THE THERMAL EXPANSION OF HUMAN LOW-DENSITY LIPOPROTEINS (LDL)

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1. Introduction

The thermal expansion of serum lipoproteins is a parameter which has received little attention. Toro-Goyco [1] reported that low density lipoproteins (LDL) of hydrated density 1.033 g/ml at 25°C changed density at the rate of -6.5×10^{-4} g/ml/°C over the range 5–25°C. This value is considerably greater than that of the aqueous solvents in which lipoproteins are usually isolated by centrifugation, and failure to allow for this difference in expansivity leads to considerable uncertainty over the exact density of the product. This is important when the objective is the precise characterisation of lipoproteins which differ only slightly in hydrated density. The dependence of the density of LDL in temperature has therefore been re-investigated over the temperature range 2–46°C.

2. Experimental

2.1 *Isolation of human serum LDL*

Blood was drawn from apparently healthy subjects of either sex and, after clotting for 2 h at room temperature, serum was isolated by centrifugation at about $2000 \times g$. A mixture of 7.5 ml serum with 1.5 ml NaCl solution, density 1.091 g/ml (final solvent density 1.021 g/ml at 20°C) was centrifuged for 18 h at 11°C in Spinco 30.2 rotor at 30 000 rev/min, and the resulting layer of lipoprotein aspirated off and discarded. The solvent density of the infranatant was then raised to 1.042 g/ml (at 20°C) by mixing it with NaCl, density 1.091 g/ml, in the proportions 6.25:2.75. The mixture was centrifuged at 11°C as before, and the LDL layer recovered by aspiration.

2.2. *Determination of hydrated density*

The isolated LDL fraction was divided into several parts, each of which was dialysed exhaustively at 4°C against a different NaCl solution of density between 1.007 g/ml and 1.063 g/ml. The sedimentation rate (S) of the lipoprotein was then determined in each solution, at a range of temperature between 2°C and 46°C, by centrifugation at 52 640 rev/min in a Spinco Model E ultracentrifuge fitted with rotor temperature control. At least four photographs of the Schlieren pattern were taken at known times (t) during the centrifugation. The radial position (x) of the peak was measured with a microscope and S determined from the slope of the best-fitting line to a plot of $\ln(x)$ versus t, as described by Schachman [2]. The density (ρ) of the dialysate against which each lipoprotein solution had been equilibrated was determined over the range of temperature 0–50°C, using a Paar density meter, Type DMA (Anton Paar, Graz, Austria). The viscosity (η) of each dialysate was also determined over this range of temperature by means of a Cannon-Ubbelohde viscometer, using water as reference fluid. The hydrated density (δ) of the lipoprotein at each temperature was determined by interpolation of a plot of ηS versus ρ to the condition $S = 0$.

3. Results and discussion

3.1. *Density of solvents*

The effect of temperature on the density of NaCl solutions is illustrated by fig.1, which refers to the solvents of highest and lowest density used in a typical experiment. In this case, the densities at 20°C were 1.0629 g/ml and 1.0078 g/ml, respectively. The

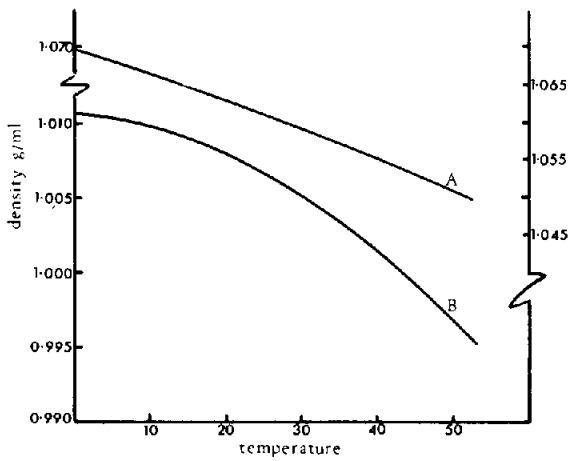

Fig.1. Change of density of NaCl solutions with temperature. Curve A (right-hand density scale) refers to a solution of density 1.0629 g/ml at 20°C. Curve B (left-hand scale) refers to a solution of density 1.0078 g/ml at 20°C.

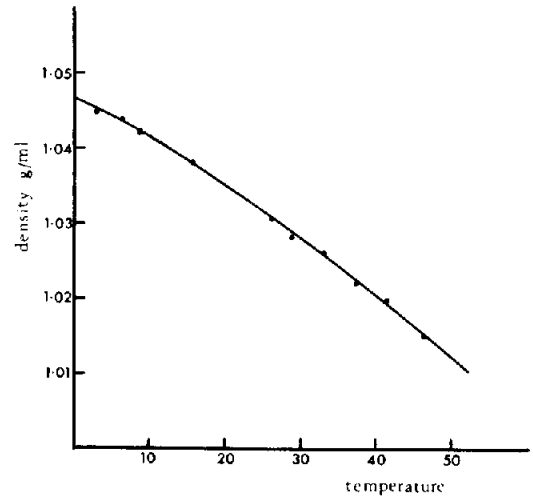

Fig.2. Change of hydrated density of human LDL with temperature.

curves shown are the best fitting regression curves of order three [3], which have the following equations:

$$\rho = 1.0696 - 3.4 \times 10^{-4}(t) + 7.18 \times 10^{-7}(t^2) - 2.78 \times 10^{-8}(t^3)$$

for the high density solution, and

$$\rho = 1.0109 - 1.0 \times 10^{-4}(t) - 1.9 \times 10^{-6}(t^2) - 3.53 \times 10^{-8}(t^3)$$

for the low density solution.

Between 5°C and 25°C these can, for many practical purposes, be approximated by straight lines of slope $(d\rho/dt)$ -3.4×10^{-4} and -1.85×10^{-4} g/ml/°C, respectively. For other solutions of NaCl, of density between 1.00 g/ml and 1.08 g/ml, the value of $(d\rho/dt)$, can be obtained to a good approximation from the equation:

$$(d\rho/dt) = (26.85 - 28.5\,\rho)10^{-4}.$$

3.2. *Hydrated density of LDL*

Figure 2 shows the relation between temperature and hydrated density, between 2.7°C and 46.6°C, for an LDL preparation of which the density was 1.035 g/ml at 20°C. The measured data can be represented with adequate accuracy by the quadratic regression equation:

$$\delta = 1.0465 - 5.17 \times 10^{-4}(t) - 3.4 \times 10^{-6}(t^2)$$

Over the range 5–25°C, this can be approximated by a straight line of slope $(d\delta/dt) = -6.18 \times 10^{-4}$ g/ml/°C. The mean value of $(d\delta/dt)$ for five different samples of LDL (mean hydrated density 1.0329 g/ml, range 1.0316–1.0348 g/ml) was -7.09×10^{-4} g/ml/°C, with a range from -6.18 to -8.07×10^{-4}. This mean value agrees satisfactorily with that of -6.5×10^{-4} reported by Toro-Goyco [1], who made only three observations, which were not all on the same sample of LDL.

There is little information available on the coefficient of expansion of lipids, but observations on olive oil and almond oil [4] lead to values of $(d\delta/dt)$ of -6.6 and -8.3×10^{-4} g/ml/°C, respectively at 20°C. Moreover, from the partial specific volumes quoted by Svedberg and Pedersen [5], it can be calculated that $(d\delta/dt)$ for an average protein is about -8.8×10^{-4} g/ml/°C. Thus the values observed for LDL are consistent with what is known of the behaviour of the separate lipoprotein constituents.

The significance of these observations lies in the fact that it is the custom to isolate lipoproteins

from solutions whose density is adjusted at 20°C, by centrifuging them at a lower temperature, which is often about 5°C. Although it is usual to describe these lipoproteins as having density limits prescribed by those of the solvents at 20°C, this is wholly misleading. This can be illustrated by the following example, which refers to LDL isolated by sequential centrifugation in NaCl solutions of densities 1.021 g/ml and 1.042 g/ml at 20°C. If the centrifugations are performed at 5°C, the solvent densities will become 1.0243 g/ml and 1.0463 g/ml, respectively, and these will define the LDL fraction isolated, at 5°C. At 20°C the limits of the LDL will have become 1.0137 g/ml and 1.0357 g/ml. To refer to this fraction as 'LDL of density 1.021–1.042 g/ml' is clearly incorrect, and leads to confusion when preparations which were centrifuged at different temperatures are compared. The most precise way of defining the LDL fraction is by reference to its range of hydrated densities at 20°C, and this should be the preferred procedure in critical studies on fractions of narrow density range. An alternative, though less satisfactory procedure would be to quote the composition and density at 20°C of the solvents used, and the temperature of the preparative centrifugations. In this context however, it must be pointed out that there are numerous reports in which the temperature of centrifugation is not quoted.

The available data suggest that $(d\delta/dt)$ is independent of δ over the range which is met in the LDL, but it has to be established whether the same value can be used for HDL and VLDL. For the HDL however, the differential effect of temperature would in any event be much smaller than for LDL, since the high density solutions of NaBr used in preparing these lipoproteins have a temperature coefficient of density which is of the order of -7×10^{-4} g/ml/°C.

References

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