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Characterization of doubly substituted polysaccharide derivatives

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Abstract

Derivatives of cellulose, amylose and chitosan, bearing simultaneously 10-undecenoyl and arylaminocarbonyl or benzoyl groups were characterized by the combined use of ^{1}H NMR and elemental analysis. The mathematical manipulation of elemental analysis data permits the calculation of the degree of substitution for each kind of substituent. The method was validated and is applicable to other derivatives.

Keywords: Polysaccharide derivatives; Cellulose; Amylose; Chitosan; Polysaccharide characterization

1. Introduction

A number of commercially available chiral stationary phases for HPLC comprise polysaccharide derivatives coated onto silica gel [1]. Their stereoselectivity depends on the polysaccharide and the kind of derivatization, but they are soluble in certain chromatographic solvents, which limits their applicability. The introduction of 10-undecenoyl groups in a double derivatization (10-undecenoate/arylcarbamate or benzoate) on the polysaccharide (Fig. 1) allows fixation on chromatographic matrices [2–8], but it complicates the chemical characterization of the polysaccharide derivatives.

Various doubly substituted derivatives of cellulose and amylose have been described [9–12] and characterized by ^{1}H NMR and elemental analysis, techniques of low sensitivity and poor selectivity. Characterization based on elemental analysis usually assumes that doubly substituted polysaccharides are completely derivatized, and the presence of free hydroxyl groups is not taken into account. Thus, if a given ratio of substituents is assumed, a theoretical composition can be calculated and the results compared with the experimental analysis. Moreover, if the doubly derivatized polysaccharide contains a heteroatom (nitrogen or chlorine) in only one of the derivatizing groups, its percentage in the elemental analysis is considered to be free of experimental error and all other percentages are referred to it in the so-called theoretical composition. The degree of substitution (DS) for the group containing this heteroatom is

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calculated directly from its percentage. The DS for the second derivatizing group is then the difference between the DS for the first substituent and the total substitution.

The need to quantify both derivatizing groups independently has led to the development of a systematic procedure that made such assumptions unnecessary. This report discusses the combined use of ^{1}H NMR and elemental analysis to characterize cellulose, amylose and chitosan derivatives and to calculate DS.

2. Results and discussion

^{1}H NMR spectra.—Polysaccharide derivatives, prepared as described [4,5] were qualitatively characterized by their ^{1}H NMR spectra, recorded at 70 °C to improve the signal resolution. Proton assignments based on COSY experiments were consistent with previous results [13–15] (Table 1). Chitosan derivatives gave broader signals than cellulose derivatives, and COSY experiments did not permit successful assignments. A monomeric unit of **3a**, 2 - deoxy - 1,3,4,6 - tetrakis - *O* - (3,5 - dimethylphenylaminocarbonyl)-2-(3,5-dimethylphenylureido)-β-D-glucopyranose, was thus prepared from 2-amino-2-deoxy-β-D-glucopyranose as a standard to facilitate assignment. The ^{1}H NMR spectrum of this compound was interpreted from the study of the coupling pattern of signals and by COSY experiments, although the comparison with the spectra of the polymers was not conclusive. A tentative assignment based on the comparison with cellulose derivatives is proposed in Table 1. Protons corresponding to substituents are less affected by the restricted motion of the macromolecule and their signals were easy to assign.

The absolute DS cannot be determined on the basis of ^{1}H NMR spectra in most cases. The broad and sometimes overlapping signals corresponding to glycosidic protons cannot always be properly integrated, although the ratio of substituents can be estimated from the integral of their signals.

^{1}H NMR spectra can also be used to assess the purity of the polysaccharide derivative, in spite of the low sensitivity inherent to the technique. Low molecular-weight impurities are detected at room temperature, even at low percentages, because of the sharp peaks that they originate. The main by-product in the preparation of carbamate derivatives is the

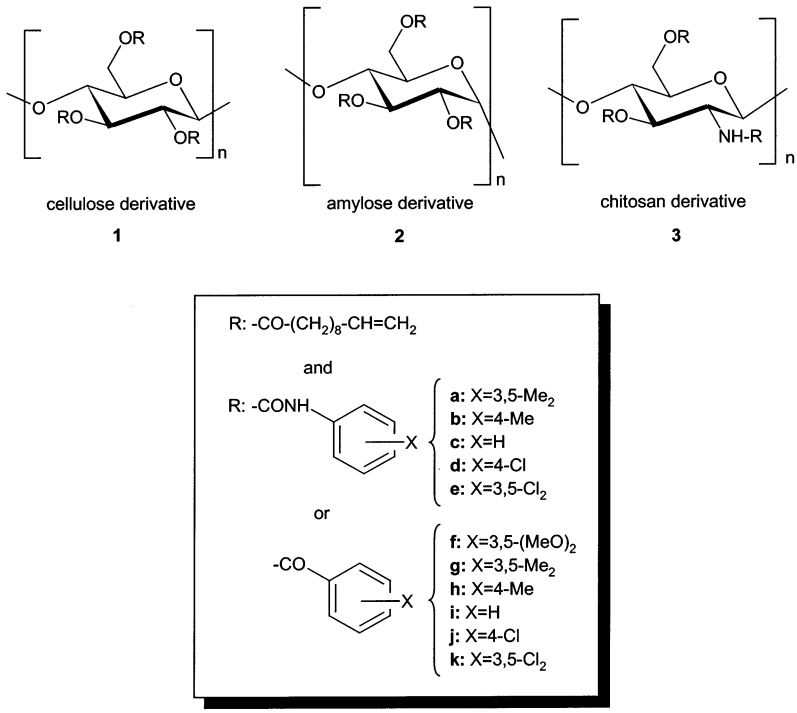

Fig. 1. Polysaccharide derivatives.

Table 1
^{1}H NMR data (300 MHz, pyridine–d_5 at 70 °C) of the polysaccharide derivatives

Derivative	Glucopyranosic protons						Aromatic protons									NH protons			
	H-1	H-2	H-3	H-4	H-5	H_2-6	Ar-$H^{2,6}$			Ar-$H^{3,5}$			Ar-H^4						
1a	4.65	5.25	5.52	3.75	3.45	4.65	7.30	7.34	7.34	–			6.40	6.61	6.71		9.24	9.33	10.27
1b	4.66	5.19	5.47	3.84	3.38	4.66	7.50–7.80			6.87	7.03	7.17	–				9.57	9.57	10.35
1c	4.66	5.20	5.48	3.84	3.40	4.66	6.70–8.00			6.70–8.00			6.70–8.00				9.68	9.75	10.47
1d	4.72	5.12	5.46	3.91	3.51	4.72	7.37	7.48	7.48	6.97	7.27	7.37	–				9.94	10.06	10.68
1e	4.50–5.00	5.00–5.30	5.52	3.95	3.75	4.50–5.00	7.42	4.56	7.59	–			6.68	7.08	7.16		9.40	9.50	10.90
1f	5.06	5.69	5.91	4.20–4.40	3.83	4.20–4.40	7.22	7.28	7.43	–			6.42	6.66	6.71			–	
1g	4.93–5.21	5.70	5.93	4.20–4.40	3.34	4.20–4.40	7.51–8.15			–			6.61–7.17					–	
1h	4.80–5.25	5.61	5.89	4.27	3.72	4.45	7.84	7.95	8.00	6.80	7.01	7.19	–					–	
1i	5.04	5.65	5.94	4.11	3.70	4.35	7.91	8.04	8.09	6.90–7.62			6.90–7.62					–	
1j	5.10	5.60	5.97	4.10–4.55	3.92	4.10–4.55	7.76	7.81	7.92	7.03	7.25	7.36	–					–	
1k	5.30	5.67	6.10	4.00–4.70	4.00–4.70	4.00–4.70	7.80	7.81	7.91	–			7.34	7.55	7.63			–	
2a	5.61 [a]	5.67 [a]	5.87	4.24	4.63	4.90, 5.05	6.97	7.05	7.71	–			6.46	6.56	6.84		9.34	9.34	9.69
2b	5.74	5.61	5.92	4.16	4.59	4.87, 5.11	7.36	7.43	7.88	6.89	6.89	7.22	–				9.30–10.00		
2d	5.65	5.43	5.87	4.25	4.46	4.83, 4.95	7.30	7.30	7.83	6.99	7.12	7.47	–				9.85	10.01	10.56
2e	4.00–6.15	4.00–6.15	4.00–6.15	4.00–6.15	4.00–6.15	4.00–6.15	6.80–8.05			–			6.80–8.05				10.28	10.63	11.08
2j	6.19	5.73	6.37	4.84	4.97	4.97, 5.23	7.64	7.82	8.27	7.15	7.27	7.53	–					–	
2k	6.28	5.64	6.45	4.57–5.55	4.57–5.55	4.57–5.55	8.05–8.35			–			8.05–8.35					–	
3a	5.48	4.50	6.55	3.99	3.65	4.87, 4.74	7.30	7.36	7.36	–			6.50	6.55	6.72	9.30	9.45	10.00	10.15
3b	5.50	4.40	6.45	3.99	3.71	4.83	7.30–8.10			7.00	7.00	7.14	–			9.00	9.62	9.75	10.09
3d	5.50	4.32	6.43	3.95	3.67	4.86	7.52	7.52	7.52	7.08	7.23	7.36	–			9.28	10.06	10.41	10.58
3e	6.50–5.70	5.40–3.60	6.50–5.70	5.40–3.60	5.40–3.60	5.40–3.60	7.60	7.70	7.73	–			7.16	7.20	7.20	10.50–11.35			11.67
3j	5.70	4.65–3.70	6.56	4.65–3.70	4.65–3.70	4.65–3.70	7.70–8.30			6.90–7.65			–				9.30		
3k	6.13	4.75 [a]	6.68	4.37	4.01	4.90 [a], 4.75	7.89	7.92	7.93	–			7.37	7.50	7.52		10.00		

[a] Assignments may have to be reversed.

urea from the decomposition of the unreacted isocyanate. *N*,*N*′-bis(3,5-dimethylphenyl)urea is formed in the preparation of **1a** (and also **2a** and **3a**). The high intensity of the singlet absorption at δ 2.26, assigned to the four methyl groups of this compound, can compensate for the low sensitivity of NMR.

Elemental analyses.—Once the absence of low-molecular weight impurities has been proved by ^{1}H NMR and thin-layer chromatography (TLC) of the washing liquors, elemental analysis becomes the technique of choice to determine the DS of polysaccharide derivatives. For example, the empirical formula for **1d** and **2d** is: $[C_6H_7O_5(C_{11}H_{19}O)_x(C_7H_5ClNO)_y(H)_{3-x-y}]_n$, where x is the number of 10-undecenoyl groups and y the number of 4-chlorophenylaminocarbonyl groups, per glucose unit. Only the extreme substituents in the polysaccharide chain (position 1 of the first and position 4 of the last unit in each molecule) are not considered. From this composition, several equations can be established to link x and y with the experimental values of the elemental analysis (Eqs. (1)–(4)).

$$\%C = \frac{12 \cdot (6 + 11x + 7y) \cdot 100}{162 + 167x + 154.5y - x - y} \quad (1)$$

$$\%N = \frac{14y \cdot 100}{162 + 167x + 154.5y - x - y} \quad (2)$$

$$\%H = \frac{(10 + 19x + 5y - x - y) \cdot 100}{162 + 167x + 154.5y - x - y} \quad (3)$$

$$\%Cl = \frac{35.5y \cdot 100}{162 + 167x + 154.5y - x - y} \quad (4)$$

This equation system has a solution when the experimental error in the determination of the elemental composition is taken into account. The considered margins of error are lower than those generally admitted for the characterization of low molecular weight organic compounds and they result in a narrow margin in the calculated DS for every substituent.

The DS was calculated using a program written in QBasic v4.5 (Fig. 2). This procedure was applied to benzoates and carbamates of cellulose and amylose (Table 2). A series of values for DS compatible with the previously mentioned equations was obtained from a single elemental analysis. All of them are included in an interval expressed by the arithmetic mean of the extreme values and its difference from them (Gaussian distribution) (column DS in Table 2). The mean of the results is used to estimate the molecular weight per glycosidic unit corresponding to each derivative (Table 2). The calculated DS is consistent with the ratio of substituents calculated from ^{1}H NMR spectra (Table 2), except for low DS in one of the substituents (derivative **1a^I**, Table 2), where the ratio of substituents cannot be determined accurately from the ^{1}H NMR spectra.

Ranges of DS in derivatives containing only C, H and O (**1f**–**i**) are broader than those of derivatives containing additional heteroatoms such as N or Cl. Thus, the more available equations, the narrower the interval of DS.

Chitosan derivatives.—The content of acetyl groups, remaining from the partial deacetylation of chitin, has to be considered when chitosan is the starting polysaccharide. Techniques such as ^{1}H NMR and IR have been used in the determination of the acetylation degree [16], but they are not free of experimental error. The measurement of the integral in broad and overlapping signals of glycosidic ring protons is the main source of error when ^{1}H NMR is used. The low sensitivity of the technique makes it difficult to determine the low content of acetyl groups. The determination of a suitable baseline to measure the relative intensity of bands (such as those at 1655 or 1560 cm^{-1}) is critical when IR is used. Moreover, depending on the degree of acetylation of the chitosan considered the relationship between relative absorption and acetylation is nonlinear [16]. In addition, water adsorbed on the highly hygroscopic chitosan may alter the absorption of certain bands [16,17].

To test the validity of elemental analysis to estimate the DS in chitosan derivatives, differently acetylated chitosans were first derivatized with 3,5-dichlorobenzoyl chloride (compounds **4**, Table 3). The presence of chlorine provides an additional criterion to determine the DS. However, the finding of lower %C and higher %Cl values than expected was attributed to the presence of inorganic mate-

Table 2
DS of the cellulose and amylose derivatives

Derivative	Elemental analysis				DS [a]		Ratio arom./10-undec.		MW per glycosidic unit [b]	Calculated elemental composition [b]				Ref.
	%C	%H	%N	%Cl	10-undec.	Arom.	From elemental analyses [b]	From ^{1}H NMR spectra		%C	%H	%N	%Cl	
1a^{I} [c]	63.70	6.37	6.26	–	0.05 ± 0.03	2.12 ± 0.11	42.4	–	479.88	63.73	6.20	6.17	–	4
1a^{II}	65.80	6.79	5.97	–	0.35 ± 0.02	2.44 ± 0.07	7.0	8.1	579.50	65.97	6.61	5.91	–	4
1a^{III}	64.60	7.05	4.57	–	0.51 ± 0.05	1.55 ± 0.07	3.0	4.0	473.37	64.64	6.98	4.57	–	4
1a^{IV}	66.10	8.04	2.49	–	1.16 ± 0.07	0.86 ± 0.06	0.7	0.6	480.29	66.09	8.04	2.48	–	4
1b	63.50	5.84	6.77	–	0.14 ± 0.04	2.41 ± 0.13	18.5	17.9	502.82	63.56	5.80	6.68	–	6
1c	62.03	5.24	7.13	–	0.16 ± 0.05	2.40 ± 0.14	15.9	14.2	471.29	61.95	5.19	7.15	–	6
1d	52.45	3.98	5.94	15.41	0.12 ± 0.02	2.27 ± 0.09	20.6	15.9	529.53	52.43	3.98	6.01	15.24	6
1e	47.50	3.70	4.88	25.82	0.31 ± 0.03	2.11 ± 0.12	6.5	9.1	608.35	47.66	3.62	4.81	24.40	6
1f	61.51	5.72	–	–	0.32 ± 0.06	2.47 ± 0.25	7.8	9.4	625.53	61.47	5.72	–	–	5
1g	70.53	6.65	–	–	0.39 ± 0.15	2.45 ± 0.23	6.3	7.2	549.53	70.54	6.66	–	–	5
1h	69.11	5.99	–	–	0.29 ± 0.06	2.43 ± 0.14	8.4	7.7	495.84	69.14	6.00	–	–	5
1I	67.91	5.40	–	–	0.30 ± 0.05	2.42 ± 0.13	8.1	7.3	462.74	67.94	5.41	–	–	5
1j	57.40	3.88	–	16.72	0.24 ± 0.04	2.67 ± 0.12	11.7	9.6	572.53	57.32	3.88	–	16.67	–
1k	49.14	2.90	–	29.30	0.19 ± 0.02	2.73 ± 0.08	14.4	13.8	667.56	49.02	2.83	–	29.14	5
2a	64.67	6.46	5.97	–	0.18 ± 0.05	2.19 ± 0.10	12.2	12.6	514.53	65.47	6.41	5.97	–	8
2b	63.56	5.78	6.75	–	0.13 ± 0.06	2.44 ± 0.16	20.3	14.1	505.68	63.60	5.77	6.75	–	8
2d	52.12	4.03	5.91	15.03	0.09 ± 0.03	2.12 ± 0.13	23.6	17.8	502.44	52.15	4.00	5.91	14.98	8
2e	46.59	3.38	5.18	25.76	0.22 ± 0.03	2.31 ± 0.10	10.2	9.1	634.38	46.72	3.32	5.10	25.85	8
2j	58.02	4.12	–	16.34	0.32 ± 0.02	2.63 ± 0.06	8.3	14.7	580.55	57.86	4.08	–	16.14	8
2k	49.64	3.01	–	28.59	0.26 ± 0.03	2.66 ± 0.10	10.7	9.0	666.74	49.48	2.98	–	28.52	8

[a] DS expressed as interval.
[b] Based on the arithmetic mean of DS.
[c] **1a$^{I–IV}$**, **1a** derivatives having different DS.

Table 3
DS of chitosan derivatives

Derivative [a]	Elemental analysis					DS		Ratio arom./10-undec.		Calculated elemental composition [b]			
	%C	%H	%N	%Cl	10-Undec.	Arom.	Ac	From elemental analyses [b]	From ^{1}H NMR spectra	%C	%H	%N	%Cl
3a^{I}	58.07	6.31	7.98	–	0.13 ± 0.06	1.38 ± 0.38	0.20	10.5	15.0	61.61	6.65	8.44	–
3a^{II}	60.04	6.30	8.09	–	0.16 ± 0.07	1.87 ± 0.57	0.13	10.9	14.9	63.73	6.64	8.55	–
3a^{III}	61.69	6.32	8.06	–	0.24 ± 0.08	2.22 ± 0.56	0–0.05	9.3	9.0	65.14	6.69	8.52	–
3b	61.30	5.92	8.69	–	0.25 ± 0.07	2.31 ± 0.38	0–0.05	9.8	14.3	64.09	6.19	9.09	–
3d^{I}	52.80	4.96	7.38	13.12	0.39 ± 0.10	1.66 ± 0.31	0.20	4.3	5.5	54.69	5.13	7.63	12.01
3d^{II}	49.55	4.15	7.90	14.93	0.16 ± 0.07	2.05 ± 0.39	0.13	12.7	16.1	52.87	4.42	8.40	14.25
3d^{III}	50.32	3.99	8.20	15.14	0.14 ± 0.07	2.50 ± 0.27	0–0.05	19.4	19.1	52.82	4.11	8.63	15.68
3e	45.16	3.48	6.94	26.14	0.27 ± 0.10	2.18 ± 0.41	0–0.05	8.4	11.2	47.17	3.63	7.23	25.09
3j	57.40	4.00	2.37	17.14	0.19 ± 0.02	2.74 ± 0.04	0–0.05	14.4	13.2	57.21	3.96	2.44	16.94
3k	49.39	3.15	2.05	28.60	0.23 ± 0.03	2.67 ± 0.06	0–0.05	11.6	14.2	49.43	3.10	2.11	28.58
4k^{I} [c]	43.12	3.80	3.99	22.91	–	0.89 ± 0.05	0.20	–	–	46.87	4.08	4.33	19.54
4k^{II}	45.64	2.88	2.70	28.57	–	1.95 ± 0.12	0–0.05	–	–	47.36	3.00	2.80	27.66
4k^{III}	45.21	3.32	3.20	26.30	–	1.43 ± 0.07	0.20	–	–	47.25	3.42	3.36	24.36
4k^{IV}	46.96	2.86	2.48	30.56	–	2.24 ± 0.09	0–0.05	–	–	47.47	2.83	2.54	28.88

[a] **3a$^{I–III}$**, **3d$^{I–III}$**, chitosan derivatives having different DS.
[b] Calculated from the arithmetic mean of DS.
[c] **4k$^{I–IV}$**, chitosan 3,5-dichlorobenzoates having different DS.

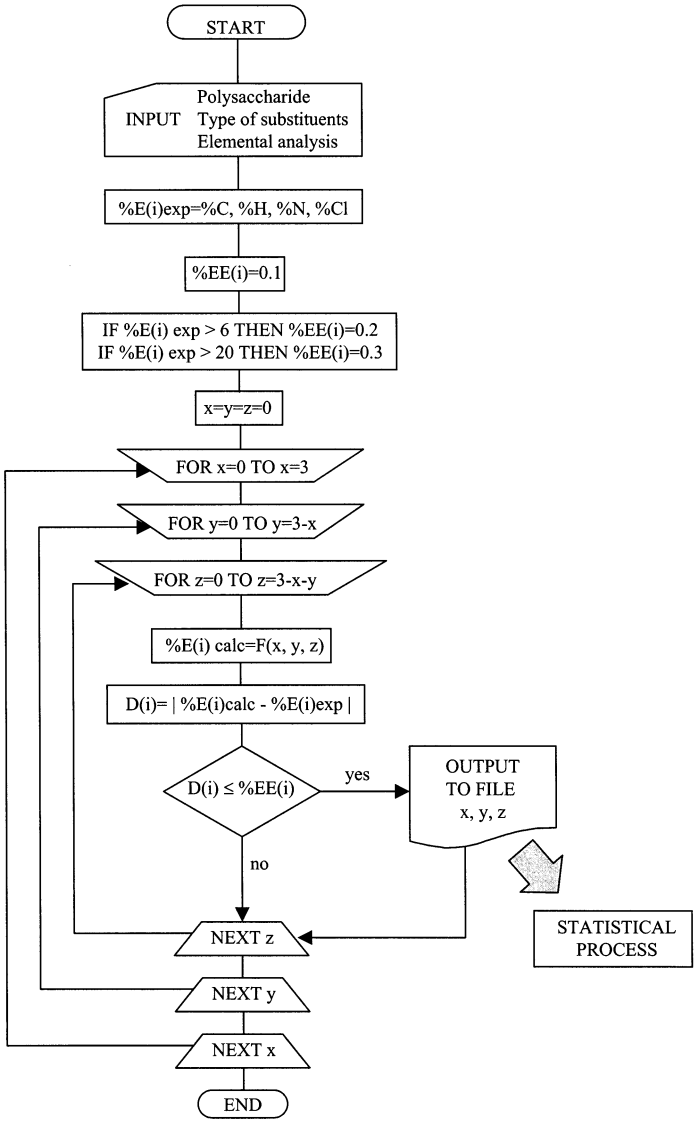

Fig. 2. Flowchart of the program to calculate DS from their elemental analyses. (%$E(i)$calc: calculated percentage of element i; %$E(i)$exp: experimental percentage of element i; %$EE(i)$: experimental error of element i).

rial, from the treatment previous to the derivatizing step [18]. The weight of residue after combustion at 450 °C confirmed the presence of variable amounts (up to 8% w/w) of inorganic materials.

Therefore, the direct determination of the DS based on the percentages of each element is not possible. However, assuming that these salts do not contain C, H or N, the ratios of these elements should be maintained, and additional equations can be used for the calculations. In the case of 3,5-dichlorobenzoylchitosan (**4**), $[C_6H_8NO_4(C_2H_3O)_z(C_7H_3Cl_2O)_y(H)_{3-z-y}]_n$, Eqs. (5)–(14) can be established, where z is the degree of acetylation.

$$\%C = \frac{12 \cdot (6 + 2z + 7y) \cdot 100}{161 + 43z + 174y - z - y} \quad (5)$$

$$\%N = \frac{14 \cdot 100}{161 + 43z + 174y - z - y} \quad (6)$$

$$\%H = \frac{(11 + 3z + 3y - z - y) \cdot 100}{161 + 43z + 174y - z - y} \quad (7)$$

$$\%Cl = \frac{35.5 \cdot 2y \cdot 100}{161 + 43z + 174y - z - y} \quad (8)$$

$$\frac{C}{N} = \frac{6 + 2z + 7y}{1} = \frac{\%C \cdot 14}{\%N \cdot 12} \quad (9)$$

$$\frac{C}{Cl} = \frac{6 + 2z + 7y}{2y} = \frac{\%C \cdot 35.5}{\%Cl \cdot 12} \quad (10)$$

$$\frac{Cl}{N}=\frac{2y}{1}=\frac{\%Cl\cdot 14}{\%N\cdot 35.5} \quad (11)$$

$$\frac{C}{H}=\frac{6+2z+72y}{11+3z+3y-z-y}=\frac{\%C\cdot 1}{\%H\cdot 12} \quad (12)$$

$$\frac{N}{H}=\frac{1}{11+3z+3y-z-y}=\frac{\%N\cdot 1}{\%H\cdot 14} \quad (13)$$

$$\frac{Cl}{H}=\frac{2y}{11+3z+3y-z-y}=\frac{\%Cl\cdot 1}{\%H\cdot 35.5} \quad (14)$$

Analogous equations can be obtained when the presence of 10-undecenoyl groups on the derivative is taken into account. These equations were also solved using a computer program (Fig. 3) and the calculation method was applied to all chitosan derivatives prepared. Although these equations are subject to the error of both elements involved, the results correlate with those of ^{1}H NMR (Table 3).

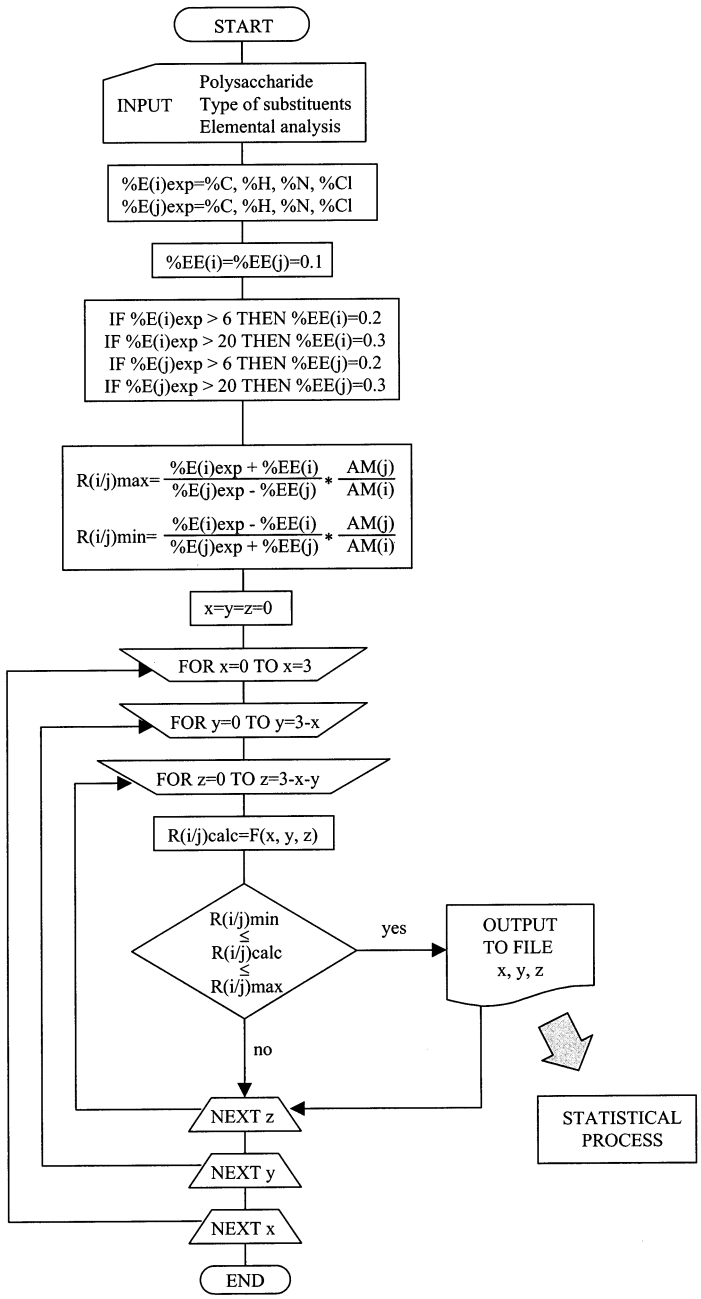

Fig. 3. Flowchart of the program to calculate DS from the ratio of elements. ($AM(i)$, $AM(j)$: atomic mass of elements i, j; $\%E(i)$exp, $\%E(j)$exp: experimental percentages of elements i, j; $\%EE(i)$, $\%EE(j)$: experimental errors of elements i, j; $R(i/j)$max, $R(i/j)$min, $R(i/j)$calc: element i/element j ratios maximum, minimum, calculated).

The acetyl content was determined on the underivatized chitosan, dried under vacuum at 60 °C, using the equations established from the ratio of elements. A content of inorganic material, matching with the experimental values, was established once the DS of chitosan derivatives was calculated. The total DS of a derivative is inversely proportional to its content in inorganic salts. Thus, the higher the DS, the lower the inorganic content in the sample. The chelating ability of chitosan [19] is decreased by derivatization.

3. Experimental

General methods.—^{1}H NMR spectra were measured using a Varian Gemini-300 spectrometer. Samples (15 mg) were dissolved in 99.6% pyridine–d_5 (0.7 mL). The downfield peak of the three solvent signals was taken as the internal standard (δ 8.73 ppm). All experiments were carried out at 70 °C. Elemental analyses were performed in a CE Instruments apparatus Mod. EA 1108 (Carlo Erba Instruments, Milan, Italy) using standard conditions by the Serveis Científico-Tècnics at the University of Barcelona (Spain). The specifications of the apparatus allow a tolerance of $\pm$ 0.3% for percentages greater than 20%, $\pm$ 0.2% for percentages ranging from 6 to 20%, and $\pm$ 0.1% for less than 6%. Four analyses were carried out on every sample and a standard of known composition was introduced systematically from time to time to test the accuracy of the results. Combustion of samples for residue weighing was performed in a Heraeus oven Mod. M – 110 at 450 °C during 12 h with a 1 °C/min gradient.

Preparation of polysaccharide derivatives.—Cellulose and amylose derivatives were obtained by the successive reaction of cellulose (Avicel®, E. Merck) or amylose (ICN Biochemicals) with 10-undecenoyl chloride followed by the appropriate isocyanate or benzoyl chloride in pyridine at 115 °C in order to obtain the corresponding carbamate (**1a**–**1e**, **2a**, **2b**, **2d** and **2e**) or benzoate (**1f**–**1k**, **2j** and **2k**).

Chitosan (Fluka, low molecular weight) previously deacetylated or not was used as starting material for the preparation of derivatives **3** and **4**. Deacetylation consisted of treating commercially available chitosan with 50% NaOH [20] until absence of amide absorptions in IR and ^{1}H NMR spectra. The deacetylated product was purified by dissolution, filtration and reprecipitation. The precipitate was dispersed and washed in MeOH and diethyl ether [21]. The chitosan was allowed to react successively with the appropriate isocyanate or benzoyl chloride followed by 10-undecenoyl chloride in pyridine at 115 °C for 24 h, yielding the corresponding carbamates (**3a^{III}**, **3b**, **3d^{III}** and **3e**) and benzoates (**3j** and **3k**). Derivatives **3a$^{I–II}$** and **3d$^{I–II}$** were analogously prepared from non-deacetylated chitosan, and derivatives **4** were obtained similarly with 3,5-dichlorobenzoyl chloride, but with different reaction times (**4k^{I}**, 20% Ac, 8 h; **4k^{II}**, 0–5% Ac, 8 h; **4k^{III}**, 20% Ac, 16 h; **4k^{IV}**, 0–5% Ac, 16 h).

The resulting polysaccharide derivatives were isolated as the fraction insoluble in MeOH. They were redissolved and reprecipitated in MeOH, and thoroughly washed with MeOH and hot EtOH.

4. Conclusions

Elemental analyses of cellulose, amylose and chitosan derivatives allows the calculation of their DS, once the presence of low molecular weight impurities has been ruled out. ^{1}H NMR can be used to confirm these calculations, except when the presence of a particular substituent is low and outside of the limits of detection of the technique.

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References

[1] Y. Okamoto, E. Yashima, *Angew. Chem., Int. Ed. Engl.*, 37 (1998) 1020–1043.
[2] A. Senso, L. Oliveros, C. Minguillón, *J. Chromatogr. A*, 839 (1999) 15–21.
[3] L. Oliveros, P. López, C. Minguillón, P. Franco, *J. Liq. Chromatogr.*, 18 (1995) 1521–1532.
[4] C. Minguillón, P. Franco, L. Oliveros, P. López, *J. Chromatogr. A*, 728 (1996) 407–414.
[5] L. Oliveros, A. Senso, C. Minguillón, *Chirality*, 9 (1997) 145–149.
[6] L. Oliveros, A. Senso, P. Franco, C. Minguillón, *Chirality*, 10 (1998) 283–288.
[7] P. Franco, A. Senso, C. Minguillón, L. Oliveros, *J. Chromatogr. A*, 796 (1998) 265–272.
[8] A. Senso, L. Oliveros and C. Minguillón, *J. Chromatogr. A*, in preparation.
[9] Y. Kaida, Y. Okamoto, *Bull. Chem. Soc. Jpn.*, 66 (1993) 2225–2232.
[10] C. Chassaing, A. Thienpont, T. Félix, *J. Chromatogr. A*, 738 (1996) 157–167.
[11] E. Francotte, T. Zhang, *Analusis*, 23 (1995) M13–M20.
[12] B. Chankvetadze, E. Yashima, Y. Okamoto, *Chem. Lett.*, (1993) 617–620.
[13] C. Dens, H. Friebolin, E. Siefert, *Makromol. Chem.*, 192 (1991) 75–83.
[14] A. Domard, C. Gey, M. Rinaudo, C. Terrasin, *Int. J. Biol. Macromol.*, 9 (1987) 233–237.
[15] C.M. Buchanan, J.A. Hyatt, D.W. Lowman, *J. Am. Chem. Soc.*, 111 (1989) 7312–7319.
[16] Y. Shigemasa, H. Matsuura, H. Sashiwa, H. Saimoto, *Int. J. Biol. Macromol.*, 18 (1996) 237–242.
[17] J.G. Domszy, G.A.F. Roberts, *Makromol. Chem.*, 186 (1985) 1671–1677.
[18] A. Senso, L. Oliveros, C. Minguillón, *Carbohydr. Res.*, (2000) in press.
[19] N.K. Mathur, C.K. Narang, *J. Chem. Ed.*, 67 (1990) 938–942.
[20] Y.C. Wei, S.M. Hudson, J.M. Mayer, D.L. Kaplan, *J. Polym. Sci. Part A*, 30 (1992) 2187–2193.
[21] G.K. Moore, G.A.F. Roberts, *Int. J. Biol. Macromol.*, 4 (1982) 246–249.