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CONTINUOUS-FLOW CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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SUMMARY

The design of a probehead for continuous-flow ^{13}C NMR spectroscopy is described and the sensitivity and resolution presently available demonstrated. Comparison of static continuous-flow measurements shows that continuous-flow ^{13}C NMR can be advantageously employed for studying reaction kinetics and for monitoring the separation of complex ^{13}C-labelled product mixtures by direct coupling with high-performance liquid chromatography.

INTRODUCTION

Continuous-flow nuclear magnetic resonance (NMR) spectroscopy has been successfully applied to the investigation of reaction pathways[1–4] as well as in direct coupling of high-performance liquid chromatography (HPLC) with ^{1}H NMR[5–13]. Although ^{1}H NMR spectroscopy is indeed helpful for structure elucidation, the information content of ^{13}C NMR spectroscopy is significantly higher, providing direct access to the structures of compounds investigated. This justifies the efforts to carry out continuous-flow measurements with ^{13}C NMR spectroscopy, despite the problems of sensitivity, relaxation time and proton decoupling. In continuous-flow NMR measurements the disadvantage of the dependence of the NMR signals of the flow-rate[13,14] is compensated by the great advantage of obtaining immediate information.

In order to obtain signals of about the same intensity as those from static measurements, the substance investigated should remain in the magnetic field for three times the longitudinal relation time, T_1. According to eqn. 1

$$M_t = M_0 (1 - e^{-t/T_1}) \quad (1)$$

the longitudinal magnetization, M_t, then reaches about 95% of the equilibrium magnetization, M_0. In the case of continuous-flow ^{1}H NMR spectroscopy, this condition is met at flow-rates up to 1 ml/min, where the relaxation times are substantially

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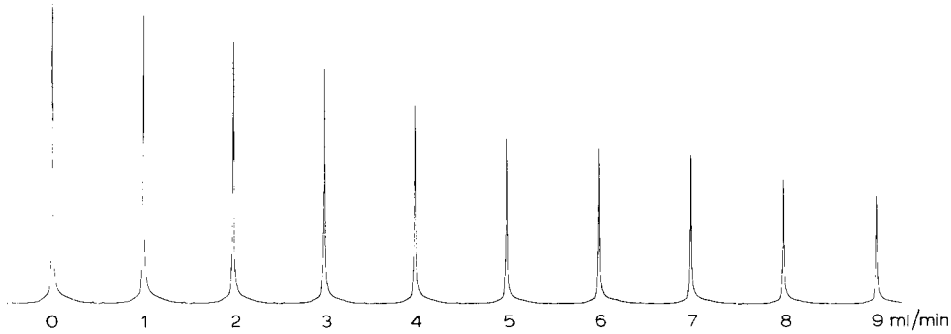

Fig. 1. Flow-rate dependent ^1H NMR signals of chloroform in [$^2H_6$]acetone. Pulse repetition time: 5.1 sec. 8K data points; one scan, spectral width 800 Hz.

smaller than the residence times in the magnetic field. In Fig. 1, the dependence of the signals for chloroform on the flow-rate under the measurement conditions is depicted. With increasing flow-rate the intensity of the signals is reduced.

In the case of nuclei with long relaxation times such as ^{13}C nuclei, this effect is stronger. In Fig. 2, the flow-rate dependence of the intensity of ^{13}C signals for benzene is shown.

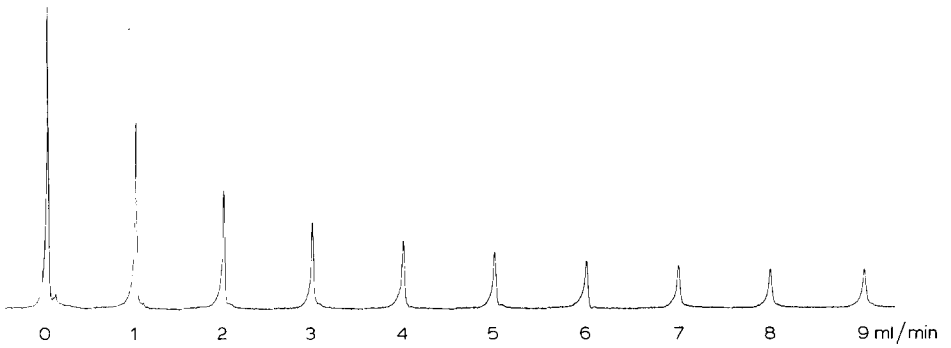

Fig. 2. Flow-rate dependent ^{13}C NMR signals of benzene in [$^2H_6$]acetone. Pulse repetition time: 13.7 sec. 8K data points; one scan, spectral width 600 Hz. Continuous-wave decoupling.

Continuous-flow spectra are not only influenced by the longitudinal relaxation time, T_1, but also by the transverse relaxation time T_2. The dependences of T_2 and the width at half-height, $\Delta\omega$, on the residence time, τ, of a substance in the magnetic field are as follows:

$$\begin{aligned} 1/T_{2,\text{flow}} &= 1/T_{2,\text{stat.}} + 1/\tau \\ \Delta\omega_{\text{flow}} &= 1/\pi \cdot T_{2,\text{stat}} + 1/\tau \\ &= \Delta\omega_{\text{stat}} + 1/\tau \end{aligned} \tag{2}$$

There is thus a linear dependence between the flow-rate and the measurement time

TABLE I

RELATIONSHIP BETWEEN THE FLOW-RATE AND THE WIDTH AT HALF-HEIGHT OF ^{1}H NMR SIGNALS

Flow-rate (ml/min)	*Residence time, τ (sec)*	*$1/\tau$ (Hz)*	*Width at half height, $\Delta\omega$ (Hz)*	
			Theoretical	*Experimental*
0	∞	0	0.55	0.55
0.5	5.2	0.19	0.74	0.75
1.0	2.6	0.38	0.93	1.05

on the one hand, and between the measurement time and the width at half-heigt of the signals on the other. Table I shows experimentally determined $\Delta\omega$ values in a ^{1}H NMR flow probehead as well as the theoretical values.

EXPERIMENTAL

The NMR spectra were measured on a Bruker WM 400 NMR instrument equipped with the computer system Bruker Aspect 2000. For the data storage a 80-MByte disk system (CDC) was employed. The chromatographic system (Bruker LC 31) was installed 2 m away from the cryomagnet. The HPLC instrument and continuous-flow NMR cell were connected to each other using PTFE tubes (1.5 mm I.D.).

The ^{13}C NMR flow probehead

The flow probehead was a non-rotating cell as depicted in Fig. 3. A measuring coil, 15 mm in length, was mounted at the inlet of a U-shaped 2 mm I.D. glass tube. The internal diameter of the glass tube was increased to 4 mm over the length of the

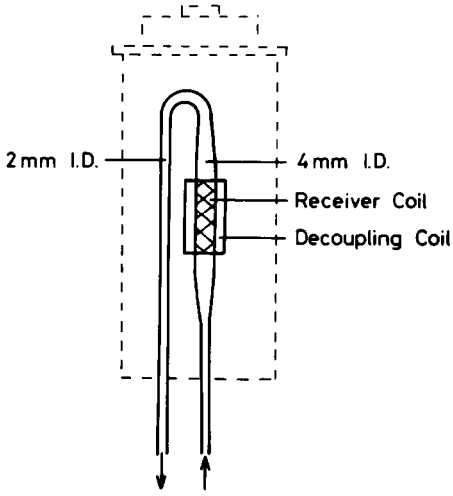

Fig. 3. Schematic diagram of the ^{13}C NMR flow probehead.

measuring section. Consequently a volume of 188.5 μl and a measurement time of 11.3 sec at a flow-rate of 1 ml/min were obtained. The decoupling coil was mounted axially to the measuring coil. The addition to and removal of the sample solution from the cell was so regulated that no disturbance of the flowing fluid inside the measuring section was incurred. This was determined visually using coloured substances. Cell-wall effects were eliminted by treatment of the cell with hexamethyldisilazane.

Arrangement for measurements

Since the ^{13}C NMR relaxation times can be of the order of several minutes, the conditions for eqn. 1 are not fulfilled. This problem can however be partially solved by pre-polarization of the flowing solution in the scattering field of the magnet. Under our experimental conditions, with a flow-rate of 1 ml/min, the pre-polarization time was 35 sec. Although it is possible to polarize all carbon atoms of molecules such as cholesteryl acetate within the above time, it is not possible to achieve the same effect for carbon atoms of smaller molecules such as ethylbenzene. Nevertheless, an acceptable signal to noise ratio can be achieved with such compounds, as is shown below.

RESULTS AND DISCUSSION

Characterization of the ^{13}C NMR continuous-flow probehead

Compensation for field inhomogeneity, usually achieved by rotation of the measuring cell, is not possible using the continuous-flow cell described here. Resolution measurements on benzene indicated a peak width at half-height of the ^{13}C signal of the order of 0.37 Hz (Fig. 4), compared with *ca.* 0.08 Hz for the corre-

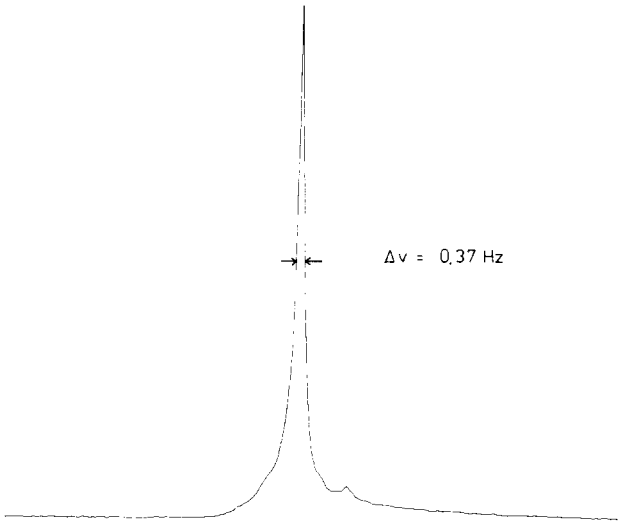

Fig. 4. ^{13}C NMR spectrum of benzene in [$^{2}H_6$]acetone obtained with the ^{13}C NMR flow probehead. Pulse repetition time: 13.7 sec. 8K data points; sixteen scans, spectral width 300 Hz, continuous-wave decoupling.

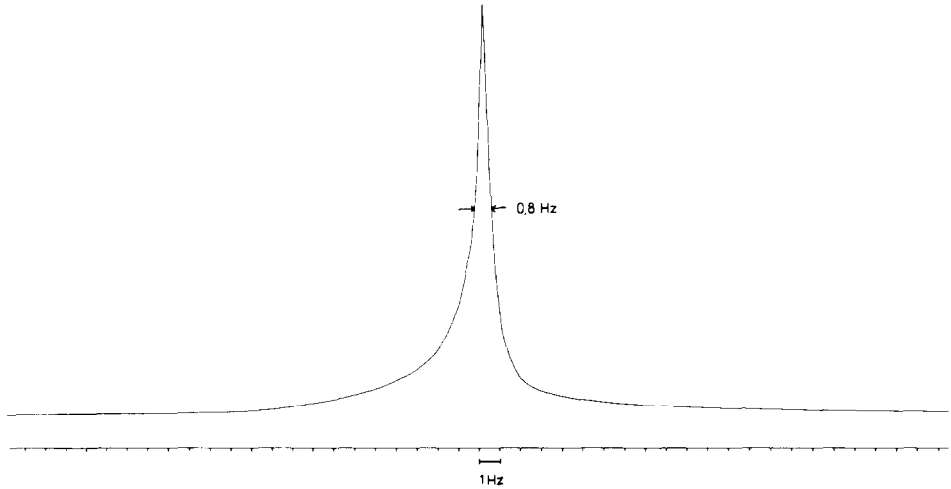

Fig. 5. ^{1}H NMR spectrum of chloroform in [$^{2}H_6$]acetone measured with the decoupling coil of the ^{13}C NMR flow probehead. Pulse repetition time: 10.2 sec. 16K data points; sixteen scans, spectral width 800 Hz.

sponding static measurement. By applying the decoupling coil to measure the ^{1}H NMR spectrum, a width at half-height of 0.8 Hz is obtained for chloroform (Fig. 5).

The sensitivity test with one scan and pulse repetition time fo 0.22 sec for 80% ethylbenzene in acetone at a flow-rate of 1 ml/min gives a signal to noise ratio of 155 (Fig. 6). The corresponding value for a static measurement is about 350–400.

A comparison of a static and a continuous-flow spectrum of 100 mg/ml cholesteryl acetate in deuterochloroform is seen in Fig. 7. Spectrum (a) is a static measurement of a degassed solution in a sealed tube, and spectrum (b) a flow measurement at a flow-rate of 1 ml/min.

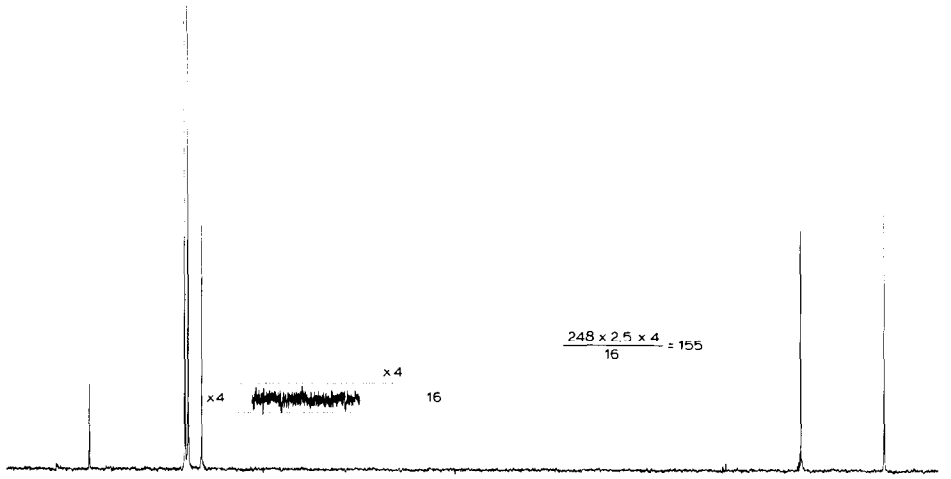

Fig. 6. ^{13}C NMR spectrum of 80% ethylbenzene in [$^{2}H_6$]acetone obtained with the ^{13}C NMR flow probehead. Pulse repetition time: 0.22 sec. 8K data points; one scan, spectral width 18,500 Hz, line broadening 4.5 Hz, pulse width 8.5 μsec. Flow-rate: 1 ml/min, signal-to-noise ratio = 155:1.

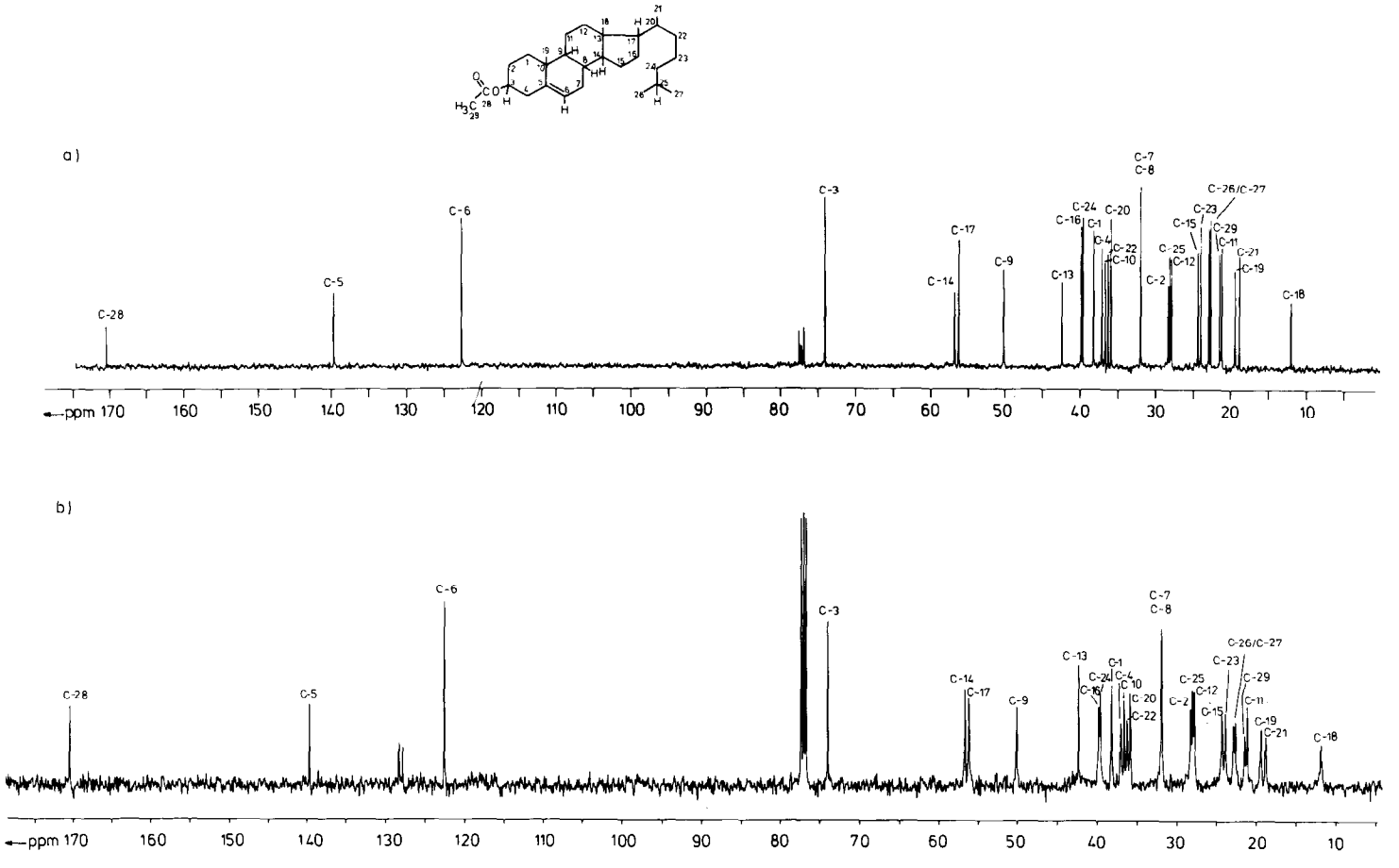

Fig. 7. ^{13}C NMR spectra of cholesteryl acetate (100 mg/ml) in deuterochloroform: a, conventional measurement (sealed NMR tube); b, continuous-flow measurement (flow.-rate 1 ml/min). Pulse repetition time: 0.34 sec. 16K data points; 256 scans, spectral width 23,800 Hz, line broadening 3 Hz. Broad band decoupling.

The presently available sensitivity is sufficient to allow *in vivo* investigations of biochemical metabolic processes[15], for studing electrochemical conversion processes which cannot be observed directly within the magnetic field or as a mass detector for complex ^{13}C labelled synthetic mixtures. In terms of the time and effort involved, the direct coupling method is preferable to off-line detection. All resonances in the complicated aliphatic range are resolved in the flow spectrum, giving full information about the structure. Modification of the measuring cells with the goal of achieving a sensitivity approaching that of static measurement is at present underway.

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