Table II. Methods for Drying Biological Tissue Samples

method	associated problems
thermal oven	high temperatures cause loss of volatile elements and alteration of sample matrix (*10*) 16-72 h needed to dry samples (*10*)
microwave oven	for effective drying samples must be minced (*9*), internal high temperature effects probable
freeze-drying	sample contamination from the metallic freezer housing (esp. Cr) is possible (*11*), cellular integrity destroyed
dry ashing	high temperatures result in a loss of elements such as As, Co, Cr, and Ni by volatilization (*12-17*)
wet ashing	loss of Se, Te, and Po and matrix interferences due to incomplete destruction of the organic matrix when samples are treated with $HNO_3/HClO_4$ or H_2SO_4/H_2O_2 (*12, 18*), possible sample contamination from reagents used in the procedure (*19-27*)

elemental losses due to volatilization were held at an absolute minimum by keeping the drying chamber and its contents at ambient temperature and at atmospheric pressure. Losses, including those resulting from the alteration of the biological matrix upon heating, or the loss, via volatilization, of elements or compounds having high vapor pressures are therefore reduced or eliminated. By use of this drying scheme, the sample is protected from sample–container interactions such as those encountered during ashing and freeze-drying. The sample is simply suspended in an inert, clean atmosphere without any solvent pretreatment, mincing, or prefreezing.

This cost-effective method of drying samples is as efficient as those methods already in existence and has been optimized with respect to maintaining sample integrity. The ability to dry entire organs and large pieces of tissue without significant change in form makes this technique particularly well suited to XRF analysis and preserves the sample for other invasive analytical examinations.

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Proton and Carbon-13 Nuclear Magnetic Resonance Spectrometry of Formaldehyde in Water

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A number of NMR studies, involving either ^1H or ^{13}C spectrometry, have been devoted to aqueous solutions of formaldehyde owing to the great industrial importance of this compound (*1-12*). In aqueous solutions, formaldehyde reacts with water to give methylene glycol which oligomerizes easily according to the following scheme:

$$CH_2O + H_2O \rightleftarrows HOCH_2OH$$

$$HOCH_2OH + HOCH_2OH \rightleftarrows HO(CH_2O)_2H + H_2O$$

$$HO(CH_2O)_{n-1}H + HOCH_2OH \rightleftarrows HO(CH_2O)_nH + H_2O$$

The structures of the different species present in the solutions have already been determined, mainly by NMR (*2-12*), but no special attention has been paid to the influence of temperature on the oligomeric distribution nor to the chemical relaxation rate of dilution, i.e., the time required for the solution to reach equilibrium when the concentration is changed. On the other hand, the quantitative determination of oligomers in solutions, which is performed by gas chromatography (GC), necessitates the substitution of the labile hydrogens by a trimethylsilyl group, and one might expect the distribution measured by GC to be perturbed by this chemical treatment. Carbon NMR spectrometry has been used in this work to obviate this difficulty and good quantitative results have been obtained. Moreover, the ^{13}C NMR spectra already published were not fully assigned, preventing any valuable quantitative application.

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Table I. Longitudinal Relaxation Times T_1 of Terminal CH_2O (A, B, C, and E) and Internal CH_2O Groups (D, F, and H) in Oligomers

procedure		A	B	C	E	D	F	H
semilog plot	T_1	4.0	3.0	2.4	1.9	2.1	2.0	1.7
with standardization[a]	Z exp[d]	1.91	1.90	1.89	1.95	1.97	1.82	1.85
	I_∞	100	100	50	50	50	25	10
	r[b]	0.99_5	0.99_5	0.99_3	0.98_3	0.99_1	0.99_7	0.99_5
exponential regression	T_1	4.1	3.1	2.45	2.05	2.1	1.8	1.6
(3 parameters)	I_∞	99.2	99.0	48.8	48.4	47.3	24.0	9.7
	Z (ν_{min})[d]	1.90	1.87	1.88	1.86	1.94	1.90	1.84
	v[c]	12.8	10.1	3.1	6.6	6.8	2.0	0.23

[a] $\ln([(I_\infty - I_\tau)/I_\infty]) = \ln(1 - \cos\alpha) - (1/T_1)\tau$ was the relation used in the semilogarithmic analysis. [b] r = correlation coefficient. [c] v = variance of the adjustment. [d] Z is defined as the Z imperfection factor of the pulse $Z = (I_\infty - I_{\tau 0})/I_\infty$. If the pulse was perfect, $Z = 2.0$, and there would be no need to iterate on Z. Since $I_{\tau 0}$ is different from I_∞, it is necessary to iterate considering Z as a variable, until the variance is minimum. It is interesting to note that the value of Z (ν_{min}) agrees quite well with the experimental one ($Z_{exp} = (I_\infty - I_{\tau 0})/I_\infty$ (16).

EXPERIMENTAL SECTION

Chemicals. Aqueous solutions were prepared from paraformaldehyde (Aldrich), depolymerized in distilled water. A sample of paraformaldehyde, containing less than 100 (CH_2O) units was introduced under nitrogen flow in a flask which was sealed and maintained for 60 h at 340–360 K. The content of the solution in (CH_2O) units is obtained from the weight percentage of paraformadehyde in the solution $W = m(CH_2O)(m\cdot(CH_2O) + m(H_2O))^{-1}\,100$.

$$[CH_2O] = \frac{dW}{3} \text{ (ref 5)} \qquad (1)$$

where the density d is linearly related to W: $d = a + bW$. Walker (1) gives approximative values for a and b, but we have computed a more precise relationship from 19 couples of d, W values measured by Auerbach and Natta, and quoted by Walker (1).

$$N = 19, \quad r = 0.9998$$

$$d(\pm 0.0005) = 0.9984(\pm 0.0002) + 0.00310(\pm 0.00001)W \qquad (2)$$

NMR Spectra. Proton spectra were recorded at 250 MHz with a Cameca 250 spectrometer in the continuous wave (CW) mode (SW 300 Hz) and at 100 MHz with a Varian XL 100 12 spectrometer in the CW mode (SW 100 Hz; integration sweep time 100 s). The signal of C_6H_6 contained in a thin coaxial tube was used for field-frequency locking. The shifts were referred to the C_6H_6 signal, measured at 6.47 ppm from TSPA (3-(trimethylsilyl)propionic acid Na salt): ^1H (TSPA)/(Me_3Si) = 0.015 ppm.

Carbon spectra were recorded with a Cameca 250 or a Bruker WH 90 spectrometer: Cameca 250 (F = 62.86 MHz, sample tube ϕ = 8 mm, SW (sweep width) = 1250 Hz, PW (pulse width) = 10×10^{-6} s, AT (acquisition time) = 6.6 s); Bruker WH 90 (F = 22.635 MHz, sample tube ϕ = 10 mm, SW = 1500 Hz, AT = 2.73 s PW = 3 to 3.5×10^{-6} s, $PW_{90} = 11.5 \times 10^{-6}$ s, T = 303 K).

In both cases, a thin 4 mm o.d. coaxial tube containing C_6D_6 was introduced in the NMR cell to provide signal locking and shift reference. The ^{13}C signal of C_6D_6 is observed at 129.56 ppm from TSPA.

Relaxation Time Determination. The T_1 values of ^{13}C signals observed in the oligomer solutions were determined with the Bruker WH 90 spectrometer according to the IRFT method (13) applied under well-defined conditions (14, 16).

The delay between 90° pulses was 25 s and τ values ranging from 0.001 to 25 s were taken into account. The signals of the external CH_2O group in the mono-, di-, tri-, and tetramer, denoted A, B, C, and E, and internal in tri-, tetra-, and pentamer, D, F, and H (see Figure 1), have been studied for 17 values of τ (0.01, 0.1, 0.25, 0.3, 0.5, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0). The result of the analysis performed by using two computation procedures is shown in Table I. For the lines I, L, and P, we obtain a null intensity for τ = 0.9 s, $T_1 \simeq 1.5$ s, with ln (1 − (I_0/I_∞)) = 1.85. Interestingly, the values of the T_1 relaxation times decrease with the degree of oligomeric condensation, but for a given oligomer the contribution to anisotropic motion is not significant.

In order to save time in the quantitative determinations the pulse width was fixed at 25° and AT = 2.73 s. Under these conditions, the difference in signal-to-noise ratios for signals having relaxation times ranging from 1.5 to 4.0 s is always smaller than 9% but the loss in sensitivity is of the order of 25/30%, with respect to the optimum S/N value computed for the Ernst angle. The S/N values were calculated according to

$$S/N = \frac{Mo}{\tau^{1/2}} \frac{(1 - \exp(-\tau/T_1))\sin\theta}{(1 - \exp(-\tau/T_1))\cos\theta}$$

Oligomeric species $HO-(CH_2O)_nH$		δ^1H (ppm/TMS)	$\delta^{13}C$ (ppm/TMS)	T_1 (s)
n = 1	A	4.80	84.70	4.1
2	B B	4.87	88.38	3.1
3	C D C	(C) 4.86 (D) 4.93	88.89 91.59	2.45 2.1
4	E F F E	(E) (a) (F) 4.93	89.15 92.12	2.05 1.8
5	G H I H G	(H) (a) (I) (a)	92.30 92.52	1.6 ≃ 1.5
6	J K L L K J	(L) (a)	92.72	≃ 1.5
7	M N O P O N M	(P) (a)	92.95	≃ 1.5
8	Q R S T T S R Q	(T) (a)	(a)	

Figure 1. Oligomer distribution of aqueous solutions of formaldehyde (35% in weight of formaldehyde) at 338 K. $\delta^1H(OH)$= 4.60 ppm/Me_3Si. (a) Signal not resolved. ^{13}C spectra show overlapping signals: E, G, J, M, and Q; H, K, N, and R; L, O, and S; P and T.

RESULTS AND DISCUSSION

Presentation of Spectra. The aqueous solutions of formaldehyde are composed of mixtures of oligomers, the composition of which depends mainly on the concentration and the temperature. It is convenient to represent the solutions in terms of the nonequivalent (CH_2O) units, denoted A, B, ..., which are found in the different oligomers (see Figure 1).

The experimental ^{13}C chemical shifts may be represented as a sum of different additive contributions

$$\left.\begin{matrix}H\\ H\text{-}O\text{-}CH_2\end{matrix}\right\}\text{-}[OCH_2O]\text{-}CH_2\text{-}O\text{-}\left\{\begin{matrix}H\\ CH_2\text{-}O\text{-}H\end{matrix}\right.$$

$$\delta\ \gamma\ \beta \qquad 0 \qquad \beta\ \gamma\ \delta\ \epsilon\ \psi$$

$$\delta\,^{13}C = A_0 + \sum_{i=1}^{n} A_i \qquad \begin{cases} r = 0.999 \quad n = 10 \quad S = 0.012 \\ A_0 = 84.55\ (\pm 0.01) \end{cases}$$

A_i:	βH	βCH_2O	δH	δCH_2O	ψH
	0.1 (±0.1)	3.5 (±0.15)	0.2 (±0.1)	1.0 (±0.1)	−0.23 (±0.007)

In fact, we observed that two identical groups, substituted in the same relative position to the group taken as an origin, do not have a purely additive contribution. We have weighted

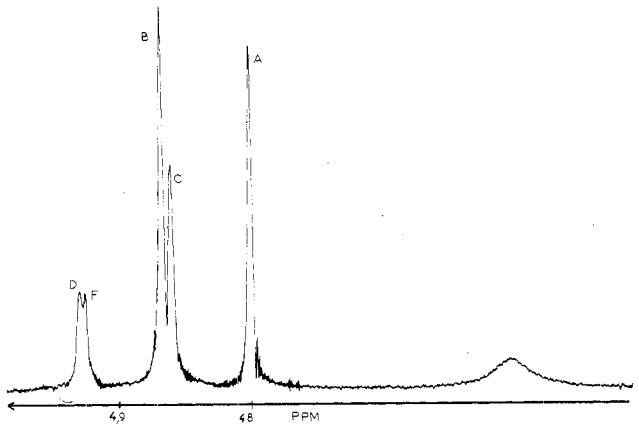

Figure 2. 100-MHz ^1H NMR spectrum of 31% formalin at 333 K (see Figure 1 for description of the signals).

the discrete independent variable 2 by a factor f which minimizes the standard deviation of the estimate ($f = 0.935$). It is interesting to note that one observes a shielding effect of the γ(OH) group, which is, however, smaller than the γ(OH) parameter calculated for alcohols (−5.8 ppm) (*15*).

Experimental conditions and spectrometer performances are important factors governing the discriminating power of the NMR method as far as the nonequivalent units A, B, ..., are concerned.

In ^1H NMR spectrometry, three (*2*, *5*, *8*, *9*), four (*3*), or five (*6*, *7*, *10*) signals have been observed under different conditions, and in ^{13}C NMR only five oligomers were identified (*11*). High-field NMR improves the resolution in terms of chemical shift differences and four oligomers may be observed at 250 MHz (^1H) or seven oligomers at 22.6 MHz (^{13}C) (see Figures 2 and 3).

Influence of Experimental Conditions (Concentration and Temperature) on the NMR Spectra. Application to the Determination of the Composition of Formaldehyde Solutions. (*a*) *Proton Spectra.* If we consider the signal of residual water, the difference $\Delta\delta = \delta_{H_2O} - \delta_A$ is a function of W and T, the absolute temperature

$$\Delta\delta(\pm 0.012) = 3.115 - [1.053\ (\pm 0.008) \times 10^{-2}]T + [0.63\ (\pm 0.02) \times 10^{-2}]W$$

$$n = 42 \qquad r = 0.999$$

However, the changes in signal intensities, which are observed when a solution of formaldehyde ($W = 35\%$) is strongly diluted, provide interesting information on the kinetics of

Table II. Variation with the Temperature of the Percentage of Monomer A[a]

		% monomer A			
signal	sample	265 K (45 days)	281 K (250 days)	293 K (15 days)	343 K (0.08 day)
A	I	65.4	64.8	66.3	70.8
	II	66.4	64.7	66.7	69.4
	III	64.1	65.1	66.2	69.4
	$\bar{X}$	65.3	64.9	66.4	69.9
	$\bar{S}$	0.9	0.2	0.2	0.6
B + C	III	33.2	31.9	32.5	28.3
		0.8	0.6	2.5	0.4
D	III	2.2	3.1	1.3	2.3
		0.3	0.2	0.3	0.1

[a] The values obtained for $T = 281$ K are the mean of 10 quantitative determinations for which the standard deviation is of the order of 0.5%. Thus, the dispersion of the determination due to the sample is smaller than that due to the measurement procedure. Comparable results were obtained when the dimer and trimer were considered ($T = 281$ K): B + C, 31.7% (0.6); D, 3.5% (0.4).

reequilibration (relaxation) of the exchanging species. At 273 K, a 3% solution prepared from a 35% solution needs 50 h for equilibrium to be reached, and at 327 K, a relaxation time of about half an hour is measured. Figure 4 shows the change in (CH_2O)% as a function of time for the mono- and dimeric species. This fact is important to consider when aqueous solutions of formaldehyde are used in fast polycondensation reactions. If that is the case, then the overall kinetics of the reaction is governed by the depolymerization step.

An increase in the temperature leads to an acceleration of the depolymerization step and concentrated solutions ($W \geq 30\%$), which always show some quantities of paraformaldehyde, depolymerize readily. The influence of the temperature on the oligomer distribution is of great practical importance but seems to have been minimized. Indeed, Moedritzer (*3*) and Jones (*8*) noted that the temperature has a significant, but small, effect on oligomeric distribution. In order to avoid anomalous results due to the preparation of the compound, we have always studied a set of three samples referenced I, II, and III prepared from the same D_2O solution of formaldehyde ($W \simeq 9\%$). The influence of the temperature is clearly evidenced in Table II which shows the change of the percentage of signal A of the monomer. Analysis of the variance indicates that the observed variations are signficant

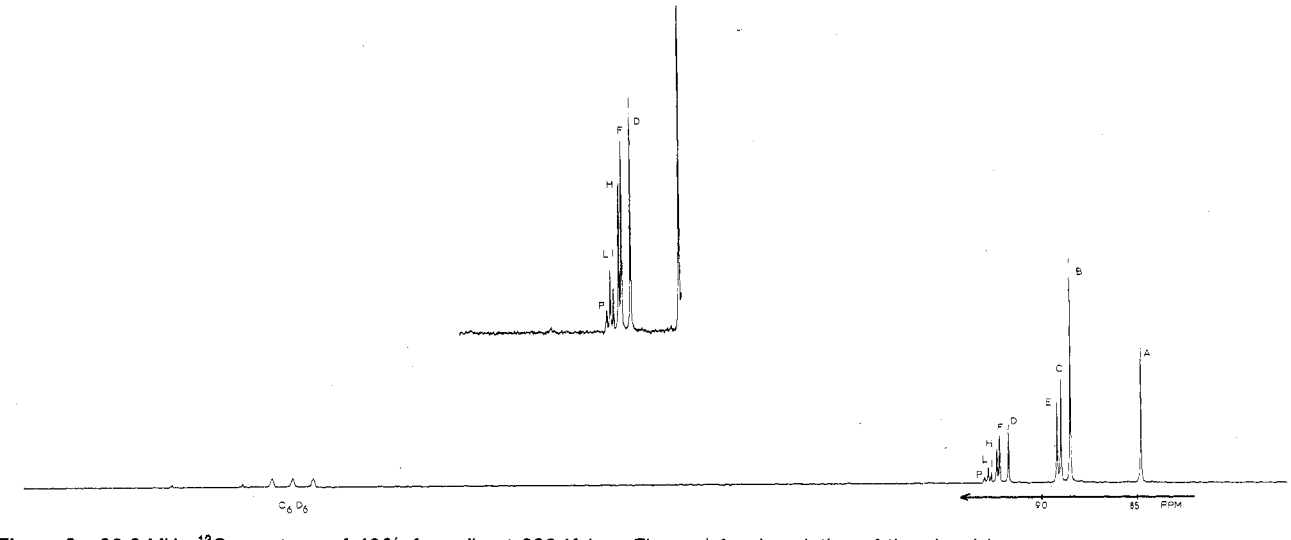

Figure 3. 22.6-MHz ^{13}C spectrum of 48% formalin at 333 K (see Figure 1 for description of the signals).

Table III. Influence of Temperature Increase (30 K) on Carbon-13 NMR Lines (see Figure 1) in Aqueous Solutions of Formaldehyde

	$\Delta\delta$ [a]									
% W	A	B	C	D	E	F	H	I	L	P
42.8	0.29	0.40	0.40	0.57	0.34	0.45	0.42	0.47	0.45	0.41
37.0[b]	0.32	0.52	0.49	0.67	0.46	0.62	0.60	0.63	0.61	0.56

[a] $\Delta\delta > 0$ paramagnetic effect (ppm). [b] Solution with 2% methanol/[CH_2O].

Table IV. Variations of Carbon-13 NMR Spectra of Aqueous Solutions of Formaldehyde as a Function of Concentration

no. of signals	1	2	4	6	8	9	10	10	10
no. of oligomers (n)	1	2	3	4	5	6	7	>7	>7
concn C, M	0.16	0.33	1.0	2.6	4.5	7.8	10.9	13.7	16.1
wt percentage W, %	0.5	1.0	3.0	7.6	13	22	30	37	42.8
monomer C_1, M	0.16	0.3	0.7	1.7	2.4	2.65	3.0	2.95	3.15

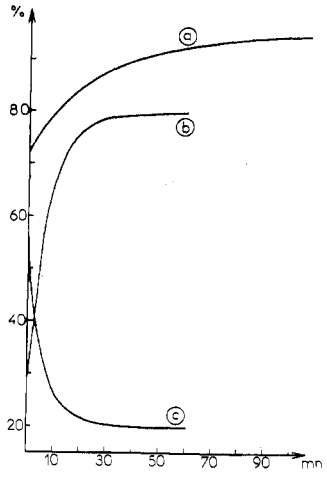

Figure 4. Time variations of the percentage of $-CH_2O-$: (a) in the monomer, T = 294 K, dilution from 8% to 1.3%; (b) in the monomer, T = 327 K, dilution from 40% to 4%; (c) in the dimer and terminal groups $-CH_2OH$ (lines B and C), T = 327 K, dilution from 40% to 4%.

assuming a normal distribution for the population and a F distribution for the sample. At the 95% confidence level, the null hypothesis H_0:T has no significant effect and can be rejected and H_0' (the three samples are equivalent) can be accepted. At temperatures above 350 K, the kinetics of the depolymerization is relatively fast since measurements performed 0.06, 0.20, 0.97, and 1.94 days after the temperature of the sample was set at 368 K give statistically equivalent results.

It is now easy to obtain the concentration of the first three oligomers, n = 1, 2, and 3, from the values collected in Table II and using eq 1 and 2, the concentration in $(-CH_2O-)$ is computed as being equal to 3.1 M. The water concentration at equilibrium $(H_2O)_e$ is then evaluated according to

$$(H_2O)_e = \left[\frac{d}{18}\left(1 - \frac{W}{1000}\right) \times 1000\right] - ((1) + (2) + (3))$$

T, K	(1)	(2)	(3)	(1 + 2 + 3)	$(H_2O)_e$
281	2.02	0.40	0.10	2.52	49.3
343	2.15	0.37	0.07	2.59	49.21

(b) Carbon Spectra. Only signal A (monomer) seems to significantly change with the dilution since a low frequency shift is evidenced for 23 different determinations

$$\delta\ ^{13}C/Me_3Si = 84.58 + 0.0055W \qquad r = 0.93$$
$$\pm 0.02 \qquad\qquad \pm 0.005$$

However an increase in temperature of 30 K leads to high frequency shifts which become even higher, the lower the concentration (T_i = 303 K). A small quantity of methanol (2%) induces a slight paramagnetic shift (Table III).

In fact, the number of signals which are detected in the experimental conditions used in this work depends on the concentration and the diagram presented in Table IV may be a useful guide.

It is clear that in order to deal with solutions containing only the monomeric unit A, it is necessary to dilute the formaldehyde down to 0.16 M. In order to establish the relative composition of the different oligomers, the results shown in Table V and Figure 5 should be considered. The reproducibility of the experiment has been checked by studying five different solutions W% = 34.7 (a two-sided 95% confidence interval is obtained for a standard deviation s = 0.5).

Table V. Composition (in Percentage) of the $-CH_2O-$ Group in the Various Oligomers as a Function of the Ponderal Concentration of the Solution

% W of the solution	% $-CH_2O-$					
	1	2	3	4	5	≥6
0.5	100	0	0	0		
1	92	8	0	0		
3	84.5	14.5	1	0		
7.6	65.2	27.9	6.0	1.0	0	0
12.0	56.0	31.5	10	2.5	0	0
17.8	46.5	37	12.5	3	0.5	0
25.0	35	35.5	17.5	7.5	4.5	
30.0	27.5	32.3	21.0	10.3	9.0	
35.0	24.2	32.4	21.3	11.0	6.9	4.2
37.0	21.4	29.6	20.2	12.1	7.2	5.7
42.8	19.5	29.0	22.0	12.6	7.2	13.3
50.0	14.6	24.0	20.5	14.7	9.4	12.7

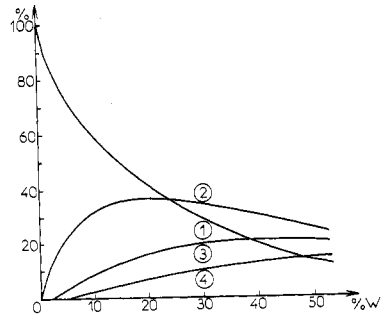

Figure 5. Change (in percentage) of the monomer (1), dimer (2), trimer (3), and tetramer (4) as a function of the ponderal concentration W.

	n = 1	2	3	4	5	≥6
%	24.8 (0.7)	32.4 (0.5)	21.2 (0.6)	10.9 (0.4)	6.6 (0.6)	4.0 (0.8)

Although ^1H NMR spectrometry has been extensively used in studying formaldehyde solutions, quantitative analysis of the proton signals remains inconclusive. We have shown that a given aqueous solution of formaldehyde may display a different number of proton lines, depending on the experimental conditions used during preparation. In ^{13}C NMR spectra, however, a greater number of species can be determined and therefore ^{13}C NMR spectrometry is a better method than ^1H NMR for the qualitative and quantitative analyses of aqueous formaldehyde solutions. In addition ^{13}C NMR spectrometry provides a good alternative to gas chromatography since no prior chemical transformation, such as silylation, is necessary and which might significantly affect the oligomer distribution.

Determination of the Equilibrium Constants of Oligomer Formation. Oligomerization may be assumed to proceed via the reaction of dihydroxymethylene (monomer unit) on each successive oligomer via the following scheme:

$$(1) + (1) \rightleftarrows (2) + H_2O \qquad K_2$$

$$(1) + (2) \rightleftarrows (3) + H_2O \qquad K_3$$

$$(1) + (n-1) \rightleftarrows (n) + H_2O \qquad K_n$$

where (1), (2), ..., (n) represent respectively the various species (mono-, di-, ..., n-mer).

The equilibrium constants are then conventionally defined as

$$K_2 = \frac{(C_2)(H_2O)}{(C_1)(C_1)} \qquad K_3 = \frac{(C_3)(H_2O)}{(C_1)(C_2)} \qquad \dots \qquad K_n = \frac{(C_n)(H_2O)}{(C_1)(C_{n-1})}$$

so then the ratio K_n/K_{n-1} does not depend on the concentration of water

$$\frac{K_n}{K_{n-1}} = \frac{(C_n)(C_{n-2})}{(C_{n-1})^2}$$

Thus using ^{13}C NMR spectrometry, it is possible to compute the values of the ratios K_n/K_{n-1}, as far as the signals corresponding to various oligomers can be resolved, and the ^{13}C data collected in Table V give the ratios at $T = 303$ K of $K_3/K_2 = 0.66$ (0.05) and $K_4/K_3 = 0.86$ (0.06). ^{13}C results provide also values of equilibrium constants: $K_2 = 6.1$ (0.6); $K_4 = 3.6$, using $(H_2O) = (H_2O)_{initial} - (oligomers)$.

In previous studies, a quantitative determination of different oligomers (*3*, *5*, *10*) was made possible on the assumption that the equilibrium constants of reactions involving the considered oligomers were constant. As pointed out by Kopf (*6*) from the results of a preliminary ^1H study at 313 K ($W = 40\%$), K_3 is significantly smaller than K_2 ($K_3/K_2 = 0.62$ and 0.71 with methanol). Our results indicate that $K_n/K_{n-1} \rightarrow 1$ when $n \geq 6$, i.e., only for highly condensed oligomers which exist in a small quantity.

Registry No. HO-(CH_2O)H, 463-57-0; HO-$(CH_2O)_2$H, 4407-89-0; HO-$(CH_2O)_3$H, 3754-41-4; HO-$(CH_2O)_4$H, 28317-12-6; HO-$(CH_2O)_5$H, 28317-13-7; HO-$(CH_2O)_6$H, 28317-14-8; HO-$(CH_2O)_7$H, 28317-15-9; formaldehyde, 50-00-0.

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Column Fittings without Ferrules for Liquid Chromatography

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A need for high-performance liquid chromatography (HPLC) columns as short as a few millimeters has arisen in our laboratory. Commercially available HPLC column end fittings with ferrules are not suitable because the end fittings occupy a space of several centimeters on the column blank and thus limit how short the column can be. This problem can be circumvented by placing a fluorocarbon insert in the column (*1*) or by using threaded columns which screw directly into the end fittings (for example, EM Science's Precolumn Assembly and Upchurch Scientific's Uptight Precolumn). Even with the latter approach it is difficult to make columns shorter than 2 cm. We present here a versatile column design which can be used for columns as short as 5 mm.

EXPERIMENTAL SECTION

Column Design. Details of the column design are shown in Figure 1. The inlet and outlet end fittings (A) are machined from 0.75 in. hexagonal type 304 stainless steel. Connections to inlet and outlet tubing are made to accept 10/32 threaded $^1/_{16}$ in. male fittings (B). Parker $^1/_{16}$ in. male fittings (Rainin, Woburn, MA) were used in our studies. A $^1/_{16}$ in. diameter hole (C) connects to a 0.0135 in. hole (D) with a tapered opening of 0.08 in. diameter and 0.015 in. depth (D). A channel (I) of depth 0.160 in. aids in removal of frits (E) and O-rings (H).

The end fittings are connected together by a 0.75 in. hexagonal brass connector (F) of length approximately 0.5 in. greater than the length of the column blank (G). The brass connector and end fittings have $^5/_8$ in.-32 threads. The 0.25 in. o.d. type 316 low

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