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A comparison of gas–liquid chromatography, NMR spectroscopy and Raman spectroscopy for determination of the substituent content of general non-ionic cellulose ethers

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Abstract

This paper describes and compares three techniques that can be used to characterize the substituent content of hydroxypropylcellulose (HPC and L-HPC) and hydroxypropyl methylcellulose (HPMC): gas–liquid chromatography (GLC) with a BP1 column and FI detection, ^{13}C-NMR spectroscopy of hydrolysed samples, and Raman spectroscopy. GLC and ^{13}C-NMR spectroscopy both allow independent quantification of hydroxypropoxyl and methoxyl contents. ^{13}C-NMR spectroscopy, though requiring lengthier sample preparation, has the advantage of also quantifying the degree of substitution at each substitutable glucopyranose hydroxyl. Raman spectroscopy may be useful for rapid approximate estimation of hydroxypropoxyl content. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Hydroxypropylcellulose; Hydroxypropylmethylcellulose; Substitution; Gas–liquid chromatography; ^{13}C-NMR spectroscopy; Raman spectroscopy

1. Introduction

Cellulose ethers (CEs) are a class of semisynthetic polymers obtained by chemical reaction of the hydroxyl groups at positions 2, 3 and/or 6 of the anhydroglucose residues of cellulose. If only a single kind of substituent group is present, they are thus random copolymers of eight possible glucopyranoses: the unsubstituted species, the three possible monosubstituted species, the three possible disubstituted species and the trisubstituted species. Their many industrial applications

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[1] include their use as excipients in drug dosage forms [2,3]. Their properties depend not only on the type of substituent(s), but also on the degree of substitution (DS, the average number of modified hydroxy groups per glucose residue) and the distribution of the substituents along the polymer chain [4–7]. The most important for pharmaceutical use have a DS of between 1 and 2.

The development of new CEs and their quality control both require reliable analytical methods for determining their DS and characterizing their substitution patterns. Early methods for anionic CEs such as sodium carboxymethylcellulose were based on acid–base reactions or precipitation, conductimetry or spectrophotometry [8–12]. For non-ionic CEs of medium-to-high DS, such as methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC), a method that has been widely used is to hydrolyse the alkoxy groups with hydroiodic acid and quantify the resulting halogenated derivatives by redox titration: this is the USP method for hydroxypropylcellulose (HPC) [13,14]. However, all these methods have limited selectivity and sensitivity, and the conductimetric and spectrophotometric methods have only a narrow range of linear response [8]. An alternative that can sometimes be used is selective enzymatic cleavage of the polymer chain at residues with no substituents, which allows determination of the length of the segments composed of substituted glucose units [15,16], but this approach is not applicable to all the common cellulose ethers and, like most of the other methods mentioned above, is laborious and time-consuming.

Most of the more accurate and more generally applicable methods that have been developed are based on nuclear magnetic resonance (NMR) spectroscopy or gas–liquid chromatography (GLC). ^{1}H or ^{13}C-NMR spectroscopy of relatively small CEs in solution is rapid and requires no prior calibration [17–21]. Direct analysis of larger CEs is more problematical: the need to use very dilute solutions (these CEs form viscous dispersions) implies very long scan times and poorly resolved spectra. This problem is particularly serious for ^{13}C-NMR methods, but can be overcome by subjecting the polymer to prior acylation [21–23], methanolysis [24] or hydrolysis [15,18,25,26]; these pretreatments allow most CEs to be dissolved in common solvents, regardless of their DS [27]. GLC methods for determining the DS of CEs involve preparation of the sample by modified versions of the Zeisel alkoxy reaction. Previously published GLC methods use packed columns and thermal conductivity detection [28–32], and this is the prescribed USP method for low-DS hydroxypropylcellulose (L-HPC) and HPMC [13], but better results should in principle be obtained using more modern types of column and detector [33].

In this work we determined the substituent contents of HPMCs, HPCs and L-HPCs jointly covering a wide range of DS by a ^{13}C-NMR method with a simplified preparation stage, a GLC method using a capillary column and flame ionization detection, and a method based on Raman spectroscopy [34]. Our objectives were to assess the scope and limitations of each technique, and the extent to which their results are interchangeable.

2. Materials and methods

2.1. Polymers and reagents

L-HPC: varieties LH-11 (batch 503078), LH-20 (batch 405117), LH-21 (batch 506157), LH-22 (batch 301018) and LH-31 (batch 502032) from Shin-Etsu Chemical (Tokyo, Japan). HPC of medium-high DS: varieties Nisso® M (batches BJ, DC and JD) and Nisso® H (batches BJ and BE) from Nippon Soda (Tokyo, Japan), and Klucel® GF (batch FP10-10293) and Klucel® MF (batch 7857) from Aqualon (Hercules, Wilmington DE, USA). HPMC: Methocel® Premium varieties K4M (batch MM87050902K), K15M (batch 89110712), E4M (batch 87061702) and F4M (batch 89020706) from Dow Stade GmbH (Stade, Germany).

Analytical grade barium carbonate, chromium(III) acetylacetonate, adipic acid and sulphuric acid were supplied by Merck (Darmstadt, Germany), HPLC grade toluene and *o*-xylene by Scharlau (Barcelona, Spain), and GC grade 2-iodopropane, methyl iodide and hydroiodic acid by Riedel de Haën (Seeize, Germany).

2.2. Procedures

2.2.1. Gas–liquid chromatography

The GLC method was based on D-3876-79 [32] and USP23-NF18 [13]: the CE is reacted with hydroiodic acid in the presence of adipic acid, producing 1 mol of methyl iodide per mole of methoxy substituent and 1 mol of isopropyl iodide per mole of propyloxy substituent, and these products are then extracted from the reaction mixture with *o*-xylene and quantified by GLC using toluene as internal reference.

The toluene reference standard (12.5 mg toluene ml^{-1} *o*-xylene) was prepared by adding 3.125 g of toluene to 10 ml of *o*-xylene in a 250-ml volumetric flask and making up to the mark with *o*-xylene. Mixed PrI/MeI calibration standards were prepared by accurately weighing between 50 and 55 mg of adipic acid into 10 ml glass vials, adding 3.0 ml of 57% (w/w) hydroiodic acid and 3.0 ml of the toluene standard, reweighing, and adding 5, 15, 30, 45 or 60 μl of isopropyl iodide and respectively 5, 15, 30, 50 or 80 μl of methyl iodide using a Hamilton microsyringe; the vials were re-weighed again, sealed, shaken to mix the contents, and stored in the dark pending use.

Polymer samples were oven-dried at 105°C for 30 min and stored over silica gel in a desiccator. Samples of between 50 and 55 mg were accurately weighed into 10 ml vials together with approximately the same mass of adipic acid. To each vial was added 3.0 ml of 57% (w/w) hydroiodic acid and 3.0 ml of toluene standard, and the vial was then sealed, reweighed, shaken to mix its contents, and incubated at 150°C for 1 h (with shaking after the first 20 min). Once cool, the vials were reweighed with a view to rejecting any with a mass loss greater than 10 mg.

GLC was performed on a Hewlett-Packard Series II Mod. 5890 chromatograph (Hewlett Packard, Avondale, PA, USA) equipped with a split/splitless injector, a 25 m × 0.53 mm i.d. BP1 fused silica dimethylsiloxane-coated capillary column (Scientific Glass Engineering, Australia) and a flame ionization detector linked via an HP ChemStation to an HP 3365A PC. The injector, detector and oven temperatures were 200, 250 and 60°C, respectively. Nitrogen (C-55, Carburos Metálicos, Spain) was used as both carrier gas (flow rate 5.5 ml min^{-1}) and make-up gas (flow rate 44 ml min^{-1}). In each run, a 1-μl subsample of the organic phase of the standard or sample was injected manually with a split ratio of 10:1. With these conditions, the retention times of methyl iodide, isopropyl iodide, toluene and *o*-xylene are 1.5, 2.2, 3.4 and 8.0 min, respectively.

The stability of standards and sample preparations was evaluated by determining their methyl iodide and/or isopropyl iodide contents after (standards) or before and after (duplicate sample preparations) 1 week storage in the dark at room temperature.

2.2.2. ^{13}C-NMR spectroscopy

Prior to spectroscopy the polymers were hydrolysed by a method simplifying those described by Parfondry and Perlin [15] and Zadorecki et al. [26]. A 1-g polymer sample was stirred in 30 ml of 6 M sulphuric acid for 1.5 h at 20°C. This solution was made up to 90 ml with deionized water, refluxed in an autoclave at 2 atm for 1 h, cooled, and neutralized with barium carbonate. The resulting suspension was left to settle, and the supernatant was then decanted, filtered through Albet No. 242 filter paper and concentrated in a rotary evaporator at 40°C (ethanol was added to facilitate this process). A 1-ml sample of the residue was diluted with an equal volume of D_2O and centrifuged at $3575 \times g$ for 5 min, and 1 ml of the resulting supernatant was analysed by ^{13}C-NMR spectroscopy.

The room temperature ^{13}C-NMR spectra of triplicate samples were recorded at 75 MHz on a Bruker AMX-300 spectrometer (Karlsruhe, Germany) using a 3 mg ml^{-1} solution of chromium(III) acetylacetonate in dimethylsulphoxide, sealed in a capillary tube and placed in the NMR tube containing the sample, as an external standard of $\delta \approx 40$ ppm [35]. Spectra with a width of 301.12 ppm (64 K data points) were obtained using an inverse gated proton decoupling sequence suppressing nuclear overhause enhancement, a 30° carbon pulse flip angle and a relaxation time of 0.44 s; the line broadening before FT was 3 Hz. Signals were identified by comparison with those of methylated and hydroxy-

propylated glucopyranoses [36,37] and unhydrolysed hydroxypropylcellulose [18] (Table 1).

The degrees of substitution at anhydroglucose positions 2, 3 and 6 (DS_2, DS_3 and DS_6, respectively) were estimated, following Leeand Perlin [18], as follows: DS_2 as the ratio between the area of the signal at 90–91 ppm due to C1 in C2-substituted α-glucopyranoses and the sum of this area and that of the C1 signal at 93 ppm due to C2-unsubstituted α-glucopyranoses; DS_3 as the ratio a/b, where a is the area of the signals at 86–87 ppm due to substituted C3 atoms in β-glucopyranoses and b is the combined area of C1 signals from β-glucopyranoses at 96–98 ppm; and DS_6 as the ratio between the area of the signals at 71–72 ppm due to substituted C6 atoms and the total area of all C6 signals. DS was calculated as $DS_2 + DS_3 + DS_6$, and molar substitution (MS) as per Lee and Perlin [18]. In view of the finding that the hydroxypropoxyl side chains of HPMCs exhibited hardly any branching (see Results and Discussion), their total hydroxypropoxyl contents were estimated as the ratio of the area of the hydroxypropyl methyl group signal at 19 ppm to the total area of all anomeric carbon signals [26]; methoxyl content was then calculated as DS minus hydroxypropoxyl content.

2.2.3. Raman spectroscopy

Raman spectra were recorded on a Bruker IFS 66V FT-IR spectrometer (Karlruhe, Germany) equipped with an FRA 106 FT-Raman accessory incorporating an Nd:YAG laser and a germanium detector cooled with liquid nitrogen. For Raman spectroscopy, powdered samples were held in 1.6 mm i.d. glass capillary tubes or aluminium sample holders, depending on particle size; spectra with a spectral resolution of 4 cm^{-1} were recorded using an excitation wavelength of 1064 nm. Hydroxypropoxyl content was estimated following Langkilde and Svantesson (34) as the mean (over triplicate samples) of the ratio between the absorbances by side-chain C–C and anhidroglucose C–H bonds at 1260 and 1367 cm^{-1}, respectively.

3. Results and discussion

3.1. Gas–liquid chromatography

As regards the preparation of samples and standards, the GLC method used in this work differed from the ASTM/USP procedure only in that the volumes of both aqueous and organic phases were increased to improve separation. None of the sample preparations was rejected because of weight loss.

The chief differences with respect to the standard method concerned the chromatographic apparatus and conditions: a capillary column was used because capillary columns generally separate better than packed columns, and FI detection was used because it is more sensitive and less noisy

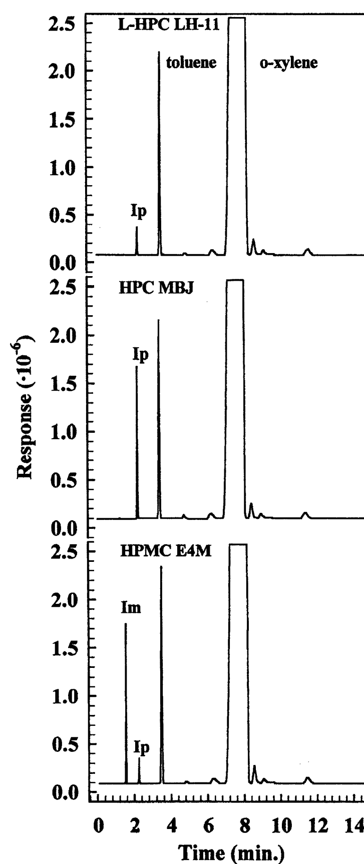

Fig. 1. Chromotagrams of the L-HPC LH-11, the HPC M-BJ and the HPMC E4M. Im, methyl iodide; Ip, isopropyl iodide.

Table 1
^{13}C-NMR signals of HPC and of unsubstituted and monomethoxy- and monohydroxypropoxy- substituted α- and β-plucopyranoses, after Usui et al. [35], Haverkamp et al. [36] and Lee and Perlin [17]

Compound	Anomer	C1	C2	C3	C4	C5	C6	OMe	C7	C8	C9
D-glucopyranose	α	93.3	73.1	74.4	71.2	72.9	62.4				
	β	97.1	75.6	77.3	71.2	77.3	62.4				
2-*O*-methyl-D-glucopyranose	α	90.7	81.9	73.5	71.3	72.8	62.4	59.3			
	β	97.1	85.2	76.8	71.3	77.3	62.4	61.7			
2-*O*-(2-hydroxypropyl)-D-glucopyranose	α	91.4	81.2	73.6	71.0	72.7	62.1		79.0	68.1	19.4
	β	97.3	84.5	76.8	71.0	77.3	62.2		76.8	68.1	19.4
3-*O*-methyl-D-glucopyranose	α	93.4	72.6	84.1	70.6	72.8	62.3	61.3			
	β	97.2	75.1	86.7	70.4	77.3	62.3	61.3			
3-*O*-(2-hydroxypropyl)-D-glucopyranose	α	93.6	72.8	83.7	70.8	71.4	62.0		79.2	68.3	19.4
	β	97.3	75.3	86.5	70.8	75.8	62.2		79.2	68.3	19.4
6-*O*-methyl-D-glucopyranose	α	93.3	73.0	74.3	71.4	71.8	72.6	60.3			
	β	97.3	75.8	77.2	71.4	76.2	72.6	60.3			
6-*O*-(2-hydroxypropyl)-D-glucopyranose	α	93.6	72.9	74.2	71.4	71.8	71.4		77.8	67.6	19.6
	β	97.4	75.6	77.2	71.4	76.2	71.4		77.8	67.5	19.6
HPC	α	104.2	83.6	84.3	80.6	75.8	72.1		75.2	76.4	17.2
									75.8	68.0	19.7
									(C10)	(C11)	(C12)

Table 2
Calibration data of the GLC method

	Methyl iodide	Isopropyl iodide
Slope	0.0947	0.2259
Standard error of slope	0.0007	0.0014
Intercept	0.0017	0.0028
Standard error of intercept	0.0018	0.0023
Correlation	0.9993	0.9995

than thermal conductivity detection, has a wider linear range, and is nowadays more common. The chromatograms obtained had little background noise and tall, narrow, well-resolved peaks (Fig. 1).

3.1.1. Calibration range and linearity

The range of calibration standards used (3.7–60.3 mg ml^{-1} for methyl iodide (equivalent to 0.808–13.2 mg ml^{-1} of methoxy substituent) and 2.8–34.0 mg ml^{-1} for isopropyl iodide (equivalent to 1.25–15.0 mg ml^{-1} of hydroxypropoxyl substituent) covers the entire range of hydroxypropoxyl and methoxyl contents currently found in commercial cellulose ethers. The method showed excellent linearity over this range ($r > 0.999$; see Table 2). For both analytes the relative standard deviation (RSD) of the slope of the calibration line was very low (3.43% and 2.09% for methyl and isopropyl iodide, respectively), and for neither was the ordinate at the origin significantly different from zero ($\alpha \leq 0.05$).

3.1.2. Precision and accuracy

For no concentration of either of the analytes did the RSD of six replicate determinations exceed 2.5% (Table 3). The mean value of these determinations was a mean 99.99% of the known value for methyl iodide (RSD 3.43%) and a mean 99.29% of the known value for isopropyl iodide (RSD 2.09%).

3.1.3. Stability

After storage for 1 week in the dark, the measured concentrations of methyl iodide in standards and samples were a mean 98.67% of the values measured or established a week earlier (RSD 1.25%), and those of isopropyl iodide were a mean 99.44% of the earlier values (RSD 0.50%).

3.2. ^{13}C-NMR spectroscopy

Solubilization by hydrolysis prior to NMR spectroscopy has previously been applied to HPCs and certain other ionic and non-ionic CEs [15,26], but not to L-HPCs or HPMCs. The complete hydrolysis of the CEs studied in this work was shown by the absence of any signal for glucoside-linked C1 at 100–105 ppm in their spectra (Fig. 2 shows the spectrum of one member of each class of CE studied). The spectra furthermore show no signals suggesting loss or alteration of any of the substituent groups [18,25]; they were interpreted with the aid of published signal assignments for HPC and for the unsubstituted and monosubstituted monomers (Table 1).

Table 3
Precision (RSD,%) and accuracy (recovery,%) of the GLC method, as estimated by determination of six replicates of each standard

Methyl iodide			Isopropyl iodide		
Concentration (mg·ml^{-1})	% RSD	% Recovery	Concentration (mg·ml^{-1})	% RSD	% Recovery
3.67	1.456	96.79	2.83	0.179	95.53
11.3	2.374	103.6	8.50	0.523	101.0
22.4	1.853	100.9	17.0	2.292	101.1
37.7	2.313	100.9	25.5	1.399	99.76
60.3	2.388	99.63	34.0	1.954	99.99

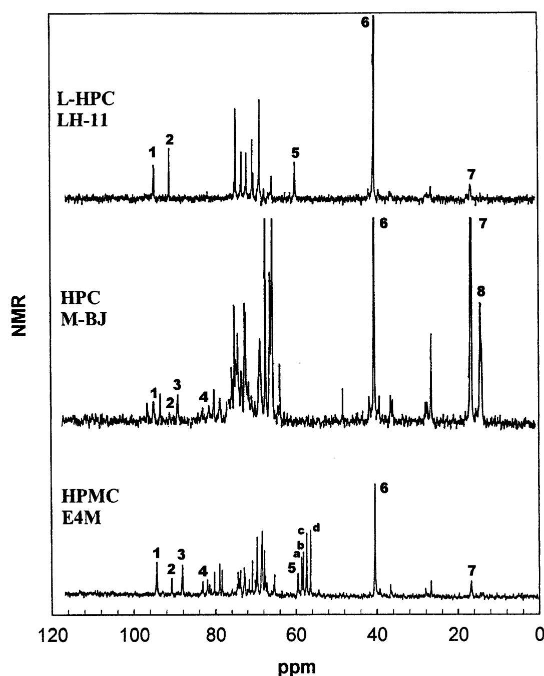

Fig. 2. ^{13}C-NMR spectra of the L-HPC LH-11, the HPC M-BJ and the HPMC E4M. (1) C1β with and without substituents in positions 2, 3 or 6; (2) C1α without substituents in position 2; (3) C1α with substituents in position 3.; (4) C3β with substituents in position 3; (5) C6α and β without substituents in position 6; (6) reference; (7) terminal methyls of hydroxypropoxyl substituent; (8) internal methyls of hydroxypropoxyl substituents; (a) C2β methoxyl; (b) C3α and β methoxyl; (c) C6α and β methoxyl; (d) C2α methoxyl.

3.2.1. L-HPCs

The spectra of the varieties of L-HPC studied show three singlets due to the anomeric carbon (C1): two at 95 and 96 ppm due to substituted and unsubstituted β-glucopyranoses respectively, and one at 92 ppm due to C2-unsubstituted α-glucopyranoses. The absence of signals at 90–91 ppm (for C1 of 2-substituted glucopyranoses) and at 81–84 ppm (for substituted C2) shows that DS_2 is negligible, and the absence of any signal for substituted C3 at 83–87 ppm that DS_3 is likewise negligible. All the L-HPC spectra show both a signal at 60–62 ppm due to unsubstituted C6 and a signal at 69–71 ppm attributed to substituted C6 (a glucopyranose carbon bearing an alkoxy group usually appears 8–11 ppm downfield of the signal due to the corresponding hydroxy-substituted carbon [36], and the assignment of the signal at 69–71 ppm is further supported by the fact that the sum of the peak areas of this signal and the signal at 60–62 ppm is practically the same as the total area of the signals due to C1 atoms [19]); DS_6 values (which in view of the absence of substitution at positions 2 and 3 are identical to DS values) are listed in Table 4.

The 67–77 ppm region shows signals for unsubstituted non-anomeric carbons and for the methines and methylenes of the hydroxypropoxyl group; the latter are the more intense due to their greater mobility [18,19]. A low-intensity peak at 19 ppm is attributed to 'external' hydroxypropoxyl methyls (the terminal methyls of any mono- or poly(hydroxypropoxyl) side chain), and the spectrum of LH-20 also shows a weak signal at 17 ppm for the 'internal' (non-terminal) methyls of poly(hydroxypropoxyl) side chains; LH-20 is thus the only L-HPC studied for which MS differs from DS (Table 4).

3.2.2. HPCs

Unlike those of the L-HPCs, the spectra of the HPC hydrolysates include signals due to substituted C2 and C3 atoms, and their hydroxypropoxyl signals are more intense than those of the L-HPC spectra. The HPC varieties have very similar partial and total degrees of substitution (Table 5), the values of which indicate that positions 2 and 6 of the anhydroglucose are much more reactive than position 3. MS varies more widely among varieties or lots than does DS, which must reflect appreciable differences as regards the mean number of hydroxypropoxyl groups per side chain (Table 5).

Table 4
Degree of substitution (DS) and molar substitution of L-HPCs, as determined by ^{13}C-NMR spectrometry (means of three replicate samples and standard deviations)

Polymer	DS	MS
LH-11	0.255 (0.007)	0.255 (0.007)
LH-20	0.300 (0.008)	0.320 (0.010)
LH-21	0.247 (0.003)	0.247 (0.003)
LH-22	0.175 (0.007)	0.175 (0.007)
LH-31	0.255 (0.007)	0.255 (0.007)

Table 5
Partial (DS_i) and total (DS) degrees of substitution, and molar substitution (MS), of HPCs as determined by ^{13}C-NMR spectrometry (means of three replicate samples and standard deviations)

Polymer	DS_2	DS_3	DS_6	DS	MS
KGF	0.91 (0.01)	0.50 (0.01)	0.89 (0.02)	2.30 (0.04)	3.86 (0.05)
MBJ	0.90 (0.02)	0.49 (0.01)	0.81 (0.01)	2.20 (0.04)	3.48 (0.04)
MDC	0.90 (0.01)	0.51 (0.01)	0.80 (0.03)	2.21 (0.05)	3.56 (0.06)
MJD	0.89 (0.01)	0.51 (0.01)	0.80 (0.01)	2.21 (0.03)	3.37 (0.04)
HBJ	0.89 (0.02)	0.52 (0.02)	0.89 (0.01)	2.30 (0.05)	3.42 (0.06)
HJE	0.90 (0.01)	0.49 (0.01)	0.82 (0.03)	2.21 (0.05)	3.44 (0.06)
KMF	0.91 (0.03)	0.59 (0.02)	0.90 (0.01)	2.40 (0.06)	3.89 (0.07)

3.2.3. HPMCs

The HPMC spectra show two singlets at 95 and 97 ppm attributed to β-glucopyranose C1; a singlet at 92–93 ppm attributed to C1 in α-glucopyranoses with no C2-substituent; and a singlet at 89–90 ppm due to C1 in C2-substituted α-glucopyranoses. Of the signals appearing between 78 and 85 ppm, the six most intense are attributed, in order of increasing δ, to hydroxypropoxyl-substituted C2 in α-glucopyranose, methoxyl-substituted C2 in α-glucopyranose, C3 with either substituent in α-glucopyranose, hydroxypropoxyl-substituted C2 in β-glucopyranose, methoxyl-substituted C2 in β-glucopyranose, and C3 with either substituent in β-glucopyranose. Chief among the many signals in the 68–78 ppm region are those of the methine and methylene carbons of the hydroxypropoxyl groups and the signal at 70–71 ppm due to substituted C6. The signal for unsubstituted C6 at 62 ppm is accompanied by signals due to methoxyl groups at C2 (one for α- and one for β-glucopyranose), C3 and C6 [38]. The hydroxypropyl methyl signals appear at 19 ppm, and the absence of any signal at 17 ppm due to 'internal' methyls shows that there is little or no side chain branching.

As in the HPCs, C2 and C6 are the most reactive positions (Table 6). The lower reactivity of C3 has been attributed to steric hindrance and to hydrogen bonding between the C3 hydroxyl and the ring oxygen of an adjacent monomer [24]. The fact that in the most highly substituted lot, E4M, DS_3 is also higher, may be due to substitution of C2 and C6 having eliminated competition from these substitution sites, but mathematical models of substituent distribution during CE formation [24,39] suggest that substitution at C2 may have an inductive effect activating the C3 hydroxyl.

3.2.4. Precision and accuracy

Method precision was evaluated by determination of the substituent contents of triplicate samples; for none of the CEs studied was any of the corresponding RSDs greater than 1.5%. For HPMCs, the mean values of the determinations of hydroxypropoxyl content were all within 2% of the values obtained by GLC (Table 8), for L-HPCs they were all within 2.5% of the GLC values (Table 7) and for HPCs they were within 4% of the GLC values (Table 7); accuracy relative to GLC thus fell slightly with increasing hydroxypropoxyl content. For low hydroxypropoxyl content, the accuracy of the NMR method relative to the GLC method is sufficient for the two methods to rank L-HPCs and HPMCs in virtually the same order with respect to hydroxypropoxyl content, as is reflected by the close correlation shown in Fig. 3 (upper left inset):

$$OPrOH_{NMR} = -0.254 + 1.030 \cdot OPrOH_{NMR}$$

$(r^2 = 0.9957)$.

For high hydroxypropoxyl contents, the error of the NMR method relative to GLC is too large to preserve the ordering of HPCs (Fig. 3, lower right inset). The HPMC methoxyl contents determined by NMR were all within 2% of the GLC values, and the corresponding regression lines (Fig. 4) has a slope of near unity:

$$OMe_{NMR} = 1.009 \cdot OMe_{GLC}.$$

Table 6
Partial (DS_i) and total (DS) degrees of substitution of HPMCs and their total degree of substitution by hydroxypropoxyl groups (DS_{PrOH}) as determined by ^{13}C-NMR spectrometry (means of three replicate samples and standard deviations)

Polymer	DS_2	DS_3	DS_6	DS	DS_{PrOH}
K4M	0.73 (0.02)	0.23 (0.01)	0.69 (0.01)	1.65 (0.04)	0.21 (0.01)
E4M	0.89 (0.02)	0.46 (0.01)	0.77 (0.02)	2.13 (0.05)	0.23 (0.01)
F4M	0.90 (0.01)	0.30 (0.01)	0.75 (0.01)	1.95 (0.03)	0.17 (0.01)
K15M	0.72 (0.02)	0.24 (0.01)	0.73 (0.01)	1.69 (0.04)	0.22 (0.01)

3.3. Raman spectroscopy

Fig. 5 shows the Raman spectrum of one sample of each type of CE. The bands for the ether linkages appear between 820 and 890 cm^{-1}. The fact that the band around 850 cm^{-1} is more intense for the HPC than for the L-HPC or the HPMC may be attributed to only HPCs having branched side chains with internal ether linkages. The relatively high MS of the HPCs is shown by the band around 927 cm^{-1}, which is due mainly to C–C bonds in alkyl chains, and by the absence of the band around 1097 cm^{-1} that is characteristic of unsubstituted cellulose sugar rings.

The spectrum of unsubstituted cellulose also has bands in the C–H vibration region, 1200–1500 cm^{-1}: specifically, a medium-intensity band at 1338 cm^{-1}, a strong band at 1380 cm^{-1} and a weak band at 1462 cm^{-1} [34]. Hence the band at 1367 cm^{-1} in the spectra of the CEs studied may be attributed to the C–H bonds of the anhydroglucose skeleton, and may, therefore, be used as a reference for quantification of substitution. This reference may be compared with the bands attributed to alkoxyl side chains: the band at 1260 cm^{-1} due to C–C bonds and the band at 1458 cm^{-1} in the methyl/methylene region (1440–1475 cm^{-1}). In keeping with the NMR results listed in Tables 4–6, in Fig. 5 the intensity of the band at 1458 cm^{-1} relative to the 1367 cm^{-1} band increases in the order L-HPC $<$ HPMC $<$ HPC. Fig. 6 shows that the ratio $R_{12/13}$ between the absorbances at 1260 and 1367 cm^{-1} (Tables 7 and 8) can be used to obtain a rough estimate of hydroxypropoxyl content by means of the equation

$$OPrOH_{GLC} = -49.71 + 145.5\ R_{12/13}.$$

The $R_{12/13}$ method is accurate enough to distinguish between low- and high-OPrOH CEs, but is not sufficiently accurate to distinguish among different CEs within those two groups.

3.3.1. Precision

Method precision was evaluated by determination of the $R_{12/13}$ values of triplicate samples; for none of the CEs studied was the corresponding RSD greater than 2.1%.

4. Conclusions

All three methods examined are potentially useful in appropriate circumstances. The most accurate quantification of hydroxypropoxyl and methoxyl contents is probably achieved by the GLC method, closely followed by NMR spectroscopy, which for low hydroxypropoxyl CEs

Table 7
Hydroxypropoxyl contents (%) of L-HPCs and HPCs, as determined by GLC and ^{13}C-RMN spectrometry, and $R_{12/13}$ values obtained by Raman spectroscopy (standard deviations)

Polymer	^{13}C-RMN	GLC	$R_{12/13}$
LH-11	10.89 (0.27)	10.84 (0.12)	0.43 (0.01)
LH-20	13.48 (0.17)	13.15 (0.03)	0.46 (0.01)
LH-21	10.60 (0.14)	10.61 (0.05)	0.41 (0.01)
LH-22	7.62 (0.23)	7.50 (0.08)	0.39 (0.01)
LH-31	10.89 (0.27)	11.05 (0.06)	0.43 (0.01)
KGF	64.49 (0.09)	62.11 (0.34)	0.80 (0.01)
MBJ	62.11 (0.12)	61.40 (0.24)	0.72 (0.01)
MDC	62.68 (0.13)	61.24 (0.28)	0.73 (0.01)
MJD	61.40 (0.12)	61.31 (0.16)	0.73 (0.01)
HBJ	61.68 (0.10)	60.90 (0.27)	0.76 (0.01)
HJE	61.91 (0.15)	60.96 (0.19)	0.77 (0.01)
KMF	64.67 (0.11)	63.02 (0.17)	0.81 (0.01)

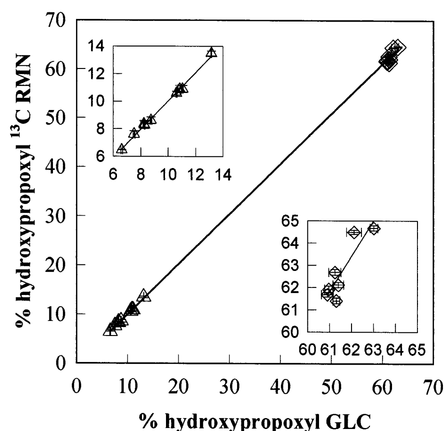

Fig. 3. Correlation between ^{13}C-NMR and GLC measurements of hydroxypropoxyl contents. Upper left inset: L-HPCs and HPMCs; lower right inset: HPCs.

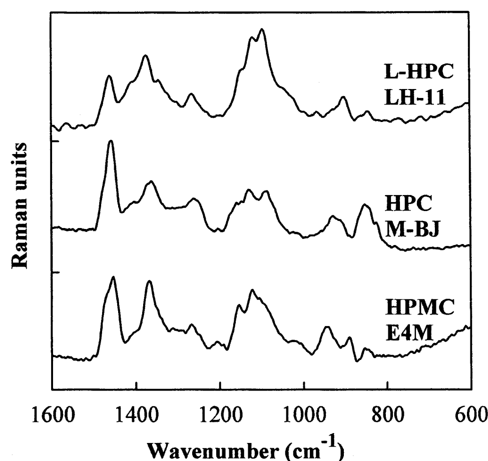

Fig. 5. Raman spectra of the L-HPC LH-11, the HPC M-BJ and the HPMC E4M.

affords almost the same results as GLC. Since the GLC method involves relatively little sample preparation and quite short chromatographic runs, it allows analysis of numerous samples in a short time and can be recommended for routine use. The NMR method involves rather lengthier sample preparation, but has the advantage of also allowing quantification of the degree of substitution at each substitutable glucopyranose hydroxyl. The Raman method is inaccurate in comparison with the other two, but since it involves virtually no sample preparation is much faster, and may therefore be useful in circumstances in which what is desired is a rapid rough estimate of hydroxypropoxyl content, sufficient to distinguish between low- and high-OPrOH CEs.

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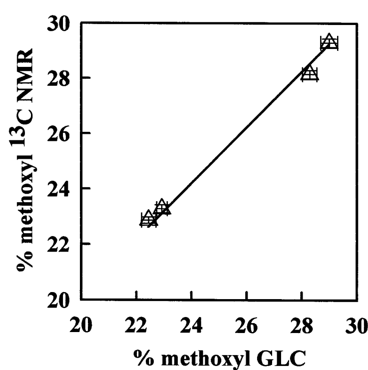

Fig. 4. Correlation between ^{13}C-NMR and GLC measurements of methoxyl contents.

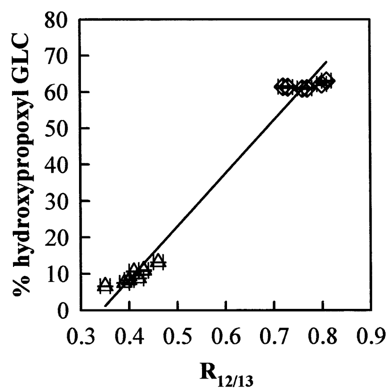

Fig. 6. Regression of the ratio between absorbance at 1260 and 1367 cm^{-1} of Raman spectra ($R_{12/13}$) on the GLC-determined hydroxypropoxyl content of L-HPCs, HPCs and HPMCs.

Table 8
Hydroxypropoxyl and methoxyl contents (%) of HPMCs, as determined by GLC and ^{13}C-NMR spectrometry, and $R_{12/13}$ values obtained by Raman spectroscopy (standard deviations)

Polymer	Methoxyl content		Hydroxypropoxyl content		
	^{13}C-RMN	GLC	^{13}C-RMN	GLC	$R_{12/13}$
K4M	22.88 (0.11)	22.44 (0.26)	8.27 (0.25)	8.27 (0.03)	0.40 (0.01)
E4M	29.29 (0.16)	28.99 (0.31)	8.36 (0.24)	8.23 (0.06)	0.40 (0.01)
F4M	28.15 (0.15)	28.38 (0.26)	6.49 (0.01)	6.60 (0.06)	0.35 (0.01)
K15M	23.29 (0.14)	22.92 (0.19)	8.62 (0.25)	8.74 (0.06)	0.42 (0.01)

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