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DETERMINATION OF THE CHEMICAL STRUCTURE OF CELLULOSE-BASED BIOPOLYMERS

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ABSTRACT

Cellulose-based biopolymers are used by pharmaceutical industries for drug delivery. In the case of cellulose ethers, the drug delivery function is highly impacted by the molecular structure, namely the degrees of substitution of the polymer chains. The complexity of hydroxypropyl methylcellulose (HPMC) structure and resulting properties is due to the presence of two grafting groups, the methoxy group (OCH_3) and the hydroxypropyl group (OC_3H_6OH). The goal of this work is to present an original way to characterize and determine the structure of HPMC based on ^{13}C-Nuclear Magnetic Resonance (NMR). Quantitative determination of the degrees of substitution is achieved.

Keywords: chemical structure, degree of substitution, HPMC, cellulose-based polymers, ^{13}C-NMR.

1. INTRODUCTION

Hydroxypropyl methylcellulose (HPMC) is a cellulose-based biopolymer that presents great interest for pharmaceutical industries as drug delivery support or matrix [1-5]. Important molecular variables that control the drug delivery function are the nature of the monomers and monomer linkers, monomer sequence distribution along chains, the average molecular weight and molecular weight distribution, molecular conformations and molecular architecture. For drug delivery systems, the important polymer bulk properties, which derive from the polymers molecular properties, are solubility, biocompatibility, biodegradability, and stability [6]. The usefulness for drug delivery of these nonionic cellulose ethers [7] such as HPMC is also based on four key attributes: efficient thickening, surface activity, film-forming ability, and the ability to form thermal gels that melt upon cooling [8]. Suppliers of HPMC and other cellulose ethers produce it with different properties and dissimilar purity [9] due to broad source of cellulose. For this reason, pharmaceutical industries are interested in high performance characterization techniques of HPMC before using it in formulated systems. The most important microstructural parameter of HPMC is the density of grafting groups on polymer chains [10]. The goal of this work is to present an original way to characterize and determine the structure of hydroxypropyl methylcellulose (HPMC) based on ^{13}C Nuclear Magnetic Resonance (NMR) [11].

2. MATERIALS AND TECHNIQUES

2.1 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC) were manufactured by Dow Chemical Company and kindly supplied by Dow Chemical Company-Colorcon (France). The complexity of HPMC structure is due to the presence of two grafting groups on different sites on the polymer chains. In order to propose a precise characterization of HPMC polymers, preliminary analyses were done using model polymers intervening in the manufacture of HPMC, like cellulose (C), methyl cellulose (MC), and hydroxypropyl cellulose (HPC). These three polymers have an increasing complexity in term of lateral substituents. Cellulose ethers are obtained by chemical reaction of the hydroxyl groups at positions 2, 3 and/or 6 of the anhydroglucose residues of cellulose, which is made of D-glucopyranose units of conformation chair, bonded through β(1→4) glycosidic linkages. Hydroxypropyl methylcellulose (HPMC), one of the cellulose ether, contains two types of substituents: the methoxy group (OCH_3) and the hydroxypropyl group (OC_3H_6OH).

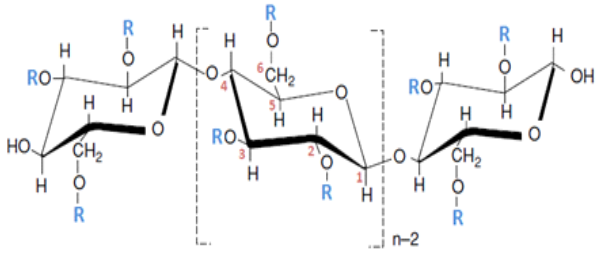


Figure-1. Chemical structure of hydroxypropyl methylcellulose (HPMC); R = H, $-CH_3$ or $-(OCH_2CHCH_3)OH$

The chemical structure of HPMC is shown in Figure-1. The physicochemical properties of HPMC polymers are strongly affected by the methoxy group content and the hydroxypropoxy group content [12]. However, these products are described by the degree of substitution (DS) and the molar substitution (MS). Each anhydroglucose unit in the cellulose chain has three hydroxyl groups available for modification. Thus, if all three available positions on each unit are substituted, the DS is designated as 3, if average of two on each ring is reacted, the DS would be 2. The term DS is reserved for substituents that block reactive hydroxyl groups (methoxy groups). The substitution is described by the MS. i.e., the

number of moles of hydroxypropyl group per mole of anhydroglucose in the chain.

Along the cellulose backbone, methyl substitutes constitute hydrophobic zones whereas hydroxypropyl groups are more hydrophilic. The introduction of these groups allows HPMC to behave as a surfactant. Thus HPMCs are adsorbed at fluid interfaces lowering the surface tension. By increasing the degree of substitution, the polymer hydrophobicity and then the interfacial activity increase [13]-[15].

2.2 Nuclear Magnetic Resonance (^{13}C - NMR)

In the case of cellulose based polymers where the crystalline structure plays an important role in its final properties (solubility, thermal properties …), solid-state NMR can offer information about structural properties such as degree of crystallinity, nature of allomorphs and their proportion. This technique gives information on the presence of specific organization into cellulosic chains of HPMC polymer. The advantage is the direct use of samples without passing through a dissolved state. In our study, we have used the Cross-Polarization Magic Angle Spinning (CP-MAS) technique. Cross polarization uses the proximity between the abundant spins nuclei (^{1}H) and rare spins nuclei (^{13}C) and their coupling by dipolar interaction. The abundant spins are magnetized under B_0 and then one part of their magnetizing is transferred to the rare spins during the CP step. Therefore, the CP experiment improves carbon sensitivity using the magnetization of protons [11]. The analysis was carried out on a Bruker Avance II (400MHz) Spectrometer with Bruker CP-MAS BB 2.5 mm probes. The rotational frequency was fixed at 10,000 Hz.

3. RESULTS AND DISCUSSIONS

3.1 Determination of the substitution degrees (DS and MS) by ^{13}C-NMR

Type and amount of substituted grafted groups along the polymer chain can influence on drugs release [16], [17]. This fact is due to solubility of cellulose ethers according to the substitution degrees. The substitution degree value affects also HPMC polymer properties such as the thermal gelation [18]. For this reason, the microstructure investigation is required before using HPMC in pharmaceutical formulations. To be precise, the term "DS" represents the number of methoxy groups attached on a glycosidic unit, and the term "MS" represents the number of moles of hydroxypropoxy group per mole of anhydroglucose. The term "Total DS" means the number of hydroxyl groups of the glycosidic unit (attached with C2, C3 and C6) substituted by the methoxy and the hydropropoxy groups.

In this original work, NMR spectroscopy was used for the quantitative determination of substitution degrees (DS and MS) which allows a fine describing of the pattern of cellulose ethers structure [10]. Enzymatic treatment of the polymer is needed before analysis. This step requires the cleavage of the glycosidic units. Figure-2 shows an example of ^{13}C-NMR spectrum of hydrolyzed HPMC. The spectrum of Figure-2 shows a complete hydrolysis of glycoside-linked. This result was necessary to realize thereafter a good NMR analysis. The determination the substitution degree was carried out as follows: the first step is to quantify the total degrees of substitution (DS_T), the second is to quantify the MS values and by subtracting the MS to the DS_T, the DS values can be obtained.

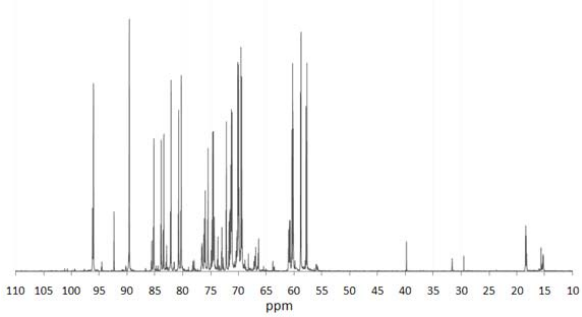
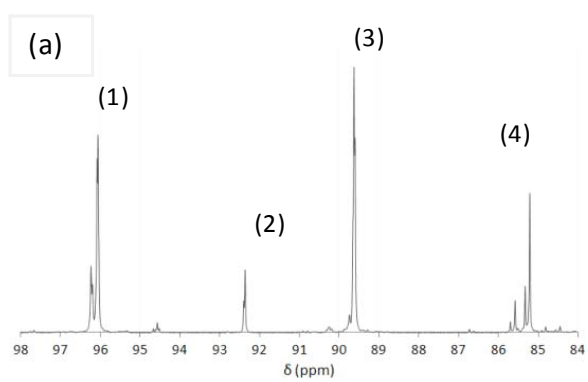


Figure-2. ^{13}C-NMR spectrum of hydrolyzed HPMC.

3.2 Quantitative determination of the total Degree of Substitution (DS_T)

As mentioned above, the DS_T represents the number of hydroxyl groups substituted on the glycosidic unit ($DS_T \leq 3$). The spectrum of hydrolyzed HPMC (Figure-2) seems complex. However, it presents many peaks similar to the spectra of hydrolyzed MC and HPC (not shown). They were interpreted with the aid of published assignments related to unsubstituted and monosubstituted anhydroglucose units [20]-[22]. On this basis, the spectra obtained on hydrolyzed cellulose ethers were interpreted for determination of the total DS_T, as follows:

The hydrolyzed cellulose ethers present three singlets due to the anomeric carbon (C1) (Figure-3a): the first at 96-97 ppm (1) due to C1 of β-glucopyranoses. A singlet at 92.4 ppm (2) attributed to C1 in α-glucopyranoses with no C2-substituent and a singlet at 89-90 ppm (3) due to C1 in C2-substituted α-glucopyranoses.



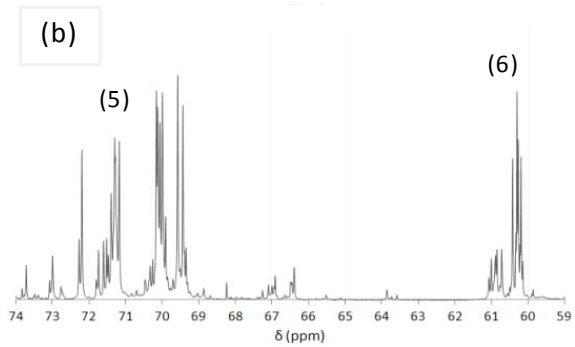


Figure-3. Peak assignments in two interesting regions of ^{13}C-NMR spectrum of hydrolyzed HPMC.

The signals at 71-71.5 ppm and at 60-61.5 ppm are due to substituted C6 (5) and to all C6 carbons (6) respectively (Figure-3b). The numerous signals in the range 79-86 ppm are due to ring carbons (C2 and C3 of α and β-glucopyranoses). The other signals present in the 68-78 ppm region are due to the methine and methylene carbons of the hydroxypropoxy groups.

The degrees of substitution for anhydroglucose positions 2, 3 and 6 (DS2, DS3 and DS6, respectively) were determined as follows:

DS2 is the ratio between the area of the signal at 89-90 ppm due to C1 in C2-substituted α-glucopyranoses and the sum of this area and that of the C1 signal at 92.4 ppm due to C2-unsubstituted α-glucopyranoses.

DS3 is the ratio between the area of the signals at 85-86 ppm (peak 4 in Figure-3.a) due to substituted C3 atoms in β-glucopyranoses and that of C1 signals from β-glucopyranoses at 96-97 ppm.

DS6 is the ratio between the area of the signals at 71-71.5 ppm due to substituted C6 atoms and the total area of all C6 signals (60-61.5 ppm). The total DS_T is the sum of DS2, DS3 and DS6.

$$DS_T = DS2 + DS3 + DS6 \quad (1)$$

Table-1 shows the results obtained for MC and three types of HPMC.

Table-1. Partial and total degrees of substitution of MC and various HPMCs as determined by ^{13}C-NMR.

Samples	DS2	DS3	DS6	DS_T
MC	0.82	0.42	0.65	1.89
HPMC A	0.89	0.46	0.77	2.12
HPMC B	0.82	0.43	0.67	1.92
HPMC C	0.75	0.33	0.58	1.66

In this case, the total DS_T represents not only the number of carbons substituted by methoxy groups on glycosidic unit, but also contains carbons substituted by hydroxyproxy groups. The values of DSi indicate that the positions 2 and 6 of the anhydroglucose are much more substituted than position 3. To determine only the DS of methoxyl group, we must know the values of Molar Substitution (MS).

3.3 Quantitative determination of the Molar Substitution (MS)

MS represents the amount of hydroxypropoxy groups $-OCH_2(CHOH)CH_3$ on the anhydroglucose unit of cellulose. The internal and external MS were determined as follows [23]: $MS_{(int)}$ and $MS_{(ext)}$ are respectively the ratio between the area of the signals at 15-16 ppm (1) (due to methyl CH_3 of internal hydroxypropoxy group) and the area of the signals at 18-19 ppm (2) (due to CH_3 of external hydroxypropoxy group) (Figure-4) and that of total area of all anomeric carbons C1 signals.

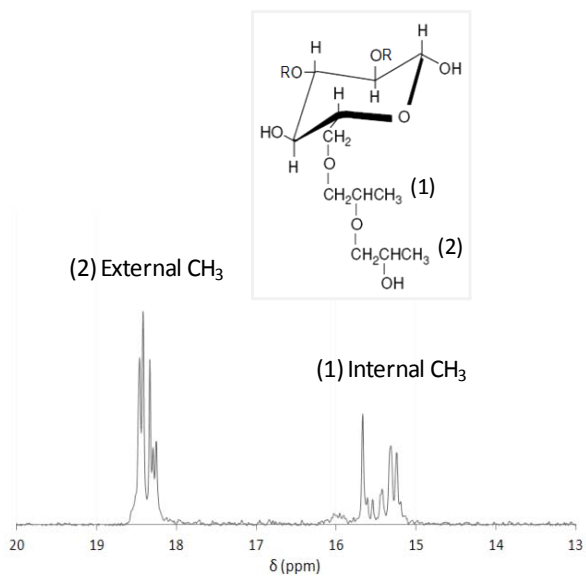


Figure-4. Peak assignments in the region of hydroxypropoxy chemical shifts

The total Molar Substitution MS was calculated by the sum of $MS_{(int)}$ and $MS_{(ext)}$. Knowing MS, the degree of substitution (DS) of methoxy groups was thus calculated as total DS_T minus total MS (Table-2). Since the hydroxypropoxy may polymerize during synthesis, one can define a degree of polymerization for this group as:

$$Dp = MS / (3 - DS) \quad (2)$$

The term (3-DS) represents the number of carbons substituted only by hydroxypropoxy groups. Thus in this case, D_P represents the average number of repeat units of hydroxypropoxy groups.

Table-2. Internal MS, external MS, total MS, degree of substitution (DS) and degree of polymerization of HPC and various HPMCs as determined by ^{13}C-NMR.

Samples	MS_{int}	MS_{ext}	Total MS	DS	Dp
HPC	2.67	3.34	6	0	2
HPMC A	0.10	0.15	0.25	1.87	0.22
HPMC B	0.05	0.08	0.13	1.79	0.11
HPMC C	0.08	0.15	0.23	1.43	0.15

The results show that the HPC polymer possesses high MS value (6 hydroxypropoxy groups per anhydroglucose unit), and then the possibility of duplication is important. Note that HPC used in our study are considered as witness in order to define the signature peaks of their functional groups. However, the HPMC polymers present a low content of hydroxypropoxy groups relative to methoxy groups. In addition, the $MS_{(ext)}$ values are higher than those of $MS_{(int)}$, and this result seems logical. In order to evaluate the degree of polymerization D_P of hydroxypropoxy groups of HPMC types, the ratio between MS and the number of substitution sites of this group is calculated. The degree of polymerization of hydroxypropoxy groups depends on the accessibility of unsubstituted hydroxyl groups of methylcellulose during HPMC synthesis. In other words, D_P increases with DS and the hydroxypropoxy groups tend to polymerize for the HPMC with high content of OCH_3 (HPMC A, $D_P = 0.22$) relative to HPMC with low content of OCH_3 (HPMC C, $D_P = 0.15$).

3.4 Carbon reactivities into cellulosic chains

The substitution reaction (etherification) in cellulosic unit depends on the accessibility of three positions (C2, C3, and C6) and their reactivities. However, the partial degrees of substitution of these positions provide the possibility to know how the grafted groups are distributed. The amount of substituents on each carbon in MC and HPMC polymers was determined using the following equation:

$$\text{Reactivity of } C_i = 100 \times (DS_i / DS_T) \quad (3)$$

The results obtained indicate that 43% of the substituents are on C2, 35% on C6 and 22% on C3. Thus C2 and C6 are the most reactive positions. Indeed, position C3 possesses the lower reactivity, which is due to steric hindrance and to intermolecular hydrogen bonding between the C3 hydroxyl (OH) and the ring oxygen (O) of an adjacent monomer. In addition, the formation of intermolecular hydrogen bonds between C6-OH and bridged oxygen lower the reactivity of this position [24]. It is important to note that the methoxy and hydroxypropoxy groups are distributed randomly on C2, C3, and C6 positions. But there is a great possibility that C2 and C6 are substituted rather by OCH_3 than by $OCH_2(CHOH)CH_3$, because during HPMC synthesis, methyl chloride CH_3Cl reacts preferentially with C2 and C3 as propylene oxide is not still present in solution.

4. CONCLUSIONS

In the present work, the complex molecular structure of cellulose ethers in general and particularly of HPMC was determined. To achieve a goal an original ^{13}C-NMR spectroscopic analysis was conducted on hydrolyzed HPMCs. Precise peak assignments allows for the determination of the degrees of substitution for

Anhydroglucose in positions 2, 3, and 6 (DS2, DS3, and DS6, respectively) as well as total substitution DS_T. Quantitative determination of molar substitution (MS) that represents the amount of hydroxypropoxy groups $-OCH_2$ (CHOH) CH3 on the anhydroglucose unit of cellulose was also realized. Thus, the weight percent of substituents into HPMC polymers was determined using the DS and MS values. Finally, the knowledge of degrees of substitution provides the possibility to know how the grafted groups are distributed and thus the reactivities of the substituted carbons. The results indicate that C2 and C6 are the most reactive positions. Given the importance of substitution degrees as a characteristic factor for HPMC polymers, this ^{13}C-NMR spectroscopic methodology offers a new route of precise analysis of these key parameters.

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