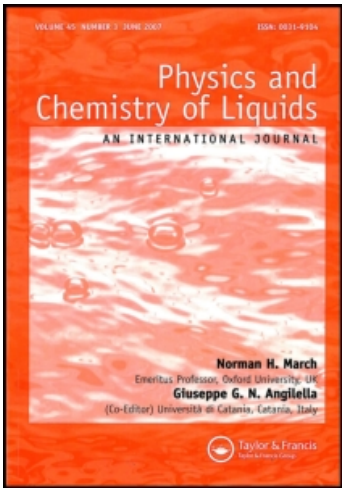

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X-RAY STRUCTURAL ANALYSIS OF HUMAN SERUM

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The structure of human serum was studied by X-ray diffraction method and the patterns were referred to those of pure water. For two groups of serum samples, the patterns differed significantly from those of pure water. One of the distinguished groups came from people suffering from neoplasmic diseases – the samples were studied directly after centrifugation. The other distinguished group included the samples studied after precipitation of proteins. The results obtained confirmed the earlier supposition that the dominant interactions in water low-concentration solutions of proteins in the serum of healthy people are those between water molecules and non-polar chains of side amino acids. For the samples of serum with precipitated proteins or obtained from hemolysed blood, the diffraction pattern shows a sharp maximum, testifying to the formation of a significant number of water–ion complexes disturbing the structure of pure water. The results have confirmed a possibility of using serum diffraction patterns as a diagnostic tool.

Keywords: X-ray diffraction patterns; Human serum; Intermolecular interactions

1. INTRODUCTION

The human blood serum is the blood plasma devoid of anticoagulant proteins. A high content of water, reaching 92% of the sample volume [1] means that the samples studied are much diluted water solutions of proteins. The presence of ions in the amount of 0.3035 mol/L [2], means that these samples are electrolytes. For over 60 years it has been known that molecules of pure water tend to assume such a configuration in which a central molecule is surrounded by four nearest neighbours, as it occurs in the structure of ice [3]. The tetrahedral structure of water has been confirmed by many authors [4–8].

In the samples studied, water molecules interact with molecules and ions of proteins. It is known that non-polar side chains of amino acids from the proteins have a stabilising effect on the structure of water [9]. The interactions between water molecules and ions known as ions' hydration [10] are much complex. The dominant ions in blood serum are sodium cations Na^+ (0.142 mol/L, which take about 47% of all ions) and chlorine anions Cl^- (0.102 mol/L, making about 34% of all ions) [2]. Assuming that

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Na^+ ions hydrate with four molecules of water, they take positions at the apices of a tetrahedron. The water molecules approach the cations with their electronegative side, i.e. the oxygen atom. The chlorine ions can be surrounded by an octahedron of six water molecules approaching these ions with protons forming hydrogen bonds. Such a distribution of water molecules undoubtedly disturbs the structure of water in the neighbouring layers. It has been established that hydration ions disturb the structure of water and the degree of this disturbance depends on the ions concentration, their charge and the size and kind of electron configuration [11]. In particular large radius and electric field of ions affect the orientation of water molecules. Already Bernal and Fowler [3] have shown that the effect of large ions on the water structure is analogous to that of an elevated temperature. The shoulders of the maximum of a typical angular distribution of scattered X-ray radiation, corresponding to the tetrahedral configuration of water molecules, disappears. The structure of dilute water solutions of electrolytes does not differ much from that of pure water. When the concentration of the electrolyte increases, (above 1 mol/L), the distribution of ions is no longer random because of the electrostatic interactions among the ion charges [10,11]. The structure of such an electrolyte is similar to that of ionic crystals.

2. EXPERIMENT

Diffraction patterns were recorded on a typical X-ray diffractometer equipped with a special cell for liquid samples [12,13]. A layer of the liquid studied restricted by mica windows was placed between the source of monochromatic X-rays MoKα and a counter probe. The pulses were counted within 120 s, in the angular range $2^\circ \leq \Theta \leq 40^\circ$, with a varying accuracy of $0.005^\circ \leq \Delta\Theta \leq 0.02^\circ$. The sample of pure water was three times distilled, while the samples of serum were defrosted prior to the measurement. A few samples were stored at 293–298 K for about 2–3 weeks in order to precipitate proteins and they were used for X-ray experiment after removal of the precipitate.

3. RESULTS

Figure 1 presents a comparison of the shapes of mean angular distributions of X-ray radiation scattered by the sample of pure water (broken lines) and those corresponding to different samples of serum from healthy and neoplasmic subjects numbered from 1 to 8.

The angular distributions of X-ray scattered radiation intensity were normalized to electron units $I_{eu}/N \pm 0.4$ eu per one molecule relative to pure water [9]. For the clarity of the picture, subsequent curves are vertically shifted by 20 I_{eu}/N. The main maxima of the curves from Fig. 1 correspond to the angles from the range $6.6^\circ \leq \Theta_{max} \leq 6.9^\circ$. The side maxima corresponding to the angles Θ in the range $8.5^\circ \leq \Theta \leq 10^\circ$, curves 1–4 in Fig. 1, are related to the characteristic tetrahedral arrangement of water molecules [3,14,15]. The other curves do not show distinct main and side maxima, they are merged into different shapes. Fig. 2 presents the angular distributions of X-ray scattered intensity for samples of human serum from healthy and neoplasmic patients studied immediately after centrifugation of blood (thin curves) and after precipitation of proteins (bold lines).

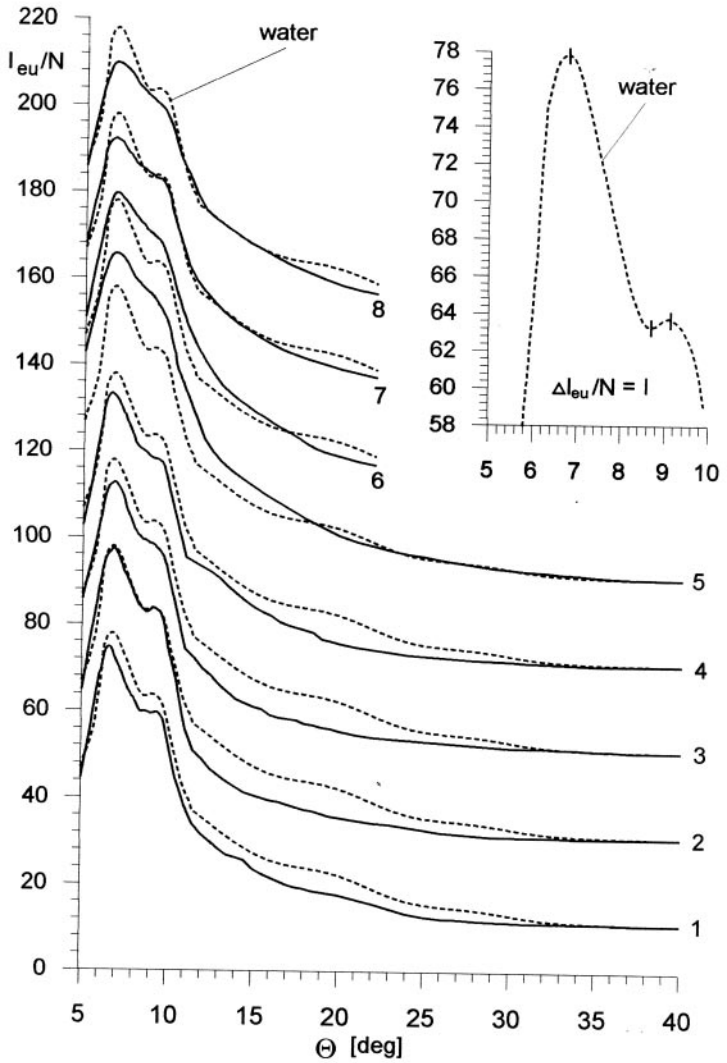

FIGURE 1 The mean angular distribution of X-ray scattered radiation intensity I_{eu}/N in electron units for pure water (broken lines) and for human serum from healthy subjects (curves 1–4) and neoplasmic patients (curves 5–8). The error is marked in the insert $\Delta(I_{eu}/N) = \pm 0.4$ eu.

The curves taken for the samples studied after precipitation of proteins (bold lines 1, 2, 3 in Fig. 2) as well as those recorded for hemolysed blood samples (curves 4 and 5, Fig. 2) reveal a sharp peak at about 9.60 corresponding to the side maximum for pure water.

4. DISCUSSION AND CONCLUSIONS

The character of curves 1, 2, 3 and 4 obtained for the samples of human serum from healthy subjects is similar to that for pure water (broken lines, Fig. 1). As has been

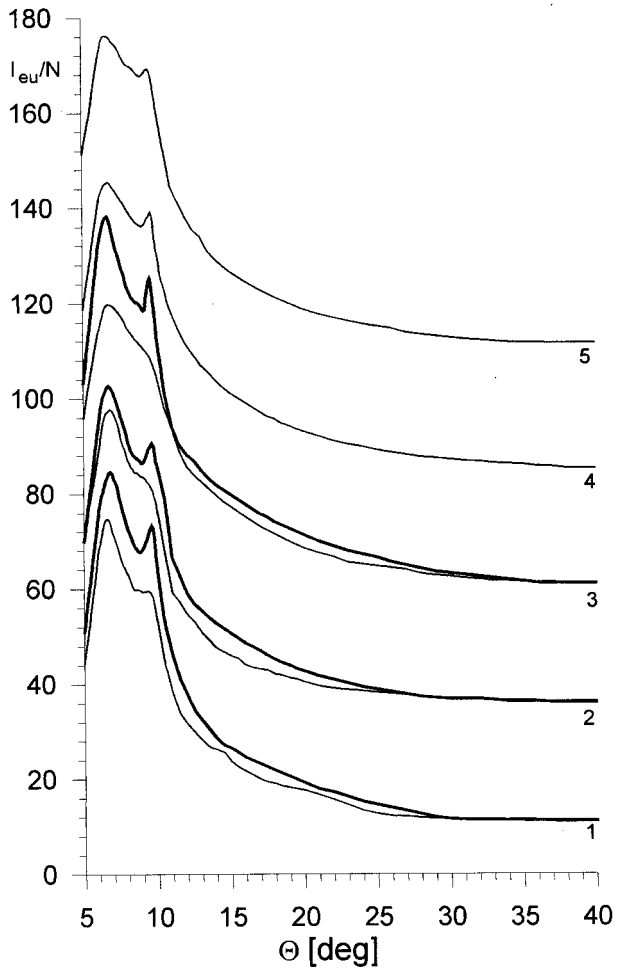

FIGURE 2 Angular distribution of X-ray radiation intensity scattered in samples of human serum of healthy people (1, 2, 4, 5) and for a neoplasmic patient (curve 3). Thin lines correspond to the samples studied immediately after centrifugation and bold lines to the samples studied after precipitation of proteins. The thin and bold curves 1, 2 and 3 have been determined for the same individuals, curves 4 and 5 represent results obtained for samples from healthy subjects after hemolysis.

established earlier [9], the water from human serum preserves the structure of pure water because of the stabilising effect of the non-polar amino acid chains of proteins. The ordering effect of non-polar groups on the structure of water has been already presented [16,17]. It is also known that the greatest stabilising effect of the amino acid groups occurs in the range of small and medium concentrations of proteins, up to 5%, and at higher concentrations it is not observed [18]. At higher concentrations of proteins the conformations of proteins changes, e.g. small polypeptide chains agglomerate, forming helices or globules characteristic of the tertiary structure of proteins [19,20]. This process can also be a result of disease caused changes. In the blood of patients with neoplasmic disease, the so-called acute phase proteins appear [2], whose concentration can reach even to about 50%. In such circumstances, the polar side amino acid chains on the surface of the globules interact through hydrogen bonds

with water molecules and anions, disturbing the structure of water [19,21]. This disturbing effect is reflected in curves 5, 6, 7 and 8, in Fig. 1, recorded for samples from neoplasmic patients.

The diffraction patterns recorded for human serum of healthy subjects and neoplasmic patients differ from those obtained for the samples studied after precipitation of proteins, Fig. 2. In the patterns of the latter, a sharp maximum appears in the range $8^\circ \leq \Theta \leq 10^\circ$, reflecting the ordering typical of pure water, Fig. 2, bold curves 1, 2 and 3. Most probably in the samples after precipitation of proteins the ions have been hydrated. The facilitated access of ions to water molecules disturbs the water structure. The ion–water complexes become distinct scattering objects and their presence is responsible for a sharp maximum at about 9.6°. It is known that the presence of cations (e.g. Na^+) and anions (e.g. CL^-) disturbs the structure of water [6], but when they are present at low concentration in healthy human serum their effect is negligible, because they are uniformly distributed in the vicinity of polar chains of proteins with which they can form hydrogen bonds. The diffraction patterns of healthy human serum are similar to those of pure water, the structure of water is disturbed by the growth and disappearance of protein in serum. The bold curves in Fig. 2 illustrate the effect of newly formed water-ionic complexes on the structure of water. As has been established earlier [2], in the hemolysed blood and in the blood of patients suffering from certain neoplasmic diseases, the concentration of ions in blood serum increases, e.g. that of K^+ ions increases ~ 10 times. A high concentration of ions can induce the formation of ordered microregions because of the Coulomb interactions. The diffraction pattern of blood serum is a result of the appearance of these ordered microregions or ionic complexes and the preserved fragments of the structure of pure water.

The above-discussed results suggest that diffraction patterns of properly prepared and stored blood serum carry important information about the health status of patients.

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