MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2002; **40**: S83–S88
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mrc.1121

Use of ^{1}H NMR-determined diffusion coefficients to characterize lipoprotein fractions in human blood plasma

Maili Liu,[1,2]* Huiru Tang,[2] Jeremy K. Nicholson[2] and John C. Lindon[2]

[1] Laboratories of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China.
[2] Biological Chemistry, Biomedical Sciences Division, Faculty of Medicine, Imperial College of Science, Technology and Medicine, Sir Alexander Fleming Building, South Kensington, London SW7 2AZ, UK

Received 28 June 2002; Revised 30 August 2002; Accepted 2 September 2002

The ^{1}H NMR spectra of human blood plasma show, *inter alia*, prominent resonances from the methyl and methylene groups of the fatty acyl chains from the triglycerides, phospholipids and cholesteryl esters of lipoproteins. Each band shows some degree of peak resolution due to chemical shift differences between the lipoprotein fractions, namely very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). In order to assign resonances within the NMR bands to individual lipoproteins, previous studies relied upon the measurement of pure fractions obtained by ultracentrifugation. Here a new approach was used where the lipoprotein peaks in the NMR spectrum were deconvolved mathematically and the area of each sub-peak was monitored using an NMR pulse sequence to yield the diffusion coefficient relating to each sub-peak and hence to each type of lipoprotein. The validity of the deconvolution results was confirmed by comparison of ^{1}H NMR spectra measured at 600 and 800 MHz. The diffusion coefficient is directly related to the hydrodynamic radius of the particle and hence provides an unambiguous assignment. The derived diffusion coefficients of the lipoprotein fractions therefore provide a direct assignment of the NMR bands and also give a measure of lipoprotein particle size, the results being in agreement with those from conventional measurements. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; lipoproteins; diffusion; blood plasma

INTRODUCTION

Lipids in the diet are absorbed and transported in the blood as lipoprotein complexes. These are classified by their size and density and this in turn is determined by the type and ratio of protein and fats. The largest lipoprotein complexes are chylomicrons and very low-density lipoproteins (VLDLs). They consist largely of a lipid core of non-polar triacylglycerides and cholesteryl esters surrounded by more polar phospholipids, cholesterol and apoproteins. The most predominant lipids in high-density lipoprotein (HDL) and low-density lipoprotein (LDL) are phospholipids, especially phosphatidylcholine, and cholesterol, respectively. HDL, synthesized and secreted by the liver and the intestine, is the smallest lipoprotein and also the most soluble and protein-rich lipoprotein. The levels of the various lipoproteins in blood serum or plasma have been related to risk of cardiovascular disease and the measurement of their levels and assessment of the risk involves a comparison relative to age- and sex-specific normal values using samples from fasted subjects.[1] The levels of LDL, specifically oxidized LDL, in the blood correlate with the severity of atherosclerosis.[2,3] Some physicochemical properties of the lipoproteins are given in Table 1, together with the typical proportions of those components which constitute lipoproteins.[1]

The measurement of serum or plasma lipoproteins can be a time-consuming process and has been summarized by the NIH in the USA.[4] The traditional basis for accurate lipoprotein measurement is based on combined ultracentrifugation and polyanion precipitation. LDL-cholesterol is calculated by difference from total cholesterol and HDL-cholesterol

*Correspondence to: Maili Liu, Laboratories of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China.
E-mail: ml.liu@wipm.ac.cn
Contract/grant sponsor: National Natural Science Foundation of China; Contract/grant numbers: 29925515; 20175035.
Contract/grant sponsor: Multidisciplinary Research Program of the Chinese Academy of Sciences.
Contract/grant sponsor: Metabometrix Ltd.
Abbreviations: CHD, coronary heart disease; *D*, diffusion coefficient; *G*, magnetic field gradient in gauss; HDL, high-density lipoprotein; HPLC, high-performance liquid chromatography; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LED, longitudinal eddy current delay; NIH, National Institutes of Health; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; T_D, diffusion time; T_E eddy current recovery time; T_R, relaxation delay; VLDL, very low-density lipoprotein.

Table 1. Definition and composition of lipoproteins from human blood plasma[1]

Property	Chylomicrons	VLDL	LDL	IDL	HDL
Density (g cm^{-3})	<0.950	0.950–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Radius (Å)	400–2500	150–400	125–175	90–140	25–60
Apolipoproteins (wt%)	2–3	5–10	15–20	20–25	40–55
Triglycerides (wt%)		39	27	6	3
Phospholipids (wt%)		17	26	24	24
Cholesterol (wt%)		7	8	9	3
Cholesteryl esters (wt%)		13	23	41	21
Apolipoprotein (wt%)		17	16	21	49

levels. For routine patient evaluation and for monitoring non-laboratory-based measurements and in situations where ultracentrifugation is impractical, LDL-cholesterol can be estimated from direct measurements of total cholesterol, triglycerides, and HDL-cholesterol using the Friedewald equation:[1]

$$[\text{LDL-cholesterol}] = [\text{total cholesterol}] - [\text{HDL-cholesterol}] - [\text{triglycerides}]/5 \quad (1)$$

where all concentrations are expressed in mg dl^{-1}. The Friedewald equation cannot be used when chylomicrons are present, when the plasma triglyceride concentration exceeds 400 mg dl^{-1} or in patients with certain types of lipoproteinemia.[4]

A number of approaches to measure plasma and serum lipoprotein concentrations have been proposed and one of the most promising methods is the use of ^{1}H NMR spectroscopy of whole blood plasma. The ^{1}H NMR spectra of whole blood plasma contain a variety of signals from low and high molecular weight compounds and complexes including lipoproteins.[5–7] The predominant features in the ^{1}H NMR spectrum are composite bands for the various types of hydrogen in the fatty acyl chains of the lipoproteins, although other parts of the lipoprotein complexes can also been seen, such as the characteristic signal from the N^+Me_3 groups of choline-containing phospholipids. The features of the spectra which have been used for lipoprotein characterization have been the signals from the terminal methyl groups and the long-chain methylene groups at around δ 0.8 and 1.3, respectively.[8] The relative proportions of the various lipoproteins are usually obtained by curve fitting the observed bandshape based on known band positions and line widths for individual lipoproteins measured from purified fractions.

An alternative approach based on physical separation of the lipoproteins has been proposed based on directly coupled HPLC–NMR spectroscopy on whole plasma samples using a hydroxyapatite column, with elution by pH 7.4 phosphate buffer.[9] Using a chromatographic run time of 90 min with stopped-flow chromatography, 600 MHz ^{1}H NMR spectra of each lipoprotein could be collected in 7 min. The lipid signal ^{1}H NMR chemical shifts were identical with those in conventional NMR spectra of available standard lipoprotein fractions, confirming that the lipoproteins were not degraded by the HPLC separation. Co-elution of HDL with plasma proteins such as albumin occurred and this limited the quantitation of that species by HPLC peak integration.[9]

Here a new approach to characterizing lipoprotein NMR resonances is described. This involves first the deconvolution of the methyl (CH_3) and long-chain methylene [$(CH_2)_n$] bands in the diffusion-edited ^{1}H NMR spectrum of plasma into resonances from individual lipoproteins. Next, the sample is subjected to a pulsed field gradient NMR experiment which is used to calculate diffusion coefficients of molecules based on changes in area of the NMR peaks as a function of the applied gradient strength.[10,11] The changes in area in the above NMR bands are interpreted by deconvolving the bands into the same sub-peaks derived earlier and identifying area changes for each sub-peak. By this means, it is possible to derive a diffusion coefficient corresponding to each NMR sub-peak and, given the relationship between diffusion coefficient and particle hydrodynamic radius, to assign each peak to a lipoprotein fraction.

EXPERIMENTAL

A sample of human blood was obtained from a healthy female volunteer and the plasma was prepared conventionally. This was stored frozen at −80 °C until required for NMR spectroscopic analysis. Standard ^{1}H NMR spectra were measured at 600 and 800 MHz using Bruker Avance DRX-600 and DRX-800 spectrometers. The 600 MHz spectrometer was equipped with an actively shielded gradient unit with a maximum gradient-strength output of 58 G cm^{-1}. The diffusion coefficients were determined using a bipolar gradient LED pulse sequence[10] with solvent pre-saturation pulses being incorporated.[11] All measurements were made at 298 K and used a diffusion time (T_D) of 100 ms, an eddy current recovery time (T_E) of 50 ms and a relaxation delay (T_R) of 2 s. A series of 64 spectra were measured with values of gradient strength linearly increasing from 30 to 95% of the maximum output using sine-shaped gradients of 5 ms duration at the base of the pulse. This gradient strength was chosen because the NMR peaks from fast-diffusing, low molecular weight metabolites are drastically attenuated and thus do not cause interference in the deconvolution process. On the other hand, the intensities of the peaks from the lipoproteins are not affected at this gradient strength. Typically, 64 transients were acquired into 16K data points over a spectral width of 7200 Hz. These data were multiplied by a cosine ($0-\pi/2$)

function to improve the signal-to-noise ratio and zero-filled by a factor of two prior to Fourier transformation. The bands of the $(CH_2)_n$ protons over the region δ 1.40–1.20 and the CH_3 protons over the region δ 0.97–0.75 were digitized and transferred to a PC for deconvolution using ORIGIN software (Microcal Software, Northampton, MA, USA, version 3.54). A Lorentzian lineshape was used for the deconvolution. It was found that six Lorentzian lines were the best fit to the spectra for both the $(CH_2)_n$ and CH_3 bands. Using the spectrum obtained with the lowest gradient strength (30%) and hence with the highest signal-to-noise ratio, the position and width at half-height of each peak were then optimized. The output parameters (peak position, width and areas) were used as initial input to fit the next two spectra in the gradient strength series. The average values of the line position and width at the half-height from these three fits were now fixed and used to fit all 64 spectra with the areas of the components as the only variables. The areas of the individual components were used to derive the diffusion coefficients using the equation

$$\ln[A(b)/A(0)] = -bD \qquad (2)$$

where $b = (2a\gamma\delta G)^2(\Delta - 2\delta/3 - \tau/2)$, γ is the ^{1}H magnetogyric ratio, δ (5 ms) is the length of base of the gradient pulse, Δ is the effective diffusion time (100 ms), G is the gradient strength, $a = 2/\pi$ is a gradient shape factor for the sine-bell-shaped gradient, τ (100 μs) is a short delay, $A(b)$ and $A(0)$ are the NMR signal areas in the presence and absence of the gradient pulses, respectively, and D is the diffusion coefficient.[10]

The experiment was repeated at 600 MHz and 298 K using a sample of LDL (Sigma, Poole, UK) to test the results.

RESULTS AND DISCUSSION

A typical 600 MHz diffusion-edited ^{1}H NMR spectrum of human blood plasma is shown in Fig. 1(a). In this spectrum, obtained with a relatively low gradient strength of 30%, the peaks from the small molecule metabolites have been suppressed,[7,11–13] but there has been no significant effect on the intensities from bands arising from macromolecules and lipoprotein particles. It is noteworthy that whereas the peaks from other small molecule metabolites have been largely eliminated, the sharp peaks from citrate are still visible, thus indicating that citrate in blood plasma must have a significant proportion which is bound to macromolecules, principally albumin, hence lowering its diffusion coefficient. In the region around δ 1.3 there is a broad band with some structure. This band comprises a superposition of separate peaks arising from the long-chain methylene protons of fatty acyl groups in different types of lipoprotein in the plasma. The fatty acyl chains are moieties of lipids such as triglycerides, phospholipids and cholesteryl esters. The component peaks arise from different types of lipoprotein in the plasma and the chemical shifts are due to a magnetic susceptibility effect caused by the different radii of the particles.[14] From previous measurements using purified lipoprotein fractions, it has been shown that this band can be deconvolved into a number of peaks, namely two from HDL, three from LDL and two from VLDL,[8] but it has not been

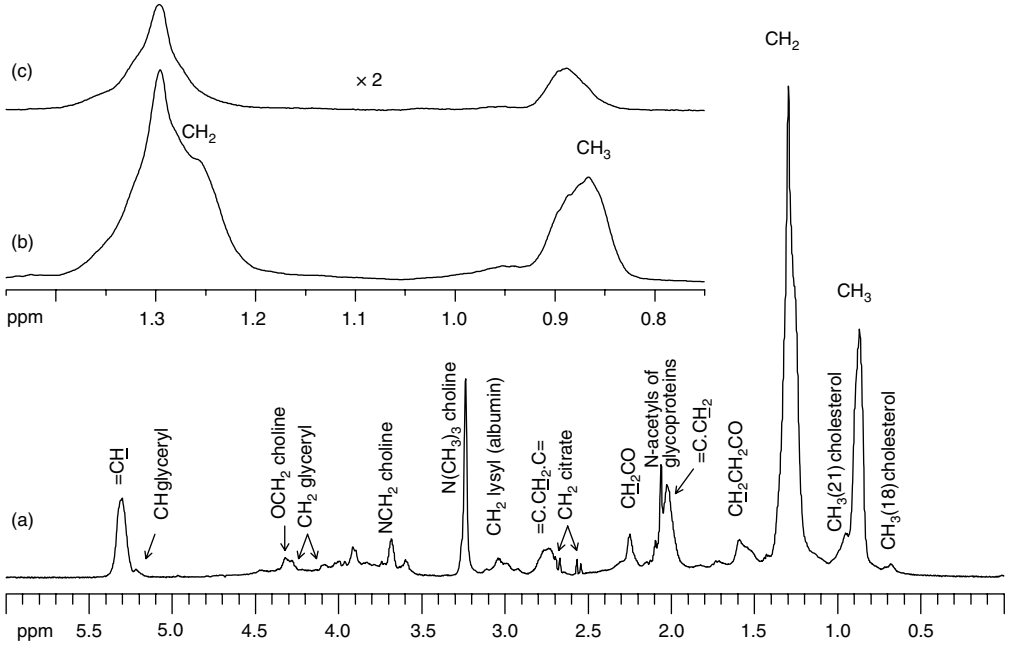

Figure 1. (a) 600 MHz ^{1}H NMR spectrum of human blood plasma measured using the bipolar LED pulse sequence for editing based on molecular self-diffusion, at gradient strength of 30%. At this strength, the peaks from fast-diffusing, low molecular weight metabolites have been attenuated, but the peaks from the lipoproteins are not affected. The assignments of the major peaks from the lipid moieties are given based on the literature.[12,15] In addition, peaks from the mobile lysyl groups of albumin, from the methyl protons of the *N*-acetyl groups of glycoproteins and from the methylene protons of citrate can also be seen. The insets show the regions of the $(CH_2)_n$ and CH_3 bands from lipoproteins at gradient strengths of (b) 30% and (c) 95% showing the differential decay profile across each band because of the different diffusion coefficients of the lipoproteins.

possible to separate individual peaks arising from different types of fatty acyl group.

A series of ^{1}H NMR spectra of human blood plasma were measured using the bipolar LED pulse sequence as a function of the applied magnetic field gradient strength.[10,11] The region of the spectrum between δ 0.75 and 1.45 is shown in Fig. 1(b) and (c). Figure 1(b) is the spectrum at a gradient strength of 30% and is an expansion of Fig. 1(a), and Fig. 1(c) shows the result at a gradient strength of 95%. The fatty acyl group terminal CH_3 signals appear near δ 0.85 and show a different profile at the two gradient strengths, indicating that the band is made up from a number of peaks from protons in lipoprotein particles with different diffusion coefficients. The fatty acyl chain $(CH_2)_n$ protons appear as a band near δ 1.3 and this also shows a change in profile as the gradient strength is increased.

These two bands were each deconvolved into six peaks of various intensities. The intensities of the sub-peaks were monitored in the bipolar-LED diffusion experiment and the diffusion coefficients corresponding to each of the six peaks determined as in the Experimental section. The fits to the overall bands are shown in Fig. 2(a) for the (CH_2) proton resonances and Fig. 2(b) for the CH_3 proton signals. The chemical shifts, linewidths, intensities and corresponding diffusion coefficients are summarized in Table 2. The assignments of the individual lipoprotein bands were made on the basis of the derived diffusion coefficients. For the $(CH_2)_n$ peak, it is possible to calculate the proportions of HDL, LDL and VLDL in the sample and these are 27.8, 17.8 and 54.3%, respectively. Using the results from the CH_3 band, the proportions for HDL and LDL are in good agreement at 31.0 and 16.7%, respectively. The value for VLDL from the CH_3 peak can only be in agreement with that from the $(CH_2)_n$ peak if the band at δ 0.956 is taken to arise from VLDL, when the total proportion becomes 52.3%. However, the derived diffusion coefficient for this peak is too fast for it to arise only from VLDL. On examination of the spectrum, this peak is seen to be well resolved from the rest of the lipoprotein band and the increased diffusion coefficient could be a result of overlap with small peaks of more rapidly diffusing molecules. Considering the differences in the composition of the different lipoprotein fractions, the ratios of the peak areas of HDL, LDL and VLDL may be different for the $(CH_2)_n$ and CH_3 bands.

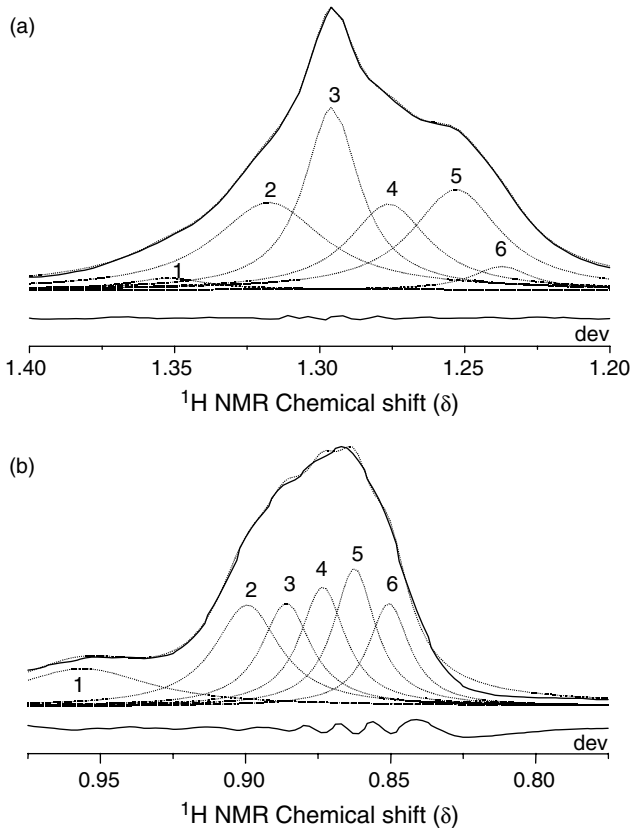

Figure 2. Partial 600 MHz ^{1}H NMR spectra of human blood plasma measured using the bipolar LED pulse sequence for editing based on molecular self-diffusion, showing the bands from the (a) $(CH_2)_n$ and (b) CH_3 protons of fatty acyl chains in lipoproteins. Both bands were fitted to six Lorentzian components as shown. The differences between the experimental and simulated spectra are also given.

Confirmation of the validity of the peak deconvolution process was achieved by measuring the same sample at the same temperature at both 600 and 800 MHz observation frequencies. This is demonstrated in Fig. 3, which shows the $(CH_2)_n$ and CH_3 proton peaks at both observation frequencies. The spectrum at 600 MHz was deconvolved into the six components as described above and the frequencies and linewidths determined. The same process was also applied to the 800 MHz NMR spectrum and the relevant parameters were determined. Figure 3 shows the fit to the two bands using the derived parameters which are also listed in Table 3. This demonstrates that the deconvolution process results in peaks in both cases at the same chemical shifts on the ppm scale, although obviously the 800 MHz NMR peak position separations are larger in frequency terms by the correct proportion. The linewidths of the component peaks at 800 MHz are on average larger by 29% than at 600 MHz, indicating that the peaks still contain a range of chemical shifts. The increase in linewidths is close to the ratio of observation frequencies and thus is probably due to variations in fatty acid composition. Given the very small anisotropies in ^{1}H NMR chemical shifts of methyl groups, it is unlikely that this factor, which would be in proportion to the square of the observation frequency ratio (ca 1.8), contributes to the spin–lattice relaxation rates, and hence observed linewidths.

Based on the measured diffusion coefficients, it is possible to calculate the radius of the particles assuming that they are spherical. If the average radius of LDL is taken to be 150 Å from Table 1 and with an average diffusion coefficient of 3.11×10^{-7} cm^2 s^{-1} (excluding that for the very small peak, CH_2 peak 1) taken from Table 2, this allows the calculation of the radii of VLDL and HDL particles. These are calculated to be approximately 255 and 80 Å, respectively. All values are in good agreement with the values given in Table 1 and hence the NMR-based diffusion approach confirms the assignment of the ^{1}H NMR peaks to the various lipoproteins. This approach also helps to validate the use of mathematical deconvolution of the full bands as a means of

Table 2. Chemical shifts, linewidths and diffusion coefficients of the component peaks obtained by deconvolution of the lipoprotein methyl and methylene bands in the ^{1}H NMR spectrum of human blood plasma

Peak	δ (ppm)	Width (Hz)	D (cm^2 s^{-1} $\times 10^7$)[a]	Area (%)[b]	Assignment
$(CH_2)_n$ 1	1.353	19.7	2.72	2.3	LDL + VLDL
$(CH_2)_n$ 2	1.317	28.0	1.98	26.4	VLDL
$(CH_2)_n$ 3	1.296	14.0	1.85	27.9	VLDL
$(CH_2)_n$ 4	1.276	17.8	3.15	15.5	LDL
$(CH_2)_n$ 5	1.255	20.5	5.19	19.9	HDL(60.6%) + LDL(39.4%)
$(CH_2)_n$ 6	1.240	18.4	5.96	7.9	HDL
CH_3 1	0.956	33.9	3.77	15.8	VLDL + HDL
CH_3 2	0.899	16.4	1.70	20.6	VLDL
CH_3 3	0.886	12.4	1.84	15.9	VLDL
CH_3 4	0.873	11.3	3.07	16.7	LDL
CH_3 5	0.862	10.5	4.51	17.9	HDL(40.8%) + LDL(59.2%)
CH_3 6	0.851	10.2	7.11	13.1	HDL

[a] The correlation coefficient (r) for the linear fit to Eqn.(2) is in the range 0.987–0.999. The estimated error on D is approximately 4% from the fitting process, and a maximum of 10% including errors from the deconvolution process.

[b] Relative areas were obtained from the spectrum with a gradient strength of 30%. This may not be directly related to the relative amounts of the lipoprotein since the relaxation times (T_1, T_2) may different for the different components. The larger variation in the diffusion coefficients measured for HDL may be caused by possible overlap with albumin resonances.

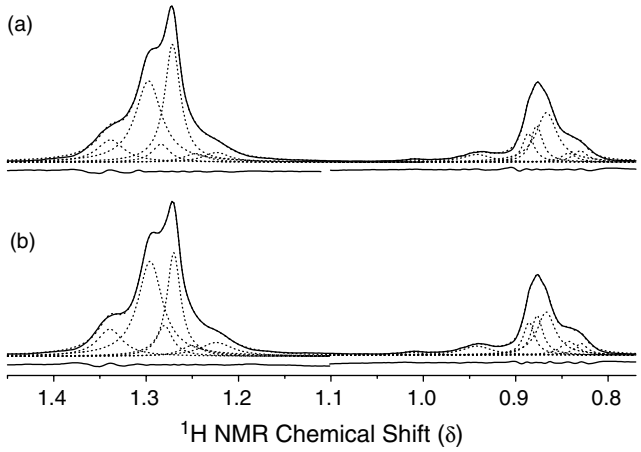

Figure 3. ^{1}H NMR spectra of the $(CH_2)_n$ and CH_3 bands from lipoproteins in human blood plasma at (a) 600 and (b) 800 MHz, indicating the similarity of the positions of the deconvolved sub-peaks.

Table 3. Chemical shifts and linewidths of the component peaks obtained by deconvolution of the lipoprotein methylene and methyl bands in the ^{1}H NMR spectrum of human blood plasma at 600 and 800 MHz

	600 MHz		800 MHz	
Peak	δ (ppm)	Width (Hz)	δ (ppm)	Width (Hz)
$(CH_2)_n$ 1	1.339	21.2	1.339	26.3
$(CH_2)_n$ 2	1.297	20.4	1.296	25.4
$(CH_2)_n$ 3	1.284	13.9	1.280	16.5
$(CH_2)_n$ 4	1.271	12.2	1.270	14.3
$(CH_2)_n$ 5	1.247	18.3	1.250	23.5
$(CH_2)_n$ 6	1.224	20.3	1.224	34.6
CH_3 1	0.942	22.4	0.942	29.9
CH_3 2	0.887	8.7	0.886	11.6
CH_3 3	0.877	9.1	0.877	12.0
CH_3 4	0.867	16.3	0.867	18.9
CH_3 5	0.843	11.5	0.842	16.9
CH_3 6	0.830	11.9	0.829	13.6

determining lipoprotein concentrations in blood plasma. The use of a diffusion-edited NMR spectrum using a low gradient strength, rather than a simple water-suppressed spectrum, is proposed to improve the deconvolution by minimizing artifacts in the lineshape analysis produced by the presence of overlapping resonances from small molecules such as lactate or threonine.[15]

CONCLUSION

It is demonstrated that the sub-peaks obtained by deconvolution of the methyl, CH_3 and long-chain methylene, $(CH_2)_n$, bands in the diffusion-edited ^{1}H NMR spectra showed typical diffusion coefficient corresponding to the fractions of the lipoproteins, HDL, LDL and VLDL. The experiments at 600 and 800 MHz showed good consistency. This approach may be used as an alternative way to characterize the lipoproteins in blood plasma. Owing to the complexity in composition, physical and chemical properties of the lipoproteins, the quantitative correlation between the sub-peaks areas and the concentrations of HDL, LDL and VLDL needs to be further studied on a larger cohort of samples and therefore is beyond

the scope of this study. However, different correlations are expected for the methyl and methylene bands, respectively, since the area ratios of the two bands are different for different fraction of the lipoproteins. It is likely that in order to obtain a reliable quantitative correlation, a study over a temperature range (277–313 K) may be necessary owing to lipid composition-dependent liquid crystal phases affecting the NMR visibility of the lipid protons, particularly in the core regions of the LDL particles. These studies are now under way.

Acknowledgment

M.L. thanks the National Natural Science Foundation of China (Grant Nos 29925515, 20175035), the Multidisciplinary Research Program of the Chinese Academy of Sciences and Metabometrix Ltd. (UK) for financial support. We acknowledge Dr J. Brindle for provision of the plasma and Dr J. C. Yang (LMB, Cambridge, UK) for access to 800 MHz NMR facilities.

REFERENCES

1. Illingworth DR, Duell PB, Conner WE. In *Endocrinology and Metabolism*. (3rd edn), Helig P, Baxter JD, Frohman LA (eds). McGraw-Hill: New York; 1995; 1315–1406.
2. Brown MS, Goldstein JL. *Science* 1986; **232**: 34.
3. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. *N. Engl. J. Med.* 1989; **320**: 915.
4. *National Cholesterol Education Program Recommendations on Lipoprotein Measurement, from the Working Group on Lipoprotein Measurement, National Institutes of Health, National Heart, Lung, and Blood Institute*. NIH Publication No. 95–3044. National Institutes of Health: Washington, DC, 1995.
5. Nicholson JK, Buckingham MJ, Sadler PJ. *Biochem. J.* 1983; **211**: 605.
6. Nicholson JK, O'Flynn MP, Sadler PJ, Macleod AF, Juul SM, Sonksen PH. *Biochem. J.* 1984; **217**: 365.
7. Nicholson JK, Foxall PD, Spraul M, Farrant RD, Lindon JC. *Anal. Chem.* 1995; **34**: 793.
8. Ala-Korpela M. *Prog. Nucl. Magn. Reson. Spectrosc.* 1995; **27**: 475.
9. Daykin CA, Corcoran O, Hansen SH, Bjørnsdottir I, Cornett C, Connor SC, Lindon JC, Nicholson JK. *Anal. Chem.* 2001; **73**: 1084.
10. Wu DH, Chen AD, Johnson CS Jr. *J. Magn. Reson. A* 1995; **115**: 260.
11. Liu M, Nicholson JK, Lindon JC. *Anal. Chem.* 1996; **68**: 3370.
12. Liu M, Nicholson JK, Parkinson JA, Lindon JC. *Anal. Chem.* 1997; **69**: 1504.
13. Zhang X, Li CG, Ye HH, Liu ML. *Anal. Chem.* 2001; **73**: 3528.
14. Lounila J, Ala-Korpela M, Jokisaari J, Savolainen MJ, Kesaniemi YA. *Phys. Rev. Lett.* 1994; **72**: 4049.
15. Lindon JC, Nicholson JK, Everett JR. *Annu. Rep. NMR Spectrosc.* 1999; **38**: 1.