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Positional and Quantitative Characterization of the Hydroxyethyl Groups in Hydroxyethyl Starch by GC/MS or NMR

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Abstract: The position and quantity of the hydroxyethyl groups in hydroxyethyl starch (HES) were studied by GC/MS and NMR. Quantitative characterization was carried out based on the results of both methods.

Keywords: Hydroxyethyl starch, GC/MS, NMR.

Hydroxyethyl starches (HES) have been used medically as plasma volume expanders. They are composed of a starch backbone and substituted hydroxyethyl groups. The backbone is α-(1,4)-glycosidic-linked anhydroglucose units, which has branches formed by α-(1,6)-glycosidic bonds. Hydroxyethylation can take place at the C-2, C-3 and C-6 sites of the glucose rings, as well as at the hydroxyl groups of the substituted hydroxyethyl groups. Variation of the position and quantity of the substituted hydroxyethyl groups profoundly influence the relevant pharmacokinetic parameters, *e.g.* the circulation time[1]. It is clinically desirable to have a precise knowledge of the position and the quantity of the substituted hydroxyethyl groups[2]. The widely-used hydroiodic acid titration method[3] can only give information on the total substituted hydroxyethyl groups in HES. We used GC/MS and NMR to determine the position and the quantity of the substituted hydroxyethyl groups in HES.

Experimental

The HES was synthesized by General Hospital of PLA. In the GC/MS study, all free hydroxyls in the original HES were first converted into methoxyl groups, using "sequential" methylation method with sodium hydroxide and methyl iodide in dimethyl sulfoxide[4]. The permethylated HES was hydrolyzed with trifluoroacetic acid (2 mol/L) at 120°C for 1 hr, followed by reducing of the resulting monomers with $NaBH_4$. After removing borate and water, the residue was peracetylated by acetic anhydride and pyridine (1:1 v/v) at 100°C for 30 min. The derivatives were analyzed by Qp5050A GC/CIMS spectrometer and AutoSpec-Ultima ETOF GC/EIMS/MS spectrometer.

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The ^{13}C-NMR spectrum was taken on a Bruker AVANCE DRX-500 NMR spectrometer after dissolving the HES (20 mg) in deuterium oxide. The inverse gated decoupling method was applied in order to enable the quantitative evaluation of the NMR data. The relaxation delay was 10 s and the flip angle was 50°.

Figure 1 Total ion chromatogram of partially methylated alditol acetates of HES

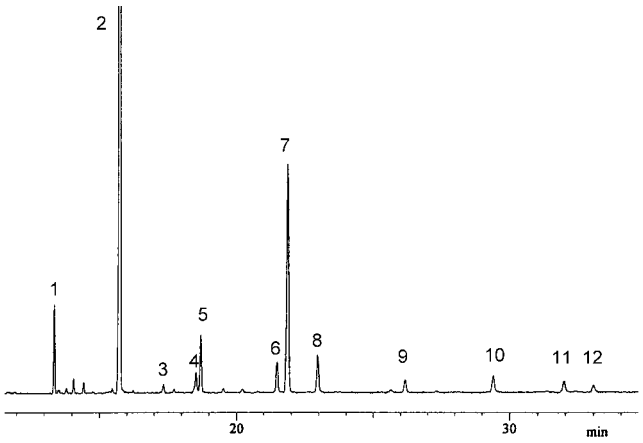


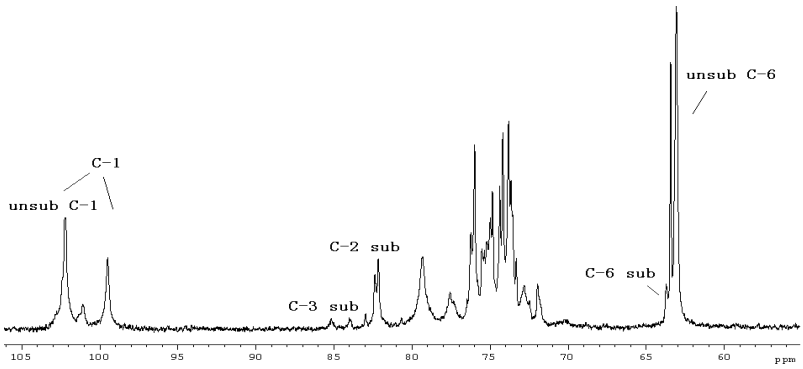
Table 1 Identification of the peaks in the total ion chromatograph of HES

No.	Derivatives of glucose
1	1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylglucitol
2	1,4,5-Tri-O-acetyl-2,3,6-tri-O-methylglucitol
3	1,3,4,5-Tetra-O-acetyl-2,6-di-O-methylglucitol
4	1,5-Di-O-acetyl-2-O-methoxyethyl-3,4,6-tri-O-methylglucitol
5	1,4,5,6-Tetra-O-acetyl-2,3-di-O-methylglucitol
6	1,4,5-Tri-O-acetyl-3-O-methoxyethyl -2,6-di-O-methylglucitol
7	1,4,5-Tri-O-acetyl-2-O-methoxyethyl -3,6-di-O-methylglucitol
8	1,4,5-Tri-O-acetyl-6-O-methoxyethyl -2,3-di-O-methylglucitol
9	1,4,5,6-Tetra-O-acetyl-2-O-methoxyethyl-3-O-methylglucitol
10	1,4,5-Tri-O-acetyl-2,3-di-O-methoxyethyl-6-O-methylglucitol
11	1,4,5-Tri-O-acetyl-2,6-di-O-methoxyethyl-3-O-methylglucitol
12	1,4,5-Tri-O-acetyl-2-O-methoxyethoxyethyl-3,6-di-O-methylglucitol

Results and Discussion

The signals in the total ion chromatogram (**Figure 1**) were obtained from GC/CIMS, and GC/EIMS/MS. CI measurements permitted the recognition of the introduced hydroxyethyl groups in the glucose ring by detection of $(M+1)^+$-60 signals, which increased by 44 or 88 in mass corresponding to the introduction of $-CH_2CH_2O-$ or $-CH_2CH_2OCH_2CH_2O-$ groups. Investigations concerning the EI schemes allowed the determinations of the positions (C-2, C-3, C-6) of the substituted hydroxyethyl groups by analyzing the fragment process. GC/EIMS/MS measurements confirmed our deduction. The assignment of the signals in the total ion chromatogram was presented in **Table 1**. The quantity of substitution at the C-2, C-3, C-6 of the glucose rings in this method were determined according to the relative signal areas in the total ion chromatogram (**Figure 1**).

Figure 2 ^{13}C-NMR spectrum of HES in deuterium oxide



unsub=unsudstituted, sub=substituted

The position and quantity of substitution of HES could also be obtained by NMR spectroscopy. The signals concerning the positions of substitutions in ^{13}C-NMR spectrum (**Figure 2**) were assigned by comparing with the spectra of non-derivatived starch[5] and hydroxyethyl-cellulose[6]. Quantitative evaluation was carried out by matching the areas of the relevant peaks in ^{13}C-NMR spectrum of HES, because the signal areas here are directly proportional to the number of carbon atoms observed.

The results of both methods were compiled in **Table 2** (S, S_2, S_3, S_6 represent the total quantity of substitution and the substitution at C-2, C-3, C-6 of the glucose rings respectively). From **Table 2** we can see that the difference of results for these two methods lies within the limits of measuring accuracy.

Table 2 Comparison of the quantitative results determined by GC/MS and NMR (%)

Method	S_2	S_3	S_6	S
GC/MS	28.5	4.7	4.5	37.7
NMR	30.8	4.3	6.0	41.1

With the deepening of the physicochemical study on HES, the titrimetric method by hydroiodic acid which is used to determine the total quantity of substitution can not be satisfied. In our experiment, comprehensive information of the structure of HES including the position and the categories of substitution of hydroxyethyl groups, as well as the total and partial quantity of substitution, has been obtained by GC/MS or NMR. We also established the standard mass spectrometry of partially methylated alditol acetates derived from hydroxyethyl glucose in this experiment and developed a practical method to detect the structure of derivatives of polysaccharides.

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