CHAPTER 9

VICINAL HYDRATION OF BIOPOLYMERS:
CELL BIOLOGICAL CONSEQUENCES

W. DROST-HANSEN*

Laboratorium Drost, 5516 N.Mallard Run, Williamsburg, VA 23188, USA

Abstract: A novel type of hydration of macromolecules in aqueous solution was first suggested by Etzler and Drost-Hansen (1983). This hydration, observed for all macromolecules with a critical mass of >2000 Daltons (MW_C), seems identical with vicinal hydration of solid surfaces, possessing the same characteristics, e.g., thermal anomalies at the same temperatures [T_k] and similar shear rate dependence, as well as slow reforming after shear. Furthermore, the vicinal hydration is independent of the detailed chemistry of the macromolecules and of the presence of other solutes, electrolytes and non-electrolytes alike. Evidence for this poorly recognized and often overlooked hydration is presented

Keywords: Vicinal hydration; Thermal anomalies; Shear rate effects; MW dependence; Anomalous viscosities; Anomalous diffusion coefficients; Hydrodynamic radii; Biophysical implications; Sources of variability

1.   INTRODUCTION

Water adjacent to a solid surface is structured differently from that of the bulk phase, but the burning question of the extent of this water structuring has been a highly contentious issue for ~100 years. Few disagree that the first two or three molecular layers of water will be strongly influenced by the specific chemical nature of a solid surface, particularly when ionic sites are present (as well as strongly polar groups). However, many reports indicate that this structured interfacial water can be altered over far greater distances from, say, 10 molecular layers to 10,000. Currently, more realistic estimates are in the range of 10 to 100 (ca. 5 to 50 nm). Such distances approach the (probable) maximum 'free distances' within a typical

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* Correspondence to Professor Walter Drost-Hansen: wdrosthans@aol.com

biological cell (Clegg 1984a, 1984b). Much of the evidence for extensive modifications of interfacial water has been less than convincing. However, over the past 20–30 years substantial evidence now makes it more certain that extensive restructuring does occur, and such is generally referred to as 'vicinal water' [hereafter *VW*] – a summary of the unusual properties of *VW* is in Section 2. In view of the fact that the structure of bulk water continues to escape a complete and rigorous description, it is not surprising that far less is known about *VW*.

One highly characteristic feature of *VW* is the occurrence of thermal anomalies, which exist at no less than 4 distinct temperatures [T_{ks}] at ∼15, 30, 45 and 60 °C. This paper largely addresses newer evidence that strongly suggests – indeed proves – that sufficiently large macromolecules in aqueous solution are also vicinally hydrated; indeed, *all* macromolecules of >2000 Da (MWc) will be vicinally hydrated. Thermal anomalies are also properties of aqueous macromolecular solutions at the T_k values just mentioned. In addition, vicinal hydration structures are highly shear rate dependent, and once sheared, the time to reform *VW* structures close to the original state may be minutes or even hours. Thus these properties exhibit notable hysteresis that can account for the apparent lack of reproducibility in much of the data.

2.   WATER AT SOLID INTERFACES: PROPERTIES OF VICINAL WATER

2.1   Density of VW

Etzler, Conners and Ross (1990) have contributed greatly to the delineation of the properties of *VW*, in particular to its density and specific heat. Etzler and Fagundus (1987) explored the density of water in a number of porous silica gels of controlled pore diameters. Invariably densities were observed of less than that of bulk water, decreasing smoothly with decreasing pore diameter. For pores with an average diameter of 10nm, the density at room temperature was ∼0.97 g/cm^3 (see Table 3). Until fairly recently, there was uncertainty regarding the density of interfacial water, but this data puts it now above reproach, although it is easy to see how vastly different estimates might previously have been reported. For water near an ionic solid, or a solid with numerous surfaces charges, it seems obvious that the innermost 2 or even 3 layers of water might have a notably greater density than the bulk because of electrostriction of the water molecules near the surface charges. However, even if densities as high as, say, 1.05 g/cm^3 prevailed over a distance of 3 molecular layers from the surface, this contribution to the net density of the combined innermost layers plus the *extended VW* structures is far too little to bring average value to the density of the bulk. With regard to thermal expansion coefficient, Shoufle et al. (1972) measured this for water in narrow capillaries of different diameters over a wide range of temperatures. One interesting result was that the temperature of maximum density [T_{MD}] of water decreases essentially linearly with decreasing diameter of the capillaries (and T_{MD} also decreases for

D_2O, although apparently not in a linear manner). According to Deryaguin, a decrease in T_{MD} in narrow pores of TiO_2 and silica gel. The data of Shoufle et al., were reanalyzed and it was found that the thermal expansion coefficient of the capillary-confined water was *notably* larger than the corresponding bulk value (see Drost-Hansen, 1982).

The density of *VW* is distinctly less than that of bulk water; in other words, the specific partial molar volume of *VW* is larger than that of bulk. Hence, by LeChatelier's principle, we can predict that increased pressure will tend to 'squeeze out' *VW* (presumably converting it to more bulk-like water). As discussed below, the same type of vicinal hydration at solid surfaces also occurs at water/macromolecule interfaces. Hence, the density data may be of particular interest to studies of the hydration of macromolecules in aqueous solutions by ultracentrifugation. In principle, the high pressure in the centrifuge tube may indeed diminish or completely eliminate *VW*. On the other hand, this fact may conceivably be useful in delineating the likely total contribution to the hydration of macromolecules if, for instance, low shear-rate and low-pressure means of determining the total amount of hydration are available. One such determination might be by measurements of diffusion coefficients that may be assumed to affect neither the 'classical' types of hydration nor the hydration due to the *VW*. The difference between the two values may directly relate to the amount of vicinal hydration. Interesting consequences of the volume-pressure effects on living cells, as mediated by the hydrated macromolecules, can be found in Mentre and Hui (2001).

An interesting confirmation of the low density of *VW* was provided in some high-precision dilatometric studies in our laboratory (Drost-Hansen et al., 1987). We showed that when a suspension of polystyrene spheres (of highly controlled particle diameter) settled, a distinct contraction in volume was observed, which was interpreted as the result of increasing overlap of the less dense *VW* of hydration of the particles. The compaction process thus 'squeezes' out *VW* hydration 'hulls' and this water then 'reverts' to the higher density bulk water, leading to a decrease in total volume. The same effect is seen in the sedimentation of a suspension of silica spheres ('Minusil'; radii ∼5 microns); in other words, the same effect is observed for *VW* adjacent to polystyrene and silica (examples of the 'Substrate-Independence Effect'; see below).

Low et al. (1979) found densities of water near clay surfaces were lower than the bulk phase value (see also Viani et al., 1983). However, Low apparently believed that the effect was specifically due to the chemical nature of the clays employed, whereas it now appears that *VW* with low density is indeed induced by proximity to *any* solid surface (Table 1).

2.2   Specific Heat

One of the most characteristic properties of bulk water is its high specific heat of 1.0 cal/°K gram. We have made extensive measurements of the specific heat of vicinal water, measurements further perfected by Etzler and White (1987). In *all*

Table 1. Densities of vicinal water

T (°C)	$\rho\,pore(kg^{-1}\,m^3)$	SD	mm
10	949.8	3.0	1.25
	983.55	1.8	5.85
	986.7	2.0	7.00
	993.2	2.2	12.1
	999.7	–	bulk
20	952.9	3.6	1.25
	983.3	7.9	5.85
	985.2	2.1	7.00
	991.3	3.0	12.1
	998.2	–	bulk
30	949.0	5.8	1.25
	981.3	1.0	5.85
	981.8	1.2	7.00
	990.7	1.7	12.1
	995.6	–	bulk

cases, values for the specific heat were notably larger than that of bulk water, which must surely have important implications for the structure of *VW* and merits attention by those modelling the structure of bulk water by computational means (Table 2).

Values for the specific heat of water adjacent to chemically different surfaces are all higher than for bulk water, clustering around 1.2 cal/°K gram, which is consistent with the concept of the Substrate-Independent Effect ('Paradoxical Effect'). This further endorses the conclusion that *VW is induced by proximity to any surface, regardless of the specific detailed chemical nature of the solid*. More recently, Etzler and co-workers have greatly improved on the measurements of c_p for water in dispersed systems and invariably obtained values larger than that for bulk (see under thermal anomalies; Section 3.4).

Table 2. Heat capacity of vicinal water (cal^{-1} g @ 25 °C)

Confining material	Observed value	SD	Corrected value	SD
Physical				
Bulk	1.08	0.08	1.00	–
Porous glass	1.37	0.20	1.27	0.20
Activated charcoal	1.38	0.03	1.28	0.03
Zeolite	1.31	0.08	1.21	0.03
Diamond	1.30	0.08	1.20	0.08
Biological				
Collagen			1.24	
Egg albumin			1.25	0.02
DNA			1.26	0.06
Artemia cysts			1.28	0.07

2.3 Viscosity of VW

Much data has accumulated on the viscosity of water at interfaces, usually confined in capillaries or between highly polished plates. As early as 1932, Deryaguin (1932, 1933) reported elevated values for the viscosity of water between glass plates, as determined with a torsion viscometer, using a highly polished glass plate and a slightly convex plate free to oscillate above the base plate (reviewed in Henniker (1949), Clifford (1975) and Drost-Hansen (1968). Elevated viscosity values of confined water have frequently been reported, but none has proved entirely satisfactory. For example, it is very difficult to rule out spurious effects of a speck of dust or, in some cases, the postulated existence of 'swollen' surface layers (especially where the confining solid is glass). However, Peschel and Belouschek (1976) showed in a series of elegant studies of water confined between highly polished quartz (and sapphire) plates, that *VW* had a higher viscosity than the bulk water (by a factor of 2–10). The reproducibility of Peschel's data strongly argues against both spurious dust contamination and alleged 'swollen layers'. The latter is further substantiated by results with sapphire being similar to those obtained with the highly polished quartz plates (Peschel and Aldfinger, 1969; see also Section 2.7.2).

2.4 Overview of Some Properties of VW

The properties of VW are listed in Table 3.

Table 3. Summary of some of the properties of vicinal water

Property	Bulk water	Vicinal water
Density ($g\,cm^{-3}$)	1.00	0.96–0.97
Specific heat ($cal\,kg^{-1}$)	1.00	1.25
Thermal expansion coeff. ($°C^{-1}$)	250.10^{-6}	$300–700.10^{-6}$
Adiabatic compressibility ($coeff.\,atm^{-1}$)	7.10^{-17}	35.10^{-17*}
Heat conductivity ($cal\,sec^{-1}\,°C^{-1}\,cm^{-1}$)	0.0014	0.010–0.050
Viscosity (cP)	0.89	2–10
Activation energy, ionic conduction ($kcal\,mole^{-1}$)	4	5–8
Dielectric relaxation frequency (Hz)	19.10^{9}	2.10^{9}

Etzler et al. (1983, 1987, 1990, 1991).
Shoufle et al. (1976).
*Drost-Hansen (unpublished).

2.5 Historical Aspects; on the Geometric Extent of VW

The question of possible long-range structural effects in water near interfaces, as well as the question of the occurrence of thermal anomalies in the properties of aqueous systems, have had a long and contentious history. It appears that many

investigators have – either by design or inadvertently – adopted an attitude of 'maximum intellectual economy', and on that basis ignored the available evidence for long-range structural effects on water at interfaces. The same holds true for thermal anomalies of aqueous systems – thermal anomalies having often been referred to as 'kinks' or 'discontinuities'. For clay-water systems, Low et al. (1979) have long argued for the existence of modified water structures at clay surfaces, and Clifford (1975) published a critical review of evidence for structural effects in water at solid surfaces. More than anyone, Deryaguin has advocated structural effects at interfaces or 'boundary layers' (Deryaguin, 1964, 1977; see also Churev and Deryaguin, 1985. But the most definitive studies of VW have probably come from Etzler's laboratory (discussed below). Very significant studies of VW have also been reported by Alpers and Hühnerfuss (1983), who eloquently argued for the existence of truly long-range structural effects at solid interfaces; they also demonstrated thermal anomalies in vicinal water. Table 4 is from a paper by Hühnerfuss (for references in this Table, see the original paper). We do not subscribe to some of the proposed estimates for the geometric extent of the vicinal hydration – especially not values above one micron. However, there seems to be sufficient evidence to accept distances of the order of *many* molecular diameters and for the purposes of this paper the suggestion is made that the vicinal hydration is likely to be several tens of molecular layers, but probably not >100–200; or in other words, the likely extent of vicinal water lies somewhere between 5 and 50 nm.

At this time, very little can be said for certain about the structure of *VW*. However, Etzler (1983) and Etzler et al. (1990) have speculated on structural aspects of *VW*., of which one feature appears clear; the degree of H-bonding in *VW* is probably greater than in the bulk, as reflected in the more open structure (the density being less than that of the bulk). As for thermal transitions, one likely explanation is that these reflect transitions (akin to higher order phase transitions) from one type of structure to another. However, in the theory of Kaivarainen (1995), the temperatures of the T_k are seen merely as those at which some structural parameter assumes an integral value.

2.6   The 'Paradoxical Effect' (Substrate Independence)

The properties of water near a solid interface do not appear to depend on either the detailed chemistry of the adjacent surface, or the presence (or absence) of most solutes. Because this is highly unexpected, the 'Substrate Independence Effect' is also sometimes referred to as the 'Paradoxical Effect'. Vicinal water is induced by mere proximity to *any* solid surface, and also by proximity to any sufficiently large macromolecule (see below). Thermal anomalies have been seen at identical temperatures in the properties of water adjacent to glass, quartz, clays, diamond, metals, polystyrene, cellulose and a large number of other organic, polymeric materials (Drost-Hansen, 1965; 1976; Kurihara and Kunitake, 1992; Lafleur et al., 1989). Thermal anomalies of *VW* are seen also in aqueous solutions (at solid surfaces) of both electrolytes and non-electrolytes, up to 1–2M. They also occur at the same

Table 4. Experimental evidence for 'long range ordering effects' within interfacial water layers at the boundary of solid surface and water

Reference	Method	Solid boundary	Penetration depth (μm)
Etzler and Lilies (1986)	Dielectric constant	Sheets of mica	2–5
Drost-Hansen (1976)	Adhesion at glass	Glass	1.5
Henniker (1949)	Disjoining pressure	Mica or steel plates	<1
Mastro and Hurley (1985)	Surface conductivity	Glass tube	0.3–0.4
Peschel and Aldfinger (1976)	Conductivity	Quartz particles	0.2–0.3
Peschel and Aldfinger (1969)	Conductivity	Quartz particles	0.2–0.3
Falk and Kell (1966)	Viscosity	Glass plates	0.25
Montejano et al. (1983)	Conductivity	Pyrex glass	0.05–0.2
Drost-Hansen (1969)	Viscosity	Pyrex glass	~0.2
Steveninck et al. (1991)	Viscosity	Quartz plates/convex	0.16
Braun and Drost-Hansen (1981)	Modulus of rigidity	Glass plates/convex	0.15
Bailey and Koleske (1976)	Disjoining pressure	Fused silica convex plates	~0.1
Nir and Stein (1971)	Disjoining pressure	Quartz plates	0.1
Deryaguin (1933)	Modulus of rigidity	Glass	0.1
Antonsen and Hoffman (1992)	Viscosity	Glass plates	~0.1
Clifford (1975)	Air-bubble flow	Glass tubes	~0.1

temperatures for acidic and alkaline solutions, at least within the range of pH from 1–2 to 12–13. *In other words, the establishment of vicinal water structures is a proximity effect and the underlying cause must therefore be sought solely in the unique properties of hydrogen bonding systems.* Surely, *VW* structures do *not* reflect epitaxial ordering due to some specific surface features. This effectively invalidates the basis, for instance, of the so-called Association-Induction hypothesis (Ling 1962; 1979), and some (but not all) of the alleged specific effects of clay surfaces (based on crystallographic similarities between the clay structure and the molecular arrangement in Ice-Ih). Vicinal hydration structures appear unaffected by the ionic strength of the aqueous phase – and thus *are* not dependent on the presence or absence of electrical double layers.

If VW was indeed the result of charge-water-charge interactions, as suggested by Ling, one would expect values for the density of VW to be greater than the value for the bulk, due to electrostriction. Furthermore, as proposed by Alpers and Hühnerfuss (1983), vicinal water exists beneath an insoluble

monolayer at the air/water interface and as discussed by Drost-Hansen (1978), a distinct thermal anomaly at 15 °C is observed in the case of a monolayer of myristic acid on the surface of water (data of Phillips and Chapman, 1968). In other words, vicinal water exists beneath an insoluble monolayer and in the case of monolayers there are obviously no 'polarizing' effects from separated charges of opposite sign, as required by the Ling Association-Induction 'theory'. Vicinal water is induced merely by proximity to *any* interface; see postscript.

2.7   Thermal Properties of Vicinal Water

The idea that the properties of water exhibit thermal anomalies has been hotly debated for about a century. A distinct thermal anomaly may be seen in some very old data; for instance, the surface tension data of Brunner (1847) shows a very definite anomaly near 30 °C, although he did not comment on it. The idea that thermal anomalies might occur in the properties of (bulk) water (and aqueous systems) was mentioned by Dorsey (1940) in his monumental book on water properties. An even more extravagant claim was made by Drost-Hansen and Neill (1955), who suggested the occurrence of anomalies at no less than 4 different temperatures. At the time, it seemed that the anomalies were a manifestation of some unexpected property of bulk water and only later was it realized that the anomalies were in fact strictly associated with interfacial water (Drost-Hansen, 1968). Others have considered the occurrence of thermal anomalies, including Bernal, Magat, Forslind, Krone and several others (historical data can be found in Drost-Hansen (1971, 1978, 1981). Among some other earlier papers regarding thermal anomalies, it was proposed by the present author (Drost-Hansen, 1956, 1965, 1971) that the thermal anomalies might have been responsible for the selection of body temperatures in mammals (and birds) during the process of evolution – an idea that became firmly established in later years (Drost-Hansen, 1971, 1978, 1985). However, many authors have vigorously disputed the existence of thermal anomalies in (bulk) properties of water (and aqueous solutions), including Young (1966). Falk and Kell (1966) reviewed some of the evidence for 'kinks' and dismissed these as all falling within the limits of experimental error. Spurred on by these criticisms, we undertook a very high precision study of the viscosity of bulk water as a function of temperature (Korson et al., 1969), and observed *no* thermal anomalies whatsoever. In other words, for bulk systems the thermal anomalies are simply 'spurious effects' due to the properties of the water at the confining surfaces of the samples studied.

   In the remainder of this section, some typical results demonstrating the thermal anomalies of *VW* adjacent to solid surfaces will be discussed, whereas Section 3 deals with the evidence for the thermal anomalies (and the existence of *VW*) at the macromolecular/water interface. The temperatures at which the thermal anomalies $[T_k]$ occur are close to 15, 30, 45 and 60 °C, and are sometimes referred to as the 'Drost-Hansen Thermal Transition Temperatures'. The thermal transitions at T_k are relatively abrupt. However, they are generally not as sharp as expected of a first-order phase transition. The transitions generally occur over a narrow range (within 1–2 °C), e.g., 14–16, 29–32, 44–46, and 59–62 °C.
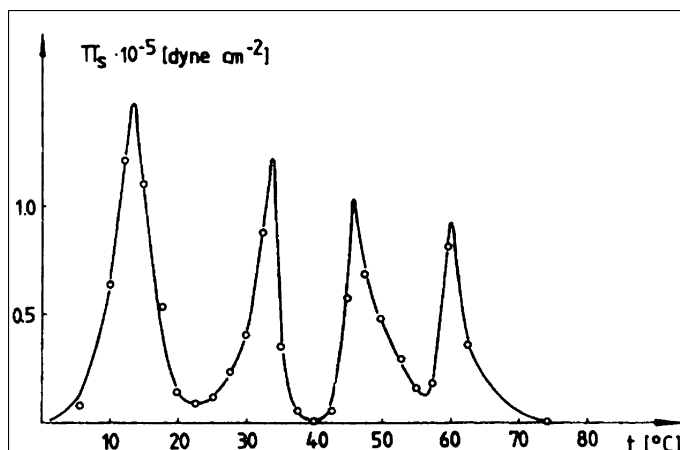
Figure 1. Disjoining pressure [π] of water (10^{-5} dynes/cm^2) between quartz plates separated by 50 nm as a function of temperature (°C). Data from Peschel and Belouschek (1979)

2.7.1   Disjoining pressure

Peschel and co-workers (1970, 1979) constructed an early form of a high-precision force-balance, allowing them to measure the disjoining pressure of water in thin films of water between two highly polished quartz plates, one being optically flat and the other essentially – but not quite – flat, but with a radius of curvature of ~1 meter. Typical results obtained as a function of temperature for a different plate separation of 50 nm are given in Figure 1. The results are truly remarkable, with the disjoining pressure [π] going through a series of maxima and minima. Note that the peaks in π occur at 15, 30, 45 and 60 °C – i.e., at T_k in perfect agreement with the temperatures of the thermal anomalies first postulated by Drost-Hansen and Neill (1955). Similar results were obtained using sapphire plates instead of quartz (Peschel, personal communication).

2.7.2   Viscosity measurements

In an alternative use of the force-balance, the dynamics of the top plate 'settling' on to the bottom plate were followed, and from these data one could calculate the viscosity of the water squeezed from between the plates. Peschel's data Peschel and Adlfinger's (1970, 1971) data are shown as Arrhenius' plots of the viscosity as a function of the reciprocal of the absolute temperature (Figure 2). Like the disjoining pressure, the viscosity goes through a number of maxima and minima.

2.7.3   Specific heat measurements

Etzler and Conners (1990) measured the heat capacity of water in narrow pores of silica gels (diameters of 4, 14 and 24.2 nm). In all their experiments, distinct 'heat capacity spikes' were observed in their thermograms, and the peaks of the spikes
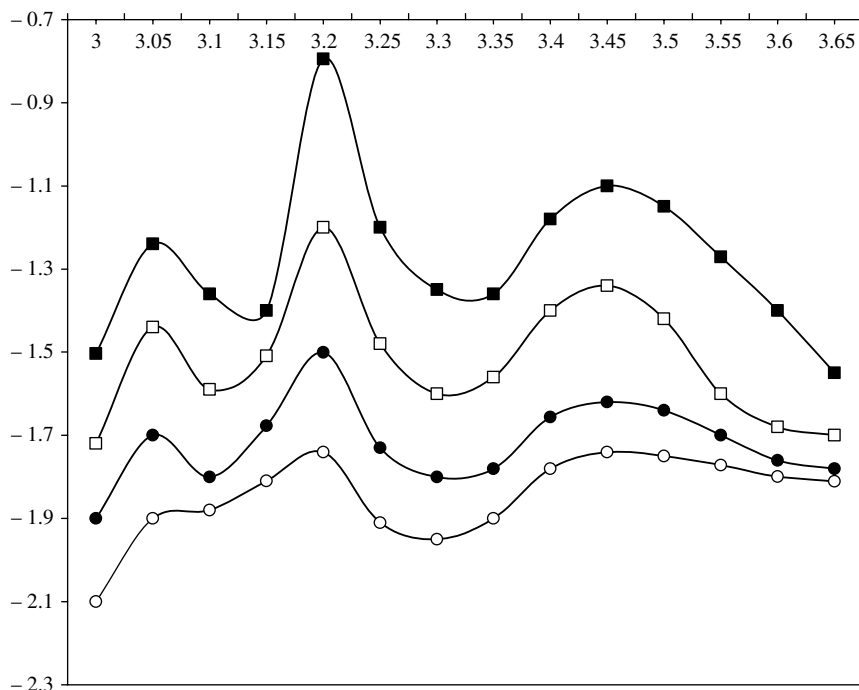
Figure 2. Arrhenius graph: log (viscosity; poise) of water between quartz plates versus 1/T (°C). Plate separations, top to bottom: 30, 50, 70 and 90 nm. Data from Peschel and Aldfinger (1969)

were at the thermal transition temperatures of *VW*. In all cases, the observed heat capacity values were above that of bulk water, consistent with the findings of Braun and Drost-Hansen (1976) and Braun (1981; Section 2.2) Figures 3 and 4 show enlargements of some of the data obtained by Etzler and Conners; the anomalies near T_k are very distinct at both the lower and the higher temperatures.

In preliminary studies, (Drost-Hansen, unpublished), distinct thermal anomalies in DSC measurements have frequently been seen on a variety of aqueous interfacial systems (similar to the characteristic peaks in the DSC curves observed by Etzler and Conners, 1991). A pronounced thermal anomaly occurs at 60 °C in thermograms of highly dilute aqueous solutions of cetyl-trimethyl ammonium salicylate $(C_{16}H_{33}(CH_3)NH_4^+ - C_6H_4(OH)COO^-)$. Even at concentrations as low as 0.01%, the anomaly remains very distinct. This quaternary amine forms very large micelles that are presumably vicinally hydrated, suggesting that VW plays a role in the highly unusual rheological properties of solutions of this quaternary amine, including their viscoelastic behavior.

The distinct peaks in the specific heat curves reported by Etzler and Conners (1991) on water in porous silica particles have been seen in many other studies, including an early study of Braun and Drost-Hansen (1976), and more recently in a DSC study of 10% polystyrene sphere suspensions. In the latter study,
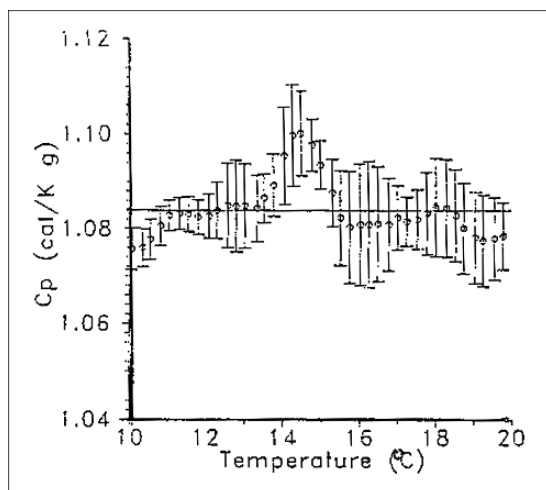
Figure 3. Specific heat of water in small pores; lower temperatures. Data from Etzler and Conners (1990)
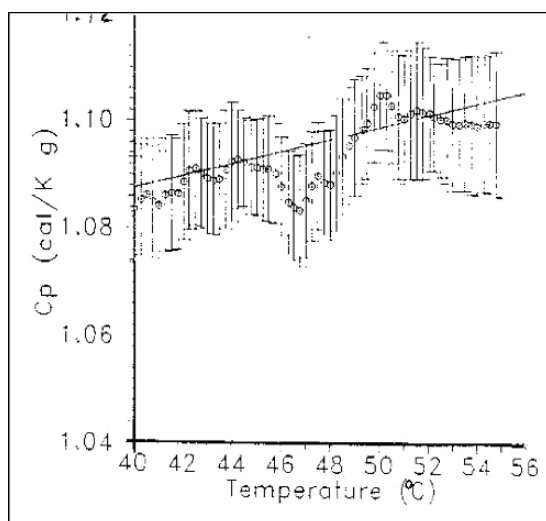

Figure 4. Specific heat of water in small pores; higher temperatures. Data from Etzler and Conners (1990)

heat flow (in a Perkin-Elmer DSC7 instrument) showed a pronounced peak near 28 °C. This is important, since it demonstrates that interfacial water does not have to be confined on all sides, as in a capillary or between plates, to exhibit *VW* characteristics. That the polystyrene spheres should be vicinally hydrated agrees with the volume contraction data for the settling of suspensions of these spheres, discussed in Section 2.1.

2.7.4   Solute distributions

Wiggins (1975) published a groundbreaking paper describing highly anomalous distributions of ions between bulk solutions of mixed electrolytes (equimolar with respect to K^+ and Na^+) and a silica gel. Three series were made with equimolar cation concentrations present as Cl^-, I^- or $SO_4{}^{2-}$. Measurements were made as a function of temperature and graphs of the selectivity coefficients between potassium and sodium ions plotted as a function of temperature. Distinct, sharp peaks were seen in the data, occurring at 15, 30 and 45 °C, i.e. exactly where thermal anomalies had been predicted by Drost-Hansen and Neill (1955), and had been observed by Peschel and Adlfinger (1971). Because of the potentially revolutionary importance of these measurements to cell physiology, we decided to repeat Wiggins' measurements (on a slightly more limited scale, using only equimolar solutions of NaCl and KCl). Our results (Hurtado and Drost-Hansen, 1979) agree *quantitatively* with Wiggins' data (Figure 5), making it exceedingly unlikely that the results reported by Wiggins are spurious or unreliable.

Further confirmation of distinct peaks in ion distribution in vicinal as a function of temperature were obtained by Etzler and Lilies (1986), using Li^+ and K^+ (as their chlorides; Figure 6A and B). This study is also important because it includes measurements of the same ion pairs in D_2O. Not surprisingly, similar and in fact dramatic and distinct peaks in the selectivity coefficients were also observed in this case, but at slightly different temperatures.
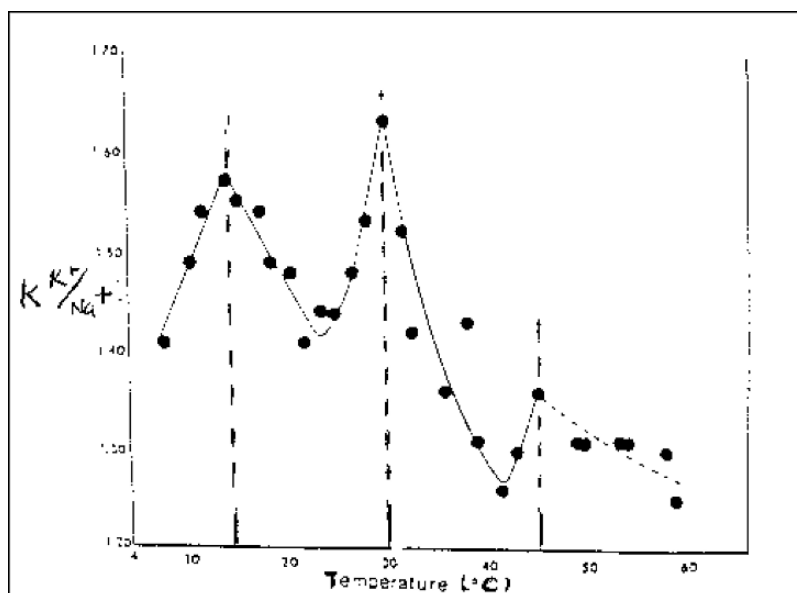
Figure 5. Selectivity coefficient, $K_{K/Na}$, as a function of temperature (°C) for potassium/sodium ions between the pores of a silica gel and the bulk solution, as a function of temperature. Data from Hurtado and Drost-Hansen (1979)
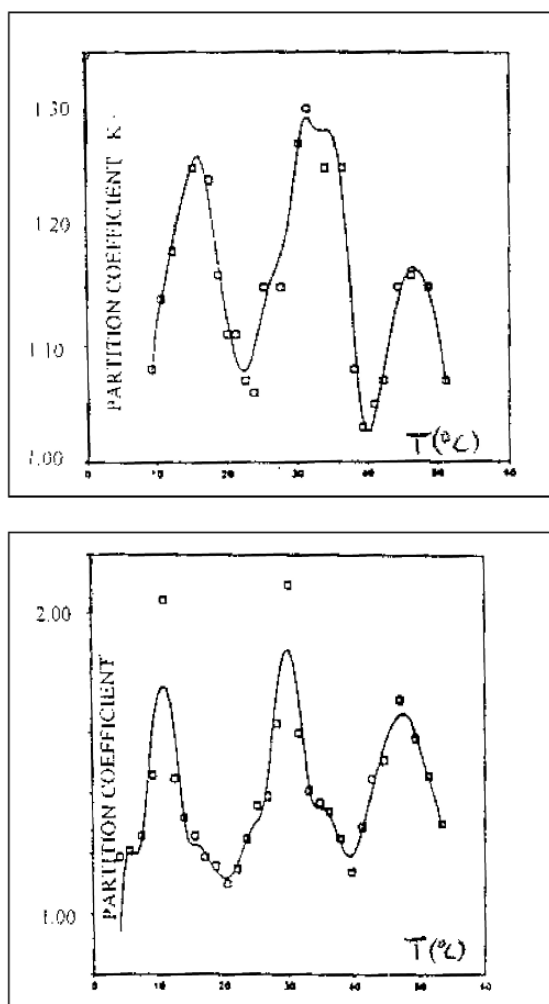
Figure 6. Selectivity coefficient, $K_{K/Li}$, as a function of temperature for a solution of pH $= 2.9$. Smooth curve represents a computer smoothing and interpolation of the data. Data by Etzler and Lilies. (1996). Selectivity coefficient, $K_{K/Li}$ as a function of temperature for D_2O solution, pD $= 4.4$ [pD $=$ pH(meter reading) $+ 0.4$]

Anomalous solute distributions are observed not only with ionic solutes. van van Steveninck et al. (1991) have made a series of measurements on non-electrolytes. Some of their results are shown in Figures 7 and 8. In Table 5 are listed the temperatures at which changes in slope were observed. Again the critical temperatures match those observed with ionic solutes, the T_{ks}. It is difficult to escape the conclusion that the same underlying mechanism must be operating, namely thermal transitions in the interfacial *VW.*
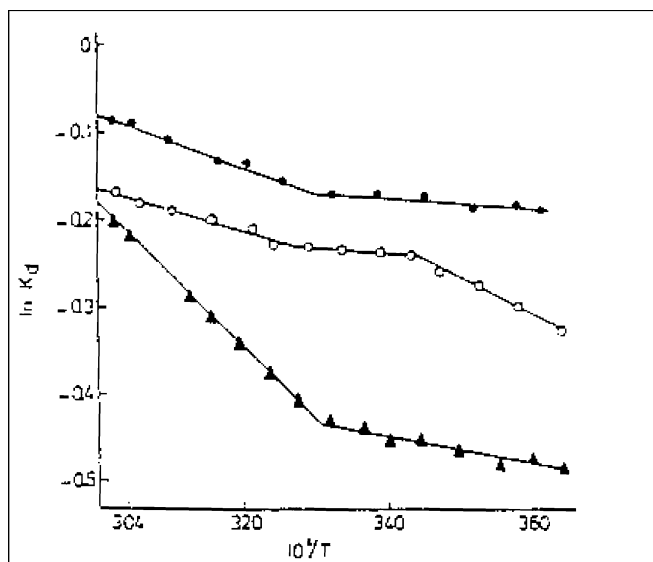
Figure 7. Log$_e$ (selectivity coefficient) as a function of 1/T for some low-molecular weight solutes of Sephadex G10 columns. Filled circles: methanol; open circles: DMSO; triangles: galactose. Data from van Steveninck et al. (1991)
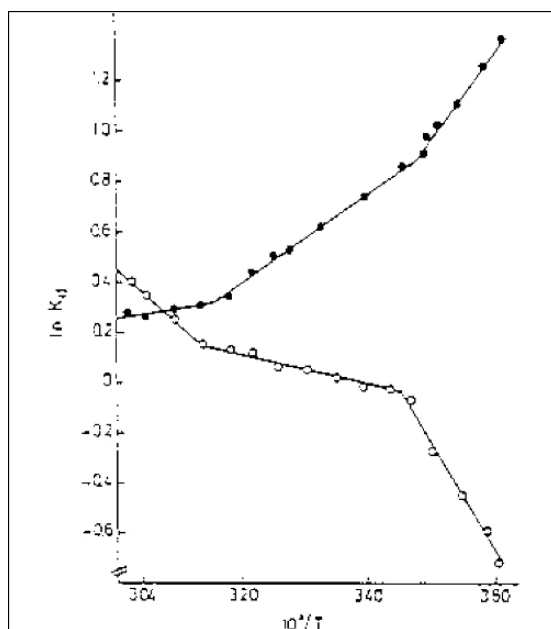
Figure 8. Log$_e$ (selectivity coefficient) as a function of 1/T for thiourea (solid circles) and t-butanol (open circles) on Sephadex G10 columns. Data from van Steveninck et al. (1991)

Table 5. Breaking points in the curves of the partitioning coefficient (Kd) for small solutes on Sephadex columns

Solute	Breaking points (°C)		
Thiourea	13.9		43.4
Galactose		28.9	
t-Butanol	16.6		47.7
Dimethylsulfoxide	16.1	31.5	
Methanol		30.4	

2.7.5 *'Missing anomalies'*

In some experiments carried out over a sufficiently large temperature interval, all 4 thermal anomalies (at T_k) can be observed in any given run. However, in other experiments, only one or two thermal anomalies may be observed. The reason is not fully understood, but the explanation may well be related to the slow rate of forming of stable vicinal structure above any given transition. For instance, in experiments where measurements are made continuously as the temperature is increased, a sample initially at room temperature may exhibit an anomaly at 30 °C, but the rate of forming the new vicinal hydration structure that is stable between say 30 and 45 °C may be too low, so that when the sample temperature reaches 45 °C, the intervening stable structure may not yet have been fully formed and hence the anomaly is overlooked at that next expected T_k. (As discussed below, experiments in which measurements are made continuously while temperature is *decreasing* frequently fail to show thermal anomalies.) In addition to the anomalies reflecting the *VW* transitions, the overwhelming effects of temperature on many very large macromolecules (such as denaturation – although such processes are rarely very abrupt) must be remembered. On the other hand, great abruptness – generally not at T_k – must of course also be expected in the case of lipid phase transitions. Many of these factors may contribute with the result that nearly all biological responses to temperature inevitably become highly complicated. The purpose of this paper is to call attention to the *specific contributions one can expect from VW* in the cell.

3. VICINAL HYDRATION OF MACROMOLECULES

Since *VW* definitely exists at all solid/water interfaces, it is perhaps not too surprising that individual large molecules in aqueous solution also induce vicinal hydration structures. Obviously, small solutes are not vicinally hydrated (although, in general, both ions and non-electrolyte have their own distinct types of hydration). However, over the years we have observed thermal anomalies in the properties of most solutions of macromolecules, and indeed these thermal anomalies occur at the characteristic temperatures, T_k, Hence we have pursued the idea that *VW* does

indeed occur at the macromolecule/water interface, as first proposed by Etzler and Drost-Hansen (1983) and at the same time, we began to explore the question of how large a macromolecule needs to be to become vicinally hydrated.

3.1   Critical Molecular Weight

Although a lot is known about various types of hydration/solvation of macro-molecules, little attention has been paid to the possibility that this is vicinal hydration. About 20 years ago, I began investigating the properties of various macro-molecules in aqueous solutions from the perspective of the existence of pronounced vicinal hydration structures at solid interfaces. Two striking observations had been reported: the first was a graph of the diffusion coefficients for 32 different solutes in water as a function of their MW (data from Stein and Nir, 1971). In Figure 9, one sees a distinct change in slope at ~1000 Daltons, and our tentative interpretation was that this MW corresponds to the onset of vicinal hydration (Etzler and Drost-Hansen, 1983; see also Drost-Hansen, 1997). The other interesting finding was the intrinsic viscosity of aqueous polyethylene oxide (PEO) solutions as a function of MW. The data in Figure 10 comes from Bailey and Koleske (1976). A distinct change in slope is seen at a MW of ~2000–3000 Daltons.
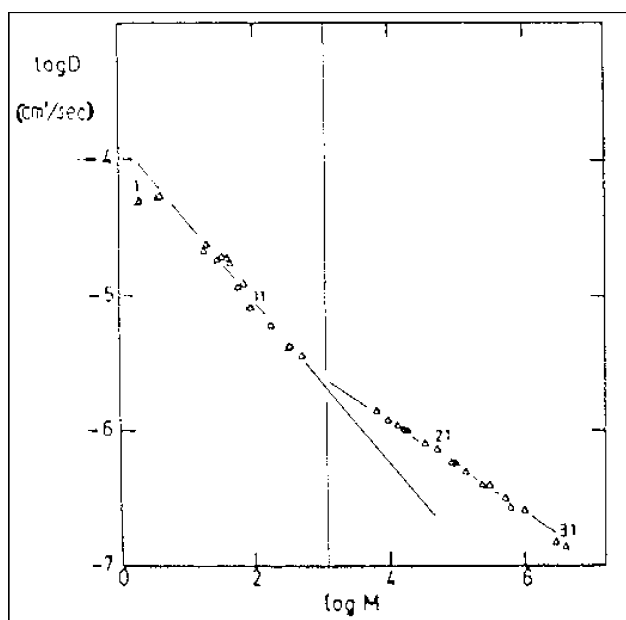
Figure 9. Log (diffusion coefficient) for solutes in water as a function of the MW of the solute at 20 °C. Solutes ranging from #1 (H$_2$) up to #31 (hemocyanin component, *Helix pomatia*). See original paper for detailed data, Nir and Stein (1971)
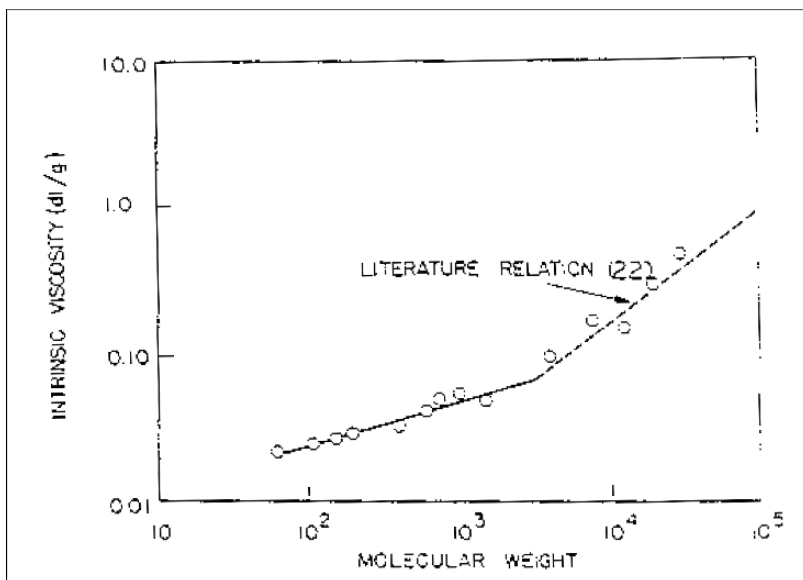
Figure 10. Intrinsic viscosity of polyethylene oxide in water as a function of MW. Data from Bailey and Koleske (1976)

3.2   Rheology

Over the years, we have studied the viscous properties of water and aqueous solutions, at first primarily of pure water and electrolytes solutions (Korson et al., 1969, Dordick et al., 1979, Dordick and Drost-Hansen, 1981), having developed a very high precision technique for such measurements. For example, we have determined the temperature dependence of the viscosity of water over a wide range with the highest precision and, as mentioned in Section 2.7, one of the important conclusions from this study was the confirmation that – contrary to earlier assertions, for instance by Magat, Bernal, Forslind, Krone, the present author and others – there were no thermal anomalies in the viscosity of pure bulk water. In part as the result of this study, it was subsequently proposed (Drost-Hansen, 1968) that the frequently reported thermal anomalies ('kinks' or 'discontinuities') in the properties of the bulk water were caused by spurious influences of the surfaces of the confining containers, as can readily be expected, for instance, from the viscosity experiments using very narrow capillaries. It was also stressed that the thermal anomalies appear to be the direct result of changes in the structural properties of the interfacial water, i.e. caused solely by its proximity to the interface. Later this was indeed expanded to mean *any* adjacent surface, regardless of the detailed, specific chemical nature of the solid (The 'Substrate Independence' or 'Paradoxical' Effect, see below; and Drost-Hansen, 1965, 1976, Kurihara and Kunitake, 1992). Lafleur et al., 1989).

   More recently, it became of interest to obtain rheological data at far lower shear rates than those found in typical capillary viscometers (which are of the order of 1000–3000 sec^{-1}). For the study of the viscosities of dilute aqueous suspensions of polystyrene spheres and other suspensions, and of the rheology of aqueous macromolecular solutions, we primarily employed a Brookfield plate-and-cone variable shear viscometer (Model LVTDV-22). Because of space limitations, the results of such experiments on a wide variety of polymers will be exemplified in just one graph (Figure 11) [Note: some of these studies have not been published, although the majority have appeared in Final Grant Reports to the US Air Force, Office of Scientific Research, Bolling AF Base, Washington, DC, USA]. Our viscosity data for diverse polymers in aqueous solutions all showed distinct thermal anomalies throughout and almost invariably at the *VW* T_k values. From frequency with which these changes occur at $\sim T_k$, it is difficult to escape the conclusion that the polymers are vicinally hydrated (in particular see Drost-Hansen, 2001). In these experiments, the rate of heating in the viscometer was usually relatively low. For example, a measurement might be made at some constant temperature and after a constant viscosity value had been recorded, the temperature of the circulating bath controlling the viscometer sample cup would be increased, by $\sim 1\,°C$. The system was then left for a period of time (a few minutes or longer) and the next viscosity measurement taken. In no case were measurements made while the temperature was being lowered, and if a repeat run was to be made on any given sample, sufficient time was allowed for the vicinal water structures to reform. The time required for most systems to revert to their initial state has been from a few hours up to a day. Measurements were rarely made at shear rates above 225 sec^{-1} and many rates were as low as 11 sec^{-1}.
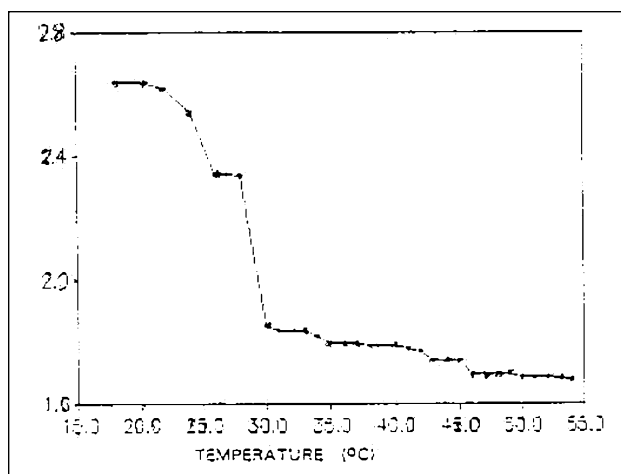

Figure 11. Viscosity versus temperature for a 5% Dextran solution. Shear rate: 90 sec^{-1}. Data from Drost-Hansen and Gamacho (unpublished)
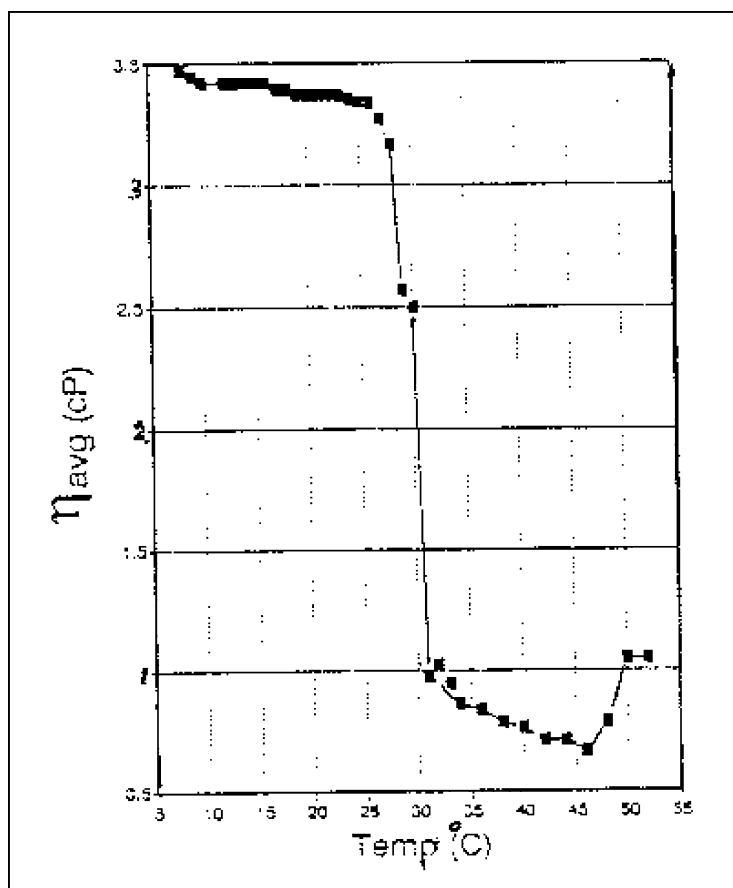
Figure 12. Viscosity versus temperature of blood plasma, Habor Seal

While *all* macromolecules in aqueous solution are vicinally hydrated (Figure 12) many biochemically important macromolecules are partly embedded in cell membranes. Again, it must be expected that those parts of such molecules which extend away from the membrane are also vicinally hydrated. Not surprisingly, thermal anomalies are also very frequently – but not invariably – seen in Arrhenius graphs of enzyme reactions (see in particular Drost-Hansen, 1971, 1978, 2001; see also Etzler and Drost-Hansen, 1979).

Anomalies are seen also in the viscosities of suspensions of solid particles., Figure 13, for example, shows the viscosity of a 0.1% polystyrene sphere suspension (particle size 0.17 microns) as a function of temperature. An anomaly is seen at 30 °C consistent with the expectations based on the evidence of vicinal hydration of such particles discussed above: the volume contraction upon settling, Section 2.1.1; the anomaly in index of refraction (see below); and the Bragg scattering results
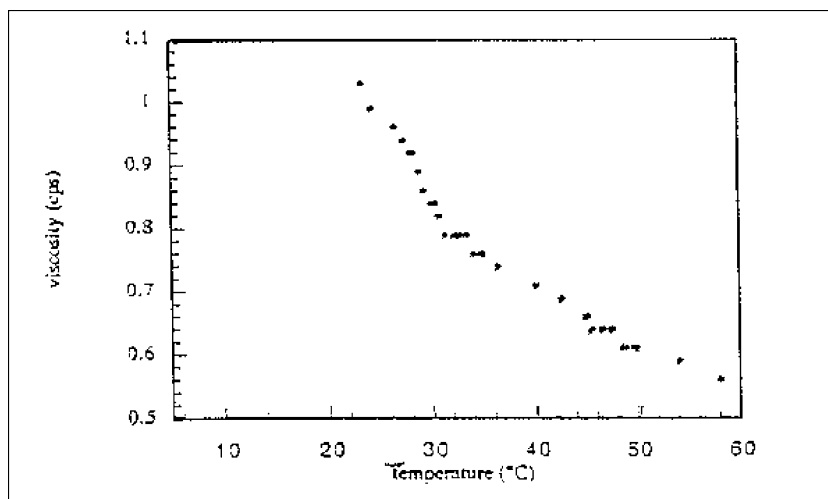
Figure 13. Viscosity versus temperature of 0.1% polystyrene sphere suspension; particle diameter 0.17 micron. Shear rate 90 sec^{-1}

(Section 3.7). The effects of vicinal hydration quite naturally become more pronounced the more concentrated the suspension, and in the case of, say, 10% kaolinite, very large anomalies are seen in the rate of sedimentation and compaction of such systems (Drost-Hansen, 1981).

3.3   Other Rheological Studies

A large number of rheological studies exist on far more complex systems than those examined so far in the present paper. Thus data ranging from cell physiology to food science and food technology have provided considerable insight into likely effects of *VW* in very complex systems. Because of space limitations, only a few examples will be discussed, e.g., Figure 14 shows the viscosity of a 1.4% actinomyosin solution.

Considering the thermal anomalies discussed above for relatively simple biopolymers, it is not surprising that complex protein systems also show distinct anomalies. In Figure 14, for instance, it is difficult to envision any obvious molecular mechanisms to explain such dramatic changes in this system over such narrow temperature intervals, except in terms of *VW* and the thermal anomalies (see also Drost-Hansen, 2001).

Finally, Figure 15 shows the viscosity of the protoplasm of *Cumingia* eggs as a function of temperature. Note the distinct (and abrupt) peak near 15 °C, and the even more dramatic change near 31°C. It seems inescapable that these effects must be due to the cell-associated *VW*.
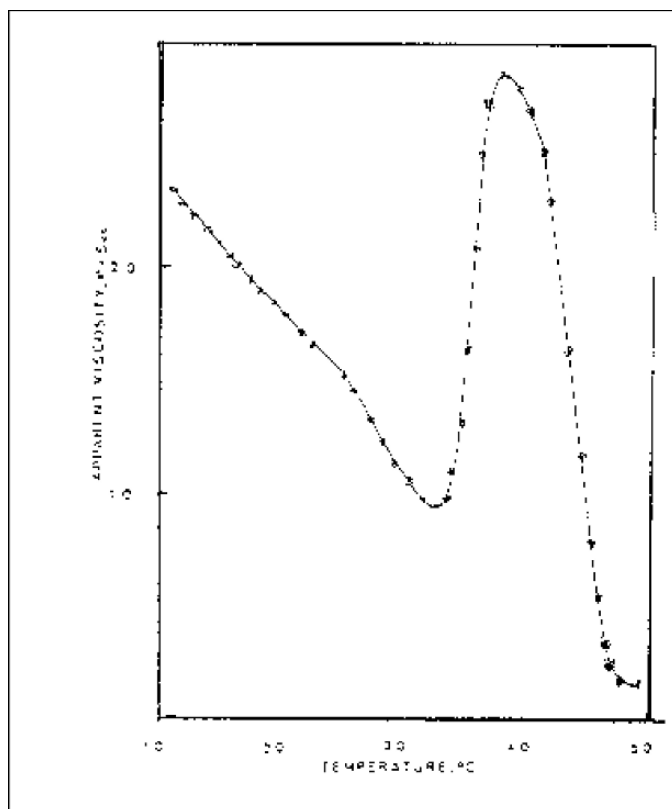
Figure 14. Viscosity versus temperature of actomyosin in aqueous solution. Continuous heating; rate = 1 °C min^{-1}. Shear rate 1.02 sec^{-1}. Data from Wu et al. (1985)

3.4 Diffusion Coefficients

Anomalous temperature dependencies of diffusion coefficients were reported as early as 1969 by Dreyer and co-workers (see, for instance, Drost-Hansen, 1972). In view of the distinct anomalies in the viscosity data discussed above, anomalies should also be expected in diffusion coefficient data, for both suspensions of particulate matter, such a polystyrene spheres, and in macromolecular solutions. The diffusion coefficient data shown here were obtained using a Photon Correlation Spectrometer (Coulter Counter Particle Analyzer Model CN4 SD). In a series of measurements, the temperature would be increased step-wise, usually in 1°C increments, allowing sufficient time (for instance, 2–5 min) between each reading to ensure that the sample had come to thermal equilibrium with the sample chamber. Figure 16 shows data obtained on rather highly diluted suspensions of polystyrene spheres. Anomalies are seen at 30 °C and in some cases at 45 °C, the spheres behaving like any other 'solid' surface (see also Sections 3.6 and 3.7).
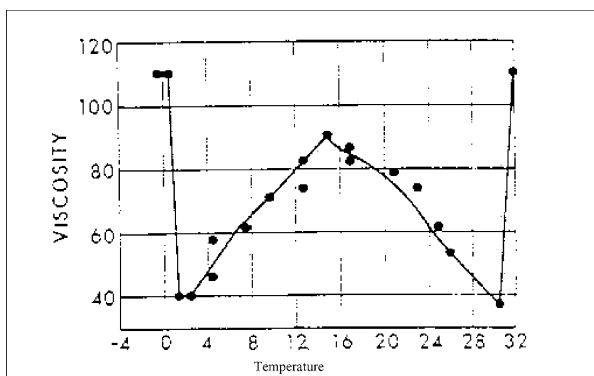
Figure 15. Viscosity versus temperature for protoplasma in *Cumingia* egg. Data of Heilbrunn, quoted in Johnson, Eyring and Polissar (1954)
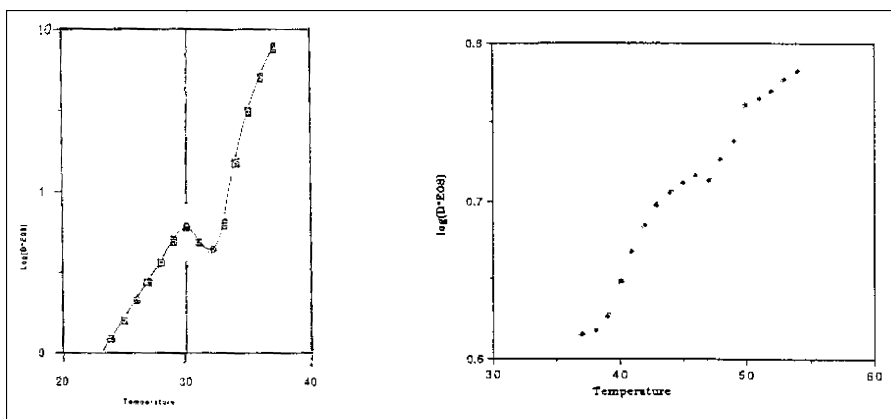

Figure 16. Log$_e$ (diffusion coefficient) versus temperature of polystyrene spheres in water, given as a 3-point moving average. Particle diameter 0.17 micron

Recall also the volume contraction data observed upon settling of the suspensions (Section 2.1) and the DCS data (Section 3.5). Figures 17A and B give diffusion coefficients for a variety of macromolecules in water. Again distinct thermal anomalies at T_k occur. In view of the effects of viscosity on diffusion coefficients (as, for instance, implied in the Einstein relationship), the anomalies seen in diffusion coefficients should indeed be expected. Once again, the conclusion must be drawn that, as far as water structure is concerned, sufficiently large macromolecules in water behave as a 'solid surface' to promote the formation of vicinal hydration. In some cases, the anomalies are more pronounced than in other cases, this variability probably being due to slight differences in sample preparation and
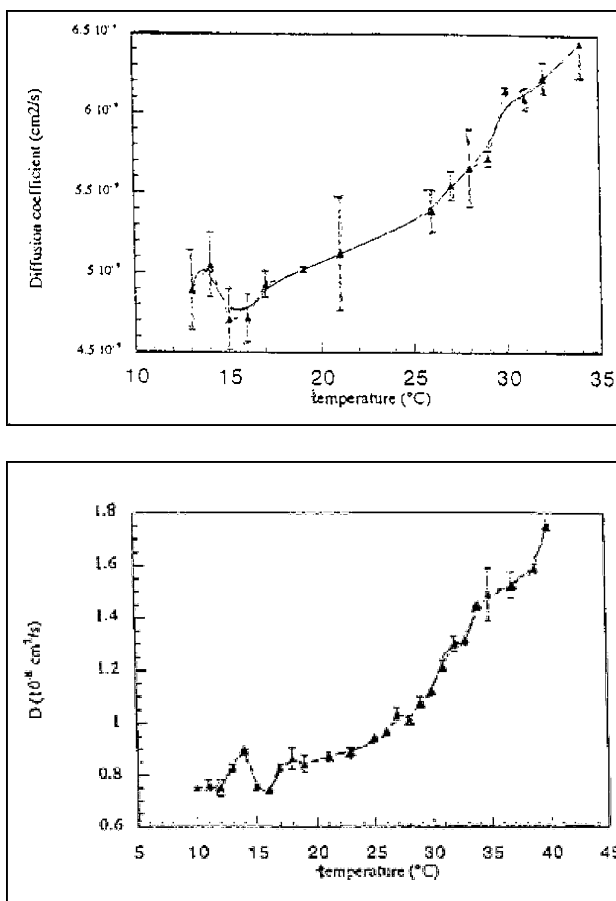
Figure 17. (A) Diffusion coefficient versus temperature of Dextran, MW = 40.8 kDa. Concentration 9.1%. (B) Diffusion coefficient versus temperature of Dextran, MW = 515 kDa. Concentration 8.18%

particularly in the time that had elapsed since the samples were last stirred and placed in the sample cuvette (see Section 3.11 on hysteresis).

3.5   Calorimetric Data

Effects similar to the distinct peaks in the specific heat curves reported by Etzler and Conners on water in porous silicate particles have been seen in many other studies, including Braun and Drost-Hansen (1976), and more recently in a DSC study of 10% suspension of polystyrene spheres (particle size 0.22 microns). The viscosity anomaly is shown in Figure 13, and the Bragg scattering results discussed in Section 3.7.

3.6   Index of Refraction

An equilibrium property has been studied in a preliminary manner, namely index of refraction, as a function of temperature. Measurements were made with an Abbe refractometer (Bausch and Lomb, Abbe 3L), with the temperature controlled by a circulating constant temperature bath. Again, data have been collected for a number of macromolecules in aqueous solution and typical results are shown
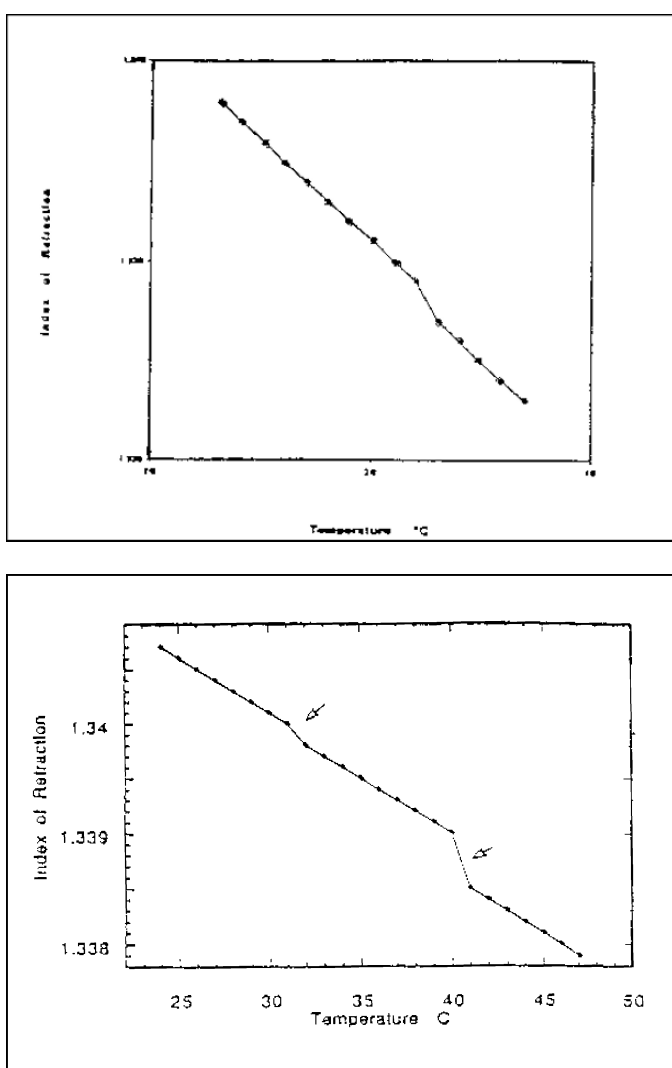

Figure 18. (A) Index of refraction of aqueous solution of Dextran in water versus temperature. Concentration 5%. (B) Index of refraction of γ-globulin in water versus temperature. Concentration 5%

in Figures 18A and B, where the expected, distinct (albeit small) anomalies are observed at T_k, regardless of the specific nature of the solutes.

3.7   Bragg Scattering from Suspensions

It has been anticipated for many years that ions in sufficiently concentrated solutions may form crystalline lattices and this has indeed been observed visually for 'giant ions' (macroions), such as negatively charged polystyrene spheres in suspension. A similar effect has also been reported for relatively concentrated solutions of turnip yellow mosaic virus. In a particularly interesting study, Daly and Hastings (1981) studied the Bragg scattering from crystallized suspensions of the readily available 'macroions', polystyrene spheres. It is important to stress that the 'crystallization process' takes place only very slowly and depends on the nearly complete absence of any mechanical shear [see also Section 3.11].

Figure 19 shows the results of Daly and Hastings (1981); in addition to the data points the calculated curves based on the theory developed in the paper are also shown. At a first approximation, the experimental points follow the trends of
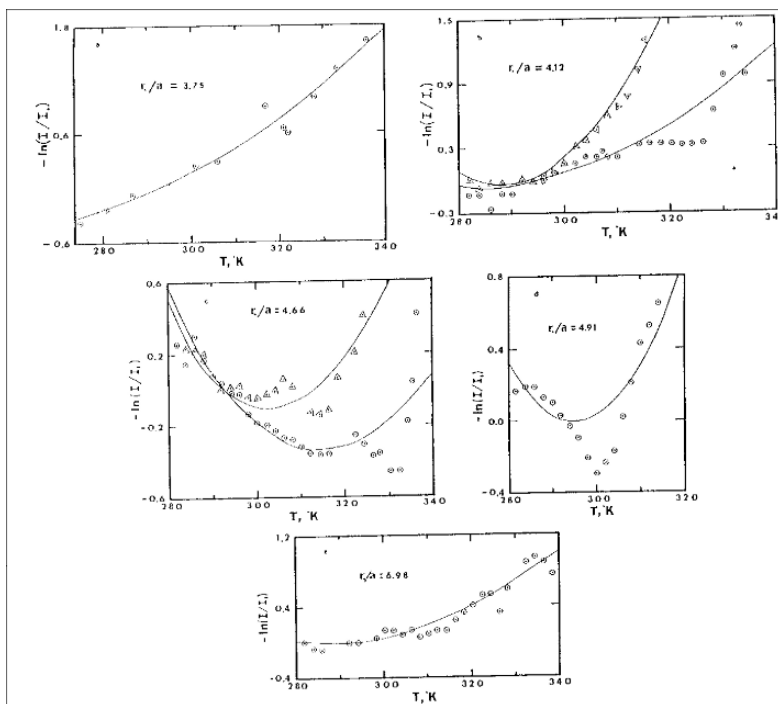

Figure 19. First-order Bragg scattering intensity versus temperature of 'crystallized' suspensions of 'macroions' (i.e., polystyrene spheres.) Data from Daly and Hastings (1981); see this reference for the experimental details and first approximation theory
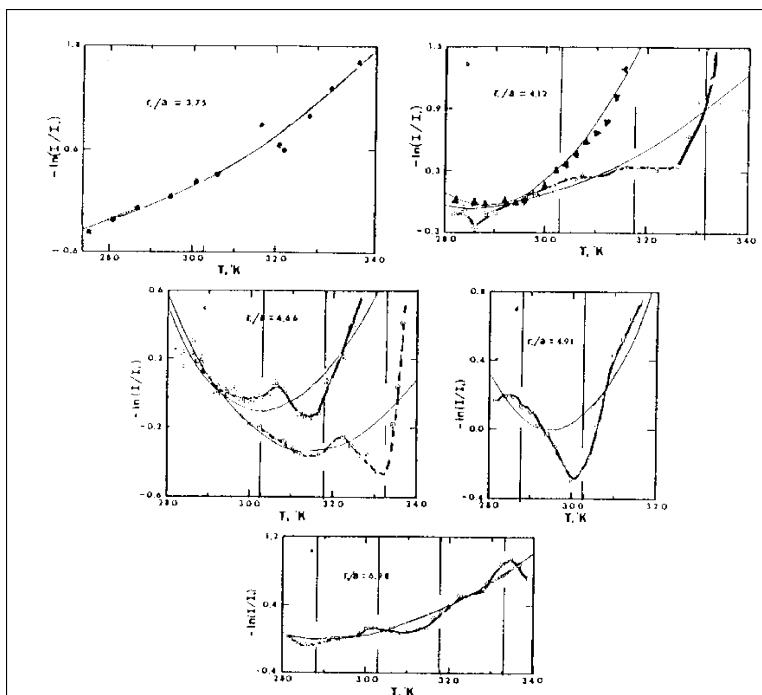
Figure 20. First-order Bragg scattering intensity versus temperature; data as in Figure 19, but with curves redrawn ('freehand') by the present author; also indicated are the temperatures of T_k (vertical lines)

the calculated curves. However, reasonably good-fit, continuous curves have been drawn in Figure 20, as well as vertical lines to indicate the temperatures of the thermal anomalies. Notable differences clearly exist between the calculated curves and the observed points. These differences demonstrate a likely role of the *VW* transitions at T_k.

3.8   Critical Molecular Weight Dependence, MWc: Detailed Considerations

Electrolytes and small non-electrolytes are obviously not vicinally hydrated, but the evidence presented in the preceding sections suggests that larger macromolecules are indeed vicinally hydrated. This poses the question: is there a critical Molecular Weight range (MWc) below which no vicinal hydration exists, but above which all molecules are vicinally hydrated? The answer seems to be that indeed there exists such a critical size, discussed in Section 3.1. As illustrated below, the MWc falls in

a range 1,000 and 5,000 Daltons. Note also the *great diversity* of types of molecules with >MWc included in these graphs, consistent with the idea of the 'Substrate Independence Effect' (the 'Paradoxical Effect').

Sometimes the abruptness of the change at MWc is very pronounced. Thus Gekko and Noguchi (1971) measured the MW dependence of a number of properties of oligodextrans with different MW. Figures 21a-d show respectively:-: a Stockmayer-Fixman plot; a derivative plot of the sound velocity (with respect to concentration);
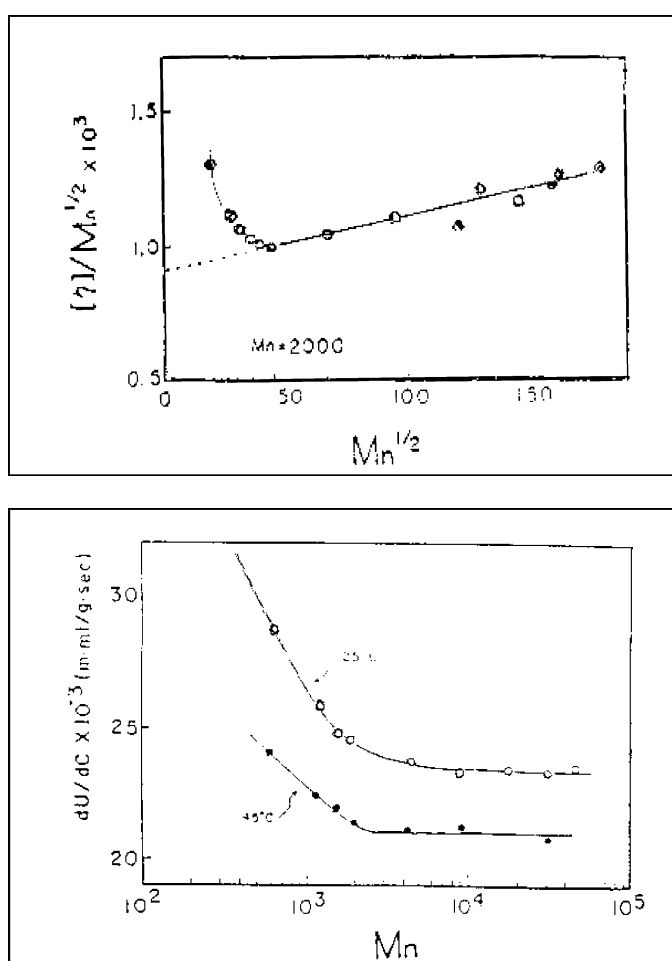

Figure 21. (A) Stockmayer-Fixman plot for aqueous solutions of Dextran versus square root of MW of the polymer. (B) Derivative of ultrasound velocity with respect to concentration for aqueous Dextran solutions versus MW. (C) Partial specific compressibility, $\beta_t{}^\circ$ of Dextran in water at 25 °C versus MW. (D) Fraction of 'bound water' in aqueous Dextran solutions versus MW Data from Gekko and Noguchi (1971)
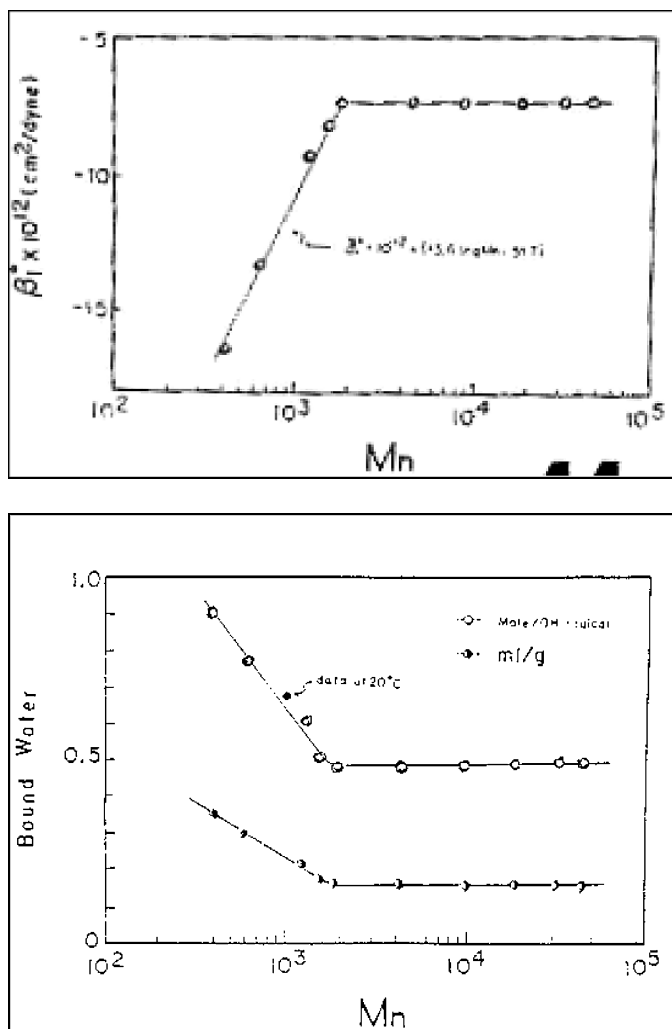
Figure 21. (Continued)

partial specific compressibilities; and the amounts of bound water – all as a function of MW. In these graphs, changes from one functional dependency to another is remarkably abrupt and always in the vicinity of a MW of $\sim 2,000$ Daltons.

As discussed in Section 3.11, vicinal hydration structures are highly shear-rate dependent. For this reason we mostly made measurements with a variable shear-rate instrument (a Brookfield cone-plate rheometer), generally using shear-rates of $100\ sec^{-1}$ or lower. However, in some cases it was also possible to use capillary viscometers. Figure 22 shows some apparent energies of activation for viscous flow
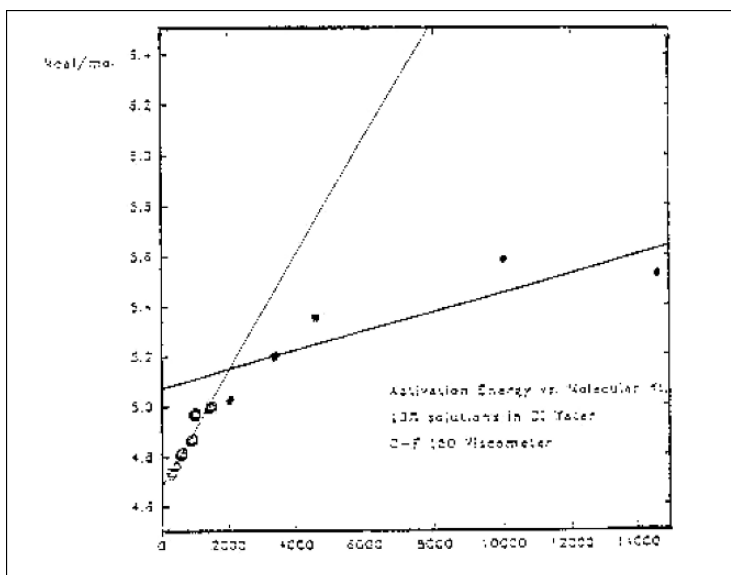
Figure 22. Apparent energies of activation for viscous flow of polyethylene oxide solutions [10%] versus MW. Data by Drost-Hansen and Vought (unpublished). See also Drost-Hansen (1992)

of 10% PEO solutions as a function of the MW of the polymer (Cannon-Fenske capillary viscometer, size 150; Drost-Hansen, 1992). A change in slope occurs at a MW of ~2,000 to 4,000 Daltons, in agreement with the general idea that a critical MW range exists.

Antonsen and Hoffman (1992) followed the properties of PEO solutions as a function of MW. Again a small but distinct anomaly was found at ~1,000 Daltons. A far more dramatic change is seen in the transition temperature for the 30% PEO solutions as a function of MW (Figure 23). Here the abrupt change is observed for a MW of 1,200 Daltons. Antonsen and Hoffman (1992) also measured the total heat required to melt frozen 30% PEO solutions, and found a distinct change at a MW of ~1,000 Daltons.

Related to cell functioning, Mastro and Hurley (1985) have compiled data for viscosities and diffusion coefficients and viscosities of a number of 'markers' in the cytoplasm of cells. By the Walden rule, the product of viscosity and diffusion coefficients should be a constant for any given solute. Figure 24a shows the data of Mastro and Hurley, which on replotting in terms of the product of the diffusion coefficient (D) and the viscosity (η), gives the results shown in Figure 24b. In Figure 24a, a distinct inflection point is observed ~2,500 Daltons. However, Figure 24b indicates that a *very* marked change in slope occurs at or just above 1,000 Daltons – consistent with the assumption that this is the critical mass for vicinal hydration.
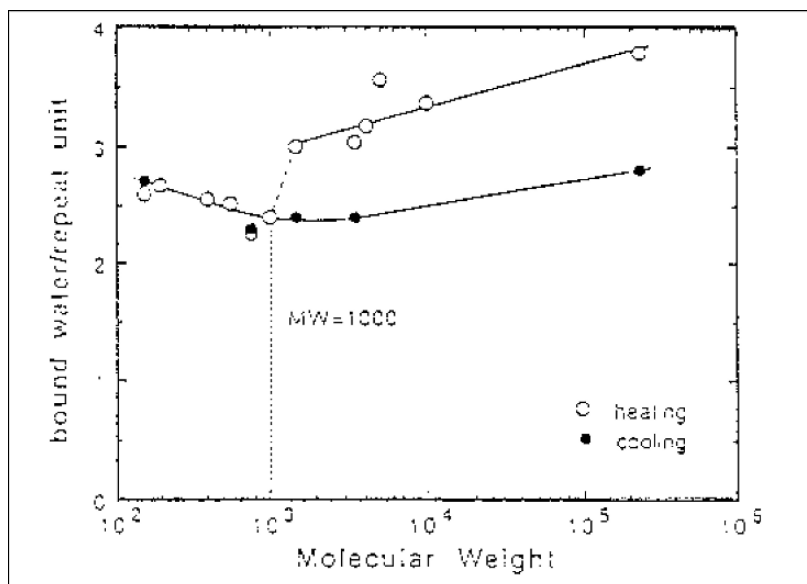
Figure 23. Amount of 'bound water' per repeat unit in 30% PEO solution as a function of MW. Data from Antonsen and Hoffman (1992)

The abrupt transition between vicinally hydrated biomacromolecules and smaller solutes finds a dramatic manifestation in cell biochemistry in terms of the normal composition of eukaryotic cells. Thus, based on data from Antonsen and Green (1975), Clegg (1979) first called attention to the remarkable coincidence of the onset of vicinal hydration and the MW of solutes in the cell. In Figure 25, the concentration of various solutes in the cell [the ordinate is the relative occurrence of solutes] are seen as a function of MW. Note the conspicuous absence of solutes in the cell in the range 1,000–10,000 Daltons. It seems as if cells, in the process of evolution, have selected for solutes that are either definitely *not* vicinally hydrated *or* distinctly vicinally hydrated. Note also that these small polypeptides (usually in very low concentrations) can have dramatic physiological effects (such as endorphins), and these are often in the range of 1,000–10,000 Daltons.

Based on the rheological data and the evidence discussed above, one must conclude that as far as water structure is concerned, a molecule with a MW of ∼2,000 Daltons is 'mechanically' as 'substantial' as a 'solid surface'. Surely this information must be important to those investigators modeling the structure of water at interfaces and the idea of a 'critical MW range' must have implications for the idea of 'soft interfaces', [see De Gennes, 1997]. In this connection, see also the recent papers of Etzler et al. (2005) on particle-particle adhesion, especially in the way it is influenced by trace amounts of water at the particle interfaces.
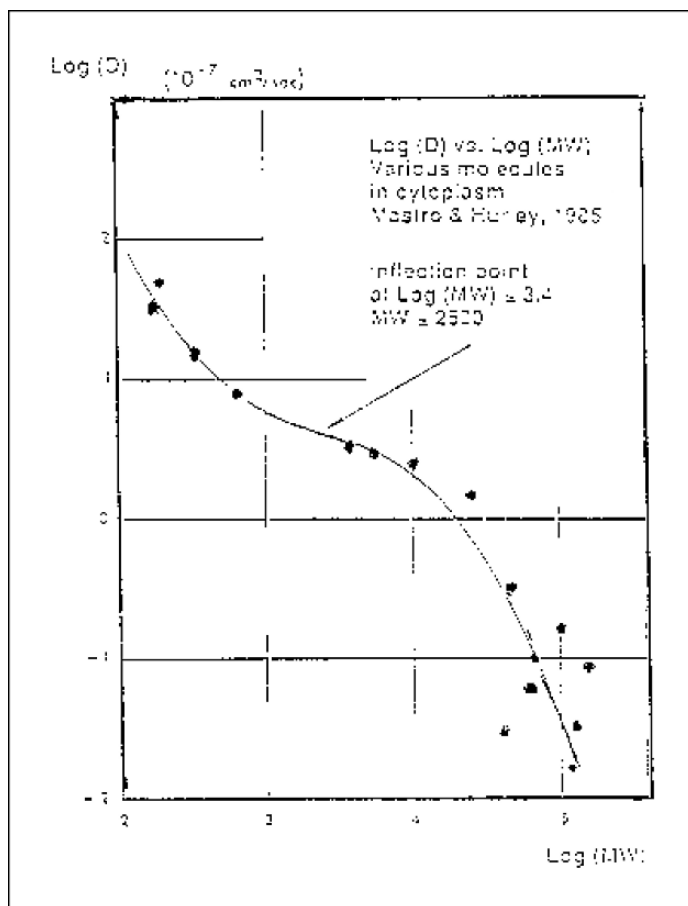
Figure 24. (A) Log (Diffusion coefficient) versus log (MW) for various molecules in the cytoplasm. (B) Product of viscosity and diffusion coefficient versus log (MW) for the molecules from Figure 24A. Data redrawn from Mastro and Hurley (1985)

3.9   Shear Rate Effects

VW is shear rate dependent and once destroyed by shear, the time to reform the vicinal hydration layers (when the shear stops) may be very long; of the order of minutes, hours or even a day. This finding has been corroborated many times. Kerr (1970) and Drost-Hansen (1976) studied the internal damping of a vibrating hairpin capillary (vibrating in a vacuum). The damping is caused primarily by the energy dissipation at the water/quartz interface of the hairpin element, showing a notable reduction at 30 °C, which we have ascribed to the breakdown of the extended interfacial water structures at the capillary surface at this temperature. In other words, the coupling between the water molecules and the confining surface
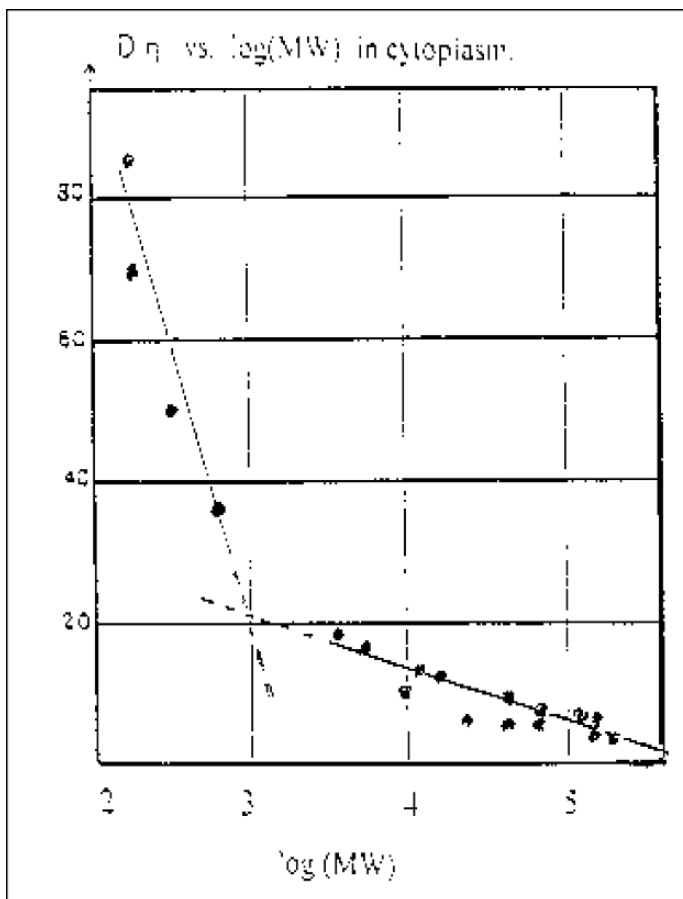
Figure 24. (Continued)

changes drastically at T_k. Kerr (1970) also noted that it was not possible to obtain reproducible results if another run was initiated immediately after completion of the previous run. The system had to be left undisturbed for as long as 24 h before reproducible results could be obtained. Likewise, Braun and Drost-Hansen (1976) carried out a number of DCS experiments with water in a porous silica gel. While each new run would show a distinct anomaly at T_k, such results could not be repeated unless the sample was allowed to sit at a lower temperature overnight. We return to the shear rate and temperature induced hysteresis in Section 3.11, which deals with rheological properties of aqueous macromolecular solutions, where the effects appear to be even more pronounced.

The effects of shear on some micellar systems were studied by Shephard et al. (1974), who measured the viscosity of various solutions prepared using some sulfonated alkanes [of the type considered for use in tertiary oil recovery
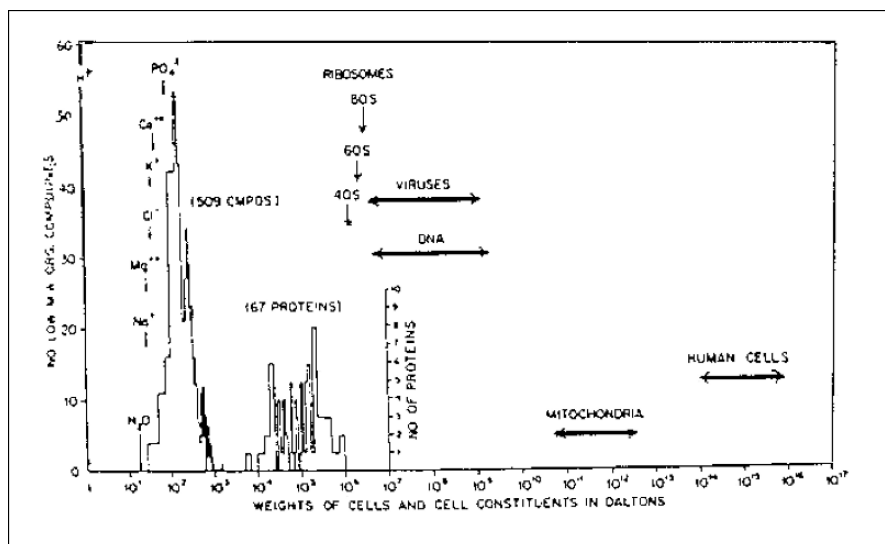
Figure 25. Solute concentrations in a typical eukaryotic cell versus MW of the solute. Data from Anderson and Green (1975), as presented in Clegg (1984a)

processes] in a brine solution (2.5% NaCl plus smaller amounts of $CaCl_2$ and $MgCl_2$). Figures 26A and B show some of their results, from which it is clear that the thermal anomalies near both 45 °C and ~60-62 °C rapidly become less prominent with increasing shear rate, consistent with the idea that the vicinal hydration is indeed sensitive to shear effects. It is interesting to speculate that the shear rate effect may ultimately have a bearing on the overall rheological properties of many, or most, aqueous suspensions, as well as macromolecular and micellar systems. Thus the transient properties of *VW* under conditions of shear may conceivably play a role in non-Newtonian behavior of such systems as dough; heavily hydrated clay deposits (as seen in mud slides and turbidity currents in marine environments), or the large micelles in such solutions as cetyltrimethyl ammonium salicylate (strongly elastic solutions, even at dilutions as high as 0.01%! – see also the mention of these micelles in the discussion of DCS studies of such solutions).

3.10   Possible Thermodynamic Implications of Vicinal Hydration

From the examples discussed above, it is clear that mechanical shearing notably affects the viscosities of macromolecular solutions. However, if a transport property is affected by shearing, it seems logical that thermodynamic properties may also be affected. Thus the proposal is made that in an osmometer with identical aqueous solutions (of a high MW polymer) on either side of a semi-permeable membrane, at least a transient osmotic pressure difference might be created if the contents on one side of the osmometer are suddenly stirred sufficiently vigorously. By
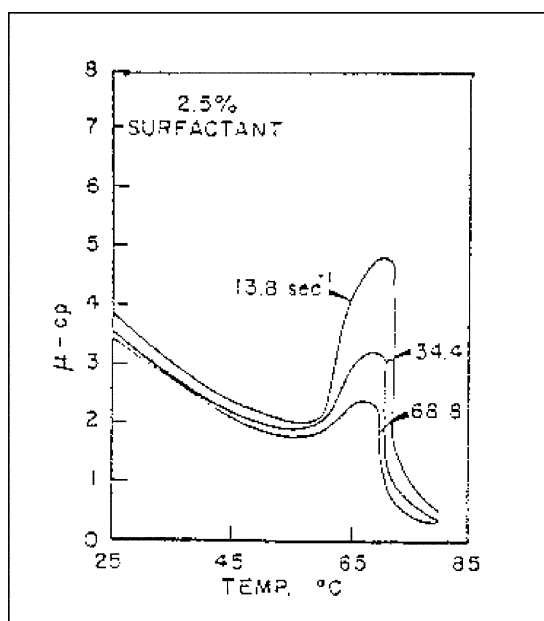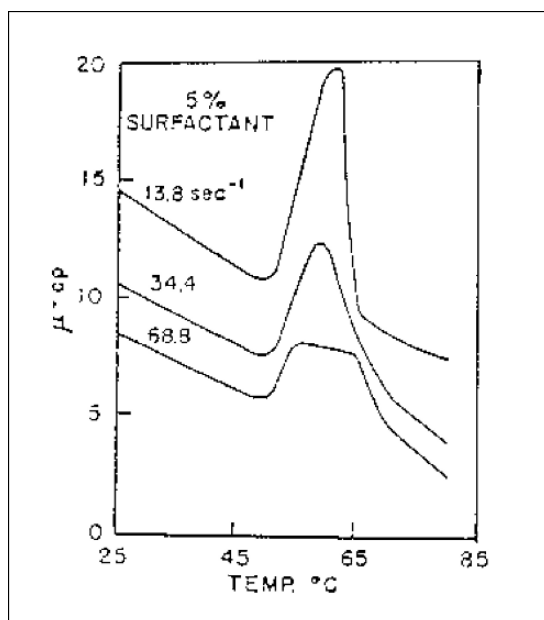
Figure 26. (A) Viscosity versus temperature of a surfactant dispersion (SS-5) in a dilute electrolyte solution, measured at 3 different shear rates. (B) Viscosity versus temperature of a surfactant dispersion (SS-2.5) in dilute electrolyte solution, measured at 3 different shear rates. Data from Shephard et al. (1974)

a similar argument, if a stagnant solution of a sufficiently concentrated macro-molecular solution (or suspension of particles) is stirred, a (transient) vapor pressure difference should develop. Indeed, about 40 years ago, I did exactly this kind of experiment using 5 and 10% suspensions of montmorillonite: a (transient) vapor pressure increase was indeed been observed upon suddenly stirring the suspen-sion! However, because this was a highly unexpected finding – and for lack of a deeper understanding of *VW* at that time – I was dissuaded from publishing the results.

Returning to transport phenomena: if the above arguments can be corrobo-rated, one may conceivably expect differences as well in reaction rates involving solutes that are highly vicinally hydrated. As we have repeatedly shown, enzymes in solution are vicinally hydrated, as evidenced by the occurrence of thermal anomalies in Arrhenius graphs of enzymatic reaction rates (see Etzler and Drost-Hansen, 1979, 1983). Hence it is postulated that differences in rates of reaction involving sufficiently large enzymes may exist between reactions taking place in stagnant solutions compared to vigorously stirred systems. This may be particularly pronounced if the enzyme acts upon a relatively large substrate molecule with its own vicinal hydration.

In hemodynamics, it has long been known that shear may profoundly affect some of the dynamic properties of the circulating blood. One obvious site of such shear effects is the deformable particles of the blood, notably the platelets, leucocytes, and, in particular, the erythrocytes (Dintenfass, 1981; Uijttewaal, 1990). However, in the case of a more-or-less suddenly created stenosis, the locally increased shear rate due to increased velocity at the point of constriction may not only affect the geometric shapes of the deformable circulating cells, but might also affect the dynamic properties of some of the circulating macromolecules. Essentially 'stripped' of the 'protective' vicinal hydration hulls, due to the increased shear rate, some macromolecules may become more reactive and thus alter the homeostatic dynamic equilibria of the circulating blood.

3.11   Mechanistic Aspects

In considering slope changes in Arrhenius graphs of rate processes, it is important to recognize that distinct changes in slope may mean very significant differences in energies of activation for the processes (below and above a critical temperature, T_k). In other words, the energetics may differ notably – implying not merely a small change in the overall molecular dynamics, but sometimes possibly a very dramatic change in the underlying mechanisms of the reactions. This might be brought about by changes in a relatively small number of water molecules in the hydration shell, if these are very strongly bound, or, in the case where the energetics of the *VW* does not differ significantly from that of the bulk water, very *large* numbers of water molecules must be involved. The latter situation is by far the more probable.

3.12   Hysteresis

The fact that heating past one of the critical temperatures, T_k, destroys the vicinal hydration structure stable below that point, together with the very low rate of reforming of the hydration structures (after the temperature range has been decreased to a range below T_k) results in notable hysteresis. In addition, at constant temperature, shearing of the solutions will also destroy the vicinal hydration structures and again the time for reforming the appropriate hydration structure is long (minutes or hours). These facts, combined with the fact that many studies of polymer viscosities have been made in relatively high shear instruments, such as capillary viscometers – may offer an explanation why the effects described in this paper have rarely been noted or explored before. The formation of a 'stable' vicinal hydration structure will often be fairly fast: in a continuous heating run, once the temperature has exceeded T_k, the 'new' vicinal hydration structure stable between T_k and T_{k+1} may form sufficiently quickly so that another thermal transition is seen at T_{k+1}. However, in some cases of relatively fast heating – and depending on the nature of the solid interface or the specific type of polymer used – there may be insufficient time for the stable vicinal hydration structure to form, and in such cases only one (or perhaps two) of the thermal transitions may be seen in any given run.

3.13   Conformational Changes and Pre-denaturation

In view of the ubiquitous presence of *VW*, it may prove useful to review a large number of previously published data on biomacromolecular solutions. Studies where a parameter has been measured as a function of temperature might reflect previously overlooked influences of *VW* and the thermal anomalies. As an example, Lopez-Lacomba et al., 1989) used a DSC method to follow the thermal unfolding of myosin rod, light meromyosin and subfragment 2. An inspection of their data (notably their Figures 1-3) suggests that the thermal transitions of *VW* may play a role in the unfolding process, considering the events in the DSC curves that appear to occur near T_k. Likewise, the study by Urbanke et al. (1973) of conformational changes in tRNA[phe] (yeast) by a differential melting technique, suggests that some elements of transitions may be caused by – or at least be influenced by – the structural transitions of the *VW* of hydration of the nucleic acid. By the same token, the concept of 'pre-denaturation' may be influenced by – or simply reflect – the thermal transitions of the *VW*.

4.   ROLE OF VICINAL WATER IN BIOPHYSICS
AND CELL BIOLOGY

In view of the inevitable vicinal hydration of all solid surfaces and of large macromolecules, it is hardly surprising that *VW* plays a critical role in cell biology. Space limitations do not allow a review of the large amount of information now available on this subject, but below a number of physiological elements and processes are

enumerated for which *VW* has been found to play an important – and sometimes a controlling – role. The reader is encouraged to check the various references listed, especially those of Clegg, Etzler, Mentre, Nishiyama, Wiggins, and the authors who contributed to the Symposium Proceeding: 'Cell-Associated Water' (Drost-Hansen and Clegg, 1979). More recently, the present author has discussed in some detail various aspects of the role of VW in cell biology (Drost-Hansen, 2001). Among the topics discussed in that paper from the point of view of *VW* and the thermal anomalies are:

- Enzyme kinetics and anomalies in Arrhenius graphs
- Membrane functioning
- Cell volume control
- Erythrocyte sedimentation rates
- Chromosome aberrations
- Seed germination
- Multiple growth optima
- Upper thermal limits for growth and Pasteurization temperature
- Body temperature selection in mammals and birds
- Thermal stress and hyperthermia therapy

5.   SUMMARY AND CONCLUSIONS

It has long been apparent that solid surfaces induce *VW* structures. It now appears that sufficiently large macromolecules in aqueous solution are also vicinally hydrated. There exists a 'critical MW range' [MWc] above which all dissolved macromolecules are vicinally hydrated while molecules with lower MW are not vicinally hydrated: the transition appears to fall between 1,000 and 5,000 Daltons. Like solid surfaces, the vicinal hydration of macromolecules is essentially independent of the specific chemical details (the 'Substrate-Independent Effect' or 'Paradoxical Effect'). In the case of the hydration of solid surfaces, the geometric extent of the *VW* is probably in the range of several tens of molecular layers and possibly as many as 100 layers and *it is likely that the vicinal hydration of macromolecules is of the same order of magnitude.*

The most notable characteristics of vicinal hydration are the highly anomalous temperature dependencies: anomalous changes are seen over four narrow temperature ranges (T_k) centered around 15, 30, 45 and 60°C. Apparently different geometric structures are stable between each of these intervals; heating a sample above any one of the characteristic temperatures destroys the vicinal hydration structure stable below the transition temperature and a new structure becomes stable. Upon lowering the temperature below the previous critical temperature, the vicinal hydration structures that are stable below the critical point become re-established. However, the reforming of the new structures takes time (depending on circumstances, from minutes to hours), and the properties may thus exhibit notable hysteresis.

The vicinal hydration structures are highly shear-rate dependent. This is clearly evidenced by the progressive decrease of change seen in the thermal anomalies

(at T_k) as shear rate is increased. The reforming of the vicinal hydration structures may be slow, similar to the hysteresis observed upon heating and cooling a sample past any one of the critical temperatures, T_k. Furthermore, high pressure, as might be found in ultracentrifuge measurements, is also likely to reduce the amount of *VW* of hydration of macromolecules. In addition, if the temperature of a sample has exceeded one of the critical temperatures, T_k, just before an experiment is begun, then the intrinsic vicinal hydration may have been destroyed and may reform only very slowly. In other words, such parameters as intrinsic viscosities, diffusion coefficients and sedimentation coefficients will depend strongly on the experimental protocol. In view of these facts, it is perhaps not surprising that estimates of hydrodynamic radii (and other transport properties, as well as some thermodynamic properties) may differ, even notably, from one investigator to another.

Since vicinal hydration can occur at all solid interfaces (including membranes) and with all large macromolecules in solution, it is little wonder that all cellular systems (i.e., all living systems) also show the effects of VW. Any biophysical or molecular biology theory that does not allow for – and specifically includes – VW must be judged to be incomplete.

6.   POSTSCRIPT

Even the most casual reading of this paper must surely suggest to the reader that thermal anomalies are an important and intrinsic characteristic of VW. Note that *their existence cannot be predicted from Ling's Association-Induction hypothesis for interfacial water* and in fact it does not appear that his hypothesis can ever accommodate, let alone explain, thermal anomalies. The *'Substrate Independence' effect is, of course, also inconsistent with Ling's theory, nor is the shear rate dependence a natural part of it*.

The Vicinal Water hypothesis, as it currently stands, is strictly empirical and does not allow for any detailed quantitative predictions or calculations. Progess in this direction will almost certainly have to await great advances in the statistical description of cooperative phenomena involving hydrogen bonding. On the other hand, Ling's Association-Induction hypothesis allows for semi-quantitative estimates of many parameters – even if some of these efforts may seem more like clever data-fitting than genuine theory. Furthermore, Ling's hypothesis critically depends on the existence of suitably located positive and negative charges to induce the alignment of water molecules, and the energetics of such geometries remain highly uncertain. However, is it possible that both Ling and I are 'partially blinded'? Do we merely describe 'different parts of the elephant'? In other words, is it possible that all interfaces of solids or large macromolecules induce VW (with all its attendant implications for density, heat capacity, entropy, transport properties, etc., and thermal anomalies), but within this framework of modified water structures the Association-Induction mechanisms may operate – assuming the presence of the requisite distribution of charges of opposite signs?

7. ACKNOWLEDGEMENTS

REFERENCES

Alpers W, Hühnerfuss H (1983) Molecular aspects of the system water/monomolecular surface film and the occurrence of a new anomalous dispersion regime at 1. 43 GHz. J Phys Chem 87:5251–5258

Anderson NG, Green JG (1975) The soluble phase of the cell. In: Roodyn (ed), Enzyme Cytology, Academic Press, New York, pp 475–490

Antonsen KP, Hoffman AS (1992) In: Harris JM (ed), Poly(ethylene glycol) Chemistry, Plenum Press, New York, pp 15–28

Bailey FE, Koleske JV (1976) Poly(ethylene oxide), Academic Press, New York

Braun CV Jr (1981) Calorimetric and dilatometric studies of structural properties and relaxations of vicinal water, MSc Thesis, Univ Miami at Coral Gables

Braun CV Jr, Drost-Hansen W (1976) A DSC study of the heat capacity of vicinal water in porous materials. In: Kerker M (ed), Colloid and Interface Science, Academic Press, New York, vol 3, pp 533–541

Brunner C (1847) Untersuchubg uberr die cohesion der flussigkeiten. Ann der Physik und Chem (Pogendorff's Annals) 70:481

Churev NV, Deryaguin BV (1985) Inclusion of structural forces in the theory of stability of colloids and films. J Colloid Interface Sci 103:542–553

Clegg JS (1979) Metabolism and the intracellular environment: the vicinal water network model. In: Drost Hansen W, Clegg JS (ed), Cell-associated Water, Academic Press, New York, pp 363–413

Clegg JS (1984a) Properties and metabolism of the aqueous cytoplasm and its boundaries. Am J Physiol 246:R133–R151

Clegg JS (1984b) Intracellular water and the cytomatrix: some methods of study and current views. J Cell Biol 99:167–171

Clifford J (1975) Properties of water in capillaries and thin films. In: Franks F (ed), Water – A Comprehensive Treatise Plenum Press, New York, pp 75–132

Daly JG, Hastings R (1981) Temperature dependence of Bragg scattering from crystallized suspensions of macroions. J Phys Chem 85:294–300

De Gennes PG (1997) Soft interfaces. The 1994 dirac memorial lecture. Cambridge University Press, Cambridge, p 117

Deryaguin BV, Physik Zeit. D Sowj. (1932) 4:431–432

Deryaguin BV, Physik Z (1933) 84:657–670

Deryaguin BV (1964) Recent research into the properties of water in thin films and in microcapillaries. The state and movement of water in living organisms, In 19th Symposium of Soc Exp Biol, Cambridge University Press, Cambridge, pp 55–60

Deryaguin BV (1975) Physik Zeit. D Sowj. 1932, also Zeitung für Physik 1933; 84:657–670 [For an extensive list of references, see list in 'Water – a comprehensive treatise' ('Water in disperse systems')], Plenum Press 5:335–336

Deryaguin BV (1977) Structural components of the disjoining pressure of thin layers of liquids. Croatica Chem Acta 50:187–195 (see also Clifford 1975)

Deryaguin BV, Churev NV (1987) Structure of water in thin layers. Langmuir 3:607–612

Dintenfass L (1981) Hyperviscosity in hypertension Pergamon Press, Sydney p 250

Dordick R, Drost-Hansen W (1981) High precision viscosity measurements. 2. Dilute aqueous solutions of binary mixtures of the alkali metal chlorides. J Phys Chem 85:1086–1088

Dordick R, Korson L, Drost-Hansen W (1979) High precision viscosity measurements on aqueous solutions of single and mixed electrolytes. 1. Alkali chlorides. J Colloid Interface Sci 72:206–214

Dorsey NE (1940) Properties of ordinary water substances, Rheinhold, New York

Dreyer G, Kahrig E, Kirstein D, Erpenbeck J, Lange F (1969) Structural anomalies of water. Naturwiss 56:558–559

Drost-Hansen W (1956) Temperature anomalies and biological temperature optima in the process of evolution. Naturwiss 43:512

Drost-Hansen W (1965) Aqueous interfaces – methods of study and structural properties. Part 1. Ind Eng Chem Res. March issue: 28–44, and Part 2 April issue:18–37

Drost-Hansen W (1965) The effects on biological systems of higher-order phase transitions in water. NY Acad Sci Ann, Art B 125:471–501

Drost-Hansen W (1968) Thermal anomalies in aqueous systems – manifestations of interfacial phenomena. Chem Phys Lett 2:647–652

Drost-Hansen W (1969) Structure of water near solid interfaces. Ind Eng Chem Res 61:10–47

Drost-Hansen W (1971) Role of water structure in cell-wall interactions. Fed Proc 30:1539–1548

Drost-Hansen W (1971) Structure and properties of water at biological interfaces. In: Brown HD (ed), Chemistry of the Cell Interface, Part B, Chapter 6, Academic Press, New York, pp 1–184

Drost-Hansen W (1972) Effects of pressure on the structure of water in various aqueous systems. The effect of pressure on organisms, vols XXVI, Symposia, Society for Experimental Biology, Cambridge University Press, pp 61–101

Drost-Hansen W (1976) The nature and role of interfacial water in porous media. Am Chem Soc, Div Petroleum Chem 21:278–280

Drost-Hansen W (1976) Structure and functional aspects of interfacial (vicinal) water as related to membranes and cellular systems. Colloq Internat du CRNS (L'eau et les systemes biologique) 246:177–186

Drost-Hansen W (1977) Effects of vicinal water on colloidal stability and sedimentation processes. J Colloid Interface Sci 58:251–262

Drost-Hansen W (1978) Water at biological interfaces – structural and functional aspects. Phy Chem Liquids 7:243–346

Drost-Hansen W (1981) Gradient device for studying effects of temperature on biological systems. J Wash Acad Sci 71:187–201

Drost-Hansen W (1982) The occurrence and extent of vicinal water. In: Franks F, Mathias S (ed), Biophysics of Water, Wiley and Sons, New York, pp 163–169

Drost-Hansen W (1985) Anomalous volume properties of vicinal water and some recent thermodynamic (DSC) measurements relevant to cell physiology. In: Pullman A, Vasilecu V, Packer L (ed), Water and Ions in Biological Systems, Plenum Press, New York, pp 289–294

Drost-Hansen W (1991) Temperature effects on erythrocyte sedimentation rates, cell volumes and viscosities in mammalian blood. Final grant report to USAF Office of Scientific Research, Bolling Air Force Base, Washington DC, p 29

Drost-Hansen W (1992) Rheological, biochemical and biophysical studies of blood at elevated temper-
atures Final grant report to US Office of Scientific Research, Boilling Air Force Base, Washington
DC, p 40

Drost-Hansen W (1996) Biochemical and cell physiological aspects of hyperthermi Final grant report to
US Office of Scientific Research, Boilling Air Force Base, Washington DC, p 20

Drost-Hansen W (1997) Long-range hydration of macromolecules in aqueous solutions. 2. 214th ACS
National Meeting, Las Vegas, Nevada, Abstract 274

Drost-Hansen W (2001) Temperature effects on cell functioning – a critical role for vicinal water. In
L'eau dans la cellule. J Cell Mol Biol 47:865–883

Drost-Hansen W, Neill H (1955) Temperature anomalies in the properties of liquid water. Phys rev, vols
100, Abstract 1800

Drost-Hansen W, Clegg JS (1979) Cell-associated water, Academic Press, New York, p 440

Drost-Hansen W, Lin Singleton J (1995) Our aqueous heritage: evidence for vicinal water in cells.
In: Bittar EE, Bittar N (ed), Principles of Medical Biology, vol 4, JAI Press Inc, Greenwich, CN,
pp 171–194; and 195–215

Drost-Hansen W, Braun CV Jr, Hochstim R, Crowther GW (1987) High precision dilatometry on aqueous
suspensions: volume contraction upon settling. In: Ariman T, Nejat Veziroglu T (ed), Particulate and
multiphase processes, vol 3, Hemisphere Publishing Corporation, Springer-Verlag, Berlin, pp 111–124

Etzler FM (1983) A statistical and thermodynamic model for water near solid surfaces. J Colloid Interface
Sci 92:94–98

Etzler FM (1991) A comparison of the properties of vicinal water in silica gel, clay, wood, cellulose,
and other polymeric materials. In: Levine H, Slade L (ed), Water Relationships in Foods, Plenum
Press, New York, pp 805–821

Etzler FM, Drost-Hansen W (1979) A role for water in biological rate processes. In: Drost Hansen W,
Clegg JS (ed), Cell-associated Water, Academic Press, New York, pp 125–164

Etzler FM, Drost-Hansen W (1983) Recent thermodynamic data on vicinal water and a model for their
interpretation. Croatica Chem Acta 56:563–592

Etzler FM, Lilies TL (1986) Ionic selectivities by solvents in narrow pores: Physical and biophysical
significance. Langmuir 2:797–800

Etzler FM, Fagundus D (1987) The extent of vicinal water. J Colloid Interface Sci 115:513–519

Etzler FM, White PJ (1987) Heat capacity of water in silica pores. J Colloid Interface Sci 94:98–102

Etzler FM, Conners JJ (1990) Temperature dependence of the heat capacity of water in small pores.
Langmuir 6:1250–1253

Etzler FM, Conners JJ (1991) Structural transitions in vicinal water: pore size and temperature depen-
dence of the heat capacity of water in small pores. Langmuir 7:2293–2297

Etzler FM, Conners JJ, Ross RF (1990) The structure and properties of vicinal water. In: Passeretti JD,
Caulfield DF (ed), Materials Interactions Relevant to the Pulp, Paper and Wood Industries, Material
Research Society Publishers

Etzler FM, Deanne R, Ibrahim TH, Burk TR, Neuman RD (2002) Direct adhesion measurements between
pharmaceutical materials. Particles on Surfaces, VSP Utrecht, Netherlands, pp 7–16

Etzler FM, Ibrahim TH, Burk TR, Wiulling GA, Neuman RDv (2005) The Effect of the acid-base
chemistry of lactose on its adhesion to gelatin capsules; conclusions from contact angles and other
surface chemical techniques. Contact Angle, Wettability and Adhesion vol 2, VSP Utrecht

Falk M, Kell GS (1966) Thermal properties of water: discontinuities questioned. Science 154:1013–1015

Gekko K, Noguchi H (1971) Physicochemical studies of oligodextrans. 1. Molecular weight dependence
of intrinsic viscosity, partial specific compressibility and hydrated water. Biopolymers 10:1513–1524

Henniker JC (1949) Rev Mod Phys 2:322–341

Hühnerfuss H (1987) Molecular aspects of organic surface films on marine water and the modification
of water waves. La Chemica e L'Industria 107:97–101

Hurtado RM, Drost-Hansen W (1979) Ionic selectivities of vicinal water in pores of a silica gel. In:
Drost Hansen W, Clegg JS (ed), Cell-associated Water, Academic Press, New York, pp 115–123

Johnson FH, Eyring H, Polisar MJ (1954) The kinetic basis of rheology, Wiley Sons, New York

Kaivarainen A (1995) Hierarchic concept of matter and field. Water, biosystems and elementary particles Privately published ISBN 0-9642557-0-7, p 483

Kerr J (1970) Relaxation studies on vicinal water. Dissertation, University of Miami, Coral Gables, FL

Korson L, Millero F, Drost-Hansen W (1969) Viscosity of water at various temperatures. J Phys Chem 73:34–38

Kurihara K, Kunitake T. (1992) Submicron-range attraction between hydrophobic surfaces in monolayer-modified mica in water. J Am Chem Soc 114:10927–10933

Lafleur M, Pigeon M, Pezelot M, Caille J-P (1989) Raman spectrum of interstitial water in biological systems. J Phys Chem 93:1522–1526

Lin GN (1965) The physical state of water in living cells and model systems. Ann New York Acad Sci, vols 125, pp 402–417 (article 2)

Ling GN (1962) A physical theory of the living state: the association-induction hypothesis, Blaisdell, Waltham, MA

Ling GN (1979) The polarized multilayer theory of cell water according to the adsorption-induction hypothesis. In: Drost Hansen W, Clegg JS (ed), Cell-associated Water Academic Press, New York, pp 261–269

Ling GN (1992) A revolution in the physiology of the living cell, Krieger Publishing Co, Malabar FL, p 378

Ling GN (2003) A new theoretical foundation for the polarized-oriented multilayer theory of cell water and for inanimate systems demonstrating long-range dynamic structuring of water molecules. Physiol Chem Phys Med NMR 35:91–130

Ling CS, Drost-Hansen W (1975) DTA study of water in porous glass. Adsorption at interfaces. ACS Symp Ser 8:129–156

Lopez-Lacomba JL, Gutzman M, Cortijo M, Mateo P, Aquirre R, Harvey SC, Cheung HC (1989) Differential scanning calorimetric study of the thermal unfolding of myosin rod, light meromyosin, and subfragment 2. Biopolymers 28:2143–22159

Low PF (1979) Nature and properties of water in Montmorillonite-water systems. Soil Sci Soc Am J 43:651–658

Lowe GDO (1987). Thrombosis and hemorheology. In: Cien S, Dormandy J, Ernst E, Matrai A Dordrecht (ed), Clinical hemorheology, Martinus Nijhof Publishers, pp 195–226

Mastro AM, Hurley DJ (1985) Diffusion of a small molecule in the aqueous compartment of mammalian cells. In: Welch R, Clegg JS (ed), Organization of cell metabolism, Plenum Press, New York, pp 57–74

Mentre P, Hui BH (2001) The effects of high hydrostatic pressures on living cells: A consequence of the properties of macromolecules and macromolecular associated water. Int Rev Cytol 201:1–84

Montejano JG, Hamann DD, Lanier TC (1983) Final strength and rheological changes during processing of thermally induced fish muscle gels. J Rheology 27:557–579

Montejano JG, Hamann DD, Lanier TC (1984) Thermally induced gelation of selected comminuted muscle systems – rheological changes during processing, final strengths and microstructure. J Food Sci 49:1496–1505

Nir S, Stein WD (1971) Two modes of diffusion. J Chem Phys 55:1598–1603

Okano M, Yoshida Y (1994) Junction complexes of endothelial cells in atherosclerosis-prone and atherosclerosis-resistant regions on flow dividers of brachiocephalic bifurcation in the rabbit aorta. Biorheology 31:155–169

Peschel G, Adlfinger KH (1969) Temperatur abhängigkeit der Viskosität sehr dünner Wasserschichten Quartzglasoberflächen. Naturwiss 58:558–559

Peschel G, Adlfinger KH (1970) Viscosity anomalies in liquid surface zones. 3. The experimental method. Ber Bunsen-Gesellschaft 74:351–357

Peschel G, Adlfinger KH (1971) Thermodynamic investigation of the liquid layers between solid surfaces. II. Water between entirely hydroxylated fused silica surfaces. Z Naturfor 26a:707–715

Peschel G, Adlfinger KH (1971) Viscosity anomalies in liquid surface zones. 4. The apparent viscosity of water in thin layers adjacent to hydroxylated fused silica. J Colloid Interface Sci 34:505–510

Peschel G, Belouschek P (1976) Eine neue Messmethode zur Untersuchung der Struktur dunner Elektrolytschichten zwischen Festkörperoberflächen. Prog Colloid Polym Sci 60:108–119

Peschel G, Belouschek P (1979) The problem of water structure in biological systems. In: Drost Hansen W, Clegg JS (ed), Cell-associated Water, Academic Press New York, pp 3–52

Phillips MC, Chapman D (1968) Biochim Biophys Acta 75:301

Rhykerd Jr CL, Cushman JH, Low PF (1991) Application of multiple-anlge-of-incidence ellipsometry to the study of thin films adsorbed on surfaces. Langmuir 7:2219–2229 [with references to Low's numerous earlier papers]

Sato M, Ohshima N (1994) Flow-induced changes in shape and cytoskeletal structure of vascular endothelial cells. Biorheology 31:143–155

Shoufle JA, Huang S-Y (1972) Tex J Sci 24:197 [see also J Geophys Res 1968; 73:3345]

Shoufle JA, Huang CT, Drost-Hansen W (1976) Surface conductance and vicinal water. J Colloid Interface Sci 54:184–202

Shephard J, Malmberg E, Logerot D (1974) Some rheological properties of an aqueous surfactant oil recovery agent. Preprint 48th Nat Colloid Symp, Austin TX, 191–196

Streekstra GJ (1990) The deformation of red blood cells in coquette flow. Dissertation, University of Utrecht, p 113

Uijttewaal W (1990) On the motion of particles in bounded flow: Applications in hemorheology. Dissertation, University of Utrecht, p 128

Urbanke C, Romer R, Maass G (1973) The binding of ethidium bromide to different conformations of tRNA: Unfolding or tertiary structure? Eur J Biochem 33:511–516

van Steveninck J, Paardekooper M, Dubbleman TMAR, Ben-Hur E, Leddeboer AM (1991) Anomalous properties of water in macromolecular gels. Biochim Biophys Acta 1115:96–100

Viani BE, Low PF, Roth CB (1983) Direct measurements of the relation between interlayer force and interlayer distance in the swelling of montmorillonite. J Colloid Interface Sci 96:229–234

Wiggins P (1975) Thermal anomalies in ion distribution in rat kidney slices and in a model system. Clin Exp Pharmacol Physiol 2:171–176

Wu MC, Lanier TC, Hamann DD (1985) Rigidity and viscosity changes of croaker actomyosin during thermal gelation. J Food Sci 50:14–19

Young TF (1966) Paper presented at Tetrasectional ACS meeting. Santa Fe, New Mexico