TABLE II

N.M.R. SPECTRAL PARAMETERS FOR CCl_2F–CCl_2F AND $C^{13}Cl_2F$–CCl_2F

Parameter	90 vol. % in CCl_3F, 21.0°	Neat, 24.5°
ϕ^*, CCl_2F–CCl_2F	$+67.842 \pm 0.007$[a]	.....
$\Delta\phi$ for impurity, CCl_3–$CClF_2$	-2.755 ± 0.010[b]	-2.819 ± 0.005[b]
$\Delta\phi$ ($C^{13}F$–$C^{12}F$)	.....	$+0.157 \pm 0.003$[b]
$\Delta\phi$ ($C^{13}CF$–$C^{12}CF$)	$+0.008 \pm 0.018$[b]	.....
$J(C^{13}F)$, c./sec.	.....	-307.26 ± 0.24[c,d]
$J(C^{13}CF)$, c./sec.	$+34.9 \pm 0.3$[c]	.....
$J(FF')$, c./sec.	15.6 ± 0.3[e]	15.40 ± 0.08[e]

[a] $\phi^*(20\%) = +67.806 \pm 0.004$ and $\phi^*(5\%) = +67.802 \pm 0.004$, from which (at inf. diln. and 21.0°), $\phi = +67.798 \pm 0.003$. [b] In p.p.m., relative to the strong line due to CCl_2F–CCl_2F. [c] Corrected for AB analysis as described in the text. [d] Sign of $J(C^{13}F)$ arbitrarily taken as negative, to emphasize reversal of sign for $J(C^{13}CF)$. [e] Sign not determined.

Discussion

It is seen from the spin-decoupling frequencies of Table I that for the saturated molecule, CCl_2F–CCl_2F, the "direct" and "distant" couplings of carbon-13 to fluorine are opposite in sign, just as had been found for its dechlorinated derivative.[1] One may predict that this reversal of sign will be found to be quite general for fluorine compounds, as is the closely related alternation of sign recently reported for fluorine–fluorine coupling constants.[13–15] The relative signs of $J(C^{13}F)$ and $J(FF')$ cannot be decided from the present evidence, but such information would be accessible by a rather similar spin decoupling technique which has been described recently.[16]

The "direct" isotope shift, $\Delta\phi(C^{13}F–C^{12}F)$, is slightly larger for the $-CCl_2F$ group than for those previously reported,[1,2,12,17] but further studies will be required to establish the generality of such structural correlations. A similar situation exists with regard to $J(C^{13}CF)$, the "distant" coupling constant, while for the "direct" one, $J(C^{13}F)$, Harris[18] has demonstrated that useful correlations can be found.

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THE PARTIAL SPECIFIC VOLUME OF PROTEINS BY A MAGNETIC BALANCE TECHNIQUE[1]

BY A. M. CLARKE, D. W. KUPKE, AND J. W. BEAMS

Department of Physics and the Department of Biochemistry, School of Medicine, University of Virginia, Charlottesville, Virginia

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Two ways of determining the density of solutions by the magnetic balance principle have been described recently.[2] Because the determinations are more rapid and the volumes of solution are much smaller than those by conventional pycnometric methods, a study of the partial specific volume, $\bar{v}$, of a number of proteins has been initiated. The possibility of achieving greater over-all accuracy by magnetic means was a further incentive, because values of $\bar{v}$ in the literature for given proteins often are so different that the corresponding molecular weights by ultracentrifugation are 10% apart or more. This report concerns data obtained with two well known proteins, ribonuclease and tobacco mosaic virus (TMV), which are near the extremes of the molecular weight spectrum for proteins. The first apparatus described in the original report was used throughout.

Experimental

Two samples of ribonuclease were measured, the Sigma Chemical Co. Type II, Lot R101B-67 (52 Kunitz units per mg.) and Type III, Lot R111B-51 (56 Kunitz units per mg.). The protein was dissolved in 0.15 *M* KCl–0.00625 *M* K_2HPO_4–0.00075 *M* KH_2PO_4, pH 7.7,[3] and the resulting solution was dialyzed against this solvent overnight before use. The molecular weight of the Type III sample has been compared in this solvent in two types of equilibrium ultracentrifuges and essentially linear plots of the logarithm of the concentration *vs.* the square of the distance to the axis of rotation were obtained.[4]

Four samples of TMV were used. These were grown by Dr. R. L. Steere of the U. S. Department of Agriculture, Beltsville, Md., and purified by the method of Boedtker and Simmons.[5] For the determination of $\bar{v}$, the virus solutions were dialyzed against 0.01 *M* ethylenedinitrilotetraacetate (Na), pH 7.5. Velocity sedimentation studies showed that dimers and higher aggregates formed with time if the virus was stored in dilute phosphate buffer or in water. The aggregates dispersed completely to yield a clean monomer boundary (181 S at 0.12 g./100 ml.) when the virus was transferred to the sequestering medium even after several weeks storage.

The concentration of the proteins was estimated at 20° in a differential refractometer at 546 mμ with the dialysate as reference solution. The refractometer was calibrated regularly before use with a sample of sucrose obtained from the National Bureau of Standards. The refractive increment for the Type III ribonuclease was obtained by determining the dry weight of equal volumes of protein solution and of dialysate after the refractometric measurements. Two separate experiments each gave a value for the increment, in this solvent, of 0.1850 ml./g.; the last digit probably is of little significance. This value was assumed for the Type II sample. For TMV, the increment of 0.194 ml./g. was used as determined by Boedtker and Simmons. Excellent agreement was obtained from the absorbancy at 265 mμ using their optical density value of 3.06 for 1 mg. of virus per ml.

For the estimation of $\bar{v}$, the densities of 0.2-ml. samples of each solution were determined several times and the averages were plotted against concentration. The precision of the readings was within a range of ±0.0002 g./ml. All observations were made at 25 ± 0.01°. The solutions were enclosed in a tightly covered, thermostated chamber containing the small permalloy float (0.042 g.) which was raised magnetically to a preselected level when viewed through a permanently mounted telescope. The support current values were translated into densities from calibration curves on liquids of known density (glass-distilled water and solutions of sucrose, NaCl and KCl) all of which were internally consistent. These currents were measured with a Leeds and Northrup Type K potentiometer with readings accurate to within 20 μv.

Results and Discussion

The densities of the ribonuclease solutions *vs.* concentration are shown in Fig. 1. The value at zero concentration is that of the dialysate; the undialyzed solvent gave the same readings within experimental error. Since the data from the two samples of ribonuclease appeared to be indistinguishable, a straight

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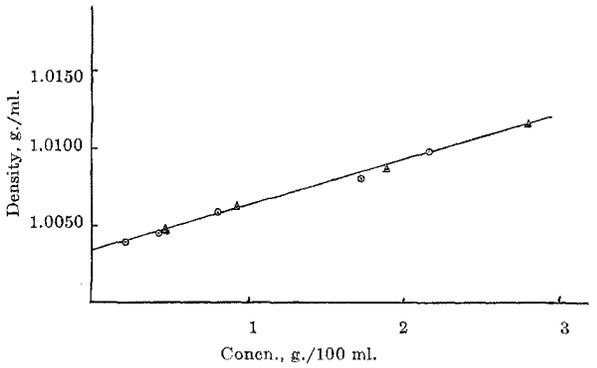

Fig. 1.—Density of ribonuclease solutions *vs.* protein concentration in 0.15 *M* KCl–0.007 *M* (K)PO_4, pH 7.7, at 25°: ▲, Type II sample; ⊙, Type III sample (see text).

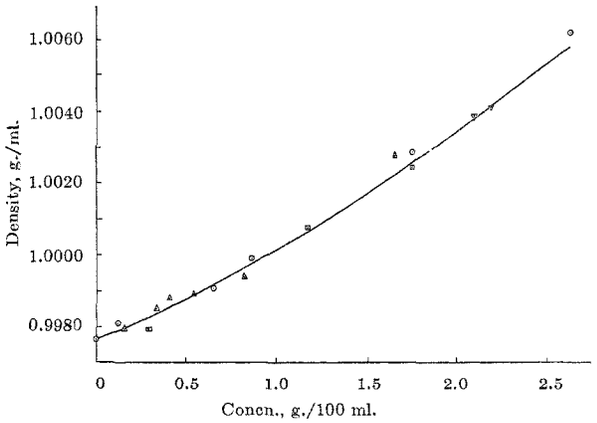

Fig. 2.—Density of TMV solutions *vs.* protein concentration in 0.01 *M* (Na)EDTA, pH 7.5, at 25°. Each symbol refers to a different preparation. A fit of data to a cubic expression is shown by the solid line.

line was fitted by the method of least squares to all the points. From the calculated slope (22.8), the value of $\bar{v}$ by the equation

$$\bar{v} = 1/\rho - 1/\rho^2 d\rho/dc$$

is 0.709 ± 0.002 ml./g. In this equation ρ is the density at $c = 0$, where c is grams of protein per 100 ml. A least squares fit to the data from the two separate samples gave values within 0.001 ml./g. of one another. This value is identical with that of Rothen[6] determined pycnometrically and with that of Brunish and Högberg[7] determined in density gradient tubes. Other values of $\bar{v}$ recorded for this protein vary from 0.693 to 0.728 ml./g.[8–10] McMeekin has suggested that this large difference in values may not reflect experimental error so much as real differences in the respective preparations.[11]

The densities for different concentrations of TMV are shown in Fig. 2. The entire curve best follows a cubic equation, both quadratic and fourth power expressions showing greater deviation. The least squares fit to the points below $c = 1.17\%$ gives a line of slope 24.5 which intercepts the observed density of the dialysate. The value of $\bar{v}$ at this limiting slope is 0.738 ± 0.002 ml./g. The slope from the least squares fit above $c = 1.2\%$ yields a value for $\bar{v}$ at the latter concentration of 0.66 ml./g. Because of the paucity of data at the higher concentrations, no limits have been assessed. Several attempts were made to obtain readings on solutions at the highest concentration indicated, but, except for the one experiment shown, the densities decreased with time; probably the TMV rods were forming an ordered lower phase as they are known to do in dilute salt solutions at these virus concentrations.[12] Values in the literature for TMV range from 0.646 to 0.77 ml./g.[13,14] Our value from the limiting slope agrees best with the average value of 0.73 ml./g. obtained by Lauffer[15] and by Bawden and Pirie.[16] It is quite possible that a dependence on concentration in the various solvents used, or the state of aggregation of the TMV, is reflected in the wide range of reported values. An abbreviated series of determinations in 0.005 *M* ethylenedinitrilotetraacetate exhibited curvature somewhat more marked than that shown in Fig. 2, whereas a series at 0.05 *M* showed no obvious curvature. Both series were carried out over the same protein concentration range as that shown in the figure. Whether the lower values of $\bar{v}$ reflect association of virus with exclusion of hydration water in solutions where the chelating agent is in limited supply must be left open. Polydispersity at the high virus concentrations could not be demonstrated clearly by velocity sedimentation. Other means are required to give a more definitive answer.

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SPECIFIC RATE CONSTANT FOR URETHAN CLEAVAGE

BY A. V. TOBOLSKY AND E. PETERSON

Department of Chemistry, Princeton University, Princeton, N. J.

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In a recent study of the stress relaxation of urethan rubbers, Colodny and Tobolsky[1] found that stress decay at constant extension in the temperature range 100–140° could be expressed by the formula

$$f(t)/f(0) = \exp(-t/\tau_{ch}) \qquad (1)$$

In eq. 1 $f(t)$ is the stress measured at time t, $f(0)$ is the stress measured initially, and τ_{ch} is a constant of the system which depends on temperature. It was deduced that in the rubber network obtained by treating a hydroxy terminated ethylene–propylene adipate polyester with methyl triphenyl triisocyanate (sample II of ref. 1) the stress decay in the temperature range 100–140° was due to a cleavage of urethan linkages.[1,2]

From the data in ref. 1, it can be shown that for sample II

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