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RHEOLOGY OF BIOLOGICAL AND BIO-ARTIFICIAL FLUIDS

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ABSTRACT

Although rheology is not widely utilized in medical research as compared to polymer science, it can provide valuable contributions wherever biological fluids are studied. Rheology applies a certain mechanical load to the sample and the receiving flow properties are measured. This can help to investigate the flow behavior of biological fluids under simulated "natural" conditions and achieve a better understanding of the flow behavior of these fluids.

This study focused on investigating the flow behavior of cerebrospinal fluid (CSF, human) and synovial fluid (SF, horse). A closer look at CSF and SF shows that these biological fluids exhibit certain similarities to polymer solutions. CSF and SF are aqueous solutions which contain certain amounts of macromolecules, such as different proteins. These macromolecular components are only present in low concentrations; however, they are expected to be responsible for the specific flow behavior. In order to support this relation, corresponding artificial bio-fluids were prepared and rheologically characterized following the same procedure as for the biological fluids. Specific interactions in such biological fluids can be reflected by viscoelasticity. Therefore CSF, SF and the relevant artificial fluids were compared based on their viscoelastic behavior.

The overall goal is to gain a better understanding of the rheological behavior of these biological fluids and their response to applied mechanical load in shear flow. Additionally, it is investigated whether rheological measurements can aid clinical practice for disorders involving CSF or SF.

INTRODUCTION

Studying the flow behavior of materials is a cornerstone of polymer science as it allows for the mechanical characterization of polymer samples. Rheology is comparatively underrecognized in the medical sciences[1, 2]. Biological fluids are typically dilute aqueous matrices that contain macromolecular components, such as proteins, which are expected to modulate their flow and viscoelastic properties[3-5]. Biological materials rarely behave as ideal elastic solids or ideal viscous liquids. Instead, constituent interactions in synovial (SF) and cerebrospinal fluids (CSF) lead to viscoelasticity[6]. Viscoelasticity can be investigated using oscillatory rheometry, which quantifies the elastic component (storage modulus, G′) and the viscous component (loss modulus, G″)[7]. Rheology may provide a positive contribution to characterizing the flow

behavior of biological fluids and further on to evaluating whether rheological parameters may provide an addition to the treatment decisions in the clinical routine.

This study focused on equine SF, CSF from human hydrocephalus patients, and artificial alanogues to determine how individual components influence the specific flow behavior. SF acts as a lubricant, supplies the avascular articular cartilage with nutrients and removes metabolic waste[8]. CSF serves to protect the brain and spinal cord and therefore must exhibit properties that enable effective mechanical cushioning and regulatory properties[9]. Some studies have already been focused on the investigation of artificial fluids. Bortel et al.[10] formulated artificial SF to make joint prosthesis wear testing more physiologically realistic. Artificial CSF has also been investigated by Zheng et al.[11] to replicate the ionic composition of native CSF, thereby providing a controlled, physiologically relevant medium for reproducible experiments. Therefore all samples the biological, as well as the bio-artificial samples were rheologically investigated with a rotational rheometer, equipped with a measuring geometry especially for low viscosity fluids[12].

MATERIAL AND METHODS

SF samples were drawn postmortem from horses of the vet clinic Tillysburg. The CSF samples were drawn from human hydrocephalus patients via an external ventricular drainage places for routine treatment after intracranial hemorrhage at the Department of Neurosurgery, Kepler University Hospital and Johannes Kepler University Linz.

To enable a more detailed investigation, artificial analogues were prepared from its principal composition and most relevant constituents. CSF consist of proteins, glucose, lactate and electrolytes[3, 4]. Given that albumin is reported to account for more than 50% but less than 75% of total protein, albumin was assumed to represent the protein fraction[3]. For the synovial fluid analogue, a hyaluronan solution was applied[10]. Human Albumin (20%), D(+)Glucose, Sodium L-Lactate and a ringer solution were used for preparation.

Table 1 presents the sample composition of the artificial CSF which was prepared in 100 mL ringer solution [3, 4]. Additionally an albumin solution (19 g/L) and a hyaluronic acid (HA) solution (3 g/L) were prepared in ringer solution.

TABLE 1: Compostion of artificial samples[3, 4].

Sample	Albumin	Glucose	Lactate
aCSF	180 µL	68.7 mg	18.6 mg

For the rheological measurements a rotational rheometer Physica MCR702 (Anton Paar Ltd., Graz, Austria) with a double gap geometry (DG26.7/T200/SS). Rotational and oscillatory rheological tests were conducted to characterize the steady-shear and viscoelastic behavior. The measurement parameters were applied as shown in **Table 2.**[12].

TABLE 2: Measurment parameters for rheology[12].

measurement	shear rate / s^{-1}	Angular fregency / rad s^{-1}	deformation / %
Rotational test	1-700	-	-
Amplitude test	-	10	0.01-100
Frequency test	-	0.1-628	10

To simulate relevant conditions in human and veterinary contexts, measurements were conducted at defined temperatures. For human CSF, the setpoints were: 5°C (storage), 35°C (hypothermic), 37°C (physiological), and 40°C (elevated body temperature). For equine synovial fluid, measurements were performed at 5°C (storage), 20°C (room temperature), 36°C (hypothermic), 37°C (physiological), and 39°C (elevated body temperature).

RESULTS AND DISCUSSION

The roatational test showed a non-Newtonial behavior in the low shear rate range for both the human CSF and the artificial CSF, meaning the viscosity is changing with varying shear rate (**Fig. 1.**). The higher the shear rate the more unaffected the viscosity is and resulting in an Newtonial behavior.

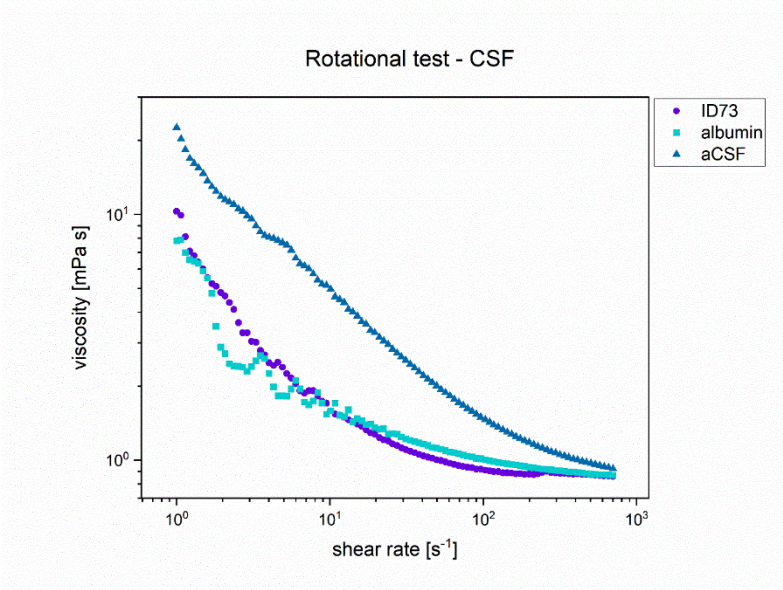

FIGURE 1: Rotational test comparision of albumin solution, artificial CSF and human CSF ID73. Non-Newtonian behavior in the low shear rate range, but with increasing shear rate viscosity is getting constant.

In **Fig. 2**. The rotational test of equine SF shows decreasing viscosity with increasing shear rate over the whole measurement range. In contrast to the hyaluron solution which exhibits a long Newtonian plateau.

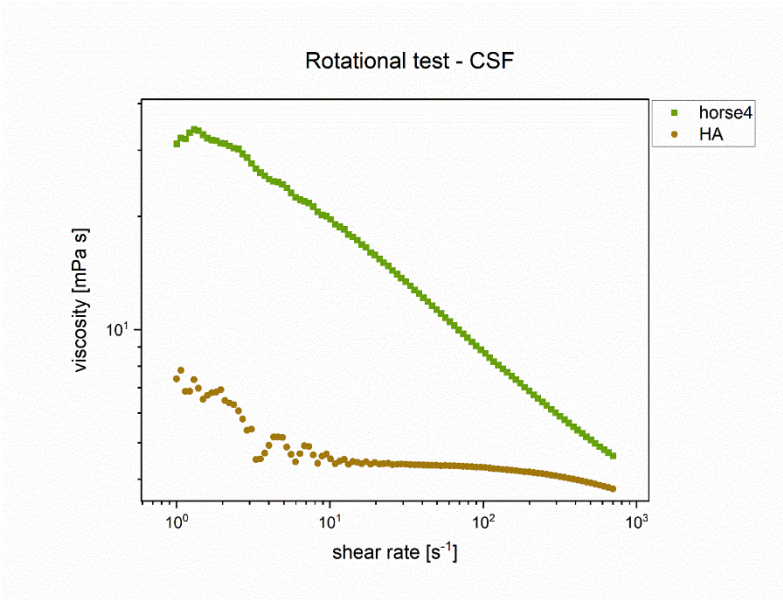

FIGURE 2: Rotational test comparison of HA and equine SF.

Prior to the frequency test amplitude tests were performed to define the linear viscoelastic region of the samples to ensure reversible deformation. In **Fig. 3.** and **Fig. 4.** the amplitude tests are shown. For all samples 10% deformation were preset for the frequency test.

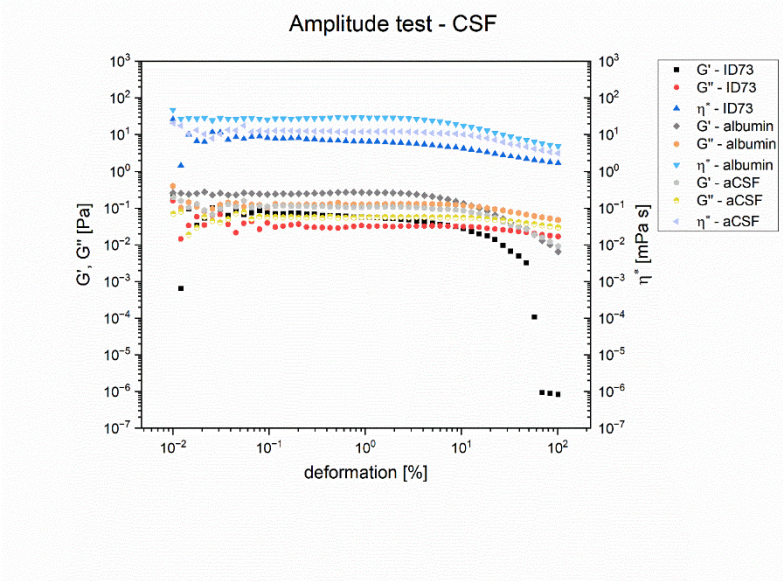

FIGURE 3: Amplitude test comparision of albumin solution, artificial CSF and human CSF ID73.

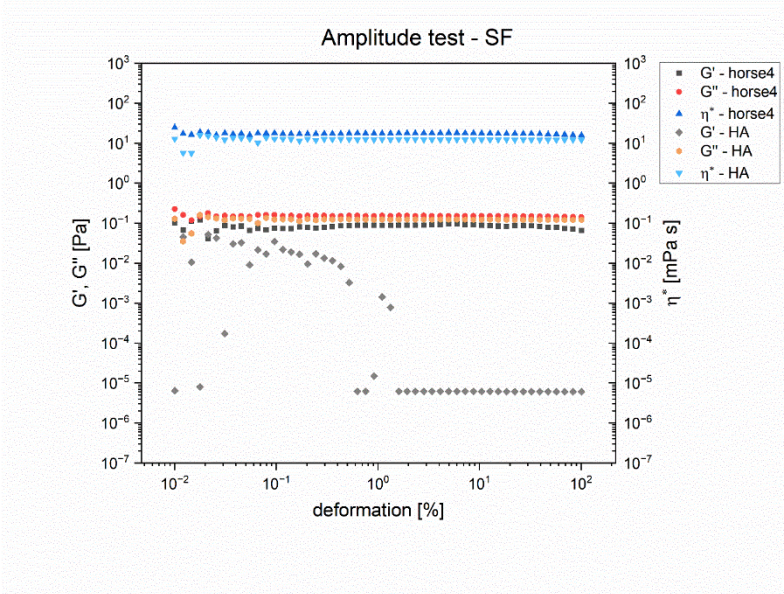

FIGURE 4: Amplitude test comparision of hyaluronic acid and equine SF.

In the case of CSF, an albumin solution, the artificial CSF and human CSF (ID73) were compared in the frequency test, shown in **Fig. 5.** Albumin is showing the lowest curves and storage modulus (G') curve is even lower than the loss modulus (G'') curve different from the artificial CSF and the human CSF. This suggests that the albumin solution is more viscose dominated compared to the other two. The complex viscosity shows shear thinning behavior as the viscosity decreases with increasing angular frequency. Human CSF shows the highest viscosity compared to the artificial CSF and the albumin solution.

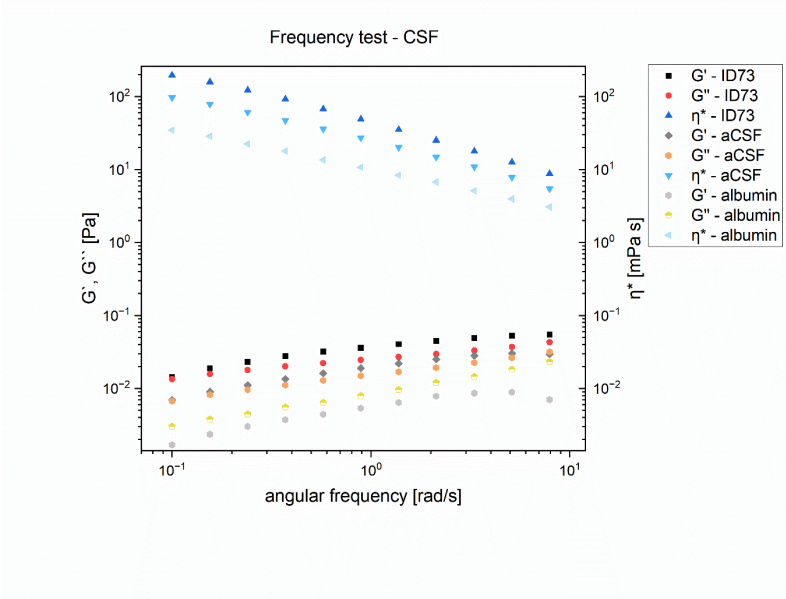

FIGURE 5: Frequency test comparison albumin solution, artifical CSF and human CSF ID73.

The horse SF and the hyaluronic acid solution are much more different compared to the CSF system, which can be seen in **Fig. 6.** For the horse SF and the albumin solution the loss modulus curve is higher than the storage modulus curve intending higher viscous amount in the flow behavior. The G' and G'' curves of the horse SF are much more steep, resulting in an increased frequency dependancy. That contrasts with the complex viscosity showing a flatter, near-Newtonian plateau.

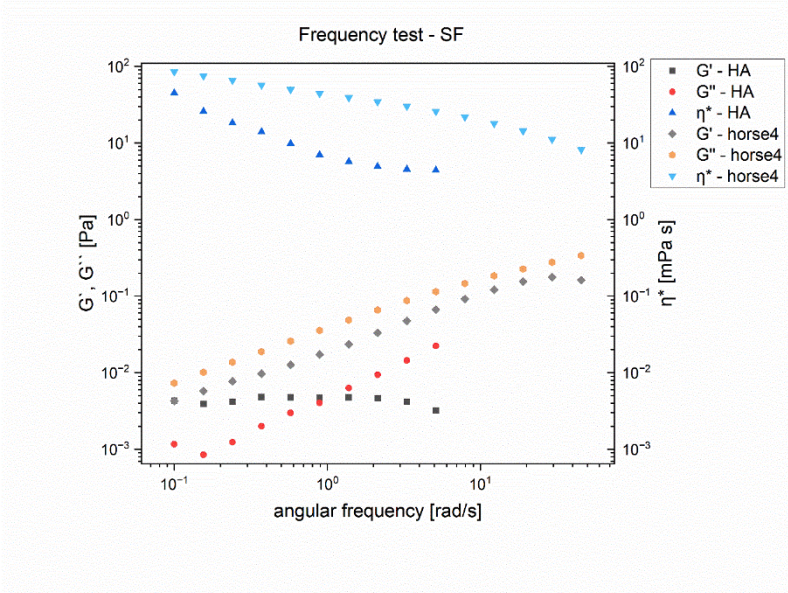

FIGURE 6: Frequency test comparision HA solution and equine SF.

Comparing the different temperature conditions applied for the frequency test is shown in **Fig. 7.** and **Fig. 8.** Both figures show the same tendency with inceasing temperature the values for storage, loss modulus and complex viscosity are increasing. This is acting against the natural trend that would be expacted to be decreasing viscosity with increasing temperature. For proteins it is known that at higher temperature thermical induced structural changes can appear[13]. This could be one reason for the unexpected trend. **Fig. 8.** shows the frequency test of the hyaluron soluction with defined measurement temperatures. The complex viscosity curve changes the shape between the low temperature measurements (5°C, 20°C) and the higher temperature measurements (35°C, 37°C, 40°C). At low temperatures the complex viscosity curve has a Newtonian shape, but with increasing temperature a shear thinning behavior appears.

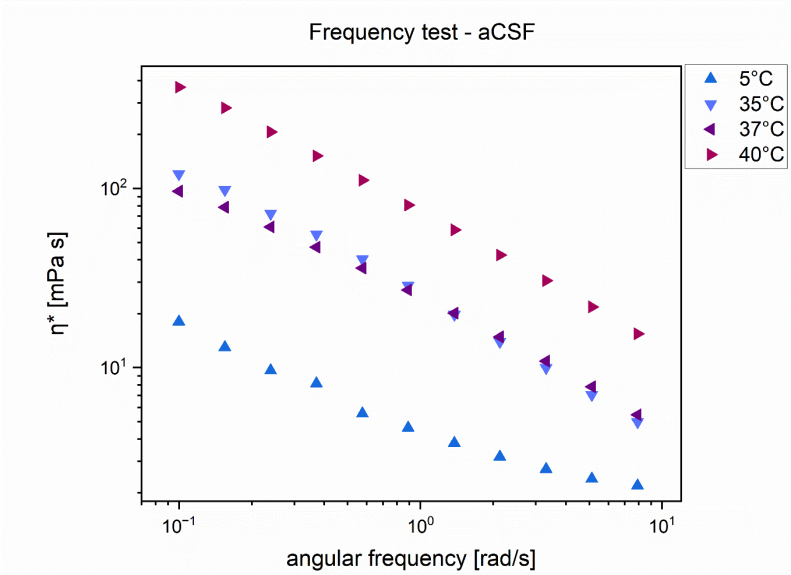

FIGURE 7: Frequency test of artificial CSF over defined measurment temperatures.

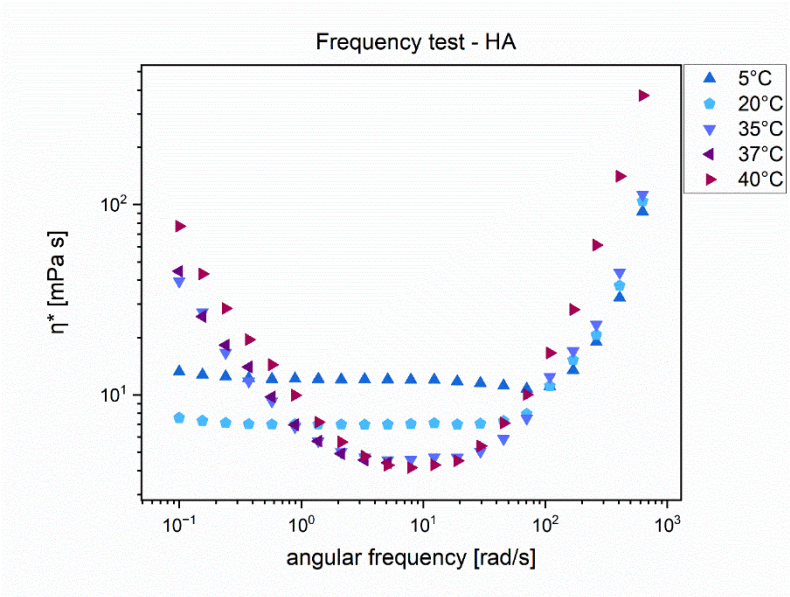

FIGURE 8: Frequency test of hyaluron solution over defined measurement temperatures.

CONCLUSION

The rheological measurements show that albumin and hyaluronic acid have a big influence on the flow behavior of the CSF and SF and potentially provide an effort for a better understanding of the rheological behavior of these biological fluids and their response to applied mechanical force. Further investigations will focus on the temperature trend and the influence of different components of the flow properties to reach the overall goal to evaluate if rheological parameters can positively contribute to clinical practice for CSF and SF disorders.

Ethical Statement: This study involving human samples was approved by the Ethics Committee, Faculty of Medicine, Johannes Kepler University Linz, MED Campus I, ADMBuilding 7th Floor, Krankenhausstaße 5, 4020 Linz (approval No. 1025/2022) on 1 July 2022. This study was conducted in accordance with local legislation and institutional requirements. The participants provided written informed consent to participate in this study.

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Conflicts of Interest: The authors have no conflicts to disclose.

REFERENCES

1. Bloomfield, I. G.; Johnston, I. H.; Bilston, L. E. Effects of proteins, blood cells and glucose on the viscosity of cerebrospinal fluid. *Pediatric neurosurgery* **1998**, *28*, 246–251. https://doi.org/10.1159/000028659.
2. Hollister, J. C. P.; Wang, A. C.; Kim, W.; Giza, C. C.; Prins, M. L.; Kavehpour, H. P. Shear thinning behavior of cerebrospinal fluid with elevated protein or cellular concentration. *Frontiers in Physics* **2023**, *11.* https://doi.org/10.3389/fphy.2023.1308136.
3. Wilhelmy, F.; Krause, M.; Schob, S.; Merkenschlager, A.; Wachowiak, R.; Härtig, W.; Meixensberger, J.; Gburek-Augustat, J.; Wende, T. Cerebrospinal Fluid Protein Concentrations in Hydrocephalus. *Children* **2023***, 10*(4), 644. https://doi.org/10.3390/children10040644.
4. Czarniak, N.; Kamińska, J.; Matowicka-Karna, J.; Koper-Lenkiewicz, O.M. Cerebrospinal Fluid–Basic Concepts Review. *Biomedicines* **2023**, 11, 1461. https://doi.org/10.3390/biomedicines11051461.
5. Bauer, T.-C.; Bradt, E.; Hild, S.; Gruber, A.; Rossmann, T.; Ruiz-Navarro, F.; Oberndorfer, J.; Stefanits, H.; Kracalik, M. Rheology of Cerebrospianl Fluid under Different Temperature Conditions. *Annu. Trans. Nord. Rheol. Soc.* **2023**, *31*, 87–92.
6. Michieletto, D.; Marenda, M. Rheology and Viscoelasticity of Proteins and Nucleic Acids Condensates. *JACS Au* **2022**, 2, 1506–1521. DOI: 10.1021/jacsau.2c00055.
7. Kracalik, M. Recycled clay/PET nanocomposites evaluated by novel rheological analysis approach. *Appl. Clay Sci*. **2018**, 166, 181–184. https://doi.org/10.1016/j.clay.2018.09.007.
8. Edwards, N.L. Cellular Components of Synovial Fluid in Health and Disease. In: Mandell, B.F. (eds) Synovial Fluid Analysis and The Evaluation of Patients With Arthritis. *Springer, Cham.* **2022**. https://doi.org/10.1007/978-3-030-99612-3_5..
9. Hladky, S.B.; Barrand, M.A. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. *Fluids Barriers* **2014**,CNS 11, 26. https://doi.org/10.1186/2045-8118-11-26.
10. Bortel, E.L.; Charbonnier, B.; Heuberger, R. Development of a Synthetic Synovial Fluid for Tribological Testing. *Lubricants* **2015**, 3, 664-686. https://doi.org/10.3390/lubricants3040664.
11. Zheng, W. H.; Yan, C.; Chen, T.; Kang, D. Z. New scheme for the preparation and use of artificial cerebrospinal fluid. *Journal of physiology and pharmacology* **2020**, 71(6), https://doi.org/10.26402/jpp.2020.6.15.
12. Bauer, T.-C.; Bradt, E.; Hild, S.; Gruber, A.; Rossmann, T.; Ruiz-Navarro, F.; Oberndorfer, J.; Stefanits, H.; Kracalik, M. Rheological Characterization of Cerebrospinal Fluid Under Different Temperature Conditions. *Fluids* **2026**, 11, 38. https://doi.org/10.3390/fluids11020038.
13. Anomalous change of viscosity and conductivity in blood plasma lipoproteins in the physiological temperature range. *Int. J. Quantum Chem.* **2001**, 81, 348–369. DOI: 10.1002/1097-461X(2001)81:5<348:AID-QUA1004>3.0.CO;2-H.