Research Article

Differential Analysis of O-(2-hydroxypropyl) cellulose by Using Two-Dimensional 1H-NMR Spectroscopy

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Abstract

Two-dimensional ^{1}H-NMR is used to determine the intra-molecular interactions of O-(2-hydroxypropyl) cellulose (HPC) in aqueous (D_2O), DMF and DMSO solutions. Four grades HPC with different molecular weights are analyzed by using NOESY (Nuclear Overhauser Effect Spectroscopy) for proton-proton cross-interactions. A strong dependence of the polymer chain structure on the HPC Molecular Weight (MW) is overserved. The lower MW HPCs exist in solutions as a more linear chain showing less proton-proton interactions whereas the higher MW HPCs are more twisted and bended and form a tangled molecule mess with very intensive interactions between the -CH_3, -CH_2- and -C-H protons. From all the grades, the ultra-low molecular weight HPC-UL (MW 20,000) revealed the weakest proton-proton cross-relaxations and exists in solutions probably only as an almost linear chain polymer.

Introduction

The solubility enhancement of poorly soluble drugs is known to be one of the biggest challenges in the pharmaceutical technology. The permanently increasing number of APIs (Active Pharmaceutical Ingredient) with low solubility creates the need of new polymers that enhance the API solubility and bioavailability. Usually used as a binder, the O-(2-hydroxypropyl) cellulose (Hydroxypropyl Cellulose, HPC) revealed a new and unexpected activity as solubility enhancer for poorly soluble drugs. Recent papers described the successful application of HPC as solubilizing polymer for nano-suspensions and amorphous solid dispersions [1-3]. Ito and Konnerth [1,2], described in two papers the application of HPC and copovidone for the preparation of nano-suspensions where the best results were achieved with the low molecular weight HPC-SSL. Zecevic et al., [3], reported the application of HPC-SSL in combination with HPMCAS as a solubilizer for Dipyridamole and Griseofulvin, two poorly soluble drugs with rash precipitation under certain pH conditions. Prodduturi et al., [4], reported an investigation of different molecular weight HPCs in Hot Melt Extrusion (HME) with clotrimazole where the

lowest MW HPC demonstrated better dissolution results than the higher MW HPCs. This was explained with the possible increased entanglement of the polymer chain and a slower polymer erosion. Ten years later Osawa et al., [5] and Sarode et al., [6], gave the same explanation for the better solubility and permeability results of sulindac [5] and felodipine [6], in HPC-SSL containing solid dispersions. A short time ago, some of us published the efficient performance of the ultra-low molecular weight HPC-UL as best milling aid and solubilizer for Izraconazole in aqueous nano-suspensions [7].

The reason for the much better functionality of the low mwt HPC as solubilizer for poorly soluble drugs is still not well clarified and a close look at the HPC structure in solution could give the answer of this question.

The chemical structure of hydroxypropyl cellulose was already investigated per ^{1}H and ^{13}C-NMR [8,9]. Kimura et al., [8], described detailed the Degree of Substitution (DS), Molecular Substitution (MS) and even the different reactivity of the hydroxyl groups in the cellulose molecule based on the ^{13}C-NMR in D_2O peaks integrals. By using the same carbon NMR method, Desai et al., [9], explained the different performance

of HPCs with different cloud points also giving details on the ratio outer/single carbons vs inner carbons. These fundamental works assume the HPC molecular structure as independent of molecular weight and that the grade examined in the studies was assumed to be representative of the entire family of HPC molecular weights.

Based on the published information we cannot explain why only the low molecular weight HPC works as a drug solubilizer. No meaningful differences in the physico-chemical properties (solubility, DS, MS and inner/outer carbon ratio) between the different grades of this cellulosic ether can be found. In order to get better understanding in this phenomenon, we decided to investigate what happens with the dissolved HPC polymer chain in the space by using a special two-dimensional ^{1}H-NMR analysis - Nuclear Oherhauser Effect (NOE). Standard ^{1}H-NMR spectra show the proton spin-spin coupling due to chemical bonds, whereas NOE occurs through the space when the distance between two protons is less than 4.9 Å. Thus, by using NOESY (Nuclear Overhauser Effect Spectroscopy) we can gain information on atoms and groups that are in close proximity to each other in the same molecule. This NMR method has been already successfully used for the investigation of three-dimensional structures of many proteins and other macromolecules [10-14].

In the present work, we describe a differential analysis of four grades hydroxypropyl cellulose with different molecular weights (viscosities) by using NOESY in DMSO-d_6, DMF-d_7 and D$_2$O.

Experimental

Chemicals

Hydroxypropyl cellulose (HPC) with average molecular weight of 140,000 (HPC-L), 100,000 (HPC-SL), 40,000 (HPC-SSL) and 20,000 (HPC-UL) were provided by Nippon Soda, Japan. All the HPC grades have similar physico-chemical characteristics like Molecular Substitution (M.S.), Hydroxypropoxy Content (HPO) and outer methyl+single methyl to inner methyl ratio [6] (outer/inner) were selected for the measurements (Table 1). The only relevant difference is in the Molecular Weight (MW).

Deuterated dimethyl sulfoxide (DMSO-d_6, 99.9% D) and deuterated dimethyl formamide (DMF-d_7, 99.5% D) were purchased by FUJIFILM Wako Pure Chemical Corporation, Japan. Deuterium oxide (D$_2$O, 99.8% D) was purchased by Tokyo Chemical Industry, Japan.

Sample preparation

For DMSO-d$_6$ and DMF-d_7, in order to avoid the OH groups exchange with residual H$_2$O, the samples were prepared as follow: a. preliminary weighted amount of HPC was dried at 45°C overnight, then placed in a desiccator over CaCl$_2$ for 30min under vacuum; b. the sample was then dissolved in the deuterated solvent (0.5%) at room temperature under nitrogen atmosphere. For deuterated water (D$_2$O), where the residual H$_2$O doesn't play an important role, the sample preparation involves weighting and preparing of a 0.5% solution.

Equipment & measurement conditions

Bruker Biospin AVANCE III HD 500 Onebay was used for the measurements: mixing time 0.3s; number of scans 8. Tetramethyl silane (Me$_4$Si) was used as an internal standard.

Results and discussion

The hydroxypropyl cellulose is a cellulosic ether that is composed of approximately 25-30% cellulose and 70-75% polypropylene oxide (HPO content Table 1, Figure 1).

Table 1: Physico-chemical Characteristics of HPC.

HPC	MW^a	M.S.	HPO(%)^b	Outer/Inner
L	1,40,000	3.83	74.8	1.11
SL	1,00,000	3.62	73	1.13
SSL	40,000	3.67	73.4	1.1
UL	10,000	3.35	70.5	1.07

^{a}GPC method; average value. ^{b}JP17 method.

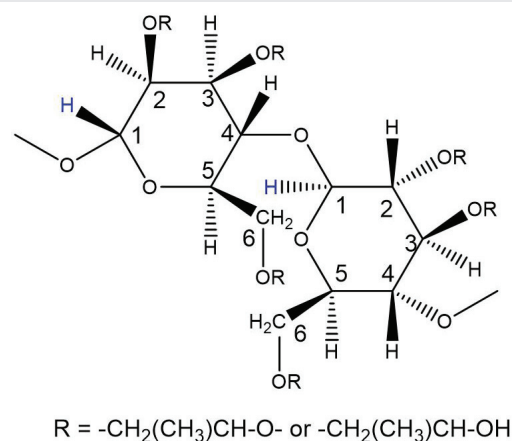R = -CH$_2$(CH$_3$)CH-O- or -CH$_2$(CH$_3$)CH-OH

Figure 1: Idealized chemical structure of HPC.

The cellulose bone of the polymer chain is responsible for some rigidity and hydrophilicity of the molecule, whereas the side polypropylene oxide chain for some hydrophobicity and flexibility.

The possible theoretical proton-proton cross-relaxations in HPC-solutions are summarized in Figure 2.

Depending on the linearity of the chain and its position in the space, the intensity of the interactions can vary.

^{1}H-NMRs for HPC-L, SL, SSL and UL

The chemical shifts of the ^{1}H-NMR spectra for all HPC grades measured in DMSO-d_6, DMF-d_7 and D$_2$O are given in Table 2.

Firstly, we have measured the ^{1}H-NMRs in deuterated organic solvents that have no exchangeable protons: Dimethyl Sulfoxide (DMSO) and Dimethyl Formamide (DMF), where the O-H protons cannot be exchanged and show own peak in the spectrum. The samples were preliminary dried down to avoid

any O–H exchange with the residual water. Figure 3 shows the ^{1}H-NMR spectrum of HPC-L in DMSO-d_6 (as example) with the peaks of the different protons.

The methyl groups appear as singlet at 1.02ppm whereas the methylene groups (-O-CH_2–; -O-CH(CH$_3$)-CH_2-O-, cel-CH_2-O-), inner and outer alpha-protons (CH$_3$-C-H, HO-CH(CH$_3$)-CH$_2$-) and cellulose protons (cel-H from C2, C3 and C4) are low-field shifted as a multiplet at 3.05-3.89ppm. For this study, it was not necessary to distinguish between the protons of tertiary and secondary carbons in this multiplet since their interaction with the protons of the other functional groups is identical. The C1 cellulose protons appears as a broad singlet at 4.39ppm. The hydroxyl protons, exchangeable with D$_2$O, appear as two broad singlets: one stronger at 4.42ppm and one weaker at 4.22ppm. In order to define the exact position of the hydroxyl protons, deuterated water (D$_2$O) was added to the sample solution. After the D$_2$O addition, the peaks at 4.22 and 4.42ppm disappeared while the peak for the C1 proton remained unchanged at 4.39ppm (Figure 4).

The other HPC grades (SL, SSL, and UL) have identical ^{1}H-NMRs with HPC-L in both DMSO-d_6 and DMF-d_7.

The ^{1}H-NMR spectrum of HPC in aqueous solution looks slightly different compared to those in DMSO and DMF. The methyl groups appears as a sharp singlet at 1.18ppm. The methylene groups (-O-CH_2–; -O-CH(CH$_3$)-CH_2-O-, cel-CH_2-O-), inner (CH$_3$-C-H) and outer (HO-CH(CH$_3$)-CH$_2$-) alpha-protons as well as the cellulose protons (cel-H from

Figure 3: ^{1}H-NMR spectrum of HPC-L in DMSO-d_6.

Figure 4: ^{1}H-NMR spectrum of HPC-L in DMSO-d_6 after addition of D$_2$O.

C2, C3 and C4) are all low-field shifted as multiplet at 3.24-3.98ppm. The C1 cellulose protons appears as a broad singlet at 4.53ppm. The hydroxyl protons cannot be observed because of H-D exchange in solution with D$_2$O. Figure 5 shows the ^{1}H-NMR of HPC-L as example.

All other HPC grades (SL, SSL, and UL) have identical ^{1}H-NMRs with HPC-L.

NOESYs of HPC-L, SL, SSL and UL NOESYs in DMSO-d$_6$

The HPC with the highest molecular weight (L) showed multiple H-H interactions between the functional groups (Figure 6). The strongest was the one between the protons of CH, CH_2 and CH_3 groups (III and IV). This interaction was very strong, whereas it is independent on which proton was is the source. This speaks for a close proximity pf those groups in the space. The methyl protons demonstrated also interaction with the C1 protons form the cellulose moiety (II) as well as with the protons of the hydroxyl groups (I). This speaks for close distance between the inner and outer propoxyl groups and cellulose skeleton.
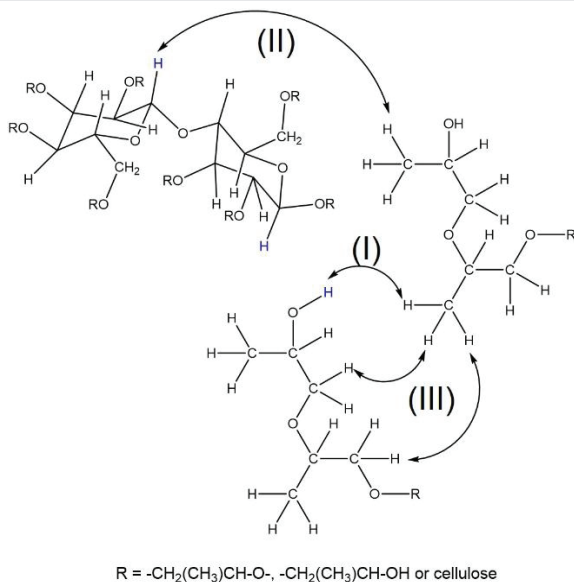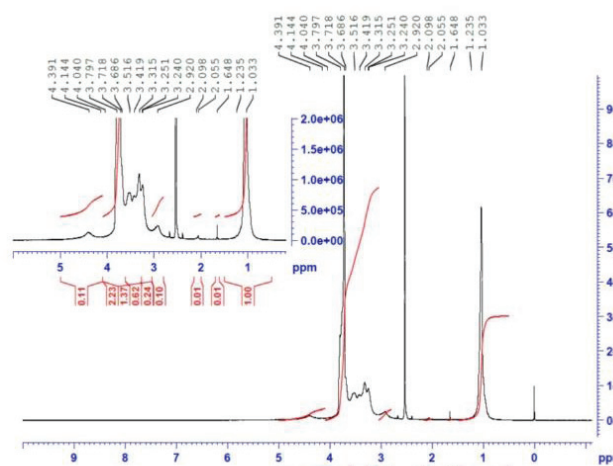
Figure 2: Possible proton-proton cross-relaxations between the functional groups in the HPC molecule.

Table 2: ^{1}H-NMR spectra of all grades HPC.

Solvent	δ in ppm			
	CH$_3$	CH_2, CH	H C1	OH
DMSO-d_6	1.02, s	3.05-3.89, m	4.39, br. s.	4.22, 4.42, br. s.
DMF-d_7	1.07, s	3.05-3.88, m	4.56, br. s.	4.32, 4.56, br. s
D$_2$O	1.18, s	3.24-3.98, m	4.53, br. s.	not observable

The lower molecular weight grades HPC, revealed the same interactions however with decreased intensity, proportionally to the decrease of their molecular weight (Figure 7).

Generally, the intensity of all observed cross-relaxations (I–IV) was decreased with the decrease of the HPC molecular weight. The biggest difference was observed in the cross-relaxations between the hydroxyl and C1 cellulose protons and methyl group protons. We can conclude that the distance between the end-propoxy groups and cellulose skeleton is bigger at the lower molecular weight HPC. At HPC-UL (MW 10,000), the cross-relaxation was with the lowest intensity speaking for an almost linear, not much bended structure in DMSO solution.

NOESYs in DMF-d₇

The ¹H-NMRs in DMF-d_7 were almost identical with those in DMSO-d_6. Differences were observed in the NOE spectra where the proton-proton cross-relaxations in DMF were much stronger (Figure 8).

In DMF-solutions, the HPC molecule seems to be more tangled than in DMSO-solutions. Thus, in DMF, the functional groups appear to be in closer proximity in the space and interact stronger compared to DMSO. This is also confirmed by the NOESYs of the other HPC grades (SL, SSL and UL). As shown in Figure 9, also in DMF, a significant decrease of the H–H-cross-relaxations by decreasing the HPC MW was observed: HPC-UL (black) vs. HPC-SL (red).

NOESYs in D₂O

In deuterated water (D₂O), all the observed proton-proton cross-relaxations were generally weaker compared to DMSO and DMF (Figure 10, example HPC-L).

As expected, proton-proton cross-relaxation between the hydroxyl and methyl group protons (I) was missing because of the exchanged O-H proton. The intensity of the cross-relaxations III and IV in aqueous solutions are the strongest
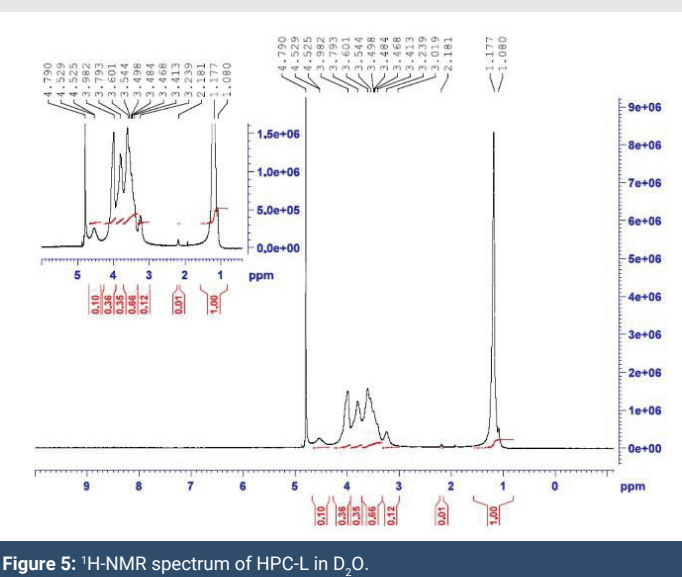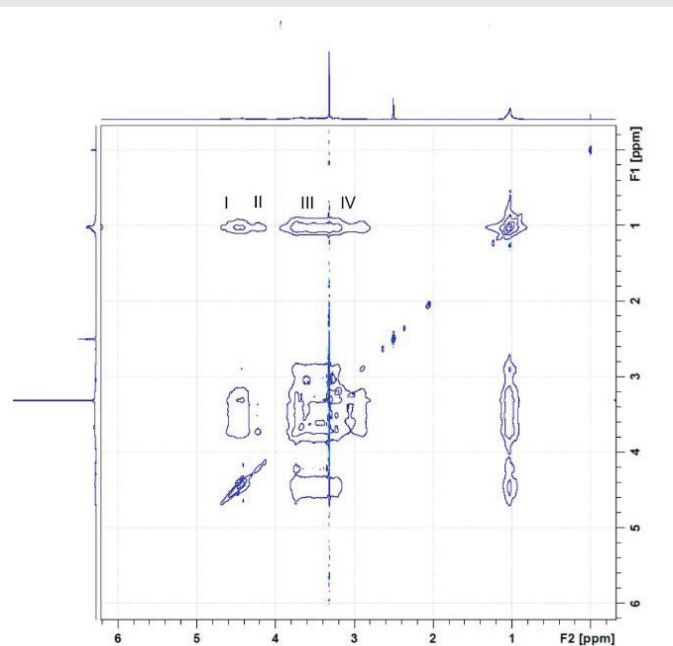

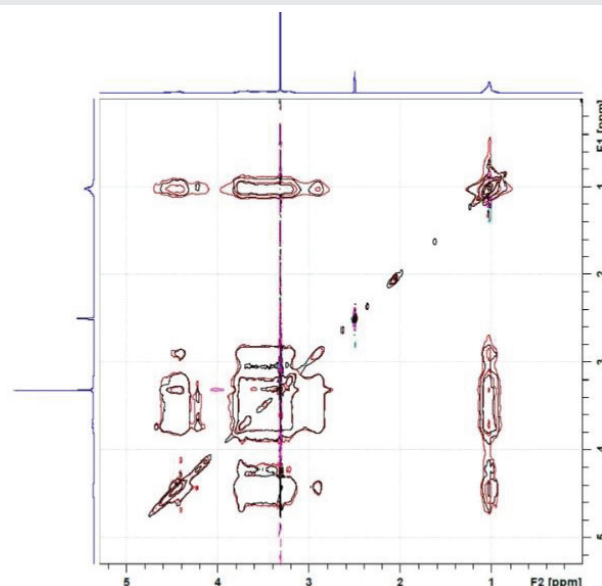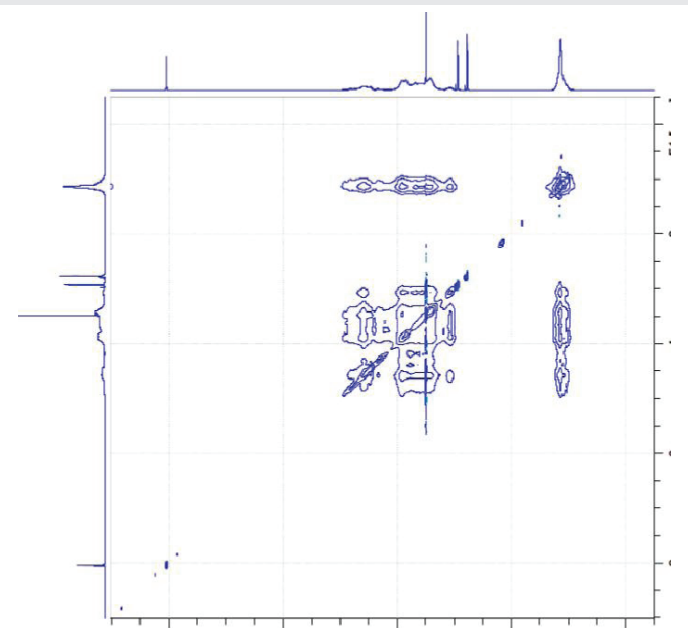**Figure 6:** NOESY of HPC-L (140,000) in DMSO-d_6.

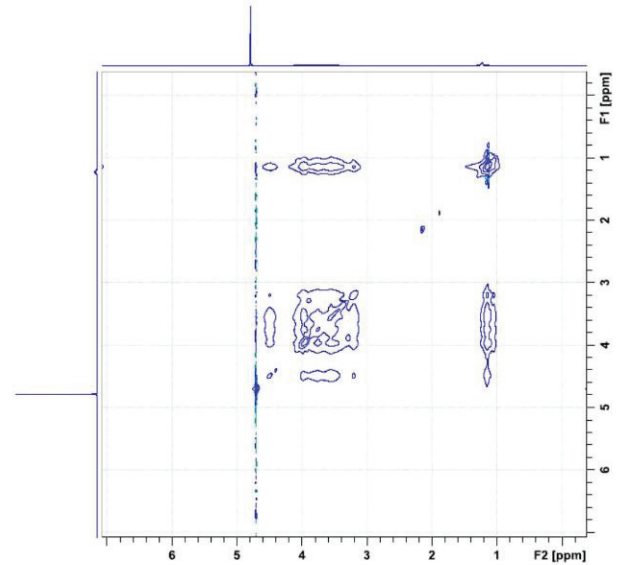**Figure 7:** NOESY of HPC-SL (100,000, red) and UL (20,000, black) in DMSO-d_6 (as example).

like in DMSO and DMF whereas cross-relaxation II (C1 cellulose protons and methyl group protons) is weaker (Figure 10). Obviously in water, the HPC molecule is better dissolved, fully hydrated and stretched compared with the organic solvents (DMSO and DMF). The polymer chain is open giving a less tangled structure. Additionally, the distances between the methyl group protons and C1 cellulose protons (II) is bigger in water solutions than in DMSO and DMF that could facilitate interaction with other small molecules.

Like in the organic solvents DMSO and DMF, in aqueous solution all other HPC grades revealed a decrease of the proton-proton cross-relaxations proportional to the decrease of their molecular weight/molecule length (Figure 11).

Figure 5: ¹H-NMR spectrum of HPC-L in D₂O.

In all solvents used, the ultra-low molecular weight HPC-UL revealed the lowest intensity of the proton-proton cross-relaxation confirming a less tangled and bended structure in solutions compared with the other, higher MW HPC grades (L, SL and SSL, Figure 12).

In aqueous solutions, the observed differences in the intensity of the CH_3-CH_3 interactions (proton-proton cross-relaxations) at 1.1ppm caused by closer proximity of this functional groups in aqueous solution, confirm the observed minor differences in the cloud point of the different HPC grades. A stronger CH_3-CH_3 interaction with increase of the HPC molecular weight corresponds to a decrease of the cloud point (Figure 13).

Figure 10: NOESY of HPC-L (140,000) in D_2O.

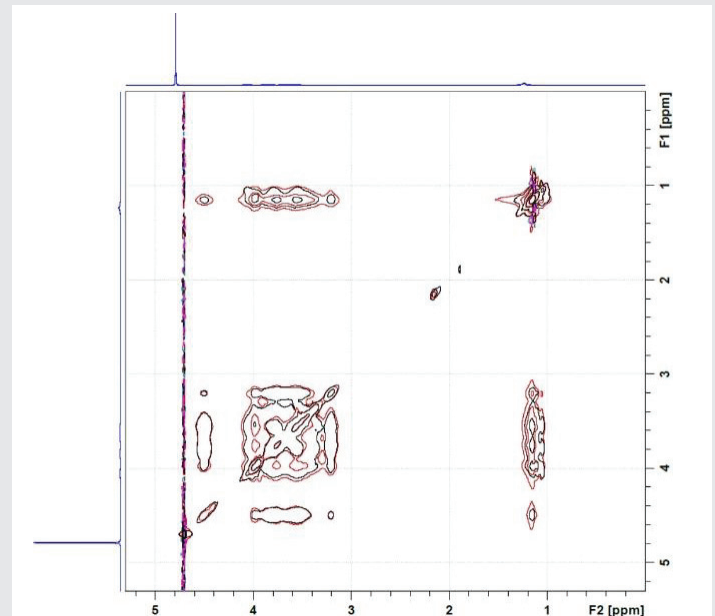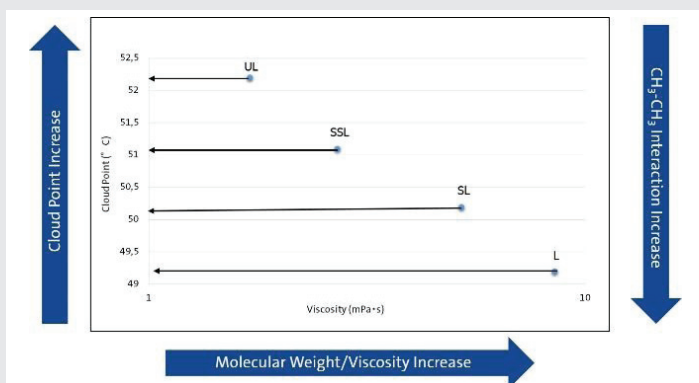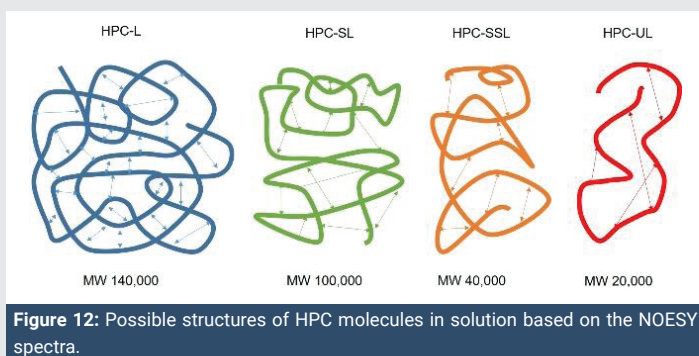**Figure 8:** NOESY of HPC-L (140,000) in DMF-d_7.

Figure 11: NOESY of HPC-SL (100,000, red) and UL (20,000, in black) in D_2O (example).

Conclusions

NOESY analysis of HPC solutions in organic solvents (DMSO and DMF) and aqueous solution revealed a relation between molecular weight (polymer chain length) and structure: low MW HPC exhibits more linear structure with low intensity proton-proton cross-relaxations between the functional groups protons. The aqueous HPC solutions favor a more "relaxed" and less tangled polymer chain with bigger space between the functional groups. In organic solvents, the HPC chain is more tangled and bended with multiple cross-relaxations. The differences in the HPC grades cloud point caused by the interactions between the methyl groups in the space is also proportional to their molecular weight. The

Figure 9: NOESY of HPC-SL (100,000, red) and UL (20,000, black) in DMF-d_7 (as example).

Figure 12: Possible structures of HPC molecules in solution based on the NOESY spectra.

Figure 13: Molecular weight – cloud point dependence at the tested HPC grades.

ultra–low molecular weight HPC–UL demonstrated the lowest strength of molecular interactions due to a more linear polymer structure.

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