solid reflects packing heterogeneity and differences in crystallinity, regardless of whether these differences lead to the formation of different phases, or not. Differences in flexibility at the submolecular level were investigated by comparing two types of $^{13}$C ss-NMR spectrum for the same solid sample; the $^{13}$C-CPMAS spectrum based in cross-polarization between protons and $^{13}$C, and the $^{13}$C-PARIS spectra based in direct polarization from protons to $^{13}$C. As is explained in the Supporting Information section, the former spectrum is more sensitive to $^{13}$C atoms in rigid environments typical of a crystalline, and relatively well-ordered phase, while the latter is more sensitive to the effects of mobility and/or conformational flexibility, typical of a semi-fluid and relatively disordered phase.

The $^{13}$C-CPMAS and $^{13}$C-PARIS spectra for the ASD HPC-UL and FEN mixture (Sample 5), represented at a comparable scale are shown in Figure 3A,B, respectively, and the signal assignment was obtained from the spectra of the pure FEN and HPC-UL compounds (Figures S3 and S4). The spectra of Figure 3 clearly demonstrate that a number of carbon signals are substantially attenuated, or invisible, in the $^{13}$C-PARIS spectrum (Figure 3B) compared to the reference $^{13}$C-CPMAS spectrum (Figure 3A), which contains the expected signals at a semi-quantitative intensity in relationship to the sample composition. Additionally, the signals detected in $^{13}$C-PARIS spectra correspond to the most flexible carbons in a mobile or semi-fluid phase, and represent the pendant chains of the hydroximethyl (Signal 6) and hydroxymethylpropyl groups (Signals 7, 8, and 9), certain carbons of the HPC pyranose ring (Signals 2 to 5), and the methyl groups at the terminal portion of FEN (Signals n and o). Meanwhile, all of the signals expected for the two aromatic rings of FEN, as well as most of the HPC pyranose ring signals (i.e., Signals 1 to 5) are not detected in the $^{13}$C-PARIS spectrum, indicating that they are in a rigid and structured environment. Similar results were observed for the comparisons of the $^{13}$C-CPMAS and $^{13}$C-PARIS spectra for any of the other samples (Figures S5 and S6), indicating that for HPC, the high flexibility of the carbons in the pendant chain, as well as the rigidity of the pyranose ring, are independent of the differences in HPC grade, or the sample preparation method.

2.5 | Proton relaxation study in the solid state

To study the formation of different phases, proton T$_1$ and T$_{1r}$ relaxation times were measured for Samples 5 to 8 corresponding to HPC and FEN solid mixtures; (Figure 4 and Figure S7). In all cases FEN and HPC signals had T$_1$ values considerably larger than T$_{1r}$, indicating low crystallinity.[1]

Proton T$_1$, corresponding to the two aromatic protons c and g of FEN, decreased in the four mixtures relative to those in the pure fenofibrate (Figure 4A), which reflects a decrease in the local crystallinity ordering of FEN in the mixtures. Moreover, a consistent decreasing trend was observed for the proton T$_1$ of FEN with an increase in the chain length, or HPC grade according to the following order: UL > SSL > SL > L, which can be associated with the more tangled and banded structure of the long chain HPC grades that favor the stable drug amorphous state. These results likely denote an intermolecular interaction occurring between HPC and FEN in the solid state involving the diphenylketone moiety of FEN, similar to what has been described above in the solution state. Moreover, a substantial decrease in the T$_{1r}$ values of the FEN peaks (Figure 4B) was observed compared to the pure fenofibrate, which also indicates the aforementioned intermolecular interaction and low crystallinity. The trend in the T$_{1r}$ values of FEN reflect differences in mobility in the range of the spin-locking field strength ($\gamma B_0/2\pi$), which correspond to correlation times ($\tau_c$) in the submicrosecond range (i.e., maximum sensitivity of the proton T$_{1r}$ data for motions at $\tau_c = 0.13$ μs), typical of ring flips and domain motions. Sample 6 has the highest contribution of motions in the referred time scale.

A substantial difference was observed in the proton T$_1$ and T$_{1r}$ of HPC intra- and inter-sample signals depending on the proton site (Figure 4C,D). These differences, however, cannot be attributed to the presence of FEN because of its relative low abundance in these mixtures (FEN abundance is only 15% wt), and must, therefore, be related to subtle differences in the local mobility caused by the combined effect of the HPC grade and/or the influence of the preparation method. However, the
Figure 3 Solid NMR spectra of the hydroxypropyl cellulose (HPC)-UL (20 kDa) and fenofibrate (FEN) 15 wt% amorphous solid dispersions (ASD) mixture (Sample 5). (A) $^{13}$C-CPMAS; (B) $^{13}$C-PARIS. The letter and number codes used to identify the protons are the same as those presented in Figure 1. The signal assignment is based on the ss-NMR spectra of the pure FEN and HPC-UL compounds given in Figures S3 and S4, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4 Bar-plots of the proton NMR relaxation data obtained for the four mixtures prepared (Samples 5 to 8). (A) Direct detection of the proton $T_1$ (s) of fenofibrate (FEN). (B) Direct detection of proton $T_{1r}$ (s) of FEN. (C) Inverse detection of proton $T_1$ (s) of hydroxypropyl cellulose (HPC). (D) Inverse detection of proton $T_{1r}$ (s) of HPC. In (A) and (B) the analyzed signal between 7.5 and 9 ppm corresponds to those assigned to protons c and g of FEN in the spectrum presented in Figure S3b. The values of the pure FEN (Sample 9) are included for comparison. In (C) and (D) the analyzed signals of HPC correspond to those assigned in the $^{13}$C CPMAS spectrum presented in Figure S4a. [Color figure can be viewed at wileyonlinelibrary.com]
influence of the preparation method in the $T_1$ of HPC is modest, at least for the three samples prepared with HPC-SSL (Samples 3, 4 and 6), as their values are similar (Figure S8). Additionally, the $T_{1r}$ of HPC signals (Figure 4D) show a clear trend for protons in the hydroximethylpropyl and hydroxymethyl pendant groups (protons 6, 8, 9 and possibly 7) to be substantially larger than other types of protons of the sugar pyranose ring (protons 1 to 5). This is consistent with their increased flexibility at a scale faster than microseconds, and agrees with the stronger contribution made by their attached carbons in the $^{13}$C-PARIS spectra (Figure 3). Finally, the $T_{1r}$ of HPC signal 9, but not of the other signals, is highly sensitive to the sample preparation method, as is evident for the three samples prepared with HPC-SSL in Figure S8, and the four mixtures of Figure 4D. Hence, the sample preparation method impacts the flexibility of the hydroximethylpropyl pendant group of HPC.

The enhanced flexibility found herein for the pendant chains of HPC compared to other fragment of this polymer is in concordance with the ss-NMR results obtained in a previous study for the pendant chain of diethyl-amino-ethyl cellulose which were described to be in a gel-like environment. However, for the HPC samples studied herein, the flexibility of these pendant chains is clearly more modest as they provide much stronger signals in the $^{13}$C CP-MAS spectrum, and therefore should be better described by a semi-rigid environment.

2.6 | Detection of HPC and FEN binding interaction in the solid state

To further test the type of intermolecular interaction occurring between HPC and FEN in its ASD mixture, a new ss-NMR experiment called 2D PARIS-(13C-selective)-HSQC-PDSD is proposed (Figure S9a). The experiment was developed to discern if the flexible and rigid parts of HPC and FEN (see section 2.4) are in close proximity, or if they are well separated in the solid and therefore, they would be forming an independent phase. The experiment of Figure S9a contains a preparation period to appropriately select (invert) the magnetization of a specific proton signal from the flexible environment. The final step is a PDS (Proton Driven Spin Diffusion) is reminiscent of the STD experiment used in liquids, in the sense that it relies in spin diffusion to transfer the magnetization from a selected proton to other protons that may reside either in the same or a different molecule.

The ASD mixture of HPC-FEN of Sample 5 was analyzed by the 2D PARIS-(13C-selective)-HSQC-PDSD experiment. The protons of the methyl group (H-9) in the flexible pendant chain of the HPC component were selected (inverted) in the experiment by PARIS irradiation, $^{13}$C selectivity on the methyl carbon C-9 and back cross-polarization from $^{13}$C to $^1$H. In Figure S9b is shown the reference spectrum measured with mixing time 0 ms which only contains the HSQC peak H-9/C-9 and is in agreement with the desired selectivity. In Figure S9c it is shown the spectrum measured with mixing time 110 ms. It contains a continuous band of peaks between 1 and 7 ppm that denotes the transfer of magnetization by spin diffusion from proton H-9 (ca. 1 ppm) to many other protons in this system. They include the pyranose protons of HPC (between 3 and 5.2 ppm) and to the aromatic protons b, c, g and h of FEN (ca. 7 ppm), all of them located in a rigid environment. An scheme of the referred transfer of magnetization by spin-diffusion between HPC and FEN is given in Figure S9d.

Overall, the results of this section show that there exist intermolecular interactions between HPC and FEN in the solid state and that in the complex that is formed, the flexible and rigid parts of HPC and FEN are in close proximity.

3 | CONCLUSIONS

In this work we provide evidence of intermolecular interactions between HPC and FEN in aqueous mixtures and solid state. In the liquid state, the aromatic rings of FEN contact the protons in the pyranose rings of HPC and are relatively accessible to the bulk external water. We found evidence of interaction between the CH$_3$ groups of FEN and HPC in aqueous solution, these groups represented the least hydrated portions of both polymer (HPC) and drug (FEN) molecules. A hydrophobic interaction between drug and polymer may exist to sustain drug crystallization.

In the solid state ASD mixtures of HPC and FEN there is a binding interaction between FEN and HPC similar or identical to what it occurred in solution. These samples have very low crystallinity and high rigidity in terms of their molecular mobility. Their most flexible portions were detected by $^{13}$C-PARIS spectra, as well as proton $T_1$ and $T_{1r}$ relaxation analysis and correspond to the pendant chains of HPC. However, the high intensity signals detected by $^{13}$C-CPMAS spectra suggest only moderate flexibility, indicating that a semi-liquid phase does not exist. Meanwhile, the ss-NMR proton relaxation study indicates that the distribution of components in the solid mixtures has low crystallinity and is homogeneous leaving the rigid and flexible parts of HPC and FEN in close proximity, a model which agrees with recently published results.
Our results also probe that the stability of HPC and FEN mixtures in water and in the solid state are different in what refers to the effect of the HPC chain length and stability. Water solutions are metastable, they eventually aggregate and precipitate. In the solid state, HPCs with or without the non-polar drug FEN were seen to have excellent stability regardless of the chain length. In solution, low molecular weight HPCs are preferable because they are more capable to solubilize non-polar drugs such as FEN and therefore they must be the first choice.

The versatile NMR methodology proposed in this work for the characterization of HPC-FEN systems which derive properties such as the drug-polymer interactions, molecular hydration and flexibility can be used to study other polymer-drug systems. These properties may contribute to understand relevant functional aspects of these systems such as the rate of drug-delivery and their stability.

4 | EXPERIMENTAL

4.1 | Samples

The samples studied consisted of pure HPC prepared as spray dried powder from the solvents acetone or ethanol, as well as ASD of 85 wt% HPC and 15 wt% FEN, spray dried from ethanol.[14] HPC samples were procured from Nippon Soda (Japan), for this study. FEN (98% purity) was provided from VWR International (Germany). The HPC component in each sample is denoted by its molecular weight that was determined by GPC; that is, HPC-UL, HPC-SSL, HPC-SL and HPC-L correspond to 20, 40, 100 and 140 kDa, respectively. The following nine samples were studied by NMR and are referred to in the text by their number: (1) pure HPC-UL spray dried from acetone, (2) pure HPC-UL spray dried from ethanol, (3) pure HPC-SSL spray dried from acetone, (4) pure HPC-SSL spray dried from ethanol, (5) ASD mixture of HPC-UL and FEN 15 wt%, (6) ASD mixture of HPC-SSL and FEN 15 wt%, (7) ASD mixture of HPC-SL and FEN 15 wt%, (8) ASD mixture of HPC-L and FEN 15 wt%, (9) pure FEN. The ASD mixtures were measured 8 weeks after preparation and stored at room temperature in closed vessels.

4.2 | Liquid nuclear magnetic resonance

4.2.1 | Sample preparation

Samples 1–9 were studied by liquid NMR. They were prepared by dissolving 10 mg of the solid product in 0.6 ml of D2O (D 99.9%; Eurisotop, Inc.). The samples were dissolved with stirring and the overnatant liquid was transferred to a 5 mm NMR tube for analysis. Similarly, Samples 5 to 8 were also prepared in 0.6 ml in the mixture of solvents H2O:D2O 90:10.

4.2.2 | NMR spectrometry

Liquid NMR spectra were measured in a Bruker NEO-750 spectrometer (750 MHz proton frequency) using the TopSpin 4.0 control software. All spectra were processed with MestreNova software v14.0 (Mestrelab Research, Inc.). Signal assignment of the 1H resonances of HPC was made based on previous work.[16] The 1H signals of FEN were assigned based on their multiplicity and relative integral in the 1H spectrum of FEN in D2O. The later assignment is compatible with that reported by Salama et al. for FEN dissolved in CDCl3.[20] Finally, the 1H assignments reported for HPC and FEN were consistent with those predicted by ChemDraw v14.0 (Perkin-Elmer, Inc) on the basis of their covalent structure.

The one-dimensional 1H spectrum (1H_noepresat) was measured for samples prepared in D2O with 128 scans, a relaxation delay (d1) of 2 s and a fid acquisition time (aq) of 2.75 s. The fid was acquired with 32 k complex data points and was processed with zero-filling and Fourier Transformation (FT) to obtain a spectrum with 131 k data points.

The one-dimensional 1H noe-presaturation spectrum (1H_noepresat) (sequence noesygppr1d of the Bruker library) was measured for Samples 5 to 8 prepared in H2O:D2O 90:10. The presaturation was applied over the strong H2O peak at ~4.7 ppm by a continuous wave pulse of low power and 4 s duration. The spectrum was acquired with 128 scans, mixing time of 12.8 ms, FID acquisition time (aq) of 2.22 s, and spectral width of 20 ppm.

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The one-dimensional saturation transfer difference 1H spectrum (STD) was measured for Samples 5 to 8 prepared in 99.9% D2O. Selective saturation consisted of a train of soft Gaussian shaped pulses of 50 ms duration with a 1 ms interpulse delay. The selective saturation was applied during 2 s at a specific frequency of the 1H spectrum, covering a ± 125 Hz spectral region around the chosen frequency (i.e., ± 0.17 ppm in a 750 spectrometer). The STDoff saturation was applied at 20 ppm. The STDon saturation was applied at the frequency of one specific signal of FEN separated by more than 300 Hz from any HPC signals. The STDon and STDoff scans were measured in alternate scans and subtracted by the phase cycling, providing the subtracted STDoff-on spectra. Four STD spectra were obtained by STDon saturation of the FEN...
signals at 6.52, 6.94, 7.27 and 7.33 ppm, respectively. Each spectrum was acquired in 15 min with 128 scans and a 6.75 s total scan duration consisting of a 2 s prescan delay ($d_1$), 2 s STD saturation time and a 2.75 s fid acquisition.

A 1D WaterLOGSYP\textsuperscript{[18]} spectrum (pulse sequence WaterLOGS of the Bruker library) was measured for Samples 5 to 8 prepared in H$_2$O:D$_2$O 90:10. The experiment used a $sinc$ shaped selective 180° inversion pulse applied over the H$_2$O signal (~4.7 ppm) with a 7.5 ms duration and band width of 75 Hz. The strong water peak was suppressed before detection with a pair of excitation-duration and band width of 75 Hz. Samples 5 to 8 prepared in H$_2$O:D$_2$O 90:10. The experiment used a $sinc$ shaped selective 180° inversion pulse applied over the H$_2$O signal (~4.7 ppm) with a 7.5 ms duration and band width of 75 Hz. The strong water peak was suppressed before detection with a pair of excitation-duration and band width of 75 Hz.

4.3 | Solid state nuclear magnetic resonance

4.3.1 | Sample preparation

Samples 1–9 were studied in powder form by ss-NMR without any further treatment.

4.3.2 | NMR spectrometry

Solid NMR spectra were measured using a Bruker NEO-750 spectrometer (750 MHz proton frequency) with TopSpin 4.0 control software. All spectra were processed with MestreNova software v14.0 (Mestrelab Research, Inc.). Two types of solid probes were used to measure the spectra. The Varian T3 solid triple resonance probe 1H/X/Y uses a zirconia rotor with an outer diameter of 3.2 mm and has an effective sample capacity of 22 μL, corresponding to approximately 30 mg of powder sample. The probe was adapted to the Bruker spectrometer by a A2B conversion kit (Revolution NMR, LLC) and the spectra were measured at 298 K. Meanwhile, the trigamma 1H/13C/15 N probe employs 1.3 mm rotors with a sample volume of 3 μL in which the spectra were measured at a temperature of 278 K. Carbon-13 chemical shifts were referenced to the carbonyl signal of solid glycine that resonates at 4.1 ppm. The assignment of $^1$H and $^{13}$C solid resonances of FEN are given in Figure S3 and are based on the consistency between the assignment obtained in this work for FEN in D$_2$O solution and the values reported by Salama et al. for FEN dissolved in CDCl$_3$.\textsuperscript{[24]} The assignment is also consistent with the predictions made using ChemDraw v14.0 software (Perkin-Elmer, Inc). Signal assignment of the $^1$H and $^{13}$C resonances of HPC in the liquid and solid state were obtained from previous works.\textsuperscript{[15,16]} The assignment of the solid $^{13}$C and $^1$H resonances of HPC is provided in Figure S4.

1D $^{13}$C cross-polarization spectra ($^{13}$C CPMAS) were measured for the nine samples with the 3.2 mm probe (sequence “h.c.cp” of the Bruker library) with an inter-scan delay ($d_1$) of 2 s, minimum number of scans of 512, and MAS rate of 20 kHz. Cross-polarization was applied during 2 ms with a constant carbon field strength of 63.1 kHz, the power on the $^1$H nucleus was linearly ramped from 70% to 100% with a peak field strength of 59.0 kHz. Heteronuclear decoupling during FID acquisition was performed with Spinal-64 with a proton field strength of 79.4 kHz.

1D $^{13}$C direct polarization spectra ($^{13}$C PARISxy) were measured for the nine samples with the 3.2 mm probe at a MAS rate of 20 kHz with the PARIS.\textsuperscript{[23,25]} During the inter-scan relaxation, immediately before the $^{13}$C excitation pulse, a train of PARISxy 25 ms pulses with 12.5 kHz field strength were applied to introduce modulation sidebands at half the rotation rate causing $^{13}$C enhancement. The total duration of PARISxy irradiation was 3 s delay. The $^{13}$C excitation pulses have a tilt angle of 90° and was applied with a B$_1$ field strength of 63.1 kHz. Heteronuclear decoupling during acquisition of the FID was performed with SPINAL-64 with a proton field strength of 79.4 kHz. The spectrum was acquired with 512 scans.

Indirect detection proton T$_1$ relaxation times were measured for the HPC-FEN mixtures (samples 5 to 8) and pure fenofibrate with the 3.2 mm probe at a MAS rate of 20 kHz. The experiment (sequence “cphirt1” of the Bruker library) combines proton inversion-recovery and CPMAS to transfer polarization to the carbons. The conditions for CPMAS were identical to the $^{13}$C CPMAS experiment described above. Sixteen points were measured with an inversion-recovery delay duration between 1 and 6 s. The inter-scan delay ($d_2$) was 10 s and each point was acquired with 32 scans.

Indirect detection proton T$_{1p}$ relaxation times were measured for the HPC-FEN mixtures (samples 5 to 8) and pure fenofibrate with the 3.2 mm probe at a MAS rate of 20 kHz. The experiment (sequence “cphitrho” of the Bruker library) combines proton inversion-recovery and CPMAS to transfer polarization to the carbons. The conditions for CPMAS were identical to the $^{13}$C CPMAS experiment described above. Sixteen points were measured with a variation in the duration of the spin-lock pulse from 0.001 to 0.050 s and a constant field strength
of 12.5 kHz. The inter-scan delay ($d_1$) was 10 s and each point was acquired with 32 scans.

Direct detection proton spin–lattice $T_1$ relaxation times were measured for the HPC-FEN mixtures (samples 5 to 8) and pure fenofibrate with the 1.3 mm probe at a MAS rate of 50 kHz. The experiment applied pulses and performed FID acquisition exclusively on the proton nuclei. The inversion-recovery sequence was followed by a short $T_2$ filter double-spin echo period of 2 ms to filter very broad signals. Sixteen points were measured with variable durations of the inversion-recovery delay between 1 and 8 s. The inter-scan delay ($d_2$) was 20 s and each point was acquired with 32 scans.

Direct detection proton $T_{1p}$ relaxation times were measured for each HPC-FEN mixture (samples 5 to 8) and pure fenofibrate with the 1.3 mm probe at a MAS rate of 50 kHz. The experiment applies pulses and perform FID acquisition exclusively on the proton nuclei, comprising a 90° hard pulse followed by a spin-lock pulse with a field strength of 42.3 kHz. Sixteen points were measured with the duration of the spin-lock pulse from 1 to 150 ms. The inter-scan delay ($d_1$) was 12 s and each point was acquired with 32 scans.

2D PARIS($^{13}$C-selective)-HSQC-PDSD spectra (sequence of Figure S9a) were measured for sample 5 (mixture HPC-FEN) with the 3.2 mm probe at a MAS rate ($\omega_m$) of 20 kHz. The interscan relaxation delay $d_1$ was 0.5 s and it is followed by PARIS[23,25] irradiation that was applied with a field strength of 42.3 kHz. Sixteen points were measured with the duration of the spin-lock pulse from 1 to 150 ms. The inter-scan delay ($d_2$) was 12 s and each point was acquired with 32 scans.

Selective excitation on $^{13}$C was applied at the frequency of the carbon methyl signal C-9 of HPC and performed FID acquisition exclusively on the proton nuclei. Heteronuclear decoupling during acquisition of the FID was performed with SPINAL-64 with a proton field strength of 79.4 kHz. The number of scans per $t_1$ increment was 32. Two spectra were acquired with a PDSD mixing time of 0 and 110 ms.

**ORCID**

Edmont Stoyanov © https://orcid.org/0000-0002-1571-9259

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